

**Appendix A. Supplemental Tables:**  
**Sequence reads and expression.**

**Supplemental Table A1: Summary of the library preparation, sequencing and quality filtering of the sequence data of *R. appendiculatus*.**

| Dataset                                    | Library preparation (concentration of starting total RNA) | Library preparation (RNA fragmentation time) | Library preparation (number of amplification cycles) | Library preparation (size selection by excision from agarose gel) | Illumina instrument used for sequencing | Sequence read length | Number of raw sequence reads (read 1/ read 2) | Size of raw sequence reads (bp) | Number of quality filtered sequence reads (read 1/ read 2) | Average size of quality filtered sequence reads (bp) | Percentage of reads discarded (read 1/ read 2) |
|--|---|--|--|---|---|----------------------|---|---------------------------------|--|--|--|
| <b>HiSeq 2000 generated sequence reads</b> |   |  |  |   |   |                      |   |                                 |  |  |  |
| HiSeq                                      | 4 ug  | 8 min  | 15   | ±300 bp   | HiSeq 2000                              | 100 x 100            | 413 323 262/<br>413 323 262                   | 100                             | 366 810 605/<br>338 340 792                                | 20-100   | 11.3/<br>18.1                                  |
| <b>MiSeq generated sequence reads</b>      |   |  |  |   |   |                      |   |                                 |  |  |  |
| MiSeq SE*                                  | 4 ug  | 8 min  | 15   | ±300 bp   | MiSeq                                   | 240 (SE)             | 3 855 867                                     | 240                             | 2 961 283  | 20-240   | 23.2   |
| MiSeq PE*                                  | 3.1 ug  | 3 min  | 12   | ±600 - 1200 bp  | MiSeq                                   | 250 x 250            | 13 216 382/<br>13 216 382                     | 250                             | 8 781 175/<br>6 297 010                                    | 20-250   | 33.6/<br>52.4                                  |
| <b>Total MiSeq data</b>                    |   |  |  |   |   |                      | <b>17 072 249/<br/>13 216 382</b>             | <b>150-250</b>                  | <b>12 565 276/<br/>5 474 192</b>                           | <b>20-250</b>  | <b>26.4/58.6</b>                               |
| <b>Total generated sequence reads</b>      |   |  |  |   |   |                      |   |                                 |  |  |  |
| Total sequence data (HiSeq and MiSeq)      |   |  |  |   |   |                      | 430 395 511/<br>426 539 644                   | 100-250                         | 379 375 881/<br>343 814 984                                | 20-250   | 11.9/19.4                                      |

