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Evaluation of microsatellite markers for parentage verification in South African Angora goats

By

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DECLARATION

I, Henriëtte Friedrich, do hereby declare that the research presented in this dissertation, was executed by myself, and apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this University or any other University.

This dissertation is presented in partial fulfilment of the requirements for the degree MSc (Agric) in Animal Breeding.

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Date……..

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Proverbs 12:10 A righteous man regards the life of his animal, but the tender mercies of the wicked are cruel.

ABSTRACT

South Africa is currently the world leader in mohair production and emphasis is placed on mohair traits to ensure the production of a high quality clip. Accurate and complete pedigree information is a prerequisite for accurate selection and estimation of breeding values that in turn can improve the traits associated with fleece quality and yield. South African Angora goats are farmed under extensive breeding conditions in relatively large herds. As a result breeders make use of mating systems that tend to limit accurate parentage recording. Incorporation of genetic parentage testing into the breeding plan can improve the accuracy of pedigree records and lead to increased selection accuracy. Microsatellite markers were evaluated for inclusion into a parentage verification panel, to be applied in the South African Angora goat population. The panel of 18 microsatellite markers was constructed, optimized and tested in 200 South African Angora goats. These goats represented different family structures, including candidate parents alone as well as known and candidate parents. The microsatellite markers were evaluated based on the number of alleles, allele frequency, PIC, H_E, H_O and CPE of each individual marker and as a panel. Four microsatellite markers were excluded from the panel based on their poor performance for the above mentioned parameters. The panel of 14 markers allowed a cost effective panel with the highest exclusion power. The CPE_1 of the 14 microsatellite marker panel was 99.73%. The use of molecular parentage verification may aid Angora goat breeders in improving the accuracy of the parentage records of their animals.

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LIST OF ABBREVIATIONS

BLUE	Best linear unbiased estimation
BLUP	Best linear unbiased prediction
cM	Centimorgan
CPE	Combined Exclusion Probability
DFP	DNA fingerprints
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleotide triphosphate (A, C, G, T)
EBVs	Estimated Breeding Values
FABI	Forestry and Agricultural Biotechnology Institute
FAO	The Food and Agriculture Organisation
F_{Null}	Null allele frequency
GADI	Grootfontein Agricultural Development Institute
GDP	Gross Domestic Product
H_E	Expected heterozygosity
H_O	Observed heterozygosity
IBD	Identical by descent
IBS	Identical by state
ISAG	International Society of Animal Genetics
LOD	Natural logarithm ($\log_e$) of a combined likelihood
mM	Millimolar
ng/µl	Nanograms per microlitre
nM	Nanomolar
PCR	Polymerase Chain Reaction
PE_1	First parent exclusion probability
PE_2	Second parent exclusion probability
PE_P	Parent pair exclusion probability
PE_{ID}	Exclusion probability of identity
PE_S	Exclusion probability of sib identity
PIC	Polymorphic Information Content
pmol/µl	Picomol per microlitre

QTL	Quantitative trait loci
R	South African Rand
RFLP	Restricted fragment length polymorphism
SNP	Single Nucleotide Polymorphisms
Tm	Annealing temperature
µl	Micro litre

CHAPTER 1: INTRODUCTION

1.1 History of the Angora goat and the South African Mohair Industry

Goats provide useful products to man, such as meat, milk, skin and fibre (MacHugh & Bradley, 2001; Malan, 2000; Sung *et al.,* 1999 and Shelton, 1981). These traits were probably the driving forces that resulted in the domestication of goats. Archaeological findings suggest that goats were the first wild herbivore to be domesticated from its ancestor the bezoar (*Capra aegagrus*) (MacHugh & Bradley, 2001). Some literature suggests that domestication of goats (*Capra hircus*) began around 8000 B.C. in more than one site in Asia and Africa (Shelton, 1993; Zeder & Hesse, 2000) whereas other scientists suggest that this occurred between 1571 and 1451 B.C. (Briggs, 1970; Yalçin, 1986).

It is generally accepted that the Angora goat was domesticated in the region of Angora (now the city of Ankara) in Central Anatolia, Turkey (Yalçin, 1986; Shelton, 1993). The Angora goat is the only breed that produces mohair. From as early as the sixteenth century Angora goats were exported to European countries in an attempt to establish a mohair industry outside of Turkey. These attempts were mostly unsuccessful because the climates in these countries were unfavorable for Angora goat farming (Shelton, 1993).

During the late eighteenth and early nineteenth centuries the industrial revolution in England brought about many changes in the textile industry (Deane, 1979). By 1835 mohair spinning had developed into a significant industry and this led to a high demand for mohair (Van der Westhuysen *et al.,* 1985). The climate in Britain was unfavourable for Angora goat production and therefore British breeders could not meet this demand. South Africa, a British colony at the time, had regions with a similar climate to Turkey and was identified as a favourable region for Angora goat production. During 1837 a South African, Colonel John Henderson, persuaded the Turkish authorities to allow the exportation of Angora goats to South Africa. The exact number of goats that were imported is unclear from historical documents. Upon the goats' arrival in Port Elizabeth it was discovered that all the bucks had been castrated to prevent the establishment of a rival market (Pringle, 1989; www.bkb.co.za). One ewe was however pregnant with a

male kid which resulted in the introduction of the Angora goat to South Africa. During 1856 to 1896 over 3000 Angora goats were imported to South Africa and this resulted in the establishment the breed in South Africa (Pringle, 1989; Shelton, 1993). Today, Angora goats are still farmed mainly in the Karoo region (which means "thirsty land") as well as parts of the Eastern Cape with an arid and harsh climate. This region is encircled and marked "A" on the map of South Africa shown in Figure 1.1.

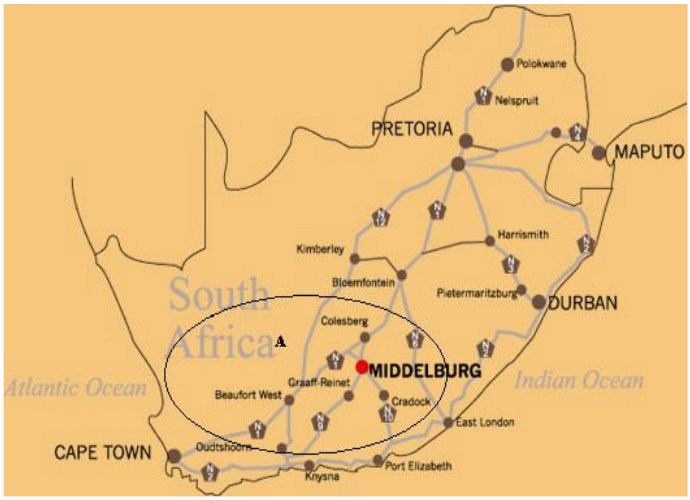

Figure 1.1 The Karoo region in South Africa (http://www.karoocountryinn.co.za/images/map1.jpg)

The modern South African Angora goat is a small sized goat that stands 55 – 58 cm high at the withers and is 60 – 62 cm long. Mohair is a long, strong and relatively uniform lustrous fibre (Yalçin, 1986) as can be seen in Figure 1.2.

Figure 1.2 South African Angora goat ewe and kid

Over the past 150 years the South African Mohair industry has experienced changes in production and relative importance. During the first decade of the twentieth century approximately 5.44 million kilograms mohair was produced in South Africa annually and this number reached a record in 1912 when 9.21 million kilograms mohair were produced by 4.4 million Angora goats (Pringle, 1989). During the Great Depression and World War II the mohair industry shared in the effects of the severe economical recession. This lead to a sharp decline in South African mohair production with output levels as low as 1.4 million kilograms in 1945. By 1950, wool and mohair prices began to rise and textile farmers received better returns on their products. In the mid-1960's South Africa experienced a devastating drought that placed enormous financial strain on Angora goat farmers. As a result the number of Angora goats in the country decreased from 1.8 million to 0.6 million between 1965 and 1971 and a sharp decline in production followed (Pringle, 1989). Many Angora goat farmers diverted to mutton sheep breeding, as meat production was more profitable than mohair production (Pringle, 1989). By 1988 the South African mohair industry had recovered to approximately 2.9 million Angora goats producing an average of 12.2 million kilograms of mohair per annum (www.bkb.co.za).

From 1988 to 2003 the world mohair production decreased by almost 70% from 26 million kg to 6.6 million kg per annum (Van der Westhuysen, 2005). A similar trend was observed in South Africa where production declined from 10.1 million kilograms in 1990 to 4.0 million kilograms in 2003/2004 and 3.4 million kilograms in 2005/2006 (http://www.nda.za/). The major reason for the decline in the world and South African production of mohair was a change in fashion trends and a shift towards synthetic fibres compared to expensive natural fibres (Humphries, 2004). In 1950 approximately 19% of the world textiles were produced synthetically and 81% consisted of natural fibres. This proportion changed drastically over the years and by 2000, 57% of the world's textiles were synthetically produced and only 43% was natural fibres of which approximately 40% was cotton and 0.03% was produced by goats (Humphries, 2004; van der Westhuisen, 2005). Two additional causes led to the decline in the mohair output in South Africa. Meat prices increased, providing breeders with a better return when farming with meat producing animals than fibre production animals (Mangxamba, 2005).

The other contributing factor to the decrease in production was the change in land use from farming with small stock to more profitable enterprises such as game farming (Mangxamba, 2005).

Despite these factors South Africa had become the world leader in mohair production. By 1995 South Africa produced 43.6% of the worlds' mohair. This contribution increased to 61.3% in 2001 (www.agrimark.co.za/mohair.htm). Today South Africa is the highest producer of mohair clip in the world and during 2007 produced approximately 54% of the world's mohair (Retief, 2008). During 2007 South Africa exported 3 477 630 kg mohair to 18 countries worldwide (Retief, 2008), including Taiwan, France, India, Korea, Japan, China, Germany, Turkey, USA, UK, Australia, Spain and Italy (van der Westhuysen, 2005; Retief, 2008).

The demand for mohair in the fashion industry remains difficult to forecast. The effect of the world-wide financial crisis and economical recession that commenced in 2008 is a further factor that will have an impact on the demand. Despite this bleak forecast, as a result of the global phenomenon of the prevention and regulation of global warming, fashion houses may include more natural fibres in their ranges (Angora goat and Mohair Journal, Autumn 2008). The Food and Agriculture Organisation (FAO) has declared 2009 as International Year of Natural Fibres. This might shift the emphasis once again to the importance of natural fibres and as mohair is one of the world's fifteen major natural fibres, might highlight this fibres importance once again (http://www.naturalfibres2009.org/).

1.2 South African Angora goat breeding practices

There are currently approximately 2.9 million Angora goats in South Africa (http://www.nda.za/) which are mostly farmed under extensive or semi-extensive production systems. Herd sizes vary between 1000 and 2500 goats per herd. The majority of South African Angora goat breeders have both a commercial and a stud herd.

Only a few South African Angora stud breeders make use of artificial insemination. This is not a common practice and many breeders will only inseminate ewes if a buck has exceptional breeding values (Personal communication: Dr M.A. Snyman; Grootfontein Agricultural Development Institute, Jansenville, Private Bag X529, Middelburg Cape, 5900, South Africa; January 2009). This would be the best system with the lowest error margin for parentage allocation (Ganai & Yadav, 2005). The large herd sizes and extensive farming conditions make the use of hand mating unpractical and too laborious for South African Angora goat breeders.

Breeders often use group mating and/or flock mating for their commercial herds. During group mating 25 to 35 ewes are mated with one buck. This mating system, when used exclusively, should ensure correct parental allocation. Recording errors are however still possible when a kid is allocated to a dam by the breeder. Twins, triplets and kids that are "stolen" by other ewes could also increase the error rate. The popular alternative mating system used in the commercial herds is flock mating where approximately 200 ewes are mated to four to six bucks. The result is that accurate parentage recording is impossible for the breeder.

As breeders want to ensure that all the ewes are impregnated at the end of the mating season, almost all breeders use overmating in their commercial herds. This method combines group mating with a complete flock mating at the end of the mating season. Kids born first during the kidding season are presumed to have been conceived during the group matings and the kids born later in the season are said to have unknown parentage, thus conceived during overmating (Personal communication: Dr. M.A. Snyman; Grootfontein Agricultural Development Institute, Jansenville, Private Bag X529, Middelburg Cape, 5900, South Africa; April 2007). This creates an immense problem to identify the sire of each kid and to perform accurate selection.

To ensure accurate kidding records, intensive field observations are required. This invasive management practice creates strain on the ewes during parturition as they feel threatened by the presence of the humans and could result in a ewe abandoning her lamb.

However, if the intensity of the field observations is relaxed, it can increase the parentage error rate even further (Dodds *et al.*, 2005).

In a recent study it was found that approximately 23% of the South African Angora goats born from the commercial herds between 2000 and 2004 had incomplete pedigree records as the sire was unknown (Personal communication, Dr. M Snyman; Grootfontein Agricultural Development Institute, Jansenville, Private Bag X529, Middelburg Cape, 5900, South Africa; April 2007). In both the commercial and stud herds the pedigree records are not only incomplete but also inaccurate in many cases. As a result, phenotypic selection and accurate estimation of EBVs are extremely limited, which in turn may result in a lower genetic gain. Given the major role South Africa plays in the world mohair industry it is critical that Angora goat breeders need to produce animals with a high quality clip. Genetic parentage testing is more practical and accurate than field observations and can ensure accurate pedigree data that will increase the genetic gain and quality of the clip through more efficient selection.

1.3 Aim of the study

South African Angora goat breeders face practical challenges in terms of large herd sizes and extensive breeding conditions that limit accurate animal recording. One of the recordings neglected is parentage allocation. Field recordings often lead to ewes abandoning their kids and certain mating systems makes accurate recording of the sire impossible. To ensure that a high quality clip is produced to meet the global demand, breeders need to select superior producing animals based on the performance of their parents. This can only be done if parentage records are complete. Accurate parentage records are also essential if breeders wish to participate in the National Small-stock improvement scheme.

Mohair South Africa identified the need for more accurate parentage recording. A project based on genetic technology was listed as part of their research priorities to alleviate the problem. Genetic technology is less invasive than field observations and more accurate than conventional management practices. It might assist breeders in assigning the correct sire and dam to the kids. The Department of Animal and Wildlife Science at The

University of Pretoria conducted the study to evaluate the use of microsatellite markers for parentage verification.

Over the years, genetic parentage verification has been performed with various genetic markers of which microsatellite markers is currently the most popular. The International Society of Animal Genetics (ISAG) recommended two panels of microsatellite markers suitable for parentage verification of goats. These panels and panels used in previous literature were used as a guideline to construct a panel of microsatellite markers for evaluation in South African Angora goats.

The aim of the study was to evaluate a panel of microsatellite markers and to assess the panel for parentage verification in South African Angora goats.

The following objectives were set in this study:

1. Selection of 18 microsatellite markers from the ISAG panels and previous literature based on individual criteria including PIC, H_E, H_O, allele frequencies and exclusion probabilities.
2. Selection of animals based on available pedigree data and DNA samples
3. Optimizing panel of selected microsatellite markers
4. Testing panel of markers in 200 selected Angora goats of different family structures
5. Evaluation of the panel to compile the most cost effective panel with the highest exclusion power.

CHAPTER 2: LITERATURE OVERVIEW

2.1 Introduction

The South African Angora goat industry was established in 1837 and has since grown to become the largest producer of mohair in the world. In order to ensure that the best quality clip is produced, breeders need to be able to accurately select breeding animals for production of high quality fleece traits. Selection is based on the performance of the animals' parents and thus requires accurate and complete pedigree data. Practical constraints e.g. herd sizes and extensive production systems often lead to incomplete or inaccurate pedigree information. Genetic parentage testing and verification provides a non-invasive method of accurate parental allocation.

The aim of this chapter was to provide an overview on the development of genetic parentage verification and the application of genetic markers to verify parentage in farm animal species with special reference to goats.

2.2 Selection practices

For centuries conventional selection of animals was based on the phenotype of the animal itself and on that of the relatives of the animal (Bourdon, 2000). Conventional selection entails selecting animals with parents with superior performance for a certain trait as breeding animals even before the animal itself have expressed the trait. In the event of incorrect pedigree information the breeder might select an animal from parents with inferior performance. This kid might not perform as well as expected, while the offspring of the superior parents may be culled (Bourdon, 2000).

As selection tools like EBVs became available, breeders were able to predict breeding values of an animal based on the additive genetic relationships between related individuals (Ganai & Yadav, 2005; Bovenhuis *et al.*, 1997). To perform an accurate analysis, the full pedigree of the animal is needed. If the pedigree records are inaccurate or incomplete it will create a bias towards a certain sire by inducing errors in estimates of heritability and breeding values (Davis & DeNise, 1998, Ganai & Yadav, 2005). The

participation of South African Angora goat breeders in the small stock recording scheme is limited.

In a study by Geldermann *et al*. (1986) it was reported that, based on previous literature, between four and 23% of the paternal assignations of European dairy cattle were false. Erroneous parentage assignation for a trait with a heritability of 0.5 and a parental misidentification rate of 15% may result in an estimated reduced genetic gain of 8.7%. The gain for a trait with a heritability of 0.2 and a parental misidentification rate of 15% will be reduced with an estimated 16.9% (Geldermann *et al.*, 1986). A study conducted on the Israeli-Holstein breeding programs concluded that paternity testing could generate profits of more than US$ 2 million over a period of 20 years due to the increase in the efficiency of the selective breeding program (Ron *et al.*, 1996). The study further indicated that parental testing could lead to a 5% increase in annual genetic gain. In a more recent study, Visscher *et al.* (2002) estimated the percentage error rate of parents that are incorrectly assigned to progeny in UK dairy cattle to be 10%. A loss of 2 – 3% in genetic gain based on this error rate was predicted.

A serious consequence of incorrect parentage is possible inbreeding (Luikart *et al.*, 1999). The mating of related animals may cause the appearance of deleterious phenotypes e.g. kids born dead or with severe abnormalities. Of greater concern is the appearance of inbreeding depression (Fairbanks & Andersen, 1999). In cases where parentage records are incorrect, the frequency of inbreeding can easily escalate. Genetic parentage verification can be incorporated into the breeding plan to limit this problem by ensuring that the correct pedigree of the animals is known.

2.3 Markers used in parentage verification

Different markers have been applied to verify the parentage of humans and animals. Markers that were mainly used over the years to infer parentage include blood group antigens, DNA fingerprinting (DFPs), Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP), microsatellite markers and Single Nucleotide Polymorphisms (SNPs) (Silver, 1989; Mitra *et al.*, 1999; Gerber *et al.,*

2000; Thomsen *et al.*, 2002; Jones & Arden, 2003). Microsatellite markers are currently most widely used because of the ease of use and the degree of information obtained from these markers. SNPs are gaining popularity as they present certain advantages over those of microsatellite markers (Lopez-Herráez *et al.,* 2005).

During the 1940's Irwin and co-workers made use of blood group antigens in Holstein Friesians for parentage verification (Oosterhoff, 1998; Hines, 1999). During the following decade Stormont *et al.* (1951) investigated cattle blood group systems for their use as a molecular marker to infer parentage. Blood typing was based on the principle that a genetic profile generated followed the Mendelian rules of inheritance (Thomsen *et al.*, 2002). Despite the availability of a number of blood group variants the major limitation of the use of blood group systems was the lack of information and low exclusion power (Oosterhoff, 1998, Mitra *et al.*, 1999). Molecular markers show a much greater exclusion power (>90%) compared to blood groups (70 – 90%) (Mitra *et al.*, 1999).

The use of RFLPs, DFPs and AFLPs to infer parentage gained popularity in the late 1980's. The limitations when using RFLPs included non-standardized techniques, difficulties in interpretation of fragment sizes, mutation rates and low exclusion power (Silver, 1989). DFPs are highly polymorphic markers that make use of multi-locus probes to detect the DFPs when hybridized to hyper-variable loci in the genome. The limitation with this technique was that limited probes are available for the different farm animal species (Haberfeld *et al.,* 1993). Another molecular marker used for parentage verification was AFLPs. AFLP and DFPs are dominant markers and cannot distinguish between heterozygote and homozygote individuals that lead to less information which is a major limitation when using these markers (Gerber *et al.*, 2000).

During the last decade of the previous century microsatellite markers, that is multi-allelic, co-dominant molecular markers, gained popularity. Microsatellites are tandem repeats of between one and six base pairs that can be repeated up to 60 times. These markers are co-dominant and found in both coding (<10%) and non-coding (>90%) regions throughout the genome. Microsatellites are highly polymorphic due to the variation in the number of

repeats and this variation is detected by the polymerase chain reaction (PCR) (Beuzen *et al.*, 2000; Turner *et al.*, 2003; Van Marle-Köster & Nel, 2003). The polymorphic nature of the markers and the ease of use make them highly suitable for an array of diagnostic and forensic projects. These projects include animal species identification in meat products (Partis *et al.*, 2000; Saez *et al.*, 2004), forensic investigations, identity and parentage verification (Luikart *et al.*, 1999; Hoff-Olsen *et al.,* 2001; Ganai & Yadav, 2005; Aronson, 2005; Glowatzki-Mullis *et al.*, 2007), genetic diversity studies (Baumung 2004), genome mapping and quantitative trait loci (QTL) studies (Maddox, 2005; Cano *et al.*, 2007). The limitations of microsatellite markers are that they are not as stably inherited and not as abundant as SNP markers.

Since the discovery of SNPs in the late 1990s and the application of the SNP panels it became evident that SNPs would revolutionize animal genetics. SNPs are the substitution, addition or deletion of one nucleotide (Beuzen *et al.*, 2000). SNPs are more stably inherited than microsatellites and because they are found more frequently in the coding region of the gene, they can be directly responsible for phenotypic variation among individuals (Beuzen *et al.,* 2000; Heaton *et al.,* 2002). The potential genotyping errors when using SNPs tend to be lower than when scoring microsatellite markers (Anderson & Garza, 2006). SNPs can be suitable in projects where more than one laboratory collaborate on a project as they perform more consistent in different amplification reactions compared to microsatellite markers (Anderson & Garza, 2006). The greatest limitation when using SNPs is their bi-allelic nature and the limited information provided per marker. In contrast to this, microsatellites are multi-allelic and can have up to 30 or more alleles in a population. In an investigation by Weller *et al.* (2006) it was established that 2 to 2.25 SNPs have the same exclusion power as one microsatellite with five alleles and a minimum of eight SNPs are required to achieve a 99% exclusion probability for a match between two individuals. A large-scale public SNP project hopes to produce a 20K sheep SNP chip, but a chip for goats still need to be developed (Madox & Cockett, 2007). SNPs in goats have not been researched as widely as in other farm animal species. This makes the current use of SNPs for goat parentage verification impossible.

2.4 Parentage verification studies using microsatellite markers

A large number of microsatellite markers have been mapped for humans, cattle, sheep and goats (Fadiel *et al.*, 2005). More than 2400 microsatellite markers have been mapped for cattle (BOVMAP, http://dga.jouy.inra.fr/cgi-bin/lgbc/summary.operl?BASE=cattle) whereas in the sheep genome approximately 1400 markers have been mapped (Maddox & Cockett, 2007). Currently there are 423 microsatellite loci in the INRA Goatmap database (http://dga.jouy.inra.fr/cgibin/lgbc/summary.operl?BASE=goat) and the goat genome spans 2737 cM (Maddox & Cockett, 2007). Even though the goat genome has not been studied as extensively as other farm animal species, there are a sufficient number of microsatellite markers available for development of a panel for parentage verification.

Various studies have been conducted to compile accurate, cost efficient panels of microsatellite markers for parentage verification in animal species. Panels for cattle, horses, dogs, sheep and goats have been constructed and approved by the International Society of Animal Genetics (ISAG) in 2001/2002 and are listed in Table 2.1. (ISAG website: http://www.isag.org.uk/journal/comparisonguide.asp and http://www.isag.org.uk/ISAG/all02_PVpanels_LPCGH.doc). The recommended panels are regularly updated but the majority of markers remained unchanged for all species. During 2005 two additional microsatellite markers, namely HSC and ILSTS19, were added to the first Multiplex of the 2001/2002 goat panel. During 2009 the FAO released a list of microsatellite markers recommended for use in molecular genetics characterization. The panel constructed for goats consisted of 30 microsatellite markers of which 11 corresponded with the markers used in the current study (http://dad.fao.org/cgi-bin/getblob.cgi?sid=-1,50005882).

Table 2.1 Microsatellite markers recommended for parentage verification of cattle, sheep, pigs, dogs, horses and goats (ISAG 2001/2002)

Species	Number of markers	Microsatellite markers and multiplex
Cattle	9*	Multiplex 1: BM1824, BM2113, INRA023, SPS115, TGLA122, TGLA126, TGLA227, ETH10, ETH225
Sheep	19	Multiplex 1: CSRD247, HSC, INRA63**,** MAF214, OarAE129, OarCP49, FCB11, FCB304
		Multiplex 2: D5S2, INRA005**,** INRA023, MAF65, MCM527, OarFCB20, SPS113
		Multiplex 3: BM1258, BM1329, BM1818, INRA231
Pigs	15	Multiplex 1: S0005, S00090, S0101, S0155, S0355, S0386, SW240, SW857, SW951
		Multiplex 2: SW72, SW936, SW911, S0228, S0227
Dogs	23	Multiplex 1: AHT121, AHT171, AHT253, C22.279, FH2001, FH2054, FH2164, FH2611, FH2247, FH2289, INRA21, PEZO8
		Multiplex 2: AHTk211, LEI2D2, FH2326, FH2328, FH2361, PEZ12, PEZ22, FH2305, PEZ03, PEZ10, PEZ11
Horses	9	Multiplex 1: AHT4, AHT5, HMS6, HMS7, HTG4, VHL20
		Multiplex 2: ASB2, HMS3, HTG10
Goats	16	Multiplex 1: **INRA005, INRA063, MAF65, SRCRSP5, SRCRSP8, SRCRSP24**
		Multiplex 2: **CSRD247**, OarFCB20, **ILSTS87**, INRA023, **McM527,** SRCRSP23
		Multiplex 3: **BM1258, BM1329, BM1818**, INRA132

*: The panel recommended for cattle consisted of nine international markers and an additional three to five markers that varied among laboratories and were amplified in one or two multiplex reactions
Markers printed in **bold** corresponds to the markers used in the current study

Microsatellite markers have been used for parentage verification of cattle, especially beef cattle, since the mid 1990's (Glowatzki-Mullis *et al.*, 1995; Usha *et al.*, 1995; Heyen *et al.,* 1997; Vankan & Faddy, 1999; Curi & Lopes, 2002). Most dairy breeders make use of artificial insemination and therefore have a higher degree of accuracy regarding the parentage of their animals compared to beef breeders who make use of artificial insemination as well as natural breeding. The ISAG panels were mostly developed in European breeds (*Bos taurus*) and when tested in Zebu breeds (*Bos indicus*) a lower exclusion probability was recorded (Curi & Lopes, 2002). This indicates that all microsatellite markers are not equally polymorphic and informative in all breeds.

