Hereditary paraganglioma in South Africa: An investigation of the Succinate-ubiquinone Oxidoreductase subunit genes, *SDHB*, *SDHC* and *SDHD*

BY

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Abstract

Paraganglioma (PGL) are tumours occurring in the head-and-neck -, intra-abdominal - and thoracic paraganglia. Germ-line mutations in genes encoding the subunits of mitochondrial succinate dehydrogenase complex II (*SDHB*, *SDHC*, *SDHD*) and the *SDHAF2* gene are involved in hereditary paraganglioma. Our aim was to identify mutations within these genes in ten South African PGL families. Individuals were screened for mutations in *SDHAF2* using Sanger sequencing and Multiplex Ligation-dependent Probe Amplification was utilised to investigate large rearrangements in these genes. A 7905bp *SDHB* exon 3 deletion [c.201-4429_287-933del], was identified in all SA families. The same deletion is reported as a founder mutation in Dutch PGL families. Genotype analysis revealed a common haplotype at the *SDHB* locus between SA and Dutch patients, indicating common ancestry. This is the first Afrikaner *SDHB* founder mutation. These results now enable predictive testing of other family members and allow better clinical management of the families.

SUMMARY

Paragangliomas (PGL) are neuro-endocrine tumours occurring in the head-and-neck -, intra-abdominal - and thoracic paraganglia. These tumours may lead to significant morbidity due to compromised function of cranial nerves and some may also be malignant and aggressive. It has been shown that familial paraganglioma is caused by germline mutations in one of ten susceptibility genes, namely *RET* , *VHL,NF1,SDHA, SDHB,SDHC, SDHD, SDHAF2, TMEM127* and *MAX* genes. The three most common PGL susceptibility genes are *SDHB*, *SDHC*, and *SDHD* encoding subunits of the mitochondrial succinate dehydrogenase (SDH) complex II. Mutations in the *SDHAF2* gene encoding the SDH assembly factor 2 are also involved in paraganglioma formation. Previously, the *SDHB*, *SDHC* and *SDHD* genes were screened for point mutations in 11 South African paraganglioma families. Only one family carried a disease-causing *SDHB* mutation in exon 4.

The aim of this study was to investigate the remaining 10 families for large rearrangements in the *SDHB*, *-C*, *-D* and *SDHAF2* genes using Multiplex Ligation-dependent Probe Amplification (MLPA) and bi-directional Sanger sequencing. These families predominantly presented with head-and-neck PGL with three families presenting with abdominal tumours. Five of the families had malignant tumours.

A 7905bp deletion [c.201-4429_287-933del.] removing exon 3 of *SDHB* was detected in all of the families. A common haplotype was identified between the South African patients. The SA patients thus share a common ancestor. Interestingly, this deletion has been shown to be a founder mutation in the Dutch population. Haplotype analysis of the South African and Dutch patients revealed a common core haplotype at the *SDHB* locus. The identical exon 3 deletions and common haplotype in the Afrikaner patients indicates that this deletion is the first Afrikaner *SDHB* founder mutation, possibly introduced into SA by the Dutch.

At least half of the Afrikaner families carrying the *SDHB* deletion showed non-penetrance which is also apparent in the Dutch families carrying the same mutation. Previous studies reported a family history of PGL in only 31% of mutation-positive cases which is much lower than patients carrying *SDHD* (61%) and *SDHC* (62.5%) mutations. The presence of an *SDHB* mutation is associated with an excess mortality and it is thus expected for *SDHB* mutation carriers to have a decreased life expectancy in comparison to other *SDH* gene mutation carriers. Ultimately, detection of disease-causing mutations will enable predictive testing of other family members and allow better clinical management of these families.

KEYWORDS: Familial paraganglioma, pheochromocytoma, South Africa, Dutch, MLPA, large rearrangements, *SDHB*, Founder mutation, common haplotype, predictive genetic testing.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation/Symbol	Meaning
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
bp	basepairs
CT	Computed Tomography
Cys	Cystine
ddH_2O	Double distilled water
dNTP	Deoxy-nucleotide triphosphate
DQ	Dosage Quotient
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
FAD	Flavin adenine dinucleotide
^{18}F-FDG	2-deoxy-2-[fluorine-18]fluoro-D-glucose
FADH2	Flavin adenine dinucleotide (reduced)
FMTC	Familial medullary thyroid cancer
fs	frameshift
g	Gravitational acceleration
GDNF	Glial cell line derived neurotropic factor
GISTs	Gastrointestinal stromal tumours
HGT	Hereditary glomus tumour
HIF1	Hypoxia inducible factor 1
His	Histidine
kb	kilobases
KIF1B	Kinesin family member 1B encoding gene
KIF1Bα	Kinesin family member 1B alpha
KIF1Bβ	Kinesin family member 1B beta
LDL	Low Density Lipoprotein
M	Molar
MAX	MYC-associated factor X
Mb	Megabases
MEN2	Multiple EndrocrineNeoplasia type 2
$MgCl_2$	Magnesium Chloride
min	Minutes
ml	millilitre
MLPA	Multiplex Ligation-dependent Probe Amplification
MRI	Magnetic Resonance Imaging
mTOR	Mammalian target of rapamycin
NaCl	Sodium Chloride
$NACIO_4$	Sodium perchlorate
NADH	Nicotinamide adenine dinucleotide hydrate
NF1	Neurofibromatosis type 1

ng	nanogram
nm	nanometer
NMD	Nonsense-mediated mRNA decay
nt	nucleotides
PCC	Pheochromocytoma
PCR	Polymerase chain reaction
PDGF- β	platelet-derived growth factor beta
PGL	Paraganglioma
PGL1	Paraganglioma type 1
PGL2	Paraganglioma type 2
PGL3	Paraganglioma type 3
PGL4	Paraganglioma type 4
PHD	Prolyl hydroxylase
PHD2	Prolyl hydroxylase domain protein 2 encoding gene
RCC	Renal cell carcinoma
ROS	Reactive oxygen species
rpm	revolutions per minute
SA	South Africa(an)
SD	Standard deviation
SDH	Succinate Dehydrogenase
SDHA	Succinate dehydrogenase subunit A encoding gene
SDHA	Succinate dehydrogenase subunit A protein
SDHB	Succinate dehydrogenase subunit B encoding gene
SDHB	Succinate dehydrogenase subunit B protein
SDHC	Succinate dehydrogenase subunit C encoding gene
SDHC	Succinate dehydrogenase subunit C protein
SDHD	Succinate dehydrogenase subunit D encoding gene
SDHD	Succinate dehydrogenase subunit D protein
sec	seconds
SNP	Single nucleotide polymorphism
TGF- α	Transforming growth factor alpha
TMEM127	Transmembrane protein 127 encoding gene
Tris-EDTA	Trisaminomethane- Ethylenediaminetetraacetic acid
Tris-HCL	Trisaminomethane-Hydrochloric acid
V	Volt
VEGF	Vascular endothelial growth factor
VHL	Von HippelLindau
WT	Wildtype
α	Alpha
β	Beta
µM	Micro Molar

Chapter 1: Literature Review

1.1 INTRODUCTION

Paraganglioma are rare tumours of neuroendocrine origin occurring sporadically or as part of hereditary tumour syndromes (1).The tumours develop in the paraganglia which are organs consisting mainly of neuroendocrine cells and are able to synthesise and secrete catecholamines including adrenaline and noradrenaline (3). Paraganglioma (PGL) may originate in the neural crest cells located in the adrenal medulla or along the sympathetic and parasympathetic ganglia. Intra-adrenal PGL are known as pheochromocytomas (PCC) and usually secrete catecholamines (4) whereas extra-adrenal PGLs are divided into those that develop in the sympathetic ganglia and those developing in the parasympathetic ganglia. Sympathetic PGL develop mostly in the abdomen, chest and pelvis, as shown below (figure 1.1), and can also be functional catecholamine secreting tumours. These tumours are usually more aggressive than parasympathetic PGL which are mostly benign and functional in only 1 – 5% of cases (5). Parasympathetic PGLs are also known as head-and-neck PGL and mostly develop in the vagus nerve, inner ear with the most common location being the carotid body (figure 1.2) which functions as an oxygen sensor in peripheral blood (6).

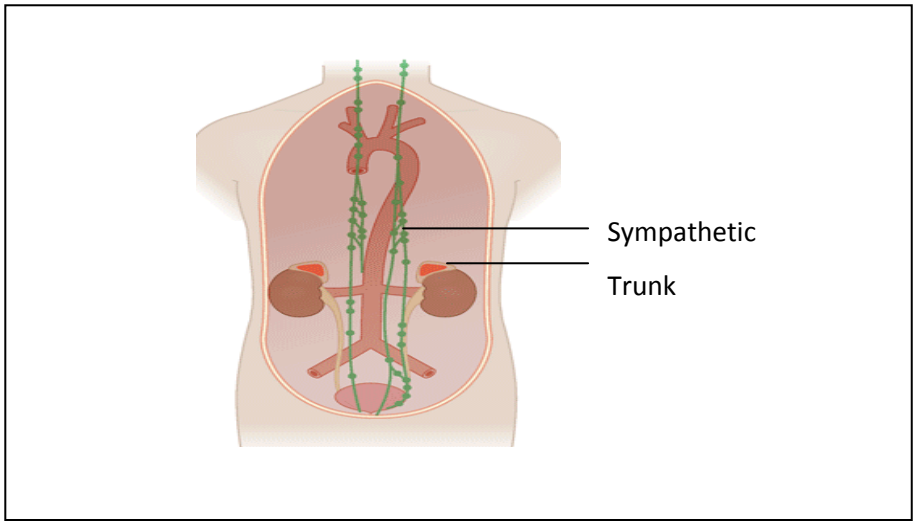

Figure 1.1: Common sites of sympathetic PGL

Image Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: Harrison's Principles of Internal Medicine, 18th Edition: www.accessmedicine.com

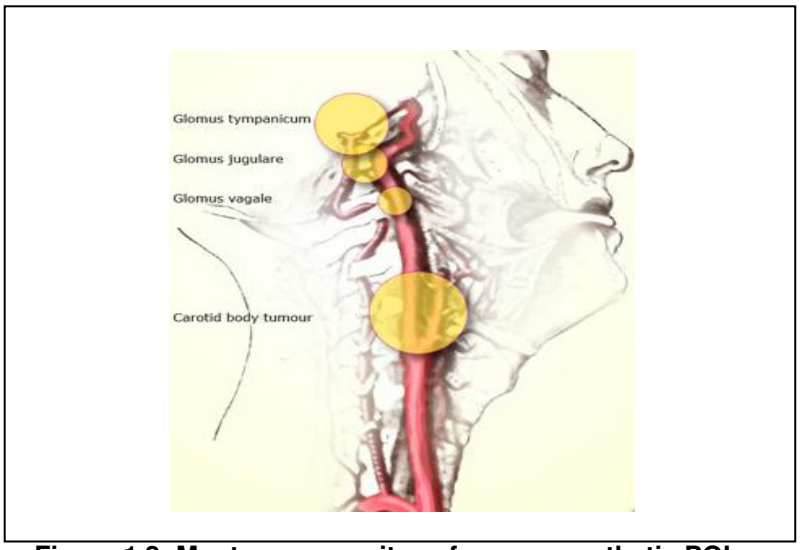

Figure 1.2: Most common sites of parasympathetic PGL

Image Source - A: Image created by Frank Gaillard-http://radiopaedia.org/images/11077

Paragangliomas are thus named according to their origin and classified as functional or non-functional depending on whether they produce catecholamines. These catecholamines help to regulate the blood stream in the arterioles and will cause an increase in blood pressure if in excess. One of the symptoms of active paraganglioma is thus hypertension (7). Different symptoms are associated with different tumours depending on localisation, although a number of tumours may show no symptoms at all. The estimated annual incidence of PGL has been reported to be 3-8 cases per million in the general population (8). Although these tumours are rare and mostly benign, they may still lead to significant morbidity. Approximately 10% of PCC and 20% of abdominal secreting PGLs are malignant (9). Patients with malignant PCCs and PGLs have a 5 year mortality rate of greater than 50% (10). Most malignancies are identified in vagal PGLs (16-19%) as opposed to carotid body tumours (6%) and jugulotympanic PGLs (2-4%) (11, 12). Factors which seem to be associated with an increased risk of malignant PGL/PCC include young age at diagnosis, large tumour size as well as a fast growing tumour and extra-adrenal abdominal or mediastinal tumours (13-15).

The majority of PGLs are sporadic with ~35% of all paragangliomas predicted to be hereditary and caused by germline mutations in several susceptibility genes (16). Ten loci are associated with the formation of PGL and PCC.

A mutation in any of the ten genes coding for these loci will lead to an increased susceptibility for the development of hereditary PGL syndromes: Multiple endocrine neoplasia type 2 (MEN2), Neurofibromatosis type 1 (NF1), Von Hippel-Lindau (VHL) and familial paraganglioma.

1.2 SUSCEPTIBILITY TO PARAGANGLIOMA AND PHEOCHROMOCYTOMA

Pheochromocytoma and paraganglioma may arise as a result of mutations in the following genes: *RET* (MEN2); *VHL* (VHL); *NF1* (NF1); *SDHA*, *-B* *-C* or *-D*, *SDHAF2*, *TMEM127* and *MAX* genes in familial PCC/PGL syndrome. All ten loci involved in the development of these tumours are inherited in an autosomal dominant manner with variable expressivity and reduced penetrance. Three loci show an inheritance pattern similar to that of maternal imprinting and thus the mutated gene appears to be expressed only when inherited from the father. Maternal transmission of these genes rarely, if ever, leads to tumour development thus presenting an inheritance pattern seemingly consistent with genomic imprinting (17). All ten genes and resulting syndromes are listed and compared to one another in table 1.1.

Table 1.1: Summary of the ten currently known PGL/PCC susceptibility genes

Gene	Chromosome	Syndrome	Inheritance	Freq.	Most common tumour location	Imprinting	Clinical manifestations
RET	10q11.2	MEN2A MEN2B	AD	~5%	Intra-adrenal (PCC)	No	PCC, medullary thyroid carcinoma
VHL	3p25-26	VHL	AD	~9%	Intra-adrenal (PCC)	No	Hemangioblastoma,clear- cell renal cell carcinoma, endolymphatic sac tumours, retinal angiomas, serous cystadenomas, PCC
NF1	17q11.2	NF1	AD	~2%	Intra-adrenal (PCC)	No	Café au lait spots, Lisch nodules of iris, gliomas of optic pathway and brainstem, neurofibromas, soft-tissue sarcomas, chronic myeloid leukemia in children, astrocytomas, PGL and PCC
SDHD	11q23	PGL1	AD + Pi	~5%	Head-and-neck	Maternal	PGL, PCC and GIST
SDHAF2	11q13.1	PGL2	AD + Pi	<1%	Head-and-neck	Maternal	PGL and PCC
SDHC	1q21	PGL3	AD	~1%	Head-and-neck	No	PGL, PCC and GIST
SDHB	1p36.1	PGL4	AD	~5%	Abdomen	No	PGL, PCC, renal cell carcinoma and GIST
SDHA	5p15	-	AD	<1%	Extra-adrenal	No	PGL, PCC, Leigh syndrome(homozygous)
TMEM127	2q11	-	AD	~2%	Intra-adrenal (PCC)	No	PCC
Max	14q23.3	-	AD + Pi	<1%	Intra-adrenal (PCC)	Maternal	PCC

AD = Autosomal Dominant; Pi = Parental inheritance; GIST = Gastro-intestinal stromal tumours Freq.= Frequency of a mutation reported to all patients with PGL or PCC.
This table was adapted from Galan et al., 2013 (2)

The transcription profiles of the PCC and PGL tumours can be divided into two signalling pathways. The first is a pseudo-hypoxic cluster containing tumours with VHL and SDH mutations and is associated with angiogenesis, hypoxia as well as reduced oxidative response. The second cluster contains tumours with *RET* (MEN2), *NF1*, *TMEM127* and *MAX* mutations and is associated with abnormal activation of kinase-signalling pathways (2, 18).

1.2.1 Multiple endocrine neoplasia type 2 (MEN2)

Multiple endocrine neoplasia (MEN2) is a hereditary syndrome characterised by the onset of various tumours in different locations. The prevalence of MEN2 is approximately 1/40 000 individuals (19). MEN2 can be divided into three types according to clinical manifestation namely, MEN2A, MEN2B and familial medullary thyroid cancer (FMTC). MEN2A occurs most often with a frequency of 55% in individuals followed by FMTC (35 – 40%) and lastly MEN2B with a 5 – 10% frequency. The most common manifestation of MEN2 is medullary thyroid cancer, however, approximately 5 – 10% of patients with PCC can be ascribed to MEN2. Individuals with MEN2A or MEN2B have a 40 - 50% chance of developing PCC. Patients frequently develop bilateral and recurrent PCCs, but rarely develop malignant tumours. Previous studies indicated that approximately 63% of patients with MEN2 and PCC displayed bilateral PCC and only 3% were malignant. The mean age at presentation of disease was 36 years (4). Presentation of PGLs in MEN2 patients is very rare and only few have been reported (5, 20-22).

MEN2 syndromes are inherited in an autosomal dominant manner and mutations in the *RET* proto-oncogene predispose individuals to this syndrome. This gene consists of 21 exons and is located on chromosome 10q11.21 (23). The RET protein is a tyrosine kinase receptor for members of the glial cell line derived neurotropic factor (GDNF) family (24-26) and is activated by binding of one of its ligands, inducing dimerization (4, 27). This leads to the activation of multiple intracellular pathways involved in cell growth, proliferation and differentiation (28, 29).

The RET protein plays an essential role in kidney development as well as development of the sympathetic -, parasympathetic - and enteric nervous system (30). Genetically gain-of-function mutations are the underlying cause of MEN2 and are mostly missense mutations located in exons 10, 11, 13, 14, 15 and 1 (4, 19).

1.2.2 Von Hippel-Lindau (VHL)

Von Hippel-Lindau is characterised by a range of benign and malignant tumours affecting 1 in 36 000 individuals (31). Several different tumours form part of this disease namely renal cell carcinomas, PCC's, PGL's, pancreatic islet cell tumours, lymphatic sac tumours and hemangioblastomas. VHL can be clinically classified as individuals with PCC (type 1) and individuals without PCC (type 2). Type 2 individuals can further be classified as type 2a who have a low risk of developing renal cell carcinoma or type 2b with a high risk. Patients who only develop PCC without other tumours associated with VHL can be classified as type 2c (2, 32). Approximately 10 – 20% of individuals with VHL develop PCC with the mean age of presentation at ~30 years. It is estimated that 10-26% of VHL patients develop pheochromocytomas or paragangliomas with the risk varying between different families (4).

Multiple and bilateral PCCs and even extra-adrenal PGL may arise in individuals with VHL. Previous studies investigating patients with VHL associated PCC and PGL, showed that 90% of these patients had PCC whereas only 19% had PGL. Furthermore 44% of patients presented with bilateral PCCs and 3% with malignancies. In 30-50% of all patients with VHL, the first manifestation of this disease was the onset of PCC or PGL (4).

The VHL gene is a tumour suppressor gene consisting of three exons, 213 amino acids and is located on chromosome 3p25.3 (33). A number of different germ-line VHL mutations, inherited in an autosomal dominant manner, have been reported

(34, 35). Approximately two thirds of VHL patients carry missense, nonsense and splice-site mutations as well as deletions and insertions, whereas the remaining one third of VHL families carry large deletions. Missense mutations are more frequently identified in VHL families with pheochromocytomas and paragangliomas (34). Individuals with VHL develop pheochromocytomas more often than sympathetic - or parasympathetic PGL. Missense mutations at codon 167 are associated with a particularly high risk of PCC (over 80% by age 50) (36). Whole deletion or truncating VHL mutations are found quite often in renal carcinoma-related disease whereas it is very rare in PCC cases. It seems as if the chromaffin cells and their precursors cannot tolerate complete loss of the VHL protein in contrast to renal cells (37).

The VHL protein targets a range of proteins involved in tumourigenesis including the hypoxia-inducible factor-1 (HIF-1) and regulates the levels of these proteins by means of proteasomal degradation (38). The HIF-1 protein induces the transcription of mRNA which codes for erythropoietin and regulates vascular endothelial growth factor (VEGF), platelet-derived growth factor PDGF-beta and transforming growth factor TGF-alpha (39).

An absent or abnormal VHL protein will result in decreased proteasomal degradation of these growth factors and decreased erythropoietin mRNA transcription. This will result in an increase of angiogenic - and growth factors leading to a growth spurt and ultimately tumour formation.

1.2.3 Neurofibromatosis 1 (NF1)

Neurofibromatosis type 1 is the onset of a range of tumours that may develop throughout a patient's lifetime including gliomas, astrocytomas, soft-tissue sarcomas, chronic myeloid leukemias of childhood as well as PCC (2).Symptoms of this disease include, neurofibromas, cafe´ au lait patches, skinfold freckling, iris Lisch nodules, optic pathway gliomas, and bone dysplasia (40).

Patients with NF1 do not develop PCCs or PGLs very often with the incidence being roughly 0.1-5.7% in patients (4). The risk of NF1 patients developing only PCC is between 1 and 5% (1). In previous studies, 95% of all the patients with NF1 and PCC or PGL had PCC and 6% had sympathetic PGL (4). Fourteen percent of the patients displayed bilateral PCC and 9% malignant tumours. The mean age at diagnosis was 42 years of age. Malignant tumours are slightly more frequent in NF1 than in VHL or MEN2.

Mutations in the *NF1* gene, located on chromosome 17q11.2, lead to the onset of NF1. The discovery of this gene was made in 1990 (41).The *NF1* gene is a large gene consisting of 60 exons and encodes the protein neurofibromin, expressed mainly in the nervous system (40). Cell proliferation is suppressed by Neurofibromin through the activation of RAS which in turn inhibits the oncogenic signalling cascade RAS/RAF/MAPK (42, 43). *NF1* is inherited in an autosomal dominant manner although 30-50% of all mutations occur sporadically (44). The mutations identified in this gene include missense, nonsense and splice-site mutations as well as insertions, deletions and chromosomal rearrangements.

New *NF1* mutations also occur quite often with the rate being approximately 50%. All individuals with NF1 are heterozygous for the *NF1* mutation due to the fact that homozygosity is lethal to embryos (45). Mutation positive patients normally display one germline mutation and one acquired mutation thus altering both alleles which implies that *NF1* is a tumour suppressor gene (40, 46). The majority of *NF1* gene mutations result in neurofibromin truncation leading to decreased levels of this protein, increased cell proliferation and inhibition of apoptosis (2, 47). Specific *NF1* mutations causing an increased risk of PCC have not yet been identified due to the fact that patients are diagnosed based on clinical parameters.

1.2.4 Familial paraganglioma and pheochromocytoma

It is estimated that up to 24% of patients with apparent sporadic presentation of PCC actually have familial PCC (48). Four loci have originally been implicated in the pathogenesis of hereditary paraganglioma, namely PGL1, PGL2, PGL3 and PGL4. The *SDHD (PGL1), SDHC (PGL3)*, and *SDHB (PGL4)* genes encode subunits of the mitochondrial succinate-dehydrogenase (SDH) complex II, involved in the respiratory chain as well as the tricarboxylic acid cycle. The more recently identified *SDHAF2 (PGL2)* gene is responsible for the flavination of the SDHA subunit. Previously no genetic link between *SDHA* and paraganglioma could be established, but the first germline *SDHA* mutation associated with paraganglioma was identified in 2010 (49). An additional four genes have also been linked to PCC susceptibility, namely *TMEM127*, *MAX*, *KIF1B* and *PHD2*. Paraganglioma is inherited in an autosomal dominant manner, although the inheritance pattern of the *SDHD* and *SDHAF2* genes is suggestive of maternal imprinting (50).

An inherited predisposition to PGL/PCC should be suspected if the patient has a family history of the disease, tumours develop at a young age or multiple tumours are present. Another syndrome associated with *SDHB*, *SDHC* and *SDHD* mutation carriers is the Carney-Stratakis dyad in which patients develop PGLs/PCCs as well as gastrointestinal stromal tumours (GISTs) (51). This syndrome is inherited in an autosomal dominant manner with incomplete penetrance and a mean age of onset between 28 and 33 years (51, 52). A family history of GISTs should be recorded and regarded in patients with germline mutations in the succinate dehydrogenase (SDH) genes. It has also been shown that patients with mutations in the *SDHB* gene have an increased risk of developing renal cell carcinoma (RCC). Multiple genes are thus associated with hereditary paraganglioma and pheochromocytoma. The *SDHD* gene was first to be identified as a PGL susceptibility gene which established the link between neuroendocrine tumours and succinate dehydrogenase (SDH) (53).

1.2.4.1 The *SDHD* gene

The *SDHD* (PGL1) gene is located on chromosome 11q23 (53, 54), contains four exons extending over 19kb (55) and encodes 160 amino acids. It plays a role in anchoring the SDH complex and helps with ubiquinone binding. The inheritance pattern of *SDHD* is autosomal dominant, but also indicative of maternal imprinting. The first evidence of imprinted transmission of *SDHD* mutations was described in 1989 (56).This means that patients are only at risk of the disease if the mutation was inherited from the father. These individuals, however, still have a 50% chance of passing the mutation on to their children. Several studies have argued that *SDHD* cannot show maternal imprinting as it does not belong to a chromosomal region known to be involved in genomic imprinting (57). Although *SDHD* is not imprinted, the main cluster of imprinted genes of the human genome is located on the short arm of chromosome 11 at 11p15.5. There were two isolated cases in which PGL developed after maternal transmission of *SDHD* mutations.

A previous study (57) has reported the first occurrence of paraganglioma in which the *SDHD* mutated alleles were maternally transmitted. This led to speculation that the parent-of-origin effect might be caused by another mechanism than genomic imprinting. In a previous study, *SDHD* showed bi-allelic expression in brain -, kidney - and lymphoid tissues (53). The wild-type maternal allele is also lost in *SDHD*-linked tumours suggesting that the maternal *SDHD* allele is expressed in normal paraganglioma (58). Evidence thus suggests that an alternative mechanism than imprinting seems to be responsible for the parent-of-origin effect. Another study hypothesised that a somatic genetic mechanism (figure 1.3) in which the *SDHD* gene (11q23) and a hypothetical paternally imprinted tumour suppressor gene located within the region of 11p15.5 are targeted, is responsible for the exclusive paternal transmission of the disease (58). Only the combined loss of the wild-type *SDHD* allele and the maternal tumour suppressor allele located within the 11p15.5 region will initiate tumour formation. The hypothetical tumour suppressor gene would thus be active if the mutation is derived from the mother, but inactive if derived paternally. Loss of the entire maternal chromosome 11 in tumours will cause the non-imprinted *SDHD* allele

and the active tumour suppressor to be lost. This will lead to tumour formation. No tumour will be initiated if the mutation is transmitted maternally, leading to the loss of the paternal chromosome 11 and thus the tumour suppressor will stay active.It seems as though it is not imprinting of *SDHD* responsible for the exclusive paternal transmission of the disease but rather a somatic genetic mechanism which targets the *SDHD* gene and the paternally imprinted gene on 11p15.5 (58).

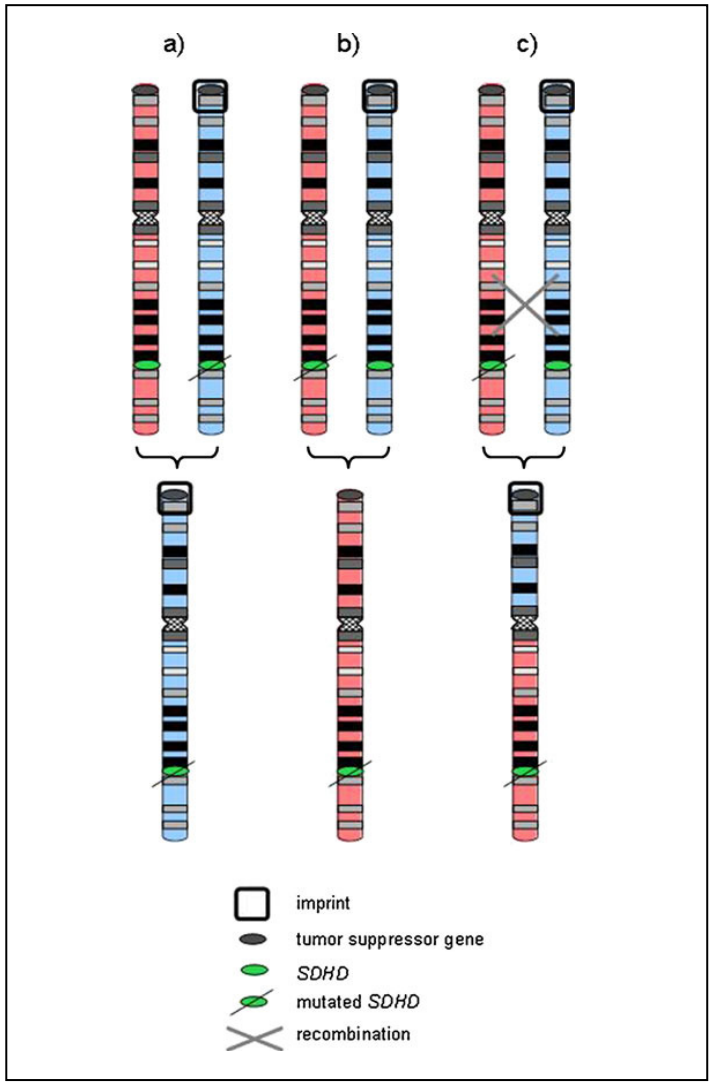

Figure 1.3: PGL1 tumour formation

Maternally derived chromosome is red and paternally derived is blue. (a) Paternal transmission of SDHD results in tumour formation due to loss of maternal copy. (b) Maternal transmission does not lead to tumour formation due to the active tumour suppressor gene. (c) Maternal transmission will only lead to tumour formation if a number of events occur including recombination between the homologous arms of chromosome 11. Figure from Müller et al,(59).

Two events will have to occur in order for a maternally inherited mutation to lead to tumour formation. Recombination between the long arms of maternal and paternal chromosome 11 has to occur and non-disjunction of the recombinant chromosome 11 should occur.

This will result in an allele with the inactivated hypothetical tumour suppressor gene and the maternally derived mutation in 11q. This model, however, inadequately explains why PGL develops frequently after transmission of a single mutation in the *SDHB* and *SDHC* genes which are located on chromosome 1. Mutations of *SDHB* and *SDHC* should thus also be practically non-penetrant due to the requirement for a third hit that targets the presumed imprinted gene on chromosome 11 (17).

Another model was proposed, assuming the partial inactivation of the maternally derived *SDHD* gene (59). In this model, residual SDH activity is present in cells with a paternally derived mutation due to the partial inactivation of the *SDHD* gene. Normal function of paraganglia cells is possible for a considerable length of time before the cells become hypoxic due to accumulation of reactive oxygen species (ROS) and succinate. Non-disjunction is favoured and the maternal chromosome 11 or parts of it gets lost leading to insufficient amounts of SDH and ultimately tumour formation. A maternally derived mutation will not lead to tumour formation because of sufficient SDH activity in the cells which ensures normal functioning. The level of SDH activity is very similar to the wild-type and thus non-disjunction as well as loss of the wild-type allele is not favoured. This model also allows for exceptions to the rule as it is possible that on rare occasions maternal inheritance of the mutation might lead to tumour formation. In this case, loss of heterozygosity is most probably a random event not favoured by hypoxia.

Although it is still unknown what exactly causes the seemingly imprinted transmission of *SDHD*, data suggests that maternal and paternal copies of *SDHD* are functionally unequal and thus indicative of a genomic imprinting inheritance pattern.

It is still suggested that individuals with the mutation, even if transmitted maternally, are at risk of paraganglioma and should be monitored in order to identify any possible tumours.

A database specifically for the purpose of reporting variations in the SDH-genes has been created (60, 61). The total number of *SDHD* unique variants reported on the database is 131 (figure 1.4). The mutations identified include Missense, frameshift, nonsense and splicing variants as well as large deletions. Most of the mutations are frameshift mutations followed by missense - and nonsense mutations and only a small proportion of the mutations are accounted for by large deletions. No large duplications have been identified to date. A summary of all the unique variations reported in *SDHD* is shown in the pie chart below.

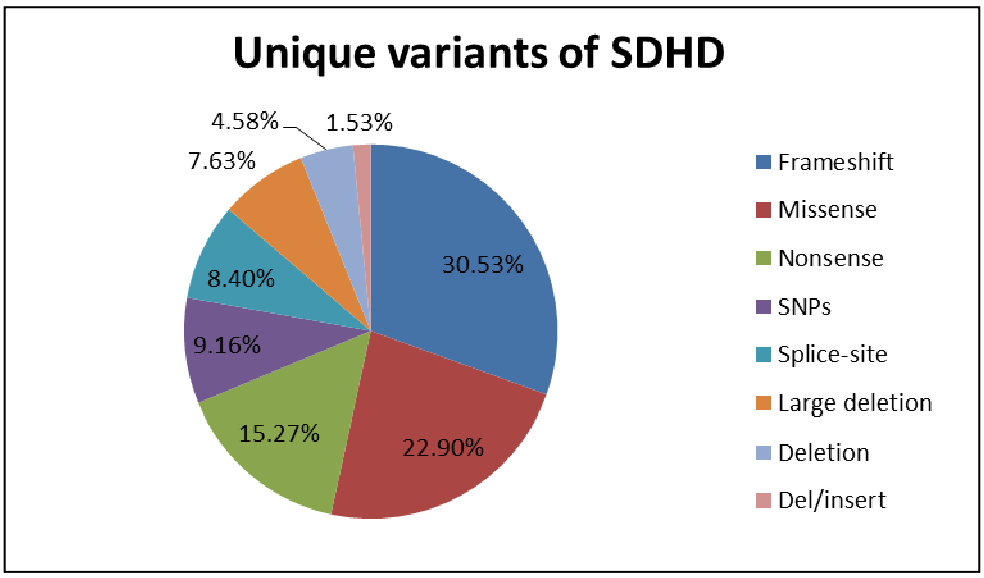

Figure 1.4: Distribution of unique variants reported in *SDHD*
Adapted from Pasini & Stratakis 2009 (50)

Tumours usually present in the head and neck but may also arise in the abdomen or thorax as PGL or PCC. These individuals should also be evaluated for multifocal tumours, malignancies, and extra-adrenal paraganglioma (62).

The PCC/PGL penetrance for *SDHD* mutation carriers is 90% or even higher by the age of 70 years (63, 64). The risk of developing a head-and-neck tumour by age 60 is 71% and the mean age is 40 years.
Furthermore, the risk of developing an extra-adrenal PGL by age 60 is 29% with the mean age of onset 21 years (64). Pheochromocytomas may develop but is mostly one of multiple tumours throughout the body. The risk of malignancy for *SDHD* mutation carriers is <5% (65).

1.2.4.2 The *SDHAF2* gene

The *SDHAF2* gene (succinate dehydrogenase assembly factor II), formerly known as *SDH5,* was shown to be associated with the PGL2 locus on chromosome 11q13.1(66).
This gene contains 4 exons and is a cofactor of flavin adenine dinucleotide (FAD) which plays a role in the flavination of SDHA and is crucial for the succinate dehydrogenase complex to function correctly (66). A mutation of *SDHAF2* will lead to decreased SDH activity and stability of the enzyme complex (67).The disease phenotype expressed in *SDHAF2*-linked families is consistent with maternal imprinting (66-68).

Even before the *SDHAF2* gene was linked to the PGL2 locus, a study showed that the inheritance pattern of PGL2 is similar to that of the *SDHD* gene (68). The same mechanism is most probably involved in the inheritance of the *SDHD* and *SDHAF2* genes. Mutations of *SDHAF2* appear to be very rare and cause mainly head and neck PGL (67). Development of pheochromocytoma or sympathetic PGL of the abdomen and thorax have not yet been linked to mutations in this gene. Only missense and nonsense point mutations were identified in the *SDHAF2* gene and no large rearrangements have been identified to date (figure 1.5). Most of the mutations reported in *SDHAF2* were identified in the Dutch and were predicted to be founder mutations, but variants have also been reported in the Spanish and Italian populations. Only four unique variants have been reported

in this gene of which three are missense mutations and one is a nonsense mutation.

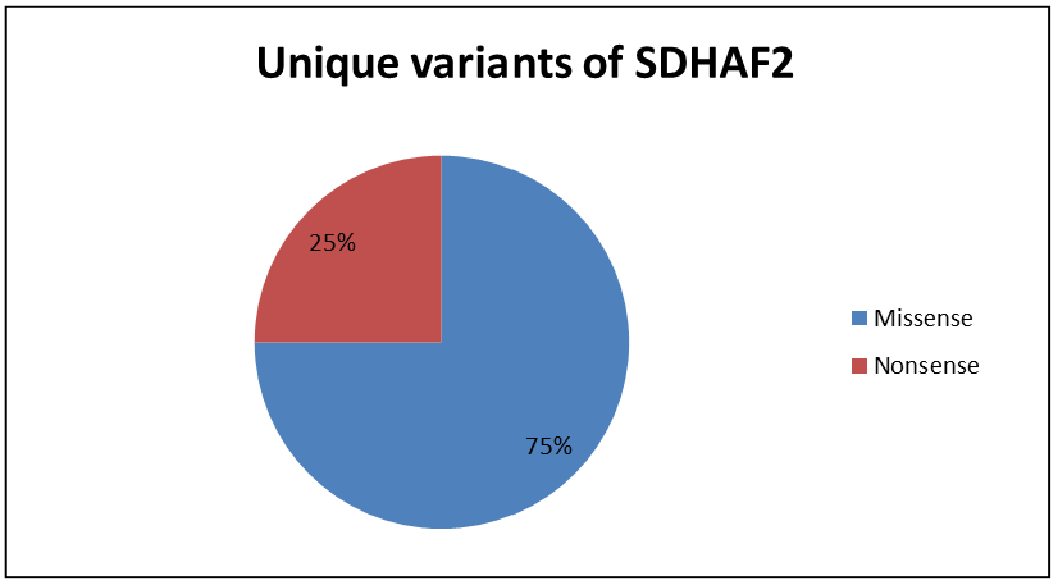

Figure 1.5: Distribution of unique variants reported in *SDHAF2*
Adapted from Pasini & Stratakis 2009 (50)

1.2.4.3 The *SDHC* gene

Mutations in the *SDHC* gene are the underlying cause of PGL 3. The *SDHC* gene is located on chromosome 1q21, spans 50.3kb and contains 6 exons (69, 70). It plays an important role in ubiquinone binding and anchoring the SDH complex in the mitochondrial membrane (70). This gene follows an autosomal dominant inheritance pattern and is not associated with imprinting. Mutations of *SDHC* are less common than *SDHB* and *SDHD* gene mutations. There are 47 unique variants reported on the database of which most are point mutations as shown on the pie chart in figure 1.6 (60, 61).

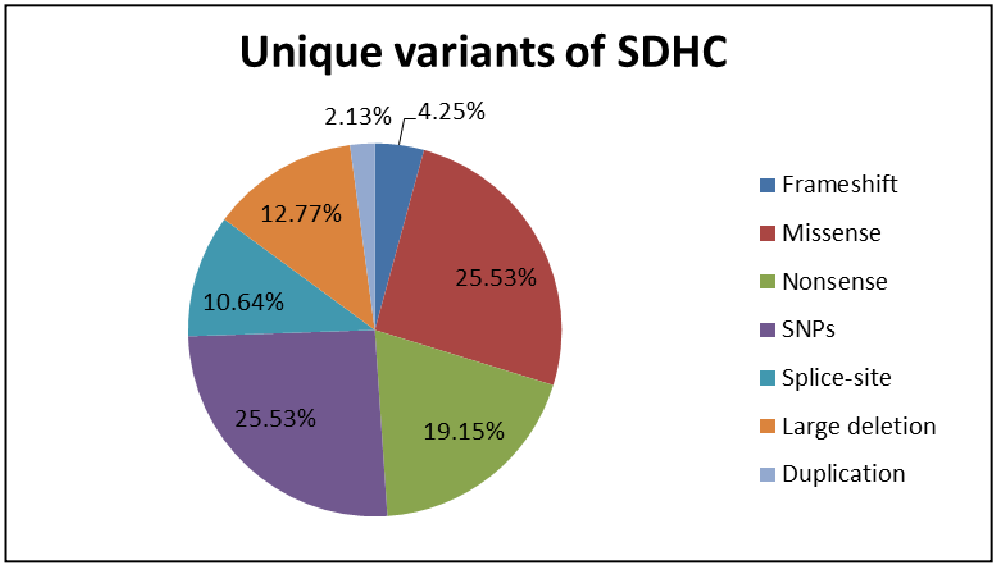

Figure 1.6: Distribution of unique variants reported in *SDHC*
Adapted from Pasini & Stratakis 2009 (50)

Large deletions account for only 12.77% of all the mutations reported on the database and no large duplications were reported in this gene.

Mutations of *SDHC* were exclusively associated with head and neck tumours up until 2007. The first case of a patient who developed pheochromocytoma and carried an *SDHC* mutation was reported (71). Another report of an *SDHC* mutation identified in a patient presenting with PCC followed (72). These findings demonstrate that *SDHC* mutation carriers may have a more diverse clinical presentation than previously expected. Non-secreting head-and-neck tumours may arise more often than PCC or functional extra-adrenal PGL, but tumours may still develop in other areas such as the adrenal medulla. One case of malignancy associated with an *SDHC* mutation have been reported (73).

1.2.4.4 The *SDHB* gene

The *SDHB* gene (PGL4) spans 35.4kb, contains 8 exons (74) and is located on chromosome 1p36.1-p35 (75). This gene encodes the iron sulphur protein of the succinate dehydrogenase enzyme complex II of the respiratory chain. Mutations of *SDHB* are inherited in an autosomal dominant manner with no imprinting

involved. Mutations of *SDHB* are very common (50) and a total of 218 unique variants have been reported on the SDH database (60, 61) (figure 1.7).

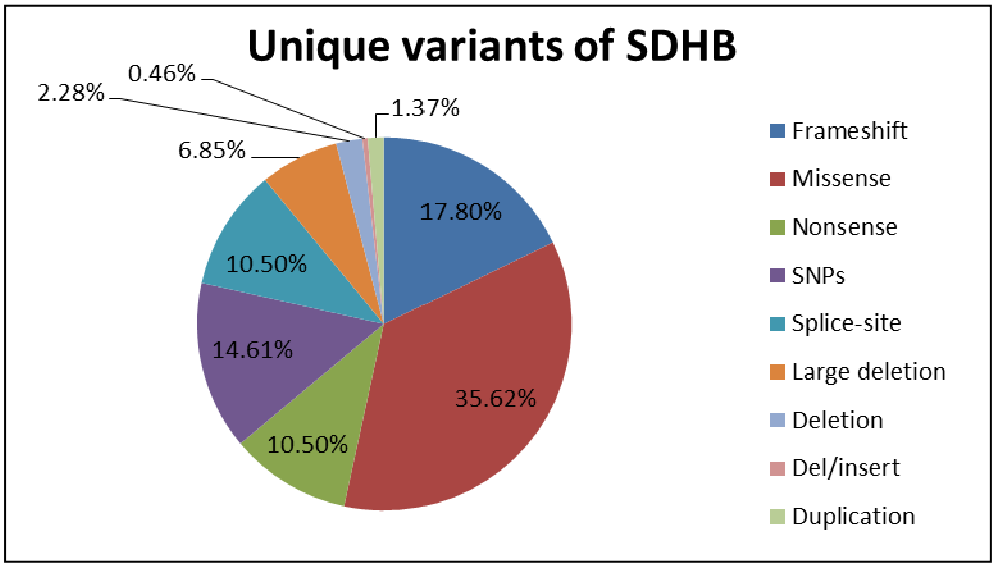

Figure 1.7: Distribution of unique variants reported in *SDHB*
Adapted from Pasini & Stratakis 2009 (50)

The most predominant phenotypes associated with mutations of *SDHB* are abdominal, pelvic and thoracic secreting PGL (63). It was reported that patients with *SDHB* gene mutations are expected to have a decreased life expectancy in comparison to other mutation carriers (62). Studies show that malignant tumours are more often associated with patients carrying *SDHB* mutations than patients carrying mutations in any of the other *SDH* genes (50). It is estimated that half of the patients presenting with malignant PGL are carriers of an *SDHB* mutation with a penetrance of 45% by the age of 40 (76). Malignant PGL tumours were identified in 41% of *SDHB* mutation carriers in previous studies (50). These patients also have an increased risk of developing kidney cancers.
In a previous study the risk of developing kidney cancer was estimated to be as high as 14% by the age of 70 (64). It is thus advised that patients presenting with an *SDHB* mutation undergo a full-body screening to identify any tumours.

Age related penetrance for *SDHB* mutation carriers is predicted to be 29% by age 30 rising to 45% by age 40 and 80% - 100% penetrance by age 70 (62). The risk for malignancy range between 31% and 71% although it is difficult to determine

the absolute risk of malignancy due to the fact that metastasis can occur up to 20 years after diagnosis of the primary tumour (65).