\* SE = single end sequencing; PE = paired end sequencing

**Supplemental Table A2: Top expressing transcripts in the *R. appendiculatus* transcriptome.**

| Expression rank * | Transcript ID | ORF ID           | Annotation   | TPM value | Percentage of transcriptome |
|-------------------|---------------|------------------|--|-----------|-----------------------------|
| 1                 | c33374_g1_i1  | Rapp_Mc2208      | Glycine rich superfamily: RIM36                                    | 43 988    | 4.40                        |
| 2                 | c53945_g1_i1  | No ORF predicted | 16S ribosomal RNA  | 40 496    | 4.05                        |
| 3                 | c43993_g1_i2  | Rapp_Mc13679     | Glycine rich superfamily   | 33 290    | 3.33                        |
| 4                 | c15622_g1_i1  | Rapp_Mc13680     | Unknown function   | 28 031    | 2.81                        |
| 5                 | c22478_g1_i1  | Rapp_Mc950       | Glycine rich superfamily   | 25 459    | 2.55                        |
| 6                 | c53938_g1_i1  | Rapp_Mc8886      | Lipocalin family: Male-specific histamine-binding salivary protein | 21 780    | 2.18                        |
| 7                 | c46457_g2_i1  | Rapp_Mc13681     | Glycine rich superfamily   | 20 255    | 2.03                        |
| 8                 | c37026_g1_i1  | Rapp_Mc13629     | Glycine rich superfamily   | 16 012    | 1.60                        |
| 9                 | c43993_g1_i1  | Rapp_Mc13812     | Glycine rich superfamily   | 15 173    | 1.52                        |
| 10                | c41649_g1_i1  | Rapp_Mc12875     | Unknown function   | 13 758    | 1.38                        |
| 11                | c41162_g1_i1  | Rapp_Mc4548      | Lipocalin family: Female-specific histamine-binding protein 1      | 13 316    | 1.33                        |
| 12                | c41649_g1_i2  | Rapp_Mc12173     | Unknown function   | 13 177    | 1.32                        |
| 13                | c43993_g1_i3  | Rapp_Mc10553     | Glycine rich superfamily   | 13 151    | 1.32                        |
| 14                | c48158_g1_i1  | Rapp_Mc8700      | No hit   | 12 495    | 1.25                        |
| 15                | c36384_g1_i1  | Rapp_Mc13682     | Glycine rich superfamily   | 12 489    | 1.25                        |
| 16                | c17798_g1_i1  | Rapp_Mc774       | ML domain: Immunoglobulin G binding protein C                      | 11 353    | 1.14                        |
| 17                | c39014_g2_i1  | Rapp_Mc9768      | Glycine rich superfamily   | 10 864    | 1.09                        |
| 18                | c36396_g1_i1  | Rapp_Mc9443      | No hit   | 9 103     | 0.91                        |
| 19                | c50957_g1_i1  | Rapp_Mc13626     | Unknown function   | 8 589     | 0.86                        |
| 20                | c1612_g1_i1   | Rapp_Mc13700     | Energy metabolism: Cytochrome c oxidase subunit 1                  | 8 340     | 0.83                        |

\* Transcripts ranked based on TPM (transcripts per million) value

**Supplemental Table A3: Characterisation of the tick secretory protein family expression in the *R. appendiculatus* transcriptome.**

| Secretory protein family                                | Number of family members | Proportion of the total number of secretory proteins represented by this family (%) | Protein family average TPM value | Proportion of the secretory protein class expression represented by this family (%) | ORF ID of the top expressing member in the family | TPM value of the top expressing member in the family | Proportion of the protein family represented by the top expressing member (%) |
|---|--------------------------|---|----------------------------------|---|---|--|---|
| Lipocalin   | 516                      | 24.18   | 133.12                           | 12.47   | Rapp_Mc8886                                       | 21 779.87  | 31.71   |
| Bovine pancreatic trypsin inhibitor                     | 236                      | 11.06   | 58.43                            | 2.50  | Rapp_Mc8896                                       | 2447.72  | 17.75   |
| Reprolysin  | 133                      | 6.23  | 34.93                            | 0.84  | Rapp_Mc5881                                       | 628.36   | 13.52   |
| Glycine rich superfamily                                | 119                      | 5.58  | 3072.04                          | 66.34   | Rapp_Mc2208                                       | 43 988.23  | 11.55   |
| TIL domain  | 108                      | 5.06  | 164.00                           | 3.21  | Rapp_Mc1646                                       | 2898.99  | 16.37   |
| 8.9 kDa family  | 102                      | 4.78  | 84.00                            | 1.55  | Rapp_Mc13118                                      | 1185.58  | 13.84   |
| Basic tail secreted protein                             | 90                       | 4.22  | 75.77                            | 1.24  | Rapp_Mc4488                                       | 879.84   | 12.90   |
| Evasin  | 68                       | 3.19  | 55.54                            | 0.69  | Rapp_Mc9039                                       | 619.77   | 16.41   |
| Ixodegrin B   | 57                       | 2.67  | 30.13                            | 0.31  | Rapp_Mc823  | 450.18   | 26.21   |
| Gluzincin   | 52                       | 2.44  | 7.63                             | 0.07  | Rapp_Mc4972                                       | 115.13   | 29.02   |
| Mucin   | 52                       | 2.44  | 44.87                            | 0.42  | Rapp_Mc417  | 876.51   | 37.57   |
| Digestive system (including Serine proteases)           | 50                       | 2.34  | 25.01                            | 0.23  | Rapp_Mc1191                                       | 137.69   | 11.01   |
| Cystatin  | 47                       | 2.20  | 54.35                            | 0.46  | Rapp_Mc13730                                      | 776.50   | 30.40   |
| Folding, sorting and degradation (including Cathepsins) | 40                       | 1.87  | 96.78                            | 0.70  | Rapp_Mc945  | 1498.52  | 38.71   |
| 28 kDa Metastriate family                               | 31                       | 1.45  | 50.68                            | 0.29  | Rapp_Mc2646                                       | 557.06   | 35.46   |
| Chitin-binding proteins                                 | 30                       | 1.41  | 23.00                            | 0.13  | Rapp_Mc9698                                       | 223.55   | 32.39   |
| Serpin  | 27                       | 1.27  | 8.98                             | 0.04  | Rapp_Mc5185                                       | 74.71  | 30.81   |