Since the dawn of the new millennium several studies using SNP markers as an alternative to microsatellite markers to verify parentage were conducted on cattle breeds (Heaton *et al.,* 2002; Werner *et al*., 2004). In a study by Lopez-Herráez *et al.* (2005) the

combined exclusion power of three sets of microsatellite markers consisting of 10, 14 and 17 microsatellite markers each and a set of 43 SNPs were compared in a population of Galloway cattle. The combined exclusion power was estimated to be higher than 99% for all three microsatellite marker sets and approximately 98% for the SNP set. The difference in exclusion power was accounted for due to the bi-allelic nature of SNPs and the multi-allelic nature of microsatellite markers, thus allowing the microsatellite sets to provide more information than the SNP set (Lopez-Herráez *et al.*, 2005).

In the past sheep breeders made limited use of genetic parentage testing as it was not cost effective (Dodds *et al.,* 2007). However in recent years cost were reduced and a French laboratory claims to evaluate 2500 – 3000 parental disputes on sheep per year (Amigues *et al*., 2003), whereas a New-Zealand company verify the parentage of around 8000 sheep yearly (Crawford *et al.*, 2006). In two parentage verification studies conducted on different sheep breeds with 20 and 19 microsatellite markers respectively, the combined exclusion probability of the set when both parents were unknown was between 99.86% and 99.9%, with the exception of one breed that had an exclusion probability of 98.6% (AiBao & DengJun, 2005; Glowatzki-Mullis *et al.,* 2007).

Commercial pig and horse breeders primarily make use of artificial insemination and therefore very limited paternity disputes arise. A panel of ten polymorphic microsatellite markers was constructed by Nechtelberger *et al.* (2001) for parentage verification in pigs. The combined exclusion probabilities of the panel when a known parent and a putative parent was analysed ranged between 99.18% and 99.76% among the different breeds studied. An additional multiplex of five microsatellite markers was also developed that can be used in cases where the exclusion power of the first ten markers was not powerful enough to exclude an individual. Parentage verification studies on horses using microsatellite markers have been conducted since the early 1990's (Marklund *et al.,* 1994; Binns *et al.,* 1995; Bowling *et al.,* 1997, Jakabova *et al.*, 2002, Cho & Cho, 2004). The combined exclusion power ranged between 98.20% and 98.88% when one known parent was included in the different studies.

Parentage testing has also become more popular among dog and cat breeders. Studies on dogs by Koskinen & Bredbacka (1999) and DeNise *et al.* (2004) have estimated exclusion probabilities above 99% when a panel of 17 microsatellite markers were evaluated for parentage verification of dogs. Among cat breeds exclusion probabilities between 90.08% and 99.87% were recorded when a panel of 19 microsatellite markers were used (Lipinski *et al.,* 2007).

The goat genome has not been studied as extensively as the genomes of cattle, swine and sheep (Maddox & Cockett, 2007) but as mentioned previously over 400 microsatellite markers have been mapped on the caprine genome (Maddox & Cockett, 2007). Parentage verification studies on goats have been conducted on different breeds including Angora goat, Cashmere goat (Luikart *et al*., 1999; Bolormaa *et al.,* 2008), Saanen goat, Murciano-Granadina goat (Jiménez-Gamero *et al.,* 2006), three indigenous Indian goat breeds (Ganai & Yadav, 2005) and ten additional European goat breeds (Glowatzki-Mullis *et al.,* 2007) and with different number of markers in each panel.

The accuracy and effectiveness of a parentage verification panel does not entirely depend on the number of markers used, but rather the informativeness of these markers. This is measured by parameters like Polymorphic Information Content (PIC), Heterozygosity (H) and Exclusion probability (PE) which in turn is dependent on the number of alleles and allele frequencies of the markers and varies among populations (Curi & Lopes, 2002). Even though the number of microsatellite markers used in the studies ranged widely (between 22 and nine), the combined exclusion power of the different panels when one known parent, the putative parent and the offspring were analysed simultaneously exceeded 99% in all the studies. Whether the genotypes for both or only one parent are tested also influence the exclusion power of the test (Ganai & Yadav, 2005). In the panel constructed by Ganai & Yadav (2005) 12 markers were needed to obtain an exclusion probability exceeding 99% when only the offspring and putative parents were tested, however only six markers were needed to obtain the same probability when a known parent was also included. If both the dam and sire were unconfirmed, but

all possible biological parents of both sexes were included in the test, only four markers were needed to obtain a similar exclusion probability.

Six of the 22 microsatellite markers used by Luikart *et al.* (1999) corresponded with the later published ISAG panels (2001/2002 and 2005) whereas nine of the microsatellite markers evaluated by Glowatzki-Mullis *et al.* (2007) corresponded with the ISAG panels and five markers used by Bolormaa *et al*. (2008) and only two included by Jimenez-Gamero *et al.* (2006) corresponded to the markers used in these panels. None of the microsatellite markers used by Ganai & Yadav (2005) were recommended in either of the ISAG panels. In the studies where fewer or none of the markers overlapped with the recommended panels, the exclusion probability of the panel was still high and non-parents could be excluded.

2.5 Accuracy of parentage tests

The accuracy of a parentage test is defined by Vankan (2005) as its ability to exclude an incorrect parent. This is influenced by the number of markers tested, the polymorphism of each marker and other factors such as species studied and the degree of relatedness of the animals being tested. A major source of error is genotypic errors (Hoffman & Amos, 2005). The computer software package Cervus 3.0 (Marshall *et al.*, 1998) takes genotypic errors into account and use a likelihood-based approach which allows for genotypic error and can assign paternity to the most likely male if more than one male are not excluded (Slate *et al.,* 2000). The incorporation of the likelihood equations in Cervus 3.0 increases the number of paternities that can be assigned at a higher level of confidence (Kalinowski *et al.,* 2007).

Even though exclusion success of putative parents can be 100%, the inclusion of a putative parent is closer to 99%. Many human genetic testing laboratories acknowledge that their tests are 99.9% accurate in including a father and 100% accurate in excluding a non-father (http://www.dnadiagnostics.com; http://www.delphitest.com/content/Quality.html; http://www.easydna.co.za/content/;

http://www.dnatest.co.za/paternity.html). Similarly, animal genetic testing laboratories also claim that their test results are 100% accurate when non-parents are excluded and around 99.9% accurate when a parent is included (http://www.gtg.com.au/animalDNATesting/index.asp?menuid=080.100; http://www.metamorphixinc.com).

The relatedness of the animals tested may cause the parentage allocation to be false and there are two factors to take into account. Firstly the probability that two random individuals have the exact same genotypes (identity by state, IBS) and that two related individuals has the same genotypes should be taken into account. Secondly that an offspring and parent have the same heterozygous genotype (identity by descent, IBD) should also be considered. In a study by Du Plessis (2002) 4731 South African cattle individuals from 13 different breeds were analysed with ten microsatellite markers as an aid in forensic analyses and parentage verification. It was found that the random match probability were 1 in 180 916 847. This indicates that the chance to find an unrelated animal in the population with the exact same genotype as the individual under investigation is more than 1 in 180 million. However with the exclusion-based paternity analysis it might happen that more than one male may remain non-excluded or that the true father may be excluded because of typing errors, null alleles or mutations. By using the likelihood approach developed by Marshall *et al.* (1998) the effect of typing or genotypic errors, null alleles and mutations are taken into account which increases the accuracy and reliability of the test.

2.6 Software for statistical analyses of parentage verification

Several software packages are available to verify parentage making use of the different methods of parentage analysis. These methods include exclusion, categorical allocation, fractional allocation and parental reconstruction (Jones & Arden, 2003). The exclusion method makes use of genotypic mismatches between parents and offspring and individuals where many mismatches are rejected. The categorical allocation is based on the principle of a likelihood-based approach that select the most likely parent from all the non-excluded parents, while fractional allocations assigns a fraction of each offspring to

all non-excluded candidate parents and this fraction is proportional to its likelihood of parenting the offspring compared to all other putative parents. The parental reconstruction method incorporates the multilocus genotypes of the known parent and offspring to reconstruct the genotypes of the unknown parent (Jones & Arden, 2003). Table 2.2 summarizes the different methods and lists the packages available for each method.

Table 2.2 Methods for parentage verification and associated statistical software packages

Method	Software packages	Reference
Exclusion	Probmax	Danzmann (1997) http://www.uoguelph.ca/~rdanzmann/software/PROBMAX/
	Newpat	Worthington Wilmer *et al*. (1999) http://www.zoo.cam.ac.k/zoostaff/amos/newpat.html
	Kinship	Goodnight & Queller (1999) http://gsoft.smu.edu/
Categorical allocation	Cervus	Marshall *et al.* (1998) http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html
	Papa	Duchesne *et al.* (2002) http://www.bio.ulaval.ca/contenu-fra/professeurs/Prof-l-bernatchez.html
	Famoz	Gerber *et al.* (2000) http://www.pierroton.inra.fr/genetics/labo/Software/Famoz/
	Parente	Cercueil *et al.* (2002) http://www2.ujf-grenoble.fr/leca/membres/manel.html
Fractional allocation	Patri	Signorvitch & Nielsen (2002) http://www.biom.cornell.edu/Homepages/Rasmus_Nielsen/files.html
Parental reconstruction	Gerud	Jones (2001) http://www.biology.gatech.edu/professors/labsites/jones/parentage.html

In the current study Cervus 3.0, a Windows-based software package developed to infer paternity in natural populations was used to analyse the samples (Marshall *et al.,* 1998; http://helios.bto.ed.ac.uk/evikgeb/cervus/cervus.html). This software package offers the added benefit that multiple non-excluded males can be statistically distinguished. Laboratory typing error is also considered and statistical confidence is determined for assigned paternities through simulation. CERVUS 3.0 is considered to be an accurate predictor in assigning confidence in paternity (Slate *et al.*, 2000).

2.7 Commercial testing

Commercial parentage testing of cattle, sheep, goats, horses and dogs are gaining in popularity worldwide. Commercial kits are available for cattle, horse and canine parentage verification

(www.appliedbiosystems.com and http://diagnostics.finnzymes.fi/index.php?lang=_en) and there are a number of laboratories providing this service worldwide (www.vetgen.com; www.laboklin.co.uk). One of the prerequisites of the marker set used in routine parentage testing is that the markers should be a set of independently segregating markers that is appropriate for all breeds of that species (Jamison & Taylor, 1997). There are however no commercial kit available for goat parentage testing.

In South Africa a limited number of laboratories perform parentage testing on farm animals, compared to the rest of the world. Primarily beef cattle, dairy cattle and horses are tested with some laboratories conducting canine tests as well (www.unistel.co.za; www.arc.agric.za; www.inqababiotec.co.za; www.mdsafrica.net). The price charged by these laboratories per animal tested range between R120-R300 (US$17-US$30). To date goat parentage testing has not been performed routinely. This highlights the importance of a genetic parentage verification test for South African goats that can be applied in commercial testing.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

In the current study 18 microsatellite markers were evaluated for their suitability to verify parentage of South African Angora goats. DNA samples from 200 Angora goats as well as a small population of 16 Saanen goats were tested.

3.2 Material

3.2.1 Angora population

The blood samples of 200 Angora goats were obtained from the DNA bio-bank for South African small-stock, which was established for long term genetic research on South African Angora goats during 2006. It is situated at Grootfontein Agricultural Development Institute (GADI), Middelburg, Eastern Cape Province, South Africa and is maintained by the National Department of Agriculture. The DNA bio-bank stores blood, DNA and tissue samples of Angora goats from seven different Angora stud herd breeders (Personal communication, Dr. M Snyman; Grootfontein Agricultural Development Institute, Jansenville, Private Bag X529, Middelburg Cape, 5900, South Africa; April 2007). Phenotypic data of economically important traits as well as pedigree information that is required by the National Small Stock performance testing scheme, are also stored at GADI for the individuals sampled in this study. The goats used in this study were selected on the basis of relatedness in the different family structures studied, availability of blood samples and completeness of pedigree data. The Angora goats included in this study were bred by four different breeders.

The blood samples were collected by personnel from the National Department of Agriculture. For each individual, 5ml blood was collected in EDTA tubes. Half of the sample was used for DNA extractions while a duplicate sample was stored at -40ºC for future use.

The 200 Angora goats were genotyped for 18 microsatellite markers. Firstly a family of 44 goats (Family A1) consisting of one sire, 17 dams and 26 halfsibs was tested. All the halfsibs had complete pedigree information. An additional four bucks (E1, E2, E3 and E4) that were kept by the same breeder and were used in the breeding season were included. A second Angora goat family (family A2) consisting of one buck and 20 halfsib offspring were included. These halfsibs had only paternal pedigree information available and the breeder considered the buck as the sire of all the kids. Thirdly DNA samples were obtained from Angora goats of two different breeders (AM group, breeder 1 and AM group, breeder 2). The kids in these groups had only maternal pedigree information available. Finally DNA samples of 93 Angora goats were included. These animals were kept by three different breeders who did not know the paternity of the animals, but in most cases the maternity was known (TA groups). These animals were divided into three groups (TA1, TA2 and TA3), according to their breeder. Refer to Table 3.1 for more detail.

Table 3.1 Angora goat genotypes included in the study

Family	Sires	n sires	Ewes	n ewes	Kids	n kids
A1	C1,E1,E2,E3,E4	5	C21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 37, 42, 44	17	C2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 35, 36, 38, 39, 40, 41, 43	26
A2	B1	1	-	0	B2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 20, 21, 22, 23	20
AM1	-	0	AM1, 2, 3, 4, 5, 6, 7, 8, 9	9	AM10, 11, 12, 14, 15, 17, 18, 19, 20	9
AM2	-	0	AM34, 35, 36, 37, 38, 39, 40, 41, 42, 43	10	AM21, 23, 24, 25, 27, 28, 29, 30, 31, 32	10
TA1	TA48, 67, 68, 16, 65, 6	6	TA28, 27, 45, 51, 10, 5, 63, 85, 82	9	TA98, 52, 12, 7, 20, 90, 92, 47, 18, 97	10
TA2	TA93, 37, 41, 26, 23	5	TA62, 36, 32, 80, 14, 84, 38, 2, 57, 72, 1	11	TA35, 64, 70, 55, 91, 33, 81, 71, 95, 11, 103, 34	12
TA3	TA58, 99, 17, 89, 24, 30, 15, 42, 61, 59, 87, 8, 22, 21, 43, 40	16	TA46, 9, 102, 3, 100, 31, 94, 4, 96, 19, 44, 25	12	TA83, 53, 56, 101, 66, 74, 86, 39, 13, 60, 69, 54	12

The different family structures were tested in order to comply with practical scenarios experienced by the breeders.

3.2.2 Saanen population

Whole blood samples from 16 Saanen goats were collected from the Small Stock Unit of the University of Pretoria's experimental farm, Pretoria, South Africa, to serve as a population to verify the individual marker results obtained from testing the Angora goats. 5ml blood samples were collected in EDTA tubes from the Jugular vein and stored at -5˚C. The family structure of this population is given in Table 3.2. All halfsibs had complete pedigree information available.

Table 3.2 Saanen goat individuals included in the study

Family	Sire	n sires	Ewes	n ewes	Kids	n kids
S1	S1	1	S2, 3, 4	3	S5, 6 7 8	4
S2	S9	1	S10, 11, 12	3	S13, 14, 15, 16	4

3.3 Methods

3.3.1 Genomic DNA extraction

The DNA extractions were performed at the University of Pretoria, Department of Animal and Wildlife Sciences, Animal Breeding and Genetics laboratory and at the laboratory facilities at GADI. Family A1, A2 and the Saanen families were extracted at the University of Pretoria from wholeblood samples using the Qiagen DNeasy Tissue kit© (Qiagen - Southern Cross Biotechnology (Pty) Ltd, Cape Town, South Africa) according to the protocol of the manufacturer. DNA from the AM groups and the TA families was extracted at GADI using the Roche DNA Isolation Kit for Cells and Tissues (Roche Applied Science) according to the protocol of the manufacturer.

For a crude estimation of DNA quality, a mixture of 3µl extracted DNA and 2.5µl loading buffer was ran on a 1% agarose gel using a Hoefer HE 33 Mini Horizontal Submarine Unit© (Amersham Pharmacia Biotech Inc.).

3.3.2 Microsatellite optimization and PCR amplification

Seventy three microsatellite markers, originating from previous studies and ISAG panels were available for selection. Characteristics of these markers have been published in various parentage verification studies. From these markers a set of 21 markers was selected based on polymorphism and fragment size. Optimization of the microsatellite markers was performed using DNA samples of five randomly chosen Angora goat kids from the A1 family. The microsatellite markers were amplified singly and not in a multiplex. The forward primers were labelled with a fluorescent dye, either red (PET®), blue (6-FAM®), green (VIC®) or yellow (NED®). The labelling of these was done in order to compile a cost efficient set for genotyping on an ABI sequencer. These markers were arranged into two genotyping sets based on PCR product size and dye colour.

Previous literature and the ISAG panels were used as guidelines to decide on the Annealing temperature (T_m) of each marker. Three of the 21 selected markers did not amplify satisfactory for the five selected animals under the PCR conditions used and were ultimately discarded. In Table 3.3 the characteristics of the 18 microsatellite markers that gave consistent results for the 200 animals are summarized.

The PCR mastermix consisted of 1.5 µl 5x Colourless GoTaq Flexi Buffer (Promega – Whitehead Scientific Inc. South Africa), 0.3 µl 0.25mM $MgCl_2$, 0.75µl 10 nM dNTP's and 6.45µl deionized water. 0.3 µl each forward and reverse primer with a concentration of 10 pmol/µl and 0.4 µl (1.5 U) Taq was added to the mastermix. The complete mastermix was then added to 5 µl DNA with a concentration of between 50ng/µl and 100ng/µl. The amplification was performed by using a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, USA). The PCR program was as follows: 10 minutes at 94ºC, followed by 33 cycles of 45 seconds at 94ºC, 80 seconds at the annealing temperature and 60 seconds at 72ºC. The amplification was ended with a final extension step of five minutes at 72ºC.

Table 3.3 Characteristics of microsatellite markers: se[illegible] nber, annealing temperatures, fluorescent labels used, expected ranges and Genebank accession numbers

Microsatellite	Sequence	Chromosome	Annealing Temperature (C°)	Fluorescent Label	Expected range	Genebank accession number
BM 1258	F: GAGTCAGACATGACTGAGCCTG R: GTATGTATTTTTCCCACCCTGC	Bos taurus: 23 Ovis aries: 20 Capra hircus: 23	58	Pet®	100 – 130	G18385
BM 1329	F: TTGTTTAGGCAAGTCCAAAGTC R: AACACCGCAGCTTCATCC	BTA: 6 OAR: 6 CHI: 6q15	54	Vic®	160 – 180	G18422 AF394444
BM 1818	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	BTA: 23 OAR: 20 CHI:23	58	6-Fam®	240 – 270	G18391
BM 7160	F: TGGATTTTTAAACACAGAATGTGG R:TCAGCTTCTCTTTAAATTTCTCTGG	BTA: 7 CHI:22	55	6-Fam®	160 – 180	G18819 UniSTS: 253556
CSRD 247	F: GGACTTGCCAGAACTCTGCAAT R: CACTGTGGTTTGTATTAGTCAGG	OAR: 14	55	Vic®	200 – 260	UniSTS: 251420
HSC	F: CACTGTGGTTTGTATTAGTCAGG R: GGACTTGCCAGAACTCTGCAAT	BTA: 23 OAR: 20 CHI: 23q22	55	Vic®	270 – 310	L23481
ILSTS 5	F: GTCTGTCTCCTGTCTTGTCATC R: CTGCCAATGCAGAGACACAAGA	BTA: 10 OAR: 7 CHI: 10	52	Pet®	180 – 220	L37279 UniSTS: 251792
ILSTS 87	F: AGCAGACATGATGACTCAGC R: CTGCCTCTTTTCTTGAGAGC	BTA: 6 OAR:6	56	6-Fam®	130 – 160	X63793
INRABERN 192	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	CHI: 7	56	Pet®	170 – 200	X71507
INRA 5	F: CAATCTGCATGAAGTATAAATA R: CTTCAGGCATACCCTAGACC	BTA: 12 OAR: 10 CHI: 12	55	6-Fam®	130 – 140	M67437
INRA 63	F: ATTTGCACAAGCTAAATCTAACC R: AAACCACAGAAATGCTTGGAAG	BTA: 18 OAR: 14 CHI: 18q22	54	6-Fam®	160 – 170	L34277
MAF 65	F: TTGTTTAGGCAAGTCCAAAGTC R:AACACCGCAGCTTCATCC	BTA: 15 OAR: 15 CHI: 15	55	Vic®	110 – 140	M82875
MCM 527	F: GTCCATTGCCTCAAATCAATTC R: AAACCACTTGACTACTCCCCAA	OAR: 5 CHI:7	55	Ned®	150 – 170	L22197
OarFCB 48	F: TTCAGGCATACCCTACACCACATG R:AAATATTAGCCAACTGAAAACTGGG	BTA: 17 OAR: 17 CHI:17q15	60	Pet®	150 – 170	L22193
SRCRSP 5	F: GACCACAAAGGGATTTGCACAAGC R: AAACCACAGAAATGCTTGGAAG	OAR: 18 CHI:21q14	55	Ned®	160 – 190	L22200 UniSTS: 254136
SRCRSP 8	F: CCACTCCTCCTGAGAATATAACATG R: AAAGGCCAGAGTATGCAATTAGGAG	Unknown	55	6-Fam®	210 – 240	Not available
SRCRSP 9	F: AGAGGATCTGGAAATGGAATC R: GCACTCTTTTCAGCCCTAATG	CHI: 12q22 OAR: 2	58	Vic	110 – 150	Not available
SRCRSP 24	F: GTCCATTGCCTCAAATCAATTC R: AAACCACTTGACTACTCCCCAA	Unknown	56	Pet®	130 - 180	Not available

After each PCR amplification a mixture of 5µl PCR product and 3µl loading dye were mixed and ran on a 3% agarose gel for an indication whether amplification was successful. In the event that the microsatellite marker did not amplify successfully the amplification was repeated at least once for optimal amplification success. In the AM and TA groups the DNA quantity was limited and therefore it was not possible in all cases to repeat a poor amplification resulting in less data for some of the markers.

3.3.3 Genotyping

PCR products were diluted with distilled water in a 1:5, 1:10 or 1:20 dilution and prepared for genotyping. Amplicons were genotyped using an ABI PRISM ® 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, USA) at the University of Pretoria's sequencing laboratory in the Forestry and Agricultural Biotechnology Institute (FABI). The dilutions for genotyping consisted of one µl diluted PCR product and nine µl of a 1000: 14 solution of Formamide to LIZ size standard. Genemapper™ software (Applied Biosystems, Foster City, USA) was used for allele calling.

3.4 Data analysis

Genotypic data was edited and prepared for statistical analyses. Allele frequencies, polymorphic information content (PIC) and Heterozygosity (H_E and H_O) were calculated using the Microsoft Excel-based program, Microsatellite toolkit (Park, 2001) and were verified using Cervus 3.0 (Marshall *et al.*, 1998). The computer program GenAlEx 6 (Peakall & Smouse, 2006) was used to determine whether the microsatellite markers were in Hardy-Weinberg equilibrium. The exclusion probability as well as the LOD and Delta scores and confidence levels were calculated using Cervus 3.0. GenAlEx 6 was used to verify the exclusion probabilities generated by Cervus 3.0.

3.4.1 Allele diversity information

Allele frequency is defined as the proportion of a particular allele among all other alleles in a population (Fairbanks & Anderson, 1999). Allele frequencies were calculated with the following formula:

$$\hat{x}_i = \frac{\left(2n_{ii} + \sum_{j \neq i} n_{ij}\right)}{(2n)}$$

Where $\hat{x}_i$ also represents the maximum likelihood estimate of x_i (allele frequencies of locus A_i) (Nei, 1987). The total number of individuals for the genotype A_iA_j is n_{ij} and the total number of individuals in n, i.e. $\sum n_{ij} = n$.

The expected heterozygosity (H_E) was calculated using the formula from Nei (1978). The rule of thumb is that loci with expected heterozygosity of 0.5 or less are in general not very useful for large-scale parentage analysis (Marshall *et al.,* 1998). The PIC value is generally smaller than that of the expected heterozygosity. The observed heterozygosity (H_O) was calculated by dividing the number of heterozygotes at a certain locus in the population by the total number of individuals in the population (Hedrick, 2000) and was calculated using Microsatellite toolkit.

Polymorphic information content (PIC) is a measure of informativeness related to the expected heterozygosity and is calculated from allele frequencies (Marshall *et al.,* 1998). Microsatellite toolkit and Cervus 3.0 computes this value by using the formula from Botstein *et al.* (1980).

3.4.2 Exclusion probability

Exclusion probability is defined as the probability to exclude a random (non-parent) individual as the parent (Luikart *et al.,* 1999). There are three formulae for three different parentage exclusion scenarios as described by Jamieson & Taylor (1997). The computer software program Cervus 3.0 was used to calculate these probabilities using the formulae by Jamieson & Taylor (1997) and were verified using the program GenAlEx (Peakall & Smouse, 2006).

The first scenario is when the genotypes of the offspring and a group of alleged parents are tested, but no genotypes are available for a known parent. This scenario held true for family A2 and the AM groups. Scenario two is when the offspring's genotype and one

confirmed parents' (in most cases the dam) relationship with the offspring in question is known and the other parents' (in most cases the sire) relationship is unknown. This was the case for family A1 and all three the TA families. Scenario three is when all individuals' relationships are unknown. This is very rare in livestock breeding and no such data were used in this study.

The formulas were described by Jamieson & Taylor (1997)
The following formula is used for scenario one:

$$P = 1 - 2\sum_{i=1}^{n} p_i^{\,2} + \sum_{i=1}^{n} p_i^{\,3} + 2\sum_{i=1}^{n} p_i^{\,4} - 3\sum_{i=1}^{n} p_i^{\,5} - 2(\sum_{i=1}^{n} p_i^{\,2})^2 + 3\sum_{i=1}^{n} p_i^{\,2} \sum_{i=1}^{n} p_i^{\,3}$$

Where: P = probability of exclusion of wrong parent at l^{th} locus;

p_i = frequency of the i^{th} allele at l^{th} locus;

p_j = frequency of the j^{th} allele at l^{th} locus;

i = 1…n (number of alleles at i^{th} locus);

j = 1…n-1

The following formula is used for scenario two:

$$P = 1 - 4\sum_{i=1}^{n} p_i^{\,2} + 2(\sum_{i=1}^{n} p_i^{\,2})^2 + 4\sum_{i=1}^{n} p_i^{\,3} - 3\sum_{i=1}^{n} p_i^{\,4}$$

Where: P = probability of exclusion of wrong parent at l^{th} locus;

p_i = frequency of the i^{th} allele at l^{th} locus;

p_j = frequency of the j^{th} allele at l^{th} locus;

i = 1…n (number of alleles at i^{th} locus);

j = 1…n-1

When combining the probability over a number of (k) loci, the following equation is used $P = 1 - (1 - P_1)(1 - P_2)(1 - P_3)\ldots(1 - P_k)$ where P could be computed from any of the above formulas.

For all microsatellite markers the probability to distinguish between related and non-related animals was also computed. The probability to distinguish between two randomly

chosen animals in a population which is identical by state (P_{ID}) is calculated by the following formula:

$$P_{ID} = \sum p_i^4 + \sum\sum (2 p_i p_j)^2$$

Where p_i and p_j are the frequencies of the *i*th and *j*th alleles and $i \neq j$ (Paetkau & Stobeck, 1994).