1.2.4.5 The *SDHA* gene

This gene is located on chromosome 5p15 and consists of 15 exons. It encodes the flavoprotein subunit of the SDH complex II and contains a covalently attached flavine adenine dinucleotide (FAD) cofactor. Although the *SDHA* gene was never shown to be involved in the formation of paraganglioma, the first *SDHA* germline mutation associated with PGL have been identified (49). This gene was identified as a tumour suppressor gene associated with paraganglioma after a heterozygous germline mutation was identified in a patient who suffered from abdominal catecholamine-secreting paraganglioma (49). This mutation, leading to structural alteration of the protein, was not identified in any of the control samples tested. Individuals with homozygote germline mutations of *SDHA* develop Leigh syndrome which is an early onset encephalopathy (77). Two more *SDHA* mutations were identified in SDHA-negative tumours and germline DNA of patients which also show loss of the wild-type allele (78). These mutations leading to a protein truncation was also identified in healthy control individuals at a very low frequency. This suggests low penetrance of PGL in patients with *SDHA* mutations and most healthy individuals with *SDHA* mutations will thus most likely not develop the disease (78).

All of the *SDHA* mutation carriers in this study lacked a family history of PGL. Considering current results there also seems to be no specific phenotype associated with *SDHA* mutation carriers. Mutations in the *SDHA* gene are not common, however, they can occur and patients with mutations in this gene may develop PGL. Mutations in the susceptibility genes, MAX, TMEM127 as well as KIF1B/PHD, are also very rare, but should not be ruled out as mutations in these genes might also lead to tumour formation.

1.2.4.6 The *MAX* gene

Germline mutations of *MAX* were identified and segregate with the disease in families with pheochromocytoma. This led to the identification of *MAX* (MYC-associated factor X) as a pheochromocytoma susceptibility gene (79). It is still unclear exactly how *MAX* mutations contribute to the formation of pheochromocytoma. This gene consists of five exons and is located on chromosome 14q23.3. It encodes the transcription factor MAX which belongs to the helix-loop-helix family and plays an important role in the regulation of cell proliferation, - differentiation and - death (80).

A recent study concluded that mutations in *MAX* are responsible for 1.12% of PCC in patients with no mutations in other susceptibility genes (81). All patients identified with a mutation in the *MAX* gene presented with pheochromocytomas. The inheritance pattern of MAX gene mutations seem to be similar to that of *SDHD* and *SDHAF2*. This is due to the paternal origin of the mutated allele in investigated cases as well as the fact that individuals who inherited the mutant allele from their mother did not develop pheochromocytoma (79).

In a previous study 12 patients with PCC were identified with *MAX* mutations of whom eight presented with bilateral PCC with the mean age at presentation being 32 years (79). The mutations identified were distributed along the gene but especially prevalent in exons 3 and 4. Most of the mutations lead to protein truncation and one mutation (c.97C>T) was identified as the first hot spot mutation of *MAX* (79, 81). It was also suggested that *MAX* mutations are associated with a higher risk of malignancy due to the fact that 25% of the patients showed metastasis at diagnosis (82).

1.2.4.7 The *TMEM127* gene

The *TMEM127* gene, located on chromosome 2q11, was identified as a pheochromocytoma susceptibility gene (83) and functions as a tumour suppressor gene. This gene encodes a 238 amino acid transmembrane protein associated

with kinase receptor signals and is a negative regulator of mTOR which promotes cell growth and protein translation. A very critical cell proliferation and cell death signalling pathway is thus linked to the development of pheochromocytoma. Mutations of *TMEM127* were identified in 30% of familial tumours and 3% sporadic PCCs in a cohort of 103 samples (83).

The only phenotype previously associated with mutations in this gene was pheochromocytoma, however, germline mutations in *TMEM127* were identified in patients with paraganglioma of the head and neck and extra-adrenal abdominal sites (84). Only a fourth of the patients with *TMEM127* mutations presented with a clear family history of PCC/PGL thus suggesting incomplete penetrance. The penetrance in one family was 64% by the age of 55 (85). In total, 23 patients were reported and all but one (96%) had PCC including 39% who had bilateral PCC. Two patients presented with PGLs of which one also had bilateral PCC. The mean age of presentation is 43 years of age (86).

Mutations were detected in all three of the coding exons of this gene but no large gene deletions or duplications have been identified. Malignancy is very rare in patients with *TMEM127* mutations and unilateral as well as bilateral tumours may arise (2).

1.2.4.8 The *KIF1B* and *PHD2/EGLN1* genes

Mutations in two genes, *KIF1B* and *PHD2*, have been identified and seem to be associated with the development of PGL and PCC. The *KIF1B* gene is very large with approximately 50 exons and is located on chromosome 1p36.22 (87). Two splice variants are involved namely, KIF1Bα and KIF1Bβ. The *KIF1B* gene functions as a tumour suppressor necessary for neuronal apoptosis. Two different *KIF1Bβ* mutations were identified in patients presenting with PCC and no mutations in any of the other PGL/PCC susceptibility genes. Three other mutations were also identified in patients with neuroblastoma (87). No other cases of patients with PCC and *KIF1B* mutations have been reported and no patients with PGL have been identified who carry mutations in this gene.

The *PHD2* gene, also known as *EGLN1,* contains 5 exons, is located on chromosome 1q42.1 and encodes the Prolyl hydroxylase domain protein 2 (88). There are 3 PHD proteins (PHD1, PHD2, PHD3) encoded by three different genes that play a role in the regulation of hypoxia inducible factor (HIF) which is involved in angiogenesis, erythropoiesis, cell metabolism and proliferation. This gene seems to function as an oxygen sensor.

Mutations in *PHD2* have previously been reported in patients with erythrocytosis, but were not associated with tumours. A germline mutation was identified in a patient with isolated erythrocytosis and recurrent para-aortic PGL (88). No tumours have been identified in any of the relatives of this patient and there has not been a description of any syndrome yet. Very stringent follow-up of *PHD2* mutation carriers is recommended as they may have an abnormally elevated risk of PGL (88).

Mutations in the *KIF1B* and *PHD* genes may lead to the development of PGL and PCC, but are not a frequent cause of familial PGL. Although mutations in the MAX, TMEM127 and *KIF1B/PHD* genes may lead to the onset of PGL and PCC, mutations in the *SDHA*, *SDHB*, *SDHC* and *SDHD* genes are the most frequent cause of familial paraganglioma. These four genes are also the only susceptibility genes encoding subunits of the mitochondrial succinate-dehydrogenase (SDH) complex II of the electron transport chain.

1.3 ELECTRON TRANSPORT CHAIN

Five protein complexes together form the electron transport chain responsible for electron transport along the affinity gravity chain (figure 1.8). This assists in transferring hydrogen ions from the mitochondrial matrix to the intermembrane space.

The first complex (NADH dehydrogenase) donates electrons to the chain after NADH was accepted by the citric acid cycle. The second complex (Succinate

Dehydrogenase) accepts electrons from FADH2 and transports them to complex III (cytochrome c reductase) via ubiquinone.
Electrons are then transported to complex IV (cytochrome-c oxidase) via cytochrome c. Lastly Adenosine diphosphate (ADP) is converted to Adenosine triphosphate (ATP) by complex V (ATP synthase) (89).

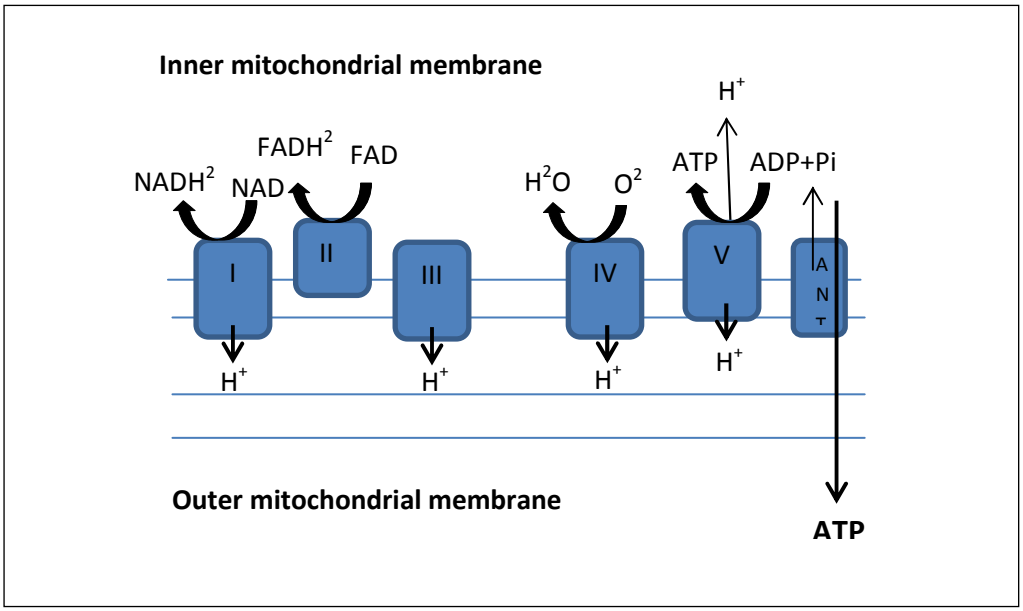

Figure 1.8: Five complexes (I,II,III,IV,V) of the ATP producing Electron Transport chain
ANT = Adenine nucleotide translocator. Image redrawn and adapted (89).

SDH is the only complex that cannot transfer hydrogen ions through the inner membrane into the intermembrane space and is the only non-transmembrane complex. It is, however, the only member of the chain that participates actively in the tricarboxylic acid cycle (8). Electrons are transferred from the tricarboxylic acid cycle to the terminal acceptor ubiquinone which plays a role in the prevention of reactive oxygen species (90).

The tricarboxylic acid cycle together with the electron transport chain thus produces ATP which is the source of energy for most biological processes. Inactivation of any of the SDH-proteins will lead to insufficient production of ATP and thus loss of energy production (91).

1.3.1 Normal physiology of the SDH complex II

SDHA, *-B*, *-C* and *-D* genes encode subunits of the heterotetrameric succinate dehydrogenase (SDH) mitochondrial complex II, a component of the respiratory chain and the tricarboxylic acid cycle illustrated below (figure 1.9). The SDH enzyme is responsible for the oxidation of succinate to fumarate in the tricarboxylic acid cycle and transfers electrons to coenzyme Q in the electron transport chain (90). The four subunits that form the SDH complex (SDHA, SDHB, SDHC and SDHD) are encoded by nuclear genes.

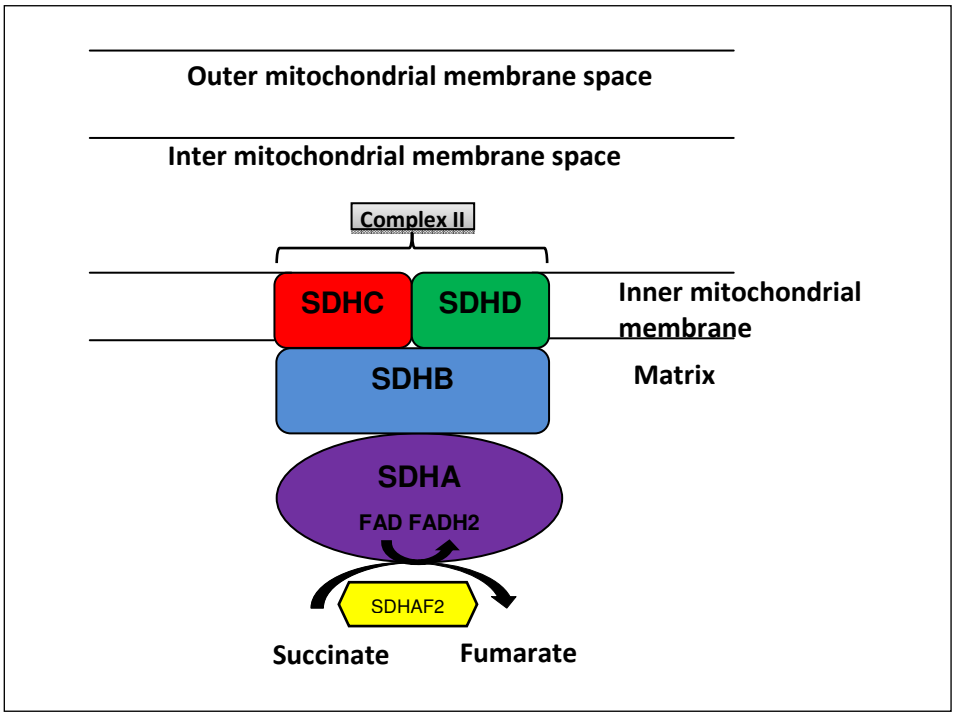

Figure 1.9: Subunits of the SDH complex II
The four subunits together form the SDH complex II and SDHAF2 interacts in order for the flavination of SDHA to occur. Image redrawn and adapted (92).

Subunits SDHC and SDHD are hydrophobic and serve as membrane anchors and a ubiquinone binding site. The catalytic domains of the complex are formed by the hydrophilic SDHA and SDHB subunits (8). The SDHA unit is a flavoprotein serving as a substrate binding site and the SDHB subunit is an iron-sulphur protein of complex II.

The SDHAF2 subunit is required for the flavination of SDHA and the stabilisation of the entire succinate dehydrogenase (SDH) complex II (67). All of the subunits are thus needed to ensure correct functioning of the complex and also in return the electron transport chain. A mutation in any of the SDH genes will lead to the inactivation of the SDH-proteins.

1.3.2 Biological effect of mutations

A mutation in any of the susceptibility genes leading to protein truncation will impair succinate-ubiquinone activity (figure 1.10). This will cause accumulation of succinate as the oxidation reaction of succinate to fumarate cannot occur. The increased succinate levels will inhibit the prolyl hydroxylase (PHD) from hydroxylating HIF1 which will lead to the stabilisation of Hypoxia inducible factors (HIFs).

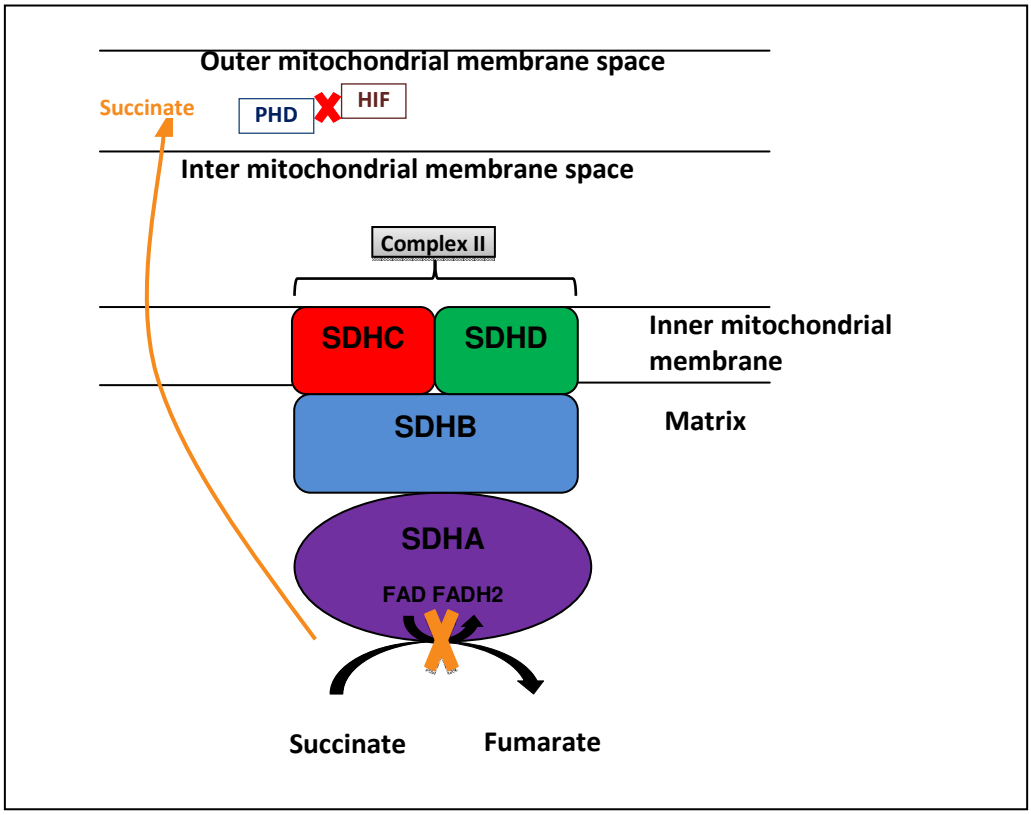

Figure 1.10: Effect of a mutation in any of the subunit genes on the complex
Image redrawn and adapted (92).

Hypoxia inducible factors are transcription factors which bind to specific DNA sequences and activate a number of genes which in turn promote adaptation and survival under conditions where oxygen is limited (4). Stabilisation of HIFs will thus lead to an up-regulation of factors responsible for angiogenesis, growth and cell division which may cause tumour formation. These factors include vasoactive endothelial growth factor (VEGF) and reactive oxygen species (ROS) responsible for cell division. Mutations in the SDH susceptibility genes can thus cause the onset of fatal tumours depending on the localization. These tumours may be fatal due to the impairment of cranial nerves and they may also become malignant. Identification of mutations in patients with PGL/PCC is thus very important for early diagnosis and treatment of patients and predictive screening of their family members.

1.4 MOLECULAR GENETIC SCREENING

Routine genetic tests were developed after the identification of susceptibility genes for paraganglioma/pheochromocytoma. Approximately 24-32% of patients with apparent sporadic PGL/PCC have, in fact, inherited a mutation in either of the susceptibility genes (93). Certain clinical predictors are associated with a higher probability of mutations. Patients with multiple or malignant tumours, pheochromocytoma, presenting with tumours at a young age or with a family history of the disease may be given priority for genetic screening (93). Mutation screening of all the PGL/PCC susceptibility genes are strongly advised especially in patients with the above-mentioned clinical predictors of the disease.

It is very important to identify any genetic predisposition to PCC/PGL in order to recognise at risk individuals before the onset of clinical manifestations which may prevent morbidity and mortality (82, 93). It has been shown that there is a correlation between genotype and phenotype due to extensive genetic screening in PGLs and thus a genetic testing algorithm was developed, based on clinical features of PGL/PCCs (figure 1.11). This allows for rapid and more cost-effective genetic screening (9). Algorithms are used in order to identify potential mutation

carriers and prioritize the order of genes to be screened based on clinical features (94). The algorithm shown here also illustrates how one should go about the genetic screening of patients with PGL and PCC according to clinical features and predictors.

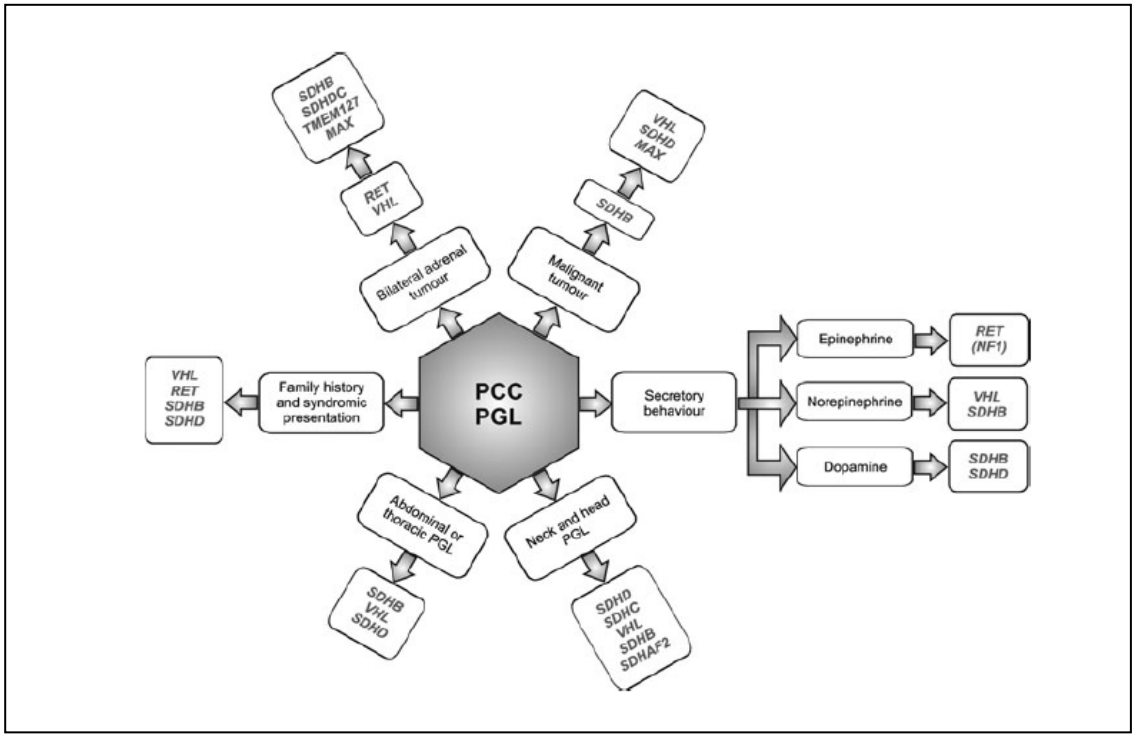

Figure 1.11: Algorithm for genetic testing for pheochromocytoma and paraganglioma susceptibility genes Figure sourced from Galan et al. (2)

Genetic testing is recommended for patients with early onset of disease (age <45), but should not be restricted to them only. NF1 is usually diagnosed on the basis of clinical features and genetic testing is only done in rare cases due to the large size of the NF1 gene. Mutations in SDHA, TMEM127 and MAX genes are still very rare and should only be analyzed if no mutations were identified in the other susceptibility genes.

Most mutations identified thus far in the PGL/PCC susceptibility genes are point mutations such as missense and nonsense mutations. It is thus advised to screen for point mutations in the susceptibility genes first, although newer technology has allowed the identification of large rearrangements in these genes.

Multiplex ligation-dependent probe amplification (MLPA) kits were developed by MRC Holland specifically for the identification of large rearrangements in the SDH and VHL genes.

The discovery of new PCC/PGL susceptibility genes and the improvement of molecular biology tools specifically for the identification of large genomic rearrangements led to the implementation of routine genetic tests for PGL/PCC patients. These tests should be accompanied by genetic counselling for PGL/PCC patients as well as familial genetic counselling specifically adjusted to their genetic risk (94).

Identification of genetic mutations in patients with PCC or PGL is crucial for early diagnosis and treatment, regular surveillance and thus a better prognosis for patients as well as their family members.

1.5 FAMILIAL PARAGANGLIOMA IN SOUTH AFRICA

DNA from eleven South African Afrikaner PGL families have been collected previously and were investigated for disease-causing point mutations in the *SDHB*, *SDHC* and *SDHD* genes. Only one family carried a pathogenic *SDHB* mutation in exon 4 namely, p.Ile127Ser. No other mutations were identified in any of the other individuals. These individuals presented predominantly with head-and-neck tumours with three families presenting with abdominal PGL. Five of the families had malignant tumours.

No other methods have been carried out to possibly identify large rearrangements in these genes or point mutations in the *SDHAF2* gene. Large rearrangements attribute ~10% of all disease-causing PGL/PCC mutations, but as the screening methods to identify large deletions and duplications are improving, this figure may increase in the future.

It is thus feasible in this case to screen for large rearrangements in the SDH genes after point mutations in all SDH genes have been ruled out.

Ultimately, detection of disease-causing mutations will enable predictive testing of other family members and allow better clinical management of these families.

To date no study has been carried out to identify possible disease-causing mutations in South African individuals with PGL.

1.6 AFRIKANER ANCESTRY

The Afrikaners are mainly descendants of Dutch, German and to a lesser extent, French immigrants to the Cape colony during the 17th century. By 1701 the population numbered 1265 (95). The offspring of these settlers remained genetically isolated due to very little further immigration and underwent rapid population increase. Thus the Afrikaner is prone to show founder effects for a number of genetic disorders (96).

1.7 AIM

The aim of this study was to identify point mutations in the *SDHAF2* gene of South African paraganglioma patients without point mutations in the *SDHB*, *-C* and *–D* genes. Furthermore to identify large rearrangements in *SDHAF2, SDHB, -C* and *-D* genes of these families without point mutations.

1.8 OBJECTIVES

- Screen the *SDHAF2* gene of 16 South African PGL patients for point mutations using PCR and bi-directional cycle sequencing
- Screen for large rearrangements in the *SDHB*, *-C*, *-D* and *SDHAF2* genes using Multiplex Ligation-dependent Probe Amplification (MLPA)
- Characterise breakpoints of large deletion/duplication if identified by the use of long-range PCR and direct cycle sequencing

Chapter 2: Materials and Methods

2.1 PATIENTS

Ethics approval for the molecular study of familial paraganglioma was obtained in 2001 (protocol number: 91/2001). Blood samples were subsequently collected after informed consent was obtained. The current study protocol was approved by the Faculty of Health Sciences, student Research Ethics Committee (protocol number: S53/2012). Sixteen patients from 10 families were included in this study. Complete pedigrees and clinical information are included in appendix B. These patients had previously been investigated for point mutations in the *SDHB*, *SDHC* and *SDHD* genes and no mutations were identified. These patients are affected with paraganglioma and/or have a family history of the disease. Fifty one sporadic PGL patients were also included in this study. Most patients were referred by private Otolaryngologist practitioners.

2.2 EXTRACTION OF GENOMIC DNA

DNA extraction was carried out by Mrs. M de la Rey as previously described (97) with a few modifications. Blood from an EDTA vacutainer was lysed with lysis buffer (0.32M sucrose; 10mM Tris-HCl, pH8; 5mM MgCl2; 1% Triton X-100) on ice for 10min.The solution was centrifuged at 8 120*g* for 30min at 4^0C (Beckman model J2-21M centrifuge, JA-17 rotor). The pellet was resuspended in suspension buffer (10mM Tris-HCl, pH8; 0.15M NaCl; 5M EDTA) followed by protein degradation by addition of 10%SDS and freshly prepared 5M sodium perchlorate ($NaClO_4$). An equal volume of chloroform:isoamylalcohol (24:1) was also added and mixed on a rotating platform for 30min. After centrifugation for 15 minutes at 20°C and 330*g*, the aqueous phase containing the DNA was removed and chloroform:isoamylalcohol (24:1) was added for a second time to remove proteins. DNA was precipitated from the aqueous phase with 2 volumes of cold absolute ethanol, spooled onto a sterile glass rod, dried under vacuum and resuspended in Tris-EDTA buffer (10mM Tris-HCl, pH8; 1mM EDTA). Sample DNA concentrations (ng/µl) were obtained by determining the absorbance at 260nm (Nanodrop ND-

1000 Spectrophotometer). The ratio of absorbance at 260 and 280nm was used to assess the purity of the DNA and the ratio of absorbance at 260 and 230nm is a secondary measure of nucleic acid purity.

2.3 SCREEN FOR POINT MUTATIONS IN THE *SDHAF2* GENE

2.3.1 Polymerase chain reaction (PCR)

The entire coding region and exon-intron boundaries of the *SDHAF2* gene were screened to identify germline point mutations. All four coding exons of the *SDHAF2* gene were amplified by using 50ng of each patient's DNA together with 0.2µM of each primer, 250µM of each dNTP and 0.5 Units Taq polymerase (Invitrogen by Life Technologies). The total reaction volume was 20µl. Primers used for the PCR are given in table 2.1. The PCR programme was as follows: 94°C for 3min then 30 cycles of 94°C – 1min, 1 minute at the annealing temperature of specific primer pair and 72°C - 1min. This was followed by 72°C for 7 minutes and lastly 6°C for 10min. The success of amplification was determined by gel electrophoresis using a 1.6% (50ml) Agarose gel (Gibco BRL, Life Technologies) with SYBR® safe DNA gel stain (Invitrogen by Life Technologies) subject to electrophoresis for 30min at 80V. Recipes for TBE Buffer, agarose loading buffer and Agarose gel are included in Appendix C.

Table 2.1: *SDHAF2* primer sequences

Primer	Sequence (5'-3')	Product Size
1F 1R	ACCTTCCGGCTCAGCTC TATCGGGCAGACGAACTC	242bp
2F 2R	GTTGACCTTCCCAGGCTC GAGGTTCAGCTGCTTTTCTG	786bp
3F 3R	GACACAGCCTTCTCAACCTC CTCAAATCAGCCTAAACTGTCC	215bp
4AF 4AR	CCCTGGTATAGGCTAACATCG TGAGTACACTTGGGCTGAGG	663bp
4BF 4BR	AGCTCTGAGCCTCAAAAGTG GAAGACTGTAGGAATGAGGGG	614bp

2.3.2 Nucleic acid purification

The PCR products were purified using Sureclean (Bioline). An equal amount of SureClean and PCR product was added and mixed together, incubated at room temperature for 10 minutes and centrifuged at 14 000g for 15 minutes (room temperature). The upper phase was removed and discarded before 70% EtOH (2x the original sample volume) was added and mixed on a vortex for 10 seconds. The centrifugation step was repeated (14 000g for 15 min) and the supernatant removed. The pellet was dried in a speed vacuum for 5 minutes, thereafter ddH_2O was added and the samples were ready for cycle sequencing.

2.3.3 Cycle Sequencing

Samples were sequenced at a concentration of 20µM each together with BigDye v.3.1 dilution. Both forward and reverse primers of each exon were used for sequencing and the sequencing temperature of each reaction was determined by each primer's specific annealing temperature.

A Veriti PCR machine (Applied Biosystems, Life Technologies, Foster city, USA) was used for amplification: 96°C-10sec, annealing temperature of each primer -5sec and 60°C -5min repeated for 25 cycles and cycle sequencing was ended with 4°C for 5min. Next, the DNA was precipitated by adding precipitation mix to the sequenced product. The final concentrations in the mix were 0.09M Sodium Acetate and 65% EtOH. Samples were incubated in the dark for 16 minutes and centrifuged at 14 000 rpm for 10 minutes, thereafter the supernatant was discarded and pellet washed with 70% EtOH.

This step was repeated twice before the pellet was dried, dissolved in Hi-Di™ formamide (Life Technologies, Foster city, USA), heated for 2 minutes at 96°C and cooled on ice. Sequencing analysis was performed on an ABI 3130 genetic analyser using POP7 polymer (Life Technologies, Foster city, USA). Sequencing traces were analysed using SeqScape v2.5 software (Life Technologies, Foster City,United States of America).

2.4 SCREEN FOR LARGE GENOMIC REARRANGEMENTS IN THE *SDHB,-C* AND *SDHD* GENES

2.4.1 MLPA analysis

Multiplex ligation-dependent probe amplification (MLPA) is a technique used for relative quantification of different DNA sequences in a single reaction. Specific SALSA MLPA kits (MRC Holland) are designed to detect deletions/duplications of one or more sequence(s) in the specific genes in a DNA sample. Probes added to the samples are amplified and quantified and not the nucleic acids itself. Probe target sequences must thus be present in the sample to ensure amplification of the probes by PCR. If one or more probe target sequence(s) are absent in the sample, no PCR amplification will occur. In case of a heterozygous deletion of target sequences, a 35 – 50% reduced relative peak area of the amplification product of that probe should be seen. The MLPA probes consist of 2 oligonucleotides (figure 2.1), one short synthetic and one long oligonucleotide, each with a primer sequence as well as a sequence complimentary to the target.

These probes hybridise to adjacent sites of the target sequence and are ligated to permit amplification (figure 2.2). Only one primer pair is needed for amplification of all ligated probes due to the fact that they all have identical end sequences. The amplification product of each probe is of a unique size, varying between 130nt and 480nt. The effective size separation of products by electrophoresis is due to a stuffer sequence on the 3' end of the long oligonucleotide which differs in length in each probe.

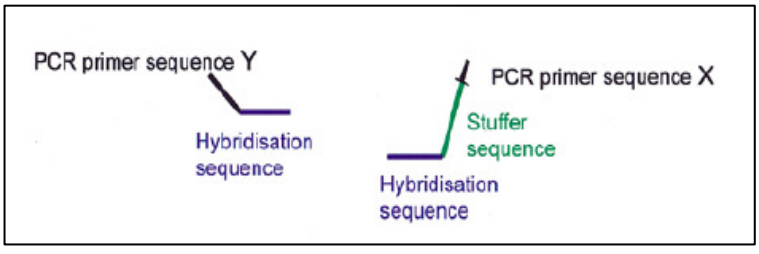

Figure 2.1: MLPA Probes. Each oligonucleotide consists of a primer sequence and hybridisation sequence complimentary to the target. A stuffer sequence is also present on each long oligonucleotide.

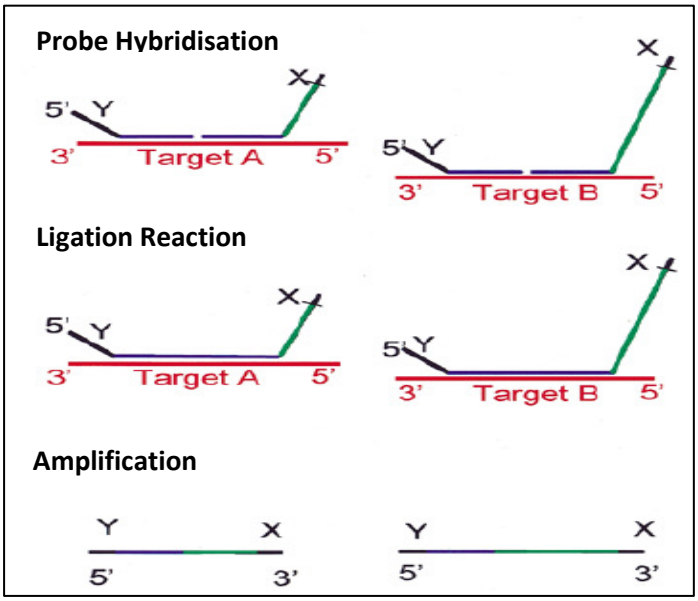

Figure 2.2: MLPA Reaction. After DNA is denatured, probes are hybridised to target sequences and ligated together before amplification with PCR.

The Salsa P226-B1 SDHB-SDHC-SDHD MLPA kit lot# 1209 (MRC Holland) was used to screen for large rearrangements. This kit contains probes for all exons of *SDHB*,-*C* and -*D* as well as probes located in the promoter region of each gene. Furthermore one probe for the *SDHAF1* gene and 3 probes for the *SDHAF2* gene are included. In total, there are 37 MLPA probes which include 10 reference probes located on different chromosomes. All probe sequences as well as ligation sites of the probes included in this kit are given in appendix E.

In addition, there are nine control fragments generating amplification products below 120nt. The control fragments include 4 quantity control fragments (Q-fragments) at 64-70-76-82nt, three DNA denaturation control fragments (D-fragments) at 88-92-96nt, one fragment recognising the X-chromosome (100nt) and one fragment recognising the Y-chromosome (105nt).

DNA was first denatured and probes hybridised to the matching sequences before the probes were ligated together and finally amplified by PCR. All reagents were used according to the manufacturer's instructions as stated in the MLPA protocol.

2.4.1.1 Hybridisation and ligation of oligonucleotides

Genomic DNA (125ng) of the 16 patients together with five unaffected individuals (negative controls) was used for MLPA analysis. The DNA was denatured for 5min at 95°C followed by oligonucleotide hybridisation. MLPA buffer and SALSA probe mix was added to the denatured DNA and heated to 95°C for 1 minute. Hybridisation followed at 60°C for 16 hours. Ligation reaction mix was added to the hybridised product whilst at 54°C. Probe ligation was allowed for 15 minutes at 54°C followed by inactivation of the Ligase-65 enzyme for 5 minutes at 98°C. All the probe sequences together with a table listing all the MLPA kit components and compositions thereof are given in appendix E.

2.4.1.2 Polymerase Chain Reaction (PCR)

The PCR buffer mix was first added to tubes before ligation mix was added. While at 60°C, the polymerase mix was added to tubes. PCR amplification occurred as follows: 1 minute at 95°C for denaturation, 32 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 1 minute at 72°C. Final extension occurred at 72°C for 20 minutes. Only one pair of primers is necessary for amplification due to the fact that all ligated probes have identical end sequences.

2.4.1.3 Fragment Separation

The PCR reaction of each sample was mixed with GeneScan-600 LIZ size standard and Hi-Di™ formamide (Life Technologies, Foster City, USA), heated for 2min at 94°C and subject to capillary electrophoresis using an ABI-3130 Genetic Analyser (Life Technologies, Foster City, USA). Peak patterns were generated and analysed using the Gene Mapper v.3.7 software (Life Technologies, Foster City, USA). The difference in relative amount of probe target sequences present in each sample results in differences in relative peak heights. These peak patterns should be evaluated to ensure that the quality of the results is reliable and adequate.

2.4.1.4 Peak pattern evaluation

Following analysis on the ABI 3130 genetic analyser, peak heights were imported into GenoTyper v3.7 for fragment analysis. The presence of a large rearrangement can be determined by either visual inspection or statistical analysis of the data, however, it is not advised to draw conclusions from only the visual peak profile inspection. False positives may occur when only visually examining the peak profiles and thus it should only be carried out to ensure that sample quality and DNA quantity was sufficient and that ligations – as well as PCR reactions were successful. Five wild-type controls were included in the MLPA reaction in order to evaluate and compare all the patients' samples.

The probe amplification products generate peaks between 130-427nt (figure 2.3). A total of 37 peaks between 130nt and 427nt should thus be present. The 4 Q-fragments or quantity fragments are located at 64-70-76-82nt and indicate whether DNA quantity was sufficient. These fragments are not ligation-dependent and are only present in small quantities. If the reaction was successful and DNA quantity sufficient, these fragments should hardly be visible or not visible at all. Should these peaks be of the same or similar height to that of the other ligation fragments, the DNA quantity is insufficient and the reaction must be repeated.

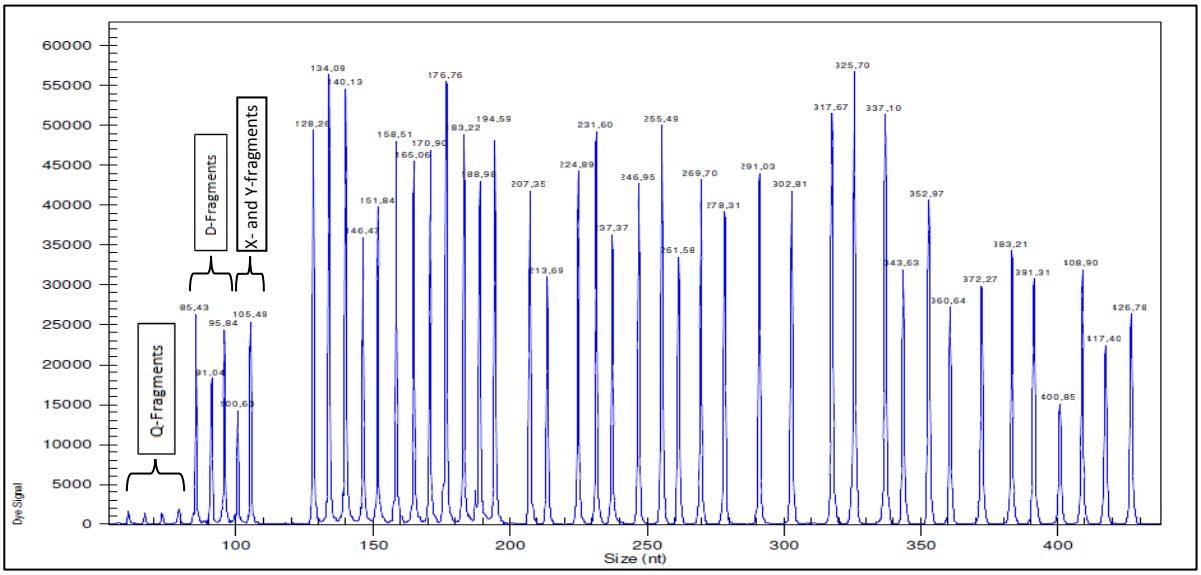

Figure 2.3: Peak profile of a successful MLPA
D-fragments and Q-fragments are indicated. The X and Y fragments are next to the Q-fragments. The remaining peaks are amplified probe ligation products each of a different exon or promoter region.

The three D-fragments at 88-92-96nt are DNA - and ligation-dependent and are visible when ligation and denaturation was successful and the quantity of DNA was sufficient (figure 2.3). The D-fragment at 92nt is also ligation dependent and if this peak is very low (lower than 1/3rd of the 88nt or 96nt fragments) ligation was unsuccessful or incomplete. In case the 88nt or 96nt fragment peak is lower than 40% the height of the 92nt D-fragment, sample DNA may have been denatured unsuccessfully. Fragments representing the X and Y chromosomes are present to show whether the sample is male or female and that there was no mix-up of samples.

Sloping of the peaks can be seen from left to right (figure 2.3) relative to increasing molecular weight of the products. Artefacts have been identified in data which causes sloping due to the differences in the electrokinetic injection sample loading process used in capillary electrophoresis. In order to correct for sloping, ten different reference (control) probes are included in the reaction. These control probes range in size and are located in 10 different autosomal chromosomal regions, not near the regions amplified by the probe ligation products used in the kit. Data is normalised during statistical analysis, using a linear regression model based on the degree of sloping of these control ligation products.

Statistical analysis of MLPA data using previously designed excel spreadsheets, was carried out. The input data on the spreadsheets is peak heights of the probe ligation products including the ten control ligation products, after the data of the nine control mix fragments (figure 2.3) was eliminated.

2.4.1.5 Data analysis

An Excel MACRO is used for analysis and is available on the National Genetics Reference Laboratory (Manchester) webpage (98) created by Andrew Wallace. Peak heights were imported into the Excel MACRO spreadsheet. Dosage quotients (DQ) or exon copy number changes were calculated in a standard manner using the Wallace method. Two additional features of analysis were also incorporated to help with the analysis.

Firstly, the quality of each individual test was assessed by determining the standard deviation (SD) of the DQs for each of the control ligation products. This standard deviation should be less than 0.1 otherwise the sample is of poor quality. In addition, a likelihood probability of concordance with one of three hypotheses is constructed, namely the occurrence of one, two or three copies of a specific site. This is determined by comparing the test samples to five unaffected control samples. A measure of variability for each ligation product is given by making use of the controls and estimating each sample's probability of deviation from expectation using the t-statistic.

The results are thus displayed in three key ways. (i) as dosage quotients for each ligation product versus each control ligation product (ii) as a mean dosage quotient for each ligation product, and (iii), as a likelihood probability and odds ratio for each ligation product calculated for one of three hypotheses: dosage that is normal (2n copies), deleted (n copies) or dosage indicating duplication (3n copies).

As illustrated in table 2.2, the sample information is given below the standard deviation in column B. The SD of the sample and control is highlighted in green indicating good sample quality (SD<0.1). The dosage quotients are given in columns D to V. This is an abbreviated table of the DQ values obtained for ligation products. Each ligation product's DQ is calculated against each control ligation product. The control ligation products (D – M on table 2.2) are located in different regions of the genome in order to correct for sloping. The mean DQ value for each ligation product is given in the last row of each of these columns.

Normal DQ values range from 0.85 – 1.15, DQ values between 0.35 – 0.65 indicates a deletion and equivocal DQ values are between 0.65 – 0.85 and >1.15. Normal values are given with a white background on the RESULTS sheet, values with an aqua background are indicative of a deletion and values with a cream background are equivocal. The two odds ratios for the alternative hypotheses are given below the mean DQ values. If these cells have a green background, the normal hypothesis is favoured.

Odds ratios with a magenta background (>1:20 in favour of a deletion or duplication) indicates that either the deleted (n) or a duplicated (3n) hypotheses is favoured. The absolute probabilities calculated by the t-statistic are given below the odds ratios. These probabilities are indicated for the normal, deleted and duplicated hypotheses. The variation between the mean DQ of that specific ligation product and the expected DQ from 5 unaffected controls is calculated. A probability of 60% for the normal hypothesis indicates that any other random wild-type sample would be expected to deviate by the same amount in 60% of the tests.

