

## **Chapter 7**

## **Appendices**

## Appendix A

Sample name	D18 F1 SS	D18 F2 SS	D18 M1 SS	D18 M2 SS	D18 M3 SS	D21 F1 SS	D21_F2 SS	D21 F3 SS	D21 F4 SS	D21 F5 SS	D21 M1 SS	D21 M2 SS	Average
<b>Total reads</b>	2080114	2062052	2040192	2309320	2148352	2119452	2009938	2120300	2152774	2123504	2048618	2096230	<b>2073810.543</b>
<b>Mapped</b>	1540672	1630884	1587154	1682538	1729541	1714133	1491835	1526086	1704036	1691104	1602862	1677186	<b>1641899.757</b>
<b>Prop. Paired</b>	1449174	1544283	1504942	1595907	1637981	1625847	1409668	1441913	1618474	1597515	1512372	1586614	<b>1549813.471</b>
<b>Unmapped</b>	539442	431168	453038	626782	418811	405319	518103	594214	448738	432400	445756	419044	<b>431910.7857</b>
<b>% Unmapped</b>	25.9	20.9	22.2	27.1	19.5	19.1	25.8	28.0	20.8	20.4	21.8	20.0	<b>20.8</b>
<b>% Mapped</b>	74.1	79.1	77.8	72.9	80.5	80.9	74.2	72.0	79.2	79.6	78.2	80.0	<b>79.2</b>
<b>% Paired</b>	69.7	74.9	73.8	69.1	76.2	76.7	70.1	68.0	75.2	75.2	73.8	75.7	<b>74.7</b>

Sample name	D21 M3 SS	D21 M4 SS	D21 F1 MS	D21 F2 MS	D21 F3 MS	D21 F4 MS	D21 M1 MS	D21 M2 MS	D21 M3 MS	D21 M4 MS	D28 F1 SS	D28 F2 SS	Average
<b>Total reads</b>	2069646	2089104	2049258	2297626	2017852	2086646	2075498	2067554	2075286	2005388	2060388	2113702	<b>2073810.543</b>
<b>Mapped</b>	1675116	1662779	1621071	1842584	1589133	1634689	1667968	1666472	1651356	1616338	1653169	1677707	<b>1641899.757</b>
<b>Prop. Paired</b>	1581325	1572093	1532912	1736812	1498166	1537935	1579703	1578906	1563079	1517476	1563201	1584245	<b>1549813.471</b>
<b>Unmapped</b>	394530	426325	428187	455042	428719	451957	407530	401082	423930	389050	407219	435995	<b>431910.7857</b>
<b>% Unmapped</b>	19.1	20.4	20.9	19.8	21.2	21.7	19.6	19.4	20.4	19.4	19.8	20.6	<b>20.8</b>
<b>% Mapped</b>	80.9	79.6	79.1	80.2	78.8	78.3	80.4	80.6	79.6	80.6	80.2	79.4	<b>79.2</b>
<b>% Paired</b>	76.4	75.3	74.8	75.6	74.2	73.7	76.1	76.4	75.3	75.7	75.9	75.0	<b>74.7</b>

Sample name	D28 F3 SS	D28 M1 SS	D28 M2 S	D28 M3 SS	D28 F1 MS	D28 F2 MS	D28 F3 MS	D28 F4 MS	D28 M1 MS	D28 M2 MS	D28 M3 MS	D28 M4 MS	Average
<b>Total reads</b>	2053026	2058386	2052242	2003982	2048124	2043236	2096656	2069750	2135820	2070970	2043092	2064518	<b>2073810.543</b>
<b>Mapped</b>	1522288	1639142	1621913	1524462	1680771	1617647	1677258	1653050	1664760	1686818	1648057	1635606	<b>1641899.757</b>
<b>Prop. Paired</b>	1428607	1548632	1529460	1433195	1599259	1525497	1585061	1554242	1565877	1595903	1557610	1541694	<b>1549813.471</b>
<b>Unmapped</b>	530738	419244	430329	479520	367353	425589	419398	416700	471060	384152	395035	428912	<b>431910.7857</b>
<b>% Unmapped</b>	25.9	20.4	21.0	23.9	17.9	20.8	20.0	20.1	22.1	18.5	19.3	20.8	<b>20.8</b>
<b>% Mapped</b>	74.1	79.6	79.0	76.1	82.1	79.2	80.0	79.9	77.9	81.5	80.7	79.2	<b>79.2</b>
<b>% Paired</b>	69.6	75.2	74.5	71.5	78.1	74.7	75.6	75.1	73.3	77.1	76.2	74.7	<b>74.7</b>

Sample name	D35 F1 SS	D35 F2 SS	D35 F3 SS	D35 M1 SS	D35 M2 SS	D35 M3 SS	D35 M4 SS	D35 F1 MS	D35 F2 MS	D35 M3 MS	D35 M1 MS	D35 M2 MS	Average
<b>Total reads</b>	2060052	2096878	2039540	2008772	2017950	2050816	2018606	2088038	2080764	2057080	2066508	2010962	<b>2073810.543</b>
<b>Mapped</b>	1545483	1704745	1629324	1600736	1635793	1632836	1610231	1710015	1668174	1669591	1641929	1622566	<b>1641899.757</b>
<b>Prop. Paired</b>	1441668	1613167	1539189	1506134	1556801	1538332	1513943	1620797	1574130	1581931	1544947	1532003	<b>1549813.471</b>
<b>Unmapped</b>	514569	392133	410216	408036	382157	417980	408375	378023	412590	387489	424579	388396	<b>431910.7857</b>
<b>% Unmapped</b>	25.0	18.7	20.1	20.3	18.9	20.4	20.2	18.1	19.8	18.8	20.5	19.3	<b>20.8</b>
<b>% Mapped</b>	75.0	81.3	79.9	79.7	81.1	79.6	79.8	81.9	80.2	81.2	79.5	80.7	<b>79.2</b>
<b>% Paired</b>	70.0	76.9	75.5	75.0	77.1	75.0	75.0	77.6	75.7	76.9	74.8	76.2	<b>74.7</b>

Sample name	D35 M3 MS	D38 F1 SS	D38 F2 SS	D38 F3 SS	D38 F4 SS	D38 M1 SS	D38 M2 SS	D38 M3 SS	D38 M4 SS	D38 M5 SS	D38 M6 SS	D38 F1 MS	Average
<b>Total reads</b>	2002350	2084778	2089938	2086154	2099738	2094136	2085988	2070088	2066106	2094480	2093118	2058490	<b>2073810.543</b>
<b>Mapped</b>	1591148	1656569	1663267	1627644	1686112	1638494	1647282	1590347	1638961	1655896	1684572	1660113	<b>1641899.757</b>
<b>Prop. Paired</b>	1495252	1563930	1570778	1528519	1593739	1543889	1549069	1496552	1544909	1560467	1589377	1574793	<b>1549813.471</b>
<b>Unmapped</b>	411202	428209	426671	458510	413626	455642	438706	479741	427145	438584	408546	398377	<b>431910.7857</b>
<b>% Unmapped</b>	20.5	20.5	20.4	22.0	19.7	21.8	21.0	23.2	20.7	20.9	19.5	19.4	<b>20.8</b>
<b>% Mapped</b>	79.5	79.5	79.6	78.0	80.3	78.2	79.0	76.8	79.3	79.1	80.5	80.6	<b>79.2</b>
<b>% Paired</b>	74.7	75.0	75.2	73.3	75.9	73.7	74.3	72.3	74.8	74.5	75.9	76.5	<b>74.7</b>

Sample name	D38 F2 MS	D38 F3 MS	D38 M1 MS	D38 M2 MS	D38 M3 MS	D49 F1 SS	D49 F2 SS	D49 M1 SS	D49 M2 SS	D49 M3 SS	Average
<b>Total reads</b>	2051724	2127108	2064036	2035976	2046108	2132220	2023748	2012990	2030664	2062238	<b>2073810.543</b>
<b>Mapped</b>	1620590	1725363	1631237	1632715	1633668	1681676	1589172	1606279	1632246	1660024	<b>1641899.757</b>
<b>Prop. Paired</b>	1525409	1634703	1531509	1543353	1539579	1584697	1493600	1514002	1541721	1568474	<b>1549813.471</b>
<b>Unmapped</b>	431134	401745	432799	403261	412440	450544	434576	406711	398418	402214	<b>431910.7857</b>
<b>% Unmapped</b>	21.0	18.9	21.0	19.8	20.2	21.1	21.5	20.2	19.6	19.5	<b>20.8</b>
<b>% Mapped</b>	79.0	81.1	79.0	80.2	79.8	78.9	78.5	79.8	80.4	80.5	<b>79.2</b>
<b>% Paired</b>	74.3	76.9	74.2	75.8	75.2	74.3	73.8	75.2	75.9	76.1	<b>74.7</b>

**Appendix A.1: Summary of sequencing statistics for sequenced RNA-Seq samples.** Samples names provide the day post infections (e.g. D18 stands for 18 d.p.i.), the gender and replicate number (e.g. F1 is female replicate 1) and the pairing status (MS for mixed sex infections, SS for single sex infection). Total reads is the number of all reads sequenced. Mapped is the total number of reads mapped to the *S. mansoni* reference genome v5.2. ‘Prop. Paired’ refers to the total number of reads mapped together with a mate-paired read in close proximity and in the correct orientation. Unmapped is the total number of reads that could not be mapped to the reference by Tophat2.

Gene ID	Product	Fold Change	Adjusted p-value
Smp_070540	Uncharacterised protein	5.58	1.62E-06
Smp_213480	Muts protein	6.54	9.36E-06
Smp_124750	Uncharacterised protein	8.53	3.93E-05
Smp_135290	Macrophage expressed gene 1 protein	3.30	3.93E-05
Smp_191690	Tumor susceptibility gene 101 protein	8.96	3.93E-05
Smp_082420	Uridine phosphorylase	2.98	3.63E-04
Smp_147320	Camp dependent protein kinase regulatory chain	5.11	5.22E-04
Smp_174530	Aminopeptidase PILS (M01 family)	5.74	5.22E-04
Smp_007760	Alanine aminotransferase 2	2.20	6.61E-04
Smp_172340	Uncharacterised protein	3.48	1.09E-03
Smp_151840	Uncharacterised protein	5.33	1.13E-03
Smp_032980	Calmodulin protein	6.17	1.49E-03
Smp_015100	Uncharacterised protein	2.32	1.60E-03
Smp_178780	Single minded	4.78	1.60E-03
Smp_042150	Tegument-allergen-like protein	3.52	1.79E-03
Smp_162840	Uncharacterised protein	3.37	1.79E-03
Smp_163170	Sodium:potassium dependent atpase beta subunit	3.61	2.31E-03
Smp_153070	Uncharacterised protein	3.43	2.32E-03
Smp_118960	Jun protein	6.60	2.60E-03
Smp_158150	Reticulocalbin 2	4.95	5.31E-03
Smp_141810	Uncharacterised protein	2.76	7.79E-03
Smp_141250	Protein jagged 1	2.59	8.74E-03

**Appendix A.2: List of the most differentially expressed genes upregulated in females compared to male worms from mixed sex infections at 21 days *post* infection.** Fold changes measure expression in female relative to male worms. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
<b>Smp_211240</b>	Beta 1,4 N acetylgalactosaminyltransferase	13.37	1.64E-07
<b>Smp_160360</b>	Sodium:chloride dependent neurotransmitter	3.54	3.36E-07
<b>Smp_074560</b>	Uncharacterised protein	3.59	9.85E-07
<b>Smp_129960</b>	Uncharacterised protein	5.07	3.52E-05
<b>Smp_195090</b>	Tegument-allergen-like protein	6.09	7.41E-05
<b>Smp_084270</b>	Rhodopsin orphan GPCR	2.92	2.28E-04
<b>Smp_153930</b>	Invadolysin (M08 family)	4.84	3.35E-04
<b>Smp_168730</b>	Carbonic anhydrase	2.35	3.63E-04
<b>Smp_105780</b>	Uro adherence factor A	6.74	8.16E-04
<b>Smp_120020</b>	Uncharacterised protein	6.44	1.13E-03
<b>Smp_212180</b>	Glucose dehydrogenase (acceptor)	5.03	1.19E-03
<b>Smp_056280</b>	Zinc finger CCCH domain containing protein 31	4.96	1.39E-03
<b>Smp_091210</b>	Uncharacterised protein	6.12	1.49E-03
<b>Smp_151660</b>	Uncharacterised protein	3.32	2.60E-03
<b>Smp_161190</b>	Uncharacterised protein	2.44	4.58E-03
<b>Smp_071810</b>	Uncharacterised protein	2.11	7.11E-03

**Appendix A.3: List of the most differentially expressed genes upregulated in males compared to female worms from mixed sex infections at 21 days *post* infection.** Fold changes measure expression in male relative to female worms. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
<b>Smp_147670</b>	Receptor kinase I-interacting protein SIP	6.49	6.85E-36
<b>Smp_159510</b>	Uncharacterised protein	6.98	1.14E-35
<b>Smp_017610</b>	amiloride sensitive amine oxidase	66.88	5.87E-33
<b>Smp_075280</b>	Uncharacterised protein	3.78	1.77E-31
<b>Smp_175090</b>	long-chain-fatty-acid--CoA ligase	5.63	3.41E-31
<b>Smp_170020</b>	tachykinin receptor protein like	9.10	2.54E-30
<b>Smp_125940</b>	Uncharacterised protein	10.53	9.28E-28
<b>Smp_168730</b>	carbonic anhydrase	9.29	4.26E-27
<b>Smp_169570</b>	glycerol 3 phosphate dehydrogenase	14.15	1.24E-26
<b>Smp_161030</b>	Uncharacterised protein	14.02	2.02E-26
<b>Smp_093210</b>	FMRFamide activated amiloride sensitive sodium	4.92	2.45E-26
<b>Smp_059570</b>	Uncharacterised protein	7.04	3.15E-26
<b>Smp_139380</b>	Spindle assembly checkpoint component MAD1	55.18	5.20E-26
<b>Smp_034550</b>	Alpha actinin	6.54	1.93E-25
<b>Smp_167160</b>	Dynamin binding protein	2.91	4.29E-25
<b>Smp_089670</b>	Alpha 2 macroglobulin	4.54	6.92E-25
<b>Smp_197860</b>	Gelsolin	11.28	4.05E-24
<b>Smp_134710</b>	Uncharacterized MFS type transporter C19orf28	16.55	4.12E-24

<b>Smp_104890</b>	Glutamate-gated chloride channel subunit 3	6.03	7.84E-24
<b>Smp_198930</b>	Uncharacterised protein	6.54	1.13E-23
<b>Smp_028670</b>	Carbonic anhydrase II	4.23	1.14E-23
<b>Smp_146200</b>	Zyxin:trip6	2.88	1.61E-23
<b>Smp_153780</b>	Glutamate receptor, ionotropic kainate 3	6.68	1.89E-23
<b>Smp_168980</b>	Adenylate cyclase	4.06	2.09E-23
<b>Smp_134130</b>	Ring canal kelch	5.60	3.63E-23
<b>Smp_068280</b>	Prolyl 4 hydroxylase subunit alpha 2	3.99	4.79E-23
<b>Smp_194050</b>	Clumping factor A	5.09	6.12E-23
<b>Smp_160360</b>	Sodium:chloride dependent neurotransmitter	9.63	9.19E-23
<b>Smp_006860</b>	PDZ and LIM domain protein Zasp	4.66	9.42E-23
<b>Smp_059170</b>	Troponin i	7.43	1.07E-22
<b>Smp_155410</b>	Coiled coil domain containing protein 102A	4.80	1.22E-22
<b>Smp_074560</b>	Uncharacterised protein	10.69	1.26E-22
<b>Smp_136930</b>	Uncharacterised protein	3.58	1.42E-22
<b>Smp_053560</b>	MAP kinase activated protein kinase 2	3.15	2.44E-22
<b>Smp_016230</b>	Protocadherin 17	12.59	4.94E-22
<b>Smp_164240</b>	Junctophilin 2	5.43	6.27E-22
<b>Smp_000170</b>	KV channel interacting protein 2 (KChIP2)	15.56	6.44E-22
<b>Smp_022340</b>	PDZ and LIM domain protein Zasp	5.83	6.54E-22
<b>Smp_126360</b>	Uncharacterised protein	10.50	8.02E-22
<b>Smp_166920</b>	PDZ and LIM domain protein Zasp	7.50	8.16E-22
<b>Smp_214190</b>	Calpain	3.03	2.08E-21
<b>Smp_121190</b>	Voltage gated potassium channel	7.48	2.44E-21
<b>Smp_133550</b>	Neuropeptide receptor type; neuropeptide ff receptor	7.09	2.79E-21
<b>Smp_072450</b>	Rhodopsin orphan GPCR	10.11	2.95E-21
<b>Smp_126320</b>	D glucuronyl C5 epimerase	2.41	3.34E-21
<b>Smp_174870</b>	Uncharacterised protein	5.16	4.25E-21
<b>Smp_046640</b>	Twik family of potassium channels	6.67	4.77E-21
<b>Smp_164960</b>	Phosphatidylinositol 4,5 biphosphate 3 kinase	1.84	5.65E-21
<b>Smp_151100</b>	cGMP dependent protein kinase	5.43	7.80E-21
<b>Smp_163090</b>	Voltage gated potassium channel	5.45	9.67E-21

**Appendix A.4: List of the most differentially expressed genes upregulated in male worms compared to female worms from mixed sex infections at 38 days *post* infection.** Fold changes measure expression in male relative to female worms. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
Smp_212820	Zinc finger protein	20.55	7.07E-67
Smp_038030	Adenylosuccinate lyase	6.35	3.02E-63
Smp_179710	Maternal Effect Lethal family member	32.86	4.82E-62
Smp_159920	Protocadherin 11	41.35	4.03E-49
Smp_090520	Purine nucleoside phosphorylase	18.90	2.20E-47
Smp_126210	Uncharacterised protein	20.18	7.25E-41
Smp_160160	Solute carrier family 17	13.56	7.68E-41
Smp_212090	Calcium dependent activator protein	13.74	3.75E-40
Smp_042700	Protein FAM81B	13.50	1.96E-39
Smp_002080	S adenosylmethionine synthetase	3.03	1.28E-38
Smp_210370	Ormdl protein	2.95	1.67E-38
Smp_148770	Ribose 5 phosphate isomerase	6.94	6.99E-36
Smp_173150	CD63 antigen	34.92	1.03E-35
Smp_041540	Hormone receptor 4	13.98	1.18E-35
Smp_080730	Cyclin dependent kinase 1	6.84	1.18E-35
Smp_056700	Membrane associated RING finger protein	36.62	1.14E-34
Smp_048430	Thioredoxin glutathione reductase	3.05	1.59E-33
Smp_092390	N acetylglucosamine kinase	5.33	5.87E-33
Smp_026400	Thyrotroph embryonic factor	23.27	7.70E-33
Smp_077900	Female specific protein 800	82.82	1.00E-32
Smp_155740	TNF receptor associated protein 1	2.22	1.79E-32
Smp_075870	Translocation associated membrane protein 1	3.65	2.47E-32
Smp_000290	Female-specific protein 800	84.02	6.32E-32
Smp_021070	Guanine nucleotide binding protein	3.79	7.89E-32
Smp_035470	Dolichyl diphosphooligosaccharide protein	3.36	7.89E-32
Smp_055800	Pescadillo	3.57	7.89E-32
Smp_146480	Uncharacterised protein	60.43	7.89E-32
Smp_198170	Protein FAM81B	13.66	1.05E-31
Smp_105680	Dolichyl diphosphooligosaccharide protein	2.97	3.41E-31
Smp_171150	Protein arginine N methyltransferase 5	3.75	5.04E-31
Smp_209060	Alpha 1,3 fucosyltransferase B	10.74	2.70E-30
Smp_148390	Uncharacterised protein	193.90	3.22E-30
Smp_142760	Centrosomal protein of 95 kDa	5.04	5.55E-30
Smp_011870	Uncharacterised protein	139.19	5.80E-30
Smp_075330	Uncharacterised protein	13.02	6.68E-30
Smp_135840	Uncharacterised protein	5.07	7.55E-30
Smp_082490	G2:mitotic specific cyclin B2	7.21	1.25E-29
Smp_062070	Sulfide quinone reductase	3.58	1.56E-29
Smp_175560	Beta site APP cleaving enzyme 1	60.07	2.12E-29
Smp_169970	Kinetochore protein ndc80	5.18	4.08E-29
Smp_076320	Uncharacterised protein	70.01	4.83E-29
Smp_083770	Anoctamin 7	14.31	6.92E-29
Smp_054010	Y+L amino acid transporter 2	35.13	2.94E-28
Smp_199540	Inositol type trisphosphate receptor	40.29	3.73E-28