|                                     |    |      |         |      |              |           |       |
|-------------------------------------|----|------|---------|------|--------------|-----------|-------|
| DA-P36 family                       | 25 | 1.17 | 23.86   | 0.11 | Rapp_Mc8808  | 340.81    | 57.13 |
| Transport and catabolism            | 25 | 1.17 | 36.14   | 0.16 | Rapp_Mc2177  | 535.87    | 59.31 |
| One of each family                  | 23 | 1.08 | 9.44    | 0.04 | Rapp_Mc3057  | 42.46     | 19.57 |
| Lipid metabolism                    | 22 | 1.03 | 4.28    | 0.02 | Rapp_Mc1456  | 12.14     | 12.89 |
| Carboxypeptidase inhibitor          | 22 | 1.03 | 42.89   | 0.17 | Rapp_Mc10222 | 388.49    | 41.17 |
| 5'-Nucleotidase                     | 16 | 0.75 | 14.33   | 0.04 | Rapp_Mc6697  | 47.63     | 20.77 |
| Microplusin                         | 16 | 0.75 | 65.37   | 0.19 | Rapp_Mc1964  | 434.68    | 41.56 |
| ML domain                           | 16 | 0.75 | 1111.82 | 3.23 | Rapp_Mc774   | 11 353.27 | 63.82 |
| Antigen 5 family                    | 13 | 0.61 | 188.87  | 0.45 | Rapp_Mc1903  | 1014.98   | 41.34 |
| Signaling molecules and interaction | 13 | 0.61 | 1.56    | 0.00 | Rapp_Mc8916  | 2.75      | 13.60 |
| Translation                         | 13 | 0.61 | 10.99   | 0.03 | Rapp_Mc13622 | 43.10     | 30.17 |
| 24 kDa family                       | 12 | 0.56 | 24.22   | 0.05 | Rapp_Mc9762  | 91.56     | 31.50 |
| Defensin                            | 12 | 0.56 | 464.44  | 1.01 | Rapp_Mc8698  | 1899.98   | 34.09 |
| 8 kDa Amblyomma family              | 11 | 0.52 | 28.78   | 0.06 | Rapp_Mc13004 | 154.27    | 48.73 |
| Glycan biosynthesis and metabolism  | 11 | 0.52 | 14.00   | 0.03 | Rapp_Mc6227  | 80.50     | 52.27 |
| Sphingomyelinase                    | 9  | 0.42 | 10.67   | 0.02 | Rapp_Mc837   | 22.89     | 23.84 |
| Signal transduction                 | 8  | 0.37 | 7.80    | 0.01 | Rapp_Mc1131  | 14.98     | 24.01 |
| Transcription                       | 8  | 0.37 | 4.42    | 0.01 | Rapp_Mc1617  | 9.48      | 26.79 |
| Carbohydrate metabolism             | 7  | 0.33 | 4.31    | 0.01 | Rapp_Mc5896  | 7.62      | 25.24 |
| Fibrinogen-related domain           | 7  | 0.33 | 52.55   | 0.07 | Rapp_Mc9028  | 248.58    | 67.58 |
| Secretory - unknown function        | 7  | 0.33 | 12.81   | 0.01 | Rapp_Mc9124  | 51.43     | 65.04 |
| Immunoglobulin G binding protein A  | 6  | 0.28 | 2051.82 | 2.23 | Rapp_Mc1190  | 4721.78   | 38.35 |
| Metabolism of other amino acids     | 6  | 0.28 | 29.87   | 0.03 | Rapp_Mc5888  | 67.37     | 37.59 |
| Phospholipase A2                    | 6  | 0.28 | 22.53   | 0.02 | Rapp_Mc8892  | 44.33     | 32.79 |
| Replication and repair              | 6  | 0.28 | 5.26    | 0.01 | Rapp_Mc2861  | 9.23      | 29.23 |
| 7DB family                          | 5  | 0.23 | 26.52   | 0.02 | Rapp_Mc5571  | 66.08     | 49.84 |
| Metalloprotease                     | 5  | 0.23 | 12.08   | 0.01 | Rapp_Mc12946 | 38.89     | 64.40 |