Table 2.2: An **abbreviated MLPA RESULTS spreadsheet of a wild-type control sample**

B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Lab No		C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8
Control3_G04_003.fsa	C1 5q31	1.00	1.02	1.07	1.05	0.99	1.08	1.07	1.00	1.06	1.00	0.96	1.00	1.03	1.09	1.06	0.95	1.04	1.01	1.01
Operator	C2 12p13	0.98	1.00	1.05	1.03	0.98	1.06	1.05	0.98	1.04	0.98	0.95	0.98	1.01	1.08	1.04	0.94	1.03	1.00	0.99
Nadja	C3 7q31	0.94	0.95	1.00	0.98	0.93	1.01	1.00	0.94	0.99	0.93	0.90	0.94	0.96	1.03	0.99	0.90	0.98	0.95	0.95
Worksheet	C4 20p13	0.95	0.97	1.02	1.00	0.95	1.03	1.02	0.95	1.01	0.95	0.92	0.95	0.98	1.04	1.01	0.91	1.00	0.97	0.96
1.1	C5 8p23	1.01	1.02	1.07	1.06	1.00	1.09	1.08	1.01	1.07	1.00	0.97	1.00	1.04	1.10	1.07	0.96	1.05	1.02	1.02
Int QC Stand Dev	C6 14q24	0.93	0.94	0.99	0.97	0.92	1.00	0.99	0.93	0.98	0.92	0.89	0.93	0.95	1.01	0.98	0.89	0.97	0.94	0.94
0.033657221	C7 12q24	0.94	0.95	1.00	0.98	0.93	1.01	1.00	0.93	0.99	0.93	0.90	0.93	0.96	1.02	0.99	0.89	0.98	0.95	0.95
	C8 8q24	1.00	1.02	1.07	1.05	0.99	1.08	1.07	1.00	1.06	1.00	0.96	1.00	1.03	1.10	1.06	0.96	1.05	1.02	1.01
	C9 15q26	0.95	0.96	1.01	0.99	0.94	1.02	1.01	0.94	1.00	0.94	0.91	0.94	0.97	1.03	1.00	0.90	0.99	0.96	0.95
	C10 7p14	1.00	1.02	1.07	1.05	1.00	1.08	1.07	1.00	1.06	1.00	0.96	1.00	1.03	1.10	1.06	0.96	1.05	1.02	1.01
	MEAN	0.97	0.98	1.03	1.02	0.96	1.05	1.04	0.97	1.03	0.97	0.93	0.97	1.00	1.06	1.03	0.93	1.01	0.98	0.98
ODDS NORMAL	:DEL	3935:1	812:1	11676:1	94627:1	5187:1	9741:1	4911:1	485:1	68144:1	3771:1	64:1	7161:1	126738:1	7099:1	58341:1	163:1	1614:1	19521:1	11393:1
ODDS NORMAL	:DUP	98:1	22:1	104:1	966:1	138:1	73:1	47:1	18:1	607:1	99:1	6:1	174:1	1745:1	43:1	528:1	12:1	27:1	353:1	228:1
PROB OF DEVIATION	NORMAL	67.7106%	89.4973%	60.0186%	66.2662%	56.1053%	49.2097%	66.1746%	80.4643%	51.8041%	63.4630%	73.8079%	58.7878%	92.5206%	39.0943%	54.5616%	61.9954%	91.2753%	74.5703%	71.2559%
PROB OF DEVIATION	DELETED	0.0172%	0.1102%	0.0051%	0.0007%	0.0108%	0.0051%	0.0135%	0.1660%	0.0008%	0.0168%	1.1481%	0.0082%	0.0007%	0.0055%	0.0009%	0.3800%	0.0565%	0.0038%	0.0063%
PROB OF DEVIATION	DUP	0.6325%	4.0513%	0.5794%	0.0686%	0.4070%	0.6740%	1.3975%	4.5713%	0.0854%	0.6408%	12.2609%	0.3378%	0.0530%	0.9011%	0.1034%	5.2589%	3.3772%	0.2110%	0.3129%

2.5 BREAKPOINT CHARACTERISATION

All the samples displaying an aberrant MLPA profile were subsequently amplified using primers flanking the deleted region. Following the PCR amplification, the samples were subjected to cycle sequencing using BigDye®Terminator v3.1 to identify the upstream and downstream breakpoints of the deletion.

2.5.1 Long range PCR analysis

Primers *SDHB* 2F and – 4R (Appendix D) were used in order to identify a possible deletion product in all patients. These primers are situated in intron 1 (2F) and intron 4 (4R) and amplify a region of 16360bp. The PCR was carried out by making use of Phusion High Fidelity DNA polymerase (Finnzymes) and High fidelity (HF) buffer (New England BioLabs Inc.). A total of 100ng or 150ng DNA was used together with 0.2µM of each primer, 250 µM of each dNTP and 0.6 Units of DNA polymerase. Reactions were repeated at different temperatures and using different PCR programmes including a stepdown PCR method. No clear results were obtained with these primers and thus new primers, *SDHB* 2162F & 2164R (99) flanking exon 3 (table 2.3) were subsequently used to amplify the deletion fragment (wildtpe = 9523bp). The total DNA concentration used per reaction was 100ng together with 1.25 Units Failsafe enzyme mix, 0.4µM of each primer and 2x premix J. The total reaction volume of each PCR was 20µl. Failsafe enzyme mix was used with PCR 2x Buffer J according to manufacturer's instructions (Epicentre Biotechnologies).

The PCR programme used was as follows: 94°C for 2min followed by 14 cycles of 98°C-10sec, 55°C-30sec and 68°C-10min. Then 98°C-10sec, 55°C-30sec and 68°C+ 15 seconds per cycle were repeated for 16 cycles.

The run was ended with 72°C for 10min. Amplification success was determined by gel electrophoresis of each PCR product using a 0.8% Agarose Gel (Gibco BRL, Life Technologies) with EtBr run for 2h at 60V.

The PCR reaction was optimised afterwards with Accuprime Taq (Invitrogen by Life Technologies) using 100ng DNA with 0.4µM of each primer and 2.5 Units Accuprime Taq together with Accuprime PCR buffer II according to manufacturer's instructions. The same PCR programme as given above was used.

The total reaction volume was also 20µl and the PCR programme was as follows: 94°C – 2min then 94°C – 30sec, 55 – 30sec and 68°C – 2min repeated for 30 cycles. The run was ended with 7min at 72°C and lastly 5min at 4°C. Gel electrophoresis was carried out in the same manner as explained above. This was followed by nucleic acid purification of the samples before cycle sequencing was carried out (Refer to section 2.3.2).

2.5.2 Cycle Sequencing

Primers *SDHB* Int2F and Int3R (table 2.3) were used for sequencing at a concentration of 20µM each together with BigDye v.3.1 dilution. The reaction containing *SDHB* Int2F primer was sequenced at 57°C and *SDHB* Int3R at 50°C. Cycle sequencing followed as explained in section 2.3.3.

Table 2.3: Long-range PCR and cycle sequencing primers for detection of *SDHB* exon 3 deletion breakpoints

Primer name	Sequence (5'-3')
SDHB_2162F	CCAGTCCATGAAAGGCA
SDHB_2164R	GCTCCATGTGTCACGTGTTT
SDHB_Int2F	GCAGGAGAATTGCTTGAGCC
SDHB_Int3R	CTAACAGACACAATACCCAAAAGT

Primer sequences obtained from a Bayley et al., (98)

2.6 GENOTYPE ANALYSIS

2.6.1 Polymerase Chain Reaction

Six microsatellite markers, three on either side of the *SDHB* gene on chromosome 1, were amplified in 6 separate fragments. Primers were obtained from http://www.ensemble.org and were optimised at specific temperatures and $MgCl_2$ concentrations. These markers span a region of ~3.2Mb. Seventeen intragenic single nucleotide polymorphisms (SNP's) were also amplified in six fragments in order to refine the haplotype. The SNP's are located in intron 1, 2, 3, 5, 6 and intron 7 of *SDHB.* These primers were designed and optimised for specific temperatures and $MgCl_2$ concentrations. The primer sequences and PCR conditions are all given (see appendix D). The same SNP's and microsatellites as described by Bayley et al., were used (99).

All the fragments were amplified using 50ng genomic DNA, 0.5 Units Taq polymerase (Invitrogen), 250µM of each dNTP, 1.5mM, 2mM or 2.5mM Magnesium Chloride ($MgCl_2$) and 0.2µM of each primer. A Veriti PCR machine was used (Life Technologies) with the programme as follows: 94°C – 3min then 94°C for 1min, annealing temperature of primer pair for 1min and 72°C for 1min repeated for 35 cycles. This was followed by 7min at 72°C and ended off at 4°C for 5min. Gel electrophoresis was performed in order to determine amplification success. A 1.6% Agarose gel with SYBR® Safe was used and subject to electrophoresis for 40min at 80V. Nucleic acid purification followed before samples were subjected to cycle sequencing (Refer to section 2.3.2).

2.6.2 Cycle Sequencing

Bi-directional cycle sequencing was carried out on the samples for all 6 microsatellite fragments. The SNP fragments were only sequenced in one direction, either forward or reverse primer. Cycle sequencing was carried out as explained in section 2.3.3, followed by precipitation of products (section 2.5.3). All sequencing reactions were carried out in a 96-well plate.

Chapter 3: Results and Discussion

Sixteen samples from ten families were analysed for point mutations in the *SDHAF2* gene by cycle sequencing. These samples were also analysed for large rearrangements in the *SDHB*, *-C*, *-D* and *SDHAF2* genes by making use of multiplex ligation-dependent probe amplification (MLPA).

3.1 SEQUENCING OF THE *SDHAF2* GENE

All patients were analysed for point mutations in the *SDHAF2* gene by means of PCR and direct cycle sequencing. No sequence variants were identified in the *SDHAF2* gene of any of the patients. MLPA was subsequently utilised to identify large rearrangements in the *SDHB*, *-C*, *-D* and *SDHAF2* genes.

3.2 SCREEN FOR LARGE DELETIONS/DUPLICATIONS IN THE *SDHB*, *-C*, *-D* AND *SDHAF2* GENES

3.2.1 MLPA Data Analysis

As explained in section 2.4, PGL patients were analysed for large rearrangements using the SALSA MLPA kit, P226-B1 SDHB-SDHC-SDHD produced by MRC Holland (www.mrc-holland.com). All probe sequences as well as ligation sites of the probes included in this kit are given in appendix E.

Following analysis on the ABI 3130 genetic analyser, peak heights were imported into GenoTyper v3.7 for fragment analysis. Visual inspection and peak pattern evaluation (section 2.4.1.4) were carried out to analyse the control mix fragments and the MLPA peak profile of each sample (figure 3.1).

a. Control mix fragment inspection

A representative example of the MLPA peak profile of a wild-type control is shown in figure 3.1A. The control mix fragments (figure 3.1B) of all samples were inspected first to ensure that the MLPA reaction was successful. The Q-fragments were barely visible on the peak profile thus confirming DNA quantity was sufficient. The D-fragments were all visible, confirming ligation and denaturation of DNA was successful. The 88nt and the 96nt fragment peaks were not lower than 40% of the 92nt D-fragment or the MLPA probes, indicating that denaturation was successful. All samples were thus of good quality and PCR as well as ligation reactions were successfully carried out.

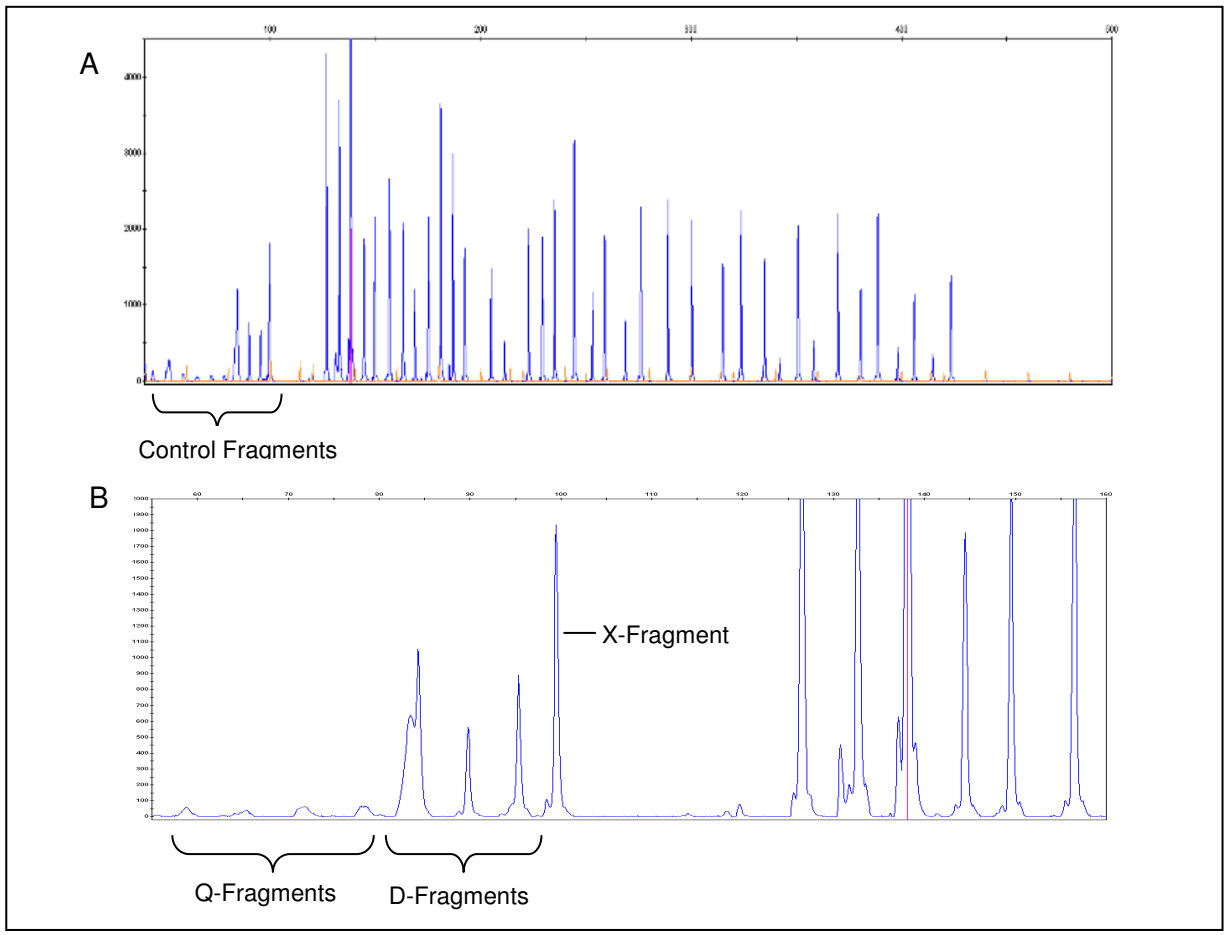

Figure 3.1: Representative electropherograms illustrating MLPA peak profiles of a wild-type sample **(A)** wild-type control; Peaks represent the amplification products of probes. Peak height is presented on the y-axis and probe position (nt) on the x-axis. Orange peaks represent the 600LIZ size standard. **(B)** Control mix fragments of a wild-type control sample.

b. Peak pattern evaluation

All control and probe ligation products have amplified successfully and are represented as peaks ranging in size (figure 3.1A). Sloping of the peaks can be seen from left to right (figure 3.1A) relative to increasing molecular weight of the products. Sloping was corrected for as explained in section 2.4.1.4. Statistical analysis of MLPA data using previously designed excel spreadsheets, was carried out. The input data on the spreadsheets is peak heights of the probe ligation products including the ten control ligation products, after the data of the nine control mix fragments (figure 3.1B) was eliminated.

3.2.2 Statistical analysis

An Excel MACRO was used for analysis and is available on the National Genetics Reference Laboratory (Manchester) webpage (98) created by Andrew Wallace. Peak heights were imported into the Excel MACRO spreadsheet. Dosage quotients (DQ) or exon copy number changes were calculated in a standard manner using the Wallace method. The MLPA results showed that all PGL patients carry an *SDHB* exon 3 deletion. A representative example of a deletion positive sample is illustrated in table 3.1. The *SDHB* exon 3 ligation products (highlighted in aqua) can be seen in column Q. The DQ values ranging from 0.43-0.50 is indicative of a deletion, together with the mean DQ value (0.47). The odds of normal:deleted is 1:394 in favour of a deletion and is indicated with a magenta background. All samples were of good quality which was confirmed by the standard deviation with a value less than 0.1. Equivocal results (cream) can be seen in this table. These values are not in the normal range, but are not abnormal either. These specific values, although equivocal, were not significant due to the fact that all the DQ values for this ligation product and the absolute probabilities were normal. Results spreadsheets for all 16 samples are included in appendix F.

Table 3.1: An **abbreviated MLPA RESULTS spreadsheet of a PGL sample**

B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Lab No		C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8
HGT4.1_F05_002.fsa	C1 5q31	1.00	1.02	0.99	1.13	1.03	1.02	1.09	0.97	1.03	1.02	0.98	0.99	1.05	0.49	1.01	0.93	1.13	1.04	0.95
Operator	C2 12p13	0.98	1.00	0.97	1.11	1.01	1.00	1.07	0.95	1.01	1.01	0.96	0.97	1.03	0.48	0.99	0.92	1.11	1.02	0.94
Nadja	C3 7q31	1.01	1.03	1.00	1.14	1.04	1.03	1.10	0.98	1.04	1.03	0.99	1.00	1.06	0.49	1.02	0.94	1.14	1.05	0.96
Worksheet	C4 20p13	0.88	0.90	0.88	1.00	0.91	0.90	0.96	0.86	0.91	0.91	0.87	0.88	0.93	0.43	0.89	0.82	1.00	0.92	0.84
1	C5 8p23	0.97	0.99	0.96	1.10	1.00	0.99	1.06	0.94	1.00	1.00	0.95	0.96	1.02	0.47	0.98	0.91	1.10	1.01	0.93
Int QC Stand Dev	C6 14q24	0.98	1.00	0.97	1.11	1.01	1.00	1.06	0.95	1.01	1.00	0.96	0.97	1.03	0.47	0.99	0.91	1.10	1.01	0.93
0.045783685	C7 12q24	0.92	0.94	0.91	1.04	0.95	0.94	1.00	0.89	0.95	0.94	0.90	0.91	0.97	0.45	0.93	0.86	1.04	0.95	0.88
	C8 8q24	1.03	1.05	1.02	1.17	1.06	1.06	1.12	1.00	1.07	1.06	1.02	1.02	1.09	0.50	1.04	0.96	1.17	1.07	0.98
	C9 15q26	0.97	0.99	0.96	1.10	1.00	0.99	1.05	0.94	1.00	0.99	0.95	0.96	1.02	0.47	0.98	0.90	1.09	1.00	0.92
	C10 7p14	0.98	0.99	0.97	1.10	1.01	1.00	1.06	0.94	1.01	1.00	0.96	0.97	1.03	0.47	0.98	0.91	1.10	1.01	0.93
	MEAN	0.97	0.99	0.96	1.10	1.00	0.99	1.06	0.94	1.00	1.00	0.96	0.96	1.02	0.47	0.98	0.91	1.10	1.01	0.93
ODDS NORMAL	:DEL	4154:1	874:1	5900:1	11518:1	12447:1	12032:1	4309:1	311:1	106288:1	7067:1	86:1	6307:1	96463:1	1:394	42882:1	120:1	1293:1	28512:1	2406:1
ODDS NORMAL	:DUP	100:1	22:1	156:1	32:1	187:1	198:1	31:1	17:1	1370:1	120:1	6:1	165:1	930:1	3:1	806:1	11:1	7:1	361:1	111:1
PROB OF DEVIATION	NORMAL	70.0548%	92.6895%	53.8029%	4.4776%	98.9229%	90.4594%	50.5047%	65.0419%	97.0511%	93.5845%	82.4595%	53.8933%	58.9443%	0.1032%	58.1540%	53.9806%	40.8890%	90.4368%	24.0733%
PROB OF DEVIATION	DELETED	0.0163%	0.1060%	0.0091%	0.0004%	0.0079%	0.0075%	0.0117%	0.2092%	0.0009%	0.0132%	0.9538%	0.0085%	0.0006%	40.6354%	0.0014%	0.4480%	0.0316%	0.0032%	0.0100%
PROB OF DEVIATION	DUP	0.7038%	4.1679%	0.3440%	0.1398%	0.5290%	0.4565%	1.6107%	3.9374%	0.0708%	0.7821%	13.4645%	0.3270%	0.0634%	0.0378%	0.0722%	4.8028%	5.7821%	0.2505%	0.2173%

The mean DQ values are used to generate a histogram for each test sample (figure 3.2). A histogram depicting the mean DQ values of a deletion positive sample and a control sample is given in figure 3.2. The dosage quotient (Y-axis) of the *SDHB* exon 3 probe for this sample (HGT 5.1) is much lower than the rest of the DQ values. All the remaining probes for this sample have normal values. Probes of the control sample have DQ values in the normal range. Exon 3 of the *SDHB* gene thus appears to be heterozygously deleted in all samples.

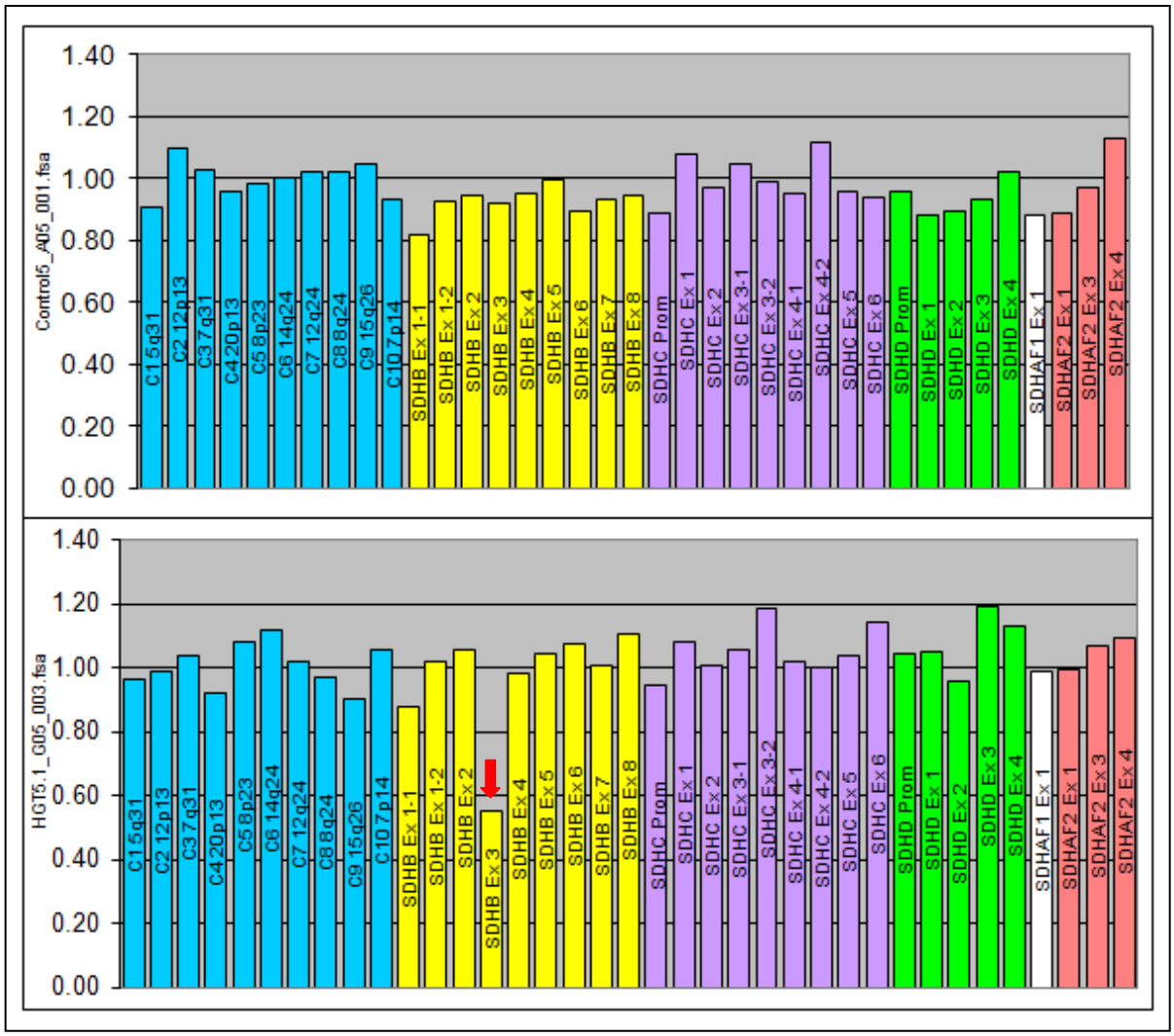

Figure 3.2: Histogram representing mean DQ values of ligation products

The mean DQ values of an unaffected control sample (top) and a PGL patient (bottom) are illustrated above. Different colours represent probes in different genes with control probes in blue. The red arrow marks the mean DQ value of the probe for *SDHB* exon 3 which is significantly decreased.

Aberrant DQ values may occur as a result of a mutation at either the hybridisation site of one of the oligos or at the ligation site. This will thus interfere with hybridisation or ligation of the two oligos. The complete probe would not be formed and therefore not be amplified, causing reduced DQ. The partial sequence of the exon 3 probe 24nt adjacent to ligation site is as follows: ATGCTTTAATCA-AGATTAAGAATG (Appendix E). The region across exon 3 was sequenced previously, and the sequences were studied to ensure that no sequence variants were present in the probe hybridisation or - ligation sites shown above. No mutations were identified in any of the patients and thus the results of the MLPA showing a deletion of *SDHB* exon 3 thus seem to be valid.

These results were subsequently confirmed and the breakpoints characterised by long-range PCR and direct cycle sequencing.

3.3 LONG RANGE PCR ACROSS EXON 3

Primers, located in intron 1 and intron 4, were used in order to identify the deletion product. The wild-type product is 16360bp with a smaller product expected to be amplified if the deletion is present. These reactions were repeated at different temperatures, using different PCR programmes. The results showed either multiple non-specific amplification products (figure 3.3A) or a complete failure to amplify any products (figure 3.3B).

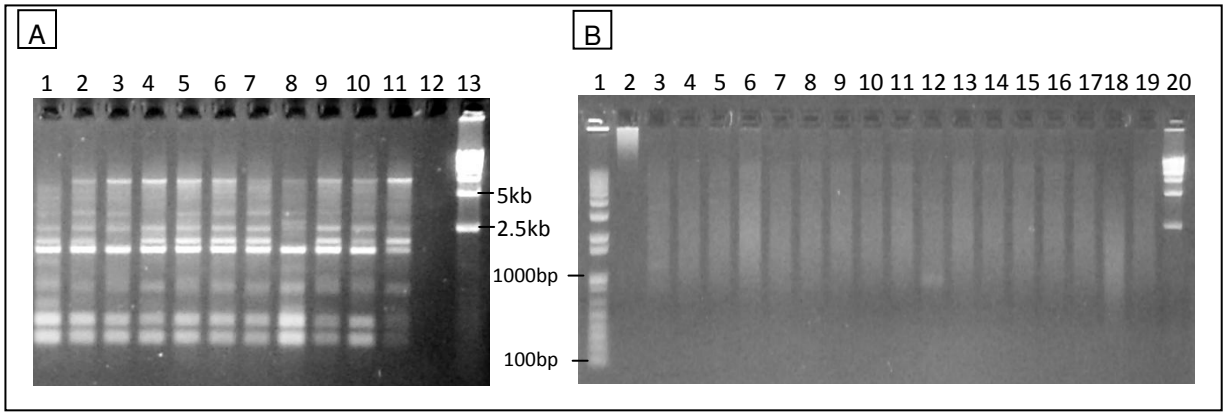

Figure 3.3 Gel electrophoreses results of two long range PCR's across exon 3

Multiple, non-specific amplification (A) or no amplification occurred and PCR resulted in only light smears (B). **Fig. A)** 0.7%gel at 60V for 2.5h; **1.**Control sample; **2-11.**PGL patient samples; **12.**Blank sample containing no DNA **13.** Molecular marker **Fig. B)** 0.8% gel at 60V for 1.5h. **1 and 20.** Molecular markers; **2.**Blank sample-no DNA; **3-4** Control samples; **5-19.** PGL patient samples

This led us to identify new primers in order to amplify this whole region successfully. The TCA Cycle Gene Mutation Database (formerly SDH complex database) (60, 61) was accessed to establish whether large deletions of *SDHB* exon 3 have been reported previously. Six large deletions have been identified to date in Spanish, Portuguese and Dutch populations and only the deletion in the Dutch population was completely characterised. The same primers (99) were thus used to attempt the identification and characterisation of the deletion breakpoints in the South African PGL patients. Long-range PCR analysis was carried out using primers *SDHB*_ 2162F (intron 2) and *SDHB*_2164R (intron 3) which yields a wild-type product of 9523bp. All the PGL patient samples showed an identical ~1.6kb product (indicated with an arrow on figure 3.4). Neither this fragment nor the wild-type fragment (9523bp) amplified in the control samples. The wild-type product can be seen faintly in the lanes containing the amplified products of deletion positive samples (Lanes 4 – 20).

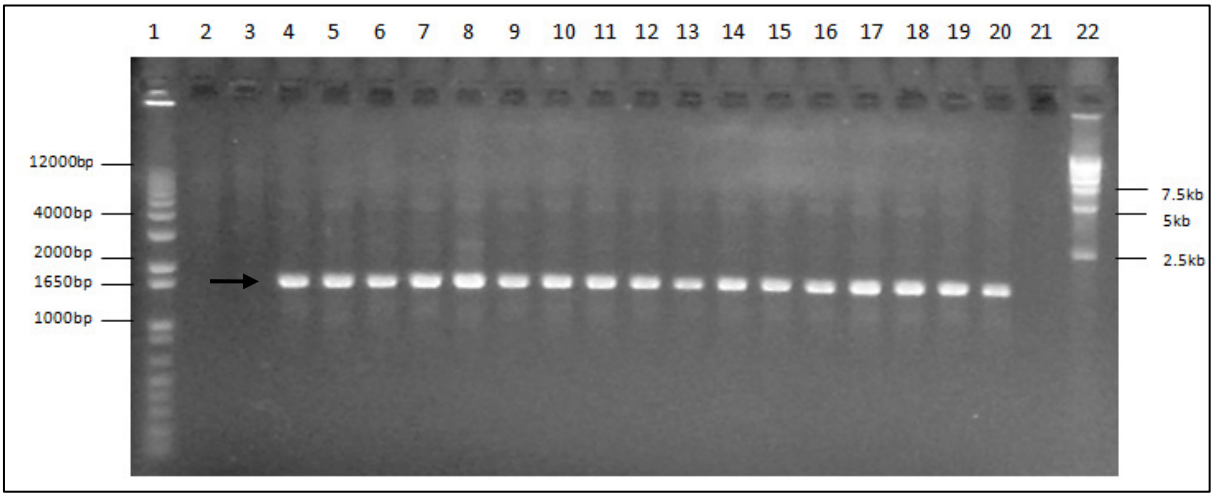

Figure 3.4: Long range PCR across *SDHB* exon 3
1% Agarose gel (EtBr) with 1kb+ ladder in lane 1 and 2.5kb ladder in lane 22. Gel electrophoresis was carried out for 1.5 hours at 60V. Lane 2 and 3: Wild-type controls; Lane 21: Blank; Lanes 4-20: PGL patients. Deletion product indicated with an arrow.

Lane 21 contains a blank control sample to which only water was added and no DNA in order to test for possible contamination. No contamination was thus present due to the fact that no amplification was seen in this lane.

The above PCR reactions were carried out using Failsafe Taq and buffer J as discussed in materials and methods (Chapter 2). The PCR reaction was, however, optimised and repeated with Accuprime Taq which yielded brighter bands and no non-specific amplification. The Accuprime Taq was used for identification of the deletion fragment from this point forward and not the Failsafe Taq (Appendix G).

The amplification products of ~1.6kb were subjected to direct sequencing in order to identify the breakpoints of this deletion.

3.4 BREAKPOINT CHARACTERISATION

Sequence traces of the ~1.6kb product were analysed and revealed identical breakpoints in all samples (figure 3.5). The two breakpoints were identified to be in intron 2 [4429bp upstream of exon 3] and intron 3 [933bp upstream of exon 4] (figure 3.6).

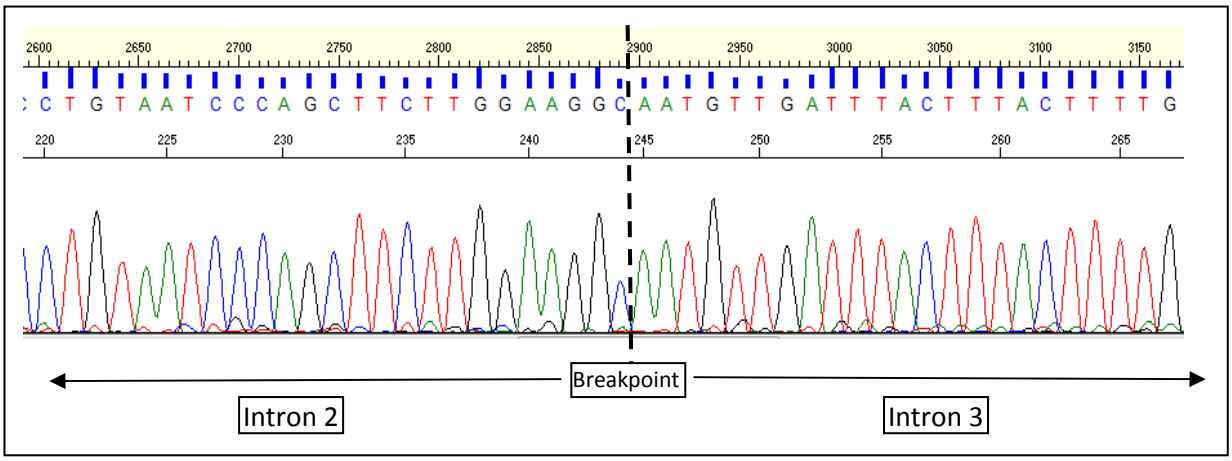

Fig 3.5. Representative example of sequence spanning the *SDHB* exon 3 deletion in a mutation positive sample

The breakpoint is indicated with a black dashed line. Sequence on the left (intron 2) is upstream of the deletion and right (intron 3) located downstream of the deletion

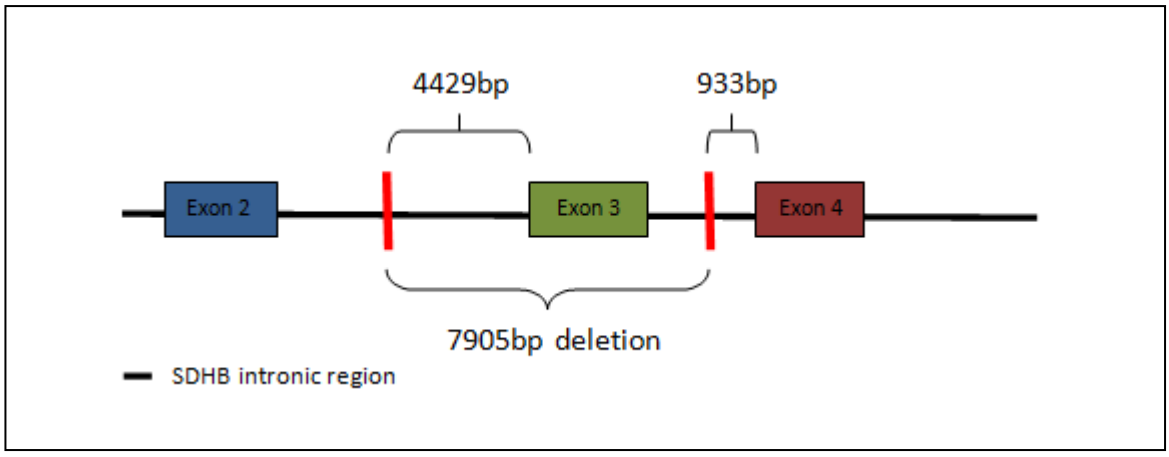

Figure 3.6: Up- and downstream breakpoints of the *SDHB* exon 3 deletion

In total, 7905bp are deleted and using the HGVS cDNA nomenclature (Reference sequence NC_000001.9), this deletion was identified as *SDHB* c.201-4429_287-933del, which is the same mutation previously identified in the Dutch population as an *SDHB* founder mutation (99).

The breakpoint in intron 2 is located in an *Alu*Sz, but the downstream breakpoint (intron 3) is not located in an *Alu* repeat nor does it have any matching or repeating sequence around the breakpoint. The mechanism of deletion is thus unknown. Interestingly, the overlap is only one basepair (Cytosine) (figure 3.7).

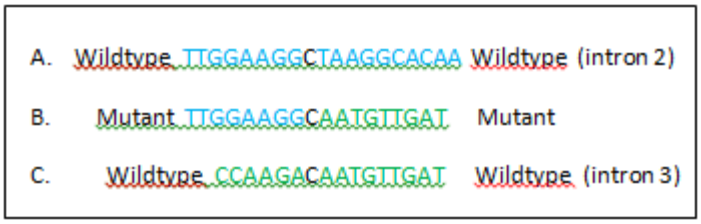

Figure 3.7: Surrounding sequence of the 7.9kb deletion breakpoints
A. Wildtype sequence of intron 2; **B.** Mutatnt sequence which is a combination of intron 2 (blue) and intron 3 (green); **C.** Wildtype sequence of intron 3.

As exon 3 is out of frame with exon 4 this deletion will cause a frameshift and the predicted protein truncation will occur 21 codons downstream from codon 68 (p.Cys68HysfsX21). This will cause a loss of 233 of the 322 amino acids. However, it is highly likely that translation of this protein will not occur and be destroyed by nonsense mediated mRNA decay (NMD). Nonsense-mediated mRNA decay is a mechanism coupled to translation and eliminates mRNAs with premature translation-termination codons (100). It was discovered upon the realisation that very low concentrations of mRNAs transcribed from alleles carrying nonsense mutations, are frequently found in cells. NMD is thought to be an mRNA-surveillance mechanism to prevent potentially harmful truncated proteins from being synthesised. In mammalian cells a premature stop codon, with distances greater than 50-55 nucleotides upsteam of the 3'-most exon-exon junction is believed to lead to NMD (101-104). At least one intron should also be downstream of the premature stop codon (105). The stop codon in exon 4 of the *SDHB* gene, caused by the deletion of exon 3, meets the above-mentioned criteria and thus NMD will most likely take place.

Although this mutation is the same in all of the SA families and the Dutch families, it is not known whether the mutations occurred due to independent events or whether it has arisen once. Genotype analysis was thus carried out in order to answer this question.

3.5 GENOTYPE ANALYSIS

Genotype analysis across the *SDHB* gene was carried out to determine whether the South African Afrikaner PGL patients share a common haplotype and thus also a common ancestor from whom the mutation originated. None of the families share a surname and they do not appear to share a common geographic location in South Africa. Six microsatellites, three on either side of the *SDHB* gene (chromosome 1) together with 17 intragenic SNPs were typed. These markers span a region of ~3,2Mb (figure 3.8). Five of the microsatellites are di-nucleotide repeats and one (D1S3669) is a tetra-nucleotide repeat. The SNPs cover a region of ~26.7 kb, spanning from intron 1 up to intron 7 of the *SDHB* gene (figure 3.9).

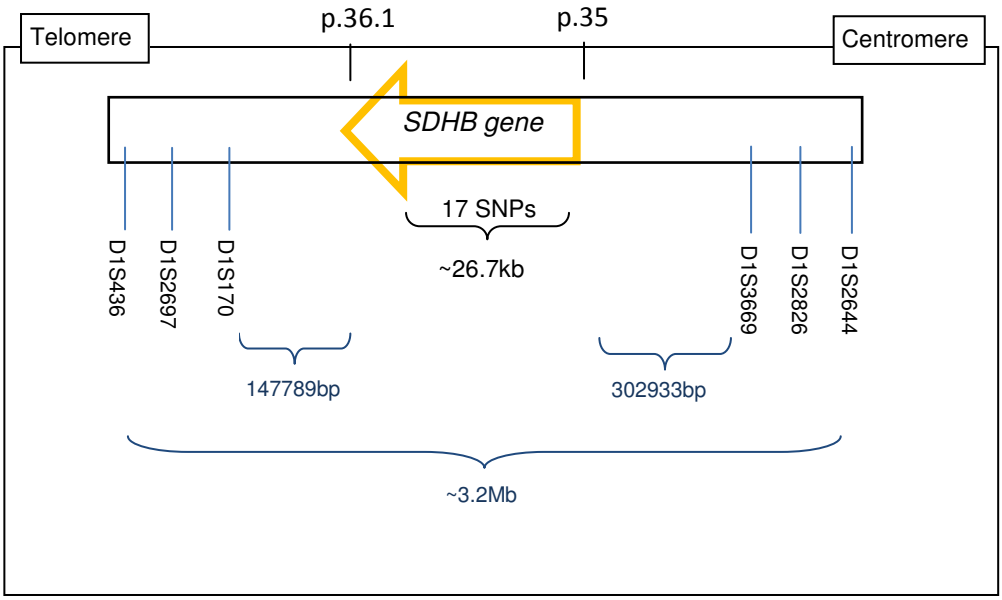

Figure 3.8: Location of microsatellite markers and SNPs spanning the *SDHB* gene on the antisense strand

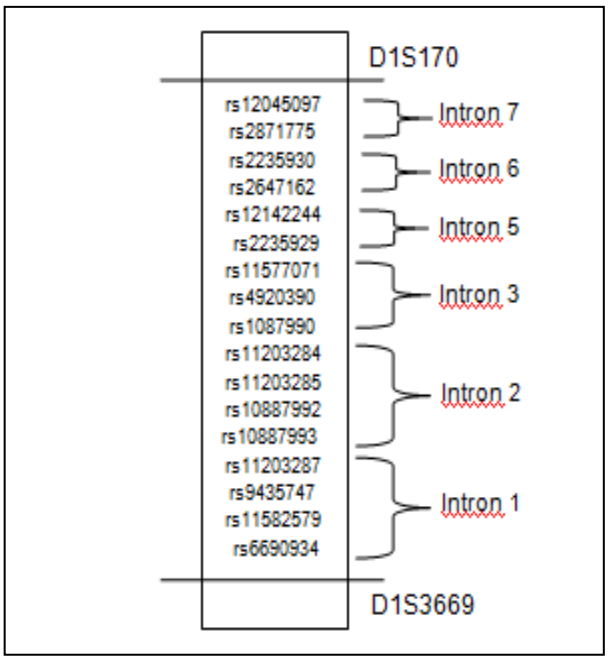

Figure 3.9: Location of the 17 SNPs in the *SDHB* gene

The microsatellite regions were amplified and subjected to gel electrophoresis to ensure that the correct fragment was amplified (figure 3.10). The products ranged from 123bp to 281bp, depending on the number of repeats.

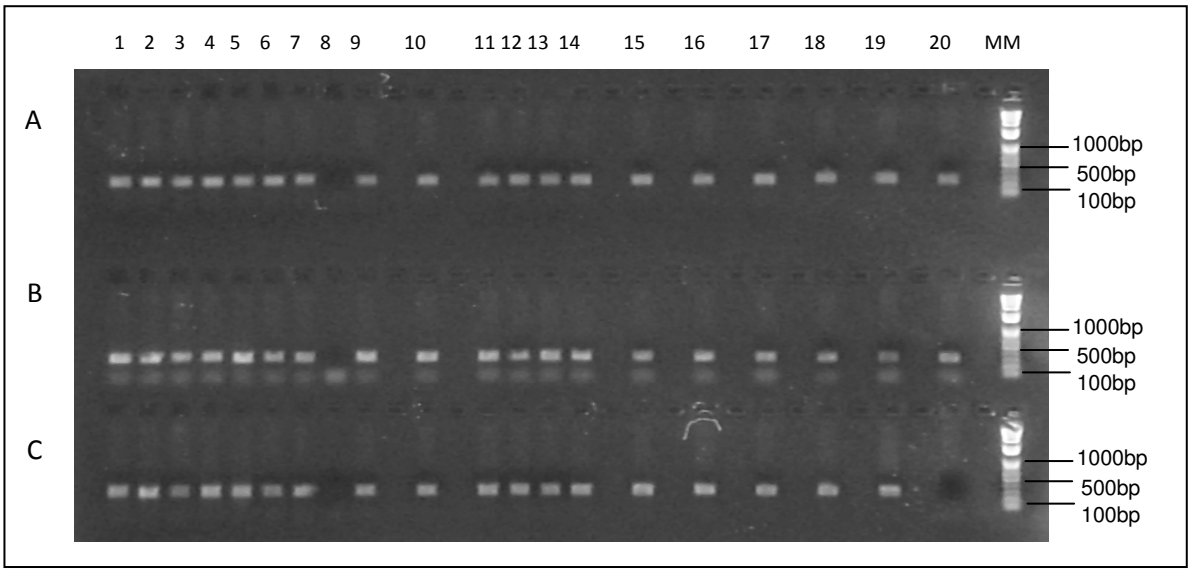

Figure 3.10: Gel electrophoresis of PCR-amplified microsatellite fragments
This image represents the amplified products of three different microsatellite marker fragments. A = D1S2644; B = D1S2826; C = D1S2697; Lane 8 = Blank; Lanes 1-20 = PGL patients MM=Molecular Marker

After cycle sequencing and capillary electrophoresis of the PCR products, the sequences were analysed and the number of repeats for each microsatellite were scored for all samples. In figures 3.11 A – F, representative examples of the scored microsatellite sequences for each marker are shown.