<b>Smp_058240</b>	Reticulon 4 (Neurite outgrowth inhibitor)	3.74	4.33E-28
<b>Smp_141160</b>	E3 ubiquitin protein ligase RNF168	11.23	5.97E-28
<b>Smp_048660</b>	Nucleolar protein 56	3.27	1.02E-27
<b>Smp_013970</b>	Uncharacterised protein	6.43	1.06E-27
<b>Smp_199830</b>	Leucine rich repeat protein	7.36	1.25E-27
<b>Smp_031730</b>	Signal peptidase 18 kDa subunit	3.43	1.34E-27

**Appendix A.5: List of the most differentially expressed genes upregulated in females compared to male worms from mixed sex infections at 38 days *post* infection.** Fold changes measure expression in female relative to male worms. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Average read count	Fold change	Adjusted p-value
<b>Smp_015630</b>	Glutamate-gated chloride channel subunit	353.74	1.07	0.929
<b>Smp_104890</b>	Glutamate-gated chloride channel subunit	244.18	0.41	5.58E-08
<b>Smp_099500</b>	Glutamate-gated chloride channel subunit	38.57	2.17	3.45E-02
<b>Smp_031680</b>	Nicotinic acetylcholine receptor	176.02	0.95	0.948
<b>Smp_012000</b>	Nicotinic acetylcholine receptor	92.58	1.18	0.779
<b>Smp_132070</b>	Nicotinic acetylcholine receptor subunit	3562.48	1.02	0.937
<b>Smp_180570</b>	Nicotinic acetylcholine receptor	132.26	1.08	0.900
<b>Smp_139330</b>	Nicotinic acetylcholine receptor non $\alpha$	811.23	0.72	0.106
<b>Smp_157790</b>	Nicotinic acetylcholine receptor subunit	121.98	2.32	4.21E-03
<b>Smp_176310</b>	Nicotinic acetylcholine receptor subunit	699.76	1.23	0.637
<b>Smp_101990</b>	Acetylcholine receptor subunit $\alpha$	23.03	0.27	9.56E-05
<b>Smp_037960</b>	Neuronal acetylcholine receptor subunit $\alpha$	1308.18	1.33	0.387
<b>Smp_096480</b>	Glycine receptor subunit $\beta$	520.33	1.03	0.951
<b>Smp_142690</b>	Neuronal acetylcholine receptor subunit $\alpha$	1875.19	1.33	5.15E-03
<b>Smp_175110</b>	Neurotransmitter gated ion channel	0	1.00	N/A
<b>Smp_197600</b>	Nicotinic acetylcholine receptor subunit	0.68	0.78	0.819

**Appendix A.6: Expression of genes encoding neurotransmitters in females compared to males from single sex infections at 38 days *post* infection.** Fold changes measure expression in male relative to female worms; that mean genes with a fold change of  $> 1$  are up regulated in males. The *p*-value has been adjusted for multiple hypothesis testing.

Pathway	Description	Total Genes	DEGs	Expected	p-value
<b>smm03010</b>	Ribosome	110	88	22.49	1.66E-43
<b>smm00190</b>	Oxidative phosphorylation	69	38	14.11	9.48E-11
<b>smm03030</b>	DNA replication	30	14	6.13	7.75E-04
<b>smm03020</b>	RNA polymerase	24	12	4.91	8.73E-04
<b>smm00240</b>	Pyrimidine metabolism	59	21	12.06	2.64E-03
<b>smm03040</b>	Spliceosome	105	32	21.47	3.93E-03
<b>smm03050</b>	Proteasome	32	12	6.54	1.20E-02
<b>smm03060</b>	Protein export	19	8	3.88	1.83E-02
<b>smm00982</b>	Drug metabolism - cytochrome P450	4	3	0.82	2.71E-02
<b>smm03008</b>	Ribosome biogenesis in eukaryotes	62	18	12.68	3.00E-02
<b>smm04122</b>	Sulfur relay system	7	4	1.43	3.06E-02

Appendix A.7: KEGG pathways which are significantly enriched amongst the genes up regulated in males from single sex infections at 18 days *post* infection compared to males at 21 days *post* infection. “Total genes” is the number of genes in the given pathway. “DEGs” provides the number of differentially expressed genes in that pathway. “Expected” provides the number of genes expected by chance to be differentially expressed in each pathway.

Comparison	Fold change	Adjusted p-value
<b>Expression in males worms at 18 d.p.i. compared to males at 21 d.p.i.</b>	5.10	6.45E-06
<b>Expression in males worms at 18 d.p.i. compared to males at 38 d.p.i.</b>	21.95	1.34E-14
<b>Expression in females worms at 18 d.p.i. compared to females at 38 d.p.i.</b>	18.16	1.30E-11

**Appendix A.8: Expression of the gene encoding the microsomal glutathion S transferase (Smp\_024010) in different gene expression comparisons of male and females worms at different time points.** The fold change provides the gene expression in the first condition relative to the second condition in each comparison. Smp\_024010 was found up-regulated in male or female worms at 18 days *post* infection (d.p.i.) compared to later time points in all the comparisons. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
<b>Smp_139380</b>	Spindle assembly checkpoint component MAD1	14.15	2.35E-14
<b>Smp_136100</b>	Kelch protein 10	8.63	3.13E-09
<b>Smp_155570</b>	Endoglycoceramidase	2.78	3.18E-08
<b>Smp_074570</b>	Uncharacterised protein	4.85	3.43E-07
<b>Smp_042160</b>	Fructose-bisphosphate aldolase	2.10	1.55E-05
<b>Smp_105410</b>	Glucose transport protein	3.14	1.55E-05
<b>Smp_169090</b>	Solute carrier family 43	3.59	1.55E-05
<b>Smp_201700</b>	Uncharacterised protein	2.68	3.77E-05
<b>Smp_017610</b>	Amiloride sensitive amine oxidase	4.95	5.81E-05
<b>Smp_133970</b>	Uncharacterised protein	5.26	7.10E-05
<b>Smp_142970</b>	Palmitoyl protein thioesterase 1	2.01	7.10E-05
<b>Smp_134710</b>	Uncharacterized MFS type transporter C19orf28	3.20	7.58E-05
<b>Smp_056460</b>	Uncharacterised protein	6.61	1.04E-04
<b>Smp_154800</b>	DC STAMP domain containing protein 2	6.54	1.23E-04
<b>Smp_128510</b>	Uncharacterised protein	7.51	1.56E-04
<b>Smp_144440</b>	Replication A protein	6.92	1.99E-04
<b>Smp_123300</b>	Follistatin	2.35	2.67E-04
<b>Smp_046790</b>	Solute carrier family 2 facilitated glucose	3.34	2.72E-04
<b>Smp_127880</b>	Uncharacterised protein	3.43	2.72E-04
<b>Smp_143590</b>	Uncharacterised protein	2.30	2.79E-04
<b>Smp_005470</b>	Dynein light chain	4.89	2.80E-04
<b>Smp_084270</b>	Rhodopsin orphan GPCR	2.80	2.80E-04
<b>Smp_169570</b>	Glycerol 3 phosphate dehydrogenase	2.86	2.80E-04
<b>Smp_104450</b>	Uncharacterised protein	4.83	3.09E-04
<b>Smp_084440</b>	Uncharacterised protein	2.32	3.23E-04
<b>Smp_045490</b>	Annexin	2.96	3.31E-04
<b>Smp_017620</b>	Uncharacterised protein	5.13	4.21E-04
<b>Smp_013040</b>	Cathepsin D	2.06	4.36E-04
<b>Smp_194950</b>	ELAV protein 2	3.57	5.00E-04
<b>Smp_212180</b>	Glucose dehydrogenase	5.23	5.43E-04
<b>Smp_091460</b>	Glutamine synthetase	2.45	5.61E-04
<b>Smp_128470</b>	Cation transporting atpase worm	2.37	5.61E-04
<b>Smp_034550</b>	Alpha actinin	2.09	6.04E-04
<b>Smp_152200</b>	Cdc25	6.03	6.11E-04
<b>Smp_161240</b>	Uncharacterised protein	3.94	6.25E-04
<b>Smp_042910</b>	Uncharacterised protein	2.08	6.41E-04
<b>Smp_091210</b>	Uncharacterised protein	5.55	6.78E-04
<b>Smp_153930</b>	Invadolysin (M08 family)	4.32	7.48E-04
<b>Smp_163360</b>	Dystrophin	2.35	7.55E-04
<b>Smp_048030</b>	Uncharacterised protein	3.05	8.06E-04
<b>Smp_007760</b>	Alanine aminotransferase 2	2.19	8.84E-04
<b>Smp_148030</b>	DC STAMP domain containing protein 1	4.24	8.84E-04

<b>Smp_161220</b>	Adipocyte plasma membrane associated protein	2.42	9.68E-04
<b>Smp_172960</b>	Serine type protease inhibitor	3.84	9.88E-04
<b>Smp_129960</b>	Uncharacterised protein	3.67	1.00E-03
<b>Smp_145900</b>	Sphingolipid delta(4) desaturase:C4 hydroxylase	2.96	1.03E-03
<b>Smp_071610</b>	Lysosomal Pro X carboxypeptidase	2.13	1.48E-03
<b>Smp_151920</b>	Paladin	3.80	1.51E-03
<b>Smp_191910</b>	Stress protein DDR48	5.82	1.52E-03
<b>Smp_033250</b>	Uncharacterised protein	5.98	1.57E-03

**Appendix A.9: Genes up-regulated in males from mixed sex infections at 28 days *post* infection (d.p.i.) compared to males at 21 d.p.i.** Fold changes provide gene expression in males at 28 d.p.i. compared to males at 21 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
<b>Smp_185130</b>	Uncharacterised protein	5.46	2.19E-03
<b>Smp_199670</b>	Cation transporting ATPase	5.09	9.85E-04
<b>Smp_141740</b>	Protocadherin 11	4.49	2.51E-04
<b>Smp_165240</b>	Uncharacterised protein	4.43	8.32E-03
<b>Smp_022740</b>	Tumor necrosis factor alpha induced protein	4.41	5.41E-03
<b>Smp_164080</b>	Bromodomain testis specific protein	4.09	4.78E-04
<b>Smp_135630</b>	Protocadherin gamma a10	4.07	1.97E-03
<b>Smp_137820</b>	Uncharacterised protein	3.80	2.89E-03
<b>Smp_012000</b>	Nicotinic acetylcholine receptor	3.55	6.18E-04
<b>Smp_198650</b>	Ankyrin :unc	3.46	6.05E-03
<b>Smp_187740</b>	Forkhead box protein j1 b	3.38	2.06E-03
<b>Smp_170270</b>	Uncharacterised protein	3.28	9.72E-03
<b>Smp_012950</b>	Cytoplasmic polyadenylation element binding	3.23	2.85E-03
<b>Smp_160590</b>	Uncharacterised protein	3.20	3.74E-07
<b>Smp_163790</b>	Nuclear receptor 2DBD gamma	3.20	2.12E-03
<b>Smp_124600</b>	Uncharacterised protein	3.15	2.10E-03
<b>Smp_193630</b>	Endoglycoceramidase	3.10	3.73E-03
<b>Smp_177020</b>	Phospholipase C Like family member (pll 1)	3.09	8.88E-03
<b>Smp_183130</b>	Liprin alpha 2	2.97	2.10E-03
<b>Smp_161640</b>	Transient receptor potential cation channel	2.84	1.43E-03
<b>Smp_203940</b>	Short chain dehydrogenase	2.81	2.06E-03
<b>Smp_126290</b>	Uncharacterised protein	2.70	5.06E-03
<b>Smp_142940</b>	Paired box protein pax	2.54	7.61E-03
<b>Smp_123300</b>	Follistatin	2.39	8.16E-04

<b>Smp_168650</b>	Microtubule actin cross linking factor 1	2.26	2.71E-03
<b>Smp_170620</b>	Rhodopsin orphan GPCR	2.26	4.44E-03
<b>Smp_174340</b>	Frizzled 9	2.22	5.41E-03
<b>Smp_158720</b>	Protein kinase	2.21	4.77E-03
<b>Smp_173450</b>	Chromosome associated kinesin KIF4A	2.19	8.60E-03

**Appendix A.10: Genes up-regulated in males from from mixed sex infections at 28 days *post* infection (d.p.i.) compared to males at 35 d.p.i.** Fold changes provide gene expression in males at 28 d.p.i. compared to males at 35 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Comparison	Fold change	Adjusted p-value
<b>Males from single sex infections at 38 d.p.i. compared to Males from single sex infections at 18 d.p.i.</b>	7.54	2.26E-06
<b>Males from mixed sex infections at 35 d.p.i. compared to males from single sex infections ar 35 d.p.i.</b>	7.45	1.23E-03
<b>Males from mixed sex infections at 38 d.p.i. compared to males from single sex infections ar 38 d.p.i.</b>	5.33	1.99E-03

**Appendix A.11: Expression of the gene encoding the aromatic-L-amino acid decarboxylase (Smp\_135230) in different gene expression comparisons of male worms at different time points.** The fold change provides the gene expression in the first condition relative to the second condition in each comparison. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
<b>Smp_191910</b>	Stress protein DDR48	165.06	1.85E-33
<b>Smp_144440</b>	Replication A protein	118.44	2.30E-33
<b>Smp_033250</b>	Uncharacterised protein	135.80	3.73E-30
<b>Smp_014610</b>	Serine:threonine kinase 1	141.61	8.74E-30
<b>Smp_129960</b>	Uncharacterised protein	27.58	2.90E-27
<b>Smp_000420</b>	Pro His rich protein	116.10	3.56E-27
<b>Smp_131110</b>	Uncharacterised protein	116.50	5.99E-27
<b>Smp_077900</b>	Female specific protein 800	41.86	2.52E-26
<b>Smp_165360</b>	Histone acetyltransferase myst4	118.07	2.52E-26
<b>Smp_151150</b>	Arabinogalactan protein	57.98	1.06E-25
<b>Smp_169090</b>	Solute carrier family 43	10.13	6.27E-25
<b>Smp_076320</b>	Uncharacterised protein	39.53	1.16E-24
<b>Smp_000290</b>	Female-specific protein 800	36.61	1.32E-23
<b>Smp_130970</b>	G2:mitotic specific cyclin B3	40.30	2.40E-23
<b>Smp_000410</b>	Trematode Eggshell Synthesis domain containing protein	64.17	2.91E-23

<b>Smp_050750</b>	Uncharacterised protein	52.20	4.24E-23
<b>Smp_138570</b>	Spore germination protein	84.73	2.53E-22
<b>Smp_077890</b>	Trematode Eggshell Synthesis domain containing protein	71.24	8.35E-22
<b>Smp_151160</b>	Stress protein DDR48	54.68	9.80E-22
<b>Smp_192220</b>	Uncharacterised protein	59.23	1.23E-21
<b>Smp_000430</b>	Eggshell protein	75.11	5.84E-21
<b>Smp_050270</b>	Tyrosinase	75.26	7.31E-21
<b>Smp_175560</b>	Beta site APP cleaving enzyme 1	21.44	2.76E-19
<b>Smp_137460</b>	Cytoplasmic polyadenylation element binding	46.34	7.38E-19
<b>Smp_202830</b>	Uncharacterised protein	52.80	1.01E-18
<b>Smp_095980</b>	Extracellular superoxide dismutase (Cu Zn)	59.48	1.72E-18
<b>Smp_000400</b>	Uncharacterised protein	58.60	1.83E-18
<b>Smp_143970</b>	Kelch protein 10	38.47	1.87E-18
<b>Smp_000270</b>	Uncharacterised protein	56.03	2.51E-18
<b>Smp_013540</b>	Tyrosinase	53.82	3.17E-18
<b>Smp_155310</b>	Tetraspanin CD63 receptor	56.65	1.23E-17
<b>Smp_000280</b>	Uncharacterised protein	54.16	1.55E-17
<b>Smp_056700</b>	Membrane associated RING finger protein	9.84	2.75E-17
<b>Smp_095350</b>	Acetyl coenzyme A carboxylase; methylcrotonyl CoA	44.73	1.00E-16
<b>Smp_125740</b>	Uncharacterised protein	3.02	1.61E-16
<b>Smp_195090</b>	Tegument-allergen-like protein	19.98	1.61E-16
<b>Smp_074710</b>	Def8 protein	11.41	6.55E-16
<b>Smp_007670</b>	Uncharacterised protein	5.83	1.36E-15
<b>Smp_058160</b>	Uncharacterised protein	24.34	1.43E-15
<b>Smp_112450</b>	Trematode Eggshell Synthesis domain containing protein	41.41	2.01E-15
<b>Smp_145490</b>	Poly(rC) binding protein 2:3:4	39.76	3.52E-15
<b>Smp_077920</b>	Uncharacterised protein	40.27	9.28E-15
<b>Smp_159920</b>	Protocadherin 11	6.03	1.57E-14
<b>Smp_211240</b>	Beta 1,4 N acetylgalactosaminyltransferase	23.57	3.77E-14
<b>Smp_196950</b>	One cut domain family member	14.57	4.00E-14
<b>Smp_136100</b>	Kelch protein 10	17.81	6.25E-14
<b>Smp_130780</b>	Monocarboxylate transporter	30.67	9.01E-14
<b>Smp_065530</b>	Acrosin	29.09	9.98E-14
<b>Smp_132800</b>	STARP antigen	18.57	1.89E-13
<b>Smp_199540</b>	Type inositol trisphosphate receptor; inositol trisphosphate receptor type	10.61	2.28E-13
<b>Smp_173150</b>	CD63 antigen	6.33	5.24E-12

