**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) |
| **HiSeq 2000 generated sequence reads** | | |  |  |  |  |  |  |  |  |  |
| HiSeq | 4 ug | 8 min | 15 | ±300 bp | HiSeq 2000 | 100 x 100 | 413 323 262/ 413 323 262 | 100 | 366 810 605/ 338 340 792 | 20-100 | 11.3/ 18.1 |
| **MiSeq generated sequence reads** | | |  |  |  |  |  |  |  |  |  |
| 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/ 13 216 382 | 250 | 8 781 175/  6 297 010 | 20-250 | 33.6/ 52.4 |
| **Total MiSeq data** | |  |  |  |  |  | **17 072 249/ 13 216 382** | **150-250** | **12 565 276/ 5 474 192** | **20-250** | **26.4/58.6** |
| **Total generated sequence reads** | | |  |  |  |  |  |  |  |  |  |
| Total sequence data (HiSeq and MiSeq) | | |  |  |  |  | 430 395 511/ 426 539 644 | 100-250 | 379 375 881/ 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 |
| **Total** | **1758** | **2346** |

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