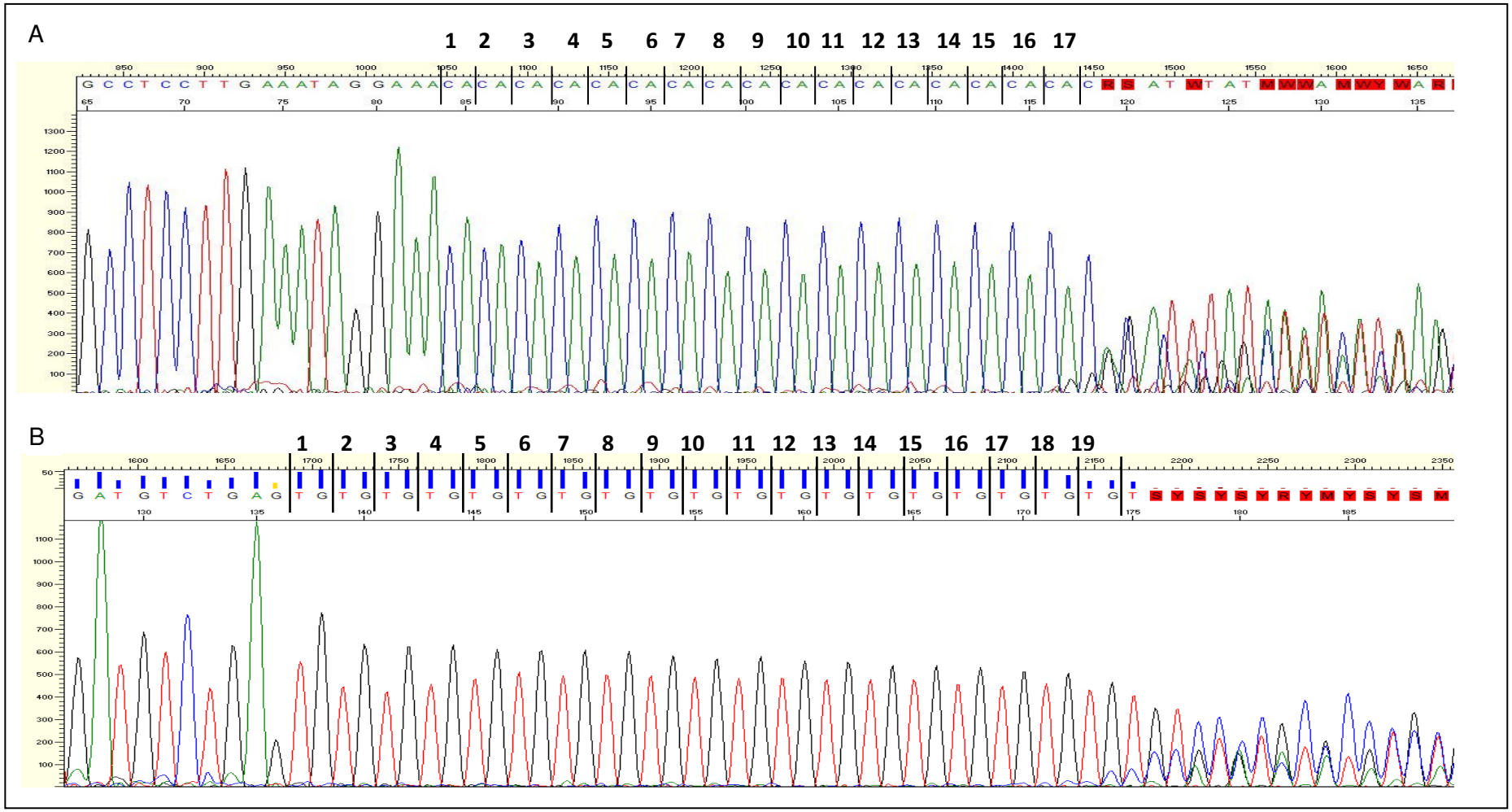

Figure 3.11A & B: Microsatellite markers D1S2644 and D1S170 partial sequence
A) The D1S2644 forward primer was used for sequencing of this dinucleotide marker (CA repeat). The first allele consists of 17 CA repeats which can be easily counted and the second allele carries 20 repeats. **B)** The reverse primer was used for sequencing of the D1S170 marker (TG repeat). The first allele consists of 19 repeats counted easily and the second allele carries 20 repeats
Images viewed and saved from Sequence Scanner v1.0 (Life Technologies)

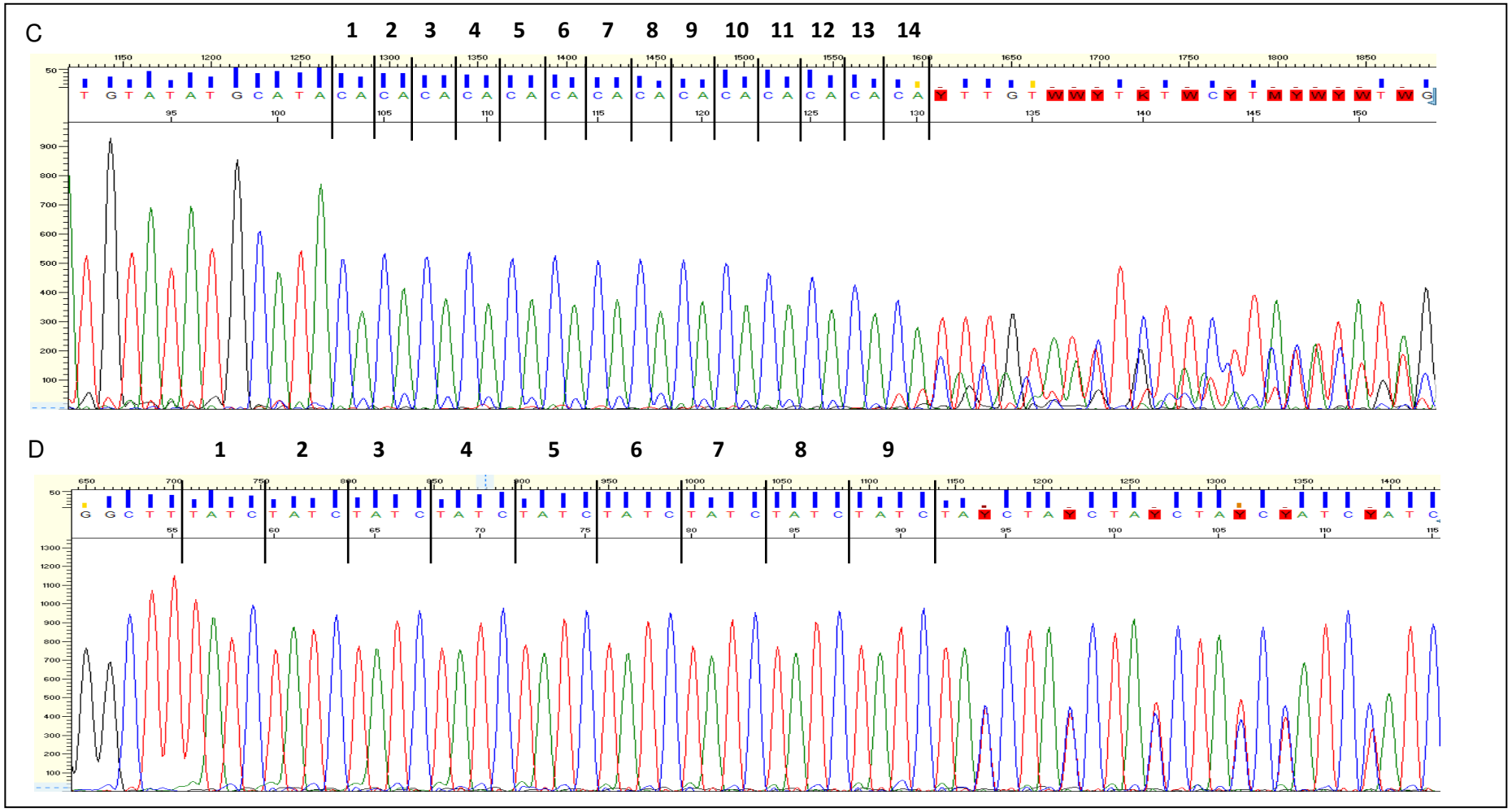

Figure 3.11C & D. Microsatellite markers D1S436 and D1S3669 partial sequence
C) The forward primer was used for sequencing of marker D1S436 (CA repeat). The first allele consists of 14 repeats counted easily and the second allele carries 17 repeats. **D)** Marker D1S3669 (TATC repeat) was sequenced using the forward primer. The first allele consists of 9 repeats counted easily and the second allele carries 14 repeats.

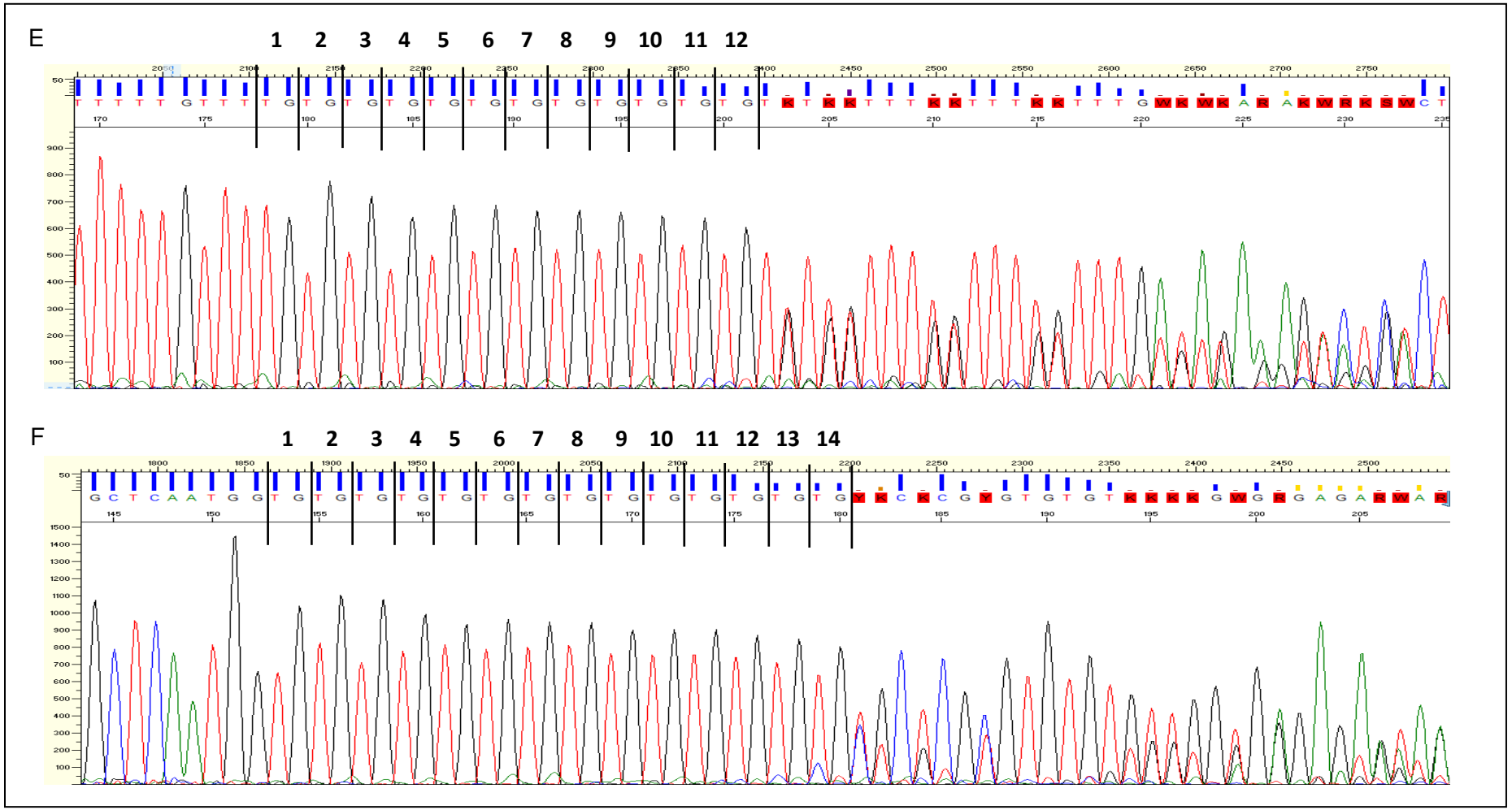

Figure 3.11E & F. Microsatellite markers D1S2697 and D1S2826 partial sequence

E) The reverse primer was used for sequencing of the D1S2697 marker (TG repeat). The first allele consists of 12 repeats counted easily and the second allele carries 14 repeats. **F)** The reverse primer was used for sequencing of marker D1S2826 (TG repeat). The first allele consists of 14 repeats counted easily and the second allele carries 15 repeats.

In the same way, the SNPs (table 3.3) were amplified, sequenced by direct cycle sequencing followed by capillary electrophoresis after which the sequences were analysed.

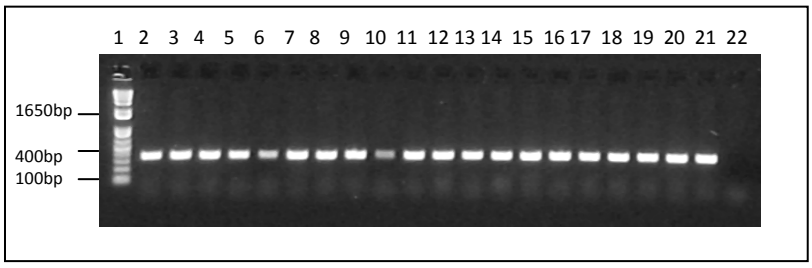

Figure 3.12: PCR amplification results of SNPs
These SNPs, **rs10887990, rs4920390 and rs11577071** were amplified in one fragment with primers B_IVS3F&R. This is a representative figure of one of the six fragments which were all amplified in the same manner.

Figure 3.13 A/B illustrates the sequence surrounding each SNP for one mutation positive sample. Only part of the sequence is shown for one person. The yellow highlighted SNPs are homozygous for a specific basepair and red is heterozygous. Two base changes are shown for SNP rs11203285, but the one change (R) is merely background interference and not a definite base change.

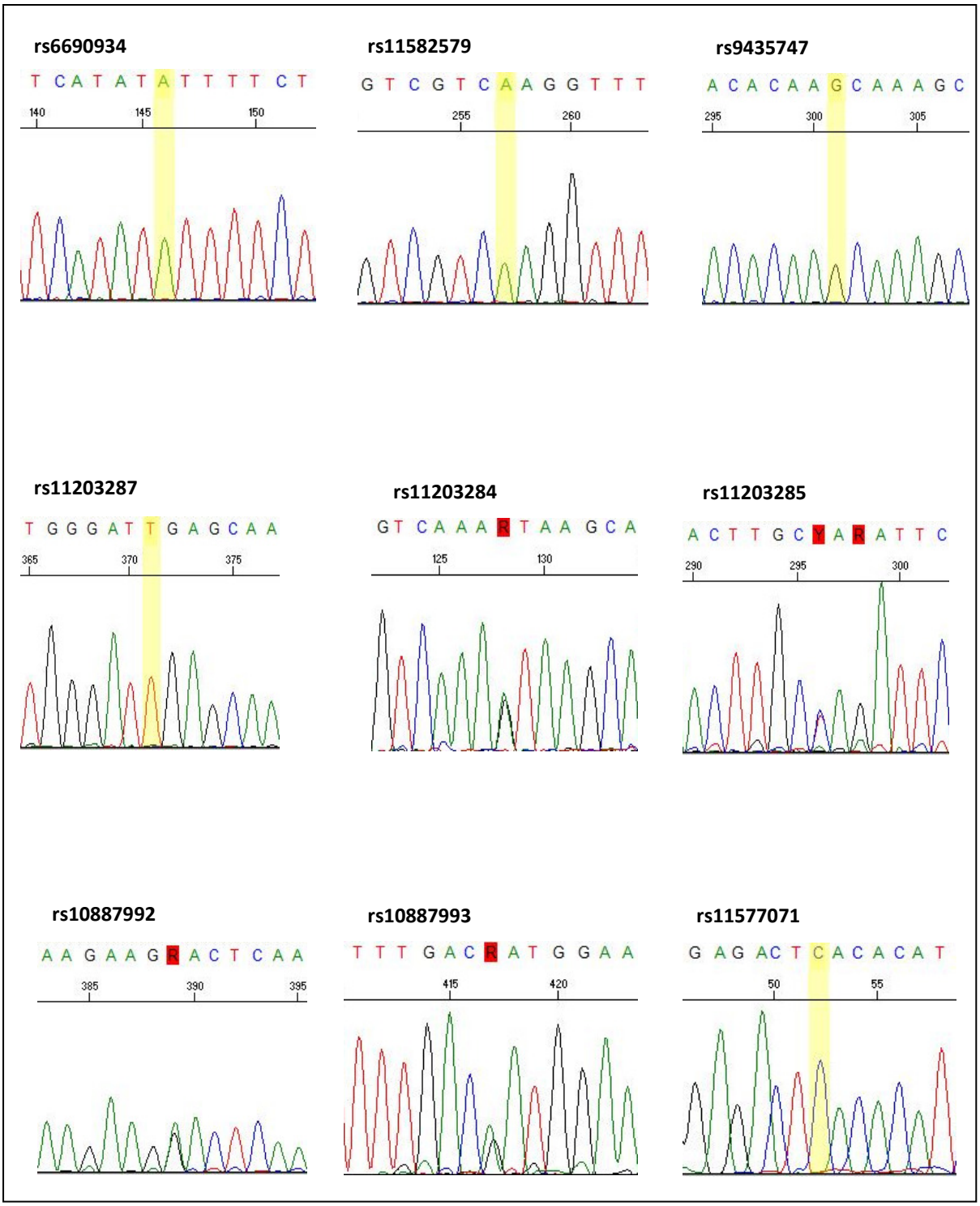

Figure 3.13 A: Representative examples of Single Nucleotide Polymorphism sequence traces of a mutation positive sample

Sequences of all 17 SNPs are given for one sample. Eight SNPs are homozygous and nine heterozygous. Homozygous alleles = yellow; Heterozygous alleles = red

R: A/G; **Y:** C/T

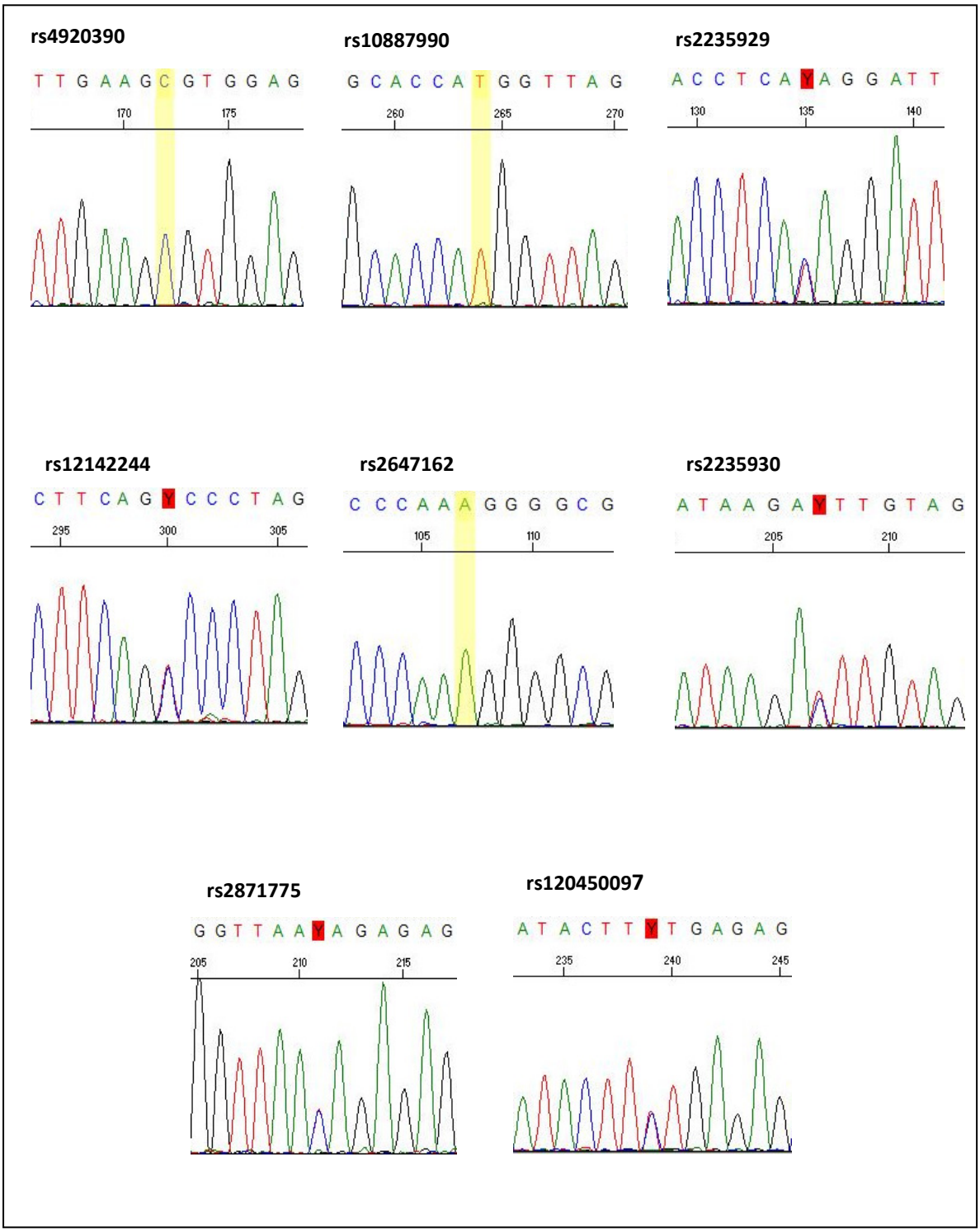

Figure 3.13B: Representative example of Single Nucleotide Polymorphism sequence traces of a mutation positive sample
Sequences of all 17 SNPs are given for one sample. Eight SNPs are homozygous and nine heterozygous. Homozygous alleles = yellow; Heterozygous alleles = red
R: A/G; **Y:** C/T

Haplotypes were constructed for eight families where phase was confirmed. For two families, probable haplotypes were constructed (phase not confirmed) that are consistent with the observed haplotype in other mutation carriers (appendix H.) A common haplotype was identified between the South African patients of nine families (figure 3.14).

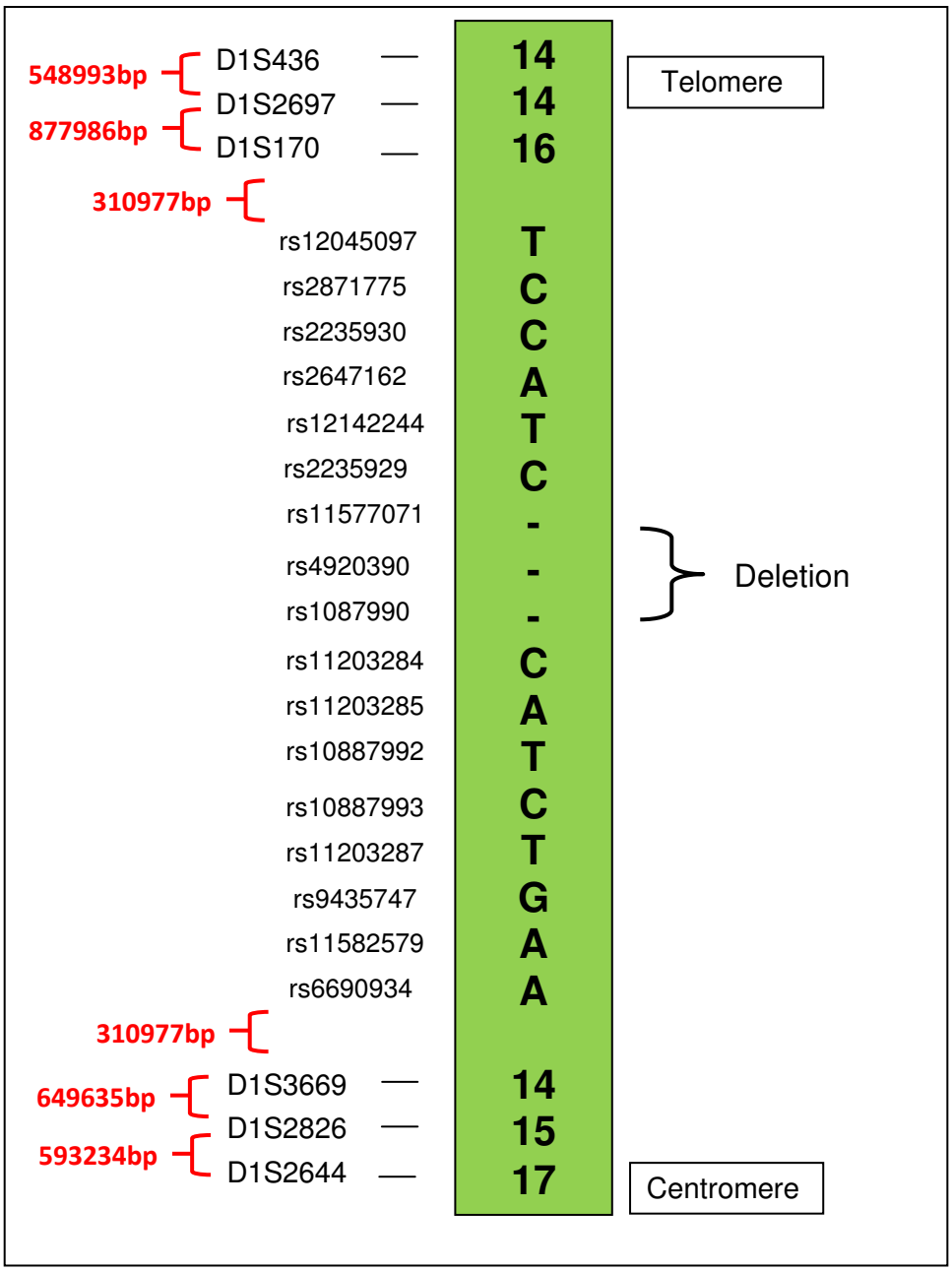

Figure 3.14: Common haplotype shared between the South African PGL patients
The number of microsatellite repeats is given for each microsatellite.
Three SNPs are in the deleted region and are thus only present on the wild-type allele.
Red brackets indicate the distance between two particular markers or between a marker and a microsatellite. The *SDHB* gene is on the antisense strand on chromosome 1.

Three of the SNPs are located in the deletion region (intron 3) and therefore typed as being "homozygous" due to the fact that only the SNPs of the remaining wild-type alleles were typed. Some of the microsatellite repeats could not be scored and this is indicated with a question mark (appendix H). In some cases the number of repeats on the first allele could be established but the number of repeats linked to the second allele could not be established successfully. In one case (HGT 8:7) the sequence was of bad quality even after it was repeated and the repeats on both alleles could not be scored accurately. The deletion haplotype consists of SNPs located on minor alleles for intron 1 and 2, whereas introns 5 to 7 are the major allele in the European population (Table 3.2).

Table 3.2: SNPs typed for genotyping analysis

SNP	Nucleotide sequence (Sense strand)	Nucleotide sequence (Antisense strand)	Minor Allele Frequency of European population (1000 genomes)
rs12045097	A/G	T/C	G (0.171)
rs2871775	G/A	C/T	A (0.471)
rs2235930	G/A	C/T	A (0.417)
rs2647162	T/C	A/G	C (0.191)
rs12142244	A/G	T/C	G (0.453)
rs2235929	G/A	C/T	A (0.227)
rs11577071	C/G	G/C	G (no results)
rs4920390	C/T	G/A	T (no results)
rs10887990	C/T	G/A	T (0.479)
rs11203284	A/G	T/C	G (0.479)
rs11203285	C/T	G/A	T (0.479)
rs10887992	G/A	C/T	A (0.479)
rs10887993	A/G	T/C	G (0.479)
rs11203287	G/A	C/T	A (0.479)
rs9435747	T/C	A/G	C (0.479)
rs11582579	G/T	C/A	T (0.479)
rs6690934	C/T	G/A	T (0.479)

Minor allele frequencies obtained from ensemble (http://www.ensembl.org/index.html) Accessed on 28/02/2014.

One family (HGT 5) shared the core haplotype from marker D1S436 to D1S2826 covering a region of ~2.56Mb. Microsatellite D1S2644 of family 5 differs from the common haplotype shared by the remaining PGL families (Appendix H).

There were, however, three families presenting with apparent recombination, although mitotic error for these microsatellite markers are also likely. In family eight (individual 8:1), a recombination event occurred between the centromeric markers D1S2826 and D1S3669, retaining the core linked haplotype of ~1.91Mb (Appendix H). A single recombination event also occurred between centromeric marker D1S3669 and rs6690934 in family 11 (figure 3.15), retaining the core haplotype of ~1.6Mb. This recombination event may have occurred in 11:2 herself or in her mother, but due to the fact that her mother is deceased, we were not able to obtain DNA for analysis.

Individual 14:8, who does not carry the *SDHB* exon 3 mutation, also presented with recombination between centromeric marker D1S3669 and SNP rs669093 (Appendix H). Even though there are three families presenting with apparent recombination events, it seems that all the South African patients share a common haplotype, with the exception of marker D1S2644 in family 5. The South African patients thus also share common ancestry and the *SDHB* exon 3 deletion is a founder mutation in the South African Afrikaner population.

Due to the origins of the Afrikaner population (see section 1.6) it is not surprising to find that familial PGL shows a founder effect. Interestingly, we have also identified founder mutations in Afrikaner breast cancer families. Three unique mutations were identified in the *BRCA1* and *BRCA2* genes (106, 107), that account for 94% of mutation positive Afrikaner families with three or more affected individuals (106).

A number of founder mutations were also identified in the low density lipoprotein (LDL) receptor gene that is responsible for more than 95% of Familial Hypercholesterolemia cases among the South African Afrikaner population (108). The frequency of Familial hypercholesterolemia in the Afrikaner population is estimated to be roughly five times that of other populations (108). Fanconi Anemia is another disease that can be contributed to founder mutations in the FANCA gene of Afrikaners (109).

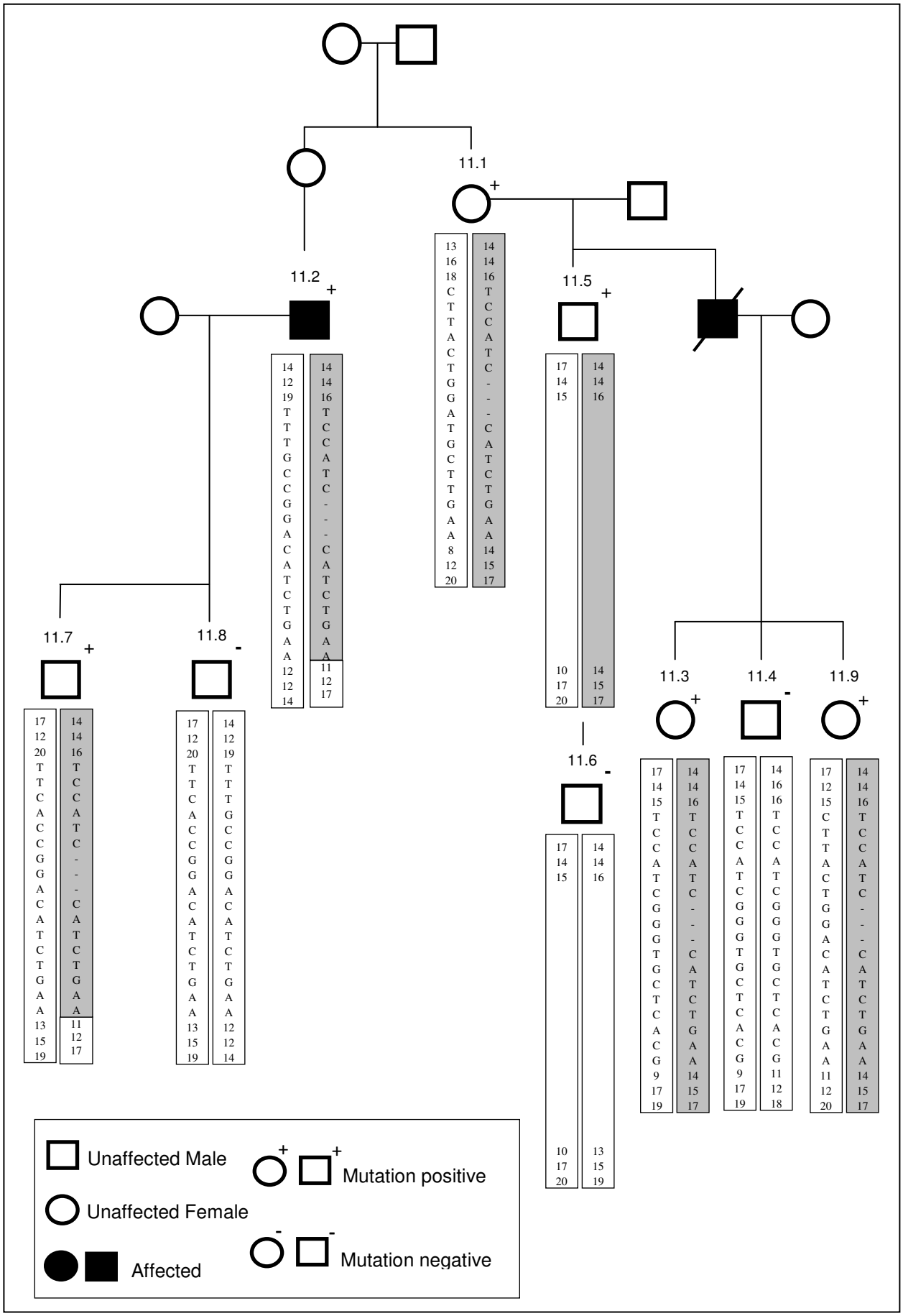

Figure 3.15: Haplotypes of family 11
Grey shaded boxes represent haplotype linked to the disease
SNPs of individuals 11:5 and 11:6 were not typed

DNA was obtained (Dr. JPL Bayley, Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands) from two unrelated Dutch individuals, both carrying the *SDHB* exon 3 deletion. These two individuals were also subjected to genotype analysis (Appendix H) in order to compare the common haplotype of the two populations and establish whether this mutation occurred only once or multiple times in the different populations. The haplotype linked to the disease was already known (91) and could be deduced. The sexes of the two individuals are not known.

Comparing the haplotypes of the Dutch and SA patients (figure 3.16), an identical haplotype was observed from marker D1S436 to D1S2826 between the populations. Only one microsatellite marker (D1S2644) differs between the two populations. Interestingly, this is the same marker that differs from the common SA haplotype in family 5. This microsatellite marker is the furthest from the *SDHB* gene of the six markers and thus a recombination event is very likely as it occurred between D1S2644 and D1S2826 in the SA patients. The core linked haplotype in the Dutch population (~2.56Mb) is still identical to the common linked SA haplotype.

According to these findings It is thus highly likely that the Dutch and SA patients share a common ancestor and that this mutation was introduced by the Dutch to the South African Afrikaner population, especially since there are a large number of Afrikaner families with Dutch ancestry in SA.

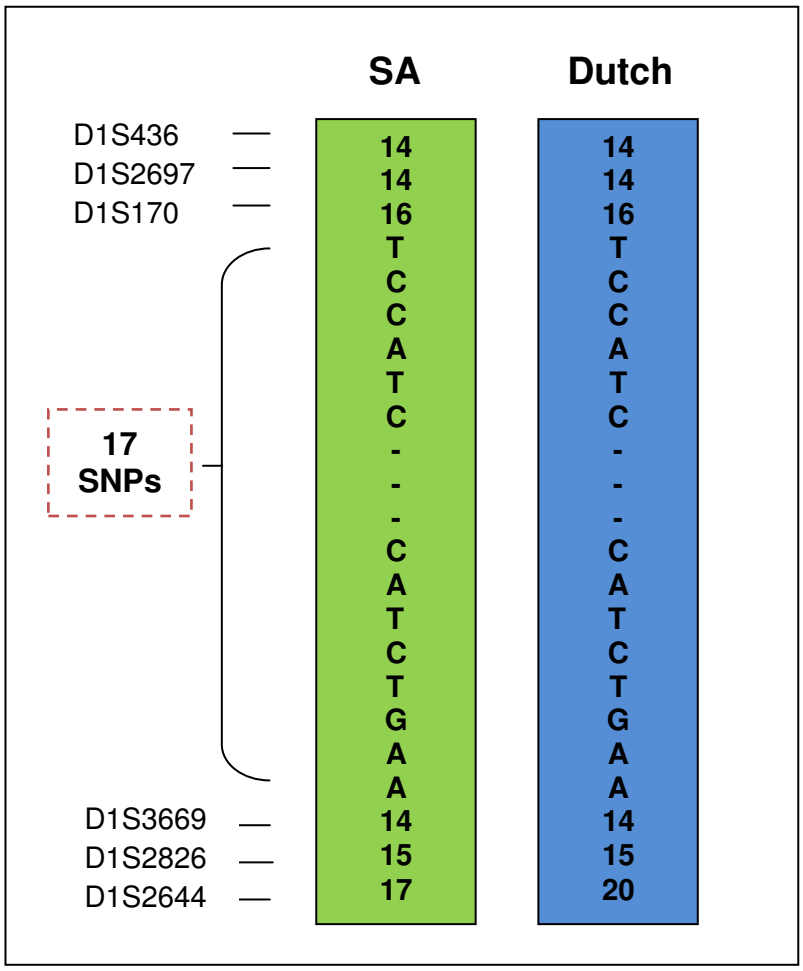

Figure 3.16: Comparison of Dutch and South African common haplotypes

3.6 NON – PENETRANCE

After the identification of this deletion, mutation analysis was also carried out on 51 seemingly sporadic cases. One of these patients (SGT 58) was diagnosed at the age of 28 years. Upon further testing of other unaffected individuals in this family, her father and son were also found to carry the deletion (appendix B). These individuals only arrived for genetic testing after this study was almost complete and no genotype analysis was carried out on the individuals in this family. It is thus not a sporadic case, but a family presenting with non-penetrance.

Reduced penetrance was also seen in 50% of the South African PGL families (HGT 3, HGT 9, HGT 11, HGT 13, HGT 14).

At least one mutation positive individual, older than the proband or other affected individuals, did not present with PGL and/or PCC. The mean age of the individuals presenting with non-penetrance is ~70 years with the ages ranging from 63 years up to 78 years. *SDHB* mutation carriers have a family history in only 31% of cases which is much lower than patients carrying *SDHD* (61%) and *SDHC* (62.5%) mutations (50). The Dutch patients carrying the same *SDHB* deletion also showed reduced-penetrance. Only two of the nine cases (22%) presented with a family history of PGL and thus 78% did not present with any family history of PGL (91). Another forty five family members of the mutation positive individuals from South Africa were screened for this mutation. Twenty three of these individuals presented with the mutation of whom only two have been diagnosed with PGL. One patient was diagnosed at the age of 29 with a carotid body tumour and the other patient also presented with a carotid body tumour (Left) at the age of 61 years. The ages of the mutation positive individuals ranged from 12 years up to 78 years with a mean age of ~41 years (SD= 18.87).

In general, abdominal tumours are predominantly the phenotype seen in patients with mutations in the *SDHB* gene. However head and neck PGL (HN-PGL) were mainly identified in the SA patients (Table 3.4). This may be due to an ascertainment bias as patients were mostly referred by otolaryngologists thus explaining the higher than expected HN-PGL frequency. The Dutch patients identified with the same deletion also showed a high frequency of HN-PGL although this might also be due to a referral bias as Leiden is a national referral centre for HN-PGL. The mean age at diagnosis of the South African individuals was 37 years (SD = 15.53) while malignant tumours were identified in five of the families (table 3.3).

Table 3.3: Clinical information of PGL families

Family number	Mean Age at Dx	Anatomical site of tumours
HGT 1	39 yrs	Glomus Jugulare and jugulotympanicum
HGT 2	13 yrs	Abdominal PGL, Bilateral Jugulare PGL, bilateral secreting carotid body, Malignant PGL
HGT 3	21 yrs	Left jugulo-tympanicum PGL, Malignant abdominal PGL
HGT 4	54 yrs	PGL of carotid body, Glomus Jugulare
HGT 5	47 yrs	Carotid body PGL, Jugulotympanicum PGL, Malignant PGL, Glomus Jugulare
HGT 8	30 yrs	Glomus Jugulare,PGL of Carotid body and - Jugulo-tympanicum
HGT 9	36 yrs	Carotid body PGL, Malignant PGL, pheochromocytoma
HGT 11	30 yrs	Abdominal PGL, Carotid body, Malignant PGL
HGT 13	72 yrs	PGL of jugulo-tympanicum, Vagus nerve
HGT 14	42 yrs	Carotid body PGL, Jugulotympanicum
SGT 58/HGT 15	28 yrs	Jugulare PGL

There seems to be a higher incidence of malignancies in patients carrying *SDHB* mutations than patients carrying mutations in the *SDHC* and *SDHD* susceptibility genes (50). These patients also have an increased risk of developing gastrointestinal stromal tumours (GISTs) as well as renal cell carcinoma (110). The presence of an *SDHB* mutation is associated with an excess mortality and it is thus expected for *SDHB* mutation carriers to have a decreased life expectancy in comparison to other *SDH* gene mutation carriers (62, 110). It is advised for patients presenting with an *SDHB* mutation to undergo an annual to bi-annual full-body computed tomography (CT) scan or magnetic resonance imaging (MRI). MRI and CT scans have excellent sensitivity, but lack specificity and thus should be followed by Positron emission tomography (PET) with 2-deoxy-2-[fluorine-18] fluoro-D-glucose (^{18}F-FDG) where indicated in order to identify any tumours or malignancies (110).

In conclusion, we have identified an *SDHB* founder mutation in South African, Afrikaner families identical to the previously reported *SDHB* exon 3 deletion in the Dutch. This mutation is the first of its kind to be reported in South Africa. The Afrikaner and Dutch deletion-positive individuals seem to share a common haplotype, with the exception of a single microsatellite marker. It is very likely that this mutation was introduced into South Africa by the Dutch.

The mutation-positive Dutch and Afrikaner families thus share common ancestry. It is possible that large genomic rearrangements might be responsible for a larger proportion of disease-causing mutations than is currently listed. Technology to identify large rearrangements should be made readily available and results listed on mutation databases. Screening for possible large rearrangements in patients with PGL is thus validated, especially for the patients without point mutations in the SDH susceptibility genes. These results hold great promise for future predictive testing of PGL in family members, facilitating early diagnosis of PGL, reducing morbidity and mortality. This will ensure better clinical management of patients and their families.

Chapter 4: Conclusions

Paraganglioma is a very rare disease with only an estimated 3-8 cases per million annually, however it may lead to significant morbidity. These tumours grow very slowly and are mostly only identified when the tumour has grown substantially, they may also become malignant. Early treatment of these tumours may result in a significant decrease of morbidity and mortality through the identification of at-risk individuals who need proper surveillance and treatment. It is thus very important to identify at-risk individuals as it may lead to early detection of the disease as well as appropriate clinical intervention for the patient and his/her family members.

It is believed that SDH-tumourigenesis is associated with the HIF/angiogenesis pathway but it is still unclear what exactly the disease-causing mechanism is (8, 18, 111). The most likely mechanism is that protein truncation causes inhibition of succinate-ubiquinone activity leading to accumulation of succinate because the oxidation of succinate to fumarate cannot occur (illustrated in section 1.3.2). The increased succinate levels block hydroxylation of HIF1 by prolyl hydroxylase which stabilises the HIF1α levels before passing through the cell nucleus and combining with HIF1β. An active HIF complex is formed which increases the expression of factors responsible for angiogenesis, growth and cell division (4). Succinate dehydrogenase is part of the Citric acid cycle as well as the electron transport chain in which electrons are transferred to ubiquinone and to the other complexes in order to produce ATP. This ATP is thus used for energy for most biological processes which will then most likely not occur due to the lack of ATP production.

Approximately 35% of all paragangliomas are predicted to be hereditary and caused by germline mutations in several susceptibility genes. A large proportion of these mutations are frameshift - , missense – and nonsense mutations with large rearrangements accounting for only a small proportion of the total mutational burden. On average, in the *SDHB*, *SDHC* and *SDHD* genes, large deletions are responsible for ~9% of all mutations. This data is based mostly on populations in Europe including Dutch, Spanish and French as well as patients from the USA, UK and China. No studies on the burden of mutations in the PGL susceptibility genes of the South African population have been carried out.

Previously, 11 South African families were screened for point mutations in the *SDHB*, *-C*, and *–D* genes and only one family carried a disease-causing point mutation in the *SDHB* gene. No other point mutations were identified.

This study aimed to identify disease-causing mutations in 10 South African PGL families. At the time *SDHAF2* was identified as a PGL susceptibility gene and these 10 families were investigated for point mutations in the gene. The exons and exon-intron boundaries of *SDHAF2* were amplified by PCR, subject to cycle sequencing and analysed for mutations. No mutations were identified in any of the individuals and thus our focus shifted to the identification of large genomic rearrangements in the susceptibility genes. Multiplex ligation-dependent probe amplification (MLPA) was used for the identification of large rearrangements in the *SDHB*, *-C*, *-D* and *SDHAF2* genes. We identified an *SDHB* exon 3 deletion in all 10 families. After breakpoint characterisation, the deletion was identified as c.201-4429_287-933del, a 7905bp deletion in *SDHB* removing exon 3. This mutation causes a truncated protein, p.Cys68HysfsX21.

An identical mutation was also previously identified as a founder mutation in the Dutch population. Interestingly, a large number of founder mutations have been identified in the SDH genes of the Dutch population. In a previous study it was shown that 88.8% of all SDH mutation carriers in the Netherland carry one of six Dutch founder mutations of which two were identified in the *SDHB* gene (112). The reason for the prevalence of founder mutations in the Dutch is thought to be due to the social segregation of the Dutch society until well into the twentieth century. Religious differences mainly caused the segregation of the Dutch society creating genetically isolated populations due to the limitation of intermarriage. This facilitated the proliferation of Dutch founder mutations.

Haplotype analysis was carried out in order to compare the haplotypes of the South African individuals to each other and to the Dutch. The results revealed a common haplotype between the South African individuals, although six individuals from 3 families seem to have undergone recombination. The South African individuals thus share a common ancestor.

The haplotype of the South Africans and the Dutch also seem to be identical apart from one microsatellite that differs between the two populations. This specific microsatellite is the furthest from the gene on the side of the centromere and thus the difference in number of repeats is likely due to recombination. This means that the SA and Dutch populations share a common haplotype the mutation occurred once and was not due to two separate events. The Dutch and SA individuals, carrying the identical *SDHB* deletion, all share common ancestry.