**Appendix A.12: The most differentially expressed genes in female worms from mixed sex infections at 28 days *post* infection (d.p.i.) compared to females at 21 d.p.i.** The fold change provides a measure of expression in females at 28 d.p.i. relative to females at 21 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold change	Adjusted p-value
Smp_131110	Uncharacterised protein	616.92	5.50E-48
Smp_077900	Female specific protein 800	504.95	4.69E-65
Smp_000290	Female-specific protein 800	463.72	5.79E-61
Smp_191910	Stress protein DDR48	431.31	3.31E-46
Smp_014610	Serine:threonine kinase 1	427.38	1.30E-43
Smp_000430	Eggshell protein	374.21	5.91E-39
Smp_151150	Arabinogalactan protein	362.41	8.12E-50
Smp_013540	Tyrosinase	353.16	1.35E-38
Smp_077890	Trematode Eggshell Synthesis domain containing protein	351.06	4.31E-40
Smp_192220	Uncharacterised protein	350.92	1.63E-40
Smp_095980	Extracellular superoxide dismutase (Cu Zn)	323.47	9.38E-37
Smp_202830	Uncharacterised protein	293.50	2.46E-37
Smp_050750	Uncharacterised protein	265.17	1.30E-43
Smp_000280	Uncharacterised protein	262.95	5.31E-34
Smp_138570	Spore germination protein	261.01	1.15E-34
Smp_000400	Uncharacterised protein	252.51	7.23E-34
Smp_000270	Uncharacterised protein	243.96	8.24E-34
Smp_050270	Tyrosinase	243.54	1.61E-33
Smp_151160	Stress protein DDR48	230.02	1.82E-38
Smp_000420	Pro His rich protein	214.78	3.81E-34
Smp_155310	Tetraspanin CD63 receptor	190.75	5.98E-30
Smp_165360	Histone acetyltransferase myst4	175.53	1.24E-30
Smp_144440	Replication A protein	169.70	2.82E-37
Smp_000370	Uncharacterised protein	152.97	5.61E-30
Smp_000410	Trematode Eggshell Synthesis, domain containing protein	146.36	2.85E-32
Smp_175560	Beta site APP cleaving enzyme 1	138.39	4.93E-45
Smp_058160	Uncharacterised protein	112.56	8.89E-33
Smp_143970	Kelch protein 10	109.35	2.49E-30
Smp_129960	Uncharacterised protein	101.63	2.15E-48
Smp_199540	Inositol type trisphosphate receptor	50.27	1.72E-34
Smp_074710	Def8 protein	38.78	5.84E-33
Smp_173150	CD63 antigen	38.76	1.62E-42
Smp_054010	Y+L amino acid transporter 2	37.52	1.66E-32
Smp_159920	Protocadherin 11	30.49	5.42E-47
Smp_169090	Solute carrier family 43	30.16	5.37E-49
Smp_212820	Zinc finger protein	19.90	1.63E-74
Smp_075330	Uncharacterised protein	19.25	4.86E-44
Smp_179710	Maternal Effect Lethal family member (mel 32)	14.46	5.02E-42
Smp_129610	Malate dehydrogenase	10.57	8.24E-34
Smp_209060	Alpha 1,3 fucosyltransferase B	9.25	1.90E-30
Smp_199830	Leucine rich repeat protein	7.03	5.61E-30
Smp_148770	Ribose 5 phosphate isomerase	6.93	4.35E-41
Smp_038030	Adenylosuccinate lyase	5.45	5.79E-61

<b>Smp_135840</b>	Uncharacterised protein	5.22	3.63E-35
<b>Smp_062070</b>	Sulfide quinone reductase	4.44	9.54E-46
<b>Smp_058240</b>	Reticulon 4 (Neurite outgrowth inhibitor)	4.00	3.24E-35
<b>Smp_011960</b>	Inositol pentakisphosphate 2 kinase	3.78	1.46E-33
<b>Smp_075870</b>	Translocation associated membrane protein 1	3.49	1.52E-34
<b>Smp_031730</b>	Signal peptidase 18 kDa subunit	3.36	2.02E-30
<b>Smp_035470</b>	Dolichyl diphosphooligosaccharide protein	3.33	7.22E-36
<b>Smp_056760</b>	Protein disulfide isomerase	3.26	1.46E-33
<b>Smp_075470</b>	Cysteine desulfurase, mitochondrial	3.21	2.74E-41
<b>Smp_048660</b>	Nucleolar protein 56	3.17	5.78E-30
<b>Smp_048430</b>	Thioredoxin glutathione reductase	2.70	1.08E-30
<b>Smp_210370</b>	Ormdl protein	2.63	1.48E-35

**Appendix A.13: The most differentially expressed genes in female worms from mixed sex infections at 38 days *post* infection (d.p.i.) compared to females from single sex infections.** The fold change provides a measure of expression in females from mixed sex infections relative to females from single sex infections. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
<b>Smp_185340</b>	Uncharacterised protein	8.98	7.08E-06
<b>Smp_121070</b>	Uncharacterised protein	8.82	2.08E-05
<b>Smp_172340</b>	Uncharacterised protein	6.87	7.56E-07
<b>Smp_141840</b>	Uncharacterised protein	6.74	8.37E-07
<b>Smp_144860</b>	Protein boule	6.61	6.34E-05
<b>Smp_012660</b>	Sushi:SCR:CCP,domain containing protein	6.15	6.52E-06
<b>Smp_191250</b>	Reverse transcriptase	6.15	6.09E-05
<b>Smp_124870</b>	Uncharacterised protein	5.99	5.42E-05
<b>Smp_145580</b>	Progesterone induced blocking factor	5.55	3.33E-06
<b>Smp_164590</b>	Fibrillar collagen chain FAp1 alpha	5.54	3.96E-11
<b>Smp_196840</b>	Collagen alpha 1(II) chain	5.21	1.06E-19
<b>Smp_123830</b>	Collagen alpha (xi) chain	4.90	3.96E-11
<b>Smp_197370</b>	Uncharacterised protein	4.66	3.96E-11
<b>Smp_135560</b>	Collagen alpha 2(I) chain	4.60	1.49E-14
<b>Smp_110280</b>	Nucleolar phosphoprotein p130	4.42	4.00E-09
<b>Smp_043390</b>	Beta D xylosidase 2	4.29	6.94E-06
<b>Smp_050520</b>	Neurogenic locus notch protein	3.97	1.14E-10
<b>Smp_166540</b>	Serine:threonine protein kinase Nek11	3.85	7.08E-06
<b>Smp_124040</b>	FMRamide activated amiloride sensitive sodium	3.72	2.10E-06
<b>Smp_062720</b>	e1b 55 kda associated protein	3.42	1.10E-06
<b>Smp_128470</b>	Cation transporting atpase worm	3.39	2.08E-05
<b>Smp_165040</b>	Tbc1 domain family member	3.36	2.08E-05
<b>Smp_133340</b>	Uncharacterised protein	3.35	1.54E-05



<b>Smp_137550</b>	Dynein heavy chain	3.27	1.40E-07
<b>Smp_168590</b>	Pema SRCR protein	3.23	5.15E-07
<b>Smp_188670</b>	Uncharacterised protein	3.23	1.14E-10
<b>Smp_062120</b>	Serine protease inhibitors serpins	3.08	7.32E-10
<b>Smp_143910</b>	Coiled coil and C2 domain containing protein 2A	2.98	3.33E-06
<b>Smp_164000</b>	Uncharacterised protein	2.94	3.50E-05
<b>Smp_123010</b>	High affinity cationic amino acid transporter 1	2.79	9.13E-06
<b>Smp_068720</b>	Formin Homology 2 Domain containing protein	2.76	4.83E-08
<b>Smp_213300</b>	Putative transcription elongation regulator 1	2.66	7.56E-07
<b>Smp_091820</b>	Bestrophin 3	2.50	4.15E-10
<b>Smp_136850</b>	Centrin 1	2.50	5.09E-05
<b>Smp_120320</b>	Splicing factor 3A subunit 1	2.43	2.34E-09
<b>Smp_189850</b>	Uncharacterised protein	2.39	2.33E-06
<b>Smp_025130</b>	RNA binding motif protein 22	2.37	4.31E-06
<b>Smp_151730</b>	Rhabdoid tumor deletion region protein 1	2.35	9.33E-09
<b>Smp_013860</b>	Glutamate cysteine ligase catalytic subunit	2.34	5.25E-08
<b>Smp_105950</b>	Armadillo repeat containing protein 3	2.33	5.36E-07
<b>Smp_021540</b>	Zinc finger protein	2.28	3.34E-05
<b>Smp_134660</b>	Radial spoke head protein 4 A	2.25	3.62E-06
<b>Smp_131890</b>	Sodium:chloride dependent transporter	2.25	2.02E-06
<b>Smp_156080</b>	Dynein heavy chain	2.24	1.48E-05
<b>Smp_175090</b>	Long-chain-fatty-acid--CoA ligase	2.20	2.19E-05
<b>Smp_180810</b>	Papilin	2.18	1.22E-05
<b>Smp_001160</b>	Sperm associated antigen 16 protein	2.18	2.08E-05
<b>Smp_005860</b>	Heterogeneous nuclear ribonucleoprotein k	2.16	2.19E-05
<b>Smp_165060</b>	Uncharacterised protein	2.16	1.04E-05
<b>Smp_136550</b>	Tyrosine protein kinase transmembrane receptor	2.08	4.83E-08
<b>Smp_067420</b>	Splicing factor 3b subunit 4	2.07	2.14E-05
<b>Smp_007640</b>	Uncharacterised protein	2.03	3.50E-05

**Appendix A.14: The most differentially expressed genes in female worms from mixed sex infections at 21 days *post* infection (d.p.i.) compared to females at 18 d.p.i.** The fold change provides a measure of expression in females at 21 d.p.i. relative to females at 18 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold Change	Adjusted p-value
Smp_150630	Dissatisfaction (Dsf)	8.56	1.43E-04
Smp_180330	MEG 2 (ESP15) family	5.90	1.74E-03
Smp_134100	G protein coupled receptor fragment	4.54	4.01E-03
Smp_138350	Zinc finger protein basoenuclin 2	4.32	1.61E-04
Smp_083880	G protein coupled receptor No9	3.89	5.00E-08
Smp_128710	G protein coupled receptor	3.86	3.85E-04
Smp_167140	WNT	3.07	6.70E-03
Smp_177670	Placental protein 11	2.79	4.84E-04
Smp_062080	Serine protease inhibitors serpins	2.75	5.57E-08
Smp_196930	WNT	2.49	2.72E-03
Smp_167400	Transcription factor sum 1	2.36	1.52E-03
Smp_062560	Secreted frizzled protein	2.21	6.67E-03
Smp_041700	G protein coupled receptor fragment	2.08	6.48E-03
Smp_157300	Basic fibroblast growth factor receptor 1 A	2.03	3.80E-05

**Appendix A.15: The most differentially expressed genes in female worms at 18 days *post* infection (d.p.i.) compared to females from mixed sex infections at 21 d.p.i.** The fold change provides a measure of expression in females at 18 d.p.i. relative to females at 21 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Fold change	Adjusted p-value	Product
Smp_062620	12.61	1.73E-07	Uncharacterised protein
Smp_138350	5.43	1.86E-05	Zinc finger protein basoenuclin 2
Smp_000620	5.13	2.15E-04	Class E basic helix loop helix protein 23
Smp_136830	4.90	3.09E-04	Subfamily A1A unassigned peptidase (A01 family)
Smp_203270	4.61	3.23E-04	Twist protein
Smp_158480	3.43	4.75E-04	AMP dependent ligase
Smp_119140	3.32	1.72E-06	Uncharacterised protein
Smp_203660	2.76	3.69E-06	Uncharacterised protein
Smp_174320	2.70	3.10E-05	Protein lozenge
Smp_201920	2.66	1.04E-04	Uncharacterised protein
Smp_124300	2.42	1.23E-04	Lamin B receptor (ERG24)
Smp_026670	2.40	1.54E-05	Transmembrane protein 26
Smp_157370	2.32	4.82E-05	Ribosomal protein s kinase alpha
Smp_202620	2.32	1.56E-04	Uncharacterised protein
Smp_202400	2.29	9.58E-06	Uncharacterised protein
Smp_130870	2.20	2.56E-04	ETS domain containing protein Elk 4
Smp_130050	2.18	5.61E-04	PDZ domain containing RING finger protein
Smp_135730	2.15	2.67E-04	Protocadherin alpha 7
Smp_086220	2.12	3.60E-04	Uncharacterised protein
Smp_137110	2.02	4.50E-05	Uncharacterised protein

**Appendix A.16: The most differentially expressed genes in male worms at 28 days *post* infection (d.p.i.) compared to males from mixed sex infections at 21 d.p.i.** The fold change provides a measure of expression in males at 28 d.p.i. relative to males at 21 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold change	Adjusted p-value
Smp_000620	Class E basic helix loop helix protein 23	3.89	0.000546398
Smp_001980	Barh 2 homeobox protein	3.98	0.000461429
Smp_009020	Zinc finger protein	2.07	0.006630352
Smp_009040	POU domain, class 4, transcription factor 2	3.16	7.04E-05
Smp_027990	Homeobox protein NK 2	2.70	0.008565627
Smp_035650	Dorsal root ganglia homeobox protein	2.73	0.001246198
Smp_045470	Prospero homeobox protein 2	9.87	1.12E-06
Smp_049160	Zinc finger protein	2.17	0.000610056
Smp_051130	ERG transcription factor	2.52	2.27E-06
Smp_060220	Lipopolysaccharide induced tumor necrosis	3.73	0.002346011
Smp_072470	Neurogenic differentiation factor	2.40	0.001806143
Smp_075230	B cell lymphoma/leukemia 11B	5.04	3.13E-05
Smp_076600	Transcription factor SOX 14	2.33	0.000577322
Smp_124090	Uncharacterised protein	2.53	0.002649938
Smp_124600	Uncharacterised protein	3.67	1.25E-06
Smp_125400	Neurogenic differentiation 6	5.89	0.001685335
Smp_126560	OTP paired class homeobox protein	9.09	3.52E-07
Smp_130870	ETS domain containing protein Elk 4	2.12	2.78E-05
Smp_134690	EMX homeobox protein	3.45	0.00905731
Smp_136120	N twist	4.37	0.009792167
Smp_136900	Homeobox protein distal less dlx	7.42	8.67E-06
Smp_138230	Doublesex and mab-3 related transcription factor 1	3.23	2.04E-05
Smp_138350	Zinc finger protein basonuclin 2	7.81	7.45E-11
Smp_140980	MDS1 and evi1 complex locus protein evi1	2.35	0.004593848
Smp_142120	Achaete scute transcription factor	9.13	4.22E-06
Smp_148110	Transcription factor SOX 14	3.50	0.008318475
Smp_153850	Zinc finger ch type domain containing protein	2.17	5.83E-05
Smp_155690	Sox family of transcription factor	3.71	0.003879486
Smp_162760	Homeobox protein prospero	3.13	4.79E-06
Smp_165410	LIM class homeodomain transcription factor	2.78	0.001109032
Smp_168600	Aryl hydrocarbon receptor	2.42	0.002250422
Smp_170750	Paired box protein pax 6	5.27	4.30E-07
Smp_171130	Glial cells missing	10.95	8.12E-07
Smp_174320	Protein lozenge	3.02	1.02E-08
Smp_175410	Zinc finger protein 398	4.26	6.23E-05
Smp_189380	Homeobox protein distal less	4.44	0.006044648
Smp_203270	Twist protein	4.33	4.67E-05

Appendix A.17: List of genes involved in the regulation of transcription (annotated with GO term G0:0006355) which were up-regulated in females from mixed sex infections at 21 days *post* infection (d.p.i.) compared to females at 28 d.p.i. The fold change provides a measure of expression in females at 21 d.p.i. relative to females at 28 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold change	Adjusted p-value
Smp_000620	Class E basic helix loop helix protein 23	1.90	0.114
Smp_001980	Barh 2 homeobox protein	2.72	0.020
Smp_009020	Zinc finger protein	1.23	0.604
Smp_009040	POU domain, class 4, transcription factor 2	1.87	0.066
Smp_027990	Homeobox protein NK 2	2.13	0.059
Smp_035650	Dorsal root ganglia homeobox protein	2.13	0.025
Smp_045470	Prospero homeobox protein 2	4.50	3.67E-03
Smp_049160	Zinc finger protein	1.74	0.026
Smp_051130	ERG transcription factor	1.90	3.68E-03
Smp_060220	Lipopolysaccharide induced tumor necrosis	4.00	1.40E-03
Smp_072470	Neurogenic differentiation factor	3.18	5.47E-05
Smp_075230	B cell lymphoma/leukemia 11B	3.70	1.87E-03
Smp_076600	Transcription factor SOX 14	2.75	5.01E-05
Smp_124090	Uncharaterised protein	1.63	0.187
Smp_124600	Uncharaterised protein	2.47	2.60E-03
Smp_125400	Neurogenic differentiation 6	7.92	1.11E-04
Smp_126560	OTP paired class homeobox protein	1.62	0.416
Smp_130870	ETS domain containing protein Elk 4	1.24	0.419
Smp_134690	EMX homeobox protein	4.15	2.58E-03
Smp_136120	N twist	0.66	0.645
Smp_136900	Homeobox protein distal less dlx	3.96	3.50E-03
Smp_138230	Doublesex and mab-3 related transcription factor 1	2.37	4.14E-03
Smp_138350	Zinc finger protein basoonuclin 2	7.94	3.98E-10
Smp_140980	MDS1 and evi1 complex locus protein evi1	1.58	0.209
Smp_142120	Achaete scute transcription factor	5.21	1.37E-03
Smp_148110	Transcription factor SOX 14	2.55	0.064
Smp_153850	Zinc finger ch type domain containing protein	1.87	2.91E-03
Smp_155690	Sox family of transcription factor	1.80	0.285
Smp_162760	Homeobox protein prospero	2.56	5.44E-04
Smp_165410	LIM class homeodomain transcription factor	2.38	8.69E-03
Smp_168600	Aryl hydrocarbon receptor	1.56	0.203
Smp_170750	Paired box protein pax 6	1.76	0.148
Smp_171130	Glial cells missing	3.05	0.051
Smp_174320	Protein lozenge	3.75	8.37E-11
Smp_175410	Zinc finger protein 398	2.84	8.82E-03
Smp_189380	Homeobox protein distal less	1.36	0.694
Smp_203270	Twist protein	2.30	0.041

Appendix A.18: List of genes from Appendix A.17 expressed in female worms from single sex infections at 21 days *post* infection (d.p.i.) compared to females at 28 d.p.i. These genes are involved in the regulation of transcription (annotated with GO term G0:0006355). The fold change provides a measure of gene expression in females from single sex infections at 21 d.p.i. compared to females at 28 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Product	Fold change	Adjusted p-value
Smp_191910	Stress protein DDR48	455.30	1.26E-41
Smp_131110	Uncharacterised protein	387.32	3.95E-38
Smp_202830	Uncharacterised protein	331.05	4.34E-31
Smp_050750	Uncharacterised protein	315.19	1.52E-39
Smp_144440	Replication A protein	281.18	2.53E-38
Smp_151150	Arabinogalactan protein	266.26	2.97E-39
Smp_014610	Uncharacterised protein	246.64	5.15E-33
Smp_033250	Uncharacterised protein	243.38	1.78E-33
Smp_077890	Trematode Eggshell Synthesis domain containing protein	224.72	8.07E-32
Smp_095980	Extracellular superoxide dismutase	213.90	1.57E-30
Smp_000410	Trematode Eggshell Synthesis, domain containing protein	203.71	2.72E-32
Smp_151160	Stress protein DDR48	203.06	1.98E-32
Smp_000420	Pro His rich protein	199.29	1.57E-30
Smp_000430	Eggshell protein	197.15	1.05E-29
Smp_192220	Uncharacterised protein	178.59	1.66E-30
Smp_076320	Uncharacterised protein	173.56	6.66E-37
Smp_050270	Tyrosinase	172.20	3.14E-28
Smp_165360	Histone acetyltransferase myst4	170.11	2.15E-28
Smp_077900	Female specific protein 800 (fs800)	168.73	4.01E-38
Smp_138570	Spore germination protein	167.61	5.00E-28
Smp_013540	Tyrosinase	165.63	4.89E-28
Smp_000280	Uncharacterised protein	155.70	4.07E-27
Smp_000270	Uncharacterised protein	147.29	9.32E-27
Smp_000400	Uncharacterised protein	143.08	2.21E-26
Smp_155310	Tetraspanin CD63 receptor	140.39	4.62E-26
Smp_148390	Uncharacterised protein	140.06	1.87E-26
Smp_000290	Female-specific protein 800 (fs800)	139.81	1.37E-34
Smp_095350	Tis11 zinc finger protein	46.36	2.34E-16
Smp_087320	TPA induced protein 11B mouse	37.10	8.14E-18
Smp_166560	Zinc finger homeobox protein 1	12.20	1.13E-10

**Appendix A.19: The most differentially expressed genes in females from mixed sex infections at 38 days *post* infection (d.p.i.) compared to females at 18 d.p.i.** Three putative transcription factor genes (Smp\_095350, Smp\_087320 and Smp\_166560) were added to this list despite ranking lower on the list of most differentially expressed genes but are in the 100 most differentially expressed genes. Fold changes provide a measure of gene expression in females at 38 d.p.i. compared to females at 18 d.p.i. The *p*-value has been adjusted for multiple hypothesis testing.