The probability to distinguish between two related animals which are identical by decent (P_S) was calculated using the following formula:

$$P_S = 0.25 + (0.5 \sum p_i^2) + [0.5(\sum p_i^2)2] - (0.25 \sum p_i^4)$$

Where p_i is the frequency of the *i*th allele (Evett & Weir, 1998).

3.4.3 Simulation parameters used by Cervus 3.0

In order to infer parentage Cervus 3.0 performs a simulation of parentage. The parameters used are shown in Table 3.4.

Table 3.4 Simulation parameters used in the study by Cervus 3.0

Parameter	Used in simulation
Number of offspring	Number of offspring analysed in specific family
Proportion of candidate fathers	60% for all families
Proportion of loci mistyped	0.01
Relaxed confidence level	80%
Strict confidence level	95%

The confidence in the simulation was determined using LOD and Delta scores.

3.4.4 Likelihood of the odds (LOD) scores

The likelihood ratio is a tool to aid in evaluating hypotheses. The likelihood (L) of a hypothesis (H) from dataset D is considered. The likelihood of one hypothesis (H_1) is written relative to another (H_2).

$$L(H_1, H_2)| = \frac{P(D|H_1)}{P(D|H_2)}$$

where H_1 and H_2 is the hypothesis that the first and second putative parent is the true parent, respectively. $P(D|H_1)$ is the probability of obtaining data D under hypothesis H_1. In parentage studies D is normally the genotypes of the known parent, offspring and the first putative parent. $P(D|H_2)$ is the probability of obtaining data D under hypothesis H_2. Under this hypothesis, D is the genotypes of the known parent, offspring and the second putative parent (Marshall *et al.*, 1998). The LOD score is estimated by calculating the natural logarithm over the overall likelihood ratio. Table 3.5 gives an interpretation of the LOD scores calculated by Cervus 3.0 (Marshall *et al.*, 1998).

Table 3.5 Interpretation of the LOD scores

LOD score	Interpretation
Positive	Individual is more likely to be the true parent
Zero	Individual is equally likely to be the parent as not to be the parent
Negative	Individual is more likely not to be the true parent

3.4.5. Delta scores

The Delta score (Δ) is defined as the difference in LOD score between the most likely and second most likely candidate parents (Marshall *et al.,* 1998). Only positive LOD scores are taken in consideration when calculating Delta. If only one candidate parent has a positive LOD score, Delta is equal to the LOD score. If no candidate parent has a positive LOD score, Delta is calculated as zero (Marshall *et al.,* 1998). The Delta score is used to assign parentage at a confidence level of 95% (*) or 80% (+).

3.4.6 Confidence levels

The confidence level gives an indication on the tolerance of false positive assignments. These two confidence levels are nested, so that parentages assigned with strict confidence are a subset of parentages assigned with relaxed confidence (Marshall *et al.,* 1998). A set of relaxed and strict LOD and Delta scores are calculated for offspring with a known parent and a second set of scores can be calculated for offspring without a known parent (Marshall *et al.*, 1998). Assignments with a strict confidence level are denoted as "*" at 95% in the parentage analysis and "+" at 80% for the relaxed confidence levels. Candidate parents with a "-" confidence level indicates that the parent is the most likely

candidate parent but could not be assigned at either confidence levels. If the candidate parent is not the most likely parent no confidence level is assigned (Marshall *et al.*, 1998). This will be the case when both candidate parents have negative LOD scores, leading to a Delta score of zero.

3.5 Selection of final panel of microsatellite markers

The statistical procedures described were performed on the data set as follows:

1. The microsatellite markers were tested individually in the entire population to evaluate the suitability of the markers for inclusion in a parentage verification panel
2. The included microsatellite markers were then evaluated as a panel in the different family structures
3. Different combinations of markers were tested and markers were eliminated with regards to allele frequencies, PIC, H_E, H_O and combined exclusion power

CHAPTER 4: RESULTS

4.1 Evaluation of individual microsatellite markers

A panel of 21 polymorphic microsatellite markers were compiled from literature on parentage verification. Three markers from this panel could not be successfully amplified and eighteen markers were evaluated in 200 Angora and 16 Saanen goats in the different family structures as described in Chapter 3. The microsatellite markers were evaluated for their suitability to infer parentage based on the allele frequencies, number of alleles, Observed and Expected Heterozygosities (H_O and H_E), Polymorphic Information Content (PIC), the exclusion probability (CPE) and ease of scoring of each marker. The null allele frequency for each microsatellite marker was included, but this parameter was not used to exclude markers from the final panel of microsatellite markers. In Tables 4.1 and 4.2 results for the parameters evaluated for all the Angora goats treated as one population are presented.

Table 4.1 Allele frequencies of 18 microsatellite markers across all families studied

Locus	k	Alleles observed	Most frequent allele	Least frequent allele
BM 1258	13	101, 103, 105, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127	105 (0.316)	115, 123, 125 (0.003)
BM 1329	8	167, 169, 171, 173, 175, 177, 179, 181	171 (0.326)	173 (0.081)
BM 1818	9	249, 251, 253, 255, 257, 259, 261, 263, 265	255 (0.375)	265 (0.013)
BM 7160	10	161, 163, 165, 167, 169, 173, 175, 177, 179, 181	163 (0.265)	161 (0.003)
CSRD 247	9	219, 229, 233, 235, 237, 239, 241, 243, 245	237 (0.425)	235 (0.005)
HSC	12	267, 269, 273, 277, 279, 281, 283, 285, 287, 289, 297, 301	277 (0.381)	297 (0.004)
ILSTS 5	4	178, 180, 182, 184	**182 (0.646)**	178 (0.007)
ILSTS 87	9	132, 136, 138, 140, 142, 144, 146, 148, 152	**140 (0.697)**	152 (0.003)
INRA 5	3	135, 137, 141	**137 (0.638)**	141 (0.006)
INRA 63	5	159, 161, 163, 165, 167	163 (0.410)	167 (0.017)
INRABERN 192	10	178, 180, 188, 190, 192, 194, 196, 198, 200, 202	188 (0.376)	180 (0.003)
MAF 65	10	117, 119, 121, 123, 125, 129, 133, 135, 137, 141	**125 (0.808)**	123, 129, 137 (0.003)
MCM 527	10	152, 154, 156, 160, 162, 164, 166, 168, 170, 172	152 (0.440)	160, 170 (0.003)
OarFCB 48	8	153, 155, 157, 159, 161, 163, 165, 167	157 (0.347)	167 (0.008)
SRCRSP 5	10	158, 160, 162, 164, 166, 168, 170, 172, 174, 176	168 (0.310)	172 (0.009)
SRCRSP 8	11	211, 215, 221, 223, 225, 227, 231, 233, 235, 239, 243	**223 (0.561)**	233 (0.006)
SRCRSP 9	9	117, 121, 123, 125, 127, 129, 131, 133, 135	133 (0.459)	123 (0.003)
SRCRSP 24	9	153, 155, 157, 159, 161, 163, 165, 167, 169	169 (0.453)	159 (0.004)

k: number of alleles
Most frequent allele with frequency exceeding 0.50: printed in **bold**

Five of the 18 microsatellite markers (ILSTS 5, ISTSTS 87, INRA 5, MAF 65 and SRCRSP 8) tested in the Angora families had one allele each with a frequency greater than 0.50 (frequent alleles are printed in bold in Table 4.1). Although these markers show a high level of polymorphism, they tend to be less informative due to the high frequency of a certain allele in the population studied. For marker MAF 65 there were ten different alleles observed with one allele having a frequency of 0.80 and the other nine alleles accounted for the remaining frequency of 0.20. The results from analysing the Saanen goats as a control population are presented in Appendix A. No additional alleles were detected for any of the microsatellite markers.

A summary of the amplification success of the markers and number of heterozygotes and homozygotes estimated at all loci for all the animals tested are shown in Table 4.2.

Table 4.2 Summary of the amplification success of the markers and number of heterozygotes and homozygotes estimated at all loci for the families tested

Locus	n animals successfully genotyped	% amplification success	n Heterozygotes	n Homozygotes
BM 1258	179	89.50	136	43
BM 1329	167	83.50	124	43
BM 1818	160	80.00	121	39
BM 7160	160	80.00	101	59
CSRD 247	173	86.50	110	63
HSC	143	**71.50**	100	43
ILSTS 5	147	**73.50**	66	81
ILSTS 87	170	85.00	83	87
INRA 5	155	77.50	68	87
INRA 63	174	87.00	114	60
INRABERN 192	191	95.50	142	49
MAF 65	172	86.00	58	114
MCM 527	189	94.50	108	81
OarFCB 48	184	92.00	130	54
SRCRSP 5	162	81.00	117	45
SRCRSP 8	155	77.50	79	76
SRCRSP 9	171	85.50	112	59
SRCRSP 24	129	**64.50**	77	52
Total no animals genotyped per microsatellite marker	200			

Three of the 18 microsatellite markers had an amplification success of less than 75% (printed in bold in Table 4.2). INRABERN 192 amplified the best with an amplification rate of over 95% whereas SRCRSP 24 had only a 64.50% success rate.

All loci were tested to conclude if the population was in Hardy-Weinberg equilibrium across all families. Seven of the 18 microsatellite markers were not in equilibrium (Table 4.3).

Table 4.3 Summary of the Hardy-Weinberg test

Microsatellite marker	Degrees of Freedom	Chi-Square	Probability	Significance
BM 1258	55	185.945	0.000	***
BM 1329	45	347.657	0.000	***
BM 1818	28	33.242	0.227	Ns
BM 7160	21	51.821	0.000	***
CSRD 247	15	17.244	0.304	Ns
HSC	78	302.007	0.000	***
ILSTS 5	6	98.574	0.000	***
ILSTS 87	36	126.553	0.000	***
INRA 5	3	1.561	0.668	Ns
INRA 63	6	9.842	0.131	Ns
INRABERN 192	28	180.376	0.000	***
MAF 65	45	51.927	0.222	Ns
MCM 527	28	33.523	0.217	Ns
OarFCB 48	78	241.300	0.000	***
SRCRSP 5	36	125.744	0.000	***
SRCRSP 8	36	73.904	0.000	***
SRCRSP 9	21	61.412	0.000	***
SRCRSP 24	28	33.840	0.206	Ns

ns: not significant
***: P<0.001

In Table 4.4 results are summarized for the population with reference to the heterozygosity, PIC values, null alleles and exclusion probabilities. H_E values ranged from 0.341 for MAF65 to 0.807 for microsatellite marker SRCRSP5. PIC values ranged between 0.331 for MAF65 and 0.780 for SRCRSP5 with 13 markers having PIC values above 0.650. H_O is defined as the proportion of individuals in a population that are heterozygous at a certain locus. H_E is defined as the estimated fraction of all the individuals that are heterozygous at any random chosen locus in a population. This is a prediction based on the allele frequencies of the microsatellite markers in the population (Nei, 1978).

Table 4.4 Summary of number of alleles per locus, number of animals tested per marker, Observed and Expected heterozygosities, PIC values, F_{Null}, PE_1, PE_2, PE_P, PE_{ID}, PE_s for 18 markers over the whole population

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_{ID}	PE_S
BM 1258	13	179	0.760	0.771	0.734	0.004	0.382	0.559	0.745	0.911	0.613
BM 1329	8	167	0.743	0.765	0.727	0.011	0.372	0.549	0.735	0.907	0.608
BM 1818	9	160	0.756	0.754	0.716	-0.017	0.363	0.541	0.733	0.903	0.602
BM 7160	10	160	0.631	0.788	0.754	**0.110**	0.408	0.585	0.769	0.922	0.623
CSRD 247	9	173	0.636	0.708	0.661	**0.054**	0.300	0.473	0.662	0.868	0.570
HSC	12	143	0.699	0.749	0.712	0.036	0.365	0.542	0.739	0.902	0.599
ILSTS 5	4	147	0.449	0.470	0.374	0.016	0.110	0.196	0.300	0.623	0.390
ILSTS 87	9	170	0.488	0.495	0.474	-0.001	0.140	0.311	0.502	0.724	0.428
INRA 5	3	155	0.439	0.468	0.363	0.029	0.109	0.185	0.281	0.612	0.386
INRA 63	5	174	0.655	0.701	0.648	0.033	0.280	0.449	0.628	0.858	0.564
INRABERN 192	10	191	0.743	0.731	0.686	-0.007	0.324	0.499	0.686	0.884	0.585
MAF 65	10	172	0.337	0.341	0.331	-0.003	0.064	0.202	0.354	0.556	0.309
MCM 527	10	189	0.571	0.701	0.654	**0.111**	0.292	0.465	0.653	0.864	0.566
OarFCB 48	8	184	0.707	0.779	0.746	0.048	0.396	0.575	0.762	0.919	0.618
SRCRSP 5	10	162	0.722	0.807	0.780	**0.054**	0.451	0.626	0.812	0.937	0.637
SRCRSP 8	11	155	0.510	0.653	0.628	**0.150**	0.268	0.458	0.673	0.855	0.539
SRCRSP 9	9	171	0.655	0.705	0.663	0.046	0.299	0.476	0.668	0.871	0.569
SRCRSP 24	9	129	0.597	0.709	0.666	0.091	0.303	0.481	0.672	0.873	0.571
Average	8.833	165.611	0.617	0.672	0.629	0.043	0.290	0.454	0.632	0.833	0.543

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

Microsatellite markers tend to present null alleles and this is usually indicated by more homozygous individuals in the population for specific markers. It often results in the parent and the offspring having a genotypic mismatch at that locus as the null allele is not amplified. The presence of the null allele is indicated by the F_{Null} values as presented in Table 4.4. In the absence of a null allele, the estimated null allele frequency will be close to zero, and may be slightly negative (negative values imply an excess of heterozygotes). A locus with a large positive estimate of null allele frequency indicates an excess of homozygotes, but does not necessarily signify that a null allele is present. A marker with a null allele frequency of more than 0.05 is not desired in parentage verification as these markers tend to indicate many individuals with homozygous alleles (Marshall *et al.,*

1998). The Null allele frequency ranged from -0.017 (BM 1818) to 0.150 (SRCRSP 8) with an average of 0.043 (Table 4.4). The null allele frequencies larger than 0.05 were printed in bold in Table 4.4. This parameter was not used to exclude microsatellite markers from the panel and was only an indication of a potential presence of a null allele.

The exclusion probabilities as shown in Table 4.4 are hypothetical values based on the allele frequencies of each of the markers alone, and can thus be computed in any family structure. PE_1 is the exclusion probability of each microsatellite marker when the genotypes of the candidate parent (most often the sire) and the offspring are known. PE_2 differs from PE_1 that the candidate parent (most often the sire), offspring, and the known parent's (most often the dam) genotypes are known. The results for all families tested were treated as one population and markers ILSTS 5, ILSTS 87, INRA 5, INRA 63, MAF 65, and SRCRSP 8 performed below average for PE_1 (0.290) and PE_2 (0.454).

PE_P is a parameter used to determine the power of each microsatellite marker to exclude the non-parent when the genotypes of the offspring and two candidate parents (thus neither the sire nor the dam is confirmed as known-parents) are analysed. The average value for PE_P across all families was 0.632. The six markers that performed poorly for PE_1 and PE_2, had a PE_P exclusion power below the population average with the exception of SRCRSP8 with a PE_P value of 67%.

Similar results were obtained for the probability to distinguish between non-related individuals (PE_{ID}) and between siblings (PE_S). Markers ILSTS 5, ILSTS 87, INRA 5 and MAF 65 performed below the average values of PE_{ID} (0.83) and PE_S (0.54). INRA 65 and SRCRSP 8 performed better than the above mentioned markers, however only slightly better than the averages of PE_{ID} and PE_S.

The remaining 12 microsatellite markers analyzed performed above average of for all exclusion probability parameters. SRCRSP5 performed the best over all the parameters (H_E, PIC, PE_1, PE_2, PE_P, PE_{ID} and PE_S) evaluated.

Evaluation of the microsatellite markers in the families treated as one population indicated that four of the markers (ILSTS5, ILSTS87, INRA5 and MAF65) were not suitable for inclusion in a parentage verification panel. To confirm this, the 18 microsatellite markers were analyzed per family. Results obtained in each separate family for the number of alleles, heterozygosity values, PIC values, F_{Null} frequencies and exclusion probabilities for each microsatellite marker are presented in Appendix B, Tables 1B – 7B. The data summarized in Table 4.1 to 4.4 (previous section) and Tables 1B – 7B (Appendix B) indicate that four markers (ILSTS 5, ILSTS 87, INRA 5 and MAF65) should be excluded from the panel of selected microsatellite markers.

A summary of the performance of these four markers are given in Figure 4.1 a-h. Figure 4.1a represents the entire population and the figures thereafter each represent a different family. In each figure the performance of the marker is shown for the parameters evaluated (H_O, H_E, PIC, PE_1, PE_2, PE_P, PE_{ID} and PE_S). The F_{Null} frequency is not shown as most of the markers had a frequency of close to zero indicating the absence of a null allele. The last set of columns is the average of all markers evaluated for each of the specific parameters in the family. The graphs in Figure 4.1 indicate that ILSTS5, ILSTS87, INRA5 and MAF65 performed exceptionally poor in most of the parameters in all of the families tested in this study. Based on these values, these markers are not suitable for inclusion in this parentage verification panel.

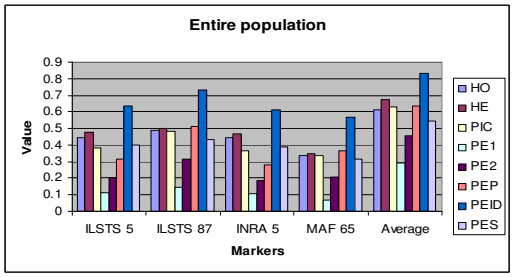

Figure 4.1a Performance of markers in entire population

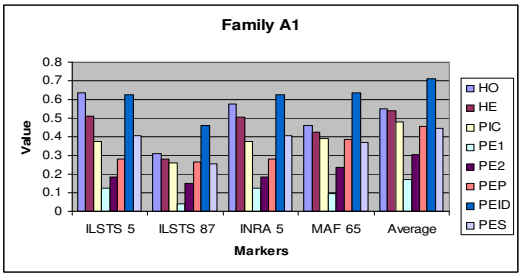

Figure 4.1b Performance of markers in family A1

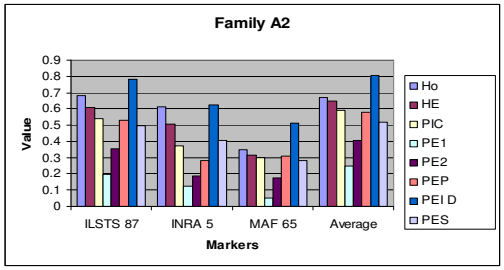

Figure 4.1c Performance of markers in family A2
*ILSTS5 not analysed

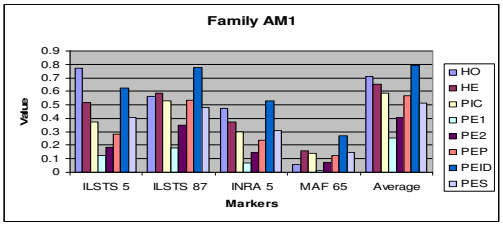

Figure 4.1d Performance of markers in AM group, Breeder 1

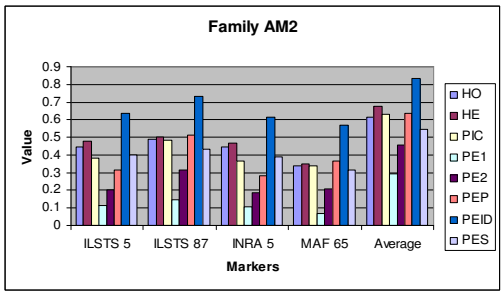

Figure 4.1e Performance of markers in AM group, Breeder 2

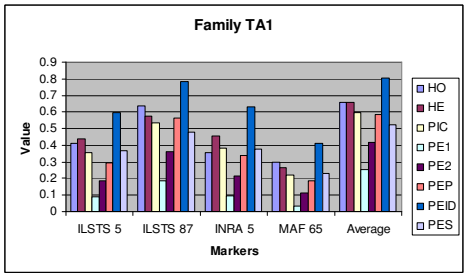

Figure 4.1f Performance of markers for test animals, Breeder 1

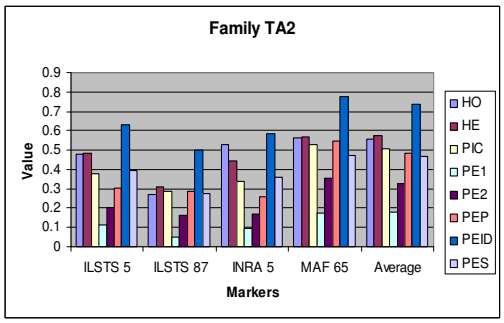

Figure 4.1g Performance of markers for test animals Breeder 2

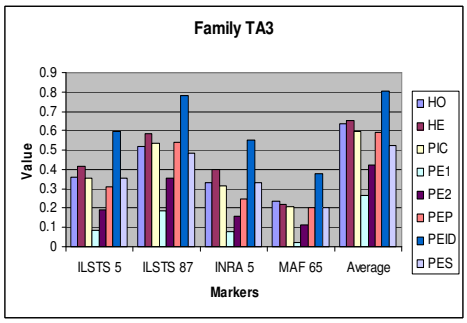

Figure 4.1h Performance of markers for test animals Breeder 3

Figure 4.1 Performance of ILSTS5, ISTST87, INRA5 and MAF65 for the parameters H_O, H_E, PIC, PE_1, PE_2, PE_P, PE_{ID} and PE_S, in the total population as well as in each family and compared to the average for all markers of each parameter in that family

The results obtained from analysing the Saanen goats are shown in Appendix A. Microsatellite markers MAF65 and ILSTS87 performed above average for all the discussed parameters in this population studied. As in the Angora population ILSTS5 and INRA5 performed poorly for all the parameters. Two additional microsatellite markers, BM1760 and INRABERN192 performed much weaker in the Saanen population compared to their performance in the Angora population. This population was however much smaller, consisting of only 16 individuals, compared to the Angora population of 200 individuals.

4.2 Optimum number of microsatellite markers for parentage verification

Microsatellite markers ILSTS5, ILSTS87, INRA5 and MAF65 were excluded from the final panel based on individual performance, but to illustrate their influence on the accuracy of the panel they were also included in this section. To improve the panel's accuracy the microsatellite markers were excluded based on their allele frequencies, the number of alleles at each locus, heterozygosity values, PIC values and exclusion probabilities (first parent, second parent, parent pair, individual-and sibling identification). Table 4.5 provides a summary of the change in the combined exclusion probability for the panel as the number of markers was decreased from 18 to eight.

After the first four markers were excluded SRCRSP 8, INRA 63 and SRCRSP 24 were the markers with the poorest performance of the remaining 14 markers. In order to compile the most powerful and cost efficient panel, these microsatellite markers were excluded to determine the effect on the exclusion power of the panel. As indicated in Table 4.5 the exclusion power was reduced even further after these markers were removed. The decrease in the exclusion power were five times as high compared to when only the first four markers were excluded. Therefore only the first four mentioned markers were left out from the final panel resulting in a final panel consisting of 14 microsatellite markers.

Table 4.5 A summary of the combined exclusion probability for different number of markers

Exclusion probability	Number of markers used	Exclusion probability	Markers excluded
CPE_1	**18 Markers**	**0.9982**	**None**
CPE_2		0.9999	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**17 Markers**	**0.9981**	**MAF 65**
CPE_2		0.9999	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**16 Markers**	**0.9979**	**MAF 65, ILSTS5**
CPE_2		0.9999	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**15 Markers**	**0.9976**	**MAF 65, ILSTS5, INRA5**
CPE_2		0.9999	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**14 Markers**	**0.9973**	**MAF 65, ILSTS5, INRA5, ISLTS87**
CPE_2		0.9999	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**13 Markers**	**0.9963**	**MAF 65, ILSTS5, INRA5, ISLTS87, SRCRSP 8**
CPE_2		0.9999	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**12 Markers**	**0.9947**	**MAF 65, ILSTS5, INRA5, ISLTS87, SRCRSP 8, SRCRSP 24**
CPE_2		0.9998	
CPE_P		0.9999	
CPE_{ID}		1	
CPE_S		0.9999	
CPE_1	**11 Markers**	**0.9927**	**MAF 65, ILSTS5, INRA5, ISLTS87, SRCRSP 8, SRCRSP 24, INRA63**
CPE_2		0.9998	
CPE_P		0.9999	
CPE_{ID}		1.0000	
CPE_S		0.9999	
CPE_1	**8 Markers**	**0.9789**	**MAF 65, ILSTS5, INRA5, ISLTS87, SRCRSP 8, SRCRSP 24, INRA63, SRCRSP 9, CSRD 247, MCM 527**
CPE_2		0.9986	
CPE_P		0.9999	
CPE_{ID}		0.9999	
CPE_S		0.9995	

CPE_1: Combined exclusion probability for one candidate parent alone
CPE_2: Combined exclusion probability for one candidate parent and one known parent of the opposite sex
CPE_P: Combined exclusion probability for a candidate parent pair
CPE_{ID}: Combined exclusion probability to exclude between two non-related individuals
CPE_S: Combined exclusion probability for distinguishing between two siblings

The combined first parent exclusion probability when using all 18 markers was 99.83% and when MAF 65 was excluded, it decreased to 99.82% as shown in Table 4.5. The next marker to be excluded was ILSTS 5 and the combined first parent exclusion probability decreased slightly to 99.79%. After INRA 5 was excluded CPE1 decreased by 0.02% to 99.77% followed by the removal of ILSTS 87 it changed to 99.73% (Table 4.5).

CPE1 decreased with a further 0.10% to 99.63% after the exclusion of SRCRSP8. The sequence of elimination of the last three markers did not have an effect. When INRA 63 (as opposed to SRCRSP 8) was removed first the CPE_1 decreased to 99.62% (opposed to 99.63%) and if SRCRSP 24 was removed first it decreased to 99.61%. The exclusion of microsatellite marker SRCRSP 24 (in addition to SRCRSP 8) led to the first parent exclusion probability decreasing to 99.47%. The other combined exclusion probabilities did not change significantly with the successive exclusion of markers. INRA63 had only five alleles and when this marker was removed from the panel (in addition to SRCRSP 8), the first parent exclusion probability decreased to 99.27%. The increments by which the exclusion probability decreased became much larger as more markers were left out.

To test the power of the panel three additional markers were excluded from the panel. These microsatellite markers were the three performing the poorest of the remaining 11 microsatellite markers namely CSRD 247, MCM 257 and SRCRSP 9. After exclusion of these three microsatellite markers, the first parent exclusion probability decreased to 97.89% (Table 4.5).

The combined exclusion probability provides an indication of how accurate the panel can exclude an individual that is not the parent. In Table 4.6 a comparison between the exclusion probabilities calculated for the original eighteen markers and the final panel of fourteen markers for the entire population, and for each family, is shown. The combined

CPE for the total population tested with the 18 microsatellite markers when the genotypes of the candidate parent alone were taken into account was 99.83%. This changed to 99.73% when 14 markers were analysed. This was the smallest decrease in CPE_1 of all the families in this study. The family with the highest difference in CPE_1 when 18 and 14 markers were used respectively was family A1 (1.53%) followed by TA2 with a difference of 1.44%. These two families also had the lowest H_E values. The largest difference in CPE_2 was for family A1 at 0.14%. The change in CPE_P, CPE_{ID} and CPE_S was small for all families.