Mutation analysis was also carried out on 51 seemingly sporadic samples in our laboratory and one patient was found to carry the *SDHB* exon 3 deletion. After genetic testing was carried out on other members of her family, the results showed that the individual's father and son carried the *SDHB* deletion. It is noteworthy that at least 50% of the SA families show non-penetrance. Two more families show possible non-penetrance although there was not enough information acquired to confirm this. The Dutch patients carrying the same mutation also show reduced-penetrance with only 22% of the patients presenting with a family history of PGL. Previous studies show that on average only 31% of *SDHB* mutation carriers have a family history of PGL and/or PCC. This is thus very similar to the SA families of whom only 30% seem to show a family history of PGL. The mean age at diagnosis of the South African individuals was 37 years.

Abdominal tumours are predominantly the phenotype seen in patients carrying mutations in the *SDHB* gene. The patients carrying the *SDHB* mutation mostly suffered from head-and-neck tumours although 3 families presented with abdominal tumours. This might be due to a referral bias due to the fact that all patients were referred to us by an otolaryngologist and may possibly explain the higher rate of head and neck tumours. Malignant tumours were also identified in 5 of the 10 families which correlates to previous findings that there seems to be a higher incidence of malignancies in patients carrying *SDHB* mutations than patients carrying mutations in any of the other susceptibility genes (50). Individuals carrying *SDHB* mutations thus have a decreased life expectancy in comparison to individuals carrying mutations in other SDH genes. An increased risk for the development of gastrointestinal stromal tumours (GISTs) as well as renal cell carcinoma is also associated with mutations in the *SDHB* gene (62, 110).

It is advisable for patients presenting with an *SDHB* mutation to undergo a full-body CT or MRI followed by FDG-PET where indicated to identify any tumours or malignancies.

It is apparent in our study that this germline deletion of *SDHB* exon 3 is linked to reduced penetrance with the age of family members presenting with non-penetrance ranging from 68 years up to 78 years and a mean age of 70 years. Patients with *SDHB*-associated PGL show an increased risk of malignancy when compared to patients with mutations in the *SDHC, SDHD* and *SDHAF2* genes. Malignancy rates in patients with *SDHB* mutations reportedly vary from 34 to 70% (110). This also seems to be apparent in the SA patients carrying the *SDHB* deletion, due to the fact that five of the ten families (50%) presented with malignant tumours. Patients without point mutations in any of the paraganglioma susceptibility genes should thus be screened for possible large rearrangements. The detection of disease-causing mutations will lead to the identification of individuals at-risk of developing PGL, facilitating early diagnosis of PGL and reducing morbidity and mortality. This is very important as it may increase a patient's chances of survival and lead to better clinical management of the patients as well as their family members.

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Appendix A

Copies of Research Ethics Committee Approval

School of Health Sciences Research
Ethics Committee
University of Pretoria
Pretoria Academic Hospital
Tel (012) 354 1560 Fax(012) 354 1831

Universiteit van Pretoria
Posbus 667 Pretoria 0001 Republiek van Suid-Afrika
Tel (012) 354-1000 Faks (012) 329-4524
http://www.up.ac.za
Fakulteit Geneeskunde

Date: **26/04/2001**

Number : 91/2001

Title : Hereditary and Sporadic Paraganglioma in South African Patients: Role of the Succinate-Ubiquinone Oxidoreductase Genes for the Large (SDHC) and Small (SDHD) Subunits.

Investigator : Prof E J van Rensburg; Department of Human Genetics; Pretoria Academic Hospital; Pretoria.

Sponsor : **None**

This Protocol and Informed Consent has been considered by the School of Health Sciences Research Ethics Committee University of Pretoria Pretoria Academic Hospital on 25/04/2001 and found to be acceptable.

Dr J.E.Davel	(female) MBChB:Hospital Superintendent
Prof A.P.du Toit	BA;DiplTheo; BA (Hons);MA;DPhil:Philosopher
Prof S.V. Grey	(female)BSc(Hons);MSc; DSc :Deputy Dean
Mrs R Jooste	(female) Dip. Pharm; M Pharm; Pharmacist.
Dr V.O.L. Karusseit	MBChB;MFGP(SA);M.Med(Chir); FCS (SA): Surgeon
Dr S.Khan	(female)MB.BCh.; Med.Adviser (Gauteng Dept.of Health).
Miss B Mullins	(female) BscHons;Teachers Diploma;
Snr Sr J. Phatoli	(female) BCur(Et.Al)Senior Nursing-Sister
Prof H.W. Pretorius	MBChB;M.Med (Psych) MD: Psychiatrist
Prof P. Rheeder	MBChB;MMed(Int);LKI(SA);MSc (KLIN.EPI): Specialist Physician
Dr C F Slabber	MB BCh, FCP (SA) Acting Head; Dept Medical Oncology
Prof De K.Sommers	BChB; HDD; MBChB; MD: Pharmacologist
Dr R Sommers	SECRETARIAT (female)MBChB; M.med (Int);MPhar.Med;

PROF. J.R. SNYMAN; MBChB;M.Pharm.Med; MD:Pharmacologist
CHAIRPERSON of the School of Health Sciences Research Ethics Committee

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

22/05/2012

Number : **S53/2012**

Title : Hereditary paraganglioma in South Africa: An investigation of the Succinate-ubiquinone Oxidoreductase subunit genes *SDHB*, *SDHC* and *SDHD*

Investigator : Nadja Roelofse, Department of Genetics, Human Genetics Section, University of Pretoria (SUPERVISOR: Prof. EJ van Rensburg)

Sponsor : The Cancer Genetics Research Group of the Department of Genetics

Study Degree: **MSc. (Human Genetics)**

This Student Protocol was reviewed by the Faculty of Health Sciences, Student Research Ethics Committee, University of Pretoria on 22/05/2012 and found to be acceptable. The approval is valid for a period of 3 years.

Prof M J Bester	BSc (Chemistry and Biochemistry); BSc (Hons)(Biochemistry); MSc (Biochemistry); PhD (Medical Biochemistry)
Prof R Delport	(female)BA et Scien, B Curationis (Hons) (Intensive care Nursing), M Sc (Physiology), PhD (Medicine), M Ed Computer Assisted Education
Dr NK Likibi	MBB HM – (Representing Gauteng Department of Health) MPH
Dr MP Mathebula	Deputy CEO: Steve Biko Academic Hospital
Prof A Nienaber	(Female) BA (Hons) (Wits); LLB (Pretoria); LLM (Pretoria); LLD (Pretoria); PhD; Diploma in Datametrics (UNISA)
Prof L M Ntlhe	MBChB(Natal); FCS(SA)
Mrs M C Nzeku	(Female) BSc(NUL); MSc Biochem(UCL,UK)
Snr Sr J. Phatoli	(Female) BCur (Et.Al); BTech Oncology
Dr R Reynders	MBChB (Pret), FCPaed (CMSA) MRCPCH (Lon) Cert Med. Onc (CMSA)
Dr T Rossouw	(Female) MBChB.(cum laude); M.Phil (Applied Ethics) (cum laude), MPH (Biostatistics and Epidemiology (cum laude), D.Phil
Mr Y Sikweyiya	MPH (Umea University Umea, Sweden); Master Level Fellowship (Research Ethics) (Pretoria and UKZN); Post Grad. Diploma in Health Promotion (Unitra); BSc in Health Promotion (Unitra)
Dr L Schoeman	(Female) BPharm (NWU); BAHons (Psychology)(UP); PhD (UKZN); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (Female) - MBChB; MMed (Int); MPharMed.
Prof T J P Swart	BChD, MSc (Odont), MChD (Oral Path), PGCHE
Prof C W van Staden	**Chairperson** - MBChB; MMed (Psych); MD; FCPsych; FTCL; UPLM; Dept of Psychiatry

Student Ethics Sub-Committee

Prof R S K Apatu	MBChB (Legon,UG); PhD (Cantab); PGDip International Research Ethics (UCT)
Mr S B Masombuka	BA (Communication Science) UNISA; Certificate in Health Research Ethics Course (B compliant cc)
Mrs N Briers	(female) BSc (Stell); BSc Hons (Pretoria); MSc (Pretoria); DHETP (Pretoria)
Prof M M Ehlers	(female) BSc (Agric) Microbiology (Pret); BSc (Agric) Hons Microbiology (Pret); MSc (Agric) Microbiology (Pret); PhD Microbiology (Pret); Post Doctoral Fellow (Pret)
Dr R Leech	(female) B.Art et Scien; BA Cur; BA (Hons); M (ECI); PhD Nursing Science
Dr S A S Olorunju	BSc (Hons). Stats (Ahmadu Bello University –Nigeria); MSc (Applied Statistics (UKC United Kingdom); PhD (Ahmadu Bello University – Nigeria)
Dr L Schoeman	CHAIRPERSON: (female) BPharm (North West); BAHons (Psychology)(Pretoria); PhD (KwaZulu-Natal); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (Female) MBChB; M.Med (Int); MPhar.Med
Prof L Sykes	(female) BSc, BDS, MDent (Pros)

DR L SCHOEMAN; BPharm, BA Hons (Psy), PhD; Dip. International Research Ethics
CHAIRPERSON of the Faculty of Health Sciences Student Research Ethics Committee, University of Pretoria

DR R SOMMERS; MBChB; M.Med (Int); MPhar.Med.
VICE-CHAIR of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

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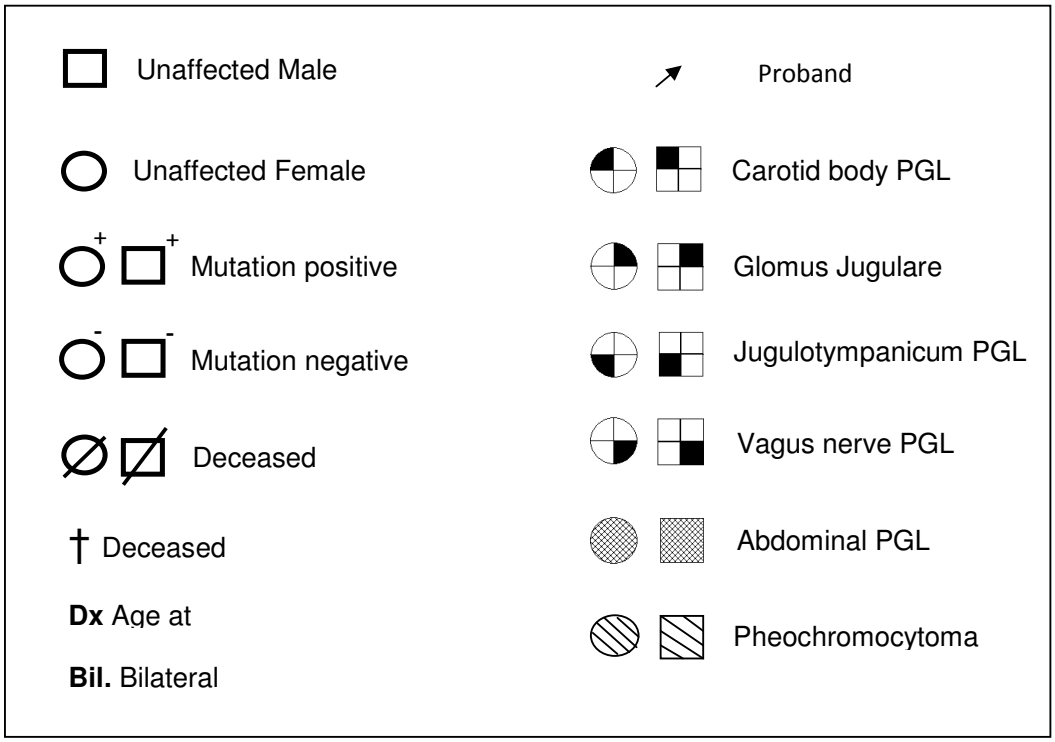
Appendix B

Family Pedigrees and Clinical Information

(Pedigrees have been abbreviated, not all spouses and unaffected children are indicated)

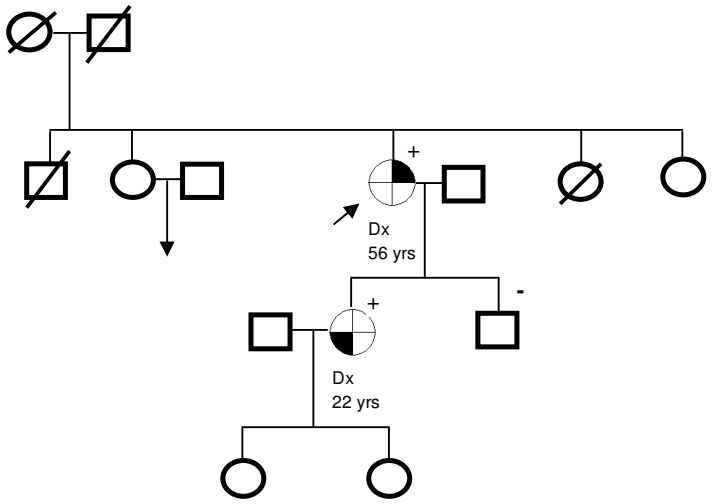
Key



Summary of clinical information

Family number	# Affected individuals	Mean Age at Dx	Anatomical site of tumours
HGT 1	2	39 yrs	Glomus Jugulare and jugulotympanicum
HGT 2	4	13 yrs	Abdominal PGL, Bilateral Jugulare PGL, bilateral secreting carotid body, Malignant PGL
HGT 3	2	21 yrs	Left jugulo-tympanicum PGL, Malignant abdominal PGL
HGT 4	5	54 yrs	PGL of carotid body, Glomus Jugulare
HGT 5	4	47 yrs	Carotid body PGL, Jugulotympanicum PGL, Malignant PGL, Glomus Jugulare
HGT 8	3	30 yrs	Glomus Jugulare,PGL of Carotid body and -Jugulo-tympanicum
HGT 9	4	36 yrs	Carotid body PGL, Malignant PGL, pheochromocytoma
HGT 11	3	30 yrs	Abdominal PGL, Carotid body, Malignant PGL
HGT 13	2	72 yrs	PGL of jugulo-tympanicum, Vagus nerve
HGT 14	5	42 yrs	Carotid body PGL, Jugulotympanicum
HGT 15	1	28 yrs	Jugulare PGL

Family HGT 1



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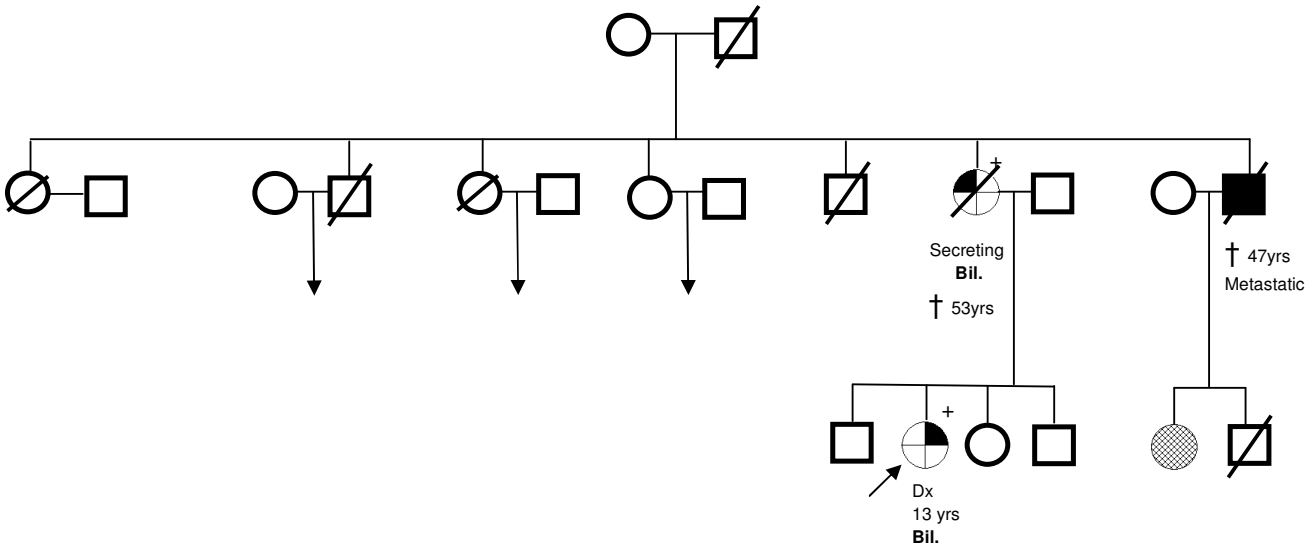
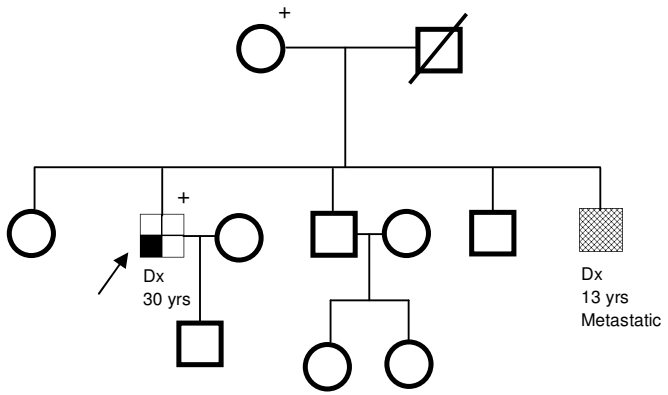


HGT 3



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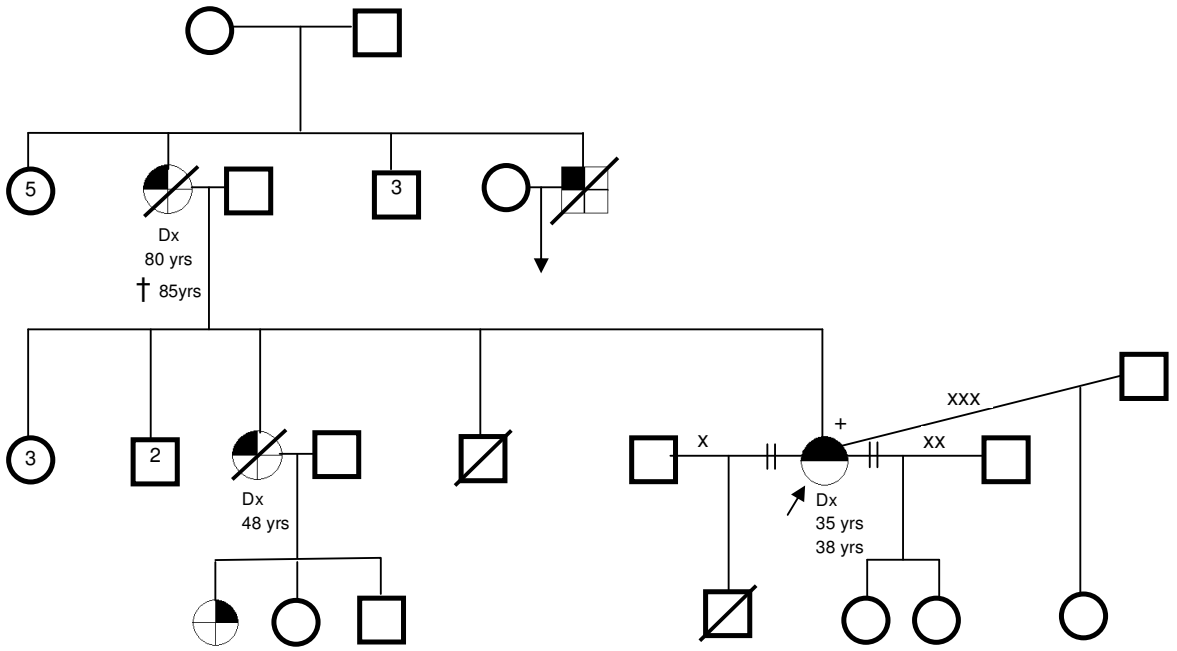

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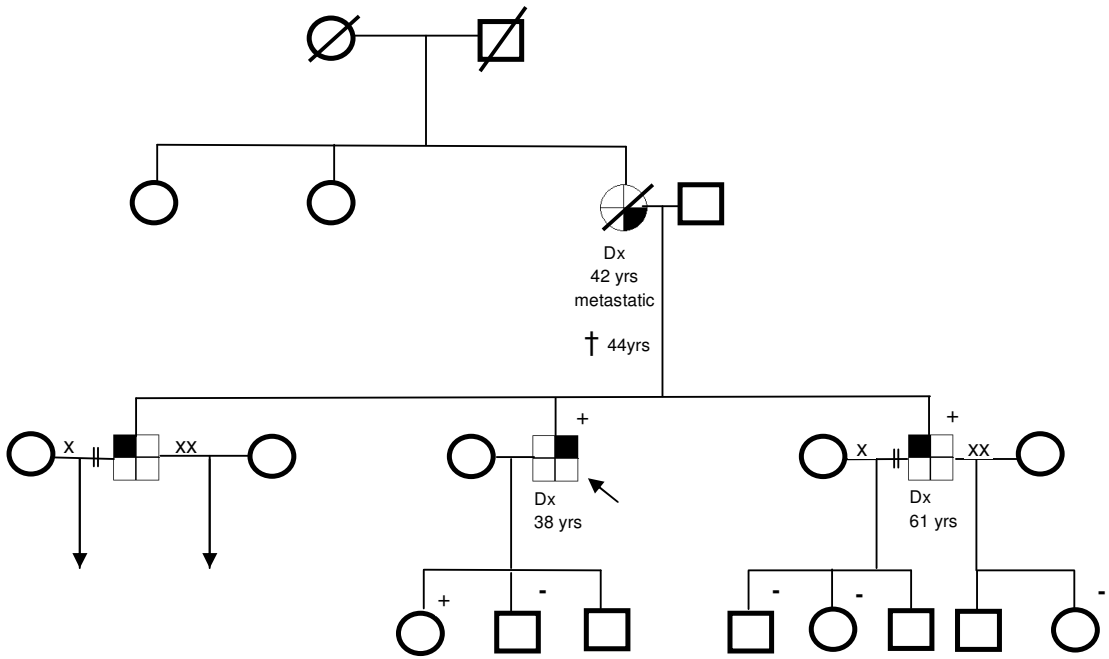
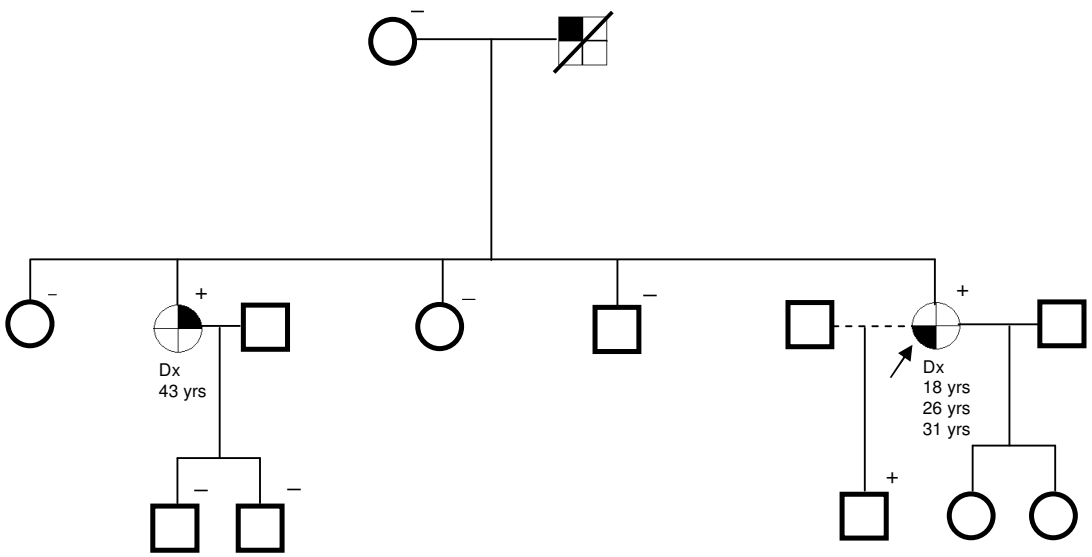


HGT 8



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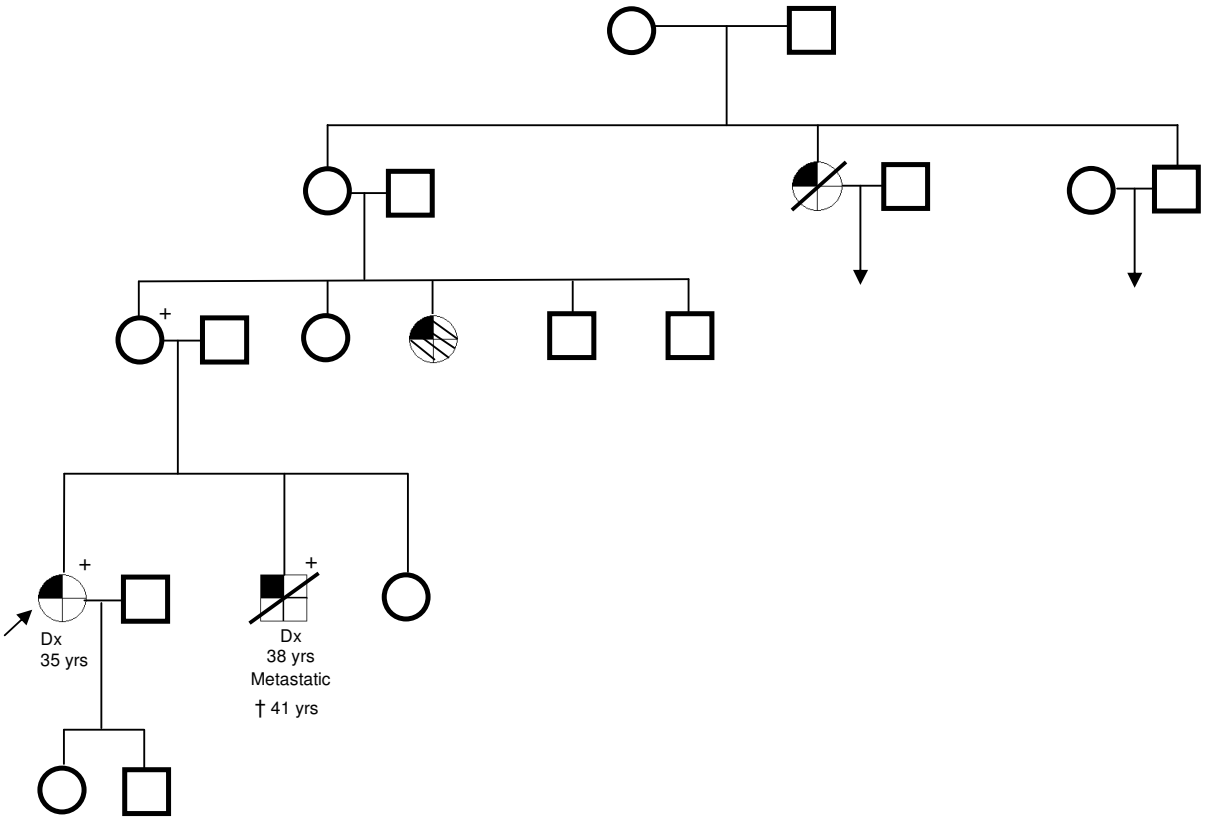

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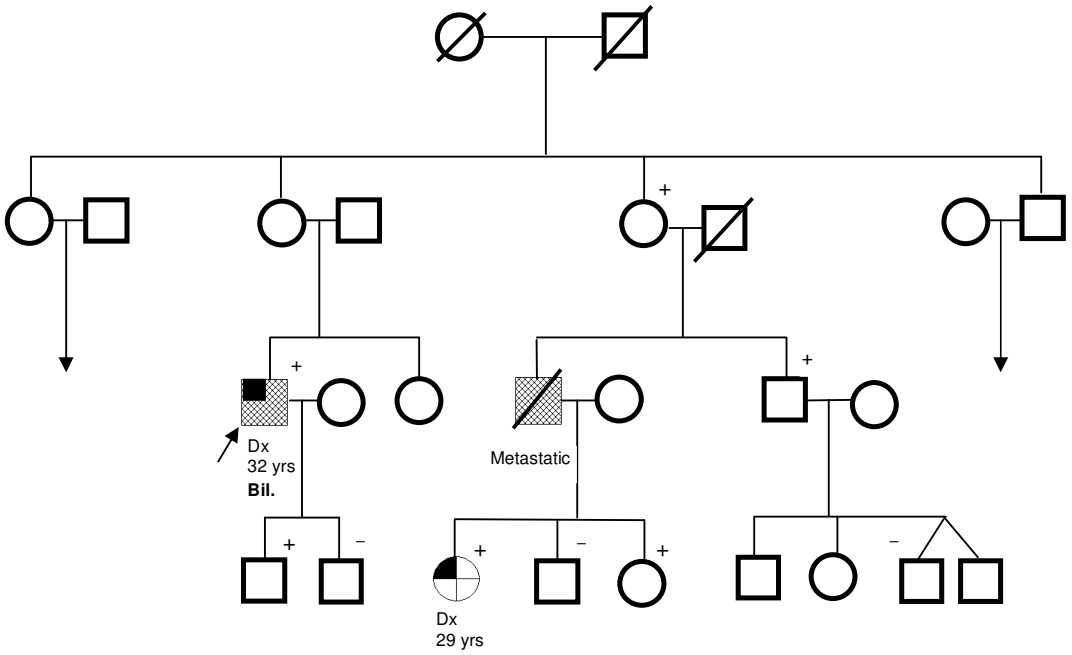

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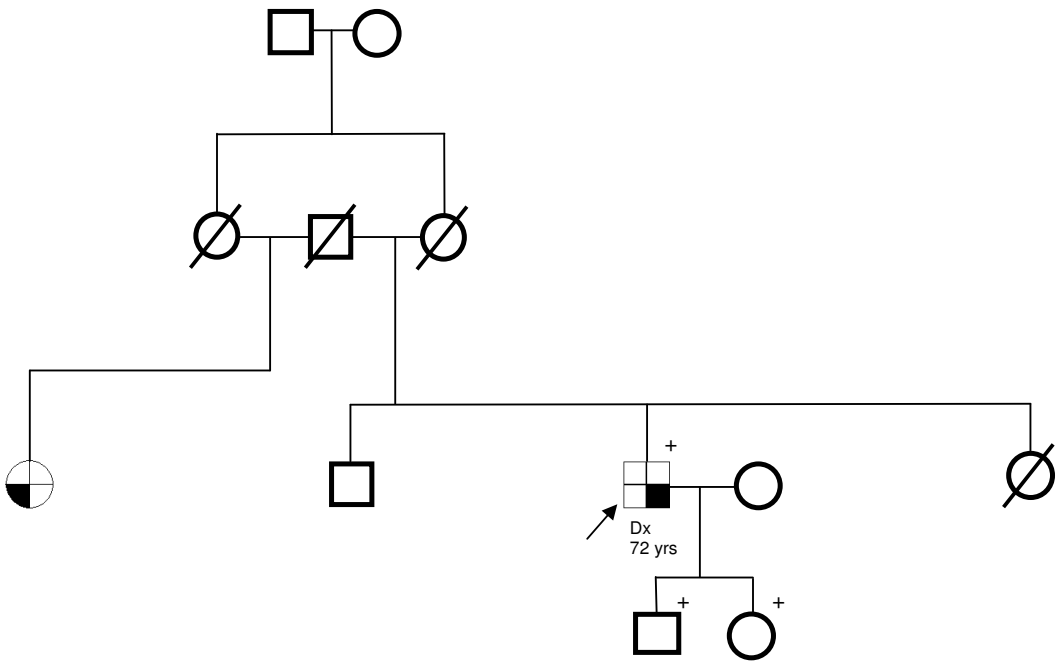

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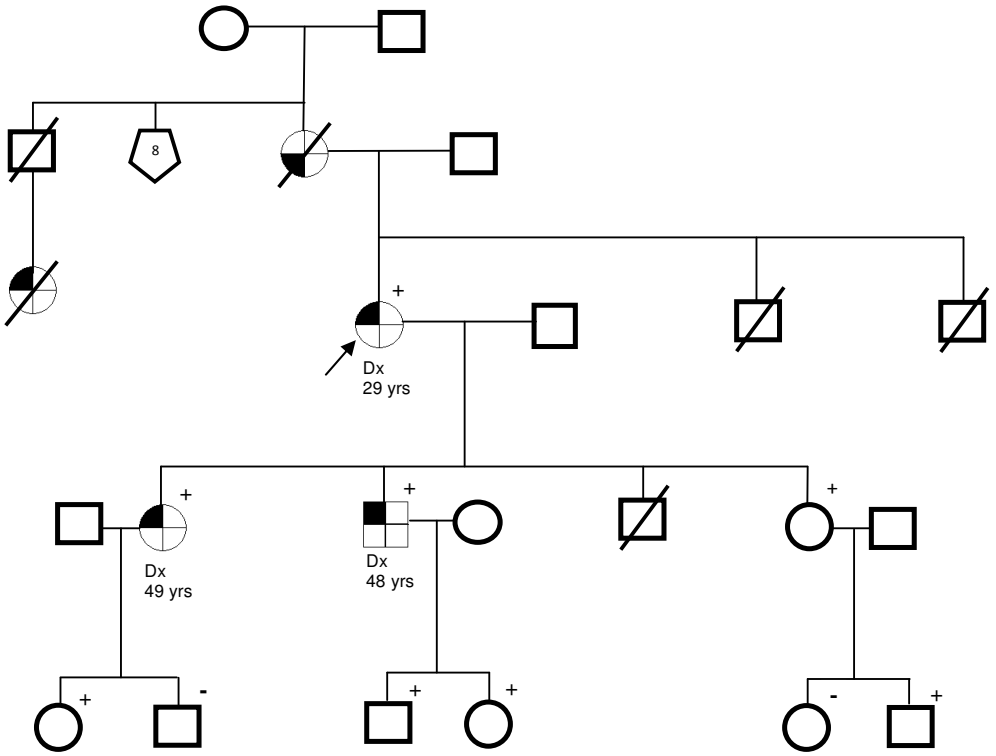
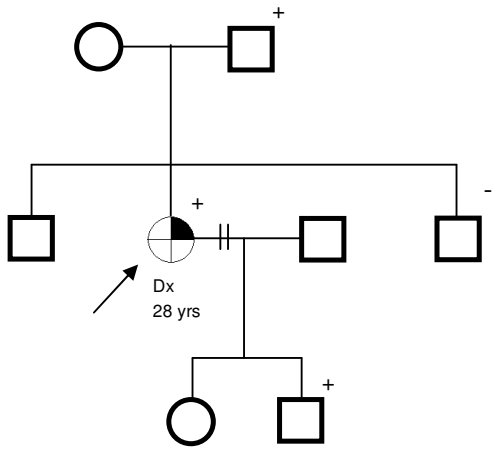


HGT 15 (SGT 58)



APPENDIX C

Detailed recipes of buffers, Agarose Gel and DNA extraction solutions

Lysis buffer

0.32M Sucrose
10mM Tris-Base
5mM MgCl2
1% Triton-x-100
(pH 8)

Suspension buffer

10mM Tris-Base
0.15M NaCl
5mM EDTA
pH8 adjusted with HCL

10% SDS

For 100ml : 10g SDS
100ml sterile H2O

Chloroform:Isoamylalcohol (24:1)

For 1000ml: 960ml Chloroform
40ml IsoAmylalcohol

Tris-EDTA buffer

10mM Tris-Base
1mM EDTA
pH8.2 adjusted with HCL

10x TBE Buffer

TRIS 216g
0.5M EDTA (pH8) 80ml
Boric acid 110g
Make up to 2l (pH8)

1x TBE Buffer

Make a 10x dilution of the 10x TBE buffer and use for gel electrophoresis

Agarose gel (1%)

1g Agarose (Gibco BRL, Life Technologies)
100ml 1x TBE buffer
100µl (1000x) EtBr/Sybr Safe

Agarose gel (1.6%)

1.6g Agarose (Gibco BRL, Life Technologies)
100ml 1x TBE buffer
100µl (1000x)EtBr/Sybr Safe

10x Agarose loading buffer

15% Ficoll (Type 400) with ddH2O made up to a volume of 10ml
0.25% bromophenol blue

Appendix D

Primer sequences and optimised PCR conditions

***SDHAF2* PCR primer sequences**

Primer	Sequence (5'-3')
1F 1R	ACCTTCCGGCTCAGCTC TATCGGGCAGACGAACTC
2F 2R	GTTGACCTTCCCAGGCTC GAGGTTCAGCTGCTTTTCTG
3F 3R	GACACAGCCTTCTCAACCTC CTCAAATCAGCCTAAACTGTCC
4AF 4AR	CCCTGGTATAGGCTAACATCG TGAGTACACTTGGGCTGAGG
4BF 4BR	AGCTCTGAGCCTCAAAAGTG GAAGACTGTAGGAATGAGGGG

***SDHAF2* PCR conditions**

Exon	[$MgCl_2$]	T_{ann}	Product Size
1	1,5mM	61°C	242bp
2	2,5mM	60°C	786bp
3	1,5mM	57°C	215bp
4A	1,5mM	58°C	663bp
4B	1,5mM	58°C	614bp

Primers used for Long Range PCR across exon 3

Primer	Sequence (5'–3')
SDHB 2F	TTTTTCCTTTTTGTGAACTTT
SDHB 4R	TGCAAATAAAAACAAAACCA
*SDHB*_2162F	CCAGTCCATGAAAGGCA
*SDHB*_2164R	GCTCCATGTGTCACGTGTTT

Long Range PCR conditions

Primers	T_{ann}	Product Size
SDHB 2F& 4R	45°C	16360bp
SDHB_2162F& 2164R	55°C	9523bp

Genotype analysis PCR Primer sequences(microsatellites)

Microsatelite	Forward Primer (5'-3')	Reverse Pimer (5'-3')
D1S436	TGAATGTGTCTCCAGTGTTAGC	CTGTAGAGCAATCTGGCAATATGT
D1S3669	TTTTGTTTCTTGATCTGGGC	TGTTAAACTTTTCACTGAGGTATAA
D1S2697	GGGCCACAGAGTGAGAC	GGCAGAGGTGGTTAAGG
D1S170	CACTCAGGCAGGTGCATG	GAATCTTGTGCATGGTGTGG
D1S2826	TCCCAGGTCCGAGAAGAACAGG	GCCCCTGGTCGTGCTGGTTTT
D1S2644	TGCAACCCACCTGAATGA	TACGTGAAGTGCCAGCACA

***SDHB*Genotype analysis PCR conditions (Microsatelites)**

Primer	[$MgCl_2$]	T_{ann}	Product Size
D1S436	2mM	54°C	200-240bp
D1S2826	2mM	60°C	288bp
D1S170	1,5mM	58°C	217bp
D1S2697	2mM	61°C	273-281bp
D1S2644	1,5mM	58°C	215-230bp
D1S3669	2,5mM	52°C	179-211bp

Genotype analysis PCR Primer sequences(SNPs)

Primer	Sequence (5'-3')
B_IVS1 F	TCCTCTCACTTCCTCCCAAATACCAC
B_IVS1 R	TCACTACAGCCTCTGATTCGTCTCG
B_IVS2 F	TGTAAGCTGAGGGCTGTTCCAAGC
B_IVS2 R	TCGGTCCAAAACTGTGGTCTGTGC
B_IVS3 F	AGCTCTCTGCTGGGTGAACTTGC
B_IVS3 R	ACATGCTGAGGGAGACACTGTACC
B_IVS5 F	TTGACCCAGTGTGGCAGGTGTG
B_IVS5 R	CCTGGAATGAGGTCAAGACGGGA
B_IVS6 F	GGGCCTGGTTTCCTTCATTGCC
B_IVS6 R	ACAGCTTCCAATCTCATAGCTGGGC
B_IVS7 F	GAGGGGAACCTTCACTAACGTC
B_IVS7 R	AGGGGTTGGGCTAAGGGCTCTCTTC

Genotype analysis PCR conditions (SNPs)

Primer	[$MgCl_2$]	T_{ann}	Product Size
B_IVS1	2mM	56°C	372bp
B_IVS2	1,5mM	58°C	517bp
B_IVS3	1,5mM	58°C	365bp
B_IVS5	1,5mM	59°C	372bp
B_IVS6	3mM	59°C	303bp
B_IVS7	1,5mM	59°C	550bp

Appendix E

MLPA probe ligation sequences and kit components

***SDHB* probes according to chromosomal location**

SALSA MLPA probe	*SDHB* Exon	Ligation site Nm_003000.2	Partial Sequence
7801-L8397	Promotor	1052 nt before exon 1	CAGGATGATT-GCTTGAGCCC
7342-L6974	Exon 1	26 nt after exon1	TCCTGACTTT-TCCCTCTCTG
7803-L6975	Exon 2	227-228	AGCCCAGACA-GCTGCAGCCA
7344-L6976	Exon 3	372-373	TGGTATTGGA-TGCTTTAATC
7345-L8400	Exon 4	461-462	TGTGCAATGA-ACATCAATGG
7804-L6978	Exon 5	619-620	AGAAGGATGA-ATCTCAGGAA
7347-L6979	Exon 6	702-701 reverse	ATGCACTCGT-AGAGCCCGTC
7348-L6980	Exon 7	910-911	AAGGACCTGT-CCTAAGGTAC
7349-L6981	Exon 8	959-960	AAGAAAATGA-TGGCAACCTA

***SDHC* probes according to chromosomal location**

SALSA MLPA probe	*SDHC* Exon	Ligation site	Partial Sequence
07350-L16209	Promotor	429 nt before exon 1 reverse	TTGGCCGGTTGA-GACCCCGAAGAG
11609-L12369	Exon 1	62 nt after exon 1 reverse	TCAGCAAAACGT-GAGGGGCCAGTT
07352-L14876	Exon 2	55-56	CTTGCAGACACG-TTGGTCGTCATT
14642-L16292	Exon 3	117-118	AGTGCTGTTCCT-TTGGGAACCACG
14641-L16291	Exon 3	91 nt after exon 3	CTTCCCTCACTT-TTACTCAACCAA
07354-L06986	Exon 4	219-220	AGTTGGTCTCTT-CCCATGGCGATG
14644-P0226-L16294	Exon 4	15 and 51 nt after exon 4	TGTATATGTGTT-36nt spanning oligo-CTGTTTCATTGG
07355-L06987	Exon 5	283-284	GGGTCTCTCTTT-TTGGCATGTCGG
07356-L06988	Exon 6	446-447	GATGTGGGACCT-AGGAAAAGGCCT

***SDHD* probes according to chromosomal location**

SALSA MLPA probe	*SDHD* Exon	Ligation site	Partial Sequence
07357-L16211	Promotor	375 nt before exon 1	TTCGTGAGGGGA-ATGGGATGCAGC
07358-L14875	Exon 1	112-113	CTAGGAGGCCGA-GGTGAGGGGTCT
07359-L06991	Exon 2	119-120	CCTCAGCTCTGT-TGCTTCGAACTC
07360-L16212	Exon 3	2 nt after exon 3	ATGGTCACTGGC-AAGTATAGCAAT
07361-L06993	Exon 4	862-863	AAGAGAATCCAA-CTTTATTACGAT

***SDHAF1* probes according to chromosomal location**

SALSA MLPA probe	*SDHAF1* Exon	Ligation site	Partial Sequence
14638-L16288	Exon 1	542-543	AGCTTGACGAAT-TGGGGATGTCAG

***SDHAF2* probes according to chromosomal location**

SALSA MLPA probe	*SDHAF2* Exon	Ligation site	Partial Sequence
14639-L16289	Exon 1	47-48	CAGTGTTCTCGA-CTTCGTCGCTGG
14643-L16293	Exon 3	328-329	GAAAAGCAGCTG-AACCTCTATGAC
14646-L16296	Exon 4	519-520	TGAAAAGCCACG-TTGAGCTGTGCT

SALSA MLPA kit component	Ingredients
SALSA MLPA buffer	KCl, Tris-HCl, EDTA and PEG-6000.pH 9.5
SALSA Ligase-65	Glycerol, BRIJ (0.05%), EDTA, Beta-Mercaptoethanol (0.1%), KCl, Tris-HCl. pH 7.5
Ligase-65 buffer A	NAD (bacterial origin). pH 3.5
Ligase-65 buffer B	Trizma, Tween-20, Nonidet-P-40, $MgCl^2$. pH 8.5
SALSA PCR buffer	Tris, KCl, Tween-20, Nonidet P-40, Trizma, $MgCl^2$. pH 8.5
SALSA PCR primer mix	FAM, Cy5.0, IRD800 or other fluorescent labelled primers, dNTPs, Tris, EDTA,. pH 8
SALSA polymerase	Glycerol, BRIJ (0.5%), EDTA, DTT (0.2%), KCl, Tris-HCL, Polymerase enzyme (bacterial origin). pH 7.5
SALSA Enzyme dilution buffer	Tris-HCl, KCl, EDTA, BRIJ (0.04%). pH 8.5
Probemix	Tris, EDTA, synthetic oligonucleotides, oligonucleotides from purified bacteria. pH 7.5