Gene ID	Fold change	Adjusted p-value	Product	Rank	Eggshell synthesis domain
Smp_000270	243.96	8.24E-34	Uncharacterised protein	17	Yes
Smp_000280	262.95	5.31E-34	Uncharacterised protein	14	Yes
Smp_000290	463.72	5.79E-61	Female-specific protein 800	3	Yes
Smp_000390	142.88	3.93E-26	Uncharacterised protein	28	Yes
Smp_000400	252.51	7.23E-34	Uncharacterised protein	16	
Smp_000410	146.36	2.85E-32	Trematode Eggshell Synthesis domain containing protein	27	Yes
Smp_000420	214.78	3.81E-34	Pro His rich protein	20	
Smp_000430	374.21	5.91E-39	Eggshell protein	6	Yes
Smp_013540	353.16	1.35E-38	Tyrosinase	8	
Smp_014610	427.38	1.30E-43	Serine:threonine kinase 1	5	
Smp_050270	243.54	1.61E-33	Tyrosinase	18	
Smp_050750	265.17	1.30E-43	Uncharacterised protein	13	
Smp_077890	351.06	4.31E-40	Trematode Eggshell Synthesis domain containing protein	9	Yes
Smp_077900	504.95	4.69E-65	Female specific protein 800	2	Yes
Smp_077920	183.03	2.02E-29	Uncharacterised protein	22	Yes
Smp_095980	323.47	9.38E-37	Extracellular superoxide dismutase (Cu Zn)	11	
Smp_112450	179.17	9.08E-30	Trematode Eggshell Synthesis domain containing protein	23	Yes
Smp_131110	616.92	5.50E-48	Uncharacterised protein	1	
Smp_138570	261.01	1.15E-34	Spore germination protein	15	
Smp_144440	169.70	2.82E-37	Replication A protein	25	
Smp_151150	362.41	8.12E-50	Arabinogalactan protein	7	
Smp_151160	230.02	1.82E-38	Stress protein DDR48	19	
Smp_155310	190.75	5.98E-30	Tetraspanin CD63 receptor	21	
Smp_165360	175.53	1.24E-30	Histone acetyltransferase myst4	24	
Smp_191910	431.31	3.31E-46	Stress protein DDR48	4	Yes
Smp_033250	96.72	4.80E-26	Uncharacterised protein	36	
Smp_152150	93.32	2.47E-22	Uncharacterised protein	38	

**Appendix A.20: Expression of genes of Cluster 13 from the Kohonen analysis in the comparison of female worms from mixed sex infections to males worms at 38 days *post* infection.** Fold changes provide a measure of gene expression in females worms relative to male worms. The *p*-value has been adjusted for multiple hypothesis testing. The Rank provides the position of the gene within the list of differentially expressed gene when ordered by fold change. All genes in Cluster 13 where amongsts the 40 most differentially expressed genes. The final column indicates the presence or absence of a trematode eggshell synthesis domain in the encoded protein.

Gene ID	Reads in females from mixed sex infections at 38 d.p.i.	Reads in females from single sex infections at 38 d.p.i.
Smp_000270	24679	14
Smp_000280	143373	49
Smp_000290	13081	17
Smp_000390	67711	14
Smp_000400	26606	14
Smp_000410	128053	501
Smp_000420	208495	299
Smp_000430	483455	97
Smp_013540	31888	8
Smp_014610	1045457	415
Smp_033250	66492	416
Smp_050270	92273	61
Smp_050750	39007	75
Smp_077890	44949	23
Smp_077900	16593	20
Smp_077920	18893	4
Smp_095980	204041	87
Smp_112450	16580	12
Smp_131110	3988553	1125
Smp_138570	229180	122
Smp_144440	129993	498
Smp_151150	5309	6
Smp_151160	44898	92
Smp_152150	10866	25
Smp_155310	84156	60
Smp_165360	50134	77
Smp_191910	1321831	1072
Sum of fertility gene reads	8536546	5203
Total of all gene reads	59621509	84076361
Percentage of reads	14.31%	0.0062%

**Appendix A.21: Numbers of reads in females from mixed and single sex infections at 38 days *post* infection for the 27 genes in Cluster 13 of the Kohonen analysis.** This is the raw read count, not normalised by gene length.



Smp_000950	Smp_075330	Smp_136870	Smp_152310	Smp_185440
Smp_005710	Smp_078200	Smp_137700	Smp_152910	Smp_186940
Smp_007670	Smp_083770	Smp_139700	Smp_154030	Smp_187360
Smp_011180	Smp_084890	Smp_139720	Smp_154830	Smp_194840
Smp_011960	Smp_085180	Smp_140570	Smp_154900	Smp_198170
Smp_012930	Smp_087760	Smp_141340	Smp_156250	Smp_198260
Smp_020840	Smp_089840	Smp_141900	Smp_158120	Smp_198900
Smp_025830	Smp_095360	Smp_142020	Smp_158360	Smp_199830
Smp_026400	Smp_101100	Smp_142620	Smp_158510	Smp_200150
Smp_029930	Smp_121070	Smp_142680	Smp_158520	Smp_200310
Smp_034680	Smp_125060	Smp_144270	Smp_159470	Smp_202450
Smp_036660	Smp_125740	Smp_145240	Smp_160160	Smp_202690
Smp_038030	Smp_126210	Smp_145290	Smp_163060	Smp_203200
Smp_038970	Smp_127130	Smp_145760	Smp_164140	Smp_204130
Smp_041540	Smp_128190	Smp_146180	Smp_170320	Smp_204250
Smp_042700	Smp_128480	Smp_146810	Smp_172830	Smp_205410
Smp_056290	Smp_129920	Smp_148770	Smp_172980	Smp_206250
Smp_058690	Smp_132660	Smp_149610	Smp_173520	Smp_206290
Smp_058700	Smp_135020	Smp_149780	Smp_175310	Smp_209060
Smp_061100	Smp_135840	Smp_151660	Smp_179710	Smp_212090
Smp_062070	Smp_136730	Smp_151860	Smp_181390	Smp_212820
Smp_063520				

## Appendix A.22: All genes belonging to Cluster 1 of the MBCluster analysis.

Smp_000500	Smp_056680	Smp_123880	Smp_141460	Smp_165500
Smp_000850	Smp_058380	Smp_124070	Smp_142490	Smp_167000
Smp_001410	Smp_059980	Smp_125070	Smp_143540	Smp_167960
Smp_004420	Smp_063530	Smp_125190	Smp_144260	Smp_169020
Smp_011270	Smp_063630	Smp_125250	Smp_144770	Smp_170560
Smp_012560	Smp_073990	Smp_126230	Smp_147450	Smp_171270
Smp_016490	Smp_074000	Smp_126820	Smp_148680	Smp_171290
Smp_018870	Smp_074570	Smp_128110	Smp_151120	Smp_172740
Smp_019030	Smp_075800	Smp_128300	Smp_153070	Smp_174500
Smp_026810	Smp_077610	Smp_128350	Smp_155510	Smp_177540
Smp_027340	Smp_089240	Smp_129270	Smp_155570	Smp_179170
Smp_030000	Smp_089290	Smp_130100	Smp_155900	Smp_181060
Smp_033930	Smp_090010	Smp_131410	Smp_157080	Smp_181070
Smp_037720	Smp_090110	Smp_131470	Smp_158620	Smp_181470
Smp_042140	Smp_091750	Smp_132800	Smp_161240	Smp_187140
Smp_042910	Smp_094500	Smp_133170	Smp_162320	Smp_191970
Smp_043120	Smp_096290	Smp_134590	Smp_162390	Smp_195070

Smp_043290	Smp_096840	Smp_137720	Smp_163310	Smp_197730
Smp_049150	Smp_102430	Smp_139160	Smp_163380	Smp_199290
Smp_052470	Smp_105420	Smp_139240	Smp_163700	Smp_201330
Smp_054620	Smp_105450	Smp_140460	Smp_163720	Smp_203910
Smp_055740	Smp_121930	Smp_140840	Smp_164320	Smp_900040
Smp_055990	Smp_123720			

### Appendix A.23: All genes belonging to Cluster 4 of the MBCluster analysis.

Gene ID	Average read count	Fold change	Gene ID	Average read count	Fold change
Smp_000270	1098.89	243.96	Smp_129960	414.79	101.63
Smp_000280	5844.21	262.95	Smp_130370	143.49	24.89
Smp_000290	520.85	463.72	Smp_130780	22.76	94.63
Smp_000370	40.72	152.97	Smp_131110	146222.11	616.92
Smp_000390	2812.64	142.88	Smp_137460	167.86	35.98
Smp_000400	1056.45	252.51	Smp_138570	10245.16	261.01
Smp_000410	5371.41	146.36	Smp_140720	336.99	22.60
Smp_000420	9619.11	214.78	Smp_141450	104.15	98.45
Smp_000430	20613.88	374.21	Smp_144440	6322.04	169.70
Smp_011870	222.98	47.15	Smp_145020	135.79	23.62
Smp_013540	1311.58	353.16	Smp_145490	63.19	113.36
Smp_014610	49190.98	427.38	Smp_146480	99.57	17.68
Smp_029620	4.49	10.18	Smp_148390	317.14	71.70
Smp_033250	2959.04	96.72	Smp_151150	237.48	362.41
Smp_041880	56.69	30.47	Smp_151160	1990.40	230.02
Smp_050270	3936.38	243.54	Smp_151850	85.29	28.57
Smp_050750	1575.47	265.17	Smp_152150	443.42	93.32
Smp_054010	876.51	37.52	Smp_154390	30.35	20.51
Smp_055260	226.77	22.05	Smp_155310	3646.08	190.75
Smp_056280	82.41	5.48	Smp_157730	9.54	24.66
Smp_056700	1482.19	17.68	Smp_159480	57.64	28.80
Smp_058160	199.83	112.56	Smp_159920	1085.76	30.49
Smp_065530	107.12	69.46	Smp_164400	424.85	40.10
Smp_070360	30.37	14.18	Smp_165360	2524.98	175.53
Smp_074710	246.16	38.78	Smp_166560	217.40	29.12
Smp_076320	648.59	25.03	Smp_167830	28.59	17.07
Smp_077890	2068.34	351.06	Smp_169090	551.51	30.16
Smp_077900	675.45	504.95	Smp_170770	11.19	21.11
Smp_077920	916.91	183.03	Smp_170840	47.48	19.16
Smp_078720	58.79	22.57	Smp_173150	3694.12	38.76
Smp_080360	54.01	41.78	Smp_175560	457.47	138.39
Smp_087320	75.30	15.68	Smp_191910	54945.48	431.31
Smp_095350	151.74	13.84	Smp_194060	6.05	4.10

<b>Smp_095980</b>	7743.79	323.47	<b>Smp_194750</b>	137.24	86.72
<b>Smp_105780</b>	68.84	1.99	<b>Smp_195090</b>	551.03	3.99
<b>Smp_112450</b>	827.17	179.17	<b>Smp_196950</b>	203.53	7.69
<b>Smp_122280</b>	66.19	44.71	<b>Smp_199540</b>	163.74	50.27
<b>Smp_124510</b>	92.96	43.69	<b>Smp_202840</b>	40.55	113.42

**Appendix A.24: Summary of the 76 genes in Cluster 7 generated by MBCluster.** The column “Average read count” provides a measure of absolute expression, the average number of reads mapped to the gene across all RNA-Seq samples used in Chapter 3. The fold changes provided reflect expression in females from mixed sex infections compared to females from single sex infections at 38 days *post* infection.

GO term	Description	Annotated	Significant	Expected	p-value
<b>GO:0006570</b>	Tyrosine metabolic process	4	2	0.02	1.40E-04
<b>GO:0046189</b>	Phenol-containing compound biosynthetic process	4	2	0.02	1.40E-04
<b>GO:0044550</b>	Secondary metabolite biosynthetic process	7	2	0.03	5.00E-04
<b>GO:0006875</b>	Cellular metal ion homeostasis	10	2	0.05	1.05E-03
<b>GO:0046148</b>	Pigment biosynthetic process	11	2	0.05	1.28E-03

**Appendix A.25: Significantly enriched GO terms amongst the genes in Cluster 7 of the MBCluster analysis.** “Annotated” provides the total number of genes in the genome annotated with a given GO term. “Significant” provides the number of genes annotated with that GO term in Cluster 9. “Expected” provides the number of genes with that GO term expected by chance. The significance cut-off was  $p = 0.01$ .

Target Gene	CD63R Replicate 1	CD63R Replicate 2	CD63a Replicate 1	CD63a Replicate 2	Control Replicate 1	Control Replicate 2	No template (negative control)
CD63R	20.86	19.52	22.05	23.04	21.51	21.27	37.15
	20.82	19.37	21.80	22.84	21.37	21.30	> 40.00
	20.80	19.41	21.90	22.83	21.49	21.44	35.97
CD63a	22.95	22.49	23.41	22.96	22.42	21.62	36.00
	22.91	22.54	23.34	23.17	22.69	21.62	35.92
	22.93	22.43	23.41	23.18	22.50	21.74	34.24
PDMS4 (House keeping gene)	17.52	16.65	17.20	18.21	16.68	16.56	> 40.00
	17.65	16.57	17.03	18.26	16.52	16.66	34.40
	17.65	16.57	17.23	18.18	16.55	16.52	35.80
No reverse transcription (negative control)	34.40	28.61	28.40	> 40.00	27.84	29.02	> 40.00
	31.00	28.56	35.50	> 40.00	28.25	33.65	37.14
	30.15	28.99	29.55	> 40.00	29.03	> 40.00	> 40.00

**Appendix A.26: This table provides the  $C_T$  for all of the qRT-PCR reactions used to measure gene expression the RNAi knockdown experiment using the soaking method.** Three PCR replicates are given for each combination of target gene and treatment replicate (e.g. CD63R 1 and CD63 R2).  $C_T$  were not determined beyond 40 cycles of replication; samples that did not show amplification were assigned “ > 40 ” as  $C_T$  value. For each sample replicate a negative control was run which had not been reverse transcribed to detect gDNA contamination in the total RNA extracted from the worms. Another negative control with out any added template was run together with the samples.

Target Gene	CD63R Replicate 1	CD63R Replicate 2	CD63R 3 Replicate 3	CD63a Replicate 1	CD63a Replicate 2	CD63a Replicate 3	Control Replicate 1	Control Replicate 2	Control Replicate 3	No template (negative control)
CD63a	24.67	23.10	23.91	23.05	22.93	22.66	23.49	22.66	23.87	31.92
	24.64	22.84	23.64	23.12	22.95	22.66	23.53	22.59	23.84	34.47
	24.41	22.78	23.63	23.00	22.76	22.60	23.34	22.66	23.79	33.22
CD63R	19.88	18.97	19.86	18.91	19.02	19.71	19.46	19.51	19.75	33.97
	19.90	18.97	19.54	18.90	19.07	19.45	19.40	19.51	19.77	22.93
	19.76	18.92	19.23	18.76	19.11	19.53	19.35	19.43	19.78	34.18
PDMS4 (House keeping gene)	19.27	18.77	18.74	18.45	18.29	18.42	16.24	15.82	16.48	> 40.00
	19.32	18.86	19.00	18.50	18.33	18.54	16.41	15.82	16.57	> 40.00
	19.38	18.97	18.90	18.57	18.30	18.53	16.43	15.91	16.59	30.63
No reverse transcription (negative control)	33.46	32.81	32.84	33.61	30.82	33.41	30.84	29.18	29.89	> 40.00
	31.79	32.79	34.81	32.90	30.52	33.94	31.28	28.99	28.44	37.10
	33.59	34.17	32.55	33.64	30.43	32.87	30.30	30.26	29.12	> 40.00

**Appendix A.27: This table provides the  $C_T$  for all of the qRT-PCR reactions used to measure gene expression the RNAi knockdown experiment using the electroporation method.** Three PCR replicates are given for each combination of target gene and treatment replicate (e.g. CD63R 1 and CD63 R2).  $C_T$  were not determined beyond 40 cycles of replication; samples that did not show amplification were assigned “ > 40 ” as  $C_T$  value. For each sample replicate a negative control was run which had not been reverse transcribed to detect gDNA contamination in the total RNA extracted from the worms. Another negative control with out any added template was run together with the samples.

## Appendix B

Samples	D0 F10	D0 F4	D0 F5	D0 F8	D0 F9	D0 M10	D0 M1	D0 M2	D0 M5	D0 M9	D4 FP10	D4 FP2	Average
<b>Total Reads</b>	42,839,264	40,885,356	40,355,812	18,025,886	49,625,790	14,063,226	42,329,640	24,937,274	27,513,990	67,601,548	23,971,200	23,260,574	36,514,504
<b>Mapped</b>	38916416	36646230	36493578	16072299	43413686	12496871	39181978	22370714	24462457	61949189	21860348	21076223	32715432
<b>Prop. Paired</b>	81.43%	78.82%	79.91%	78.76%	76.58%	79.18%	82.01%	79.81%	79.05%	82.28%	79.84%	79.27%	79.63%
<b>Unmap.</b>	3746955	4111222	3698354	1887292	6056171	1514468	2973642	2474627	2964568	5375856	2022005	2110713	3661759
<b>% Mapped</b>	90.84%	89.63%	90.43%	89.16%	87.48%	88.86%	92.56%	89.71%	88.91%	91.64%	91.19%	90.61%	89.60%
<b>% Unmapped</b>	8.75%	10.06%	9.16%	10.47%	12.20%	10.77%	7.02%	9.92%	10.77%	7.95%	8.44%	9.07%	10.03%
<b>Failed Quality Control</b>	0.41%	0.31%	0.41%	0.37%	0.31%	0.37%	0.41%	0.37%	0.32%	0.41%	0.37%	0.32%	0.38%

Samples	D4 FP3	D4 FP4	D4 FP5	D4 FP9	D4 FS10	D4 FS3	D4 FS6	D4 FS8	D4 FS9	D4 MP10	D4 MP1	D4 MP2	Average
<b>Total Reads</b>	27,937,410	38,813,550	35,647,926	48,950,808	30,331,014	20,601,208	21,341,254	38,262,878	61,440,742	39,086,800	19,033,002	41,838,666	36,514,504
<b>Mapped</b>	24681965	35272275	31809561	43934269	27024218	18506319	18967782	34423953	55229205	36277200	17194451	37390396	32715432
<b>Prop. Paired</b>	78.71%	80.24%	78.89%	78.38%	78.61%	78.84%	78.54%	78.97%	80.46%	82.61%	80.96%	80.17%	79.63%
<b>Unmap.</b>	3139249	3397391	3725939	4816482	3211224	2019624	2307076	3682758	5984085	2664807	1767972	4317276	3661759
<b>% Mapped</b>	88.35%	90.88%	89.23%	89.75%	89.10%	89.83%	88.88%	89.97%	89.89%	92.81%	90.34%	89.37%	89.60%
<b>% Unmapped</b>	11.24%	8.75%	10.45%	9.84%	10.59%	9.80%	10.81%	9.62%	9.74%	6.82%	9.29%	10.32%	10.03%
<b>Failed Quality Control</b>	0.42%	0.37%	0.32%	0.41%	0.32%	0.37%	0.31%	0.41%	0.37%	0.37%	0.37%	0.31%	0.38%