Table 4.6 A comparison of the combined exclusion probabilities between 18 and 14 markers for all the families, expressed as a percentage

Family	All families			A1			A2			AM1		
N markers	18	14	Diff	18	14	Diff	18	14	Diff	18	14	Diff
CPE_1	**99.83**	**99.73**	**0.10**	**96.91**	**95.38**	**1.53**	**99.70**	**99.17**	**0.53**	**99.55**	**99.36**	**0.19**
CPE_2	99.99	>99.99	-0.01	99.90	99.76	0.14	99.90	99.98	-0.08	99.99	99.99	0.00
CPE_P	99.99	>99.99	0.00	99.99	99.99	0.00	>99.99	>99.99	0.00	>99.99	>99.99	0.00
CPE_{ID}	>99.99	>99.99	0.00	>99.99	>99.99	0.00	>99.99	>99.99	0.00	>99.99	>99.99	0.00
CPE_S	>99.99	>99.99	0.00	99.99	99.99	0.00	99.99	100.00	-0.01	99.99	100.00	-0.01

Family	AM2			TA1			TA2			TA3		
N markers	18	14	Diff	18	14	Diff	18	14	Diff	18	14	Diff
CPE_1	**99.50**	**99.09**	**0.41**	**99.57**	**99.34**	**0.23**	**97.53**	**96.09**	**1.44**	**99.65**	**99.48**	**0.17**
CPE_2	99.99	99.98	0.01	99.99	99.99	0.00	99.93	99.81	0.12	99.99	99.99	0.00
CPE_P	>99.99	>99.99	0.00	>99.99	>99.99	0.00	99.99	99.99	0.00	>99.99	>99.99	0.00
CPE_{ID}	>99.99	>99.99	0.00	>99.99	>99.99	0.00	>99.99	>99.99	0.00	>99.99	>99.99	0.00
CPE_S	99.99	100.00	-0.01	99.99	100.00	-0.01	99.99	99.99	0.00	99.99	>0.9999	0.00

N markers: number of markers
Diff: Difference in exclusion probability when 18 and 14 microsatellite markers were used
CPE_1: Combined exclusion probability for one candidate parent alone
CPE_2: Combined exclusion probability for one candidate parent and one known parent of the opposite sex
CPE_P: Combined exclusion probability for a candidate parent pair
CPE_{ID}: Combined exclusion probability to exclude between two non-related individuals
CPE_S: Combined exclusion probability for distinguishing between two siblings

In Figures 4.2 to 4.4 the probability to exclude an individual as a true parent in different scenarios are shown. The scenarios included both parents known, one parent known, both parents unknown, distinguishing between siblings and non-siblings. For each unit on the graph, one microsatellite marker is randomly added not based on performance.

Figure 4.2 illustrates the scenario where both parents are known parents (no candidate parents included). The probability to select the correct parent in this scenario increases from 0.50 with only one microsatellite marker was tested, to greater than 0.99 when five or more microsatellite markers were tested (Figure 4.2). The red line on the graph indicates the exclusion maximum of the population, and when five markers were used, the exclusion maximum and the exclusion probability unite.

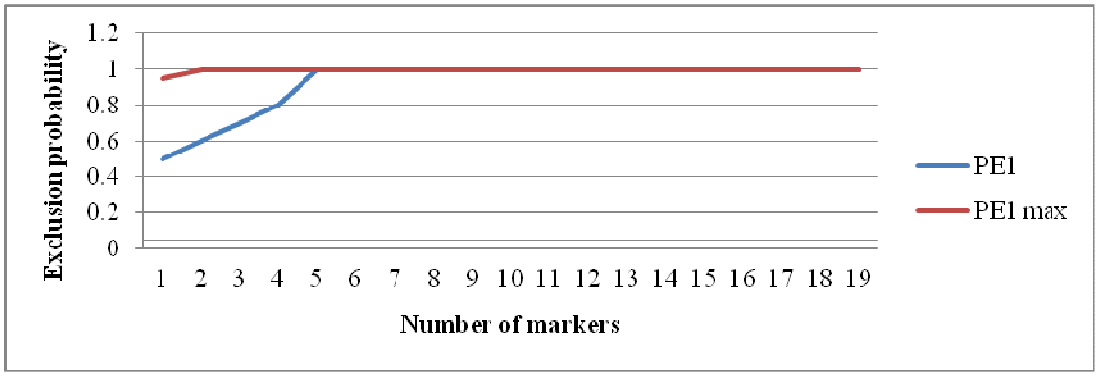

Figure 4.2 Probability of excluding the non-parent when both parents are known per microsatellite marker.

In Figure 4.3 the scenario where one parent was a confirmed parent (known parent) and the other parent was unconfirmed (candidate parent) was illustrated. The exclusion probability with only one microsatellite marker included was approximately 0.45, and increase to greater than 0.99 when another six markers were added. The maximum probability and the exclusion probability converge when seven markers were used.

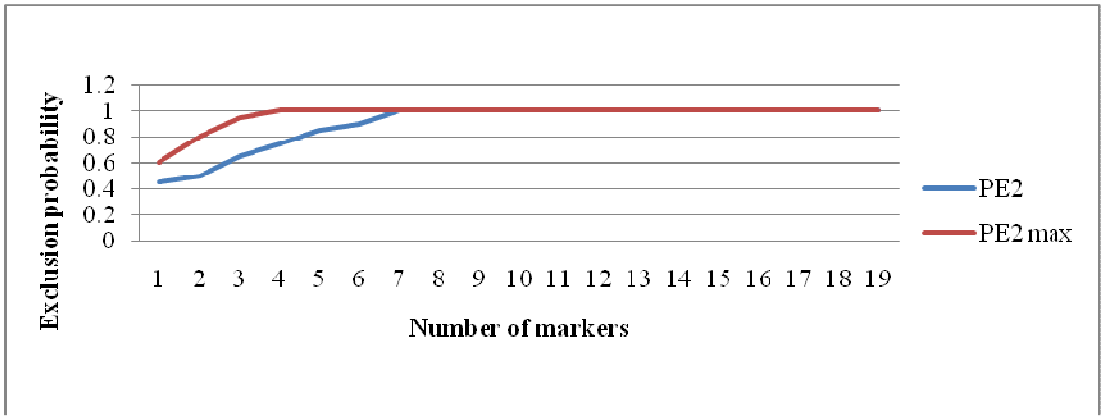

Figure 4.3 Probability of excluding the non-parent when one known parent and candidate parents are included per microsatellite marker.

The probability to exclude the non-parent when both parents were unknown (no confirmed parents) was shown in Figure 4.4. The exclusion probability was slightly above 0.60 with one microsatellite marker but increases greater than 0.99 when another two markers were added. This was also the point where the maximum exclusion probability and the exclusion probability converged.

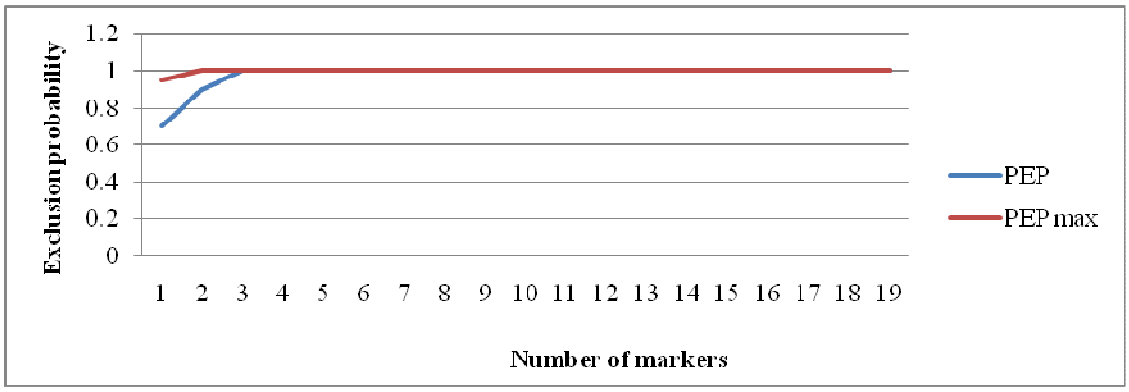

Figure 4.4 Probability of excluding the non-parent when neither of the parents is confirmed parents per microsatellite marker.

Based on all the parameters discussed only MAF 65, ILSTS 5, ILSTS 87 and INRA 5 were excluded from the final panel. The exclusion power of the panel was compromised with further exclusion of microsatellite markers. The final set of microsatellite markers with the strongest exclusion power and the least number of microsatellite markers consisted of 14 microsatellite markers namely BM1258, BM1329, BM1818, BM7160, CSRD247, HSC, INRA63, INRABERN192, MCM527, OarFCB48, SRCRSP5, SRCRSP8, SRCRSP9 and SRCRSP24. This panel had a combined first parent exclusion probability of 99.73%.

4.3 Missing genotypes and their effect on choosing the correct parent

The effect of missing genotypes on the accuracy of the panel of microsatellite markers was also evaluated. In the event of poor PCR amplification success some animals may have less genotypic information available for comparison. The analysis using the computer program Cervus 3.0 takes the effect of missing genotypes into consideration

(Slate *et al.,* 2000) by calculating the proportion of loci typed. This proportion should be an average across all the loci and individuals studied.

In order to verify whether the exclusion power of the panel will remain constant even with fewer loci to compare, a fictional marker with no genotypic information was added to the 18 and 14 marker panels and the exclusion probabilities remained unchanged in both cases. In addition the effect of the number of loci compared between the candidate parent and the offspring on the exclusion probability was analysed using only the four markers with the poorest performance and excluded from the final panel (ILSTS 5, ILSTS 87, INRA 5 and MAF 65) (Table 4.7).

Table 4.7 The change in exclusion probability if four markers with poor performance were analysed alone vs. combined with one marker with good performance

Microsatellite markers analysed	Exclusion probability	
ILSTS 5, ILSTS 87, INRA 5 and MAF 65	CPE_1	**0.3624546**
	CPE_2	0.6399852
	CPE_P	0.8383852
	CPE_{ID}	0.9821504
	CPE_S	0.8523756
ILSTS 5, ILSTS 87, INRA 5, MAF 65 and BM 1258	CPE_1	**0.6054388**
	CPE_2	0.8408732
	CPE_P	0.9585918
	CPE_{ID}	0.9984105
	CPE_S	0.9427281

CPE_1: Combined exclusion probability for one candidate parent alone
CPE_2: Combined exclusion probability for one candidate parent and one known parent of the opposite sex
CPE_P: Combined exclusion probability for a candidate parent pair
CPE_{ID}: Combined exclusion probability to exclude between two non-related individuals
CPE_S: Combined exclusion probability for distinguishing between two siblings

The combined exclusion probability of these four markers is shown in Table 4.7 and was very low at 36.24%. Thereafter, microsatellite marker BM 1258 was included in the analysis and CPE_1 increased to 60.5%.

Missing data is not linked only to the amplification success of a microsatellite marker but is a random event. Any marker, despite its performance, may have missing data for any animal. In this study the average number of loci compared between any parent and offspring was 12.07. In Table 4.8 the change in exclusion probabilities when the number

of randomly chosen microsatellite markers was increased from five to twelve were presented.

Only the fourteen microsatellite markers selected for the final panel were used. The markers were selected randomly in four consecutive tests to calculate the exclusion power of the panel when between five and twelve microsatellite markers were analysed.

Table 4.8 The change in exclusion probabilities when the number of randomly chosen microsatellite markers was increased from five to twelve

Number of microsatellite markers	CPE				
	Test 1	Test 2	Test 3	Test 4	Average
Five Randomly selected microsatellite markers					
CPE_1	**0.852982**	**0.86474**	**0.870876**	**0.852379**	**0.860244**
CPE_2	0.967931	0.970545	0.972705	0.966496	0.969419
CPE_P	0.997182	0.997427	0.997704	0.996654	0.997242
CPE_{ID}	0.999977	0.999981	0.999984	0.999975	0.999979
CPE_S	0.986562	0.987429	0.988212	0.987038	0.98731
Six Randomly selected microsatellite markers					
CPE_1	**0.912974**	**0.913837**	**0.917832**	**0.912618**	**0.914315**
CPE_2	0.98669	0.98649	0.987463	0.986094	0.986684
CPE_P	0.999349	0.999312	0.999396	0.999227	0.999321
CPE_{ID}	0.999998	0.999998	0.999998	0.999998	0.999998
CPE_S	0.99494	0.994992	0.995262	0.995119	0.995078
Seven Randomly selected microsatellite markers					
CPE_1	**0.941179**	**0.946769**	**0.948391**	**0.944394**	**0.945183**
CPE_2	0.993337	0.994043	0.994349	0.993613	0.993836
CPE_P	0.999796	0.999825	0.99984	0.999797	0.999814
CPE_{ID}	1	1	1	1	1
CPE_S	0.997902	0.998059	0.998144	0.998038	0.998036
Eight Randomly selected microsatellite markers					
CPE_1	**0.964467**	**0.964021**	**0.96223**	**0.969479**	**0.965049**
CPE_2	0.99717	0.997018	0.996936	0.997614	0.997184
CPE_P	0.999951	0.999945	0.999948	0.999962	0.999951
CPE_{ID}	1	1	1	1	1
CPE_S	0.999199	0.999195	0.999145	0.999287	0.999207
Nine Randomly selected microsatellite markers					

CPE_1	**0.980497**	**0.978703**	**0.977643**	**0.981562**	**0.979601**
CPE_2	0.998943	0.998762	0.998728	0.998987	0.998855
CPE_P	0.999991	0.999987	0.999988	0.999991	0.999989
CPE_{ID}	1	1	1	1	1
CPE_S	0.999709	0.999697	0.999678	0.999728	0.999703
10 Randomly selected microsatellite markers					
CPE_1	**0.987589**	**0.984928**	**0.984416**	**0.98842**	**0.986338**
CPE_2	0.999514	0.999338	0.999339	0.999543	0.999434
CPE_P	0.999998	0.999996	0.999996	0.999998	0.999997
CPE_{ID}	1	1	1	1	1
CPE_S	0.999883	0.999868	0.999862	0.999893	0.999877
11 Randomly selected microsatellite markers					
CPE_1	**0.991316**	**0.98897**	**0.990372**	**0.991805**	**0.990616**
CPE_2	0.999744	0.999641	0.999709	0.999756	0.999712
CPE_P	0.999999	0.999999	0.999999	0.999999	0.999999
CPE_{ID}	1	1	1	1	1
CPE_S	0.99995	0.999939	0.999947	0.999954	0.999947
12 Randomly selected microsatellite markers					
CPE_1	**0.994635**	**0.993337**	**0.994715**	**0.994287**	**0.994244**
CPE_2	0.999887	0.999848	0.999891	0.999873	0.999875
CPE_P	1	1	1	1	1
CPE_{ID}	1	1	1	1	1
CPE_S	0.999981	0.999977	0.999981	0.99998	0.99998

CPE_1: Combined exclusion probability for one candidate parent alone
CPE_2: Combined exclusion probability for one candidate parent and one known parent of the opposite sex
CPE_P: Combined exclusion probability for a candidate parent pair
CPE_{ID}: Combined exclusion probability to exclude between two non-related individuals
CPE_S: Combined exclusion probability for distinguishing between two siblings

CPE_1 did not vary much between the four different trials with the same number of markers used and the most variation was reported when five microsatellite markers were used. The average CPE_1 varied between 86.02% for five markers and 99.42% when 12 microsatellite markers were used. The other exclusion probabilities (CPE_2, CPE_P, CPE_{ID} and CPE_S) also increased as the number of microsatellite markers were increased from five to twelve.

4.4 Parentage analyses

The final panel of 14 microsatellite markers was evaluated in the different families to determine how effectively the panel can be used to infer parentage in different family structures. In families A1, A2 and the TA families, the buck suggested by the breeder to be the biological sire, as well as some of the sires used during the breeding season, were tested. In most of the cases not all of the sires used during the breeding season were tested.

For the first family (A1) both parents were known and according to the records from the breeder buck C1 was the sire to all the kids. In Table 4.9 the results of the parentage analysis for this family is shown. Each offspring occupies two lines in the table. The lines represent data of the most likely and second most likely parents, respectively. It must be highlighted that the parent chosen as the most likely candidate parent is not necessarily the biological parent. It is merely the parent in this data set that is most likely to be the correct parent, as suggested by the breeder. Column one of the table indicates the identification of the offspring and column two the identification of the known mother. Offspring C3, C38 and C39 had no maternal information available. In column three the number of loci compared between the offspring and known mother is shown. This number varied according to the genotypic data available for the parents and the offspring. In column four the number of genotypic mismatches between the offspring and known mother is indicated. The number of loci compared ranged between seven and fourteen (from a possible fourteen) and the number of genotypic mismatches did not exceed one (this will be discussed in more detail in Table 4.10). The LOD score of the known mother and offspring is shown in column five. Six of the combinations had a negative LOD score (printed in bold), indicating that the ewe is more likely not to be the true parent than to be the true parent. However, these are also the combinations with one genotypic mismatch each. Only combination C13-C21 had one mismatch and a positive LOD score.

In column six of Table 4.9 the identification of the most likely and second most likely candidate parent (sire) of each offspring is shown. Buck C1 (the sire of all the kids, according to the data from the breeder) was assigned as the most likely parent to all but

four offspring (C14, C17, C19 and C35; printed in bold). C1 was assigned the second most likely parent of C17.

Table 4.9 Parentage analysis for family A1 with 14 microsatellite markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatched	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatched	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatched	Trio LOD score	Trio Delta	Trio confidence	PE1	PE2
C2	C21	8	0	6.34	C1	10	1	-5.10	0.00		10	1	-2.86	0		0.91	0.96
	C21	8	0	6.34	E4	7	2	-5.72	0.00		7	3	-8.88	0		0.91	0.96
C3		0	0	0	C1	11	2	-5.67	0.00		0	0	0	0		0.93	0.93
		0	0	0	E4	8	4	-13.70	0.00		0	0	0	0		0.93	0.93
C4	C22	10	0	2.77	C1	12	1	-2.28	0.00		12	1	-0.85	0		0.83	0.99
	C22	10	0	2.77	E2	10	3	-10.00	0.00		10	5	-16.29	0		0.83	0.99
C5	C23	7	**1**	**-0.3**	C1	11	3	-13.00	0.00		11	4	-12.68	0		**0.98**	0.99
	C23	7	**1**	**-0.3**	E1	9	3	-12.10	0.00		9	5	-15.22	0		**0.98**	0.99
C6	C24	10	0	2.47	C1	11	2	-7.63	0.00		11	3	-9.78	0		0.95	0.99
	C24	10	0	2.47	E4	8	3	-9.78	0.00		8	3	-9.85	0		0.95	0.99
C7	C25	10	0	2.08	C1	11	1	-4.72	0.00		11	1	-2.3	0		0.71	0.94
	C25	10	0	2.08	E4	8	2	-6.33	0.00		8	2	-5.47	0		0.71	0.94
C8	C26	9	0	4.23	C1	11	2	-7.62	0.00		11	2	-5.61	0		0.87	1.00
	C26	9	0	4.23	E2	9	5	-19.50	0.00		9	6	-22.73	0		0.87	1.00
C9	C27	9	**1**	**-4.5**	C1	10	2	-8.13	0.00		10	3	-8.04	0		**0.66**	0.99
	C27	9	**1**	**-4.5**	E1	9	4	-16.40	0.00		9	4	-11.63	0		**0.66**	0.99
C10	C27	10	0	1.08	C1	10	1	-2.23	0.00		10	2	-3.9	0		0.82	**1.00**
	C27	10	0	1.08	E4	8	4	-15.10	0.00		8	5	-18.12	0		0.82	**1.00**
C11	C28	13	0	0.72	C1	14	1	-3.11	0.00		14	1	**0.01**	**0.01**	**+**	0.92	**1.00**
	C28	13	0	0.72	E4	10	4	-14.90	0.00		10	5	-17.72	0		0.92	**1.00**
C12	C29	14	**1**	**-1.4**	C1	14	1	-2.78	0.00		14	3	-3.33	0		0.94	**1.00**
	C29	14	**1**	**-1.4**	E4	10	4	-16.90	0.00		10	6	-18.19	0		0.94	**1.00**
C13	C21	11	**1**	0.63	C1	14	1	-5.15	0.00		14	2	-3.19	0		0.92	0.94
	C21	11	**1**	0.63	E4	10	3	-11.00	0.00		10	3	-7.3	0		0.92	0.94
C14	C30	13	**1**	**-1.3**	**E2**	11	5	-16.30	0.00		11	5	-12.46	0		0.95	0.99
	C30	13	**1**	**-1.3**	**E4**	9	3	-11.80	0.00		9	5	-15.09	0		0.95	0.99
C16	C31	12	0	3.34	C1	12	1	-4.63	0.00		12	3	-9.1	0		0.87	0.99
	C31	12	0	3.34	E4	8	4	-15.40	0.00		8	5	-19.68	0		0.87	0.99
C17	C32	10	0	3.03	**E4**	8	4	-14.30	0.00		8	4	-14.56	0		0.93	0.98
	C32	10	0	3.03	**C1**	11	3	-11.50	0.00		11	4	-15.16	0		0.93	0.98
C18	C33	12	0	1.81	C1	12	2	-6.36	0.00		12	3	-10.36	0		0.97	0.99
	C33	12	0	1.81	E4	9	5	-17.40	0.00		9	5	-18.38	0		0.97	0.99
C19	C33	9	**1**	**-4.4**	**E1**	6	2	-6.61	0.00		6	2	-2.95	0		0.92	0.94

	C33	9	**1**	**-4.4**	**E4**	6	2	-6.61	0.00	6	2	-2.95	0	0.92	0.94
C20	C34	10	0	3.16	C1	11	2	-8.03	0.00	11	2	-7.84	0	0.77	**0.87**
	C34	10	0	3.16	E1	8	3	-9.47	0.00	8	3	-10.83	0	0.77	**0.87**
	C34	10	0	3.16	E4	8	3	-9.47	0.00	8	3	-10.83	0	0.77	**0.87**
C35	C37	10	0	3.53	**E2**	9	3	-10.10	0.00	9	3	-9	0	0.89	0.99
	C37	10	0	3.53	**E1**	8	3	-8.24	0.00	8	4	-13.53	0	0.89	0.99
C36	C37	11	**1**	**-2.9**	C1	13	3	-10.00	0.00	13	5	-13.04	0	0.96	0.98
	C37	11	**1**	**-2.9**	E4	9	4	-15.70	0.00	9	5	-14.99	0	0.96	0.98
C38		0	0	0	C1	10	2	-5.71	0.00	0	0	0	0	0.87	**0.87**
		0	0	0	E1	8	3	-10.80	0.00	0	0	0	0	0.87	**0.87**
C39		0	0	0	C1	12	1	-3.21	0.00	0	0	0	0	0.88	0.88
		0	0	0	E4	8	3	-8.54	0.00	0	0	0	0	0.88	0.88
C40	C42	13	0	7.79	C1	14	4	-17.80	0.00	14	4	-15.28	0	0.86	0.98
	C42	13	0	7.79	E4	10	4	-17.00	0.00	10	4	-15.33	0	0.86	0.98
C41	C42	9	0	3.75	C1	9	3	-11.50	0.00	9	3	-11.5	0	0.86	0.97
	C42	9	0	3.75	E4	6	3	-12.80	0.00	6	3	-12.17	0	0.86	0.97
C43	C44	8	0	3.23	C1	10	1	-4.52	0.00	10	1	-4.76	0	0.91	0.91
	C44	8	0	3.23	E4	6	3	-9.83	0.00	6	3	-10.13	0	0.91	0.91

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

Column seven and eight of Table 4.9 indicates the number of loci compared and the number of mismatches between the offspring and candidate parent, respectively. The number of loci compared ranged between six and fourteen and the number of mismatches between one and five (most likely candidate parent and offspring combinations). Column nine of Table 4.9 depicts the LOD score of the offspring and the candidate parent (most likely candidate parent in the first line and second most likely parent in the second line for each offspring). Not one of these values (for the most likely or second most likely individuals) was positive indicating that the buck is more likely not to be the true parent

than to be the true parent. The LOD scores for the two candidate parents of offspring C19 were exactly the same. Offspring C20 had three candidate sires as E1 and E4 had the exact same number of markers compared, mismatches and LOD scores. The Delta score is the difference between the LOD scores of the most likely and second most likely candidate parents. If both the LOD scores are negative, the Delta score is zero (column ten). In column eleven the pair confidence is shown. In this family no candidate parent could be assigned at the strict or relaxed confidence levels.

Columns twelve to sixteen in Table 4.9 relates to the results when the offspring, known mother and candidate father are analysed together (trio). For the offspring without maternal information the values at these columns were zero. Column twelve indicates the number of loci compared between the three animals and range between six and fourteen whereas in column thirteen the number of mismatches between the trios ranged between one and six (most likely candidate parent). The number of mismatches of each trio is often different than the sum of the mismatches between the known mother and offspring and the candidate father and offspring. Column fourteen indicates the LOD score of each trio and all scores were negative except for offspring C11 (printed in bold). This is also the only positive Delta score (column fifteen) and the only trio that could be assigned at a confidence level (column sixteen). This trio was assigned at the relaxed confidence level of 80%.

Column seventeen (Table 4.9) indicates the first parent exclusion probability (PE_1) for each offspring if only the allele frequencies of the genotypes of the offspring are used for the calculation and compared with the exclusion probability calculated from the genotypes of the candidate parents. This value ranged between 0.66 (offspring C9, bold) and 0.98 (C5, bold). The second parent exclusion probability (PE_2) (column 18, Table 4.9) is calculated using the allele frequencies of the known parent (dam) and the offspring's genotypes and compared with the exclusion probability of the candidate parent. The second parent exclusion probability is the probability to exclude a non-parent based on the genotypes of the known parent and the offspring. It ranged between 0.87 (C20, and C38 bold) and greater than 0.99 (C10, C11 and C12, bold).

In Table 4.10 the genotypic mismatches between the known parent (dam) and the offspring for family A1 are shown. The number of mismatches between the known parent and offspring did not exceed one. Markers BM 7160, MCM 527 and BM 1329 all had one mismatch in this family. OarFCB 48 and SRCRSP 24 both had two mismatches in the family.

Table 4.10 Known parent – offspring mismatches in family A1

Locus name	Offspring ID	Offspring Allele a	Offspring Allele b	Known parent ID	Known parent Allele a	Known parent Allele b
OarFCB 48	C5	157	165	C23	153	163
OarFCB 48	C36	165	165	C37	157	163
BM 7160	C9	161	163	C27	175	175
MCM 527	C12	152	152	C29	166	172
BM 1329	C13	171	175	C21	169	179
SRCRSP 24	C14	161	161	C30	157	169
SRCRSP 24	C19	167	167	C33	161	169

For family A2 only the sire information was available and sire B1 was assigned as the true parent of all 22 halfsibs in this family. Between nine and fourteen markers were compared and there were no mismatches for any combinations between the offspring and sire B1 (Table 4.11, column four). Despite the lack of mismatches only 10 of the 20 combinations had a positive LOD score and could be assigned at the relaxed (80%, +) interval (nine combinations) and one at the strict (95%, *) interval. PE_1 ranged between 0.94 and 0.99.