APPENDIX F

MLPA ResultsSpreadsheets

HGT 1:1

TEST SAMPLE RESULTS

Test 1	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT1.1_B05_002.fsa	C1 5q31	1.00	0.97	0.89	1.02	1.01	0.91	1.00	0.98	0.97	0.96	0.82	0.95	0.96	0.53	0.88	1.07	0.97	0.76	0.93	0.80	0.97	0.95	0.96	0.99	0.95	0.95	0.93	1.04	0.98	0.98	1.03	0.90	1.03	0.94	0.90	0.95	0.79
	Operator	C2 12p13	1.04	1.00	0.92	1.05	1.04	0.94	1.04	1.01	1.01	0.99	0.85	0.98	1.00	0.55	0.91	1.11	1.00	0.78	0.96	0.83	1.00	0.98	0.99	1.02	0.98	0.99	0.96	1.08	1.02	1.01	1.07	0.93	1.07	0.97	0.93	0.99	0.82
	nadja	C3 7q31	1.13	1.09	1.00	1.15	1.13	1.02	1.13	1.10	1.10	1.08	0.93	1.07	1.08	0.59	0.99	1.21	1.09	0.85	1.04	0.90	1.09	1.06	1.07	1.11	1.07	1.07	1.04	1.17	1.10	1.10	1.16	1.01	1.16	1.06	1.01	1.07	0.89
	Worksheet	C4 20p13	0.98	0.95	0.87	1.00	0.99	0.89	0.98	0.96	0.96	0.94	0.81	0.93	0.95	0.52	0.87	1.05	0.95	0.74	0.91	0.78	0.95	0.93	0.94	0.97	0.93	0.93	0.91	1.02	0.96	0.96	1.01	0.88	1.01	0.92	0.88	0.94	0.78
	1	C5 8p23	0.99	0.96	0.88	1.01	1.00	0.90	0.99	0.97	0.97	0.95	0.82	0.94	0.96	0.52	0.88	1.07	0.96	0.75	0.92	0.79	0.96	0.94	0.95	0.98	0.94	0.95	0.92	1.04	0.97	0.97	1.03	0.90	1.03	0.93	0.89	0.95	0.79
	Int QC Stand Dev	C6 14q24	1.10	1.07	0.98	1.12	1.11	1.00	1.10	1.08	1.07	1.06	0.91	1.05	1.06	0.58	0.97	1.18	1.07	0.84	1.02	0.88	1.07	1.04	1.05	1.09	1.05	1.05	1.02	1.15	1.08	1.08	1.14	0.99	1.14	1.04	0.99	1.05	0.87
	0.044182378	C7 12q24	1.00	0.97	0.89	1.02	1.01	0.91	1.00	0.98	0.97	0.96	0.82	0.95	0.96	0.53	0.88	1.07	0.97	0.76	0.93	0.80	0.97	0.95	0.96	0.99	0.95	0.95	0.93	1.04	0.98	0.98	1.03	0.90	1.03	0.94	0.90	0.95	0.79
		C8 8q24	1.02	0.99	0.91	1.04	1.03	0.93	1.02	1.00	0.99	0.98	0.84	0.97	0.98	0.54	0.90	1.10	0.99	0.77	0.95	0.81	0.99	0.97	0.98	1.01	0.97	0.97	0.94	1.06	1.00	1.00	1.05	0.92	1.06	0.96	0.92	0.97	0.81
		C9 15q26	1.03	0.99	0.91	1.05	1.03	0.93	1.03	1.01	1.00	0.98	0.85	0.97	0.99	0.54	0.91	1.10	1.00	0.78	0.95	0.82	1.00	0.97	0.98	1.01	0.98	0.98	0.95	1.07	1.01	1.01	1.06	0.93	1.06	0.96	0.92	0.98	0.81
		C10 7p14	1.04	1.01	0.93	1.06	1.05	0.95	1.05	1.02	1.02	1.00	0.86	0.99	1.01	0.55	0.92	1.12	1.01	0.79	0.97	0.83	1.01	0.99	1.00	1.03	0.99	0.99	0.97	1.09	1.02	1.02	1.08	0.94	1.08	0.98	0.94	1.00	0.83
		MEAN	1.03	1.00	0.92	1.05	1.04	0.94	1.03	1.01	1.01	0.99	0.85	0.98	1.00	0.54	0.91	1.11	1.00	0.78	0.96	0.82	1.00	0.98	0.99	1.02	0.98	0.98	0.96	1.08	1.01	1.01	1.07	0.93	1.07	0.97	0.93	0.99	0.82
	ODDS NORMAL	:DEL	640:1	970:1	163:1	38995:1	9570:1	2668:1	5009:1	790:1	102425:1	6132:1	21:1	951:1	11717:1	1:128	2977:1	494:1	1615:1	34:1	6462:1	26:1	186:1	135387:1	17000:1	6912:1	15141:1	288:1	16555:1	12936:1	17348:1	830:1	4282:1	1060:1	3845:1	1130:1	212:1	60010:1	30:1
	ODDS NORMAL	:DUP	63:1	22:1	87:1	231:1	80:1	107:1	51:1	16:1	1269:1	115:1	5:1	195:1	1675:1	4:1	163:1	4:1	32:1	16:1	178:1	8:1	7:1	2558:1	301:1	83:1	291:1	11:1	449:1	55:1	210:1	17:1	26:1	50:1	24:1	34:1	14:1	1026:1	10:1
	PROB OF DEVIATION	NORMAL	67.2956%	97.2883%	22.4359%	21.0810%	56.5913%	32.8795%	69.1929%	93.8013%	91.6533%	85.9100%	48.5169%	71.2552%	97.1918%	0.1805%	7.3745%	49.0503%	99.5793%	1.0903%	48.1589%	28.6494%	99.9686%	37.3894%	78.9398%	81.3647%	71.4097%	91.1060%	29.7547%	16.2934%	84.7863%	93.9429%	41.9815%	41.9791%	42.5772%	75.4832%	60.4529%	66.8129%	21.6932%
	PROB OF DEVIATION	DELETED	0.0105%	0.1003%	0.0136%	0.0005%	0.0059%	0.0123%	0.0138%	0.1888%	0.0009%	0.0140%	2.2681%	0.0075%	0.0007%	23.1046%	0.0025%	0.0993%	0.0617%	0.0322%	0.0075%	1.1034%	0.5372%	0.0003%	0.0046%	0.0186%	0.0047%	0.3164%	0.0018%	0.0013%	0.0049%	0.1132%	0.0098%	0.0396%	0.0111%	0.0668%	0.2852%	0.0011%	0.7304%
	PROB OF DEVIATION	DUP	1.0741%	4.3417%	0.2568%	0.0910%	0.7057%	0.3069%	1.3636%	5.7983%	0.7723%	0.7447%	9.0045%	0.3647%	0.0521%	0.0501%	0.0452%	13.5770%	0.3474%	0.0600%	0.2707%	3.5454%	14.1448%	0.0146%	0.2624%	0.9765%	0.2456%	8.5449%	0.0662%	0.2958%	0.4036%	5.5778%	1.5899%	0.8368%	1.8022%	2.2089%	4.3121%	0.0651%	2.2137%

HGT 1:2

Test 2	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT1.2_C05_003.fsa	C1 5q31	1.00	1.10	1.00	1.07	1.07	1.09	1.06	1.04	1.07	1.02	1.04	0.97	1.03	0.56	0.99	0.99	1.13	0.95	1.11	0.98	1.24	1.08	1.20	1.01	1.02	1.01	1.03	1.18	1.13	1.09	1.08	1.05	1.01	1.07	1.02	1.07	1.13
	Operator	C2 12p13	0.91	1.00	0.91	0.97	0.97	0.99	0.96	0.94	0.97	0.92	0.94	0.88	0.93	0.51	0.90	0.89	1.02	0.86	1.01	0.89	1.13	0.98	1.09	0.91	0.92	0.91	0.93	1.07	1.03	0.99	0.98	0.95	0.92	0.97	0.92	0.97	1.02
	nadja	C3 7q31	1.00	1.10	1.00	1.07	1.07	1.09	1.06	1.04	1.07	1.02	1.04	0.97	1.03	0.56	0.99	0.99	1.13	0.95	1.11	0.98	1.24	1.08	1.20	1.01	1.02	1.01	1.03	1.18	1.13	1.09	1.08	1.04	1.01	1.07	1.02	1.07	1.12
	Worksheet	C4 20p13	0.93	1.03	0.93	1.00	0.99	1.01	0.99	0.97	0.99	0.95	0.97	0.91	0.96	0.53	0.92	0.92	1.05	0.88	1.04	0.91	1.16	1.00	1.12	0.94	0.95	0.94	0.96	1.10	1.05	1.02	1.00	0.97	0.94	0.99	0.95	1.00	1.05
	1	C5 8p23	0.94	1.03	0.94	1.01	1.00	1.02	1.00	0.97	1.00	0.95	0.98	0.91	0.97	0.53	0.93	0.92	1.06	0.89	1.04	0.92	1.16	1.01	1.12	0.95	0.96	0.95	0.96	1.11	1.06	1.02	1.01	0.98	0.95	1.00	0.95	1.00	1.05
	Int QC Stand Dev	C6 14q24	0.92	1.01	0.92	0.99	0.98	1.00	0.97	0.95	0.98	0.93	0.96	0.89	0.95	0.52	0.91	0.91	1.04	0.87	1.02	0.90	1.14	0.99	1.10	0.93	0.94	0.93	0.94	1.08	1.04	1.00	0.99	0.96	0.93	0.98	0.93	0.98	1.03
	0.024748827	C7 12q24	0.94	1.04	0.94	1.01	1.00	1.03	1.00	0.98	1.00	0.96	0.98	0.92	0.97	0.53	0.93	0.93	1.06	0.89	1.05	0.92	1.17	1.02	1.13	0.95	0.96	0.95	0.97	1.11	1.07	1.03	1.01	0.98	0.95	1.00	0.96	1.01	1.06
		C8 8q24	0.96	1.06	0.97	1.04	1.03	1.05	1.02	1.00	1.03	0.98	1.00	0.94	0.99	0.54	0.96	0.95	1.09	0.91	1.07	0.95	1.20	1.04	1.16	0.97	0.98	0.97	0.99	1.14	1.09	1.05	1.04	1.01	0.98	1.03	0.98	1.03	1.09
		C9 15q26	0.94	1.03	0.94	1.01	1.00	1.02	1.00	0.97	1.00	0.95	0.98	0.91	0.97	0.53	0.93	0.92	1.06	0.89	1.04	0.92	1.16	1.01	1.12	0.95	0.96	0.95	0.96	1.11	1.06	1.02	1.01	0.98	0.95	1.00	0.95	1.00	1.05
		C10 7p14	0.98	1.09	0.99	1.06	1.05	1.07	1.05	1.02	1.05	1.00	1.03	0.96	1.01	0.56	0.98	0.97	1.11	0.93	1.09	0.97	1.22	1.06	1.18	0.99	1.00	0.99	1.01	1.16	1.11	1.08	1.06	1.03	1.00	1.05	1.00	1.05	1.11
		MEAN	0.95	1.05	0.95	1.02	1.02	1.04	1.01	0.99	1.02	0.97	0.99	0.93	0.98	0.54	0.94	0.94	1.08	0.90	1.06	0.93	1.18	1.03	1.14	0.96	0.97	0.96	0.98	1.12	1.08	1.04	1.03	1.00	0.96	1.01	0.97	1.02	1.07
	ODDS NORMAL	:DEL	2543:1	1021:1	4580:1	84517:1	11668:1	1066:1	5528:1	641:1	85319:1	3881:1	133:1	227:1	7019:1	1:80	10998:1	201:1	1400:1	1395:1	10414:1	173:1	220:1	19557:1	3365:1	3091:1	11266:1	215:1	39703:1	5380:1	7813:1	836:1	5736:1	4144:1	2634:1	1934:1	410:1	75896:1	744:1
	ODDS NORMAL	:DUP	94:1	12:1	140:1	796:1	139:1	91:1	77:1	18:1	896:1	100:1	6:1	103:1	1249:1	3:1	357:1	12:1	10:1	92:1	63:1	11:1	1:1	1685:1	5:1	90:1	255:1	10:1	750:1	11:1	35:1	12:1	64:1	75:1	75:1	30:1	15:1	756:1	7:1
	PROB OF DEVIATION	NORMAL	51.3046%	69.0094%	45.2304%	56.9277%	82.2512%	57.2473%	89.9240%	91.5715%	70.3166%	64.6337%	96.3986%	26.5997%	58.5241%	0.1599%	20.1036%	68.2100%	50.2881%	11.1599%	35.6658%	67.0691%	37.5684%	36.1480%	5.1727%	58.4252%	58.0439%	80.5535%	58.6459%	4.9425%	23.7716%	76.2767%	75.6346%	94.7063%	85.3509%	99.5401%	82.2379%	64.2309%	60.5752%
	PROB OF DEVIATION	DELETED	0.0202%	0.0676%	0.0099%	0.0007%	0.0070%	0.0054%	0.0163%	0.1423%	0.0006%	0.0167%	0.7260%	0.0117%	0.0008%	30.5571%	0.0018%	0.3387%	0.0359%	0.0080%	0.0034%	0.3872%	0.1708%	0.0002%	0.0015%	0.0189%	0.0052%	0.3755%	0.0015%	0.0009%	0.0030%	0.0912%	0.0132%	0.0229%	0.0243%	0.0460%	0.2007%	0.0008%	0.0814%
	PROB OF DEVIATION	DUP	0.6124%	5.9673%	0.3232%	0.0716%	0.5918%	0.6317%	1.6596%	5.0660%	0.0786%	0.6461%	15.5836%	0.2581%	0.0469%	0.0487%	0.0563%	5.6189%	5.0842%	0.1207%	0.5675%	5.9281%	30.8746%	0.0217%	0.9648%	0.6517%	0.2278%	7.6905%	0.0782%	0.4566%	0.6751%	6.6130%	1.1809%	1.2711%	0.8766%	2.9453%	5.3627%	0.0949%	8.4604%

HGT 2:1

Test 10	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT2.1_D05_004.fsa	1.00	1.06	1.04	1.16	0.99	1.14	1.05	1.03	1.07	1.01	0.69	0.99	1.10	0.48	1.06	0.97	1.07	0.96	1.00	1.15	1.13	1.02	1.10	1.04	0.94	1.25	1.03	1.04	1.12	1.04	1.03	1.13	1.21	0.96	0.90	1.00	1.14
	Operator	0.95	1.00	0.98	1.10	0.94	1.08	1.00	0.97	1.02	0.96	0.66	0.94	1.04	0.45	1.00	0.92	1.01	0.91	0.95	1.09	1.07	0.97	1.04	0.98	0.89	1.18	0.98	0.99	1.06	0.98	0.98	1.07	1.15	0.91	0.86	0.94	1.08
	nadja	0.96	1.02	1.00	1.11	0.95	1.10	1.01	0.99	1.03	0.98	0.67	0.95	1.06	0.46	1.02	0.93	1.03	0.92	0.96	1.11	1.09	0.98	1.05	1.00	0.90	1.20	1.00	1.00	1.07	1.00	0.99	1.09	1.17	0.92	0.87	0.96	1.10
	Worksheet	0.86	0.91	0.90	1.00	0.86	0.98	0.91	0.89	0.93	0.88	0.60	0.86	0.95	0.41	0.91	0.84	0.92	0.83	0.86	0.99	0.98	0.88	0.95	0.89	0.81	1.08	0.89	0.90	0.96	0.90	0.89	0.97	1.05	0.83	0.78	0.86	0.99
	1	1.01	1.07	1.05	1.17	1.00	1.15	1.06	1.04	1.09	1.02	0.70	1.00	1.11	0.48	1.07	0.98	1.08	0.97	1.01	1.16	1.15	1.03	1.11	1.05	0.95	1.26	1.05	1.05	1.13	1.05	1.04	1.14	1.23	0.97	0.91	1.01	1.16
	Int QC Stand Dev	0.88	0.93	0.91	1.02	0.87	1.00	0.92	0.90	0.94	0.89	0.61	0.87	0.97	0.42	0.93	0.85	0.94	0.84	0.88	1.01	1.00	0.90	0.96	0.91	0.83	1.10	0.91	0.92	0.98	0.91	0.91	0.99	1.07	0.84	0.79	0.88	1.01
0.052756506	C7 12q24	0.95	1.00	0.99	1.10	0.94	1.08	1.00	0.98	1.02	0.97	0.66	0.94	1.05	0.45	1.01	0.92	1.02	0.91	0.95	1.10	1.08	0.97	1.04	0.99	0.90	1.19	0.98	0.99	1.06	0.99	0.98	1.07	1.16	0.91	0.86	0.95	1.09
	C8 8q24	0.97	1.03	1.01	1.13	0.96	1.11	1.02	1.00	1.05	0.99	0.67	0.96	1.07	0.46	1.03	0.94	1.04	0.93	0.97	1.12	1.10	0.99	1.07	1.01	0.91	1.21	1.01	1.01	1.09	1.01	1.01	1.10	1.18	0.93	0.88	0.97	1.11
	C9 15q26	0.93	0.98	0.97	1.08	0.92	1.06	0.98	0.96	1.00	0.94	0.64	0.92	1.02	0.44	0.98	0.90	0.99	0.89	0.93	1.07	1.06	0.95	1.02	0.96	0.87	1.16	0.96	0.97	1.04	0.96	0.96	1.05	1.13	0.89	0.84	0.93	1.06
	C10 7p14	0.99	1.04	1.02	1.14	0.98	1.12	1.04	1.01	1.06	1.00	0.68	0.98	1.08	0.47	1.04	0.96	1.05	0.94	0.99	1.13	1.12	1.01	1.08	1.02	0.93	1.23	1.02	1.03	1.10	1.02	1.02	1.11	1.20	0.95	0.89	0.98	1.13
	MEAN	0.95	1.00	0.99	1.10	0.94	1.08	1.00	0.98	1.02	0.96	0.66	0.94	1.05	0.45	1.01	0.92	1.01	0.91	0.95	1.09	1.08	0.97	1.04	0.98	0.89	1.19	0.98	0.99	1.06	0.99	0.98	1.07	1.15	0.91	0.86	0.95	1.09
	ODDS NORMAL :DEL	2409:1	1030:1	10674:1	1638:1	2771:1	6358:1	5261:1	543:1	77579:1	3493:1	1:1	3274:1	51650:1	1220	84238:1	150:1	1615:1	958:1	4808:1	463:1	4:1	2:1	86997:1	16046:1	5183:1	950:1	348:1	44428:1	32712:1	10024:1	648:1	4289:1	3233:1	177:1	379:1	61:1	12575:1	726:1
	ODDS NORMAL :DUP	82:1	22:1	199:1	33:1	105:1	28:1	90:1	16:1	757:1	96:1	3:1	122:1	344:1	3:1	1055:1	12:1	27:1	10:1	155:1	4:1	4:1	1:1	865:1	125:1	106:1	74:1	1:1	739:1	540:1	59:1	19:1	92:1	19:1	3:1	26:1	11:1	398:1	6:1
	PROB OF DEVIATION NORMAL	49.5453%	99.6134%	81.0531%	4.5382%	36.7774%	25.2118%	95.6886%	84.9492%	61.6779%	60.4468%	15.2791%	34.2595%	26.4587%	0.0910%	92.5597%	59.6387%	91.4551%	12.6050%	39.1094%	56.1376%	70.0286%	25.7001%	49.1932%	80.7388%	10.0372%	29.6285%	63.9087%	79.2749%	34.4223%	89.8685%	79.7610%	42.2470%	11.2685%	41.4062%	33.4454%	13.5646%	53.8155%
	PROB OF DEVIATION DELETED	0.0206%	0.0967%	0.0075%	0.0004%	0.0133%	0.0040%	0.0162%	0.1563%	0.0008%	0.0073%	18.1473%	0.0105%	0.0005%	19.7733%	0.0011%	0.3860%	0.0566%	0.0076%	0.0081%	0.1213%	0.3217%	0.0003%	0.0031%	0.0156%	0.0106%	0.0851%	0.0014%	0.0024%	0.0034%	0.1387%	0.0186%	0.0131%	0.0064%	0.1094%	0.5496%	0.0016%	0.0741%
	PROB OF DEVIATION DUP	0.6036%	4.4633%	0.4071%	0.1382%	0.3486%	0.8885%	1.0596%	4.7617%	0.0814%	0.6270%	4.4635%	0.2798%	0.0769%	0.0052%	0.0678%	5.1241%	3.3720%	0.1248%	0.2527%	13.2491%	19.6857%	0.0138%	0.3937%	0.7612%	0.1358%	23.5897%	0.0800%	0.1468%	0.5951%	4.8080%	0.8703%	2.1747%	3.5948%	1.5896%	3.0512%	0.0491%	9.1976%

HGT 3:1

Test 1	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT3.1_E05_001.fsa	1.00	1.02	1.06	0.99	1.08	1.03	1.15	0.93	1.01	1.05	0.84	0.92	1.01	0.48	0.95	0.83	0.98	1.02	0.99	0.92	0.99	1.04	1.08	1.16	0.91	1.14	0.97	1.06	1.01	0.94	0.88	1.00	0.80	0.95	0.86	1.00	1.02
	Operator	0.98	1.00	1.03	0.97	1.06	1.01	1.13	0.91	0.99	1.03	0.82	0.90	0.99	0.47	0.93	0.81	0.96	1.00	0.97	0.90	0.97	1.02	1.06	1.13	0.89	1.12	0.95	1.04	0.99	0.92	0.86	0.98	0.78	0.93	0.84	0.98	1.00
	Nadja	0.95	0.97	1.00	0.94	1.02	0.98	1.09	0.88	0.96	0.99	0.79	0.87	0.96	0.45	0.90	0.78	0.93	0.97	0.93	0.87	0.94	0.98	1.02	1.10	0.86	1.08	0.92	1.00	0.96	0.89	0.83	0.95	0.76	0.90	0.81	0.95	0.96
	Worksheet	1.01	1.03	1.07	1.00	1.09	1.04	1.17	0.94	1.02	1.06	0.85	0.93	1.02	0.48	0.96	0.84	0.99	1.03	1.00	0.93	1.01	1.05	1.09	1.17	0.92	1.16	0.98	1.07	1.03	0.95	0.89	1.02	0.81	0.96	0.87	1.01	1.03
	1	0.93	0.95	0.98	0.92	1.00	0.96	1.07	0.86	0.94	0.97	0.78	0.85	0.94	0.44	0.88	0.77	0.91	0.95	0.91	0.85	0.92	0.97	1.00	1.07	0.84	1.06	0.90	0.98	0.94	0.87	0.81	0.93	0.74	0.88	0.80	0.93	0.94
	Int QC Stand Dev	0.97	0.99	1.02	0.96	1.04	1.00	1.12	0.90	0.98	1.02	0.91	0.89	0.98	0.46	0.92	0.80	0.95	0.99	0.95	0.89	0.96	1.01	1.04	1.12	0.88	1.11	0.94	1.03	0.98	0.91	0.85	0.97	0.77	0.92	0.83	0.97	0.99
0.057706887	C7 12q24	0.87	0.88	0.92	0.86	0.93	0.89	1.00	0.81	0.88	0.91	0.73	0.79	0.88	0.41	0.82	0.72	0.95	0.89	0.85	0.79	0.86	0.90	0.93	1.00	0.79	0.99	0.84	0.92	0.88	0.81	0.76	0.87	0.69	0.82	0.75	0.87	0.88
	C8 8q24	1.07	1.10	1.14	1.06	1.16	1.11	1.24	1.00	1.09	1.13	0.90	0.99	1.09	0.51	1.02	0.89	1.05	1.10	1.06	0.98	1.07	1.12	1.16	1.24	0.97	1.23	1.04	1.14	1.09	1.01	0.94	1.08	0.86	1.02	0.92	1.07	1.09
	C9 15q26	0.99	1.01	1.04	0.98	1.06	1.02	1.14	0.92	1.00	1.04	0.83	0.91	1.00	0.47	0.93	0.82	0.97	1.01	0.97	0.90	0.98	1.03	1.06	1.14	0.90	1.13	0.96	1.05	1.00	0.93	0.86	0.99	0.79	0.94	0.85	0.99	1.00
	C10 7p14	0.95	0.97	1.01	0.94	1.03	0.98	1.10	0.89	0.97	1.00	0.80	0.87	0.96	0.45	0.90	0.79	0.93	0.97	0.94	0.87	0.95	0.99	1.03	1.10	0.86	1.09	0.92	1.01	0.97	0.89	0.83	0.96	0.76	0.91	0.82	0.95	0.97
	MEAN	0.97	0.99	1.03	0.96	1.05	1.00	1.12	0.90	0.98	1.02	0.82	0.89	0.98	0.46	0.92	0.80	0.95	0.99	0.96	0.89	0.97	1.01	1.05	1.12	0.88	1.11	0.94	1.03	0.98	0.91	0.85	0.98	0.78	0.92	0.84	0.97	0.99
	ODDS NORMAL :DEL	3909:1	879:1	12776:1	2454:1	9030:1	14095:1	2437:1	161:1	57852:1	7339:1	12:1	726:1	6962:1	1239	3788:1	18:1	671:1	2369:1	5925:1	83:1	12:1	1:1	36363:1	14999:1	2723:1	634:1	391:1	8269:1	32597:1	12336:1	188:1	1601:1	2734:1	21:1	468:1	39:1	33491:1	612:1
	ODDS NORMAL :DUP	98:1	22:1	130:1	63:1	70:1	206:1	7:1	15:1	1024:1	90:1	5:1	60:1	1243:1	3:1	188:1	8:1	28:1	383:1	171:1	10:1	7:1	4099:1	109:1	7:1	61:1	3:1	296:1	297:1	233:1	15:1	27:1	68:1	13:1	28:1	10:1	729:1	17:1
	PROB OF DEVIATION NORMAL	67.4266%	92.9177%	70.4032%	27.7843%	51.2275%	59.4802%	18.5113%	47.1038%	62.2249%	62.6143%	39.0099%	12.2517%	58.9480%	0.0966%	8.8458%	23.2250%	63.6424%	85.2863%	45.3222%	48.0940%	85.1443%	77.0477%	44.4051%	15.5810%	7.6298%	52.4385%	17.3490%	58.4571%	76.0612%	49.4287%	10.3895%	74.5330%	3.8501%	46.5600%	27.2322%	42.3823%	91.6978%
	PROB OF DEVIATION DELETED	0.0173%	0.1057%	0.0055%	0.0011%	0.0057%	0.0071%	0.0076%	0.2310%	0.0011%	0.0112%	3.2451%	0.0193%	0.0008%	28.9513%	0.0022%	1.2694%	0.0949%	0.0036%	0.0076%	0.5815%	0.7044%	0.0002%	0.0030%	0.0057%	0.0120%	0.1340%	0.0021%	0.0018%	0.0062%	0.2624%	0.0650%	0.0273%	0.1871%	0.0995%	0.6973%	0.0013%	0.1499%
	PROB OF DEVIATION DUP	0.6911%	4.1763%	0.5398%	0.0441%	0.7372%	0.4834%	2.6694%	3.2322%	0.0608%	0.9122%	7.6051%	0.2034%	0.0468%	0.0065%	0.0471%	2.9729%	2.2943%	0.2226%	0.2652%	4.7617%	12.1265%	0.0188%	0.4090%	2.1967%	0.1256%	15.8826%	0.0587%	0.1970%	0.3262%	3.2120%	0.3865%	1.1003%	0.2917%	1.6850%	2.7364%	0.0582%	5.2505%

HGT 4:1

Test 2	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT4.1_F05_002.fsa C1 5q31	1.00	1.02	0.99	1.13	1.03	1.02	1.09	0.97	1.03	1.02	0.98	0.99	1.05	0.49	1.01	0.93	1.13	1.04	0.95	0.80	1.07	1.02	1.22	0.86	0.90	0.90	0.91	1.04	1.11	0.99	0.96	1.09	0.99	0.96	1.02	1.09	1.38
	Operator C2 12p13	0.98	1.00	0.97	1.11	1.01	1.00	1.07	0.95	1.01	1.01	0.96	0.97	1.03	0.48	0.99	0.92	1.11	1.02	0.94	0.79	1.05	1.00	1.20	0.84	0.88	0.88	0.89	1.02	1.09	0.97	0.94	1.07	0.97	0.94	1.00	1.07	1.36
	Nadja C3 7q31	1.01	1.03	1.00	1.14	1.04	1.03	1.10	0.98	1.04	1.03	0.99	1.00	1.06	0.49	1.02	0.94	1.14	1.05	0.96	0.81	1.09	1.03	1.24	0.87	0.91	0.91	0.92	1.05	1.12	1.00	0.97	1.10	1.00	0.97	1.03	1.11	1.39
	Worksheet C4 20p13	0.88	0.90	0.88	1.00	0.91	0.90	0.96	0.86	0.91	0.91	0.87	0.88	0.93	0.43	0.89	0.82	1.00	0.92	0.84	0.71	0.95	0.90	1.08	0.76	0.79	0.79	0.80	0.92	0.98	0.88	0.85	0.97	0.88	0.85	0.90	0.97	1.22
	1 C5 8p23	0.97	0.99	0.96	1.10	1.00	0.99	1.06	0.94	1.00	1.00	0.95	0.96	1.02	0.47	0.98	0.91	1.10	1.01	0.93	0.78	1.04	0.99	1.19	0.84	0.87	0.87	0.88	1.01	1.08	0.96	0.93	1.06	0.96	0.93	0.99	1.06	1.34
	Int QC Stand Dev C6 14q24	0.98	1.00	0.97	1.11	1.01	1.00	1.06	0.95	1.01	1.00	0.96	0.97	1.03	0.47	0.99	0.91	1.10	1.01	0.93	0.79	1.05	1.00	1.20	0.84	0.88	0.88	0.89	1.02	1.08	0.97	0.94	1.07	0.97	0.94	1.00	1.07	1.35
0.045783685	C7 12q24	0.92	0.94	0.91	1.04	0.95	0.94	1.00	0.89	0.95	0.94	0.90	0.91	0.97	0.45	0.93	0.86	1.04	0.95	0.88	0.74	0.99	0.94	1.13	0.79	0.83	0.83	0.84	0.96	1.02	0.91	0.88	1.01	0.91	0.88	0.94	1.01	1.27
	C8 8q24	1.03	1.05	1.02	1.17	1.06	1.06	1.12	1.00	1.07	1.06	1.02	1.02	1.09	0.50	1.04	0.96	1.17	1.07	0.98	0.83	1.11	1.05	1.27	0.89	0.93	0.93	0.94	1.08	1.14	1.02	0.99	1.13	1.03	0.99	1.05	1.13	1.43
	C9 15q26	0.97	0.99	0.96	1.10	1.00	0.99	1.05	0.94	1.00	0.99	0.95	0.96	1.02	0.47	0.98	0.90	1.09	1.00	0.92	0.78	1.04	0.99	1.19	0.83	0.87	0.87	0.88	1.01	1.07	0.96	0.93	1.06	0.96	0.93	0.99	1.06	1.34
	C10 7p14	0.98	0.99	0.97	1.10	1.01	1.00	1.06	0.94	1.01	1.00	0.96	0.97	1.03	0.47	0.98	0.91	1.10	1.01	0.93	0.78	1.05	1.00	1.20	0.84	0.88	0.88	0.89	1.02	1.08	0.97	0.93	1.07	0.97	0.94	0.99	1.07	1.35
	MEAN	0.97	0.99	0.96	1.10	1.00	0.99	1.06	0.94	1.00	1.00	0.96	0.96	1.02	0.47	0.98	0.91	1.10	1.01	0.93	0.78	1.04	0.99	1.19	0.84	0.87	0.87	0.88	1.02	1.08	0.96	0.93	1.06	0.97	0.93	0.99	1.06	1.34
	ODDS NORMAL :DEL	4154:1	874:1	5900:1	19510:1	12447:1	12032:1	4309:1	311:1	106288:1	7067:1	96:1	6307:1	96463:1	1:394	42882:1	120:1	1293:1	28512:1	2406:1	11:1	207:1	28638:1	1838:1	116:1	492:1	54:1	946:1	39368:1	8022:1	471:1	1312:1	3484:1	2673:1	584:1	547:1	25490:1	297:1
	ODDS NORMAL :DUP	100:1	22:1	156:1	32:1	167:1	159:1	31:1	17:1	1370:1	120:1	6:1	165:1	930:1	3:1	806:1	11:1	7:1	361:1	111:1	7:1	5:1	4294:1	1:1	25:1	54:1	9:1	85:1	437:1	37:1	18:1	61:1	24:1	74:1	29:1	16:1	126:1	1:8
	PROB OF DEVIATION NORMAL	70.0548%	92.6895%	53.8023%	4.4776%	98.3229%	90.4594%	50.5047%	65.0419%	97.0501%	93.5845%	82.4595%	53.8933%	58.9443%	0.1032%	58.1540%	53.3806%	40.8890%	90.4368%	24.0733%	20.2681%	82.4917%	70.1131%	2.1162%	7.5402%	6.4391%	44.0276%	3.4480%	77.9015%	24.7106%	77.3426%	38.0276%	48.9270%	65.0392%	52.6547%	93.6049%	15.5187%	5.3426%
	PROB OF DEVIATION DELETED	0.0169%	0.0160%	0.0091%	0.0004%	0.0079%	0.0075%	0.0117%	0.2092%	0.0009%	0.0132%	0.9538%	0.0085%	0.0006%	40.6354%	0.0014%	0.4480%	0.0316%	0.0032%	0.0100%	1.7894%	0.3991%	0.0002%	0.0012%	0.0648%	0.0131%	0.8225%	0.0036%	0.0020%	0.0031%	0.1643%	0.0290%	0.0140%	0.0243%	0.0302%	0.1712%	0.0006%	0.0180%
	PROB OF DEVIATION DUP	0.7038%	4.1679%	0.3440%	0.1398%	0.5290%	0.4565%	1.6107%	3.3074%	0.0706%	0.7821%	13.4645%	0.3270%	0.0634%	0.0378%	0.0722%	4.8028%	5.7821%	0.2505%	0.2173%	2.9337%	16.9941%	0.0163%	1.5786%	0.3070%	0.1195%	5.0627%	0.0404%	0.1781%	0.6650%	4.2824%	0.6231%	2.0132%	0.8745%	1.7955%	5.9699%	0.1232%	44.4764%

HGT 5:1

Test 3	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT5.1_G05_003.fsa C1 5q31	1.00	1.03	1.08	0.96	1.13	1.16	1.06	1.01	0.94	1.10	0.91	1.06	1.10	0.58	1.02	1.09	1.12	1.05	1.15	0.98	1.12	1.05	1.10	1.24	1.06	1.04	1.08	1.19	1.09	1.10	1.00	1.24	1.17	1.03	1.04	1.11	1.14
	Operator C2 12p13	0.97	1.00	1.06	0.93	1.10	1.13	1.04	0.99	0.91	1.07	0.89	1.03	1.07	0.56	1.00	1.06	1.09	1.02	1.12	0.96	1.10	1.02	1.07	1.20	1.03	1.02	1.05	1.16	1.06	1.07	0.97	1.21	1.14	1.00	1.01	1.09	1.11
	Nadja C3 7q31	0.92	0.95	1.00	0.88	1.04	1.07	0.98	0.93	0.87	1.01	0.84	0.98	1.01	0.53	0.95	1.00	1.03	0.97	1.06	0.91	1.04	0.97	1.01	1.14	0.98	0.96	1.00	1.10	1.00	1.01	0.92	1.14	1.08	0.95	0.96	1.03	1.05
	Worksheet C4 20p13	1.05	1.07	1.13	1.00	1.18	1.22	1.11	1.06	0.98	1.15	0.96	1.11	1.15	0.60	1.07	1.14	1.17	1.10	1.20	1.03	1.18	1.09	1.15	1.29	1.11	1.09	1.13	1.24	1.14	1.15	1.04	1.30	1.23	1.07	1.09	1.17	1.19
	1 C5 8p23	0.89	0.91	0.96	0.85	1.00	1.03	0.94	0.90	0.83	0.97	0.81	0.94	0.97	0.51	0.91	0.96	0.99	0.93	1.02	0.87	1.00	0.93	0.98	1.10	0.94	0.93	0.96	1.05	0.96	0.97	0.89	1.10	1.04	0.91	0.92	0.99	1.01
	Int QC Stand Dev C6 14q24	0.86	0.88	0.93	0.82	0.97	1.00	0.92	0.87	0.81	0.94	0.79	0.91	0.95	0.50	0.88	0.94	0.96	0.90	0.99	0.85	0.97	0.90	0.95	1.06	0.91	0.90	0.93	1.02	0.94	0.94	0.86	1.07	1.01	0.88	0.89	0.96	0.98
0.063954452	C7 12q24	0.94	0.97	1.02	0.90	1.06	1.09	1.00	0.95	0.88	1.03	0.86	1.00	1.03	0.54	0.96	1.02	1.05	0.98	1.08	0.92	1.06	0.98	1.03	1.16	1.00	0.98	1.02	1.12	1.02	1.03	0.94	1.16	1.10	0.96	0.98	1.05	1.07
	C8 8q24	0.99	1.01	1.07	0.94	1.11	1.15	1.05	1.00	0.93	1.08	0.90	1.05	1.09	0.57	1.01	1.07	1.11	1.03	1.14	0.97	1.11	1.03	1.09	1.22	1.05	1.03	1.07	1.18	1.07	1.08	0.99	1.22	1.16	1.01	1.03	1.10	1.13
	C9 15q26	1.07	1.09	1.16	1.02	1.20	1.24	1.13	1.08	1.00	1.17	0.98	1.13	1.17	0.61	1.09	1.16	1.19	1.12	1.23	1.05	1.20	1.12	1.17	1.32	1.13	1.11	1.15	1.27	1.16	1.17	1.06	1.32	1.25	1.09	1.11	1.19	1.22
	C10 7p14	0.91	0.94	0.99	0.87	1.03	1.06	0.97	0.92	0.85	1.00	0.83	0.97	1.00	0.53	0.93	0.99	1.02	0.95	1.05	0.90	1.03	0.95	1.00	1.13	0.97	0.95	0.99	1.08	0.99	1.00	0.91	1.13	1.07	0.94	0.95	1.02	1.04
	MEAN	0.96	0.99	1.04	0.92	1.08	1.11	1.02	0.97	0.90	1.05	0.88	1.02	1.05	0.55	0.98	1.04	1.07	1.00	1.10	0.94	1.08	1.00	1.06	1.19	1.02	1.00	1.04	1.14	1.04	1.05	0.96	1.19	1.13	0.98	1.00	1.07	1.10
	ODDS NORMAL :DEL	2916:1	779:1	11384:1	3713:1	6075:1	4201:1	5402:1	480:1	1609:1	5903:1	30:1	14908:1	42306:1	1:82	44867:1	506:1	1430:1	29966:1	5558:1	190:1	218:1	434345:1	13481:1	1487:1	2255:1	351:1	45560:1	4296:1	13014:1	832:1	2391:1	1244:1	2330:1	1419:1	571:1	24203:1	718:1
	ODDS NORMAL :DUP	88:1	22:1	97:1	194:1	28:1	12:1	69:1	16:1	125:1	45:1	6:1	182:1	252:1	4:1	827:1	9:1	11:1	416:1	17:1	11:1	4:1	5587:1	87:1	2:1	263:1	11:1	363:1	7:1	103:1	10:1	76:1	1:1	6:1	36:1	16:1	116:1	5:1
	PROB OF DEVIATION NORMAL	56.0020%	87.7185%	57.4807%	6.2987%	27.1673%	13.5187%	33.7960%	90.1167%	4.2989%	51.9123%	55.3398%	94.8995%	20.5986%	0.1916%	60.2166%	80.0119%	53.3322%	99.6709%	14.1700%	69.8603%	70.0634%	98.3493%	37.8710%	5.8833%	82.0473%	98.7173%	43.5147%	3.6180%	51.5246%	71.9301%	55.5909%	7.9279%	17.8076%	85.1285%	95.4047%	14.5251%	51.0703%
	PROB OF DEVIATION DELETED	0.0182%	0.1126%	0.0050%	0.0017%	0.0045%	0.0032%	0.0155%	0.1688%	0.0022%	0.0088%	1.8332%	0.0057%	0.0005%	17.5907%	0.0010%	0.1569%	0.0373%	0.0033%	0.0025%	0.3880%	0.3216%	0.0002%	0.0028%	0.0040%	0.0036%	0.2814%	0.0010%	0.0008%	0.0040%	0.0864%	0.0233%	0.0064%	0.0076%	0.0600%	0.1677%	0.0006%	0.0712%
	PROB OF DEVIATION DUP	0.6356%	3.9877%	0.5503%	0.0325%	0.9647%	1.1606%	1.2215%	4.5564%	0.0337%	1.1587%	9.8620%	0.4670%	0.0816%	0.0516%	0.0728%	9.3781%	4.8986%	0.2395%	0.8158%	6.1047%	16.9277%	0.0176%	0.4336%	3.7757%	0.3121%	9.2131%	0.1200%	0.5223%	0.4998%	6.9153%	0.7300%	5.4905%	2.8021%	2.3929%	6.0716%	0.1254%	9.5373%

HGT 8:1

Test 6	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 6p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	$DHB Ex 1-1	$DHB Ex 1-2	$DHB Ex 2	$DHB Ex 3	$DHB Ex 4	$DHB Ex 5	$DHB Ex 6	$DHB Ex 7	$DHB Ex 8	$DHC Prom	$DHC Ex 1	$DHC Ex 2	$DHC Ex 4-1	$DHC Ex 4-2	$DHC Ex 5	$DHC Ex 6	$DHD Prom	$DHD Ex 1	$DHD Ex 2	$DHD Ex 3	$DHD Ex 4	$DHAF1 Ex 1	$DHAF2 Ex 1	$DHAF2 Ex 3	$DHAF2 Ex 4
	HGT8.1_B06_002.fsa	C1 5q31	1.00	1.01	1.00	1.00	1.02	1.04	1.06	0.94	1.02	1.02	0.83	0.87	0.95	0.57	0.96	0.94	0.97	0.95	0.92	0.69	1.04	0.93	0.91	1.06	0.90	1.01	0.92	0.91	0.83	1.01	0.88	0.83	0.82	1.05	1.26
	Operator	C2 12p13	0.99	1.00	0.99	0.99	1.01	1.02	1.05	0.93	1.00	1.01	0.82	0.86	0.93	0.57	0.95	0.93	0.96	0.94	0.91	0.68	1.03	0.92	0.90	1.05	0.89	0.99	0.91	0.90	0.82	1.00	0.87	0.82	0.81	1.04	1.24
	Nadja	C3 7q31	1.00	1.01	1.00	1.00	1.02	1.04	1.06	0.94	1.02	1.02	0.83	0.87	0.95	0.57	0.96	0.94	0.97	0.95	0.92	0.69	1.04	0.93	0.91	1.06	0.90	1.01	0.92	0.91	0.83	1.01	0.88	0.83	0.82	1.05	1.26
	Worksheet	C4 20p13	1.00	1.01	1.00	1.00	1.02	1.04	1.06	0.94	1.02	1.02	0.83	0.87	0.95	0.57	0.96	0.95	0.97	0.96	0.93	0.69	1.04	0.94	0.91	1.06	0.90	1.01	0.92	0.91	0.83	1.01	0.88	0.83	0.82	1.05	1.26
	1	C5 6p23	0.98	0.99	0.98	0.98	1.00	1.02	1.04	0.92	1.00	1.00	0.82	0.85	0.93	0.56	0.94	0.93	0.95	0.94	0.91	0.67	1.02	0.92	0.89	1.04	0.88	0.99	0.91	0.89	0.81	0.99	0.86	0.81	0.80	1.03	1.23
	Int QC Stand Dev	C6 14q24	0.96	0.98	0.96	0.96	0.98	1.00	1.02	0.91	0.98	0.98	0.80	0.84	0.91	0.55	0.93	0.91	0.94	0.92	0.89	0.66	1.01	0.90	0.88	1.02	0.87	0.97	0.89	0.88	0.80	0.97	0.85	0.80	0.79	1.01	1.21
	0.03210457	C7 12q24	0.94	0.96	0.94	0.94	0.96	0.98	1.00	0.89	0.96	0.96	0.78	0.82	0.89	0.54	0.91	0.89	0.92	0.90	0.87	0.65	0.98	0.88	0.86	1.00	0.85	0.95	0.87	0.86	0.78	0.95	0.83	0.78	0.77	0.99	1.18
		C8 8q24	1.07	1.08	1.07	1.06	1.09	1.10	1.13	1.00	1.08	1.09	0.89	0.93	1.01	0.61	1.02	1.01	1.04	1.02	0.98	0.73	1.11	1.00	0.97	1.13	0.96	1.07	0.98	0.97	0.88	1.08	0.94	0.89	0.87	1.12	1.34
		C9 15q26	0.99	1.00	0.98	0.98	1.00	1.02	1.04	0.92	1.00	1.01	0.82	0.86	0.93	0.56	0.95	0.93	0.96	0.94	0.91	0.68	1.03	0.92	0.89	1.05	0.89	0.99	0.91	0.90	0.81	1.00	0.87	0.82	0.80	1.03	1.24
		C10 7p14	0.98	0.99	0.98	0.98	1.00	1.02	1.04	0.92	0.99	1.00	0.81	0.85	0.93	0.56	0.94	0.93	0.95	0.93	0.91	0.67	1.02	0.92	0.89	1.04	0.88	0.99	0.90	0.89	0.81	0.99	0.86	0.81	0.80	1.03	1.23
		MEAN	0.99	1.00	0.99	0.99	1.01	1.03	1.05	0.93	1.01	1.01	0.82	0.86	0.94	0.57	0.95	0.94	0.96	0.95	0.92	0.68	1.03	0.93	0.90	1.05	0.89	1.00	0.91	0.90	0.82	1.00	0.87	0.82	0.81	1.04	1.24
	ODDS NORMAL	:DEL	6107:1	1032:1	12034:1	87492:1	12082:1	11726:1	4464:1	261:1	101076:1	7599:1	14:1	306:1	10555:1	1:43	15516:1	191:1	864:1	5991:1	1775:1	1:1	202:1	9103:1	1192:1	393:1	1421:1	41833:1	1669:1	170:1	71:1	4567:1	284:1	52:1	23:1	47775:1	430:1
	ODDS NORMAL	:DUP	112:1	22:1	206:1	1366:1	156:1	116:1	35:1	16:1	1235:1	101:1	5:1	41:1	375:1	4:1	440:1	12:1	29:1	198:1	95:1	5:1	5:1	384:1	83:1	6:1	106:1	601:1	92:1	15:1	20:1	76:1	33:1	15:1	9:1	347:1	1:2
	PROB OF DEVIATION	NORMAL	98.9983%	98.3768%	99.5659%	75.9702%	98.8182%	67.7287%	54.1454%	59.7438%	89.8288%	88.5110%	41.4643%	6.9904%	12.9529%	0.2207%	26.3130%	66.6770%	72.7875%	31.8276%	19.4710%	9.0273%	86.9498%	3.9190%	11.7411%	75.3980%	4.6148%	94.2894%	18.9106%	46.9907%	6.5398%	99.9441%	16.2444%	14.3050%	21.4576%	34.7378%	12.5761%
	PROB OF DEVIATION	DELETED	0.0165%	0.0953%	0.0072%	0.0009%	0.0074%	0.0058%	0.0121%	0.2269%	0.0009%	0.0116%	2.9327%	0.0228%	0.0012%	9.4763%	0.0017%	0.3482%	0.0842%	0.0053%	0.0110%	6.8666%	0.4294%	0.0004%	0.0099%	0.1919%	0.0032%	0.0023%	0.0113%	0.2764%	0.0925%	0.0219%	0.0572%	0.2740%	0.9319%	0.0007%	0.0293%
	PROB OF DEVIATION	DUP	0.7979%	4.5129%	0.4200%	0.0556%	0.5687%	0.5863%	1.5541%	3.7279%	0.0727%	0.8779%	8.1169%	0.1710%	0.0340%	0.0552%	0.0598%	5.5294%	2.4932%	0.1609%	0.2040%	1.9284%	16.2024%	0.0102%	0.1419%	12.0187%	0.0434%	0.1570%	0.2049%	3.1200%	0.3267%	1.3190%	0.4996%	0.9857%	2.4208%	0.1000%	24.3139%