Samples	D4 MP3	D4 MP4	D4 MP5	D4 MP8	D4 MS10	D4 MS1	D4 MS2	D4 MS3	D4 MS4	D4 MS5	D4 MS8	D4 MS9	Average
<b>Total Reads</b>	31,991,790	22,369,714	24,146,306	37,815,740	35,386,012	37,843,540	51,749,442	12,819,534	26,090,006	50,489,724	20,859,514	38,584,968	36,514,504
<b>Mapped</b>	29114450	20082407	21549878	34139827	32264435	33920979	47483220	11495623	23269207	46193807	18537694	33844860	32715432
<b>Prop. Paired</b>	81.69%	80.09%	79.23%	80.15%	81.66%	79.87%	82.06%	80.61%	78.99%	81.28%	78.98%	77.17%	79.63%
<b>Unmap.</b>	2747238	2204828	2520353	3521572	2978354	3803876	4034666	1276989	2737848	4089750	2245034	4617616	3661759
<b>% Mapped</b>	91.01%	89.77%	89.25%	90.28%	91.18%	89.63%	91.76%	89.67%	89.19%	91.49%	88.87%	87.72%	89.60%
<b>% Unmapped</b>	8.59%	9.86%	10.44%	9.31%	8.42%	10.05%	7.80%	9.96%	10.49%	8.10%	10.76%	11.97%	10.03%
<b>Failed Quality Control</b>	0.41%	0.37%	0.32%	0.41%	0.40%	0.31%	0.45%	0.37%	0.32%	0.41%	0.37%	0.32%	0.38%

Samples	D8 FP10	D8 FP2	D8 FP4	D8 FP5	D8 F51	D8 F52	D8 F53	D8 F55	D8 F58	D8 MP1	D8 MP2	D8 MP4	Average
<b>Total Reads</b>	47,008,208	24,550,604	36,397,450	44,329,112	10,712,766	31,616,670	32,081,458	68,971,632	50,614,744	25,197,978	36,872,448	35,550,458	36,514,504
<b>Mapped</b>	42150511	21965618	32047016	40412159	9292832	28375108	25469702	61525934	40167006	23003747	33246349	31627528	32715432
<b>Prop. Paired</b>	78.69%	79.58%	77.84%	81.71%	73.13%	79.92%	63.39%	79.96%	63.83%	81.14%	80.89%	79.98%	79.63%
<b>Unmap.</b>	4685762	2494367	4234879	3735666	1380139	3141867	6320535	7192351	10275620	2090259	3489994	3812064	3661759
<b>% Mapped</b>	89.67%	89.47%	88.05%	91.16%	86.75%	89.75%	79.39%	89.20%	79.36%	91.29%	90.17%	88.97%	89.60%
<b>% Unmapped</b>	9.97%	10.16%	11.64%	8.43%	12.88%	9.94%	19.70%	10.43%	20.30%	8.30%	9.47%	10.72%	10.03%
<b>Failed Quality Control</b>	0.37%	0.37%	0.32%	0.41%	0.37%	0.32%	0.91%	0.37%	0.34%	0.41%	0.37%	0.31%	0.38%

Samples	D8 MP5	D8 MP7	D8 MP8	D8 MP9	D8 MS10	D8 MS1	D8 MS2	D8 MS3	D8 MS5	D8 MS7	D8 MS8	D8 MS9	Average
<b>Total Reads</b>	42,434,848	17,753,578	25,831,508	43,743,710	38,120,912	41,733,998	45,553,098	75,767,817	42,100,608	41,931,428	55,188,534	47,965,560	36,514,504
<b>Mapped</b>	38840596	16174219	23345922	38874466	35194743	36987220	42074247	66679467	37881514	38121241	49698796	42757518	32715432
<b>Prop. Paired</b>	82.32%	81.83%	80.87%	80.44%	82.71%	78.82%	82.08%	90.16%	80.78%	81.77%	81.42%	80.16%	79.63%
<b>Unmap.</b>	3420972	1514069	2404286	4666249	2770194	4615435	3293712	8795063	4086470	3608151	5285191	5056152	3661759
<b>% Mapped</b>	91.53%	91.10%	90.38%	88.87%	92.32%	88.63%	92.36%	88.00%	89.98%	90.91%	90.05%	89.14%	89.60%
<b>% Unmapped</b>	8.06%	8.53%	9.31%	10.67%	7.27%	11.06%	7.23%	11.61%	9.71%	8.60%	9.58%	10.54%	10.03%
<b>Failed Quality Control</b>	0.41%	0.37%	0.31%	0.46%	0.41%	0.31%	0.41%	0.39%	0.32%	0.48%	0.37%	0.32%	0.38%

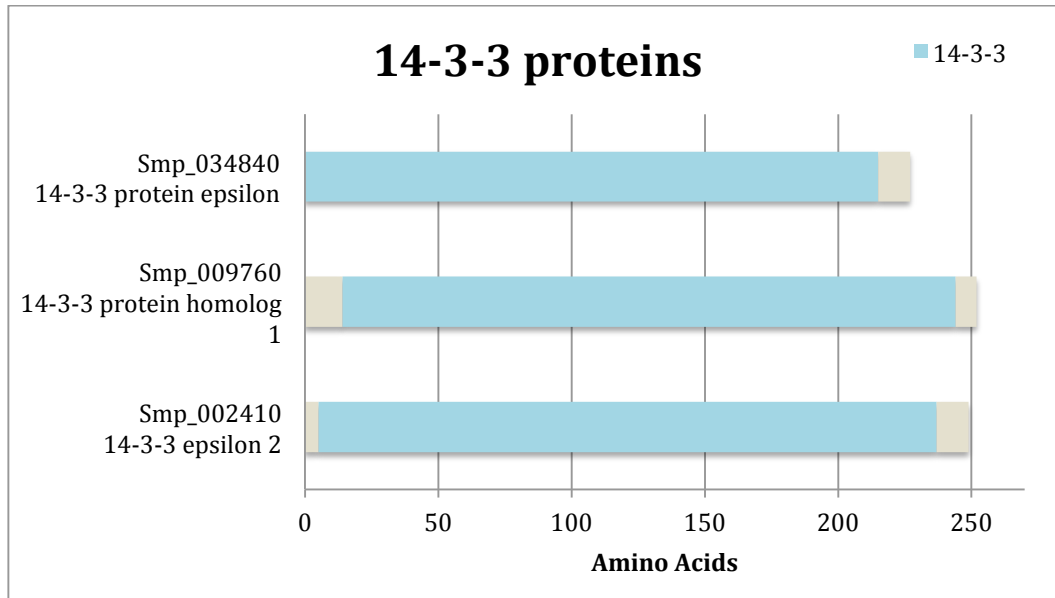
**Table B.1: Summary of sequencing statistics for sequenced RNA-Seq samples.** Total reads is the number of all reads sequenced. Mapped is the total number of reads mapped to the *S. mansoni* reference genome v5.2. 'Prop. Paired' refers to the total number of reads mapped together with a mate-paired read in close proximity and in the correct orientation. Unmapped is the total number of reads that could not be mapped to the reference by Tophat2.

## Apoptosis-related proteins

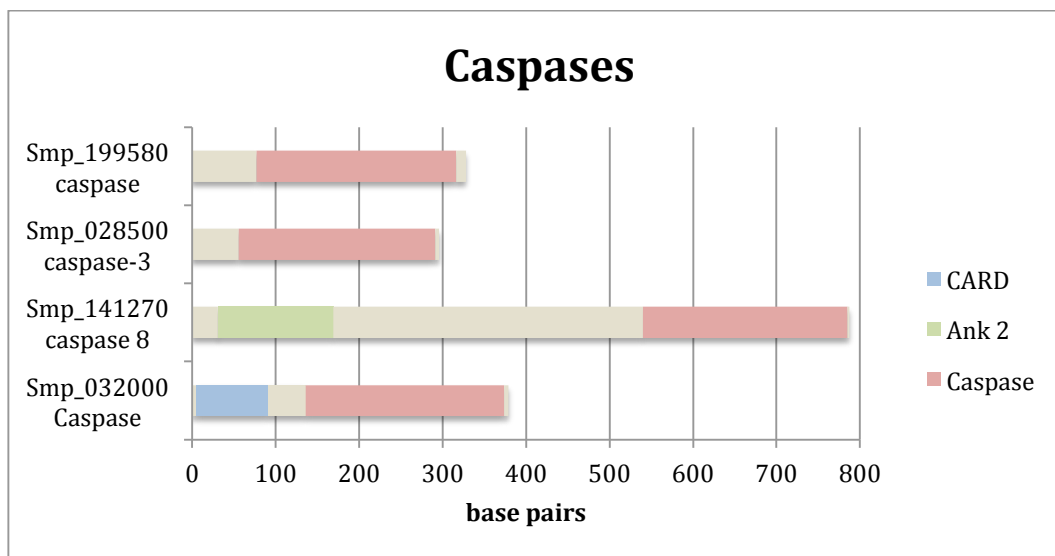
14-3-3 proteins play an important role in relaying apoptosis signals within cells and exert pro-apoptotic effects at different points of the apoptosis pathway (Rosenquist, 2003). Three 14-3-3 proteins have been annotated in *S. mansoni* all of which were found to contain a 14-3-3 Pfam domain as well as significant BLAST hits (Table 4.1) against 14-3-3 proteins of model organisms.

The next group of genes I examined are caspases which are key mediators of apoptosis as they form the apoptotic protease cascade that lead to controlled cell death (McIlwain *et al.*, 2013). Four caspase homologues have been identified in *S. mansoni*, which all contain a caspase domain. Blast results suggest caspase-3, -7 and -8 homologues are represented in the *S. mansoni* genome. Three of these caspases (Smp\_028500, Smp\_141270 & Smp\_032000) were previously described by Lee *et al.* (2014).





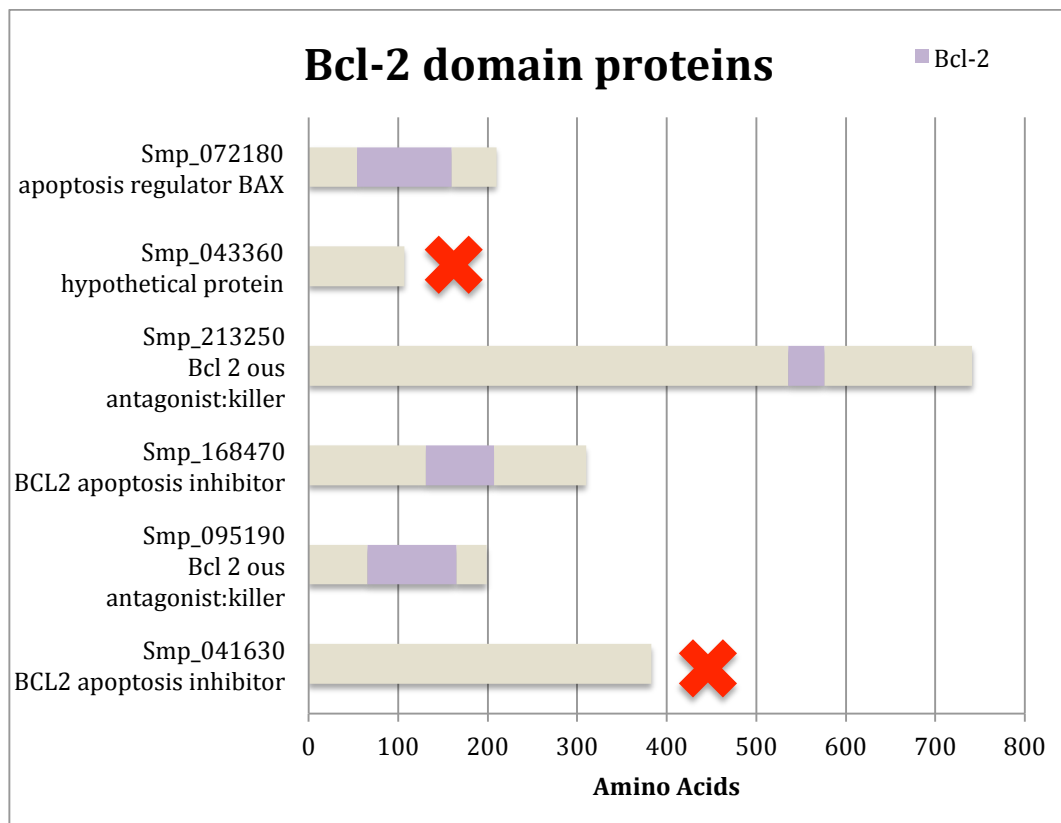
**Figure B.2:** Diagram of 14-3-3 family members showing length and presence of PFAM domains.



**Figure B.3:** Diagram of caspase family members showing length and presence of PFAM domains.

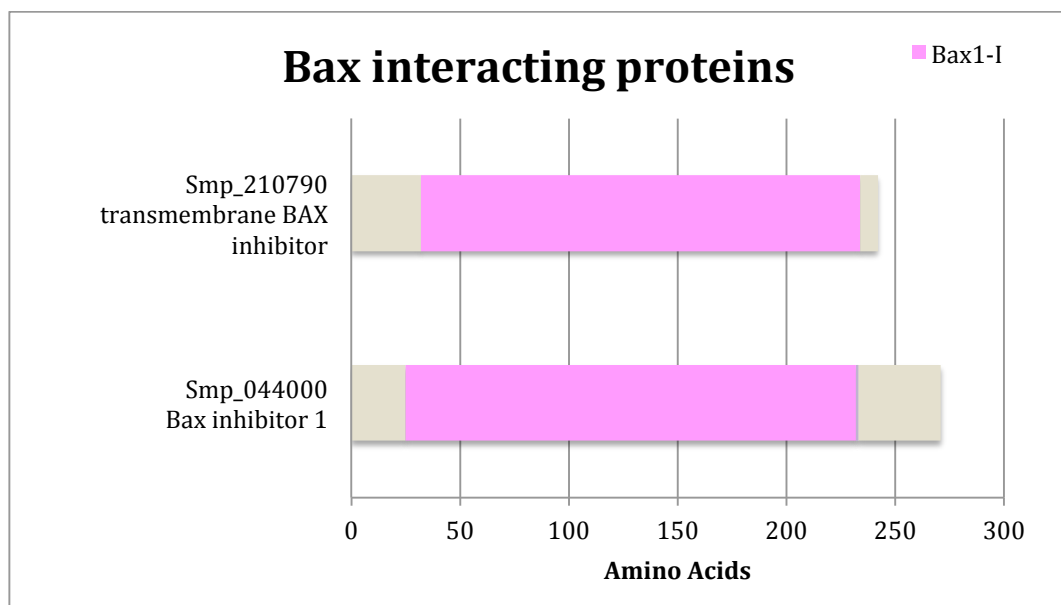
Another group of proteins that function as regulators of apoptosis, in both pro- and anti-apoptotic roles are Bcl-2 domain containing proteins. Four Bcl-2 domain containing proteins were identified by InterProScan, a further two were found to be annotated as Bcl-2 proteins, one in the genome (Smp\_041630)

(Protasio *et al.*, 2012) and the other by Lee *et al.* (2011) (Smp\_043360). However InterProScan did not identify Bcl-2 domains in the later two, nor did BLAST find significant homology to such proteins (Table B.5), which is why I decided to exclude them from the down-stream work flow of my analysis. One of the genes identified by InterProScan showed significant homology to apoptosis regulator Bax, also known as Bcl-2-like protein 4, a known pro-apoptotic member of the bcl-2 like family (Pawlowski & Kraft, 2000). Five of the Bcl-2 family proteins (Smp\_043360, Smp\_072180, Smp\_095190, Smp\_213250 & Smp\_168470) were described previously (Lee *et al.*, 2014).



**Figure B.4:** Diagram of Bcl-2 domain containing protein showing length and presence of PFAM domains. Two genes, marked with 'X', failed the quality control and were excluded from the following analysis.

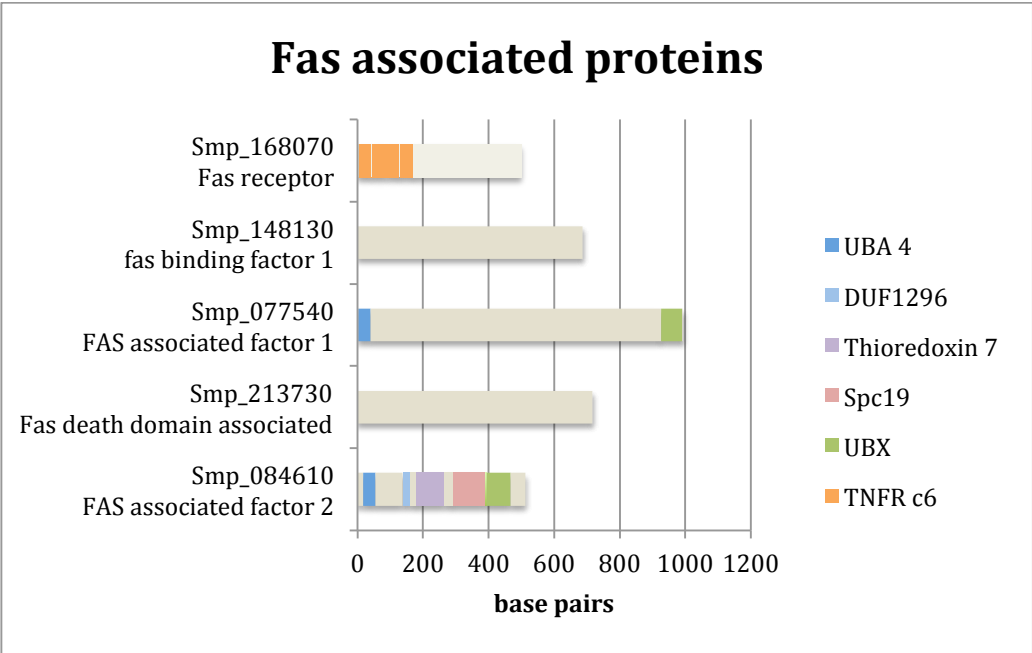
Two genes were found to contain Bax-Inhibition domains. Bax is known to have an important pro-apoptotic domain and its activity is tightly regulated by proteins such as Bax inhibitor 1. This group of proteins was first identified as an inhibitor of Bax but was later recognised to inhibit apoptosis through interactions with several other pro-apoptotic processes as well (Robinson *et al.*, 2011).



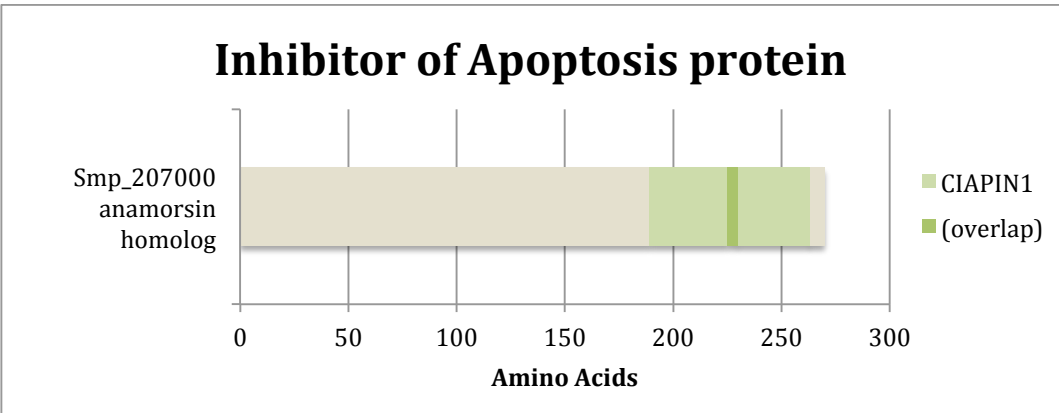
**Figure B.5:** Diagram of BAX interacting proteins showing length and presence of PFAM domains.