Table 4.11 Parentage analysis for family A2 with 14 microsatellite markers

Offspring ID	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	PE1
B2	B1	13	0	1.64	1.64	+	0.96
	E2	12	3	-10.00	0.00		0.96
B3	B1	12	0	0.83	0.83	+	0.99
	E2	10	4	-14.90	0.00		0.99
B4	B1	14	0	-1.11	0.00		0.99
	E2	12	4	-16.10	0.00		0.99
B5	B1	14	0	-0.46	0.00		0.98
	E2	12	4	-17.40	0.00		0.98
B6	B1	14	0	1.01	1.01	+	0.99
	E3	10	6	-22.60	0.00		0.99
B7	B1	13	0	1.25	1.25	+	0.99
	E3	10	6	-22.60	0.00		0.99
B8	B1	13	0	3.38	3.38	*	0.99
	E3	10	3	-7.45	0.00		0.99
B9	B1	13	0	-0.89	0.00		0.94
	E2	12	3	-12.80	0.00		0.94
B10	B1	14	0	-0.49	0.00		0.98
	E2	12	3	-11.30	0.00		0.98
B11	B1	14	0	-0.72	0.00		0.98
	E2	12	3	-10.80	0.00		0.98
B12	B1	14	0	0.84	0.84	+	0.96
	E2	12	5	-21.50	0.00		0.96
B13	B1	13	0	-1.27	0.00		0.97
	E3	9	2	-4.40	0.00		0.97
B14	B1	13	0	1.03	1.03	+	0.99
	E3	9	5	-17.20	0.00		0.99
B16	B1	13	0	-1.33	0.00		0.98
	E3	9	3	-9.17	0.00		0.98
B17	B1	14	0	0.47	0.47	+	0.99
	E3	10	4	-13.30	0.00		0.99
B18	B1	13	0	0.21	0.21	+	0.98
	E3	9	6	-22.70	0.00		0.98
B20	B1	14	0	-1.17	0.00		0.98
	E2	12	5	-20.80	0.00		0.98
B21	B1	13	0	-1.18	0.00		0.99
	E2	11	5	-21.30	0.00		0.99
B22	B1	14	0	1.38	1.38	+	0.99
	E2	12	3	-10.30	0.00		0.99
B23	B1	14	0	-2.31	0.00		0.97
	E3	10	4	-12.90	0.00		0.97

Pair Loci compared: Number of loci compared between the offspring and the candidate parent
Pair loci mismatch: Number of mismatches between the offspring and the candidate parent
* : strict confidence level, 95%
+: relaxed confidence level, 80%

In the AM1 group the number of loci compared ranged between eight and 12 (column three, Table 4.12a) and the number of genotypic mismatches did not exceed two (column four). Five of the nine most likely parent-offspring combinations had a positive LOD score indicating that for these animals the allocated ewe is more likely to be the true dam than not to be the dam. The Delta score for these animals were also positive (column six) but for the remaining four most likely candidate parent-offspring combinations (with negative LOD scores) it was zero as both of the candidate mothers had a negative LOD score. The confidence level at which the parentage could be assigned was at the relaxed (+, 80%) level. PE_1 ranged between 1.00 and 0.95.

Table 4.12a Parentage analyses for AM1 with 14 microsatellite markers

Offspring ID	Candidate mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	PE_1
AM10	**AM5**	9	0	2.57	2.45	+	0.96
	AM2	9	0	0.12	0.00		0.96
AM11	AM6	10	0	2.58	2.58	+	0.95
	AM8	11	2	-7.52	0.00		0.95
AM12	AM7	11	2	-1.13	0.00		0.99
	AM3	9	2	-8.99	0.00		0.99
AM14	AM9	12	2	-5.92	0.00		1.00
	AM5	8	2	-6.37	0.00		1.00
AM15	AM6	10	1	1.26	1.26	+	1.00
	AM8	11	1	-0.94	0.00		1.00
AM17	AM1	10	1	-2.30	0.00		0.96
	AM6	11	2	-7.23	0.00		0.96
AM18	AM5	9	0	1.82	0.71	+	0.98
	AM2	9	0	1.11	0.00		0.98
AM19	**AM1**	8	1	-3.19	0.00		0.98
	AM4	8	2	-4.30	0.00		0.98
AM20	AM3	9	0	3.95	3.95	+	0.98
	AM1	10	2	-6.62	0.00		0.98

Pair Loci compared: Number of loci compared between the offspring and the candidate parent
Pair loci mismatch: Number of mismatches between the offspring and the candidate parent
+: relaxed confidence level, 80%

In the AM1 group, offspring AM10 and AM19 (printed in bold) were allocated to different dams than the breeder suggested (Table 4.12a). In both cases the suggested dam was assigned as the ewe second most likely to be the dam. In the case of AM10 ewe AM5 was assigned with positive LOD and Delta scores at an 80% confidence level, indicating that the information received from the breeder might be incorrect. Offspring AM19 were assigned with a negative LOD score for both candidate mothers and a Delta score of zero.

The number of loci compared in the AM2 group (Table 4.12b, column three) ranged between four and eleven. Three of the most likely candidate mother and offspring combinations had one genotypic mismatch whereas the remaining seven combinations had zero mismatches (column four).

Table 4.12b Parentage analyses for AM2 with 14 microsatellite markers

Offspring ID	Candidate mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	PE_1
AM21	AM43	9	0	3.62	3.62	*	1.00
	AM41	7	1	-3.48	0.00		1.00
AM23	AM35	11	0	5.70	5.70	*	1.00
	AM41	5	2	-8.02	0.00		1.00
AM24	**AM42**	**6**	**0**	**2.11**	**2.11**	**+**	**0.96**
	AM37	**9**	**1**	**-0.96**	**0.00**		**0.96**
AM25	AM38	10	1	0.93	0.83	+	0.96
	AM36	8	0	0.10	0.00		0.96
AM27	**AM42**	**8**	**0**	**0.52**	**0.16**	**+**	**0.97**
	AM39	**11**	**1**	**0.36**	**0.00**		**0.97**
AM28	AM35	10	1	-3.77	0.00		0.94
	AM42	9	1	-3.82	0.00		0.94
AM29	**AM34**	**6**	**1**	**-1.87**	**0.00**		**0.99**
	AM43	**7**	**1**	**-3.86**	**0.00**		**0.99**
AM30	AM36	10	0	3.30	3.30	*	0.92
	AM39	9	1	-3.15	0.00		0.92
AM31	AM41	4	0	2.61	2.61	*	0.97
	AM35	7	2	-5.47	0.00		0.97
AM32	AM34	8	0	2.72	2.72	*	0.98
	AM42	8	0	-0.39	0.00		0.98

Pair Loci compared: Number of loci compared between the offspring and the candidate parent
Pair loci mismatch: Number of mismatches between the offspring and the candidate parent
* : strict confidence level, 95%
+: relaxed confidence level, 80%

The LOD score of eight of the combinations in family AM2 (Table 4.12b) was positive indicating that the ewe is more likely to be the true mother than not to be the true mother. The Delta scores of the combinations were also positive and could be assigned at a strict (95%) confidence level in five of the cases (*) and at a relaxed confidence level (80%) in three of the cases (+) (column seven, Table 4.12b). PE_1 ranged from 0.94 to 1.00.

As indicated in bold in Table 4.12b, in three of the cases in family AM2, the ewe suggested by the breeder was not assigned as the ewe most likely to be the true parent (printed in bold). For offspring AM24 the breeder suggested ewe AM37 to be the dam but this ewe was assigned as the second most likely dam, while AM42 was assigned as the most likely parent. Combination AM24-AM42 had a positive LOD score and no mismatches and could be assigned at an 80% confidence level, whereas AM24-AM37 had a slightly negative LOD score and one mismatch. The breeder recorded ewe AM39 as the dam of offspring AM27, but AM42 was assigned as the most likely dam (LOD score: 0.52) and AM39 only as the second most likely dam (LOD score: 0.36). There was one mismatch between AM27 and AM39 and zero between AM27 and AM42. The last incident was for offspring AM29 where the dam suggested by the breeder (AM40) was not allocated as the most likely or second most likely parent. AM34 and AM43 were allocated as the most likely and second most likely parents, respectively, and both these combinations had a negative LOD score (resulting in a Delta score of zero) and one mismatch each.

The final scenario in which the panel was tested was for goat samples from three different breeders where there was no confirmed paternal information available. For goats TA12, TA35, TA64, TA81 and TA86 maternal information was also absent. The columns regarding maternal or known parent data as well as the trio-data were zero for these individuals. All the animals tested from the three breeders are included in Table 4.13 a, b and c. The number of loci compared between the parents and offspring varied depending on the genotypic information generated. The quantity of DNA received for all of these animals was limited and therefore only one PCR per microsatellite marker could be performed.

In column two of Table 4.13a the number of loci compared between the known mother and offspring is indicated and ranged between five and thirteen whereas column three indicates that there was one combination with two mismatches, three with one mismatch and six with zero mismatches.

Table 4.13a Parentage analyses for Animals tested for TA1 with 14 markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
TA98	TA28	10	0	1.97	TA6	8	1	**-2.0**	0		8	3	-8.21	0		0.99	1.00
	TA28	10	0	1.97	TA48	9	3	**-11.73**	0		9	5	-16.91	0		0.99	1.00
TA52	TA27	12	0	4.52	TA6	8	0	2.14	2.14	*	8	0	3.81	3.81	*	0.99	1.00
	TA27	12	0	4.52	TA48	9	2	-5.16	0		9	4	-13.79	0		0.99	1.00
TA12		0	0	0	TA6	8	0	2.16	1.19	-	0	0	0	0		0.95	0.95
		0	0	0	TA48	7	0	0.97	0		0	0	0	0		0.95	0.95
TA7	TA45	6	1	**-3.54**	TA68	8	0	2.67	2.67	*	8	3	-4.02	0		1.00	1.00
	TA45	6	1	**-3.54**	TA48	8	2	-8.3	0		8	5	-13.69	0		1.00	1.00
TA20	TA51	10	2	**-6.15**	TA6	7	0	1.44	1.44	-	7	3	-4.51	0		0.98	1.00
	TA51	10	2	**-6.15**	TA48	7	1	-4.81	0		7	2	-6.83	0		0.98	1.00
TA90	TA10	11	0	4.25	TA65	12	2	**-4.29**	0		12	3	-6.19	0		1.00	1.00
	TA10	11	0	4.25	TA16	9	1	**-3.97**	0		9	4	-13.19	0		1.00	1.00
TA92	TA5	8	0	3.15	TA68	10	0	0.76	0.76	-	10	1	-0.55	0		0.99	1.00
	TA5	8	0	3.15	TA48	10	2	-9.95	0		10	2	-8.86	0		0.99	1.00
TA47	TA63	5	1	**-0.35**	TA68	9	0	4.62	4.62	*	9	1	6.04	6.04	*	0.99	1.00
	TA63	5	1	**-0.35**	TA67	11	4	-14.12	0		11	5	-13.72	0		0.99	1.00
TA18	TA85	11	0	3.12	TA68	8	1	**-2.76**	0		8	3	-9.12	0		0.96	1.00
	TA85	11	0	3.12	TA6	8	2	**-7.48**	0		8	4	-14.41	0		0.96	1.00
TA97	TA82	13	1	2.62	TA67	11	0	6.51	6.51	*	11	1	9.39	9.39	*	1.00	1.00
	TA82	13	1	2.62	TA16	9	2	-8.27	0		9	3	-6.51	0		1.00	1.00

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)

Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

Three of the known mother-offspring combinations had a negative LOD score (printed in bold in Table 4.13a). These included TA7 and TA47 that were only compared at six and five loci respectively and had one mismatch each and TA20 that had two mismatches.

The number of loci compared between the candidate parents and the offspring ranged between seven and twelve (column seven, Table 4.13a) and among the most likely candidate parent and offspring combinations there were seven combinations with zero mismatches, two with one mismatch and one with two mismatches (column eight, Table 4.13a).

Three of the most likely candidate parent LOD scores were negative (column nine, printed in bold), resulting in a Delta score of zero (column ten). The remaining seven combinations had positive LOD and Delta scores assigned at the strict (95%) confidence level, or were not conclusively assigned to a buck (-) (column eleven, Table 4.13a).

Column twelve to sixteen in Table 4.13a relate to data from analysing the offspring, known mother and the candidate father (trio). The number of loci compared between the trios ranged between seven and twelve whereas the genotypic mismatches ranged between three and zero (column thirteen). Out of the nine combinations (excluding TA12 because of a lack of maternal information) six had a negative LOD score resulting in a Delta score of zero (columns fourteen and fifteen, printed in bold). The remaining three combinations could be assigned at a strict (95%) confidence level. PE_1 and PE_2 ranged between 0.95 and 1.00.

In Table 4.13b the parentage analysis data for family TA2 is shown. The number of loci compared between the known mother and offspring of family TA2 (Table 4.13b, column three) ranged between five and thirteen.

Table 4.13b Parentage analyses for Animals tested for TA2 with 14 markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
TA35		0	0	0	TA93	12	0	1.24	1.24	-	0	0	0	0		0.83	**0.83**
		0	0	0	TA29	6	0	-0.1	0		0	0	0	0		0.83	**0.83**
TA64	TA62	13	**3**	**-11.79**	TA29	6	0	1.31	1.31	-	6	2	1.84	1.84	*	0.97	0.98
	TA62	13	**3**	**-11.79**	TA93	12	1	-3.08	0		12	4	-2.2	0		0.97	0.98
TA70	TA36	13	0	5.96	TA93	13	0	0.33	0.33	-	13	0	3.12	3.12	*	0.98	0.99
	TA36	13	0	5.96	TA29	6	1	-2.02	0		6	1	-2.93	0		0.98	0.99
TA55	TA32	7	0	2.05	TA23	6	0	**-0.47**	0		6	0	2.25	2.25	*	**0.77**	0.98
	TA32	7	0	2.05	TA41	6	1	**-4.81**	0		6	2	-5.93	0		**0.77**	0.98
TA91	TA80	10	2	**-3.79**	TA23	10	1	**-1.51**	0		10	3	**-3.29**	0		**1.00**	**1.00**
	TA80	10	2	**-3.79**	TA29	4	1	**-4.68**	0		4	1	-3.82	0		**1.00**	**1.00**
TA33	TA14	9	0	4.92	TA93	10	0	1.14	1.14	-	10	0	3.0	3	*	0.92	0.99
	TA14	9	0	4.92	TA29	3	0	-0.48	0		3	1	-3.36	0		0.92	0.99
TA81	TA84	5	0	3.41	TA93	12	0	0.09	0.09	-	12	0	0.66	0.66	-	0.93	0.93
	TA84	5	0	3.41	TA29	5	0	-0.86	0		5	0	-0.43	0		0.93	0.93
TA71	TA38	12	1	4.85	TA93	13	0	1.64	1.11	-	13	1	4.42	2.27	*	0.98	0.99
	TA38	12	1	4.85	TA29	5	0	0.54	0		5	1	2.16	0		0.98	0.99
TA95		0	0	0	TA93	13	0	0.71	0.29	-	0	0	0	0		0.93	0.93
		0	0	0	TA37	12	1	0.42	0		0	0	0	0		0.93	0.93
TA11	TA57	12	0	3.06	TA37	10	1	0.7	0		10	1	2.33	2.33	*	0.92	0.99
	TA57	12	0	3.06	TA93	12	0	0.6	0		12	1	-0.53	0		0.92	0.99
TA103	TA72	13	0	3.16	TA29	6	0	0.61	0.43	-	6	0	0.87	0.87	-	0.90	**1.00**
	TA72	13	0	3.16	TA41	13	0	0.18	0		13	2	-1.48	0		0.90	**1.00**
TA34	TA1	6	0	3.45	TA93	12	0	1.49	1.49	-	12	0	2.17	2.17	*	0.95	0.96
	TA1	6	0	3.45	TA37	11	1	-1.31	0		11	1	-0.78	0		0.95	0.96

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

Seven of the known mother-offspring combinations had zero mismatches, one had two mismatches and there was one with one mismatch. Between individuals TA64 and TA62 there were three mismatches (printed in bold in column four) from 13 loci compared. The known parent-offspring combination with two mismatches and the combination with three mismatches were the only two combinations with negative LOD scores between the known mother and the offspring (column five, bold).

The number of compared loci between the candidate father and the offspring ranged between six and thirteen (column seven) and the number of mismatches did not exceed one (column eight). Two most likely candidate parent and offspring combinations had a negative LOD score resulting in a Delta score of zero. The remaining combinations could be assigned as the most likely candidate parent in the data set, but could not be assigned at either confidence level (-) in column eleven. This buck is not necessarily the sire, but the most likely sire in this set. The true sire might not have been in the samples sent for testing.

In columns twelve to sixteen of Table 4.13b the trio data were analysed. The number of loci ranged between six and thirteen (column twelve) and the mismatches between the offspring, known mother and most likely candidate father ranged between zero and three (column thirteen). Only one trio combination (most likely parent) had a negative LOD score (bold) and all other combinations were assigned at either a strict (95%) confidence level or as most likely combination in the data set (-) (column sixteen). The PE_1 ranged between 0.77 and 1.00 whereas PE_2 ranged between 0.83 and 1.00.

The parentage analysis for family TA3 is presented in Table 4.13c. The number of loci compared between the known mother and offspring ranged between three and fourteen (column four) and there was two combinations with two mismatches, one with one mismatch and one with four mismatches (column four). Four of the combinations had a negative LOD score and were also the combinations with mismatches (column five, printed in bold).

Table 4.13c Parentage analyses for Animals tested for TA3 with 14 markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatchi	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
TA83	TA46	13	**4**	**-14.46**	TA22	12	0	8.48	8.48	*	12	4	6.46	6.46	*	1.00	1.00
	TA46	13	**4**	**-14.46**	TA61	13	1	-0.37	0		13	7	-4.35	0		1.00	1.00
TA53	TA9	12	0	10.3	TA75	8	0	4.77	4.77	*	8	0	6.35	6.35	*	1.00	1.00
	TA9	12	0	10.3	TA22	11	4	-12.68	0		11	4	-13.17	0		1.00	1.00
TA56	TA102	12	**2**	**-4.98**	TA40	9	0	5.08	4.91	*	9	1	5.43	5.43	*	1.00	1.00
	TA102	12	**2**	**-4.98**	TA75	8	2	-5.28	0		8	2	-6.13	0		1.00	1.00
TA101		0	0	0	TA24	2	0	1.26	1.26	+	0	0	0	0		0.94	0.94
		0	0	0	TA59	4	0	-0.62	0		0	0	0	0		0.94	0.94
TA66	TA100	10	**2**	**-6.97**	TA21	11	0	7.21	7.21	*	11	2	8.62	8.62	*	0.97	1.00
	TA100	10	**2**	**-6.97**	TA24	7	2	-4.76	0		7	4	-5.19	0		0.97	1.00
TA74	TA31	10	**1**	**-0.99**	TA21	12	0	2.87	2.87	*	12	2	0.06	0.06	+	0.99	1.00
	TA31	10	**1**	**-0.99**	TA87	7	1	-1.74	0		7	2	-2.73	0		0.99	1.00
TA86	TA94	12	0	6.38	TA21	13	1	1.31	1.31	+	13	1	2.27	2.27	*	0.99	1.00
	TA94	12	0	6.38	TA43	13	3	-8.26	0		13	3	-8.21	0		0.99	1.00
TA39	TA4	3	0	0.61	TA59	10	1	**-1.78**	0		10	2	**-4.43**	0		1.00	1.00
	TA4	3	0	0.61	TA8	9	3	**-8.1**	0		9	3	**-7.68**	0		1.00	1.00
TA13	TA96	7	0	4.66	TA40	8	0	1.12	0.77	+	8	0	2.17	2.17	*	0.87	0.96
	TA96	7	0	4.66	TA99	8	1	-3.78	0		8	1	-0.97	0		0.87	0.96
TA60	TA19	7	0	3.08	TA21	13	0	3.97	3.97	*	13	0	7.07	7.07	*	0.99	1.00
	TA19	7	0	3.08	TA17	11	2	-3.85	0		11	2	-2.63	0		0.99	1.00
TA69	TA44	7	0	2.59	TA43	9	0	3.17	2.67	*	9	0	4.86	3.53	*	0.93	1.00
	TA44	7	0	2.59	TA40	6	0	-0.02	0		6	0	1.34	0		0.93	1.00
TA54	TA25	14	0	6.88	TA43	14	0	7.17	7.17	*	14	0	9.34	9.34	*	0.99	1.00
	TA25	14	0	6.88	TA40	10	1	-2.16	0		10	3	-8.73	0		0.99	1.00

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

The number of loci compared between the offspring and candidate parent in family TA3 (column seven, Table 4.13c) ranged between fourteen and eight. The number of mismatches did not exceed one (column eight, Table 4.13c). Offspring TA101 amplified at only six loci and the number of loci compared between the two candidate parents were two and four respectively implying that the data was not sufficient to make an accurate allocation. In the entire family, only one of the LOD scores was negative (column nine, bold). The remaining eleven combinations had positive LOD and Delta scores and could be assigned at the strict of 95% (*) or relaxed 80% (+) confidence level.

The trio data in Table 4.13c indicates that the number of loci compared between the offspring, known mother and most likely candidate father ranged between eight and fourteen whereas the number of mismatches ranged between four and zero. Only one trio LOD score was negative and the remaining ten combinations with positive LOD scores could be assigned at a strict confidence level of 95% (*). PE_1 ranged between 0.87 and 0.10 whereas PE_2 ranged between 0.94 and 0.10.

Table 4.14 shows all the known parent-offspring combinations for the three TA families. One of the known mother-offspring combinations had four genotypic mismatches (TA83-TA46 (TA3)) and one had three mismatches (TA64-TA62 (TA2)). The other combinations did not exceed two mismatches. These two known parent-offspring combinations (printed in bold) had a large negative LOD score (-14.46 and -11.79 respectively).

Table 4.14 Known parent – offspring mismatches in all the TA families

Offspring ID	Locus name	Offspring Allele a	Offspring Allele b	Known parent ID	Known parent Allele a	Known parent Allele b
TA83	**BM 1329**	**169**	**175**	**TA46**	**167**	**171**
	HSC	**273**	**279**	**TA46**	**277**	**277**
	SRCRSP 5	**168**	**168**	**TA46**	**160**	**166**
	SRCRSP 8	**211**	**235**	**TA46**	**223**	**223**
TA64	**INRABERN 192**	**190**	**192**	**TA62**	**188**	**200**
	MCM 527	**152**	**154**	**TA62**	**166**	**166**
	SRCRSP 8	**211**	**239**	**TA62**	**223**	**223**
TA66	BM 1329	171	175	TA100	173	173
	OarFCB 48	157	157	TA100	155	159
TA56	HSC	281	301	TA102	277	279
	INRABERN 192	192	192	TA102	188	190
TA74	SRCRSP 9	121	121	TA31	129	131
TA71	INRABERN 192	192	198	TA38	194	200
TA7	INRA 63	161	165	TA45	159	159
TA20	CSRD 247	219	237	TA51	241	241
	SRCRSP 9	121	121	TA51	127	133
TA47	BM 7160	163	175	TA63	179	179
TA91	INRA 63	161	163	TA80	159	165
	OarFCB 48	155	159	TA80	157	161
TA97	OarFCB 48	155	155	TA82	153	163

Microsatellite markers BM 1329, INRABERN 192 and OarFCB 48 had three mismatches over all the animals of the three breeders studied. HSC, SRCRSP 8, SRCRSP 9 and INRA 63 had two mismatches whereas SRCRSP 5, MCM 527, CSRD 247 and BM 7160 had one mismatch each.e

4.5 Comparison of the 14 and 18 marker panels for parentage verification

The parentage analysis of the 14 marker panel was compared to the 18 marker analyses. As the number of markers was decreased from 18 to 14, the number of loci compared decreased and the number of mismatches tended to vary in all families. The LOD score changed slightly for almost all combinations but remained in the same range. The tables of the 18 marker analyses of each family were included in Appendix D.

4.5.1 Known mother

The decrease in the number of markers from 18 to 14 did not have an effect on the allocation of any of the known mother-offspring combinations. In the 18 marker analyses two combinations in families TA2 and TA3 had five mismatches each. This resulted in

both having negative LOD scores of -19.72 and -18.63 respectively. During the 14 marker analyses these LOD scores changed to -11.79 and -14.46 and the number of mismatches were decreased from five to four and three respectively.

4.5.2 Candidate parents

In comparison of the two panels in all families most of the allocations remained unchanged. In the entire population nine offspring were allocated to a different most likely candidate parent in the 14 marker analyses compared to the allocations made with the 18 markers. In all but one case (offspring AM15 in the AM1 group) these allocations was for offspring that were also not conclusively allocated during the 18 marker analyses. In most of these cases the LOD score was negative for both allocated parents, the allocation were not made at a confidence level and there were mismatches detected. This data indicate that even though a different most likely candidate parent was allocated no conclusive allocation could be made with either panel. In some cases the second most likely candidate parent allocation changed, but it did not have an effect on the most likely parent allocation.

All the kids from family A2 were allocated to buck B1 during both analyses. However only nine kids (during the 18 marker analyses) and 10 kids (14 markers), were assigned with a positive LOD score at either confidence level. Neither of the panels reported any mismatches with buck B1.

The most noteworthy change in allocation was in the AM1 group. Offspring AM15 were allocated to AM8 (most likely candidate parent) and AM6 (second most likely candidate parent) during the 18 marker analyses at the 80% confidence level with a LOD score of 1.23. During the 14 marker analyses the order was reversed with the offspring allocated to AM6 (most likely) and AM8 (second most likely), again at the 80% confidence level with a LOD score of 1.26.

In family TA2 the confidence levels of the allocations changed for all but two combinations. During the 18 marker analyses five offspring were allocated at the + (80%)

confidence level and three offspring were allocated at the – level. This changed to nine offspring allocated at the – level during the 14 marker analyses. This indicates that the most likely sire was allocated, but that the correct sire might not have been sampled. Among the allocations that changed during the 14 marker analysis was offspring TA101 that was typed at only 6 loci. An insufficient number of loci were compared during the 18 and 14 marker analyses to make a conclusive decision.

4.6 The final panel of microsatellite markers

The final panel of microsatellite markers consisted of two sets for cost-effective genotyping and were compiled according to size range and fluorescent labels, as shown in Table 4.15 a and b.

Table 4.15a Microsatellite markers set one

Microsatellite marker	Size range	Label
BM1258	90 – 130	Red (PET™)
OarFCB48	150 – 170	Red (PET™)
INRA63	150 – 170	Blue (6-Fam™)
BM 1818	258 – 270	Blue (6-Fam™)
SRCRSP24	120 – 180	Green (NED™)
CSRD247	200 – 260	Green (NED™)
MCM527	150 – 180	Yellow (VIC™)

Table 4.15b Microsatellite markers set two

Microsatellite marker	Size range	Label
SRCRSP9	111 – 143	Red (PET™)
INRABERN192	170 – 200	Red (PET™)
BM 7160	160 – 180	Blue (6-Fam™)
SRCRSP8	210 – 240	Blue (6-Fam™)
BM1329	160 – 180	Green (NED™)
HSC	260 – 310	Green (NED™)
SRCRSP5	150 – 190	Yellow (VIC™)

Both sets consisted of seven microsatellite markers with two microsatellite markers labelled with red (PET™), blue (6-FAM™) and green (NED™) fluorescent dye colours each and one microsatellite marker labelled with the yellow (VIC™) label in each set.