|   |   |      |         |      |              |         |        |
|---|---|------|---------|------|--------------|---------|--------|
| SALP15  | 4 | 0.19 | 10.25   | 0.01 | Rapp_Mc1541  | 28.69   | 69.99  |
| Astacin   | 3 | 0.14 | 1.77    | 0.00 | Rapp_Mc7012  | 3.39    | 63.84  |
| Cell growth and death                           | 3 | 0.14 | 6.83    | 0.00 | Rapp_Mc3897  | 17.39   | 84.91  |
| Dermacentor 9 kDa expansion                     | 3 | 0.14 | 14.72   | 0.01 | Rapp_Mc1065  | 26.04   | 58.95  |
| Histidine rich                                  | 3 | 0.14 | 2.87    | 0.00 | Rapp_Mc450   | 3.95    | 45.82  |
| 14 kDa family                                   | 3 | 0.14 | 25.10   | 0.01 | Rapp_Mc8740  | 54.41   | 72.25  |
| Kazal domain                                    | 3 | 0.14 | 21.10   | 0.01 | Rapp_Mc421   | 59.70   | 94.33  |
| Kazal/vWf domain                                | 3 | 0.14 | 18.64   | 0.01 | Rapp_Mc2515  | 35.48   | 63.46  |
| TELEM   | 3 | 0.14 | 1.99    | 0.00 | Rapp_Mc5946  | 2.32    | 38.93  |
| Thyropin  | 3 | 0.14 | 67.58   | 0.04 | Rapp_Mc1844  | 99.53   | 49.09  |
| Cysteine rich                                   | 2 | 0.09 | 1.03    | 0.00 | Rapp_Mc1691  | 1.10    | 53.66  |
| Energy metabolism                               | 2 | 0.09 | 15.83   | 0.01 | Rapp_Mc6151  | 25.10   | 79.28  |
| Hirudin   | 2 | 0.09 | 310.16  | 0.11 | Rapp_Mc11642 | 418.25  | 67.42  |
| Bovine pancreatic trypsin inhibitor - Lipocalin | 1 | 0.05 | 3.56    | 0.00 | Rapp_Mc3211  | 3.56    | 100.00 |
| Chitin deacetylase activity                     | 1 | 0.05 | 1.17    | 0.00 | Rapp_Mc2536  | 1.17    | 100.00 |
| Cell motility                                   | 1 | 0.05 | 3.36    | 0.00 | Rapp_Mc4124  | 3.36    | 100.00 |
| Cysteine rich hydrophobic domain 2              | 1 | 0.05 | 13.39   | 0.00 | Rapp_Mc7198  | 13.39   | 100.00 |
| Fatty acid-binding protein                      | 1 | 0.05 | 40.42   | 0.01 | Rapp_Mc2582  | 40.42   | 100.00 |
| Histamine release factor                        | 1 | 0.05 | 1211.56 | 0.22 | Rapp_Mc12631 | 1211.56 | 100.00 |
| Immune system                                   | 1 | 0.05 | 24.15   | 0.00 | Rapp_Mc8912  | 24.15   | 100.00 |
| 26 kDa family                                   | 1 | 0.05 | 7.18    | 0.00 | Rapp_Mc5668  | 7.18    | 100.00 |
| Kazal/SPARC domain                              | 1 | 0.05 | 64.96   | 0.01 | Rapp_Mc1895  | 64.96   | 100.00 |