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Test 7	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 6p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	$DHB Ex 1-1	$DHB Ex 1-2	$DHB Ex 2	$DHB Ex 3	$DHB Ex 4	$DHB Ex 5	$DHB Ex 6	$DHB Ex 7	$DHB Ex 8	$DHC Prom	$DHC Ex 1	$DHC Ex 2	$DHC Ex 3-1	$DHC Ex 3-2	$DHC Ex 4-1	$DHC Ex 4-2	$DHC Ex 5	$DHC Ex 6	$DHD Prom	$DHD Ex 1	$DHD Ex 2	$DHD Ex 3	$DHD Ex 4	$DHAF1 Ex 1	$DHAF2 Ex 1	$DHAF2 Ex 3	$DHAF2 Ex 4
	HGT8.2_C06_003.fsa	C1 5q31	1.00	1.09	0.95	1.06	0.92	0.97	1.07	1.02	1.04	1.00	0.90	0.98	0.98	0.57	1.01	1.13	1.04	0.89	1.03	0.87	0.98	1.10	1.02	1.28	1.00	0.92	1.02	1.03	0.97	0.99	0.92	1.07	1.03	0.96	0.92	1.00	1.15
	Operator	C2 12p13	0.92	1.00	0.88	0.98	0.85	0.89	0.99	0.93	0.95	0.92	0.83	0.90	0.91	0.53	0.93	1.04	0.95	0.82	0.95	0.80	0.90	1.01	0.94	1.18	0.92	0.84	0.94	0.94	0.89	0.91	0.85	0.98	0.95	0.89	0.85	0.92	1.06
	Nadja	C3 7q31	1.05	1.14	1.00	1.11	0.97	1.02	1.13	1.07	1.09	1.05	0.95	1.02	1.03	0.60	1.06	1.18	1.09	0.94	1.08	0.91	1.02	1.15	1.07	1.34	1.05	0.96	1.07	1.07	1.01	1.03	0.97	1.12	1.08	1.01	0.97	1.05	1.21
	Worksheet	C4 20p13	0.94	1.03	0.90	1.00	0.87	0.92	1.01	0.96	0.98	0.94	0.85	0.92	0.93	0.54	0.95	1.07	0.98	0.84	0.97	0.82	0.92	1.04	0.96	1.21	0.94	0.86	0.96	0.97	0.91	0.93	0.87	1.01	0.97	0.91	0.87	0.94	1.09
	1	C5 6p23	1.08	1.18	1.04	1.15	1.00	1.06	1.17	1.10	1.12	1.09	0.98	1.06	1.07	0.62	1.10	1.23	1.13	0.97	1.12	0.95	1.06	1.19	1.11	1.39	1.08	1.00	1.11	1.11	1.05	1.07	1.00	1.16	1.12	1.05	1.00	1.09	1.25
	Int QC Stand Dev	C6 14q24	1.03	1.12	0.98	1.09	0.95	1.00	1.10	1.04	1.06	1.03	0.93	1.00	1.01	0.59	1.04	1.16	1.07	0.92	1.06	0.90	1.00	1.13	1.05	1.31	1.03	0.94	1.05	1.05	0.99	1.01	0.95	1.10	1.06	0.99	0.95	1.03	1.18
	0.05316575	C7 12q24	0.93	1.01	0.89	0.99	0.86	0.91	1.00	0.95	0.96	0.93	0.84	0.91	0.92	0.53	0.94	1.05	0.97	0.83	0.96	0.81	0.91	1.02	0.95	1.19	0.93	0.85	0.95	0.95	0.90	0.92	0.86	0.99	0.96	0.90	0.86	0.93	1.07
		C8 8q24	0.98	1.07	0.94	1.04	0.91	0.96	1.06	1.00	1.02	0.99	0.89	0.96	0.97	0.56	1.00	1.11	1.02	0.88	1.01	0.86	0.96	1.08	1.00	1.26	0.98	0.90	1.01	1.01	0.95	0.97	0.91	1.05	1.02	0.95	0.91	0.99	1.13
		C9 15q26	0.97	1.05	0.92	1.02	0.89	0.94	1.04	0.98	1.00	0.97	0.87	0.94	0.95	0.55	0.98	1.09	1.00	0.86	0.99	0.84	0.94	1.06	0.98	1.24	0.96	0.89	0.99	0.99	0.93	0.95	0.89	1.03	1.00	0.93	0.89	0.97	1.11
		C10 7p14	1.00	1.08	0.95	1.06	0.92	0.97	1.07	1.01	1.03	1.00	0.90	0.97	0.98	0.57	1.01	1.13	1.04	0.89	1.03	0.87	0.97	1.10	1.02	1.28	1.00	0.92	1.02	1.02	0.96	0.98	0.92	1.07	1.03	0.96	0.92	1.00	1.15
		MEAN	0.99	1.08	0.94	1.05	0.91	0.96	1.06	1.01	1.03	0.99	0.89	0.97	0.97	0.57	1.00	1.12	1.03	0.88	1.02	0.86	0.97	1.09	1.01	1.27	0.99	0.91	1.01	1.02	0.96	0.98	0.91	1.06	1.02	0.95	0.91	0.99	1.14
	ODDS NORMAL	:DEL	5827:1	973:1	3494:1	42072:1	1234:1	5572:1	4084:1	783:1	71848:1	6513:1	38:1	6886:1	50224:1	1:48	97975:1	486:1	1598:1	748:1	16832:1	53:1	120:1	21311:1	23103:1	761:1	17992:1	97:1	70685:1	39725:1	5908:1	551:1	838:1	3610:1	5164:1	848:1	164:1	72995:1	632:1
	ODDS NORMAL	:DUP	110:1	9:1	124:1	260:1	73:1	149:1	27:1	18:1	664:1	117:1	6:1	171:1	1016:1	4:1	1170:1	3:1	22:1	68:1	191:1	9:1	7:1	63:1	295:1	1:4	312:1	10:1	803:1	446:1	170:1	18:1	51:1	27:1	62:1	32:1	13:1	1143:1	3:1
	PROB OF DEVIATION	NORMAL	86.2749%	55.5011%	37.4665%	23.2043%	21.2442%	54.3943%	45.5652%	97.8800%	55.6018%	89.1256%	60.4551%	57.2936%	44.8330%	0.2158%	99.6633%	45.5026%	81.9522%	7.2417%	78.5052%	39.2373%	84.9016%	2.5472%	90.5714%	1.9244%	80.4126%	57.0327%	80.3081%	79.0594%	45.8032%	83.3597%	28.6208%	52.6166%	79.5063%	64.6301%	53.5099%	77.6038%	33.4725%
	PROB OF DEVIATION	DELETED	0.0168%	0.0570%	0.0108%	0.0006%	0.0172%	0.0098%	0.0112%	0.1250%	0.0008%	0.0137%	1.5893%	0.0083%	0.0009%	10.4210%	0.001%	0.0936%	0.0513%	0.0097%	0.0046%	0.7465%	0.7077%	0.000%	0.0039%	0.0025%	0.0045%	0.5901%	0.001%	0.0020%	0.0078%	0.1512%	0.0342%	0.0146%	0.0154%	0.0762%	0.3263%	0.001%	0.0529%
	PROB OF DEVIATION	DUP	0.7838%	6.9470%	0.3030%	0.0892%	0.2908%	0.3662%	1.6970%	5.5825%	0.0838%	0.7601%	10.5108%	0.3345%	0.0441%	0.0547%	0.0952%	14.3110%	3.6645%	0.1072%	0.4119%	4.2161%	12.0958%	0.037%	0.3067%	8.5166%	0.2575%	5.9713%	0.1000%	0.1771%	0.2700%	4.5292%	0.5586%	1.9367%	1.2913%	2.0105%	3.9924%	0.0679%	12.6635%

HGT 9:1

Test 8	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT9.1_D06_004.fsa	1.00	1.14	0.93	0.92	1.03	1.01	1.10	0.99	1.01	1.03	0.69	0.93	1.05	0.53	1.03	1.01	1.04	0.90	0.96	1.03	1.05	1.07	1.02	1.01	0.92	1.12	0.97	1.04	1.00	0.91	0.87	1.12	0.97	0.92	0.88	1.08	1.05
	Operator	0.88	1.00	0.82	0.80	0.91	0.89	0.97	0.87	0.89	0.90	0.61	0.81	0.92	0.46	0.91	0.88	0.91	0.79	0.84	0.91	0.93	0.94	0.90	0.88	0.81	0.98	0.85	0.91	0.88	0.80	0.76	0.98	0.85	0.81	0.77	0.95	0.93
	Nadja	1.08	1.23	1.00	0.99	1.11	1.09	1.19	1.07	1.09	1.11	0.75	1.00	1.13	0.57	1.11	1.08	1.12	0.97	1.03	1.11	1.14	1.15	1.10	1.08	0.99	1.20	1.04	1.12	1.07	0.98	0.93	1.20	1.05	0.99	0.95	1.16	1.14
	Worksheet	1.09	1.24	1.01	1.00	1.13	1.10	1.20	1.09	1.10	1.12	0.76	1.01	1.14	0.58	1.13	1.10	1.14	0.98	1.04	1.13	1.15	1.16	1.12	1.10	1.01	1.22	1.06	1.13	1.09	1.00	0.95	1.22	1.06	1.00	0.96	1.18	1.15
	1	0.97	1.10	0.90	0.89	1.00	0.98	1.07	0.96	0.98	0.99	0.67	0.90	1.01	0.51	1.00	0.97	1.01	0.87	0.93	1.00	1.02	1.03	0.99	0.97	0.89	1.08	0.94	1.01	0.97	0.88	0.84	1.08	0.94	0.89	0.85	1.04	1.02
	Int QC Stand Dev	0.99	1.12	0.92	0.91	1.02	1.00	1.09	0.98	1.00	1.01	0.69	0.92	1.04	0.52	1.02	0.99	1.03	0.89	0.95	1.02	1.04	1.05	1.01	0.99	0.91	1.10	0.96	1.03	0.99	0.90	0.86	1.10	0.96	0.91	0.87	1.07	1.04
	C6 14q24	0.91	1.03	0.84	0.83	0.94	0.92	1.00	0.90	0.92	0.93	0.63	0.84	0.95	0.48	0.94	0.91	0.95	0.82	0.87	0.94	0.96	0.97	0.93	0.91	0.84	1.02	0.88	0.94	0.91	0.83	0.79	1.01	0.88	0.83	0.80	0.98	0.96
0.066705068	C7 12q24	1.01	1.14	0.93	0.92	1.04	1.02	1.11	1.00	1.02	1.03	0.70	0.93	1.05	0.53	1.04	1.01	1.05	0.90	0.96	1.04	1.06	1.07	1.03	1.01	0.93	1.12	0.97	1.04	1.00	0.92	0.87	1.12	0.98	0.92	0.88	1.08	1.06
	C8 8q24	0.99	1.13	0.92	0.91	1.02	1.00	1.09	0.98	1.00	1.02	0.69	0.92	1.04	0.52	1.02	1.00	1.03	0.89	0.95	1.02	1.04	1.06	1.01	1.00	0.91	1.11	0.96	1.03	0.99	0.90	0.86	1.10	0.96	0.91	0.87	1.07	1.04
	C9 15q26	0.97	1.11	0.90	0.89	1.01	0.99	1.07	0.97	0.98	1.00	0.68	0.90	1.02	0.51	1.01	0.98	1.01	0.88	0.93	1.01	1.03	1.04	1.00	0.98	0.90	1.09	0.94	1.01	0.97	0.89	0.84	1.09	0.94	0.89	0.86	1.05	1.03
	C10 7p14	0.99	1.12	0.92	0.90	1.02	1.00	1.09	0.98	1.00	1.01	0.69	0.92	1.04	0.52	1.02	0.99	1.03	0.89	0.95	1.02	1.04	1.05	1.01	0.99	0.91	1.10	0.96	1.03	0.99	0.90	0.86	1.10	0.96	0.91	0.87	1.07	1.04
	MEAN	5513:1	841:1	1509:1	2311:1	11493:1	12962:1	3388:1	576:1	91650:1	7588:1	1:1	1508:1	71198:1	1:394	67900:1	423:1	1600:1	846:1	4067:1	453:1	205:1	75567:1	22758:1	6198:1	1676:1	393:1	15402:1	34321:1	12293:1	159:1	181:1	2567:1	2220:1	327:1	72:1	26456:1	763:1
	ODDS NORMAL :DEL	108:1	4:1	84:1	146:1	133:1	203:1	16:1	18:1	1316:1	100:1	4:1	85:1	569:1	3:1	692:1	10:1	23:1	72:1	143:1	10:1	5:1	440:1	283:1	111:1	98:1	4:1	430:1	329:1	233:1	15:1	28:1	10:1	70:1	25:1	11:1	134:1	11:1
	ODDS NORMAL :DUP	83.4240%	35.5220%	21.0809%	4.4002%	79.6876%	94.8042%	32.4787%	87.2341%	88.4122%	88.1780%	17.8150%	20.0684%	39.7410%	0.1474%	67.3120%	94.5571%	82.8110%	7.8802%	34.7923%	90.4760%	84.6635%	11.6940%	87.9700%	89.9547%	14.8919%	54.6888%	28.1257%	63.0932%	75.8800%	45.4250%	11.1705%	27.6118%	58.0106%	38.1727%	36.0934%	16.2735%	78.1292%
	PROB OF DEVIATION NORMAL	0.0151%	0.0422%	0.0140%	0.0019%	0.0069%	0.0073%	0.0096%	0.1512%	0.0010%	0.0116%	10.3640%	0.0133%	0.0006%	58.0089%	0.0010%	0.2236%	0.0518%	0.0930%	0.0086%	0.1998%	0.4131%	0.0002%	0.0039%	0.0145%	0.0089%	0.1392%	0.0018%	0.0018%	0.0062%	0.2862%	0.0618%	0.0108%	0.0261%	0.1168%	0.5042%	0.0006%	0.1023%
	PROB OF DEVIATION DELETED	0.7695%	9.3114%	0.2518%	0.0301%	0.6014%	0.4673%	2.0093%	4.8678%	0.0672%	0.9798%	4.8760%	0.2365%	0.0698%	0.0453%	0.0973%	7.2924%	3.6065%	0.1097%	0.2434%	9.0420%	16.6008%	0.0266%	0.3109%	0.9083%	0.1517%	15.4031%	0.0654%	0.1920%	0.3259%	3.0606%	0.3967%	2.7119%	0.8282%	1.5282%	3.1802%	0.1216%	6.9924%
	PROB OF DEVIATION DUP																																					

HGT 9:2

Test 9	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT9.2_E06_001.fsa	1.00	1.13	1.07	1.06	1.12	1.06	1.04	0.94	1.07	1.13	0.96	0.96	1.08	0.55	1.07	1.01	1.03	1.08	1.10	1.00	1.16	1.07	1.08	1.16	0.97	0.82	1.12	1.02	1.15	1.03	1.03	0.88	1.15	1.09	1.07	1.11	0.97
	Operator	0.88	1.00	0.95	0.93	0.99	0.94	0.92	0.83	0.95	1.00	0.85	0.85	0.96	0.49	0.94	0.89	0.91	0.95	0.97	0.88	1.02	0.95	0.96	1.03	0.85	0.72	0.99	0.90	1.02	0.91	0.91	0.78	1.02	0.97	0.95	0.98	0.85
	Nadja	0.93	1.05	1.00	0.98	1.04	0.99	0.97	0.88	1.00	1.05	0.90	0.90	1.01	0.51	0.99	0.94	0.96	1.01	1.02	0.93	1.08	1.00	1.01	1.08	0.90	0.76	1.05	0.95	1.07	0.96	0.96	0.82	1.07	1.02	1.00	1.04	0.90
	Worksheet	0.95	1.07	1.02	1.00	1.06	1.00	0.99	0.89	1.02	1.07	0.91	0.91	1.03	0.52	1.01	0.96	0.98	1.02	1.04	0.95	1.09	1.01	1.02	1.10	0.92	0.78	1.07	0.96	1.09	0.97	0.98	0.84	1.09	1.04	1.01	1.05	0.91
	1	0.90	1.01	0.96	0.95	1.00	0.95	0.93	0.84	0.96	1.01	0.86	0.86	0.97	0.49	0.96	0.90	0.92	0.97	0.98	0.90	1.04	0.96	0.97	1.04	0.87	0.73	1.01	0.91	1.03	0.92	0.92	0.79	1.03	0.98	0.96	1.00	0.87
	Int QC Stand Dev	0.94	1.07	1.01	1.00	1.05	1.00	0.98	0.89	1.01	1.07	0.91	0.91	1.02	0.52	1.01	0.95	0.97	1.02	1.04	0.94	1.09	1.01	1.02	1.10	0.91	0.77	1.06	0.96	1.09	0.97	0.98	0.84	1.09	1.03	1.01	1.05	0.91
0.056569795	C6 14q24	0.96	1.09	1.03	1.01	1.07	1.02	1.00	0.90	1.03	1.09	0.93	0.92	1.04	0.53	1.02	0.97	0.99	1.04	1.05	0.96	1.11	1.03	1.04	1.11	0.93	0.79	1.08	0.98	1.10	0.99	0.99	0.85	1.11	1.05	1.03	1.07	0.93
	C7 12q24	1.07	1.20	1.14	1.12	1.19	1.13	1.11	1.00	1.14	1.21	1.03	1.03	1.15	0.59	1.14	1.07	1.10	1.15	1.17	1.06	1.23	1.14	1.15	1.24	1.03	0.87	1.20	1.08	1.22	1.09	1.10	0.94	1.23	1.16	1.14	1.18	1.03
	C8 8q24	0.93	1.06	1.00	0.98	1.04	0.99	0.97	0.88	1.00	1.06	0.90	0.90	1.01	0.51	0.99	0.94	0.96	1.01	1.02	0.93	1.08	1.00	1.01	1.08	0.90	0.76	1.05	0.95	1.07	0.96	0.96	0.83	1.07	1.02	1.00	1.04	0.90
	C9 15q26	0.88	1.00	0.95	0.93	0.99	0.94	0.92	0.83	0.95	1.00	0.85	0.85	0.96	0.49	0.94	0.89	0.91	0.95	0.97	0.88	1.02	0.95	0.96	1.02	0.85	0.72	0.99	0.90	1.01	0.91	0.91	0.78	1.02	0.97	0.94	0.98	0.85
	C10 7p14	0.94	1.07	1.01	1.00	1.05	1.00	0.98	0.89	1.01	1.07	0.91	0.91	1.02	0.52	1.01	0.95	0.97	1.02	1.04	0.94	1.09	1.01	1.02	1.10	0.91	0.77	1.06	0.96	1.09	0.97	0.98	0.84	1.09	1.03	1.01	1.05	0.91
	MEAN	2070:1	393:1	14203:1	10510:1	8302:1	12224:1	3902:1	117:1	93694:1	5017:1	48:1	1255:1	96079:1	1:402	83967:1	243:1	1011:1	25407:1	14216:1	193:1	220:1	355527:1	20747:1	3686:1	1766:1	9:1	24479:1	12242:1	7186:1	508:1	3627:1	95:1	3305:1	1887:1	644:1	38470:1	179:1
	ODDS NORMAL :DEL	77:1	9:1	177:1	1509:1	57:1	204:1	84:1	14:1	1065:1	29:1	6:1	78:1	924:1	3:1	1047:1	12:1	38:1	271:1	124:1	11:1	3:1	3964:1	222:1	15:1	101:1	6:1	129:1	326:1	30:1	18:1	87:1	21:1	16:1	24:1	15:1	245:1	15:1
	PROB OF DEVIATION NORMAL	44.9373%	80.0552%	86.6307%	87.3767%	44.5501%	95.9982%	80.3651%	40.3511%	80.3160%	38.9221%	66.3255%	17.7007%	58.6344%	0.1468%	92.0925%	74.2463%	78.9972%	73.8967%	57.3903%	70.4456%	65.3846%	74.9495%	74.3974%	25.4901%	15.4506%	20.6164%	19.2709%	38.3631%	21.0265%	80.2186%	72.0298%	10.0400%	32.3935%	77.2790%	95.8519%	26.2717%	51.0851%
	PROB OF DEVIATION DELETED	0.0217%	0.0605%	0.0061%	0.0008%	0.0054%	0.0073%	0.0206%	0.3444%	0.0009%	0.0078%	1.3677%	0.0141%	0.0006%	59.0744%	0.0011%	0.3054%	0.0781%	0.0029%	0.0041%	0.3642%	0.2967%	0.0002%	0.0036%	0.0069%	0.0088%	2.3460%	0.0008%	0.0031%	0.0029%	0.1576%	0.0199%	0.1053%	0.0098%	0.0410%	0.1489%	0.0007%	0.2653%
	PROB OF DEVIATION DUP	0.5801%	6.5820%	0.4888%	0.0579%	0.7829%	0.4711%	0.9543%	2.9635%	0.0754%	1.3276%	11.2677%	0.2276%	0.0635%	0.0452%	0.0679%	5.9776%	2.6285%	0.2728%	0.4689%	6.1420%	20.6426%	0.0189%	0.3346%	1.7275%	0.1533%	3.2943%	0.1496%	0.1178%	0.7083%	4.3992%	0.8250%	0.4787%	2.0665%	3.2754%	6.5936%	0.1074%	3.5000%

HGT 11:1

Test 4	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT11.1_G06_003.fsa	1.00	0.99	0.87	0.95	0.91	0.97	1.06	0.91	0.96	0.98	0.68	0.82	0.97	0.56	0.93	0.85	0.99	0.81	0.88	0.78	0.76	1.01	0.96	1.07	0.84	1.07	0.93	0.99	0.91	0.88	0.85	0.96	1.08	0.86	0.73	0.91	1.08
	Operator	1.01	1.00	0.88	0.96	0.92	0.98	1.07	0.92	0.97	0.98	0.68	0.83	0.97	0.56	0.94	0.85	1.00	0.82	0.88	0.79	0.76	1.02	0.97	1.07	0.85	1.08	0.93	1.00	0.92	0.88	0.86	0.97	1.09	0.87	0.74	0.92	1.08
	nadja	1.14	1.14	1.00	1.09	1.05	1.12	1.21	1.04	1.10	1.12	0.77	0.94	1.11	0.64	1.07	0.97	1.13	0.93	1.00	0.89	0.87	1.16	1.10	1.22	0.96	1.22	1.06	1.13	1.04	1.00	0.97	1.10	1.24	0.98	0.84	1.04	1.23
	Worksheet	1.05	1.04	0.92	1.00	0.96	1.02	1.11	0.96	1.01	1.03	0.71	0.86	1.01	0.58	0.98	0.89	1.04	0.85	0.92	0.82	0.79	1.06	1.01	1.12	0.88	1.12	0.97	1.04	0.96	0.92	0.89	1.01	1.13	0.90	0.77	0.95	1.13
	1	1.09	1.09	0.96	1.04	1.00	1.07	1.16	1.00	1.06	1.07	0.74	0.90	1.06	0.61	1.02	0.93	1.08	0.89	0.96	0.85	0.83	1.11	1.05	1.17	0.92	1.17	1.01	1.08	1.00	0.96	0.93	1.05	1.18	0.94	0.80	0.99	1.18
	Int QC Stand Dev	1.03	1.02	0.90	0.98	0.94	1.00	1.09	0.94	0.99	1.00	0.69	0.84	0.99	0.57	0.96	0.87	1.02	0.83	0.90	0.80	0.78	1.04	0.99	1.09	0.86	1.10	0.95	1.01	0.93	0.90	0.87	0.99	1.11	0.88	0.75	0.93	1.10
	0.054943948	0.94	0.94	0.82	0.90	0.86	0.92	1.00	0.86	0.91	0.92	0.64	0.78	0.91	0.53	0.88	0.80	0.93	0.77	0.83	0.74	0.71	0.95	0.91	1.01	0.79	1.01	0.87	0.93	0.86	0.83	0.80	0.91	1.02	0.81	0.69	0.86	1.01
	C8 8q24	1.10	1.09	0.96	1.05	1.00	1.07	1.16	1.00	1.06	1.07	0.74	0.90	1.06	0.61	1.02	0.93	1.09	0.89	0.96	0.86	0.83	1.11	1.06	1.17	0.92	1.17	1.02	1.08	1.00	0.96	0.93	1.06	1.19	0.94	0.80	1.00	1.18
	C9 15q26	1.04	1.03	0.91	0.99	0.95	1.01	1.10	0.94	1.00	1.01	0.70	0.85	1.00	0.58	0.97	0.88	1.03	0.84	0.91	0.81	0.78	1.05	1.00	1.10	0.87	1.11	0.96	1.02	0.94	0.91	0.88	1.00	1.12	0.89	0.76	0.94	1.11
	C10 7p14	1.02	1.02	0.89	0.98	0.94	1.00	1.08	0.93	0.99	1.00	0.69	0.84	0.99	0.57	0.95	0.87	1.01	0.83	0.90	0.80	0.77	1.04	0.98	1.09	0.86	1.09	0.95	1.01	0.93	0.90	0.87	0.98	1.11	0.88	0.75	0.93	1.10
	MEAN	1.04	1.03	0.91	0.99	0.95	1.02	1.10	0.95	1.01	1.02	0.70	0.86	1.01	0.58	0.97	0.88	1.03	0.85	0.91	0.81	0.79	1.05	1.00	1.11	0.87	1.11	0.97	1.03	0.95	0.91	0.89	1.00	1.13	0.90	0.76	0.95	1.12
	ODDS NORMAL :DEL	6069:1	1039:1	1279:1	93878:1	3814:1	13066:1	2869:1	354:1	104379:1	7409:1	2:1	259:1	129073:1	126	31434:1	76:1	1590:1	236:1	1602:1	211	91	70276:1	23980:1	3153:1	510:1	390:1	22571:1	32033:1	4590:1	201:1	402:1	4568:1	2296:1	258:1	91	12901:1	672:1
	ODDS NORMAL :DUP	53:1	15:1	77:1	1420:1	121:1	157:1	11:1	17:1	1319:1	91:1	4:1	38:1	1552:1	4:1	673:1	10:1	25:1	39:1	91:1	9:1	5:1	397:1	344:1	10:1	55:1	3:1	541:1	287:1	151:1	16:1	38:1	75:1	6:1	23:1	7:1	405:1	4:1
	PROB OF DEVIATION NORMAL	60.1790%	79.8231%	18.8362%	80.1875%	45.6822%	83.5422%	24.3671%	69.2617%	94.3501%	82.9090%	20.1380%	6.2816%	87.9708%	0.2436%	45.7089%	43.8342%	79.3465%	3.3888%	18.1374%	26.0352%	30.7220%	10.7280%	99.3121%	19.7379%	6.5966%	50.7120%	37.8834%	56.9794%	38.3766%	51.0082%	18.1043%	99.5974%	17.3722%	33.4729%	14.3068%	19.9631%	40.2364%
	PROB OF DEVIATION DELETED	0.0099%	0.0768%	0.0147%	0.0009%	0.0120%	0.0064%	0.0085%	0.1956%	0.0009%	0.0112%	10.5351%	0.0243%	0.0007%	6.3734%	0.0015%	0.5735%	0.0499%	0.0144%	0.0113%	1.2641%	3.4571%	0.0002%	0.0041%	0.0063%	0.0129%	0.1301%	0.0017%	0.0018%	0.0084%	0.2541%	0.0450%	0.0218%	0.0076%	0.1299%	1.6021%	0.0015%	0.0599%
	PROB OF DEVIATION DUP	1.1370%	5.3561%	0.2432%	0.0565%	0.3764%	0.5304%	2.3196%	4.1065%	0.0716%	0.9114%	5.2150%	0.1654%	0.0567%	0.0578%	0.0679%	4.2272%	3.7517%	0.0861%	0.1997%	3.3523%	5.9517%	0.0270%	0.2912%	1.9536%	0.1204%	16.2718%	0.0700%	0.1987%	0.2547%	3.2716%	0.4720%	1.3233%	2.8388%	1.4363%	1.9795%	0.0493%	11.2080%

HGT 11:2

Test 5	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT11.2_H06_004.fsa	1.00	1.03	1.03	0.97	1.01	1.03	1.10	0.93	1.02	1.04	0.60	0.94	1.00	0.52	1.05	1.04	1.01	0.92	1.06	1.07	0.71	1.07	1.06	1.18	0.94	1.27	1.07	1.05	0.92	0.86	0.99	1.04	1.18	0.92	0.87	1.03	0.96
	Operator	0.97	1.00	0.99	0.94	0.98	0.99	1.06	0.90	0.99	1.00	0.58	0.91	0.97	0.50	1.02	1.01	0.98	0.89	1.03	1.03	0.69	1.03	1.03	1.14	0.91	1.23	1.03	1.02	0.89	0.84	0.95	1.00	1.14	0.89	0.84	0.99	0.93
	nadja	0.97	1.01	1.00	0.95	0.98	1.00	1.07	0.91	0.99	1.01	0.58	0.92	0.98	0.51	1.03	1.02	0.99	0.90	1.03	1.04	0.69	1.04	1.03	1.15	0.92	1.24	1.04	1.03	0.90	0.84	0.96	1.01	1.15	0.90	0.85	1.00	0.93
	Worksheet	1.03	1.06	1.05	1.00	1.04	1.05	1.12	0.96	1.05	1.06	0.61	0.97	1.03	0.53	1.08	1.07	1.04	0.95	1.09	1.10	0.73	1.10	1.09	1.21	0.97	1.30	1.10	1.08	0.94	0.89	1.01	1.06	1.21	0.95	0.89	1.05	0.98
	1	0.99	1.02	1.02	0.97	1.00	1.02	1.09	0.92	1.01	1.03	0.59	0.94	1.00	0.51	1.04	1.03	1.00	0.92	1.05	1.06	0.70	1.06	1.05	1.17	0.94	1.26	1.06	1.04	0.91	0.86	0.98	1.03	1.17	0.91	0.86	1.02	0.95
	Int QC Stand Dev	0.97	1.01	1.00	0.95	0.98	1.00	1.07	0.91	0.99	1.01	0.58	0.92	0.98	0.50	1.03	1.01	0.98	0.90	1.03	1.04	0.69	1.04	1.03	1.15	0.92	1.24	1.04	1.03	0.90	0.84	0.96	1.01	1.15	0.90	0.85	1.00	0.93
	0.04899189	0.91	0.94	0.94	0.89	0.92	0.94	1.00	0.85	0.93	0.95	0.55	0.86	0.92	0.47	0.96	0.95	0.92	0.84	0.97	0.97	0.65	0.98	0.97	1.07	0.86	1.16	0.97	0.96	0.84	0.79	0.90	0.95	1.08	0.84	0.80	0.94	0.87
	C8 8q24	1.07	1.11	1.10	1.04	1.08	1.10	1.17	1.00	1.09	1.11	0.64	1.01	1.08	0.56	1.13	1.12	1.08	0.99	1.14	1.14	0.76	1.15	1.14	1.26	1.01	1.36	1.14	1.13	0.99	0.93	1.06	1.11	1.27	0.99	0.93	1.10	1.03
	C9 15q26	0.98	1.02	1.01	0.96	0.99	1.01	1.08	0.92	1.00	1.02	0.59	0.93	0.99	0.51	1.03	1.02	0.99	0.91	1.04	1.05	0.70	1.05	1.04	1.16	0.93	1.25	1.05	1.03	0.90	0.85	0.97	1.02	1.16	0.91	0.86	1.01	0.94
	C10 7p14	0.96	1.00	0.99	0.94	0.97	0.99	1.06	0.90	0.98	1.00	0.58	0.91	0.97	0.50	1.02	1.00	0.97	0.89	1.02	1.03	0.68	1.03	1.02	1.14	0.91	1.22	1.03	1.02	0.89	0.83	0.95	1.00	1.14	0.89	0.84	0.99	0.92
	MEAN	0.99	1.02	1.01	0.96	1.00	1.01	1.08	0.92	1.00	1.02	0.59	0.93	0.99	0.51	1.04	1.03	1.00	0.91	1.05	1.05	0.70	1.06	1.05	1.16	0.93	1.25	1.05	1.04	0.91	0.85	0.97	1.02	1.17	0.91	0.86	1.01	0.94
	ODDS NORMAL :DEL	5525:1	1042:1	14239:1	26751:1	11087:1	13186:1	3521:1	219:1	103616:1	7175:1	1:4	2494:1	96969:1	1550	43826:1	504:1	1496:1	186:1	12183:1	467:1	2:1	64336:1	14764:1	1838:1	3313:1	296:1	30138:1	26958:1	1322:1	64:1	3550:1	4314:1	1586:1	362:1	63:1	87052:1	319:1
	ODDS NORMAL :DUP	108:1	18:1	179:1	666:1	181:1	162:1	18:1	16:1	1299:1	91:1	3:1	108:1	1509:1	3:1	324:1	10:1	32:1	107:1	88:1	7:1	4:1	350:1	105:1	31:1	139:1	12	182:1	207:1	82:1	12:1	87:1	53:1	2:1	26:1	11:1	971:1	16:1
	PROB OF DEVIATION NORMAL	83.5338%	68.1385%	87.1001%	29.7678%	92.0869%	85.1638%	24.7717%	54.7714%	93.2884%	76.8182%	10.2773%	28.3721%	76.3944%	0.1380%	37.2396%	86.6288%	96.3603%	13.6818%	45.3397%	74.3267%	17.3166%	9.6585%	43.3620%	8.2896%	24.1585%	17.3443%	25.2512%	44.4974%	16.1069%	29.0328%	71.0826%	80.4448%	9.3741%	40.4036%	33.8739%	77.9995%	67.5502%
	PROB OF DEVIATION DELETED	0.0151%	0.0846%	0.0061%	0.0011%	0.0083%	0.0065%	0.0099%	0.2506%	0.0009%	0.0107%	40.7192%	0.0114%	0.0008%	75.9494%	0.0008%	0.1719%	0.0644%	0.0074%	0.0037%	0.1590%	9.5188%	0.0002%	0.0029%	0.0045%	0.0073%	0.0587%	0.0038%	0.0017%	0.0122%	0.4569%	0.0200%	0.0186%	0.0059%	0.1116%	0.5417%	0.0009%	0.2120%
	PROB OF DEVIATION DUP	0.7701%	4.9520%	0.4975%	0.0447%	0.5084%	0.5253%	1.8423%	3.5329%	0.0718%	0.9505%	3.5578%	0.2634%	0.0500%	0.0439%	0.1148%	8.7658%	3.0421%	0.1276%	0.5172%	10.6887%	4.2703%	0.0276%	0.4126%	3.0790%	0.1740%	33.8624%	0.1391%	0.2153%	0.1954%	2.4099%	0.8195%	1.5222%	3.9981%	1.5707%	3.0723%	0.0802%	4.1745%

HGT 13:1

Test 6	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT13.1_A07_001.fsa C1 5q31	1.00	1.01	0.96	0.97	0.99	0.97	1.14	0.90	0.99	1.02	0.58	0.92	1.02	0.49	0.91	0.93	0.94	0.90	1.00	0.84	0.99	1.05	0.98	0.96	0.91	1.13	0.96	1.05	0.96	0.88	1.02	0.91	1.03	0.89	0.85	1.04	1.01
	Operator C2 12p13	0.99	1.00	0.95	0.96	0.98	0.96	1.12	0.89	0.98	1.01	0.57	0.91	1.01	0.48	0.90	0.92	0.93	0.89	0.99	0.83	0.98	1.04	0.97	0.94	0.90	1.11	0.95	1.03	0.95	0.87	1.01	0.90	1.02	0.88	0.84	1.03	0.99
	nadja C3 7q31	1.04	1.05	1.00	1.00	1.03	1.01	1.18	0.93	1.03	1.06	0.60	0.96	1.07	0.51	0.95	0.96	0.98	0.93	1.04	0.87	1.03	1.09	1.02	0.99	0.95	1.17	1.00	1.09	1.00	0.91	1.06	0.95	1.07	0.92	0.89	1.08	1.05
	Worksheet C4 20p13	1.04	1.05	1.00	1.00	1.03	1.01	1.18	0.93	1.03	1.06	0.60	0.96	1.06	0.50	0.94	0.96	0.97	0.93	1.04	0.86	1.02	1.08	1.01	0.99	0.94	1.17	0.99	1.08	0.99	0.91	1.06	0.94	1.07	0.92	0.88	1.07	1.04
	1 C5 8p23	1.01	1.02	0.97	0.97	1.00	0.98	1.15	0.90	1.00	1.03	0.58	0.93	1.03	0.49	0.92	0.94	0.95	0.91	1.01	0.84	1.00	1.06	0.99	0.96	0.92	1.14	0.97	1.05	0.97	0.89	1.03	0.92	1.04	0.89	0.86	1.05	1.01
	Int QC Stand Dev C6 14q24	1.03	1.04	0.99	0.99	1.02	1.00	1.17	0.92	1.02	1.05	0.59	0.95	1.05	0.50	0.94	0.95	0.97	0.92	1.03	0.86	1.02	1.08	1.00	0.98	0.94	1.16	0.98	1.07	0.99	0.90	1.05	0.94	1.06	0.91	0.88	1.07	1.03
0.06181459	C7 12q24	0.88	0.89	0.85	0.85	0.87	0.86	1.00	0.79	0.87	0.90	0.51	0.81	0.90	0.43	0.80	0.82	0.83	0.79	0.88	0.74	0.87	0.92	0.86	0.84	0.80	0.99	0.84	0.92	0.84	0.77	0.90	0.80	0.91	0.78	0.75	0.91	0.89
	C8 8q24	1.12	1.13	1.07	1.08	1.11	1.09	1.27	1.00	1.11	1.14	0.64	1.03	1.14	0.54	1.02	1.04	1.05	1.00	1.12	0.93	1.10	1.17	1.09	1.07	1.02	1.26	1.07	1.17	1.07	0.98	1.14	1.02	1.15	0.99	0.95	1.16	1.12
	C9 15q26	1.01	1.02	0.97	0.97	1.00	0.98	1.14	0.90	1.00	1.03	0.58	0.93	1.03	0.49	0.92	0.93	0.95	0.90	1.01	0.84	1.00	1.05	0.98	0.96	0.92	1.13	0.96	1.05	0.97	0.88	1.03	0.92	1.04	0.89	0.86	1.04	1.01
	C10 7p14	0.98	0.99	0.94	0.95	0.97	0.95	1.11	0.88	0.97	1.00	0.56	0.91	1.01	0.48	0.89	0.91	0.92	0.88	0.98	0.82	0.97	1.03	0.96	0.94	0.89	1.10	0.94	1.02	0.94	0.86	1.00	0.89	1.01	0.87	0.84	1.02	0.99
	MEAN	1.01	1.02	0.97	0.97	1.00	0.98	1.15	0.90	1.00	1.03	0.58	0.93	1.03	0.49	0.92	0.94	0.95	0.91	1.01	0.84	1.00	1.06	0.98	0.96	0.92	1.14	0.97	1.05	0.97	0.89	1.03	0.92	1.04	0.89	0.86	1.05	1.01
	ODDS NORMAL :DEL	7346:1	1041:1	6943:1	44270:1	11738:1	8646:1	1955:1	157:1	10212:1	7021:1	1:5	2401:1	72910:1	1644	3810:1	186:1	648:1	146:1	17943:1	35:1	175:1	67017:1	15474:1	3201:1	2173:1	381:1	22562:1	20889:1	7548:1	119:1	5679:1	796:1	4806:1	244:1	60:1	42608:1	754:1
	ODDS NORMAL :DUP	106:1	16:1	168:1	913:1	194:1	177:1	4:1	15:1	1088:1	75:1	3:1	106:1	598:1	3:1	169:1	12:1	28:1	9:1	225:1	9:1	7:1	371:1	289:1	91:1	112:1	2:1	541:1	130:1	190:1	14:1	62:1	45:1	47:1	23:1	11:1	288:1	15:1
	PROB OF DEVIATION NORMAL	94.1589%	89.3784%	60.1011%	44.4973%	95.4135%	73.0197%	12.8257%	46.5886%	95.7871%	73.1924%	9.6290%	27.8422%	40.9675%	0.1184%	8.8853%	65.8694%	62.4650%	11.5366%	87.8353%	32.9115%	97.7701%	10.1393%	74.0100%	59.7418%	17.8895%	43.4668%	37.8709%	31.2254%	54.3327%	39.3744%	73.8600%	35.2177%	66.7067%	32.4467%	33.2603%	29.9606%	93.3412%
	PROB OF DEVIATION DELETED	0.0128%	0.0858%	0.0087%	0.0010%	0.0081%	0.0084%	0.0062%	0.2965%	0.0009%	0.0104%	46.4594%	0.0115%	0.0006%	76.2655%	0.0023%	0.3533%	0.0964%	0.0079%	0.0049%	0.9309%	0.5590%	0.0002%	0.0046%	0.0187%	0.0082%	0.1142%	0.0017%	0.0015%	0.0072%	0.3315%	0.0130%	0.0443%	0.0139%	0.1332%	0.5531%	0.0007%	0.1238%
	PROB OF DEVIATION DUP	0.8898%	4.8958%	0.3588%	0.0487%	0.5163%	0.4126%	3.2470%	3.2119%	0.0690%	0.9754%	3.4259%	0.2613%	0.0693%	0.0406%	0.0471%	5.4824%	2.2693%	0.1218%	0.3907%	3.8038%	13.0888%	0.0273%	0.2557%	0.6581%	0.1598%	18.1526%	0.0700%	0.2407%	0.2864%	2.8280%	1.1967%	0.7774%	1.4264%	1.4157%	3.0421%	0.1039%	6.0328%