CHAPTER 5: DISCUSSION

The major contribution of the South African mohair industry to the global market augments the need of producing a good quality clip. In order to produce a good quality clip, accurate selection of breeding animals and active participation in breed improvement schemes are necessary by stud and commercial breeders. In this regard accurate and complete pedigree data for all their animals will be of major importance. In a recent study (Personal communication Dr M.A. Snyman; Grootfontein Agricultural Development Institute, Jansenville, Private Bag X529, Middelburg Cape, 5900, South Africa) it was estimated that around 25% of all Angora goats born in 2005/2006 had incomplete or inaccurate pedigree records as one or both parents were unknown. This poses a major problem as breeders cannot perform accurate selection and those breeders who want to participate in performance testing schemes are limited by a lack of data. The practical and management constraints farmers often experience with parentage recording could be resolved by making use of this panel of microsatellite markers.

5.1 Evaluation of the individual microsatellite markers

In this study a panel of 18 microsatellite markers were analysed to infer parentage of goats. The panel of 18 microsatellite markers selected for this study include 11 of the markers recommended by ISAG (2005) and the other seven markers were previously used in parentage studies and a genetic diversity study on goats (Table 5.1). The complete list of microsatellite markers included in other studies is presented in Appendix E.

Table 5.1 Microsatellite markers used in previous parentage verification studies conducted on goats that corresponded with the microsatellite markers used in the current study

Microsatellite marker	Current study	Luikart *et al.*(1999)	Recommended ISAG panel (2001/2002)	Ganai & Yadav (2005)	Recommended ISAG panel (2005)	Jiménez-Gamero *et al.*(2006)	Glowatzki-Mullis *et al.* (2007)	Bolormaa *et al.*(2008)
BM1258	✔	✘	✔	✘	✔	✘	✔	✔
BM1329	✔	✘	✔	✘	✔	✘	✔	✘
BM1818	✔	✘	✔	✘	✔	✘	✘	✔
BM7160	✔	✘	✘	✔	✘	✘	✘	✘
CSRD247	✔	✘	✔	✘	✔	✘	✔	✘
HSC	✔	✘	✘	✘	✔	✘	✔	✘
ILSTS5	✔	✔	✘	✘	✘	✘	✔	✘
ILSTS87	✔	✘	✔	✘	✔	✘	✘	✘
INRA5	✔	✘	✔	✘	✔	✘	✔	✘
INRA63	✔	✔	✔	✘	✔	✘	✔	✔
MAF65	✔	✔	✔	✘	✔	✘	✘	✘
MCM527	✔	✘	✔	✘	✔	✘	✘	✘
OarFCB48	✔	✔	✘	✘	✘	✘	✘	✘
SRCRSP24	✔	✘	✔	✘	✔	✘	✘	✘
SRCRSP5	✔	✔	✔	✘	✔	✔	✘	✔
SRCRSP8	✔	✔	✔	✘	✔	✔	✔	✘
SRCRSP9	✔	✔	✘	✘	✘	✔	✘	✘

These 18 markers were evaluated for different parameters namely number of alleles, allele frequency, H_O, H_E, PIC and PE and compared to studies and the ISAG panels listed in Table 5.1. The comparison provided an indication of the performance of these markers in the population used in this study, relative to previous reports. The number of alleles detected for most of the microsatellite markers used in the other studies mentioned was comparable with the number of alleles detected in the current study as shown in Table 5.2.

The Hardy-Weinberg test indicated that seven of the eighteen markers analysed were not in equilibrium (Table 4.3). When viewed in addition to Table 4.2 the conclusion can be made that markers BM1818, CSRD247, INRA63, MCM527 and SRCRSP24 had an excess of heterozygotes and it caused the loci to be out of equilibrium. For markers INRA5 and MAF65 an excess of homozygotes were detected leading to the loci being in

disequilibrium. In the cases where there is an excess of homozygotes it is most often due to selection for a certain trait. This lowers the genetic variation of the population. Contrary to this, an excess of heterozygotes indicates a higher genetic variation in the population. As the population tested is very small the significance of the Hardy-Weinberg analysis has to be considered. One would expect markers to deviate more from the equilibrium than in a larger population.

Table 5.2 The number of alleles observed in the current study compared with other studies and the ISAG panels

Microsatellite marker	Current study	Luikart *et al.* (1999)	Saitbekova *et al.* (1999)	ISAG panel (01/02)	Ganai & Yadav (2001)	ISAG panel (2005)	Jimenez-Gamero *et al.* (2006)	Glowatzki-Mullis *et al.* (2007)	Bolormaa *et al.* (2008)
BM1258	13	NI	NI	NI	NI	NP	NI	11	9
BM1329	8	NI	NI	NI	NI	NP	NI	9	NI
BM1818	9	NI	NI	NI	NI	NP	NI	NI	9
BM7160	10	NI	NI	NI	5	NP	NI	NI	NI
CSRD247	9	NI	NI	7	NI	NP	NI	8	NI
HSC	12	NI	NI	12	NI	NP	NI	24	NI
ILSTS5	4	5	6	NI	NI	NP	NI	5	NI
ILSTS87	9	NI	NI	8	NI	NP	NI	NI	NI
INRA5	3	NI	5	4	NI	NP	NI	5	NI
INRA63	5	5	7	6	NI	NP	NI	6	4
INRABERN192*	10	NI	10	NI	NI	NP	NI	NI	NI
MAF65	10	8	NI	12	NI	NP	NI	NI	NI
MCM527	10	NI	NI	6	NI	NP	NI	NI	NI
OarFCB48	8	9	NI	NI	NI	NP	NI	NI	NI
SRSRSP5	10	10	NI	6	NI	NP	7	NI	9
SRCRSP8	11	9	NI	7	NI	NP	7	15	NI
SRCRSP9	9	9	NI	NI	NI	NP	14	NI	NI
SRCRSP24	9	NI	NI	9	NI	NP	NI	NI	NI

NI: Not included by author
NP: Not published
*: Not included in a parentage verification study

A comparison of the PIC values of the microsatellite markers in the current study with previous studies are shown in Table 5.3 where two of the microsatellite markers had lower PIC estimates than what was estimated in the current study (printed in bold in Table 5.3). For SRCRSP9 the PIC estimates found by both Luikart *et al.* (1999) (0.812) and Jimenez-Gamero *et al.* (2006) (0.781) was higher than found in the current study (0.663) whereas MAF65 showed the largest difference in PIC as it performed much better in the study by Luikart *et al.* (1999) (0.671) compared to the current study (0.339).

Table 5.3 A comparison of the PIC values of the microsatellite markers used in the SA Angora study and the studies done by Luikart *et al*.,(1999) and Bolormaa *et al*. (2008)

Microsatellite markers	PIC (Current study)	PIC (Luikart *et al*.,1999)	PIC (Jiménez-Gamero *et al*., 2006)	PIC (Bolormaa *et al*.2008)
ILSTS 5	0.385	0.433	NI	NI
INRA 63	0.656	0.645	NI	0.653
MAF 65	**0.339**	**0.671**	**NI**	**NI**
OarFCB 48	0.747	0.755	NI	NI
SRCRSP 5	0.777	0.779	0.743	0.712
SRCRSP 9	**0.663**	**0.812**	**0.781**	**NI**
SRCRSP 8	0.625	0.700	0.649	NI
BM1818	0.716	NI	NI	0.758
BM1258	0.731	NI	NI	0.840

NI: Not included in the study

PIC values are influenced by the allele frequencies found in the population and a possible reason for the variation in performance of MAF65 could be that allele 125 was correlated with a trait which was strongly selected for or against in South African Angora goats e.g. fine hair production or body weight. Phenotypic selection over years will lead to a change in genotype and thus markers linked to genes associated with economically important traits may have a higher frequency in the population due to indirect selection.

In order to construct an economically feasible panel of microsatellite markers with the highest exclusion probability and no parental allocation errors, markers that did not perform optimally were excluded from the panel. MAF 65, INRA 5, ILSTS 5 and ILSTS 87 were excluded based on their allele frequencies, number of alleles, heterozygosity values and PIC values. The parameters carrying the most weight regarding exclusion of the microsatellite markers were allele frequency and the number of alleles. If a marker had one allele with a high frequency or a low number of alleles, it affected the PIC value, $H_{E,}$ H_O and the exclusion power. All four of these microsatellite markers had one allele with an allele frequency exceeding 0.60. It should be noted that the microsatellite markers might perform better in another population where the allele frequencies might be different. However in the current study and population these markers were excluded based on their poor performance. ILSTS 87 could be considered as a possible marker to include, if an additional panel for higher exclusion power was needed.

The combined first parent exclusion power of the fourteen markers in the current study was 99.73%. The combined exclusion probability power (CPE_1) reported in previous parentage verification studies on goats was above or very close to 99% (Luikart *et al.*, 1999; Ganai & Yadav 2005; Jimenez-Gamero *et al.,* 2006; Glowatzki-Mullis *et al*., 2007 and Bolormaa *et al.,* 2008) as shown in Table 5.4. All the markers in these studies did not correspond as indicated in Table 5.1.

From Table 5.4 it can be concluded that the combined first parent exclusion probability in the current study was in the same range as the discussed studies and can be accepted as powerful enough to exclude individuals.

Table 5.4 A comparison of the Combined Exclusion probability of different parentage verification panels

	Current study	Luikart *et al*. (1999)	Ganai & Yadav (2005)	Jimenez-Gamero *et al.* (2006)	Glowatzki – Mullis *et al.* (2007)	Bolormaa *et al.* (2008)
CPE_1	0.99731	0.9999	0.989	0.9991	0.999	0.9973
CPE_2	0.9999	>0.9999	0.999	NC	NC	0.997
CPE_P	0.9999	0.99940	0.999	NC	NC	NC
CPE_{ID}	>0.9999	>0.9999	NC	NC	NC	NC
CPE_S	0.9999	>0.9999	NC	NC	NC	NC

CPE_1: Combined exclusion probability for one candidate parent alone
CPE_2: Combined exclusion probability for one candidate parent and one known parent of the opposite sex
CPE_P: Combined exclusion probability for a candidate parent pair
CPE_{ID}: Combined exclusion probability to exclude between two non-related individuals
CPE_S: Combined exclusion probability for distinguishing between two siblings
NC: Not calculated

A comparison on the effect of reducing the number of markers on CPE in a parentage verification panel was demonstrated by Ganai & Yadav (2005). A panel of twelve markers were reduced to eight, six, five and then four markers in consecutive experiments. A similar comparison was performed in this study and the results obtained for CPE_1, CPE_2 and CPE_P between the two studies were compared (Table 5.5).

Table 5.5 Comparison of the CPE between the current study and Ganai &Yadav (2005) with different number of markers expressed as a percentage

	CPE_1		CPE_2		CPE_P	
Number of markers	Ganai & Yadav (2005)	Current study	Ganai & Yadav (2005)	Current study	Ganai & Yadav (2005)	Current study
4	80.1	NC	94.5	NC	99.4	NC
5	90.0	86.0	98.2	96.94	99.9	99.72
6	92.8	91.4	99.0	98.6	99.9	99.9
8	97.8	96.5	99.8	99.7	99.9	99.9
12	98.9	99.42	99.9	99.9	99.9	99.9
14	NC	99.73	NC	99.9	NC	99.9
18	NC	99.81	NC	99.9	NC	99.9

NC: Not calculated

The change in the combined exclusion probabilities when different numbers of markers were applied was conditioned on three principles. Firstly the exclusion power was conditioned by the genotypes available for the tested parents (known and candidate parents) (Gerber *et al.*, 2000). More genotypic information was available when both parents (known or putative) were tested and this increased the statistical probability to exclude non-parents. Breeders often prefer to have only the samples of the sire and offspring analysed due to financial implications and due to the sire having a greater influence on the genetic gain in the herd compared to the dam. However, the exclusion power is higher when DNA samples of the offspring, putative (or known) dams and putative sires can be included for the analyses. It is also very important in situations where two or more of the putative parents are related to test both parents of the kid as relatives shares the same alleles inherited from their parents and will ultimately share these alleles with the offspring.

Secondly, the exclusion power was higher when the number of microsatellite markers was increased (Gerber *et al.*, 2000). It is however imperative to note that the allele frequency, number of alleles and polymorphism of the markers determines the number of markers required. After excluding ILSTS5, ILSTS87, INRA5 and MAF65, the combined first parent exclusion probability (CPE_1) decreased from by 0.1% (Table 4.5). After further exclusions the decrease was more profound and only four markers were excluded. The excluded four markers were not polymorphic and had poor performance at all

parameters. This indicates that the number of markers in a panel does not necessarily indicate the exclusion power of the panel. The performance of each individual marker as well as combined as a panel determines the power.

Results from this study indicated that markers with a poor performance for PIC, H_E, H_O, high allele frequencies and low number of alleles will lead to a panel with a low CPE. The situation changed when one marker such as BM1258 was added and the CPE of the panel increased from 0.36 to 0.61 (please refer to Table 4.7). It is therefore important that the markers have PIC, H_E and H_O values of above 0.5 and alleles with equal frequency. In a case where the amplification success may vary a parent might be included or excluded based on the performance of one or two markers with good performance. It is therefore imperative that the panel must consist of markers that all perform well at the parameters discussed.

The effect of missing genotypic data on the combined exclusion probability was evaluated by selecting between five and 12 markers (of the remaining 14 microsatellite markers) (Table 4.8, Chapter 4). The average difference in CPE_1 when using 14 and 12 microsatellite markers was 0.31%, and between 12 and 10 randomly chosen microsatellite markers it was 0.79%. Results clearly indicate that as more genotypic information per animal was available in the analysis the exclusion probability increased. This only holds true when all markers was polymorphic. In this case the four excluded markers were very low polymorphic and their inclusion had little effect on the CPE. After all four markers were excluded the CPE only decreased by 0.09%. When performing a parentage analyses each animal tested should be genotyped at as many loci as possible to ensure optimal exclusion power.

5.2 Parentage verification

The panel discussed above consisting of 14 markers were evaluated in goats for three different family structures.

Genotypic mismatches could be caused by three factors namely mutations of microsatellite markers, presence of null alleles and genotypic errors (Carolino *et al.*, 2009). The mutation rates reported for human microsatellite markers was estimated between 0.0005 and 0.007 per generation (Brinkmann *et al.*, 1998; Cifuentes *et al.,* 2006), rates of goat microsatellites mutations 0.5 x 10^{-3} to 1.10 x 10^{-4} (Luikart *et al.*, 1999, Bolormaa *et al.* 2008) and cattle 1.2 x 10^{-3} (Mukesh *et al.*, 2004). Based on these low rate mutations is not the probable cause of mismatches in this study.

Null alleles were also considered as an explanation for mismatches. A microsatellite null allele is defined as any allele at a microsatellite locus that consistently fails to amplify to detected levels via the polymerase chain reaction (Dakin & Avise, 2004). Null alleles could be caused by poor primer annealing due to nucleotide differences, as the microsatellite marker was developed in one species e.g. cattle, and analysed in another e.g. goats. A second cause of null alleles is due to the competitiveness of the PCR reaction where shorter fragment sizes will be detected more easily than longer fragments in a heterozygous animal. Finally, null alleles could occur due to inconsistent or poor quality DNA (Dakin & Avise, 2004). Despite these potential causes Dakin & Avise (2004) concluded that null alleles should not have a major influence on the exclusion probability as a parentage verification panel will consist of more than one polymorphic microsatellite marker from which the combined exclusion probability will be calculated. A microsatellite marker with a high null allele frequency will result in a higher number of homozygotes in the population and will not be suitable to use in parentage verification studies and thus be excluded from the final panel (Dakin & Avise, 2004).

In the current study, the microsatellite marker with the highest number of mismatches was OarFCB48 (five mismatches in the entire population) and a null allele frequency of 0.048 in the entire population. Contrary to this, the microsatellite marker with the highest null allele frequency in the total population was SRCRSP8 (0.150) and had only two mismatches in the total population. This marker also did not have a heterogote deficiency. In this study, it is unlikely that the primary cause of the mismatches was due to the presence of null alleles as most of the markers presenting mismatches only had one

or two mismatches in the entire population. The mismatches that occurred in family A1 for BM7160 and MCM527 may be as a result of a null allele as both had high null allele frequencies.

The third possible cause of mismatches is genotypic errors. Genotypic errors may occur in poor quality and quantity DNA that affects the reliability of the amplification (Hoffman & Amos, 2005). Poor amplification may lead to only one allele being detected for heterozygotes and amplification by-products may be interpreted as alleles. The frequency of the latter can exceed 0.25 per reaction (Hoffman & Amos, 2005). Even in perfect conditions electrophoresis artefacts and mis-scoring of allele banding patterns could lead to genotypic errors (Hoffman & Amos, 2005). Though human error can be prevented, the influence of typing errors and other administrative errors should not be overruled. Error rates of between 0.001 and 0.127 per reaction were reported (Hoffman & Amos, 2005). This means that if 12 loci are genotyped almost one in every four samples could contain one or more errors (Hoffman & Amos, 2005). Genotypic errors could lead to a parent being wrongfully excluded as the biological parent because the genotypes of the offspring and parents may differ (Morrissey & Wilson, 2005). Genotypic errors could be ruled out by testing the animals at the disputed loci again. Likelihood equations with error rates set might also reduce the effect of genotypic errors (Morrissey & Wilson, 2005; Kalinowski *et al.*, 2007).

In studies by Fang & Cheng (2002) and Cifuentes *et al.* (2006) it were concluded that a parent can still be included as the true parent even if up to three genotypic mismatches occur between the parent and offspring. In animal parentage verification studies conducted by Carolino *et al.* (2009), Bolormaa *et al.* (2008), Heyen *et al.* (1997) and Luikart *et al.* (1999) it was suggested that a parent may be included even if two mismatches occurred.

In addition to less than two mismatches a positive LOD score (resulting in a positive Delta score) is required to allocate a parent (Marshall *et al.*, 1998; Luikart *et al.*, 1999; Heyen *et al.*, 1999; Carolino *et al.*, 2009). The LOD score is influenced by the number of

mismatches and whether the offspring and the parent share alleles commonly found in the population (Marshall *et al.,* 1998). Criteria for assessing the LOD scores, as suggested by Slate *et al.* (2000), were shown in Table 5.6.

Table 5.6 Criteria to assess the LOD score (Slate et al., 2000)

LOD score	Interpretation
3.0	Candidate parent is 20 times more likely to be the true parent than not to be the true parent
>3.0	Confirmed parentage
<-3.0	Rejected parentage
-3.0 < >3.0	Inconclusive parentage

These criteria as well as the factors influencing the criteria should be taken into account before a final decision on the allocation can be made.

5.2.1 Allocation of parents

In families A1, TA1, TA2 and TA3 the known mother was included in the analysis, and there were one or two mismatches that resulted in a negative LOD score. Mismatches should however be interpreted with due consideration to the LOD score. In this study, combinations TA62-TA64 and TA83-TA46 could not be accepted as true combinations. In Table 4.13b and Table 4.13c combination TA64-TA62 had three mismatches and a LOD score of -11.79 and TA83-TA46 four mismatches and a LOD score of -14.46. This data suggests that the breeder's information was incorrect.

Most of the kids from family A1 were allocated to the suggested buck. All candidate parent-offspring combinations in family A1 had at least one mismatch. All LOD scores in this family were negative and only three combinations had a LOD score less negative than -3.0. The combinations with one or two mismatches and a LOD score more negative than -3.0 could not be accepted as a correct allocation. The three combinations with LOD scores less negative than -3.0 all had only one mismatch but the allocation was considered inconclusive. Even though buck C1 was allocated as the most likely sire to most of the offspring and suggested as the sire by the breeder, it cannot be accepted in any of the combinations as the true sire.

During the 14 marker analysis all kids from family A2 (Table 4.11) were assigned to the suggested buck B1 and there were no mismatches between any of the offspring and the suggested sire. Only half of the offspring (ten) had a positive LOD score and was assigned at the + (80%) or * (95%) confidence levels. In the cases where the LOD score was negative, it was in the range between -2.31 and -0.47, which is considered as an inconclusive assignment. This indicates that the LOD score was not affected by the number of mismatches, but rather the alleles shared among the buck and kids. The relationship of the buck and the other included candidate fathers was not known. If they were related the alleles shared among the candidate fathers will be similar and would result in a negative LOD score. The known mothers were not included and the presence of their genotypic data would have an influence on the LOD score. This family showed a high incidence of markers with one allele with an allele frequency greater than 0.4. Before a final decision can be made on the parentage of the kids the mothers should be tested and the relationships between the candidate sires should be determined. Based on the fact that no mismatches occurred between any of the offspring and the suggested sire, the outcome of the parentage test might reveal different results after the above mentioned matters have been addressed.

In the AM groups there were five combinations where the allocated dam differed from the suggested dam. Offspring AM10, AM24 and AM27 were allocated at the + or 80% confidence level to a ewe different than suggested by the breeder. This indicates that the ewe suggested by the breeder was not the true dam of these kids. Offspring AM19 and AM29 were also allocated to different ewes that suggested by the breeder. These allocations were made with negative LOD scores and one mismatch was present for both combinations. This suggests that the information received from the breeder was incorrect and that the true dam was not included in the analyses.

For the last three families, TA1, TA2 and TA3, the sire of the offspring was unknown to the breeders and thus the paternal parentage results could not be compared with the information from the breeders as in the case of family A1, A2 and the AM groups. The

limitation in this analysis was that DNA samples of all the sires used during the breeding season were not available for analysis. It was estimated by Vankan & Faddy (1999) that a parentage verification panel with an exclusion power of 0.99 is 98 – 99% reliable when 20% of the sires are unknown. In the TA2 family it was especially evident that not all sires were included. All the candidate sires were assigned as the most likely sire from this data set, but could not be assigned at either confidence level (assigned with a -). This indicates that the correct sire most possibly were not included in the analyses. In family TA1 some offspring were allocated at the 95% (*) confidence level and for others no definite allocation (-) was made, indicating that the true sires of some of the offspring were tested but that there were additional sires used in the breeding season that were not tested. Before a final decision on the parentage of these offspring can be made the other sires used during the breeding season have to be tested as well. The allocations made in TA3 were at the + or * confidence levels and only one offspring was not allocated with a positive LOD score to any of the sires. Even though not all LOD scores in this family exceeded 3.0, the allocations can be considered as true. In the test families (TA1, 2 and 3) the DNA samples provided were limited with regards to quantity and quality. This resulted in a lower number of loci amplified for inclusion in the parentage analyses.

The trio data generated for family A1 indicates that only one animal trio can be considered as a true combination (Offspring C11, Table 4.9). This LOD score was close to zero (0.011). The LOD score between the offspring and known mother was very small (0.72) and the LOD score of the candidate sire combination was more negative than -3.0. This indicates that despite the allocation being made at the 80% confidence level, C1 cannot be confirmed as the true sire and the trio data was thus inconclusive.

In family TA1 three combinations were assigned at the strict (*, 95%) confidence level and had LOD scores greater than 3.0 indicating a true trio-combination. In family TA2 combination TA64-TA62-TA29 were assigned at the strict confidence level (*, 95%). However the known mother was dismissed as a true parent (based on the number of mismatches and negative LOD score) and the candidate father could not be conclusively assigned at any confidence level. This indicates that the trio allocation was inconclusive

and that neither of the parents can be allocated as the true parent of the offspring. In all the other allocations in this family the candidate sires were allocated inconclusively (-) and thus the trio allocations cannot be accepted as conclusive. In family TA3 all but one of the allocated combinations can be accepted as true combinations. Known mother TA46 was excluded as a true dam because of four mismatches. Even though candidate father-offspring combination TA83-TA22 had a large positive LOD score the trio cannot be accepted as a true combination. All other combinations allocated at either confidence level in this family can be accepted as true.

5.2.2 Comparison between allocations made during the 18 and 14 marker panel analyses

In comparing the results generated during the analyses with the initial (18 marker) and final (14 marker) panels it was evident that the results of the 14 marker panel was as accurate as the 18 marker panel. The markers included in the 14 marker panel were all polymorphic which resulted in more information generated. In the entire population only nine offspring were allocated to different parents during 18 and 14 marker analyses. Only one of the nine offspring was conclusively assigned to a parent.

In families A1 and A2, during both analyses, all offspring were assigned to buck C1 and B1, respectively. In the first family it was concluded that C1 was not the true sire of any of the kids. All the offspring of family A2 were assigned with no mismatches to B1 during both analyses. In both of these families the same buck was assigned to nearly all offspring during both analyses, irrespective if it was the true sire. This indicates that the 14 marker panel is as powerful in assigning the most likely sire to all offspring as the 18 marker panel.

In the AM1 offspring AM15 were allocated to two different ewes during the two analyses, both at the 80% (+) confidence level. Unfortunately the relationship of these two ewes is not known, but the most likely cause of this allocation is that the two ewes were related. In order to make a final decision regarding this offspring's parentage, the sire should also be tested and the relationship of the ewes investigated.

In the test families (TA1, 2 and 3) no differences in the conclusively assigned most likely candidate parent between the two panels was reported. This indicates that the 14 microsatellite marker panel is as accurate as the 18 marker panel to assign parents accurately.

5.2.3 Potential limitations in parentage analyses in Angora goats

A number of reasons should be considered with regards to the differences in parent allocation between the breeder records and the parentage analyses. Firstly inaccurate recording may occur at farm level. Errors may have occurred in the recording of the dams or sire or group of sires. Kids might have been stolen or swapped by other ewes. Angora goats are known for their poor mothering ability and might abandon their newborn if under stress (Hafez & Hafez, 2000). Kid stealing is a common occurrence when the bonding between the dam and kid is delayed after parturition as other pregnant ewes in the flock could smell the foetal fluid and might steal the kid (Hafez & Hafez, 2000). South African Angora goats are also bred in extensive breeding conditions and it is sometimes difficult to accurately assign a kid to a dam or sire (Personal communication: Dr M.A. Snyman; Grootfontein Agricultural Development Institute, Jansenville, Private Bag X529, Middelburg Cape, 5900, South Africa; April 2007).

Close relationships in stud flocks cause ewes or bucks to share similar alleles (Sherman *et al*., 2004). It would be advisable to test the other parent or possible parents of the kids as well, or to obtain more pedigree information regarding the relatedness of the animals possible parents from the breeder before a definite decision on the parentage of the kids can be made in these cases.

In the comparison of the two panels (18 and 14 markers) it was evident that both panels were able to exclude non-parents as true parents. The four excluded microsatellite marker did not have a negative effect on the exclusion power and most of the offspring were still allocated to the same likely parents after the exclusion of the four markers. In most cases where changes in the most likely candidate parent allocations occurred the parent was

either not allocated at any confidence level or had a negative LOD score. Even though a change in allocation took place neither of the parents were thus conclusively allocated. One exceptional case was offspring AM15 where this kid was allocated to two different ewes during the different analyses, both with a positive LOD score. The reason for this allocation could be that the two ewes were related and shared similar alleles. In rare cases such as this, an additional set of markers can be added to the panel. In previous literature on pig parentage verification by Nechtelberger *et al.* (2001) a set of five microsatellite markers were added to the panel for optional use. Similarly, in the study by Luikart *et al*. (1999) a second multiplex was included if the power of exclusion of the first panel wasn't sufficient.