The Fas ligand:receptor interaction was one of the first aspects of the pro-apoptotic signalling cascade to be studied (Nagata, 1997, 1999). A homologue of the Fas receptor as well as several homologues of Fas associated proteins with putative roles in the regulation of apoptosis have been identified. Determining whether these genes should be assigned putative pro- or anti-apoptotic function based on domains was difficult as two of the genes did not contain known domains (see Figure B.7) so that their function could only be inferred by

homology to genes of model organisms. However the function of the human fas binding factor 1 (FAB1) and fas associated factor 2 (FAF2) are only poorly understood. FAF2 is thought to confer resistance to apoptosis to T cells in patients with atopic dermatitis (Koelsch *et al.*, 2013). FAB1 on the other hand was first described in 2000 but no definitive function has yet been assigned (Schmidt *et al.*, 2000).

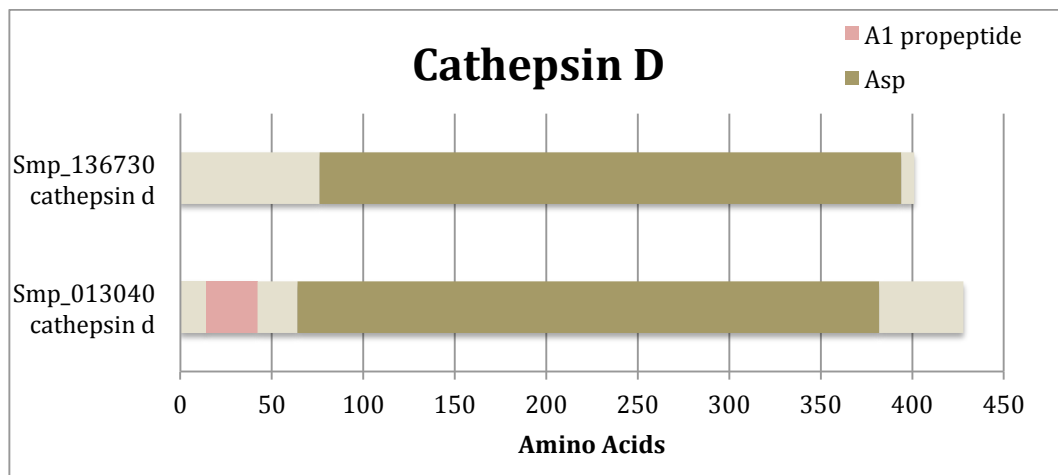


**Figure B.6:** Diagram of Fas receptor and associated proteins, showing length and presence of PFAM domains.



**Figure B.7:** Diagram of inhibitor of apoptosis protein, showing length and presence of PFAM domains.

The schistosome homologue of the Inhibitor of Apoptosis protein (IAP) was identified by Peng *et al.* (2010) and contains two overlapping “cytokine induced apoptosis inhibitor 1” domains, but lacks the Baculo virus IAP repeat (BIR) domain which is a hallmark of the family of IAPs. Rather than a member of the IAP family, Smp\_207000 shares greater sequence similarity with the human anamorsin gene, a cytokine induced inhibitor of apoptosis. Given that the schistosome homologue was shown to inhibit caspases and thus apoptosis both in human cells as well as schistosome lysate (Peng *et al.*, 2010) it can be classified confidently as an anti-apoptotic gene.

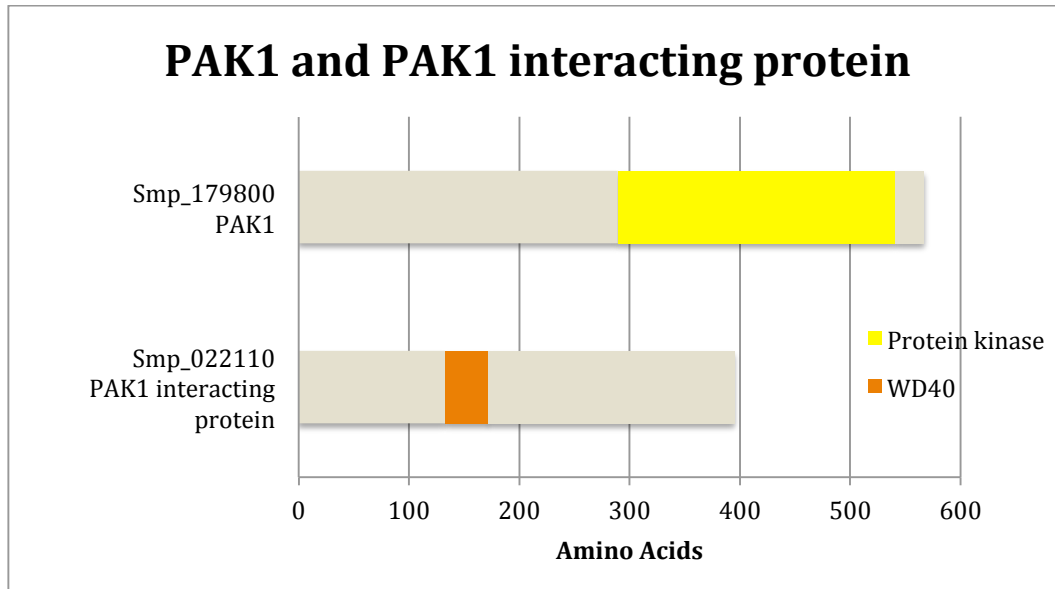


**Figure B.8:** Diagram of cathepsin D homologues, showing length and presence of PFAM domains.

Cathepsin D is a lysosomal protease that besides its role in digestion also plays a regulatory function in apoptosis. When activated, for example by ceramide (Heinrich *et al.*, 1999), cathepsin D mediates oxidative stress induced apoptosis by translocation to the cytoplasm (Watchorn *et al.*, 2001) as well as mediation of cytochrome C release and caspase activation (Conus *et al.*, 2008; Johansson *et al.*,

2003). Both cathepsin D homologues in *S. mansoni* contain aspartate protease domains as expected from cathepsins.

p21 is a cyclin dependent kinase (CDK) inhibitor and an important regulator of cell fate, controlling both division and apoptosis depending on the cell state and extracellular signals. By impeding CDKs it can arrest the cell cycle and is considered an important proliferation inhibitor (Gartel & Tyner, 2002). Despite this, p21 is a negative regulator of p53-dependent apoptosis. p21-activated kinase 1 (PAK1) are serine/threonine protein kinases serving as mediators of Rac and Cdc42 GTPase function as well as Ras-driven tumorigenesis by protecting cells from apoptosis (Ong *et al.*, 2011). As the schistosome homologue of PAK1 has a protein kinase domain as well as significant sequence similarity to the human PAK1, I assigned it a putative anti-apoptotic function. The PAK1 interacting protein (PAK1IP) homologue on the other hand contains a conserved WD40 repeat, as does the human PAK1IP. PAK1IP is a negative regulator of PAK1, binding to the N-terminal region of PAK1 to inactivate it, thus acting in a pro-apoptotic function (Xia *et al.*, 2001).



**Figure B.9:** Diagram of PAK1 and PAK1IP, showing length and presence of PFAM domains.

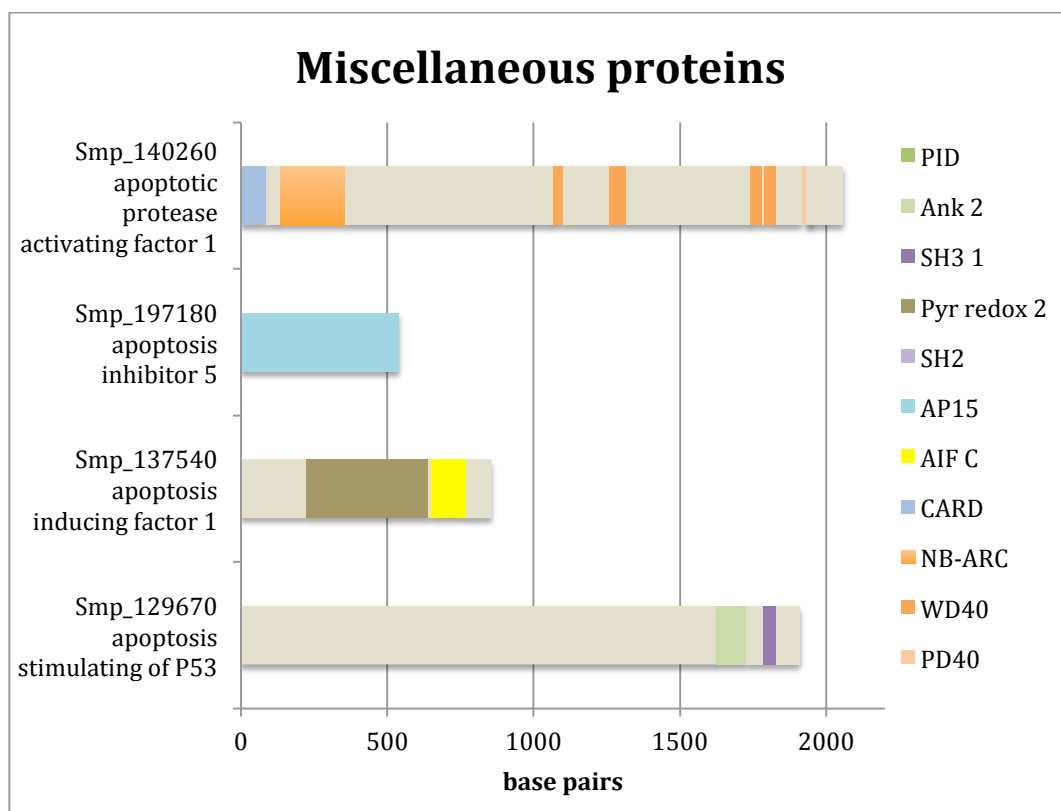
Five other *S. mansoni* genes were found to be homologous to apoptosis regulating genes of humans and *C. elegans*. These did not form a related group of genes and thus contain a range of different, unrelated protein domains, such as WD40, SH2 and Sh3 domains. The apoptotic protease activating factor (APAF1) like gene (Smp\_140260) contains several WD domains that allow its human and *C. elegans* homologues to bind to caspase proteins and activate them, thereby promoting apoptosis (Qin *et al.*, 1999; Yakovlev *et al.*, 2001). The schistosome homologue of APAF1 (Smp\_140260) was first described by Lee *et al.* (2014).

Almost the entire length of the schistosome homologue of apoptosis inhibitor 5 (AIP5) is made up of a conserved apoptosis inhibitory protein 5 domain, which, together with the sequence homology to human AIP5 suggests that it has an anti-apoptotic effect, acting as a suppressor of E2-promoter binding factor dependent apoptosis (Morris *et al.*, 2006).

In humans, the apoptosis inducing factor 1 is associated with the mitochondria and involved in a caspase independent activation of apoptosis (Joza *et al.*, 2001; Susin *et al.*, 1999). The schistosome homologue (Smp\_137540) shares a “C-terminal mitochondrion-associated apoptosis-inducing factor”-domain as well as a pyridine nucleotide-disulphide oxidoreductase domain.

Like the human apoptosis-stimulating of p53 protein (PPP1R13B), the schistosome homologue has two domains near its C-terminus: an ankyrin repeat and a SH3 domain. In humans, these domains allow PPP1R13B to interact with p53/TP53 and regulate it by enhancing the DNA binding and transactivation function of TP53 on the promoters of pro-apoptotic genes *in vivo*, thereby specifically enhancing apoptosis but not cell cycle arrest (Samuels-Lev *et al.*, 2001).





**Figure B.10:** Diagram of miscellaneous apoptosis related proteins, showing length and presence of PFAM domains.

Gene ID	Average Read Count	Fold change	Adjusted p-value	Product
Smp_107750	11120.5	4.764	0.033	Uncharacterised protein
Smp_108010	7839.4	4.696	0.033	Uncharacterised protein
Smp_116960	7850.2	4.665	0.033	Uncharacterised protein
Smp_100450	7759.5	4.605	0.033	Uncharacterised protein
Smp_074390	7340.6	4.242	0.033	Eukaryotic translation initiation factor
Smp_145370	6960.2	3.540	0.033	Uncharacterised protein
Smp_079770	41164.0	1.642	0.033	Probable protein disulfide-isomerase ER-60
Smp_049270	24812.2	0.267	0.045	Major egg antigen (p40)
Smp_131110	177674.7	4.205	0.053	Uncharacterised protein
Smp_018930	7063.7	4.011	0.053	Uncharacterised protein
Smp_023840	12609.0	3.758	0.053	Uncharacterised protein
Smp_202690	15523.4	3.599	0.053	Universal stress protein
Smp_006040	11467.1	3.563	0.053	Uncharacterised protein
Smp_054160	135853.5	3.328	0.053	Glutathione S-transferase class-mu 28 kDa isozyme
Smp_158110	9316.0	2.257	0.053	Thioredoxin peroxidase
Smp_900040	112222.7	3.631	0.064	NADH dehydrogenase subunit 2
Smp_090520	9271.9	3.434	0.064	Purine nucleoside phosphorylase
Smp_900100	57478.3	3.603	0.065	NADH dehydrogenase subunit 3
Smp_043120	41091.7	3.179	0.065	Universal stress protein
Smp_900050	227094.7	3.260	0.070	NADH dehydrogenase subunit 5
Smp_095980	30236.6	3.838	0.070	Extracellular superoxide dismutase (Cu Zn)
Smp_155310	8225.5	3.760	0.078	Tetraspanin CD63 receptor
Smp_058690	20056.6	3.022	0.088	Glutathione peroxidase
Smp_000430	75350.6	3.607	0.089	Eggshell protein
Smp_014610	82078.6	3.373	0.089	Serine:threonine kinase 1
Smp_138570	9976.6	3.364	0.089	Spore germination protein
Smp_000390	9269.4	3.361	0.089	Uncharacterised protein
Smp_191910	66486.1	3.244	0.089	Stress protein DDR48
Smp_000420	22883.9	3.184	0.089	Pro His rich protein
Smp_000280	24250.8	3.163	0.089	Uncharacterised protein
Smp_173150	7196.8	3.111	0.089	CD63 antigen
Smp_087760	15931.7	3.059	0.089	Ferritin 1 heavy chain
Smp_080920	48931.4	3.050	0.089	Uncharacterised protein
Smp_008360	21077.1	3.020	0.089	Adenosine kinase
Smp_033610	8093.4	3.007	0.089	NADH dehydrogenase (ubiquinone) 1 beta
Smp_202770	15844.8	3.002	0.089	Uncharacterised protein
Smp_071000	9542.5	2.977	0.089	26 proteasome complex subunit DSS1
Smp_016490	8570.6	2.970	0.089	Saposin B domain containing protein
Smp_900020	123222.9	2.967	0.089	NADH dehydrogenase subunit 6
Smp_077890	11865.3	2.966	0.089	Trematode Eggshell Synthesis domain containing protein
Smp_036940	8638.4	2.944	0.089	Protein transport protein Sec61 subunit
Smp_135740	8509.8	2.918	0.089	Splicing factor 3B subunit 5
Smp_900000	316106.4	2.876	0.089	Cytochrome c oxidase subunit I
Smp_066590	7573.6	2.875	0.089	LYR motif containing protein 4

Smp_105450	25570.2	2.857	0.089	Saposin containing protein
Smp_158910	6850.8	2.835	0.089	Cyclin dependent protein kinase CDC28 regulatory
Smp_028190	9228.7	2.816	0.089	Methylthioadenosine phosphorylase
Smp_053390	32861.4	2.813	0.089	Histone H4
Smp_033400	21383.4	2.755	0.089	Uncharacterised protein
Smp_079270	8197.8	2.741	0.089	LIM, zinc binding, domain containing protein
Smp_079230	21402.5	2.717	0.089	Immunophilin FK506 binding protein FKBP12, putative
Smp_004940	26544.0	2.708	0.089	60S ribosomal protein L35
Smp_036220	19780.6	2.707	0.089	Histone H2B
Smp_900110	82393.2	2.706	0.089	NADH dehydrogenase subunit 1
Smp_019060	10298.8	2.699	0.089	Sec61 beta subunit
Smp_179460	9344.1	2.683	0.089	Uncharacterised protein
Smp_007900	32378.3	2.676	0.089	Large subunit ribosomal protein 23
Smp_029820	35411.7	2.645	0.089	Ribosomal protein, large P2
Smp_205570	15821.5	2.640	0.089	Small nuclear ribonucleoprotein e
Smp_050280	25043.2	2.637	0.089	Ribosomal protein L31
Smp_066990	31353.8	2.631	0.089	40S rRNA protein homolog
Smp_146190	20760.2	2.627	0.089	40S ribosomal protein S21
Smp_035790	32243.6	2.623	0.089	60S ribosomal protein L44
Smp_074780	24348.9	2.614	0.089	Ribosomal protein S18
Smp_089570	17532.3	2.586	0.089	Stress associated endoplasmic reticulum protein
Smp_075560	12705.4	2.583	0.089	Small nuclear ribonucleoprotein Sm D2
Smp_059930	6598.7	2.568	0.089	Cytochrome b c1 complex subunit 6
Smp_035800	23949.4	2.566	0.089	60S ribosomal protein L37
Smp_041650	18343.7	2.563	0.089	Ribosomal protein S27
Smp_091640	28194.6	2.553	0.089	Ribosomal protein S24
Smp_031310	25869.9	2.553	0.089	Ribosomal protein, Small subunit
Smp_014650	20471.8	2.544	0.089	Small subunit ribosomal protein s30e
Smp_210630	8450.9	2.535	0.089	histone H2A
Smp_032060	8096.4	2.532	0.089	14 kda subunit splicing factor 3b
Smp_039400	44213.9	2.518	0.089	Eukaryotic translation initiation factor 5A
Smp_089430	30223.8	2.518	0.089	Ubiquitin (ribosomal protein L40)
Smp_113620	14145.9	2.500	0.089	Splicing factor, arginine:serine rich 2
Smp_176200	13488.6	2.484	0.089	Superoxide dismutase [Cu-Zn]
Smp_058700	8579.6	2.480	0.089	Glutathione peroxidase
Smp_001830	27123.9	2.456	0.089	Ribosomal protein L24
Smp_087550	11928.4	2.452	0.089	Uncharacterised protein
Smp_022560	15102.5	2.451	0.089	60S ribosomal protein L21
Smp_900090	167743.1	2.444	0.089	NADH dehydrogenase subunit 4
Smp_050940	24957.3	2.442	0.089	60S ribosomal protein L11
Smp_063350	23711.0	2.428	0.089	Ribosomal protein L27
Smp_054780	45354.7	2.410	0.089	40S ribosomal protein S8
Smp_060910	7628.8	2.406	0.089	Small nuclear ribonucleoprotein f
Smp_074470	26940.0	2.406	0.089	Ribosomal protein, Small subunit
Smp_019440	8101.0	2.405	0.089	Protein translation factor sui1
Smp_097380	15244.8	2.378	0.089	Heat shock 10 kDa protein 1