HGT 14:1

Test 7	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHD Ex 4	SDHD Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT14.1_B07_002.fsa C1 5q31	1.00	1.07	1.03	1.09	1.04	1.10	1.07	1.02	1.07	1.01	0.87	0.92	1.01	0.50	1.07	1.07	1.01	0.94	1.01	0.73	0.99	0.99	0.94	1.07	1.10	1.01	0.98	0.88	0.92	1.04	1.05	0.91	0.86	0.99	1.07
	Operator C2 12p13	0.93	1.00	0.96	1.02	0.97	1.03	1.00	0.95	1.00	0.95	0.82	0.86	0.94	0.47	1.00	1.00	0.94	0.88	0.94	0.68	0.92	0.93	0.88	1.00	1.03	0.95	0.92	0.82	0.86	0.97	0.98	0.85	0.81	0.93	1.00
	nadja C3 7q31	0.97	1.04	1.00	1.06	1.01	1.07	1.04	0.99	1.04	0.99	0.85	0.89	0.98	0.49	1.04	1.04	0.98	0.91	0.98	0.71	0.96	0.96	0.91	1.04	1.07	0.99	0.95	0.86	0.89	1.01	1.02	0.88	0.84	0.97	1.04
	Worksheet C4 20p13	0.91	0.98	0.94	1.00	0.95	1.01	0.98	0.93	0.98	0.93	0.80	0.84	0.92	0.46	0.98	0.97	0.92	0.86	0.92	0.67	0.90	0.91	0.86	0.97	1.00	0.93	0.90	0.81	0.84	0.95	0.96	0.83	0.79	0.91	0.98
	1 C5 8p23	0.96	1.03	0.99	1.06	1.00	1.06	1.03	0.98	1.03	0.98	0.84	0.89	0.97	0.48	1.03	1.03	0.98	0.91	0.98	0.71	0.95	0.96	0.90	1.03	1.06	0.98	0.95	0.85	0.88	1.00	1.02	0.88	0.83	0.96	1.03
	Int QC Stand Dev C6 14q24	0.91	0.97	0.93	0.99	0.94	1.00	0.97	0.92	0.97	0.92	0.79	0.83	0.91	0.45	0.97	0.97	0.92	0.85	0.92	0.66	0.90	0.90	0.85	0.97	0.99	0.92	0.89	0.80	0.83	0.94	0.95	0.82	0.78	0.90	0.97
0.034255727	C7 12q24	0.93	1.00	0.96	1.02	0.97	1.03	1.00	0.95	1.00	0.95	0.82	0.86	0.94	0.47	1.00	0.99	0.94	0.88	0.94	0.68	0.92	0.93	0.87	0.99	1.02	0.95	0.91	0.82	0.86	0.97	0.98	0.85	0.81	0.93	1.00
	C8 8q24	0.98	1.05	1.01	1.07	1.02	1.08	1.05	1.00	1.05	1.00	0.86	0.90	0.99	0.49	1.05	1.05	0.99	0.92	0.99	0.72	0.97	0.98	0.92	1.05	1.08	1.00	0.96	0.87	0.90	1.02	1.03	0.89	0.85	0.98	1.05
	C9 15q26	0.93	1.00	0.96	1.02	0.97	1.03	1.00	0.95	1.00	0.95	0.82	0.86	0.94	0.47	1.00	1.00	0.95	0.88	0.95	0.68	0.92	0.93	0.88	1.00	1.03	0.95	0.92	0.82	0.86	0.97	0.98	0.85	0.81	0.93	1.00
	C10 7p14	0.99	1.06	1.01	1.08	1.02	1.09	1.06	1.00	1.05	1.00	0.86	0.91	1.00	0.49	1.06	1.05	1.00	0.93	1.00	0.72	0.97	0.98	0.92	1.05	1.08	1.00	0.97	0.87	0.90	1.02	1.04	0.90	0.85	0.98	1.05
	MEAN	0.95	1.02	0.98	1.04	0.99	1.05	1.02	0.97	1.02	0.97	0.83	0.88	0.96	0.48	1.02	1.02	0.96	0.90	0.96	0.70	0.94	0.95	0.89	1.02	1.05	0.97	0.93	0.84	0.87	0.99	1.00	0.86	0.82	0.95	1.02
	ODDS NORMAL :DEL	2597:1	1042:1	9492:1	50635:1	9538:1	9063:1	5299:1	495:1	79785:1	3782:1	16:1	491:1	30888:1	1465	52830:1	497:1	874:1	1121:1	7347:1	2:1	90:1	24642:1	961:1	374:1	34213:1	16232:1	3047:1	50:1	307:1	3554:1	5510:1	136:1	31:1	12978:1	759:1
	ODDS NORMAL :DUP	84:1	18:1	190:1	340:1	173:1	62:1	63:1	18:1	795:1	99:1	5:1	50:1	748:1	3:1	602:1	11:1	29:1	83:1	189:1	5:1	7:1	760:1	74:1	9:1	224:1	380:1	124:1	11:1	34:1	72:1	86:1	19:1	9:1	406:1	14:1
	PROB OF DEVIATION NORMAL	51.9985%	67.6526%	74.1226%	29.3470%	80.7297%	43.8060%	79.4999%	81.2290%	64.0875%	63.5857%	43.6157%	9.4681%	30.4278%	0.1076%	60.4615%	92.3121%	73.2116%	9.6350%	52.6725%	10.3564%	76.0387%	8.8820%	10.1183%	92.8133%	29.7989%	47.3845%	28.7847%	25.9304%	15.3373%	86.8124%	97.9388%	23.6048%	24.6631%	20.0567%	89.5808%
	PROB OF DEVIATION DELETED	0.0200%	0.0843%	0.0078%	0.0006%	0.0088%	0.0048%	0.0150%	0.1642%	0.0008%	0.0168%	2.6985%	0.0150%	0.0010%	49.3722%	0.0010%	0.1853%	0.0838%	0.0085%	0.0072%	5.3040%	0.8438%	0.0004%	0.0105%	0.2480%	0.0009%	0.0029%	0.0094%	0.5169%	0.0500%	0.0244%	0.0178%	0.1738%	0.7850%	0.0015%	0.1181%
	PROB OF DEVIATION DUP	0.6159%	4.9650%	0.3911%	0.8842%	0.4842%	0.7076%	1.2603%	4.6041%	0.8906%	0.6412%	8.3986%	0.1979%	0.0407%	0.0386%	0.1004%	0.2845%	2.5005%	0.1160%	0.2793%	2.0702%	11.0111%	0.0117%	0.1361%	10.0247%	0.1330%	0.1248%	0.2326%	2.2757%	0.4446%	1.2021%	1.1331%	1.2260%	2.5994%	0.0494%	6.2514%

HGT 14:2

Test 8	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT14.2_C07_003.fsa	1.00	0.97	1.09	0.91	1.02	1.02	1.04	1.05	0.99	0.98	1.00	0.93	1.05	0.57	1.01	0.78	0.92	0.89	1.00	0.83	1.00	0.99	1.05	1.08	0.89	1.08	1.01	1.02	0.98	0.89	0.94	1.01	1.02	0.86	0.81	1.01	0.98
	Operator	1.03	1.00	1.12	0.94	1.05	1.05	1.07	1.08	1.02	1.00	1.03	0.95	1.08	0.59	1.04	0.81	0.95	0.91	1.02	0.86	1.03	1.01	1.08	1.11	0.91	1.11	1.04	1.05	1.01	0.91	0.97	1.04	1.05	0.88	0.83	1.04	1.01
	nadja	0.92	0.89	1.00	0.84	0.94	0.93	0.95	0.96	0.91	0.90	0.92	0.85	0.96	0.52	0.93	0.72	0.85	0.82	0.91	0.77	0.92	0.90	0.97	0.99	0.81	0.99	0.93	0.94	0.90	0.82	0.86	0.93	0.93	0.79	0.74	0.93	0.90
	Worksheet	1.10	1.07	1.19	1.00	1.12	1.12	1.14	1.15	1.09	1.07	1.10	1.02	1.15	0.63	1.11	0.86	1.01	0.98	1.09	0.91	1.09	1.08	1.16	1.18	0.97	1.18	1.11	1.12	1.08	0.97	1.03	1.11	1.12	0.94	0.89	1.11	1.08
	1	0.98	0.95	1.06	0.89	1.00	0.99	1.02	1.02	0.97	0.95	0.98	0.91	1.02	0.56	0.99	0.77	0.90	0.87	0.97	0.82	0.97	0.96	1.03	1.05	0.87	1.05	0.99	1.00	0.96	0.87	0.92	0.99	0.99	0.84	0.79	0.98	0.96
	Int QC Stand Dev	0.98	0.96	1.07	0.90	1.01	1.00	1.02	1.03	0.98	0.96	0.99	0.91	1.03	0.56	1.00	0.77	0.91	0.87	0.98	0.82	0.98	0.97	1.04	1.06	0.87	1.06	0.99	1.00	0.97	0.87	0.92	1.00	1.00	0.84	0.79	0.99	0.97
	0.048300475	0.96	0.94	1.05	0.88	0.99	0.98	1.00	1.01	0.95	0.94	0.97	0.89	1.01	0.55	0.98	0.75	0.89	0.86	0.96	0.80	0.96	0.95	1.01	1.04	0.85	1.04	0.97	0.98	0.94	0.85	0.90	0.98	0.98	0.82	0.78	0.97	0.94
		0.96	0.93	1.04	0.87	0.98	0.97	0.99	1.00	0.95	0.93	0.96	0.89	1.00	0.55	0.97	0.75	0.88	0.85	0.95	0.80	0.95	0.94	1.01	1.03	0.85	1.03	0.97	0.98	0.94	0.85	0.90	0.97	0.97	0.82	0.77	0.96	0.94
		1.01	0.98	1.10	0.92	1.03	1.03	1.05	1.05	1.00	0.98	1.01	0.94	1.05	0.58	1.02	0.79	0.93	0.90	1.00	0.84	1.01	0.99	1.06	1.09	0.89	1.08	1.02	1.03	0.99	0.90	0.95	1.02	1.03	0.86	0.81	1.02	0.99
		1.03	1.00	1.12	0.94	1.05	1.04	1.07	1.07	1.02	1.00	1.03	0.95	1.07	0.59	1.04	0.80	0.95	0.91	1.02	0.86	1.02	1.01	1.08	1.10	0.91	1.10	1.04	1.05	1.01	0.91	0.96	1.04	1.04	0.88	0.83	1.03	1.01
	MEAN	1.00	0.97	1.08	0.91	1.02	1.01	1.03	1.04	0.99	0.97	1.00	0.92	1.04	0.57	1.01	0.78	0.92	0.89	0.99	0.83	0.99	0.98	1.05	1.07	0.88	1.07	1.01	1.02	0.98	0.88	0.94	1.01	1.01	0.85	0.80	1.00	0.98
	ODDS NORMAL :DEL	6576:1	617:1	6357:1	2760:1	11494:1	10313:1	4994:1	798:1	67719:1	4123:1	141:1	2011:1	56567:1	143	79792:1	11:1	370:1	784:1	14960:1	29:1	168:1	164190:1	14185:1	4589:1	656:1	396:1	76143:1	39264:1	10334:1	117:1	1459:1	4500:1	5380:1	101:1	21:1	101349:1	521:1
	ODDS NORMAL :DUP	113:1	21:1	27:1	16:1	132:1	167:1	50:1	11:1	1120:1	102:1	6:1	97:1	397:1	4:1	942:1	7:1	24:1	69:1	253:1	8:1	7:1	2931:1	97:1	25:1	62:1	5:1	936:1	435:1	217:1	14:1	64:1	67:1	75:1	18:1	9:1	1317:1	17:1
	PROB OF DEVIATION NORMAL	92.7411%	78.3377%	23.0704%	5.0282%	79.5555%	86.9420%	68.7207%	76.7098%	70.2791%	67.1539%	98.5811%	24.4590%	29.6735%	0.2206%	85.1369%	19.0418%	46.1612%	7.4810%	85.5236%	30.1509%	96.3526%	44.0119%	40.8413%	36.7952%	7.8075%	67.2927%	90.0243%	77.5966%	67.4627%	39.0446%	40.6852%	92.8714%	89.7070%	20.2013%	20.4683%	97.9318%	85.1771%
	PROB OF DEVIATION DELETED	0.0141%	0.1270%	0.0036%	0.0018%	0.0069%	0.0065%	0.0138%	0.0961%	0.0010%	0.0163%	0.6970%	0.0122%	0.0065%	9.4593%	0.0011%	1.6892%	0.1248%	0.0095%	0.0057%	1.0418%	0.5732%	0.0003%	0.0029%	0.0080%	0.0119%	0.1699%	0.0012%	0.0020%	0.0065%	0.3344%	0.0279%	0.0206%	0.0167%	0.1990%	0.9892%	0.0010%	0.1636%
	PROB OF DEVIATION DUP	0.8174%	3.6850%	0.9581%	0.0300%	0.6019%	0.5198%	1.3930%	6.8417%	0.0628%	0.6576%	15.9412%	0.2514%	0.0748%	0.0553%	0.0903%	2.6795%	1.9224%	0.1081%	0.3385%	3.6325%	13.6194%	0.0150%	0.4218%	1.4522%	0.1264%	13.1668%	0.0962%	0.1783%	0.3106%	2.8152%	0.6401%	1.3885%	1.1998%	1.1448%	2.3634%	0.0744%	4.9427%

HGT 14:3

Test 9	Lab No	C1 5q31	C2 12p13	C3 7q31	C4 20p13	C5 8p23	C6 14q24	C7 12q24	C8 8q24	C9 15q26	C10 7p14	SDHB Ex 1-1	SDHB Ex 1-2	SDHB Ex 2	SDHB Ex 3	SDHB Ex 4	SDHB Ex 5	SDHB Ex 6	SDHB Ex 7	SDHB Ex 8	SDHC Prom	SDHC Ex 1	SDHC Ex 2	SDHC Ex 3-1	SDHC Ex 3-2	SDHC Ex 4-1	SDHC Ex 4-2	SDHC Ex 5	SDHC Ex 6	SDHD Prom	SDHD Ex 1	SDHD Ex 2	SDHD Ex 3	SDHD Ex 4	SDHAF1 Ex 1	SDHAF2 Ex 1	SDHAF2 Ex 3	SDHAF2 Ex 4
	HGT14.3_D07_004.fsa	1.00	1.02	1.05	0.93	0.90	0.98	1.08	0.88	1.09	0.99	0.60	0.88	1.01	0.58	0.94	0.81	0.87	0.87	0.90	1.12	0.90	1.01	0.93	0.98	1.04	1.14	0.95	0.92	0.84	0.87	1.09	0.98	1.32	0.87	0.81	0.85	0.89
	Operator	0.98	1.00	1.03	0.91	0.88	0.96	1.05	0.86	1.07	0.97	0.58	0.85	0.99	0.56	0.92	0.79	0.85	0.85	0.88	1.10	0.88	0.98	0.91	0.96	1.02	1.11	0.93	0.90	0.82	0.85	1.06	0.95	1.29	0.85	0.79	0.83	0.87
	nadja	0.95	0.98	1.00	0.89	0.86	0.94	1.03	0.83	1.04	0.94	0.57	0.83	0.96	0.55	0.89	0.77	0.83	0.83	0.86	1.07	0.86	0.96	0.88	0.93	1.00	1.09	0.91	0.88	0.80	0.83	1.04	0.93	1.26	0.83	0.77	0.81	0.85
	Worksheet	1.07	1.10	1.13	1.00	0.97	1.05	1.16	0.94	1.17	1.06	0.64	0.94	1.08	0.62	1.01	0.87	0.94	0.94	0.97	1.21	0.97	1.08	0.99	1.05	1.12	1.22	1.02	0.99	0.90	0.94	1.17	1.05	1.42	0.93	0.87	0.91	0.96
	1	1.11	1.14	1.16	1.03	1.00	1.09	1.20	0.97	1.21	1.10	0.66	0.97	1.12	0.64	1.04	0.90	0.97	0.97	1.00	1.25	1.00	1.12	1.03	1.09	1.16	1.27	1.06	1.02	0.93	0.97	1.21	1.08	1.47	0.96	0.90	0.94	0.99
	Int QC Stand Dev	1.02	1.04	1.07	0.95	0.92	1.00	1.10	0.89	1.11	1.01	0.61	0.89	1.03	0.59	0.95	0.83	0.89	0.89	0.92	1.14	0.92	1.02	0.94	1.00	1.06	1.16	0.97	0.94	0.85	0.89	1.11	0.99	1.35	0.88	0.83	0.87	0.91
	0.073823492	0.93	0.95	0.97	0.86	0.84	0.91	1.00	0.81	1.01	0.92	0.55	0.81	0.93	0.53	0.87	0.75	0.81	0.81	0.83	1.04	0.83	0.93	0.86	0.91	0.97	1.06	0.88	0.85	0.78	0.81	1.01	0.90	1.23	0.80	0.75	0.79	0.83
		1.14	1.17	1.20	1.06	1.03	1.12	1.23	1.00	1.25	1.13	0.68	1.00	1.15	0.66	1.07	0.93	1.00	1.00	1.03	1.28	1.03	1.15	1.06	1.12	1.19	1.30	1.09	1.05	0.96	1.00	1.24	1.11	1.51	0.99	0.93	0.97	1.02
		0.91	0.93	0.96	0.85	0.82	0.90	0.99	0.80	1.00	0.90	0.54	0.80	0.92	0.53	0.86	0.74	0.80	0.80	0.82	1.03	0.82	0.92	0.85	0.89	0.95	1.04	0.87	0.84	0.76	0.80	1.00	0.89	1.21	0.79	0.74	0.78	0.81
		1.01	1.03	1.06	0.94	0.91	0.99	1.09	0.89	1.11	1.00	0.60	0.88	1.02	0.58	0.95	0.82	0.88	0.88	0.91	1.14	0.91	1.02	0.94	0.99	1.06	1.15	0.97	0.93	0.85	0.88	1.10	0.99	1.34	0.88	0.82	0.86	0.90
	MEAN	1.01	1.04	1.06	0.94	0.91	0.99	1.09	0.89	1.11	1.00	0.60	0.89	1.02	0.58	0.95	0.82	0.88	0.88	0.91	1.14	0.91	1.02	0.94	0.99	1.06	1.16	0.97	0.93	0.85	0.88	1.10	0.99	1.34	0.88	0.82	0.86	0.90
	ODDS NORMAL :DEL	7297:1	1039:1	883:1	10855:1	1151:1	11346:1	3278:1	113:1	9942:1	7372:1	13	587:1	103580:1	125	11463:1	24:1	167:1	667:1	1361:1	439:1	57:1	298835:1	3609:1	5717:1	13447:1	371:1	21948:1	4274:1	197:1	111:1	3166:1	3375:1	468:1	165:1	29:1	430:1	147:1
	ODDS NORMAL :DUP	102:1	15:1	54:1	376:1	71:1	195:1	15:1	14:1	26:1	121:1	3:1	55:1	1048:1	4:1	366:1	8:1	19:1	64:1	84:1	2:1	7:1	3055:1	144:1	109:1	85:1	2:1	532:1	161:1	35:1	14:1	12:1	71:1	1:19	20:1	9:1	58:1	14:1
	PROB OF DEVIATION NORMAL	92.2623%	80.1472%	38.1088%	14.5168%	20.2860%	87.1058%	30.6526%	39.7074%	4.3561%	95.9533%	10.8943%	10.6412%	64.8136%	0.2453%	20.7635%	26.2258%	30.1063%	6.7006%	16.2007%	40.9336%	60.5424%	60.3635%	26.3056%	85.6951%	36.3048%	38.1198%	37.0660%	17.4455%	4.6284%	38.0890%	24.8719%	84.2714%	1.1252%	26.1889%	23.7499%	1.6028%	46.4028%
	PROB OF DEVIATION DELETED	0.0126%	0.077%	0.0043%	0.0013%	0.0076%	0.0077%	0.0094%	0.3502%	0.0004%	0.0130%	36.1900%	0.0181%	0.0006%	6.2059%	0.0018%	1.0890%	0.1799%	0.0100%	0.0119%	0.0935%	1.1142%	0.0002%	0.0073%	0.0150%	0.0027%	0.1023%	0.0017%	0.0041%	0.0234%	0.3431%	0.0079%	0.0250%	0.0024%	0.1591%	0.8218%	0.0037%	0.3156%
	PROB OF DEVIATION DUP	0.9010%	5.3384%	0.6999%	0.0386%	0.2865%	0.4479%	2.0680%	2.9376%	0.1688%	0.7940%	3.6783%	0.1948%	0.0618%	0.0581%	0.0567%	3.1717%	1.5585%	0.1043%	0.1301%	16.5691%	9.5758%	0.0198%	0.1826%	0.7863%	0.4274%	13.9817%	0.0697%	0.0962%	0.1340%	2.7778%	2.0663%	1.1805%	20.9861%	1.2841%	2.5495%	0.0277%	3.3089%

APPENDIX G

Long-range PCR optimisation

AccuprimeTaq was used in this reaction which yielded brighter bands and no non-specific amplification(figure S1B). The wild-type product did not amplify in the patient samples neither in the control samples. The AccuprimeTaq was used for identification of the deletion fragment from this point forward and not the Failsafe Taq.

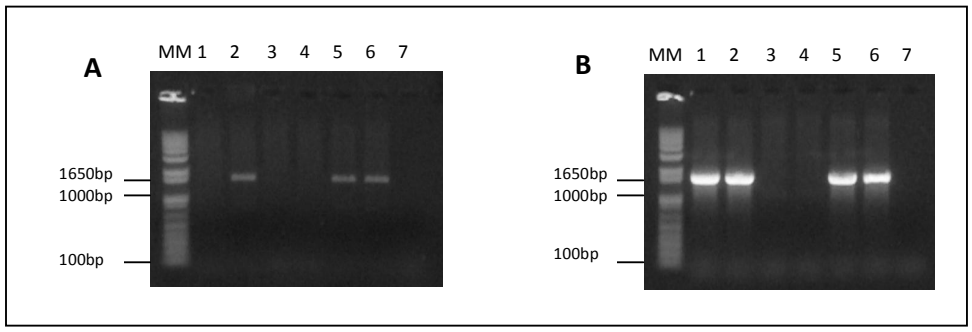


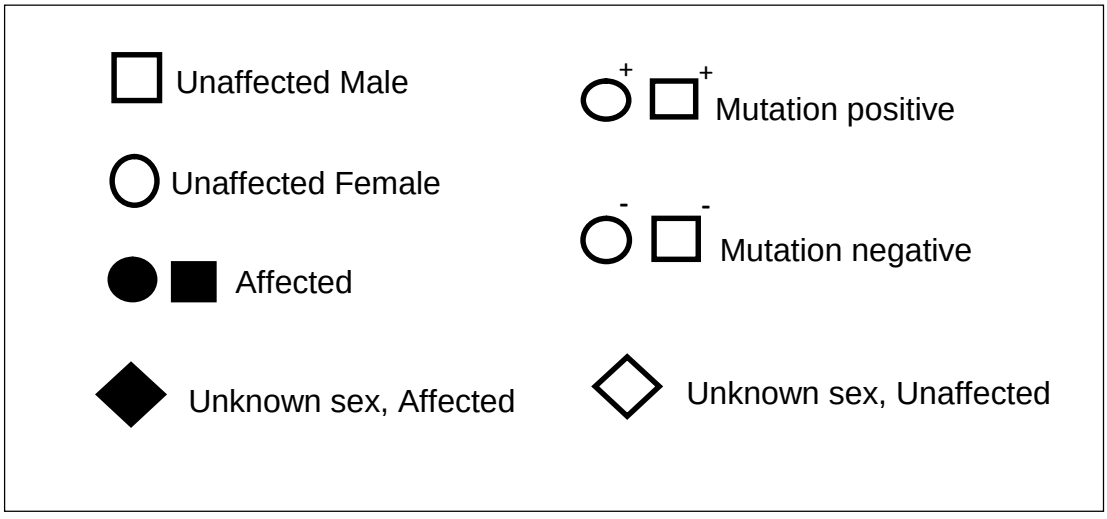
Figure S1: Comparison of the ~1.6kb pcr product yielded with Failsafe - and Accuprime Taq
Products obtained with Failsafe Taq (A) are much fainter than products obtained with AccuprimeTaq (B). MM = Molecular marker; Lanes 1-2 and 5-7: deletion positive samples; Lanes 3-4: Normal controls; Lane 7: Blank sample

Appendix H

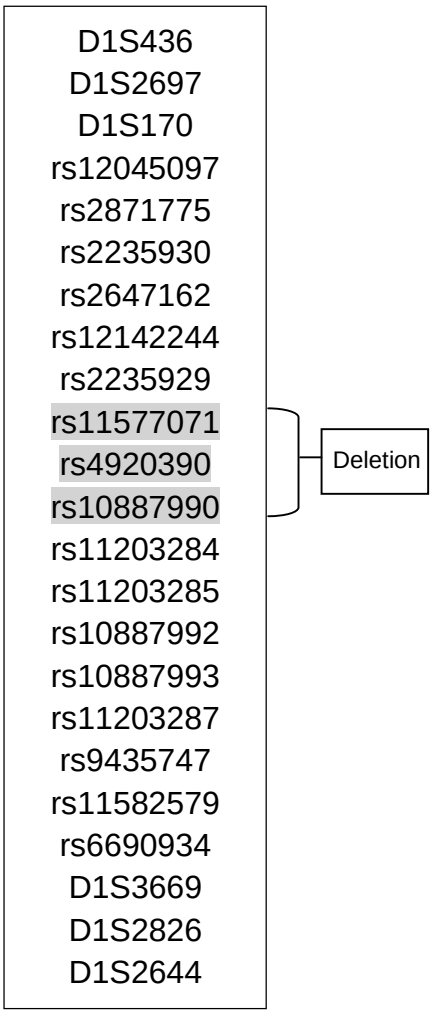
Haplotype analysis of families carrying the *SDHB* exon 3 deletion

(Pedigrees have been abbreviated, not all spouses and unaffected children are indicated)

Key

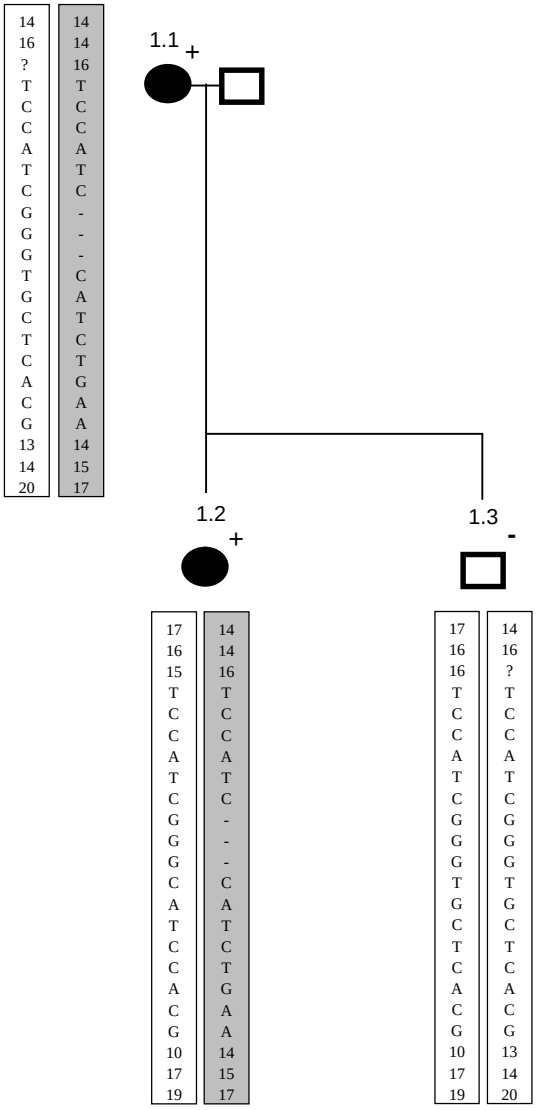


Microsatellite markers and intragenic SNPs used for haplotype analysis

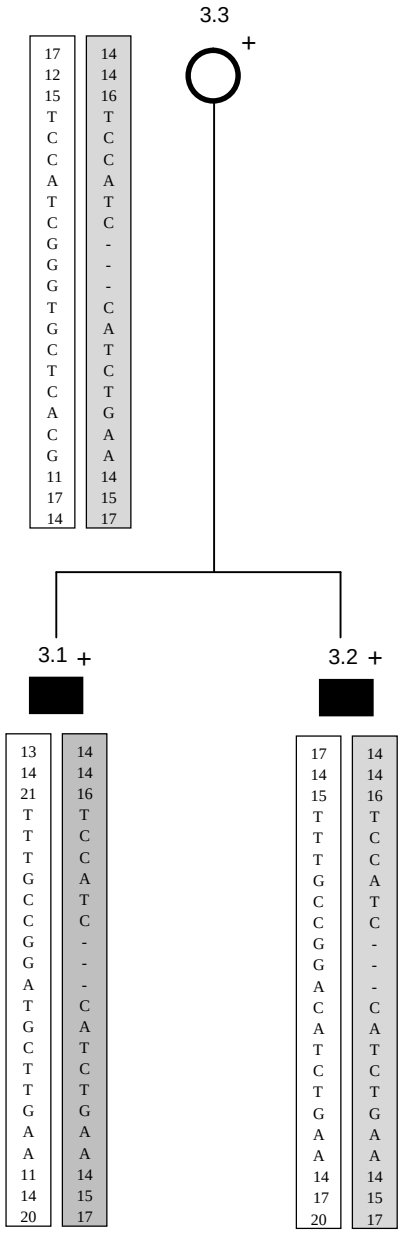


Grey shaded allele represents the common disease haplotype of the individuals. Non-shaded allele represents the non-diseased haplotype of individuals

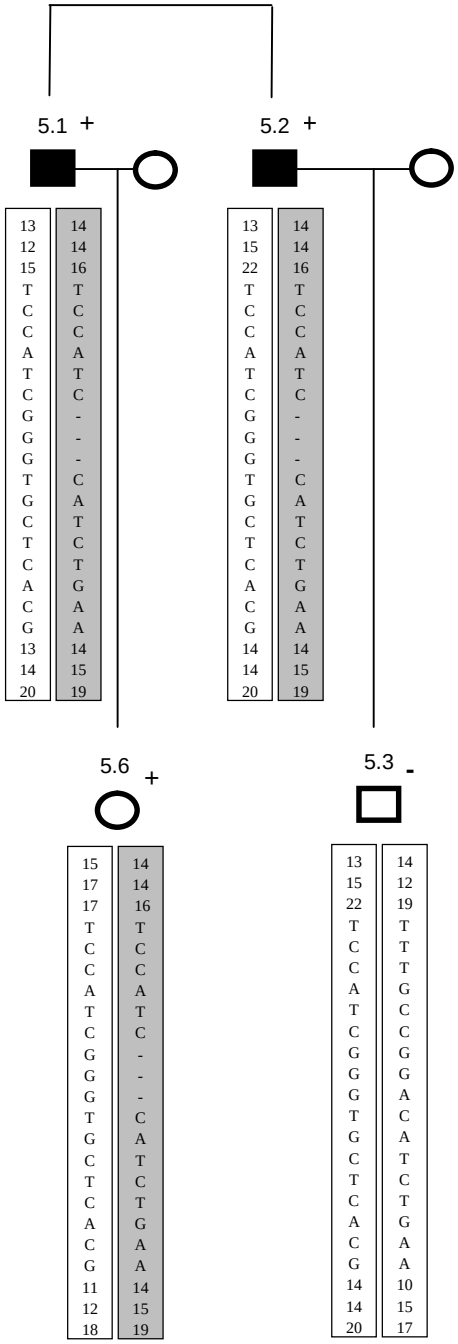
HGT 1



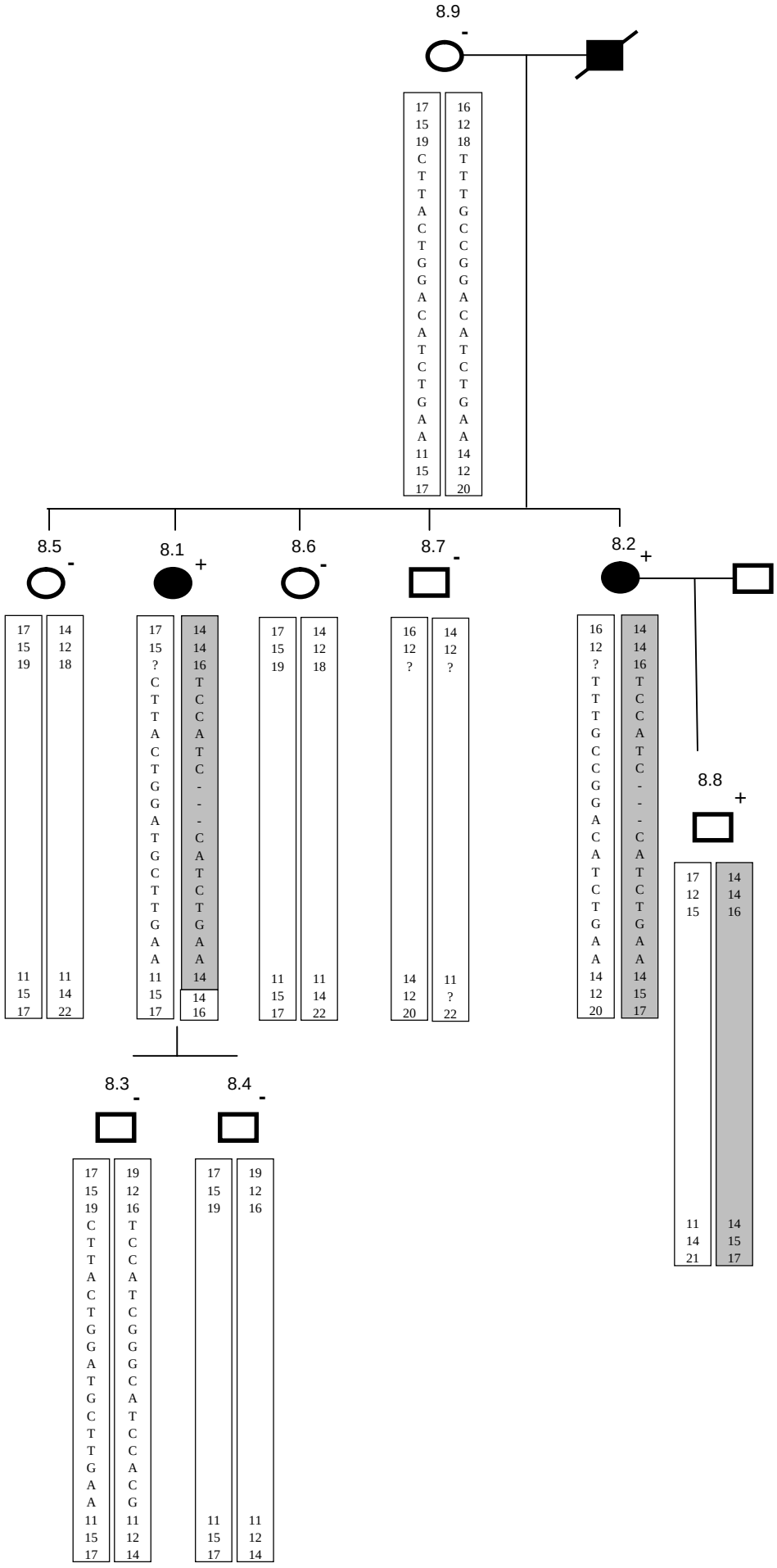
HGT 3



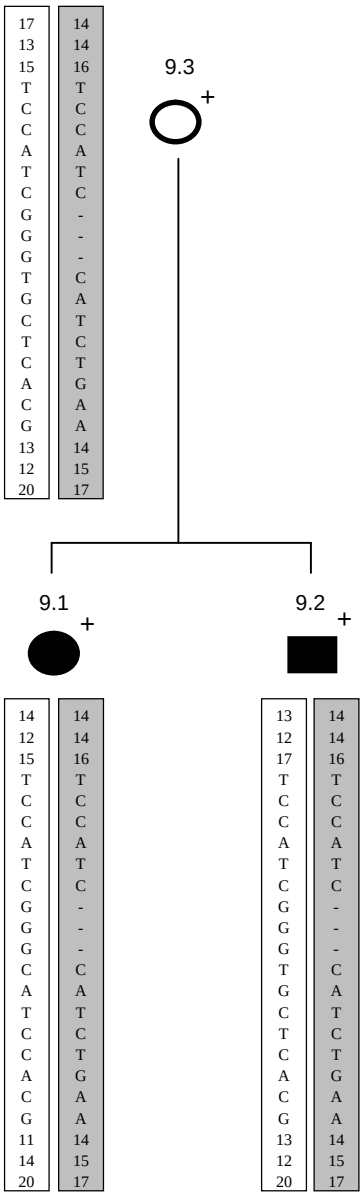
HGT 5



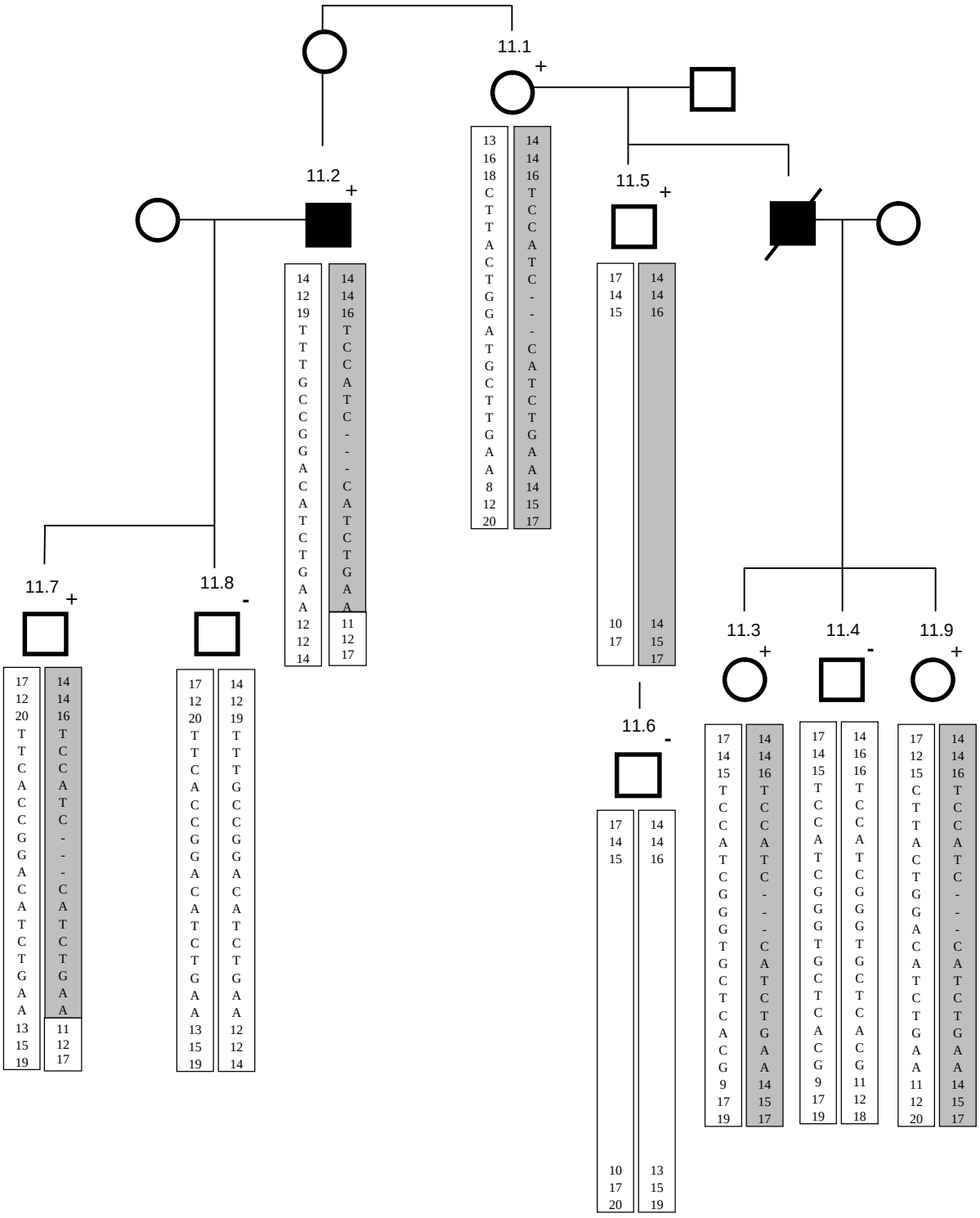
HGT 8



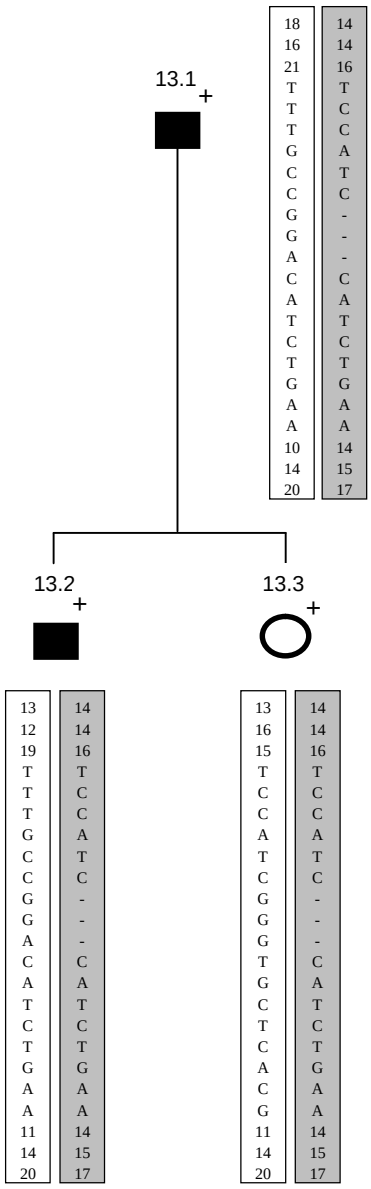
HGT 9



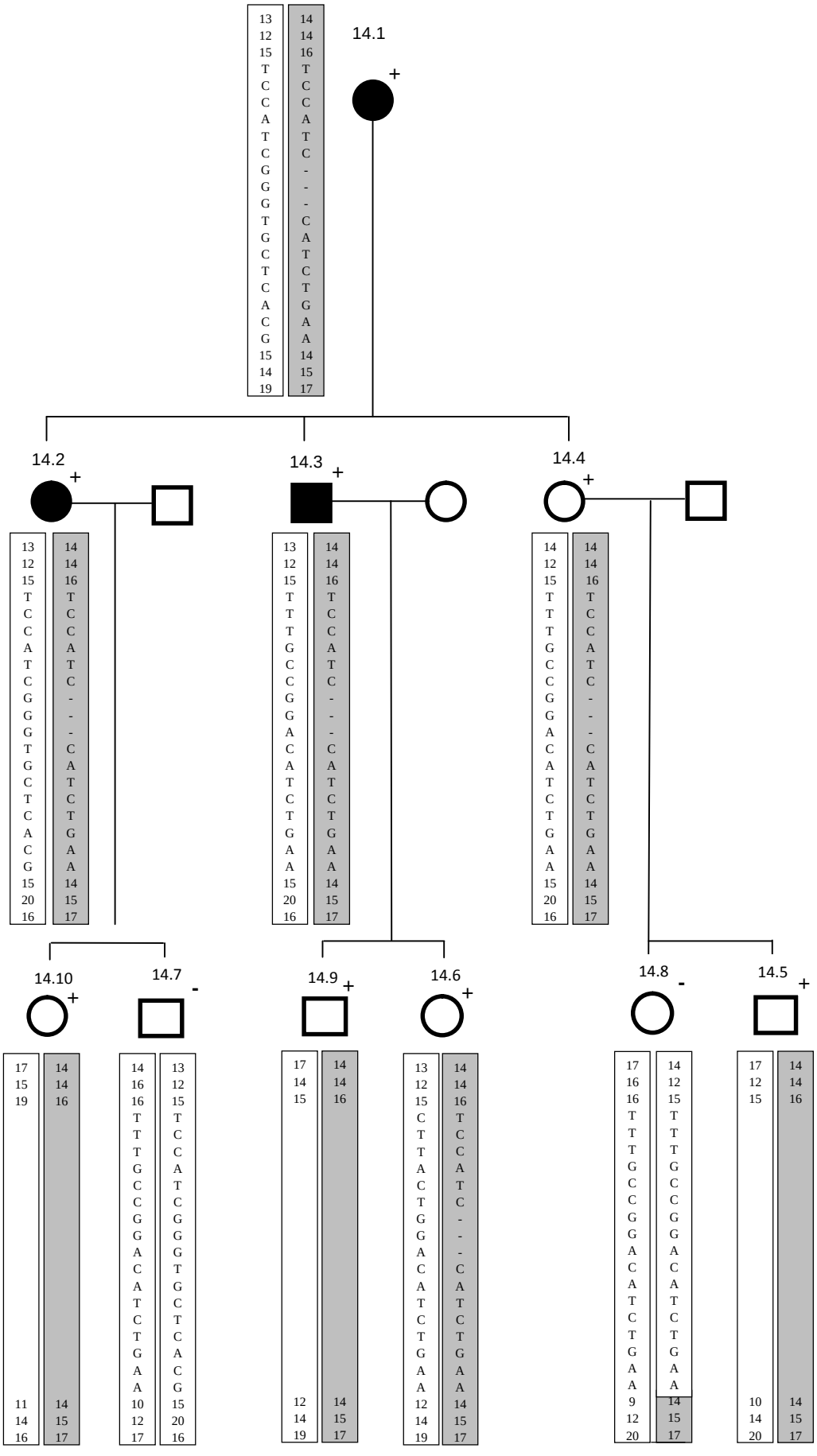
HGT 11



HGT 13

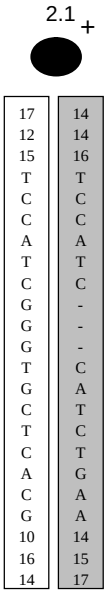


HGT 14

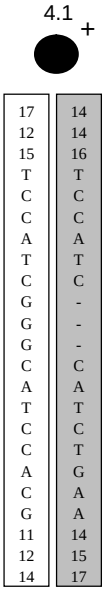


Inferred Haplotypes

HGT 2



HGT 4



Dutch Individuals