**Supplemental Table A4: Differential expression between female and male ticks in the salivary transcriptome of *R. appendiculatus*.**

| Protein families  | Female up regulated * | Male up regulated * |
|---|-----------------------|---------------------|
| Secretory protein families                              | 570                   | 553                 |
| 24 kDa family   | 3                     | 4                   |
| 28 kDa Metastriate family                               | 18                    | 4                   |
| 5'-Nucleotidase   | 3                     | 2                   |
| 7DB family  | 0                     | 1                   |
| 8 kDa Amblyomma family                                  | 2                     | 6                   |
| 8.9 kDa family  | 45                    | 28                  |
| Antigen 5 family  | 1                     | 2                   |
| Astacin   | 0                     | 2                   |
| Basic tail secreted protein                             | 36                    | 21                  |
| Bovine pancreatic trypsin inhibitor                     | 61                    | 71                  |
| Carbohydrate metabolism                                 | 2                     | 0                   |
| Carboxypeptidase inhibitor                              | 6                     | 5                   |
| Cell motility   | 0                     | 1                   |
| Chitin-binding proteins                                 | 0                     | 5                   |
| Cystatin  | 19                    | 15                  |
| Cysteine rich   | 1                     | 1                   |
| DA-P36 family   | 15                    | 2                   |
| Defensin  | 2                     | 5                   |
| Dermacentor 9 kDa expansion                             | 0                     | 3                   |
| Digestive system (including Serine proteases)           | 3                     | 25                  |
| Evasin  | 36                    | 16                  |
| Fibrinogen-related domain                               | 2                     | 0                   |
| Folding, sorting and degradation (including Cathepsins) | 1                     | 8                   |
| Gluzincin   | 2                     | 33                  |
| Glycan biosynthesis and metabolism                      | 2                     | 0                   |
| Glycine rich superfamily                                | 14                    | 31                  |
| Hirudin   | 2                     | 0                   |
| Histidine rich  | 3                     | 0                   |
| Immunoglobulin G binding protein A                      | 0                     | 6                   |
| Ixodegrin B   | 36                    | 7                   |
| Kazal domain  | 2                     | 1                   |
| Kazal/vWf domain  | 1                     | 1                   |
| Lipid metabolism  | 7                     | 5                   |
| Lipocalin   | 157                   | 154                 |
| Microplusin   | 3                     | 5                   |
| ML domain   | 1                     | 8                   |
| Mucin   | 11                    | 3                   |
| No hit  | 0                     | 1                   |
| One of each family                                      | 17                    | 1                   |
| Phospholipase A2  | 0                     | 2                   |
| Reprolysin  | 27                    | 15                  |

|                                     |             |             |
|-------------------------------------|-------------|-------------|
| SALP15                              | 0           | 1           |
| Secretory - unknown function        | 0           | 2           |
| Serpin                              | 3           | 5           |
| Signal transduction                 | 1           | 0           |
| Signaling molecules and interaction | 3           | 0           |
| Sphingomyelinase                    | 0           | 5           |
| TIL domain                          | 20          | 34          |
| Transport and catabolism            | 2           | 6           |
| Housekeeping protein class          | 220         | 413         |
| Unknown function protein class      | 130         | 288         |
| No hit protein class                | 111         | 172         |
| Transcripts without predicted ORFs  | 727         | 920         |
| <b>Total</b>                        | <b>1758</b> | <b>2346</b> |

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\* Transcripts estimated as up regulated (fold change > 2) by the edgeR (Empirical analysis of digital gene expression data in R) software package.