The panel of 14 microsatellite markers constructed in the current study was found reliable to exclude putative parents that are not the true parent. An overall exclusion probability of 99.73% was estimated using the 14 marker panel which compared well with previous studies. The parentage analyses performed in this study with the 14 marker panel has indicated inaccuracies in recorded data by the breeders. It was possible to exclude non-parents based on the mismatches and LOD scores. It was also possible to assign parents correctly at 95% and 80% confidence levels.

This study has shown the potential value of using molecular parentage analyses in South African Angora goats. The cost implication of the panel must be considered and in Table 5.7 the estimated cost of labelled primers at the small scale (10ng/µl) as the number of microsatellite markers was increased, was shown. This was compared with the CPE_1 value of each panel (Table 4.5 and Table 4.8). Please note that this calculation does not take labour, Taq polymerase or consumables into account but is only based on the cost of synthesising the primers. The quotation for the oligonucleotide synthesis was prepared by Inqaba Biotechnical Industries (Pty) Ltd., a South African based oligo-house (Personal communication: Inqaba Biotechnical Industries Pty (Ltd)., P.O. Box 14356, Hatfield, South Africa, 0028, July 2009).

Table 5.7 Estimated cost of primers and CPE_1 value of panel

Number of markers	8		10		11		12		13	
	Cost per panel	CPE1	Cost per panel	CPE1	Cost per panel	CPE1	Cost per panel	CPE1	Cost per panel	CPE1
	R 5,600	0.9789	R 7,000	0.9875	R 7,700	0.9927	R 8,400	0.9947	R 9,100	0.9963
Number of markers	14		15		16		17		18	
	Cost per panel	CPE1	Cost per panel	CPE1	Cost per panel	CPE1	Cost per panel	CPE1	Cost per panel	CPE1
	R9,800	0.9973	R 10,500	0.9976	R 11,200	0.9979	R 11,900	0.9982	R 12,600	0.9983

R/US$ exchange rate: ±$1=R7

The difference in price of synthesizing 18 vs. 14 microsatellite markers does not justify the difference of the exclusion power of the two panels (Table 5.7) and emphasize the importance of excluding the 4 markers. For the 14 microsatellite markers an estimated cost of R250 (±US$31) per goat per test was proposed and a discount rate of 15% when more than 16 goats were tested can be offered. This included labour, all reagents and consumable costs. (Personal communication: Inqaba Biotechnical Industries (Pty) Ltd., P.O. Box 14356, Hatfield, 0028, July 2009). A panel consisting of 11 or 12 microsatellite markers will have a lower exclusion power and might not be statistically powerful enough to exclude all non-parents. This is especially relevant in cases where the candidate parents may be closely related. Optimization of the 14 microsatellite marker panel to run a multiplex PCR is also a favourable option as the costs involved of running single PCR amplifications will be reduced considerably.

The use of the panel of microsatellite markers to verify parentage could be considered as an economic option in South African terms, considering the possibilities for genetic progress in application of EBV's on a wider scale in the South African Angora goat industry.

CHAPTER 6: CONCLUSION

South Africa is currently the world's largest and most reliable producer of mohair and this necessitates the production a high quality clip. There are currently around 2 million Angora goats farmed by 900 breeders in the country. In order to maintain the quality of the clip, commercial and stud breeders have to select the most superior hair producing animals for breeding purposes and stud breeders need to have complete and accurate pedigree records to take part in the National Improvement scheme and to make optimal use of EBV's in their selection programs. Due to large farms, extensive production systems and other management constraints it is often impossible to record accurate pedigree data without the use of DNA technology.

The International Society of Animal Genetics (ISAG) compiled two recommended panels for parentage verification of goats and a number of previous studies have been conducted on the subject. This provided a reference point for the microsatellite markers with regards to polymorphism and suitability for parentage verification of goats. From the microsatellite markers analysed in these studies a panel of 21 microsatellite markers were compiled of which three could not be optimized. The remaining 18 markers were evaluated with regards to number of alleles, allele frequencies, PIC, H_E, H_O and PE in 200 Angora goats and 16 Saanen goats followed by a parentage analyses. These goats were tested in different family structures as experienced by goat breeders, including both sire and dam known, only the sire known and only the dam known.

Of the 18 microsatellite markers, four were excluded based on allele frequency, number of alleles, PIC, heterozygosity and exclusion probability. The number of alleles and allele frequency ultimately influence the PIC, heterozygosity and exclusion probability values. The combined first parent exclusion probability when 14 markers were used was 99.73%. After the exclusion of more microsatellite markers the CPE_1 decreased significantly and it was decided that 14 markers was the minimum number of markers with the highest exclusion power that should be included.

This was the first attempt at constructing a panel of microsatellite markers to be used in parentage verification of South African Angora goats. This parentage verification test will be a useful tool for Angora goat breeders to incorporate in their management practices. Breeders can benefit from this by increasing the accuracy of selection of animals, higher accuracy when estimating EBVs, faster genetic gain and limited inbreeding in their herds.

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APPENDIX A

Table 1A A Summary of the number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for all 18 markers for the entire Saanen population studied

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	4	16	1	0.627	0.529	-0.266	0.193	0.328	0.483	0.767	0.496
BM 1329	5	15	0.867	0.743	0.666	-0.099	0.296	0.466	0.641	0.868	0.576
BM 1818	5	16	0.813	0.748	0.681	-0.05	0.314	0.491	0.675	0.881	0.583
BM 7160	4	16	0.438	0.47	0.425	-0.012	0.11	0.261	0.424	0.673	0.396
CSRD 247	4	16	0.688	0.708	0.62	-0.002	0.248	0.405	0.564	0.835	0.552
HSC	5	15	0.867	0.662	0.581	-0.164	0.224	0.383	0.555	0.812	0.523
ILSTS 5	2	16	0.25	0.226	0.195	-0.062	0.024	0.097	0.167	0.366	0.201
ILSTS 87	6	16	0.875	0.788	0.727	-0.071	0.369	0.547	0.732	0.908	0.609
INRA 5	2	16	0.063	0.063	0.059	-0.008	0.002	0.029	0.056	0.116	0.059
INRA 63	3	16	0.75	0.639	0.539	-0.097	0.192	0.327	0.473	0.774	0.503
INRABERN 192	3	16	0.563	0.433	0.354	-0.157	0.088	0.19	0.301	0.598	0.359
MAF 65	6	15	1	0.786	0.725	-0.15	0.366	0.546	0.733	0.907	0.607
MCM 527	3	16	0.688	0.534	0.412	-0.14	0.134	0.222	0.335	0.662	0.424
OarFCB 48	5	16	0.813	0.766	0.701	-0.059	0.335	0.513	0.696	0.892	0.594
SRCRSP 24	6	16	0.875	0.653	0.599	-0.222	0.239	0.419	0.62	0.832	0.524
SRCRSP 5	4	16	0.188	0.181	0.171	-0.04	0.016	0.093	0.173	0.316	0.167
SRCRSP 8	4	16	0.25	0.518	0.443	0.357	0.13	0.263	0.41	0.693	0.424
SRCRSP 9	5	13	0.692	0.658	0.563	-0.054	0.217	0.363	0.529	0.795	0.515
Average	4.22	15.66	0.64	0.56	0.49	-0.07	0.19	0.33	0.47	0.70	0.45

k: Number of alleles per locus
N: Number of individuals typed
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

APPENDIX B

Tables 1B to 7B presents the results obtained in each family for the number of alleles, heterozygosity values, PIC values, F_{Null} frequencies and exclusion probabilities for each microsatellite marker.

Table 1B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the A1 family

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	6	40	0.750	0.661	0.591	-0.078	0.237	0.393	0.566	0.817	0.531
BM 1329	7	41	0.707	0.699	0.635	-0.016	0.271	0.437	0.613	0.849	0.557
BM 1818	5	38	**0.842**	0.658	0.587	-0.152	0.227	0.384	0.552	0.815	0.528
BM 7160	8	34	0.618	0.739	0.686	0.080	0.329	0.504	0.695	**0.885**	0.585
CSRD 247	3	43	0.512	0.482	0.391	-0.045	0.113	0.212	0.326	0.641	0.398
HSC	7	35	0.657	0.587	0.516	-0.075	0.181	0.326	0.491	0.760	0.479
ILSTS 5	2	33	0.636	0.508	0.375	-0.120	0.125	0.187	0.281	0.625	0.406
ILSTS 87	4	39	0.308	0.279	0.262	-0.075	0.039	0.149	0.263	0.462	0.253
INRA 5	2	33	0.576	0.506	0.374	-0.072	0.124	0.187	0.281	0.624	0.405
INRA 63	5	29	0.586	0.561	0.516	-0.015	0.169	0.337	0.521	0.763	0.466
INRABERN 192	5	48	0.646	0.644	0.567	-0.009	0.218	0.365	0.529	0.798	0.518
MAF 65	4	39	0.462	0.426	0.391	-0.041	0.093	0.234	0.385	0.634	0.369
MCM 527	5	48	0.354	0.463	0.432	0.152	0.114	0.269	0.439	0.680	0.399
OarFCB 48	5	46	0.761	0.670	0.605	*-0.080*	0.243	0.405	0.579	0.829	0.538
SRCRSP 5	6	37	0.649	**0.754**	**0.705**	0.064	**0.343**	**0.521**	**0.707**	0.895	**0.596**
SRCRSP 8	5	43	0.209	0.292	0.274	0.173	0.043	0.155	0.273	0.479	0.264
SRCRSP 9	4	44	*0.250*	*0.251*	*0.238*	0.011	*0.032*	*0.134*	*0.240*	*0.424*	*0.230*
SRCRSP 24	5	45	0.356	0.566	0.508	**0.211**	0.169	0.323	0.493	0.755	0.468
Average	4.89	39	0.549	0.541	0.481	-0.005	0.171	0.307	0.457	0.708	0.444

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

Table 2B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the A2 family

Locus	k	Ho	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	7	0.846	0.750	0.696	-0.078	0.339	0.516	0.707	0.891	0.590
BM 1329	5	0.800	0.669	0.612	-0.107	0.245	0.419	0.606	0.838	0.537
BM 1818	6	0.591	0.726	0.661	0.093	0.298	0.469	0.652	0.867	0.572
BM 7160	6	0.808	**0.783**	**0.730**	-0.030	0.371	0.549	0.731	0.908	**0.611**
CSRD 247	8	0.750	0.781	**0.730**	0.006	**0.377**	**0.555**	**0.743**	**0.910**	0.610
HSC	6	0.792	0.737	0.676	-0.048	0.311	0.486	0.670	0.877	0.580
ILSTS 5	*	*	*	*	*	*	*	*	*	*
ILSTS 87	6	0.682	0.609	0.541	-0.077	0.195	0.352	0.527	0.782	0.493
INRA 5	2	0.615	0.507	0.374	-0.106	0.124	0.187	*0.280*	0.624	0.404
INRA 63	5	0.731	0.708	0.642	-0.023	0.279	0.447	0.629	0.855	0.561
INRABERN 192	8	0.731	0.582	0.532	*-0.156*	0.187	0.354	0.542	0.777	0.480
MAF 65	5	*0.348*	*0.317*	*0.300*	-0.086	*0.051*	*0.177*	0.312	*0.514*	*0.283*
MCM 527	6	0.577	0.722	0.665	**0.115**	0.300	0.476	0.664	0.872	0.572
OarFCB 48	6	0.654	0.670	0.614	-0.022	0.250	0.425	0.615	0.840	0.538
SRCRSP 5	7	0.731	0.777	0.725	0.025	0.365	0.544	0.728	0.906	0.607
SRCRSP 8	6	0.720	0.754	0.695	-0.007	0.328	0.503	0.684	0.888	0.592
SRCRSP 9	8	**0.864**	0.762	0.706	-0.077	0.347	0.524	0.712	0.896	0.596
SRCRSP 24	8	0.680	0.727	0.673	0.035	0.312	0.489	0.682	0.877	0.576
Average	6.09	0.66	0.64	0.58	-0.03	0.24	0.40	0.58	0.80	0.51

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings
*: Not computed

Table 3B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the AM: Breeder 1 group (AM1)

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	7	20	0.900	0.799	**0.747**	-0.074	**0.399**	**0.578**	**0.765**	**0.919**	**0.619**
BM 1329	6	18	0.944	0.746	0.684	-0.144	0.318	0.496	0.682	0.883	0.583
BM 1818	5	11	0.636	0.645	0.562	-0.023	0.210	0.370	0.547	0.799	0.508
BM 7160	5	19	0.737	0.727	0.653	-0.039	0.282	0.449	0.621	0.859	0.569
CSRD 247	4	16	0.813	0.706	0.625	-0.087	0.258	0.422	0.594	0.842	0.552
HSC	4	10	0.800	0.605	0.526	-0.209	0.176	0.335	0.506	0.770	0.480
ILSTS 5	2	13	0.769	0.517	0.374	*-0.215*	0.124	0.187	0.280	0.624	0.404
ILSTS 87	5	16	0.563	0.583	0.528	0.033	0.178	0.347	0.532	0.774	0.476
INRA 5	2	19	0.474	0.371	0.296	-0.134	0.065	0.148	0.234	0.527	0.312
INRA 63	4	20	0.650	0.741	0.671	0.053	0.297	0.468	0.641	0.871	0.579
INRABERN 192	6	20	0.800	0.758	0.705	-0.046	0.346	0.528	0.722	0.898	0.594
MAF 65	2	18	*0.056*	*0.157*	*0.141*	0.448	*0.012*	*0.071*	*0.126*	*0.271*	*0.144*
MCM 527	6	20	0.650	0.706	0.647	**0.036**	0.283	0.460	0.651	0.861	0.560
OarFCB 48	6	19	0.737	0.772	0.715	0.004	0.355	0.534	0.720	0.901	0.601
SRCRSP 5	5	13	**1.000**	0.788	0.719	-0.148	0.357	0.536	0.719	0.903	0.604
SRCRSP 8	4	16	0.563	0.587	0.510	0.018	0.168	0.317	0.478	0.756	0.473
SRCRSP 9	6	16	0.813	0.746	0.680	-0.072	0.317	0.492	0.680	0.880	0.581
SRCRSP 24	7	14	0.857	**0.802**	0.742	-0.049	0.394	0.573	0.763	0.918	0.616
Average	4.78	16	0.709	0.653	0.585	-0.036	0.252	0.406	0.570	0.792	0.514

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

Table 4B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the AM: Breeder 2 group (AM2)

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	6	23	0.826	0.775	0.717	-0.053	0.354	0.530	0.711	0.900	0.604
BM 1329	5	12	0.667	0.598	0.543	-0.091	0.189	0.366	0.562	0.788	0.484
BM 1818	4	16	0.813	0.627	0.529	-0.154	0.193	0.328	0.483	0.767	0.496
BM 7160	4	16	0.500	0.647	0.552	0.126	0.205	0.346	0.503	0.786	0.510
CSRD 247	4	19	0.474	0.661	0.586	0.134	0.223	0.383	0.552	0.815	0.526
HSC	7	15	0.800	**0.818**	**0.762**	-0.012	**0.418**	**0.596**	**0.781**	**0.927**	**0.627**
ILSTS 5	2	24	*0.167*	0.383	0.305	0.385	*0.070*	0.152	0.239	0.539	0.322
ILSTS 87	6	18	0.667	0.579	0.539	-0.112	0.187	0.367	0.569	0.785	0.478
INRA 5	2	22	0.364	0.495	0.367	**0.142**	0.117	0.183	0.276	0.616	0.396
INRA 63	3	22	0.682	0.647	0.554	-0.044	0.200	0.339	0.487	0.787	0.513
INRABERN 192	5	22	0.909	0.730	0.677	-0.137	0.310	0.491	0.684	0.881	0.577
MAF 65	3	21	0.190	*0.181*	*0.169*	-0.042	0.016	*0.090*	*0.166*	*0.315*	*0.167*
MCM 527	5	23	0.826	0.725	0.655	-0.078	0.284	0.452	0.625	0.861	0.570
OarFCB 48	6	21	0.667	0.783	0.727	0.072	0.369	0.546	0.730	0.907	0.609
SRCRSP 5	7	18	0.611	0.762	0.701	0.099	0.340	0.517	0.705	0.893	0.594
SRCRSP 8	4	19	0.526	0.579	0.510	0.025	0.165	0.318	0.480	0.756	0.471
SRCRSP 9	6	16	**0.938**	0.808	0.751	*-0.096*	0.400	0.579	0.762	0.921	0.622
SRCRSP 24	7	19	0.684	0.802	0.748	0.065	0.398	0.576	0.761	0.919	0.620
Average	4.78	19	0.628	0.644	0.577	0.013	0.247	0.398	0.560	0.787	0.510

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

Table 5B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the Animals tested for the breeders: Breeder1 (TA1)

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	7	20	0.700	0.755	0.694	0.014	0.331	0.507	0.691	0.888	0.590
BM 1329	6	22	0.682	0.614	0.564	-0.063	0.207	0.381	0.573	0.803	0.501
BM 1818	6	22	0.818	0.716	0.663	-0.080	0.297	0.478	0.673	0.873	0.568
BM 7160	5	21	0.762	0.708	0.635	-0.066	0.266	0.431	0.603	0.848	0.558
CSRD 247	5	21	0.762	0.655	0.572	*-0.091*	0.223	0.373	0.542	0.803	0.520
HSC	8	16	0.625	**0.806**	**0.755**	0.135	**0.414**	0.594	**0.786**	**0.926**	**0.622**
ILSTS 5	3	22	0.409	0.439	0.354	0.018	0.092	0.187	0.293	0.599	0.364
ILSTS 87	6	22	0.636	0.576	0.536	-0.071	0.184	0.362	0.560	0.782	0.477
INRA 5	3	17	0.353	0.456	0.384	0.103	0.098	0.215	0.340	0.631	0.379
INRA 63	4	24	0.583	0.691	0.616	0.075	0.252	0.413	0.584	0.835	0.547
INRABERN 192	6	23	0.826	0.771	0.716	-0.043	0.355	0.533	0.718	0.901	0.602
MAF 65	2	20	*0.300*	*0.262*	*0.222*	-0.078	*0.033*	*0.111*	*0.186*	*0.412*	*0.231*
MCM 527	5	24	0.500	0.660	0.577	**0.140**	0.222	0.372	0.535	0.806	0.524
OarFCB 48	8	22	0.864	0.771	0.714	-0.070	0.356	0.533	0.719	0.900	0.602
SRCRSP 5	6	21	0.714	0.786	0.731	0.044	0.373	**0.552**	0.735	0.909	0.611
SRCRSP 8	6	15	0.600	0.657	0.606	0.027	0.244	0.428	0.633	0.838	0.527
SRCRSP 9	5	20	0.800	0.733	0.666	-0.068	0.299	0.470	0.650	0.869	0.575
SRCRSP 24	5	9	**0.889**	0.797	0.712	NC	0.346	0.524	0.705	0.898	0.601
Average	5.33	20	0.657	0.659	0.595	-0.004	0.255	0.415	0.585	0.807	0.522

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings
NC: Not computed

Table 6B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the Animals tested for the breeders: Breeder 2 (TA2)

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	5	25	0.480	0.558	0.453	0.068	0.153	0.259	0.391	0.701	0.449
BM 1329	6	20	0.650	0.674	0.619	-0.037	0.258	0.435	0.631	0.844	0.540
BM 1818	4	25	0.760	0.692	0.610	-0.057	0.238	0.393	0.548	0.828	0.546
BM 7160	4	24	0.542	0.643	0.571	0.087	0.210	0.368	0.535	0.804	0.516
CSRD 247	3	24	0.417	0.358	0.322	*-0.108*	*0.061*	0.181	0.305	0.550	*0.313*
HSC	4	23	0.652	0.610	0.513	-0.046	0.185	0.312	0.462	0.753	0.487
ILSTS 5	3	25	0.480	0.484	0.379	-0.010	0.113	0.200	0.305	0.629	0.394
ILSTS 87	4	26	*0.269*	*0.310*	*0.287*	0.040	0.048	*0.164*	0.285	*0.499*	0.277
INRA 5	2	19	0.526	0.444	0.339	-0.098	0.093	0.169	*0.259*	0.584	0.362
INRA 63	4	26	0.577	0.695	0.624	0.087	0.257	0.421	0.593	0.841	0.551
INRABERN 192	7	27	**0.852**	**0.778**	**0.728**	-0.054	**0.374**	**0.553**	**0.740**	**0.909**	**0.609**
MAF 65	6	25	0.560	0.567	0.527	-0.010	0.177	0.352	0.546	0.774	0.471
MCM 527	5	24	0.583	0.659	0.584	0.056	0.229	0.386	0.559	0.813	0.526
OarFCB 48	7	24	0.542	0.633	0.578	0.064	0.223	0.394	0.585	0.814	0.513
SRCRSP 5	5	27	0.704	0.683	0.613	-0.029	0.252	0.415	0.591	0.834	0.544
SRCRSP 8	4	21	0.429	0.466	0.418	0.082	0.107	0.251	0.404	0.666	0.394
SRCRSP 9	5	22	0.636	0.575	0.514	-0.081	0.169	0.326	0.497	0.760	0.471
SRCRSP 24	4	15	0.400	0.513	0.442	**0.145**	0.127	0.264	0.413	0.692	0.421
Average	4.56	23	0.559	0.575	0.507	0.005	0.182	0.325	0.481	0.739	0.466

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

Table 7B Summary of number of alleles per locus, Observed and expected heterozygosities, PIC values, null allele frequencies, exclusion probabilities for the first and second parent as well as the parent pair, identity- and sibling identification for 18 markers for the Animals tested for the breeders: Breeder 3 (TA3)

Locus	k	N	H_O	H_E	PIC	F_{Null}	PE_1	PE_2	PE_P	PE_I	PE_S
BM 1258	7	36	0.833	0.754	0.702	-0.060	0.340	0.516	0.700	0.892	0.595
BM 1329	7	38	0.763	0.766	0.720	-0.019	0.368	0.546	0.737	0.905	0.604
BM 1818	6	33	0.667	0.727	0.671	0.037	0.311	0.484	0.672	0.874	0.577
BM 7160	5	28	0.500	0.618	0.538	0.107	0.196	0.340	0.502	0.777	0.498
CSRD 247	5	34	0.765	0.732	0.675	-0.025	0.308	0.482	0.664	0.876	0.580
HSC	10	28	0.643	0.785	0.740	**0.100**	**0.397**	**0.574**	0.766	0.917	0.615
ILSTS 5	4	36	0.361	0.414	0.353	0.046	0.084	0.194	0.312	0.595	0.353
ILSTS 87	7	33	0.515	0.586	0.536	0.061	0.188	0.354	0.538	0.780	0.484
INRA 5	2	30	0.333	0.398	0.315	0.080	0.076	0.157	0.245	0.553	0.334
INRA 63	4	37	0.784	0.656	0.591	-0.121	0.226	0.388	0.558	0.819	0.529
INRABERN 192	5	36	0.694	0.646	0.602	-0.048	0.237	0.417	0.613	0.833	0.527
MAF 65	5	34	*0.235*	*0.219*	*0.206*	-0.055	*0.024*	*0.113*	*0.204*	*0.375*	*0.201*
MCM 527	5	35	0.629	0.640	0.558	0.006	0.211	0.354	0.514	0.790	0.513
OarFCB 48	7	37	0.676	**0.816**	**0.778**	0.086	0.440	0.617	**0.799**	**0.935**	**0.636**
SRCRSP 5	6	30	0.733	0.739	0.694	0.014	0.332	0.516	0.713	0.893	0.587
SRCRSP 8	8	27	0.630	0.732	0.676	0.081	0.319	0.494	0.686	0.879	0.579
SRCRSP 9	7	36	0.778	0.710	0.662	-0.054	0.295	0.475	0.668	0.872	0.568
SRCRSP 24	6	12	**0.917**	0.786	0.716	*-0.101*	0.356	0.535	0.722	0.902	0.602
Average	5.89	32	0.636	0.651	0.596	0.008	0.262	0.420	0.590	0.804	0.521

k: Number of alleles per locus
H_o: Observed heterozygosity
H_E: Expected heterozygosity
PIC: Polymorphic information content
F_{Null}: Null allele frequency
PE_1: Exclusion probability for one candidate parent alone
PE_2: Exclusion probability for one candidate parent and one known parent of the opposite sex
PE_P: Exclusion probability for a candidate parent pair
PE_{ID}: Exclusion probability to exclude between two non-related individuals
PE_S: Exclusion probability for distinguishing between two siblings

APPENDIX C

Known parent – offspring combinations for family A1 and the TA families as compiled by the breeder.

Table 1C Known parent-offspring combinations for family A1

Offspring ID	Known mother ID
C2	C21
C3	
C4	C22
C5	C23
C6	C24
C7	C25
C8	C26
C9	C27
C10	C27
C11	C28
C12	C29
C13	C21
C14	C30
C16	C31
C17	C32
C18	C33
C19	C33
C20	C34
C35	C37
C36	C37
C39	
C39	
C40	C42
C41	C42
C43	C44

Table 2C Known parent - offspring combinations for the TA families

Offspring ID	Known mother ID
TA98	TA28
TA52	TA27
TA12	
TA7	TA45
TA20	TA51
TA90	TA10
TA47	TA63
TA18	TA85
TA97	TA82
TA35	
TA64	TA62
TA70	TA36
TA91	TA80
TA33	TA14
TA81	TA84
TA71	TA38
TA11	TA57
TA103	TA72
TA83	TA46
TA53	TA9
TA56	T102
TA66	TA100
TA74	TA31
TA86	TA94
TA13	TA96
TA69	TA44
TA54	TA25

Appendix D

Parentage analyses data for all families when the panel of 18 microsatellite markers were used.