Smp_151810	6755.1	2.340	0.089	Potassium voltage gated channel subfamily H
Smp_068420	7552.8	2.287	0.089	Small nuclear ribonucleoprotein sm d3
Smp_210310	18054.2	2.265	0.089	Ribosomal protein l7a
Smp_055210	9659.2	2.221	0.089	Microsomal glutathione S transferase 3
Smp_018020	6429.4	2.211	0.089	NADH dehydrogenase (ubiquinone) iron sulfur
Smp_020770	7586.2	2.095	0.089	Dolichyl diphosphooligosaccharide protein
Smp_074000	36900.7	1.742	0.089	Uncharacterised protein
Smp_049550	33633.6	1.454	0.089	78 kDa glucose regulated protein
Smp_054560	8523.3	2.363	0.089	Huntingtin interacting protein K
Smp_175740	35702.1	2.351	0.091	Ribosomal protein L14
Smp_061200	27528.7	2.469	0.092	Ribosomal protein L35A
Smp_130100	7798.6	2.830	0.092	Saposin containing protein
Smp_105320	16857.7	2.532	0.092	60S ribosomal protein L37a
Smp_213130	31795.4	2.436	0.092	Ribosomal protein S25
Smp_044580	24381.9	2.423	0.092	Ribosomal protein L30
Smp_098960	35400.1	2.408	0.092	60S ribosomal protein L26
Smp_032760	16458.3	2.369	0.092	Ribosomal protein S11
Smp_012750	39845.5	2.348	0.092	60S ribosomal protein L12
Smp_053830	29481.0	2.347	0.092	40S ribosomal protein S14
Smp_098330	6356.7	2.229	0.092	H:ACA ribonucleoprotein complex subunit 2
Smp_054240	35052.9	2.217	0.092	Translationally-controlled tumor protein homolog
Smp_082240	19731.3	2.210	0.092	Histone H3
Smp_002180	11152.9	2.068	0.092	Basic transcription factor 3 4
Smp_042400	6909.0	1.929	0.092	Arginine rich, mutated in early stage tumors
Smp_030690	16512.0	2.426	0.093	Eukaryotic translation elongation factor 1 beta
Smp_174950	28022.6	2.367	0.093	Ribosomal protein S19e
Smp_027610	32304.2	2.335	0.093	Ribosomal protein S3
Smp_022640	39721.0	2.334	0.093	60S ribosomal protein L13
Smp_036400	9180.3	2.199	0.093	NADH dehydrogenase (ubiquinone) 1 beta
Smp_027880	8201.2	2.153	0.093	Prefoldin subunit 6
Smp_138210	10112.2	2.691	0.094	Uncharacterised protein
Smp_055050	24033.0	2.372	0.094	40S ribosomal protein S17
Smp_211250	25308.8	2.343	0.094	Ribosomal protein L34a
Smp_085300	16087.7	2.260	0.094	Ribosomal protein L22
Smp_098890	6376.0	2.120	0.094	Arginine:serine rich splicing factor
Smp_079430	29778.5	1.970	0.094	GTP binding nuclear protein Ran
Smp_900030	29615.6	2.412	0.095	ATP synthase F0 subunit 6
Smp_076740	21127.7	2.344	0.095	Ribosomal protein saa; ribosomal protein rpsa
Smp_096750	14596.0	2.338	0.095	40S ribosomal protein S13
Smp_018990	24725.4	2.313	0.095	Ribosomal protein L9
Smp_194840	41848.5	2.263	0.095	Niemann Pick C2 protein
Smp_119920	21711.9	2.259	0.095	Ribosomal protein S16
Smp_037700	8014.7	2.254	0.095	Nuclear transport factor 2
Smp_066890	23966.5	2.120	0.095	40S ribosomal protein S10
Smp_055900	8220.4	2.101	0.095	Leptin receptor overlapping transcript
Smp_179650	9467.6	2.008	0.095	Chromobox protein

Smp_066940	10628.5	2.290	0.096	60S ribosomal protein L29
Smp_011570	27137.1	2.138	0.096	Ribosomal protein S4
Smp_092380	6836.2	2.042	0.096	Protein bud31
Smp_046020	10706.6	1.941	0.097	Protein phosphatase 1 regulatory subunit 7
Smp_179300	14257.1	1.915	0.097	Cellular nuclear acid binding protein
Smp_058240	6942.3	1.902	0.098	Reticulon 4 (Neurite outgrowth inhibitor)
Smp_082030	15560.6	2.169	0.098	Uncharacterised protein
Smp_041500	7421.3	2.265	0.099	Mitogen activated protein kinase kinase
Smp_130850	43065.9	2.257	0.099	40S ribosomal protein S15
Smp_008070	27008.4	2.221	0.099	Thioredoxin
Smp_089290	15876.3	2.286	0.100	Alpha galactosidase:alpha n
Smp_132300	32382.5	2.225	0.100	Kinesin protein KIF3A like
Smp_024850	26420.3	2.176	0.100	60S ribosomal protein L17
Smp_038560	20226.0	2.159	0.100	Prothymosin alpha B

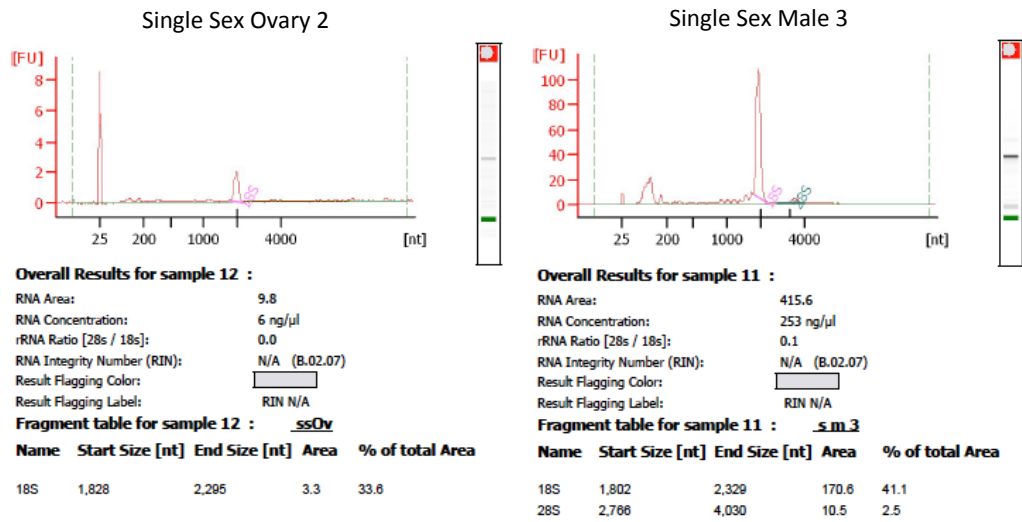
**Appendix B11: All 150 genes found to be differentially expressed between paired and separated females 8 days after perfusion.** Genes are ranked by adjusted p-value, from smallest to largest. Average read count provides a measure of absolute expression. The fold change is expression in separated females, relative to paired females. The *p*-value has been adjusted for multiple hypothesis testing.

Appendix C

Sample name	M1 MS	M2 MS	M3 MS	M1 SS	M2 SS	M3 SS	T1 MS	T2 MS	T3 MS	T1 SS	T2 SS	T3 SS	Average
Total reads	82,157,621	61,413,861	58,786,566	63,319,818	63,888,404	54,258,554	43,373,743	43,840,647	65,099,133	44,595,566	56,767,832	68,875,916	63,005,559
Mapped	71,801,485	51,649,806	51,179,327	53,852,252	53,738,964	47,564,797	38,119,993	32,048,217	48,212,772	38,876,508	49,722,004	56,457,897	53,160,332
Prop. Paired	57,615,006	42,911,100	42,452,604	45,111,588	44,763,160	38,200,040	30,926,682	26,063,698	24,158,538	29,982,900	40,360,354	46,779,174	41,746,213
Singletons	5,011,317	2,509,444	2,580,515	2,701,856	2,691,940	2,435,665	2,511,925	2,199,055	2,332,190	2,376,020	3,168,100	2,740,915	3,165,597
Unmapped	10,356,136	9,764,055	7,607,239	9,467,566	10,149,440	6,693,757	5,253,750	11,792,430	16,886,361	5,719,058	7,045,828	12,418,019	9,845,227
% Unmapped	12.6%	15.9%	12.9%	15.0%	15.9%	12.3%	12.1%	26.9%	25.9%	12.8%	12.4%	18.0%	15.7%
Mapped to feature	53,899,496	39,306,965	38,169,249	39,427,831	39,494,229	34,877,418	31,264,617	28,735,955	28,719,005	27,747,160	42,043,347	42,473,097	40,888,434
% Mapped to feature	65.6%	64.0%	64.9%	62.3%	61.8%	64.3%	72.1%	65.5%	44.1%	62.2%	74.1%	61.7%	64%

Sample name	F1 MS	F2 MS	F3 MS	F1 SS	F2 SS	F3 SS	O1 MS	O2 MS	O3 MS	O1 SS	O2 SS	O3 SS	Average
Total reads	42,049,886	56,720,483	48,461,371	62,724,794	111,348,638	80,824,593	60,987,961	83,483,600	73,599,576	62,449,538	68,107,686	54,997,636	63,005,559
Mapped	36,204,716	45,482,737	39,592,784	54,360,068	94,437,658	61,321,111	54,988,691	74,676,180	66,211,423	53,494,629	58,533,720	43,320,228	53,160,332
Prop. Paired	19,131,978	33,138,056	28,397,394	45,920,862	78,762,590	52,127,848	29,669,282	63,194,578	55,763,652	43,577,282	47,044,524	35,856,214	41,746,213
Singletons	2,683,370	4,739,835	4,281,282	2,718,634	4,987,700	2,868,193	2,960,611	4,419,900	3,971,051	3,214,019	3,714,416	2,156,378	3,165,597
Unmapped	5,845,170	11,237,746	8,868,587	8,364,726	16,910,980	19,503,482	5,999,270	8,807,420	7,388,153	8,954,909	9,573,966	11,677,408	9,845,227
% Unmapped	13.9%	19.8%	18.3%	13.3%	15.2%	24.1%	9.8%	10.5%	10.0%	14.3%	14.1%	21.2%	15.7%
Mapped to feature	23,079,604	33,100,676	28,860,010	41,880,335	74,704,667	50,759,202	33,648,187	63,218,280	56,989,452	43,844,868	49,958,368	35,120,404	40,888,434
% Mapped to feature	54.9%	58.4%	59.6%	66.8%	67.1%	62.8%	55.2%	75.7%	77.4%	70.2%	73.4%	63.9%	64%

**Appendix C.1: Summary of sequencing statistics for sequenced RNA-Seq samples.** Samples are named as follows: Sample type (Males – M; Female – F; Testes – T; Ovaries – O), replicate number (1 to 3) and infection status (Mixed sex infections – MS; Single sex infection – SS). Total reads is the number of all reads sequenced. Mapped is the total number of reads mapped to the *Schistosoma mansoni* reference genome v5.2. ‘Prop. Paired’ refers to the total number of reads mapped together with a mate-paired read in close proximity and in the correct orientation. ‘Singletons’ refers to the total number of reads with a correctly mapped mate but themselves unmapped. Unmapped is the total number of reads that could not be mapped to the reference by Tophat2. Mapped to feature is the total number of reads mapped to exons of annotated genes in the reference, excluding introns as well as 5’- and 3’-UTRs.



**Appendix C.2: Bioanalyzer data shows that ovary and whole male worm samples from single sex infections had good quality total RNA.** RNA was extracted from whole worms and isolated organs and the quality examined as described in Chapter 2.2.3. All samples showed a single, sharp ribosomal RNA peak and no signs of degradation.

Gene ID	Description	Average Gene Count	Fold Change	Adjusted p-value
Smp_173940	Frizzled	636.07	1.08	4.38E-01
Smp_175590	FGFR-A	977.81	0.16	9.34E-70
Smp_157300	FGFR-B	1537.22	0.27	2.23E-47
Smp_093700	PMRC1	1974.06	1.46	4.15E-09
Smp_050520	Notch	286.33	0.45	1.29E-06
Smp_157750	Musashi	799.75	0.73	2.66E-04
Smp_161930	Actin	2205.63	0.12	6.33E-14
Smp_156670	EIF4E	8964.66	0.92	3.27E-01
Smp_069770	NDUFV2	3069.44	1.13	1.33E-01
Smp_000740	PSMD4	7314.60	1.02	7.34E-01
Smp_047200	RPL3	46765.34	2.19	3.09E-32
Smp_091740	TPC2L	1194.72	1.30	8.45E-03
Smp_090120	TUBA	40164.97	0.85	5.61E-03
Smp_023160	ATPF	1261.13	1.08	3.68E-01
Smp_056970	GAPDH	28090.64	1.96	8.25E-45
Smp_003770	HID	21443.85	0.42	1.42E-44
Smp_007630	PABP2	3694.28	2.14	2.76E-36
Smp_002180	BTF3L4	9920.43	2.15	1.14E-54

**Appendix C.3: List of genes examined in Chapter 5.2.2.** Expression data is provided for the comparison of ovaries from females from single sex (SS) infections compared to the ovaries from mixed sex (MS) infections. Average read counts provide a measure of absolute expression for a given gene. The fold changes reflect expression in ovaries from SS females relative to ovaries of MS females. The *p*-value has been adjusted for multiple hypothesis testing.

GO term	Description	Total Genes	DEGs	Expected	topGO
GO:0007155	Cell adhesion	226	128	55.92	2.50E-14
GO:0007156	Homophilic cell adhesion	60	40	14.85	6.90E-12
GO:0007186	G-protein coupled receptor signaling pathway	161	73	39.84	2.50E-09
GO:0006811	Ion transport	389	167	96.25	2.00E-05
GO:0007165	Signal transduction	581	242	143.76	3.90E-05
GO:0006810	Transport	1184	391	292.96	0.00011
GO:0007264	Small GTPase mediated signal transduction	168	65	41.57	0.00012
GO:0006813	Potassium ion transport	77	34	19.05	0.00014
GO:0006817	Phosphate ion transport	19	12	4.7	0.00043
GO:0006897	Endocytosis	52	24	12.87	0.0006
GO:0006865	Amino acid transport	22	13	5.44	0.00061
GO:0006120	Mitochondrial electron transport	9	7	2.23	0.00124
GO:0007229	Integrin-mediated signaling pathway	9	7	2.23	0.00124
GO:0007017	Microtubule-based process	101	24	24.99	0.00196
GO:0008203	Cholesterol metabolic process	18	9	4.45	0.00257



<b>GO:0006814</b>	Sodium ion transport	64	26	15.84	0.00355
<b>GO:0007160</b>	Cell-matrix adhesion	8	6	1.98	0.00397
<b>GO:0050790</b>	Regulation of catalytic activity	60	18	14.85	0.0044
<b>GO:0006629</b>	Lipid metabolic process	241	83	59.63	0.00476
<b>GO:0006508</b>	Proteolysis	318	85	78.68	0.0055
<b>GO:0006493</b>	Protein O-linked glycosylation	12	7	2.97	0.00941
<b>GO:0051216</b>	Cartilage development	5	4	1.24	0.01499
<b>GO:0046165</b>	Alcohol biosynthetic process	13	6	3.22	0.01511
<b>GO:0031032</b>	Actomyosin structure organization	3	3	0.74	0.01512
<b>GO:0045596</b>	Negative regulation of cell differentiation	3	3	0.74	0.01512
<b>GO:0030514</b>	Negative regulation of BMP signaling pathway	3	3	0.74	0.01512
<b>GO:0030301</b>	Cholesterol transport	3	3	0.74	0.01512
<b>GO:0006855</b>	Drug transmembrane transport	3	3	0.74	0.01512
<b>GO:0015904</b>	tetracycline transport	8	5	1.98	0.02604
<b>GO:0007169</b>	Transmembrane receptor protein tyrosine signalling	31	13	7.67	0.02627
<b>GO:0022904</b>	Respiratory electron transport chain	19	12	4.7	0.03574
<b>GO:0006665</b>	Sphingolipid metabolic process	9	6	2.23	0.03606
<b>GO:0007223</b>	Wnt signaling pathway, calcium modulating...	6	4	1.48	0.03618
<b>GO:0035023</b>	Regulation of Rho protein signal transduction	23	10	5.69	0.03787
<b>GO:0042632</b>	Cholesterol homeostasis	4	3	0.99	0.04929
<b>GO:0001503</b>	Ossification	4	3	0.99	0.04929
<b>GO:0006518</b>	Peptide metabolic process	13	5	3.22	0.04931

**Appendix C.4: Expanded list of GO terms enriched amongst genes expressed more strongly in whole male, compared to testes.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in testes; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

Pathway	Description	Total Genes	DEGs	Expected	p-value
smm04142	Lysosome	47	37	14.25	5.41E-12
smm04144	Endocytosis	64	38	19.41	7.10E-07
smm00564	Glycerophospholipid metabolism	35	21	10.61	1.74E-04
smm00500	Starch and sucrose metabolism	14	11	4.25	2.35E-04
smm00010	Glycolysis / Gluconeogenesis	27	17	8.19	3.28E-04
smm00565	Ether lipid metabolism	9	8	2.73	4.37E-04
smm04145	Phagosome	38	21	11.52	7.45E-04
smm00561	Glycerolipid metabolism	17	11	5.16	2.74E-03
smm00520	Amino sugar and nucleotide sugar metabolism	24	14	7.28	2.83E-03
smm04070	Phosphatidylinositol signaling system	32	17	9.70	3.71E-03
smm04512	ECM-receptor interaction	9	7	2.73	4.06E-03
smm04310	Wnt signaling pathway	35	18	10.61	4.37E-03
smm00562	Inositol phosphate metabolism	26	14	7.88	6.81E-03
smm00052	Galactose metabolism	14	9	4.25	7.00E-03
smm04141	Protein processing in endoplasmic reticulum	89	36	26.99	1.04E-02
smm00511	Other glycan degradation	8	6	2.43	1.05E-02
smm04068	FoxO signaling pathway	38	18	11.52	1.11E-02
smm04080	Neuroactive ligand-receptor interaction	15	9	4.55	1.22E-02
smm00330	Arginine and proline metabolism	18	10	5.46	1.57E-02
smm00620	Pyruvate metabolism	21	11	6.37	1.87E-02
smm04340	Hedgehog signaling pathway	9	6	2.73	2.19E-02
smm00020	Citrate cycle (TCA cycle)	25	12	7.58	2.83E-02
smm00340	Histidine metabolism	5	4	1.52	2.93E-02
smm00190	Oxidative phosphorylation	69	26	20.93	4.18E-02
smm04320	Dorso-ventral axis formation	8	5	2.43	4.84E-02

**Appendix C.5: Complete list of KEGG pathways enriched amongst the genes up regulated the wholes males compared to testes.** The column “Total Genes” provides the number of genes in the KEGG pathway. “DEGs” provides the number differentially expressed genes (DEGs) in that pathway; “Expected Genes” provides the number of DEGs in a given pathway that is expected by chance.