Table 1D Parentage analyses for family A1 with 18 microsatellite markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
C2	C21	9	0	6.34	C1	11	1	-5.10	0.00		11	1	-2.86	0.00		0.931	0.972
C2	C21	9	0	6.34	E4	8	2	-5.72	0.00		8	3	-8.88	0.00		0.931	0.972
C3		0	0	0.00	C1	12	2	-5.67	0.00		0	0	0.00	0.00		0.947	0.947
C3		0	0	0.00	E4	9	4	-13.72	0.00		0	0	0.00	0.00		0.947	0.947
C4	C22	11	0	2.77	C1	13	1	-2.28	0.00		13	1	-0.85	0.00		0.826	0.991
C4	C22	11	0	2.77	E2	10	3	-10.03	0.00		10	5	-16.29	0.00		0.826	0.991
C5	C23	10	1	**-1.20**	C1	13	3	-12.29	0.00		13	4	-11.44	0.00		**0.986**	0.998
C5	C23	10	1	**-1.20**	E2	9	4	-14.75	0.00		9	5	-15.70	0.00		**0.986**	0.998
C6	C24	13	0	3.34	C1	13	2	-6.89	0.00		13	3	-8.54	0.00		0.955	0.997
C6	C24	13	0	3.34	E4	10	3	-10.17	0.00		10	4	-13.22	0.00		0.955	0.997
C7	C25	12	0	2.25	C1	13	1	-5.11	0.00		13	1	-2.69	0.00		0.732	0.943
C7	C25	12	0	2.25	E4	10	2	-6.04	0.00		10	2	-5.17	0.00		0.732	0.943
C8	C26	11	0	3.72	C1	13	2	-8.67	0.00		13	2	-6.66	0.00		0.881	0.998
C8	C26	11	0	3.72	E2	9	5	-19.52	0.00		9	6	-22.73	0.00		0.881	0.998
C9	C27	11	1	**-4.21**	C1	12	2	-8.51	0.00		12	3	-8.43	0.00		**0.684**	0.994
C9	C27	11	1	**-4.21**	E1	11	4	-16.07	0.00		11	4	-11.34	0.00		**0.684**	0.994
C10	C27	12	0	1.37	C1	12	1	-2.61	0.00		12	2	-4.29	0.00		0.832	**1.000**
C10	C27	12	0	1.37	E4	10	4	-14.85	0.00		10	5	-17.83	0.00		0.832	**1.000**
C11	C28	14	0	0.89	C1	14	1	-3.11	0.00		14	1	**0.011**	**0.011**	+	0.919	0.996
C11	C28	14	0	0.89	E4	10	4	-14.86	0.00		10	5	-17.72	0.00		0.919	0.996
C12	C29	18	1	0.86	C1	17	1	-1.47	0.00		17	3	-1.33	0.00		0.985	**1.000**
C12	C29	18	1	0.86	E4	13	5	-20.79	0.00		13	7	-22.51	0.00		0.985	**1.000**
C13	C21	15	1	2.30	C1	17	1	-4.53	0.00		17	2	-1.19	0.00		0.979	0.992
C13	C21	15	1	2.30	E4	13	4	-14.88	0.00		13	4	-11.65	0.00		0.979	0.992
C14	C30	17	1	**-2.23**	**E2**	12	**5**	**-16.34**	0.00		12	5	-12.40	0.00		0.956	0.998
C14	C30	17	1	**-2.23**	**C1**	16	**3**	**-10.89**	0.00		16	5	-13.13	0.00		0.956	0.998
C16	C31	15	0	3.18	C1	14	1	-4.27	0.00		14	3	-8.74	0.00		0.916	0.997
C16	C31	15	0	3.18	E4	10	4	-15.08	0.00		10	5	-19.32	0.00		0.916	0.997
C17	C32	12	0	4.55	C1	12	3	-10.77	0.00		12	4	-13.92	0.00		0.928	0.994
C17	C32	12	0	4.55	E4	9	4	-14.66	0.00		9	5	-17.94	0.00		0.928	0.994
C18	C33	14	0	0.91	C1	13	2	-6.74	0.00		13	3	-10.75	0.00		0.968	0.995
C18	C33	14	0	0.91	E4	10	5	-17.14	0.00		10	5	-18.09	0.00		0.968	0.995
C19	C33	13	1	**-5.38**	**E4**	9	2	-6.31	0.00		9	2	-2.60	0.00		0.948	0.971
C19	C33	13	1	**-5.38**	**E1**	10	2	-6.83	0.00		10	2	-3.11	0.00		0.948	0.971
C20	C34	11	0	3.32	C1	11	2	-8.03	0.00		11	2	-7.84	0.00		0.776	**0.876**
C20	C34	11	0	3.32	E4	8	3	-9.47	0.00		8	3	-10.83	0.00		0.776	**0.876**
C35	C37	13	0	3.25	**E2**	10	3	-10.08	0.00		10	3	-8.94	0.00		0.894	0.998

C35	C37	13	0	3.25	**C1**	13	3	-11.95	0.00	13	4	-13.54	0.00	0.894	0.998
C36	C37	14	1	**-3.18**	C1	15	3	-9.21	0.00	15	5	-11.06	0.00	0.966	0.997
C36	C37	14	1	**-3.18**	E4	11	4	-16.10	0.00	11	6	-18.31	0.00	0.966	0.997
C38		0	0	0.00	C1	12	2	-6.04	0.00	0	0	0.00	0.00	0.885	0.885
C38		0	0	0.00	E4	9	3	-10.77	0.00	0	0	0.00	0.00	0.885	0.885
C39		0	0	0.00	C1	14	1	-3.54	0.00	0	0	0.00	0.00	0.889	0.889
C39		0	0	0.00	E4	10	3	-8.24	0.00	0	0	0.00	0.00	0.889	0.889
C40	C42	16	0	7.57	C1	16	4	-16.97	0.00	16	4	-14.04	0.00	0.864	0.994
C40	C42	16	0	7.57	E4	12	4	-17.37	0.00	12	5	-18.71	0.00	0.864	0.994
C41	C42	12	0	3.59	C1	11	3	-10.06	0.00	11	3	-9.51	0.00	0.905	0.994
C41	C42	12	0	3.59	E3	7	4	-14.67	0.00	7	4	-15.43	0.00	0.905	0.994
C43	C44	11	0	3.01	C1	12	1	-4.91	0.00	12	1	-5.15	0.00	0.938	0.938
C43	C44	11	0	3.01	E4	8	3	-9.54	0.00	8	3	-9.84	0.00	0.938	0.938

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

Table 2D Parentage analyses for family A2 with 18 microsatellite markers

Offspring ID	Candidate father ID	Pair loci compared	Pair loci mismatched	Pair LOD score	Pair Delta	Pair confidence	PE1	PE2
B2	B1	15	0	1.48	1.48	+	0.97	0.97
B2	E2	13	3	-10.08	0		0.97	0.97
B3	B1	14	0	1.17	1.17	+	0.99	0.99
B3	E2	11	4	-14.82	0		0.99	0.99
B4	B1	16	0	-1.24	0		0.99	0.99
B4	E2	13	4	-16.16	0		0.99	0.99
B5	B1	16	0	-0.58	0		0.99	0.99
B5	E2	13	4	-17.38	0		0.99	0.99
B6	B1	16	0	1.27	1.27	+	0.99	0.99
B6	E3	11	6	-22.68	0		0.99	0.99
B7	B1	15	0	1.55	1.55	+	0.99	0.99
B7	E3	11	6	-22.64	0		0.99	0.99
B8	B1	15	0	3.69	3.69	*	0.99	0.99
B8	E3	11	3	-7.45	0		0.99	0.99
B9	B1	15	0	-1.05	0		0.96	0.96
B9	E2	13	3	-12.88	0		0.96	0.96
B10	B1	16	0	-0.19	0		0.98	0.98
B10	E2	13	3	-11.3	0		0.98	0.98
B11	B1	16	0	-0.84	0		0.99	0.99
B11	E2	13	3	-10.77	0		0.99	0.99
B12	B1	16	0	0.8	0.8	+	0.99	0.99
B12	E2	13	5	-21.46	0		0.99	0.99
B13	B1	15	0	-1.4	0		0.97	0.97
B13	E3	10	2	-4.39	0		0.97	0.97
B14	B1	15	0	1.33	1.33	+	0.99	0.99
B14	E3	10	5	-17.21	0		0.99	0.99
B16	B1	15	0	-1.46	0		0.98	0.98
B16	E3	10	3	-9.17	0		0.98	0.98
B17	B1	16	0	-0.32	0		0.99	0.99
B17	E3	11	4	-13.3	0		0.99	0.99
B18	B1	15	0	0.54	0.54	+	0.99	0.99
B18	E3	10	6	-22.64	0		0.99	0.99
B20	B1	16	0	-1.97	0		0.98	0.98
B20	E2	13	5	-20.79	0		0.98	0.98
B21	B1	15	0	-1.94	0		0.99	0.99
B21	E2	12	5	-21.23	0		0.99	0.99
B22	B1	16	0	0.59	0.59	+	0.99	0.99
B22	E2	13	3	-10.3	0		0.99	0.99
B23	B1	16	0	-1.75	0		0.99	0.99

B23	E3	11	4	-12.95	0	0.99	0.99

Pair Loci compared: Number of loci compared between the offspring and the candidate parent
Pair loci mismatch: Number of mismatches between the offspring and the candidate parent
* : strict confidence level, 95%
+: relaxed confidence level, 80%

Table 3D Parentage analyses for AM group: Breeder 1 (AM1) with 18 microsatellite markers

Offspring ID	Candidate mother ID	Pair loci compared	Pair loci mismatched	Pair LOD score	Pair Delta	Pair confidence	PE_I
AM10	**AM5**	**13**	**0**	**2.620000**	**2.625**	**+**	**0.9621**
AM10	**AM2**	**11**	**0**	**-0.612000**	0.000		**0.9621**
AM11	AM6	13	0	2.810000	**2.809**	*	0.9557
AM11	AM8	14	2	-7.590000	0.000		0.9557
AM12	AM7	15	2	-0.889000	0.000		0.9912
AM12	AM3	12	2	-8.940000	0.000		0.9912
AM14	AM9	14	2	-5.780000	0.000		0.9978
AM14	AM5	11	2	-6.040000	0.000		0.9978
AM15	AM8	14	1	1.230000	**1.229**	+	0.9996
AM15	AM6	12	2	-2.240000	0.000		0.9996
AM17	AM1	12	1	-1.580000	0.000		0.9828
AM17	AM7	13	2	-7.110000	0.000		0.9828
AM18	AM5	12	0	2.540000	**1.480**	+	0.9807
AM18	AM2	11	0	1.060000	0.000		0.9807
AM19	**AM1**	**10**	**1**	**-4.040000**	0.000		**0.9799**
AM19	**AM4**	**11**	**2**	**-4.260000**	0.000		**0.9799**
AM20	AM3	12	0	3.990000	**3.988**	*	0.9839
AM20	AM1	12	2	-7.470000	0.000		0.9839

Pair Loci compared: Number of loci compared between the offspring and the candidate parent
Pair loci mismatch: Number of mismatches between the offspring and the candidate parent
* : strict confidence level, 95%
+: relaxed confidence level, 80%

Table 4D Parentage analyses for AM group: Breeder 2 (AM2) with 18 microsatellite markers

Offspring ID	Candidate mother ID	Pair loci compared	Pair loci mismatched	Pair LOD score	Pair Delta	Pair confidence	PE_1
AM21	AM43	13	0	3.731541	3.732	*	0.998364
AM21	AM41	9	1	-3.723066	0.000		0.998364
AM23	AM35	15	0	7.735455	7.735	*	0.997860
AM23	AM41	7	2	-7.588974	0.000		0.997860
AM24	**AM42**	**9**	**0**	**2.112858**	2.113	+	**0.956885**
AM24	**AM37**	**12**	**1**	**-0.568398**	0.000		**0.956885**
AM25	AM38	14	1	1.043547	1.044	+	0.976925
AM25	AM36	11	1	-3.220017	0.000		0.976925
AM27	**AM42**	**12**	**0**	**-0.285832**	0.000		**0.976228**
AM27	**AM39**	**15**	**1**	**-1.115634**	0.000		**0.976228**
AM28	AM42	13	1	-1.909185	0.000		0.972697
AM28	AM35	14	1	-3.219879	0.000		0.972697
AM29	**AM43**	**10**	**1**	**-3.622191**	0.000		**0.992313**
AM29	**AM41**	**8**	**1**	**-4.350023**	0.000		**0.992313**
AM30	AM36	12	0	4.018625	4.019	*	0.960558
AM30	AM34	6	1	-2.153546	0.000		0.960558
AM31	AM41	6	0	3.039617	3.040	*	0.984075
AM31	AM35	10	2	-4.791266	0.000		0.984075
AM32	AM34	11	0	5.485679	5.486	*	0.991134
AM32	AM37	14	2	-6.462390	0.000		0.991134

Pair Loci compared: Number of loci compared between the offspring and the candidate parent
Pair loci mismatch: Number of mismatches between the offspring and the candidate parent
* : strict confidence level, 95%
+: relaxed confidence level, 80%

Table 5D Parentage analyses for Animals tested for Breeder 1 (TA1) with 18 markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
TA98	TA28	14	0	1.90	TA6	10	1	**-1.21**	0.00		10	3	**-7.42**	0.00		0.994	1.000
TA98	TA28	14	0	1.90	TA16	13	4	-13.86	0.00		13	6	-19.85	0.00		0.994	1.000
TA52	TA27	16	0	4.74	TA6	10	0	2.25	2.25	+	10	0	4.27	4.27	*	0.994	1.000
TA52	TA27	16	0	4.74	TA48	13	2	-5.34	0.00		13	5	-17.65	0.00		0.994	1.000
TA12		0	0	0.00	TA6	10	0	1.59	1.29	+	0	0	0.00	0.00		0.954	
TA12		0	0	0.00	TA48	9	0	0.30	0.00		0	0	0.00	0.00		0.954	
TA7	TA45	7	1	**-4.54**	TA68	10	0	0.76	0.76	+	10	4	**-8.00**	0.00		0.997	1.000
TA7	TA45	7	1	**-4.54**	TA48	10	2	-10.21	0.00		10	6	-17.67	0.00		0.997	1.000
TA20	TA51	14	3	**-9.80**	TA6	9	0	2.23	2.23	+	9	4	**-3.88**	0.00		0.985	0.999
TA20	TA51	14	3	**-9.80**	TA48	11	1	-5.56	0.00		11	3	-7.40	0.00		0.985	0.999
TA90	TA10	13	0	4.08	TA65	14	2	**-4.46**	0.00		14	3	**-6.37**	0.00		0.997	1.000
TA90	TA10	13	0	4.08	TA6	9	2	-7.51	0.00		9	4	-14.09	0.00		0.997	1.000
TA92	TA5	10	0	3.94	TA68	13	0	0.35	0.35	+	13	1	**-0.96**	0.00		0.991	1.000
TA92	TA5	10	0	3.94	TA48	13	2	-10.36	0.00		13	2	-9.27	0.00		0.991	1.000
TA47	TA63	7	1	0.15	TA68	11	0	4.54	4.54	*	11	1	5.96	5.96	*	0.992	0.998
TA47	TA63	7	1	0.15	TA16	13	3	-12.74	0.00		13	4	-15.44	0.00		0.992	0.998
TA18	TA85	14	0	4.39	TA68	10	1	**-4.18**	0.00		10	3	**-9.52**	0.00		0.967	1.000
TA18	TA85	14	0	4.39	TA6	9	2	-7.71	0.00		9	4	-14.13	0.00		0.967	1.000
TA97	TA82	17	1	5.41	TA67	15	0	7.68	7.68	*	15	1	11.85	11.85	*	0.999	1.000
TA97	TA82	17	1	5.41	TA16	13	3	-12.04	0.00		13	4	-9.15	0.00		0.999	1.000

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

Table 6D Parentage analyses for Animals tested for Breeder 2 (TA2) with 18 microsatellite markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
TA35		0	0	0.00	TA93	15	0	2.08	2.08	+	0	0	0.00	0.00		0.841	
TA35		0	0	0.00	TA29	8	0	-0.16	0.00		0	0	0.00	0.00		0.841	
TA64	TA62	17	5	**-19.72**	TA29	8	1	**-2.42**	0.00		8	4	1.26	1.26	+	0.994	0.981
TA64	TA62	17	5	**-19.72**	TA93	15	1	-1.04	0.00		15	6	-0.72	0.00		0.994	0.981
TA70	TA36	16	0	6.82	TA93	15	0	**-0.14**	0.00		15	0	3.31	3.31	*	0.984	0.997
TA70	TA36	16	0	6.82	TA29	7	1	-2.26	0.00		7	1	-2.49	0.00		0.984	0.997
TA55	TA32	9	0	1.95	TA23	8	0	0.11	0.11	-	8	0	2.69	2.69	*	0.803	0.984
TA55	TA32	9	0	1.95	TA41	8	1	-4.59	0.00		8	2	-6.17	0.00		0.803	0.984
TA91	TA80	13	2	**-4.47**	TA29	6	1	**-4.74**	0.00		6	1	-3.20	0.00		0.999	1.000
TA91	TA80	13	2	**-4.47**	TA23	13	1	-1.80	0.00		13	4	-5.59	0.00		0.999	1.000
TA33	TA14	13	0	6.35	TA93	13	0	1.53	1.38	+	13	0	3.39	3.39	*	0.948	0.991
TA33	TA14	13	0	6.35	TA29	5	0	0.15	0.00		5	1	-2.73	0.00		0.948	0.991
TA81	TA84	7	0	3.82	TA93	15	0	**-0.87**	0.00		15	0	0.19	0.16	+	0.940	0.940
TA81	TA84	7	0	3.82	TA29	7	0	-0.92	0.00		7	0	0.02	0.00		0.940	0.940
TA71	TA38	16	1	4.62	TA93	16	0	0.68	0.20	-	16	2	1.51	1.51	+	0.986	1.000
TA71	TA38	16	1	4.62	TA29	7	0	0.48	0.00		7	2	-0.58	0.00		0.986	1.000
TA95		0	0	0.00	TA37	14	1	0.31	0.31	+	0	0	0.00	0.00		0.962	0.962
TA95		0	0	0.00	TA29	8	0	-2.54	0.00		0	0	0.00	0.00		0.962	0.962
TA11	TA57	15	0	4.75	TA37	12	1	0.59	0.59	+	12	1	2.22	2.23	*	0.962	0.996
TA11	TA57	15	0	4.75	TA29	6	0	0.00	0.00		6	1	-3.03	0.00		0.962	0.996
TA103	TA72	16	0	5.93	TA29	8	0	0.56	0.22	-	8	0	1.46	1.46	+	0.922	1.000
TA103	TA72	16	0	5.93	TA41	17	0	0.34	0.00		17	2	-0.68	0.00		0.922	1.000
TA34	TA1	7	0	2.96	TA37	13	1	**-0.14**	0.00		13	1	0.95	0.95	+	0.959	0.994
TA34	TA1	7	0	2.96	TA93	14	0	0.76	0.76	+	14	1	-1.40	0.00		0.959	0.994

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

Table 7D Parentage analyses for Animals tested for Breeder 3 (TA3) with 18 microsatellite markers

Offspring ID	Mother ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Candidate father ID	Pair loci compared	Pair loci mismatch	Pair LOD score	Pair Delta	Pair confidence	Trio loci compared	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence	PE_1	PE_2
TA83	TA46	16	5	**-18.63**	TA22	15	0	9.36	9.31	*	15	**5**	7.14	7.14	*	0.999	1.000
TA83	TA46	16	5	**-18.63**	TA61	15	1	0.05	0.00		15	7	-3.93	0.00		0.999	1.000
TA53	TA9	14	0	10.72	TA75	9	0	5.07	5.07	*	9	0	6.65	6.65	*	1.000	1.000
TA53	TA9	14	0	10.72	TA22	14	5	-16.17	0.00		14	5	-16.66	0.00		1.000	1.000
TA56	TA102	16	2	**-5.11**	TA40	13	0	4.22	4.06	*	13	**2**	2.71	2.71	*	0.999	1.000
TA56	TA102	16	2	**-5.11**	TA75	9	2	-4.98	0.00		9	2	-5.83	0.00		0.999	1.000
TA101		0	0	0.00	TA59	6	0	**0.00**	0.00		0	0	0.00	0.00		0.947	
TA101		0	0	0.00	TA75	1	0	-0.62	0.00		0	0	0.00	0.00		0.947	
TA66	TA100	13	2	**-7.05**	TA21	15	0	10.45	10.45	*	15	**3**	8.34	8.34	*	0.985	1.000
TA66	TA100	13	2	**-7.05**	TA24	11	2	-3.83	0.00		11	5	-6.62	0.00		0.985	1.000
TA74	TA31	12	1	0.16	TA21	15	0	4.16	4.16	*	15	**2**	1.35	1.35	*	0.997	0.999
TA74	TA31	12	1	0.16	TA87	9	2	-5.53	0.00		9	3	-6.86	0.00		0.997	0.999
TA86	TA94	14	0	4.79	TA21	17	1	4.55	4.55	*	17	**1**	6.76	6.76	*	0.995	1.000
TA86	TA94	14	0	4.79	TA24	13	4	-15.08	0.00		13	5	-16.76	0.00		0.995	1.000
TA39	TA4	4	0	1.91	TA59	14	1	**-1.17**	0.00		14	**2**	**-3.25**	0.00		0.997	0.999
TA39	TA4	4	0	1.91	TA30	15	2	-7.23	0.00		15	3	-9.77	0.00		0.997	0.999
TA13	TA96	9	0	5.16	TA40	11	0	**-0.19**	0.00		11	0	1.28	1.28	*	**0.878**	**0.966**
TA13	TA96	9	0	5.16	TA15	10	1	-0.08	0.00		10	1	-0.92	0.00		**0.878**	**0.966**
TA60	TA19	9	0	2.33	TA21	17	0	5.37	5.37	*	17	0	9.14	9.14	*	0.995	1.000
TA60	TA19	9	0	2.33	TA17	15	2	-4.67	0.00		15	3	-6.44	0.00		0.995	1.000
TA69	TA44	9	0	3.46	TA43	12	0	3.41	3.41	*	12	0	5.52	4.89	*	0.938	0.999
TA69	TA44	9	0	3.46	TA40	9	0	-1.14	0.00		9	0	0.63	0.00		0.938	0.999
TA54	TA25	18	0	8.43	TA43	18	0	8.03	7.32	*	18	0	11.46	11.46	*	0.991	1.000
TA54	TA25	18	0	8.43	TA40	14	1	-0.68	0.00		14	3	-7.96	0.00		0.991	1.000

Column one: Offspring ID
Column two: Known mother ID
Column three: Pair loci compared: Number of loci compared between the offspring and the known parent (dam)
Column four: Pair loci mismatch: Number of mismatches between the offspring and the known parent (dam)
Column five: Pair LOD score: LOD score between known parent (dam) and offspring
Column six: Candidate father ID
Column seven: Pair loci compared: Number of loci compared between the offspring and the candidate parent (sire)
Column eight: Pair loci mismatch: Number of mismatches between the offspring and the candidate parent (sire)
Column nine: Pair LOD score: LOD score between candidate parent (sire) and offspring
Column ten: Pair Delta score: Difference in LOD scores between first and second most likely candidate parents alone
Column eleven: Pair confidence level (*: strict, 95%) (+: relaxed, 80%)
Column twelve: Trio loci compared: Number of loci compared between the offspring, known parent (dam) and candidate parent (sire)
Column thirteen: Trio loci mismatch: Number of mismatches between the offspring, known parent (dam) and candidate parent (sire)
Column fourteen: Trio LOD score: LOD score between the offspring, known parent (dam) and candidate parent (sire)
Column fifteen: Trio Delta score: Difference between the LOD score of the known parent and most likely parent and the LOD score of the known parent and second most likely parent
Column sixteen: Trio confidence level (*: strict, 95%) (+: relaxed, 80%)
Column seventeen: First parent exclusion probability (PE_1): The probability for excluding an unrelated candidate parent calculated from the genotype of the offspring
Column eighteen: Second parent exclusion probability (PE_2): The probability for excluding an unrelated candidate parent calculated from the genotypes of the offspring and the known parent

APPENDIX E

Table 1E Microsatellite markers used in previous parentage verification studies conducted on goats

Microsatellite marker	Current study	Luikart *et al.* (1999)	Recommended ISAG panel (2001/2002)	Ganai & Yadav (2005)	Recommended ISAG panel (2005)	Jimenez-Gamero *et al.* (2006)	Glowatzki-Mullis *et al.* (2007)	Bolormaa *et al.*(2008)
BM1258	✔	✘	✔	✘	✔	✘	✔	✔
BM1329	✔	✘	✔	✘	✔	✘	✔	✘
BM1818	✔	✘	✔	✘	✔	✘	✘	✔
BM5004	✘	✘	✘	✔	✘	✘	✘	✘
BM7160	✔	✘	✘	✔	✘	✘	✘	✘
BM7228	✘	✘	✘	✔	✘	✘	✘	✘
BMC5221	✘	✘	✘	✔	✘	✘	✘	✘
BMS1237	✘	✘	✘	✔	✘	✘	✘	✘
BMS332	✘	✘	✘	✔	✘	✘	✘	✘
BMS357	✘	✘	✘	✔	✘	✘	✘	✘
BMS585	✘	✘	✘	✔	✘	✘	✘	✘
BMS820	✘	✘	✘	✔	✘	✘	✘	✘
BOBT24A	✘	✘	✘	✘	✘	✘	✔	✘
BR6027	✘	✘	✘	✔	✘	✘	✘	✘
CSRD247	✔	✘	✔	✘	✔	✘	✔	✘
ChirUCO2	✘	✘	✘	✘	✘	✔	✘	✘
ChirUCO4	✘	✘	✘	✘	✘	✔	✘	✘
ChirUCO5	✘	✘	✘	✘	✘	✔	✘	✘
ETH10	✘	✔	✘	✘	✘	✔	✔	✘
HSC	✔	✘	✘	✘	✔	✘	✔	✘
ILSTS11	✘	✔	✘	✘	✘	✘	✘	✔
ILSTS19	✘	✘	✘	✘	✔	✘	✘	✘
ILSTS29	✘	✔	✘	✘	✘	✘	✘	✔
ILSTS5	✔	✔	✘	✘	✘	✘	✔	✘
ILSTS87	✔	✘	✔	✘	✔	✘	✘	✘
INRA023	✘	✘	✔	✘	✔	✘	✘	✘
INRA132	✘	✘	✔	✘	✘	✘	✔	✘
INRA172	✘	✔	✘	✘	✘	✘	✘	✘
INRA185	✘	✔	✘	✘	✘	✘	✘	✘
INRA231	✘	✘	✘	✘	✔	✘	✘	✘
INRA40	✘	✘	✘	✘	✘	✘	✔	✘
INRA5	✔	✘	✔	✘	✔	✘	✔	✘
INRA11c	✘	✘	✘	✘	✘	✔	✘	✘
INRA63	✔	✔	✔	✘	✔	✘	✔	✔
INRABERN172	✘	✘	✘	✘	✘	✘	✘	✔
INRABERN182	✘	✘	✘	✘	✘	✘	✘	✔

MAF209	✗	✓	✗	✗	✗	✗	✗	✗
MAF65	✓	✓	✓	✗	✓	✗	✗	✗
MB045	✗	✗	✗	✓	✗	✗	✗	✗
MB068	✗	✗	✗	✓	✗	✗	✗	✗
MCHII-DR	✗	✓	✗	✗	✗	✗	✗	✗
MCM527	✓	✗	✓	✗	✓	✗	✗	✗
LSCV44	✗	✗	✗	✗	✗	✗	✗	✓
OarAE54	✗	✓	✗	✗	✗	✗	✗	✗
OarCP73	✗	✗	✗	✗	✗	✗	✗	✓
OarFCB128	✗	✗	✗	✗	✗	✗	✓	✗
OarFCB20	✗	✓	✓	✗	✓	✗	✓	✓
OarFCB48	✓	✓	✗	✗	✗	✗	✗	✗
RM4	✗	✗	✗	✗	✗	✗	✗	✗
RM096	✗	✗	✗	✗	✗	✗	✗	✓
SPS113	✗	✗	✗	✗	✗	✗	✓	✗
SPS115	✗	✗	✗	✗	✗	✗	✓	✗
SRCRSP1	✗	✗	✗	✗	✗	✓	✓	✗
SRCRSP13	✗	✓	✗	✗	✗	✗	✗	✗
SRCRSP15	✗	✓	✗	✗	✗	✗	✗	✗
SRCRSP23	✗	✓	✓	✗	✓	✗	✗	✗
SRCRSP24	✓	✗	✓	✗	✓	✗	✗	✗
SRCRSP3	✗	✓	✗	✗	✗	✗	✗	✗
SRCRSP5	✓	✓	✓	✗	✓	✓	✗	✓
SRCRSP7	✗	✓	✗	✗	✗	✗	✗	✓
SRCRSP8	✓	✓	✓	✗	✓	✓	✓	✗
SRCRSP9	✓	✓	✗	✗	✗	✓	✗	✗
TGLA53	✗	✓	✗	✗	✗	✗	✗	✓