Gene ID	Description	Average read count	Fold Change	Adjusted p-value
Smp_202140	Uncharacterised protein	14.69	149.01	2.68E-06
Smp_184400	Uncharacterised protein	34.97	126.87	8.23E-12
Smp_130450	RNA-binding protein bicaudal C	500.41	111.34	1.26E-105
Smp_015190	Uncharacterised protein	10050.53	108.74	1.43E-105
Smp_205500	Uncharacterised protein	10.22	106.35	2.19E-05
Smp_193990	Uncharacterised protein	18.90	101.49	1.49E-07
Smp_138560	Uncharacterised protein	178.30	89.95	3.31E-42
Smp_194950	Putative ELAV-like RNA-binding protein	4441.96	87.64	1.93E-109
Smp_145730	Putative EGF-domain containing protein	575.65	79.13	5.22E-68

<b>Smp_135030</b>	Uncharacterised protein	97.10	78.96	3.48E-24
<b>Smp_154260</b>	Putative glioma pathogenesis-related protein	35.79	78.74	1.72E-13
<b>Smp_125190</b>	Uncharacterised protein	1628.78	77.56	7.55E-110
<b>Smp_166570</b>	Zinc finger protein	229.83	73.38	5.82E-50
<b>Smp_152310</b>	Uncharacterised protein	527.06	68.84	1.19E-92
<b>Smp_202660</b>	Uncharacterised protein	6.07	67.51	2.51E-04
<b>Smp_134110</b>	Voltage-gated hydrogen channel	12.18	67.26	3.98E-06
<b>Smp_185360</b>	Uncharacterised protein	14.76	65.70	8.63E-06
<b>Smp_161840</b>	Uncharacterised protein	5577.64	64.96	7.27E-106
<b>Smp_027290</b>	Uncharacterised protein	1467.26	64.13	2.82E-58
<b>Smp_142240</b>	Uncharacterised protein	266.80	63.42	2.37E-64
<b>Smp_062490</b>	Putative heart- and neural crest derivatives-expressed protein	421.89	60.19	1.14E-95
<b>Smp_182990</b>	Uncharacterised protein	5.84	59.98	4.56E-04
<b>Smp_123970</b>	Uncharacterised protein	103.54	59.58	1.21E-26
<b>Smp_185830</b>	Uncharacterised protein	18.29	59.28	1.07E-06
<b>Smp_205410</b>	Uncharacterised protein	7080.46	59.11	1.59E-94
<b>Smp_128140</b>	Putative ATP-dependant DNA helicase	94.14	57.69	9.08E-31
<b>Smp_203270</b>	Twist-related protein	11.16	57.14	2.55E-05
<b>Smp_168440</b>	Uncharacterised protein	16.07	56.10	4.87E-07
<b>Smp_061400</b>	Uncharacterised protein	20.61	55.82	1.46E-08
<b>Smp_161060</b>	Uncharacterised protein	1305.71	55.33	3.75E-83
<b>Smp_155350</b>	Putative coiled-coil domain containing protein	482.40	54.92	9.27E-69
<b>Smp_131240</b>	Uncharacterised protein	222.27	54.09	2.28E-32
<b>Smp_205470</b>	Uncharacterised protein	28.75	52.86	9.76E-08
<b>Smp_054680</b>	Archipelago-like protein	28.81	52.24	1.55E-11
<b>Smp_203840</b>	Uncharacterised protein	19.99	52.04	1.96E-07
<b>Smp_143940</b>	Cytoplasmic dynein-1 light intermediate chain	66.98	50.90	1.30E-17
<b>Smp_142220</b>	APOBEC1 complementation factor-like RNA-binding protein	1999.21	50.66	1.15E-96
<b>Smp_028130</b>	Uncharacterised protein	425.61	50.49	4.11E-54
<b>Smp_123300</b>	Follistatin	99.62	109.80	4.29E-11

**Appendix C.6: Expanded list of DEGs with testes-biased expression compared to whole males.** Average read counts are a measure of absolute gene expression; they are the average of normalised read counts across all RNA-seq samples used in Chapter 5. The fold changes reflect expression in the testes relative to whole male worms. The *p*-value has been adjusted for multiple hypothesis testing.

GO term	Description	Total Genes	DEGs	Expected	topGO
GO:0007018	Microtubule-based movement	60	36	9.32	3.40E-15
GO:0006941	Striated muscle contraction	145	59	22.52	1.20E-13
GO:0006270	DNA replication initiation	15	13	2.33	2.20E-09
GO:0007283	Spermatogenesis	10	9	1.55	4.40E-07
GO:0006281	DNA repair	122	46	18.95	7.00E-07
GO:0007049	Cell cycle	207	78	32.15	2.40E-06
GO:0007067	Mitotic nuclear division	88	37	13.67	3.50E-06
GO:0006260	DNA replication	161	64	25.01	7.50E-05
GO:0007126	Meiotic nuclear division	8	6	1.24	2.90E-04
GO:0006298	Mismatch repair	11	7	1.71	4.00E-04
GO:0030030	Cell projection organization	9	5	1.4	5.80E-04
GO:0000226	Microtubule cytoskeleton organization	17	7	2.64	1.65E-03
GO:0008285	Negative regulation of cell proliferation	5	4	0.78	2.54E-03
GO:0007064	Mitotic sister chromatid cohesion	3	3	0.47	3.74E-03
GO:0007076	Mitotic chromosome condensation	9	5	1.4	6.52E-03
GO:0006278	RNA-dependent DNA replication	32	11	4.97	6.67E-03
GO:0006183	GTP biosynthetic process	6	4	0.93	6.67E-03
GO:0006269	DNA replication, synthesis of RNA primer	6	4	0.93	6.67E-03
GO:0006228	UTP biosynthetic process	6	4	0.93	6.67E-03
GO:0006241	CTP biosynthetic process	6	4	0.93	6.67E-03
GO:0006486	Protein glycosylation	58	15	9.01	8.97E-03
GO:0007601	Visual perception	10	5	1.55	0.011
GO:0006310	DNA recombination	39	12	6.06	0.012
GO:0009809	Lignin biosynthetic process	4	3	0.62	0.013
GO:0006268	DNA unwinding involved in DNA replication	11	5	1.71	0.018
GO:0042026	Protein refolding	8	4	1.24	0.024
GO:0042592	Homeostatic process	58	9	9.01	0.024
GO:0016568	Chromatin modification	111	24	17.24	0.028
GO:0007059	Chromosome segregation	18	12	2.8	0.029
GO:0006955	Immune response	11	5	1.71	0.029
GO:0018298	Protein-chromophore linkage	9	4	1.4	0.038
GO:0006261	DNA-dependent DNA replication	42	24	6.52	0.050

**Appendix C.7: Expanded list of GO terms enriched amongst the testes genes.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in testes; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

Pathway	Description	Total Genes	DEGs	Expected	p-value
smm03030	DNA replication	30	24	7.00	5.80E-11
smm03430	Mismatch repair	18	16	4.20	5.93E-09
smm03440	Homologous recombination	18	15	4.20	1.07E-07
smm03010	Ribosome	110	47	25.68	1.88E-06
smm03420	Nucleotide excision repair	32	19	7.47	9.28E-06
smm03460	Fanconi anemia pathway	27	17	6.30	9.39E-06
smm03410	Base excision repair	20	12	4.67	3.71E-04
smm00230	Purine metabolism	82	30	19.14	2.15E-03
smm00240	Pyrimidine metabolism	59	23	13.77	2.70E-03
smm03008	Ribosome biogenesis in eukaryotes	62	22	14.47	9.86E-03
smm03040	Spliceosome	105	33	24.51	0.013
smm00970	Aminoacyl-tRNA biosynthesis	34	13	7.94	0.021

**Appendix C.8: Complete list of KEGG pathways enriched amongst the testes expressed genes.** The column “Total Genes” provides the number of genes in the KEGG pathway. “DEGs” provides the number differentially expressed genes in that pathway; “Expected Genes” provides the number of differentially expressed genes in a given pathway that is expected by chance.

Domain	Description	Total Genes	DEGs	Expected	p-value
PF00271	Helicase conserved C-terminal domain	75	38	17.68	2.03E-07
PF00493	MCM2/3/5 family	10	10	2.36	5.20E-07
PF00270	DEAD/DEAH box helicase	55	27	12.97	2.19E-05
PF00225	Kinesin motor domain	19	13	4.48	3.67E-05
PF08393	Dynein heavy chain, N-terminal region 2	13	10	3.06	6.67E-05
PF05186	Dpy-30 motif	6	6	1.41	1.71E-04
PF14551	MCM N-terminal domain	6	6	1.41	1.71E-04
PF13414	TPR repeat	40	20	9.43	1.73E-04
PF00852	Glycosyltransferase family 10 (fucosyltransferase)	12	9	2.83	2.18E-04
PF03028	Dynein heavy chain and region D6 of dynein motor	12	9	2.83	2.18E-04
PF12774	Hydrolytic ATP binding site of dynein motor region D1	12	9	2.83	2.18E-04
PF12775	P-loop containing dynein motor region D3	12	9	2.83	2.18E-04
PF12777	Microtubule-binding stalk of dynein motor	12	9	2.83	2.18E-04
PF12780	P-loop containing dynein motor region D4	12	9	2.83	2.18E-04
PF12781	ATP-binding dynein motor region D5	12	9	2.83	2.18E-04
PF00533	BRCA1 C Terminus (BRCT) domain	9	7	2.12	8.45E-04
PF00929	Exonuclease	7	6	1.65	9.14E-04
PF00077	Retroviral aspartyl protease	14	9	3.30	1.16E-03
PF12799	Leucine Rich repeats (2 copies)	20	11	4.72	1.85E-03
PF00400	WD domain, G-beta repeat	141	47	33.24	2.15E-03
PF07728	AAA domain (dynein-related subfamily)	10	7	2.36	2.15E-03
PF02493	MORN repeat	8	6	1.89	2.79E-03
PF00488	MutS domain V	4	4	0.94	3.08E-03
PF06220	U1 zinc finger	4	4	0.94	3.08E-03
PF08542	Replication factor C C-terminal domain	4	4	0.94	3.08E-03
PF00612	IQ calmodulin-binding motif	9	6	2.12	6.41E-03
PF03133	Tubulin-tyrosine ligase family	9	6	2.12	6.41E-03
PF03148	Tektin family	7	5	1.65	8.91E-03
PF00078	Reverse transcriptase (RNA-dependent DNA polymerase)	24	11	5.66	9.40E-03
PF00334	Nucleoside diphosphate kinase	5	4	1.18	0.012
PF01798	Putative snoRNA binding domain	5	4	1.18	0.012
PF02463	RecF/RecN/SMC N terminal domain	5	4	1.18	0.012
PF13181	Tetratricopeptide repeat	5	4	1.18	0.012
PF00385	Chromo (CHRromatin Organisation MODifier) domain	10	6	2.36	0.012

**Appendix C.9: Expanded list of Pfam domains enriched amongst the testes expressed genes.** The column “Total Genes” provides the number of genes with a given Pfam domain. “DEGs” provides the number of differentially expressed genes with that domain; “Expected” provides the number of differentially expressed genes encoding a given domain that is expected by chance.

Pathway	Description	Total Genes	DEGs	Expected	p-value
smm04142	Lysosome	47	39	13.59	9.79E-15
smm04141	Protein processing in endoplasmic reticulum	89	48	25.74	2.65E-07
smm04145	Phagosome	38	26	10.99	3.48E-07
smm04144	Endocytosis	64	35	18.51	7.26E-06
smm00520	Amino sugar and nucleotide sugar metabolism	24	17	6.94	1.98E-05
smm00510	N-Glycan biosynthesis	30	19	8.68	6.65E-05
smm00052	Galactose metabolism	14	11	4.05	1.48E-04
smm00500	Starch and sucrose metabolism	14	11	4.05	1.48E-04
smm00010	Glycolysis / Gluconeogenesis	27	16	7.81	6.85E-04
smm00511	Other glycan degradation	8	7	2.31	9.43E-04
smm00600	Sphingolipid metabolism	15	10	4.34	2.17E-03
smm04512	ECM-receptor interaction	9	7	2.60	3.03E-03
smm00051	Fructose and mannose metabolism	14	9	4.05	5.03E-03
smm04130	SNARE interactions in vesicular transport	14	9	4.05	5.03E-03
smm04320	Dorso-ventral axis formation	8	6	2.31	8.18E-03
smm00562	Inositol phosphate metabolism	26	13	7.52	1.19E-02
smm00564	Glycerophospholipid metabolism	35	16	10.12	1.44E-02
smm03060	Protein export	19	10	5.50	1.72E-02
smm04340	Hedgehog signaling pathway	9	6	2.60	1.75E-02
smm00250	Alanine, aspartate and glutamate metabolism	14	8	4.05	1.87E-02
smm04140	Regulation of autophagy	14	8	4.05	1.87E-02
smm00604	Glycosphingolipid biosynthesis - ganglio series	3	3	0.87	2.41E-02
smm00740	Riboflavin metabolism	5	4	1.45	2.47E-02
smm04070	Phosphatidylinositol signaling system	32	14	9.25	2.85E-02
smm04080	Neuroactive ligand-receptor interaction	15	8	4.34	2.86E-02
smm04068	FoxO signaling pathway	38	16	10.99	2.87E-02
smm00020	Citrate cycle (TCA cycle)	25	11	7.23	4.40E-02

**Appendix C.10: Complete list of KEGG pathways enriched amongst the genes up-regulated in whole females compared to ovaries from mixed sex infections.** The column “Total Genes” provides the number of genes in the KEGG pathway. “DEGs” provides the number differentially expressed genes in that pathway; “Expected Genes” provides the number of differentially expressed in a given pathway that is expected by chance.

Gene ID	Description	Average Gene Count	Fold Change	Adjusted p-value
Smp_054430	Scratch-family zinc finger protein	37.84	46.88	1.66E-10
Smp_136900	Homeobox protein DLX	9.77	36.34	5.04E-05
Smp_198590	Kinesin-like protein	54.56	30.95	4.24E-14
Smp_032970	Calmodium-like protein	15.05	29.95	3.45E-06
Smp_126070	Uncharacterised protein	45.96	28.29	1.16E-12
Smp_204040	Uncharacterised protein	14.31	28.25	4.59E-06
Smp_082410	Uncharacterised protein	64.87	27.36	1.36E-14
Smp_134490	Thyroid hormone receptor	265.72	26.97	4.95E-48
Smp_143820	Uncharacterised protein	6.90	26.82	3.24E-04
Smp_154860	Zinc finger CW-type protein	358.40	26.30	3.17E-42
Smp_196000	Aquaporin	20.93	26.14	2.11E-07
Smp_147880	Rootletin	41.89	24.34	2.60E-11
Smp_078590	Uncharacterised protein	5.91	22.28	9.25E-04
Smp_032990	Calmodulin-like protein	24.19	21.25	6.62E-07
Smp_145230	T-box transcription factor	108.28	19.67	1.41E-14
Smp_145570	Uncharacterised protein	418.42	19.32	9.13E-60
Smp_036660	Uncharacterised protein	2385.46	18.72	1.29E-76
Smp_193990	Uncharacterised protein	5.18	18.41	2.47E-03
Smp_043430	Uncharacterised protein	788.64	17.21	6.06E-89
Smp_160590	Uncharacterised protein	2797.25	16.88	1.09E-100
Smp_203840	Uncharacterised protein	5.71	16.71	4.25E-03
Smp_161050	Uncharacterised protein	187.69	16.44	2.26E-41
Smp_152490	E3 ubiquitin-protein ligase	301.44	16.37	3.48E-58
Smp_165230	Location of vulva defective-like protein	65.10	16.10	1.78E-18
Smp_144860	Boule-like protein	1998.21	16.06	1.34E-147
Smp_145280	NACHT and WD repeat domain-containing protein	223.85	15.80	1.71E-42
Smp_172150	Putative ATP-dependant RNA helicase	975.82	15.78	1.29E-136
Smp_170960	Uncharacterised protein	709.57	15.77	2.35E-117
Smp_141570	TWIK family potassium channel	192.15	15.34	6.45E-44
Smp_145580	Uncharacterised protein	1351.25	15.31	6.99E-58
Smp_131630	Testes expressed protein-like serine/threonine-protein kinase	1524.23	15.04	7.49E-162
Smp_132660	Rho guanine nucleotide exchange factor (RhoGEF)	2296.39	14.95	1.42E-79
Smp_165430	Sperm-tail PG-rich repeat-containing protein	81.11	14.82	2.12E-21
Smp_185360	Uncharacterised protein	3.88	14.69	6.27E-03
Smp_162740	Meiosis-specific with OB domain-containing protein	190.11	14.44	2.21E-24
Smp_129750	$\alpha$ -(1,3)-fucosyltransferase	3.70	14.35	6.92E-03

**Appendix C.11: Expanded list of differentially expressed ovary-genes compared to whole females from mixed sex infections.** Average read counts provide a measure of absolute expression across all RNA-seq samples used in Chapter 5 for a given gene. The fold changes reflect expression in ovaries relative to whole females. The *p*-value has been adjusted for multiple hypothesis testing.



GO term	Description	Total Genes	DEGs	Expected	p-value
GO:0006412	Translation	393	83	41.42	2.00E-09
GO:0042254	Ribosome biogenesis	137	31	14.44	5.90E-06
GO:0006414	Translational elongation	27	10	2.85	4.60E-04
GO:0006839	Mitochondrial transport	16	7	1.69	1.11E-03
GO:0016246	RNA interference	3	3	0.32	1.17E-03
GO:0006465	Signal peptide processing	4	3	0.42	4.29E-03
GO:0007160	Cell-matrix adhesion	8	4	0.84	6.04E-03
GO:0006446	Regulation of translational initiation	5	3	0.53	9.89E-03
GO:0007229	Integrin-mediated signaling pathway	9	4	0.95	9.98E-03
GO:0006836	Neurotransmitter transport	30	8	3.16	0.010
GO:0005975	carbohydrate metabolic process	246	30	25.92	0.011
GO:0006875	Cellular metal ion homeostasis	10	3	1.05	0.011
GO:0006413	Translational initiation	37	11	3.9	0.015
GO:0006401	RNA catabolic process	59	9	6.22	0.018
GO:0019321	Pentose metabolic process	6	3	0.63	0.018
GO:0006364	rRNA processing	108	19	11.38	0.023
GO:0008033	tRNA processing	61	10	6.43	0.026
GO:0006098	Pentose-phosphate shunt	7	3	0.74	0.029
GO:0006637	Acyl-CoA metabolic process	3	2	0.32	0.031
GO:0006855	Drug transmembrane transport	3	2	0.32	0.031
GO:0008612	Peptidyl-lysine modification to peptidyl-hypusine	3	2	0.32	0.031
GO:0006633	Fatty acid biosynthetic process	51	10	5.37	0.037
GO:0042026	Protein refolding	8	3	0.84	0.044
GO:0006821	Chloride transport	8	3	0.84	0.044
GO:0006534	Cysteine metabolic process	8	3	0.84	0.044

**Appendix C.12: Expanded list of GO terms enriched amongst the genes up-regulated in ovaries of females from mixed sex infections compared to whole females.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in ovaries of females from mixed sex infections; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

Pathway	Description	Total Genes	DEGs	Expected	p-value
smm03008	Ribosome biogenesis in eukaryotes	62	48	20.60	5.08E-13
smm03030	DNA replication	30	25	9.97	1.65E-08
smm03013	RNA transport	103	60	34.22	5.56E-08
smm03010	Ribosome	110	62	36.55	1.71E-07
smm03460	Fanconi anemia pathway	27	21	8.97	2.04E-06
smm03430	Mismatch repair	18	15	5.98	1.50E-05
smm00970	Aminoacyl-tRNA biosynthesis	34	23	11.30	2.92E-05
smm03420	Nucleotide excision repair	32	22	10.63	2.99E-05
smm03040	Spliceosome	105	53	34.89	7.50E-05
smm00240	Pyrimidine metabolism	59	33	19.60	1.49E-04
smm03440	Homologous recombination	18	13	5.98	6.55E-04
smm03020	RNA polymerase	24	15	7.97	2.20E-03
smm03018	RNA degradation	38	21	12.63	2.52E-03
smm03410	Base excision repair	20	12	6.65	8.81E-03
smm00230	Purine metabolism	82	34	27.25	0.026
smm03450	Non-homologous end-joining	5	4	1.66	0.041

**Appendix C.13: Complete list of KEGG pathways enriched amongst the genes upregulated in the ovaries of females from mixed sex infections compared to whole females.** The column “Total Genes” provides the number of genes in the KEGG pathway. “DEGs” provides the number differentially expressed genes in that pathway; “Expected Genes” provides the number of differentially expressed genes in a given pathway that is expected by chance.

Domain	Description	Total Genes	DEGs	Expected	p-value
PF00271	Helicase conserved C-terminal domain	75	58	22.29	1.36E-17
PF00270	DEAD/DEAH box helicase	55	43	16.35	1.13E-13
PF00493	MCM2/3/5 family	10	10	2.97	5.31E-06
PF00533	BRCA1 C Terminus (BRCT) domain	9	9	2.68	1.79E-05
PF00176	SNF2 family N-terminal domain	19	14	5.65	8.21E-05
PF00400	WD domain, G-beta repeat	141	62	41.91	1.02E-04
PF00856	SET domain	19	13	5.65	4.55E-04
PF00481	Protein phosphatase 2C	6	6	1.78	6.87E-04
PF14551	MCM N-terminal domain	6	6	1.78	6.87E-04
PF00852	Glycosyltransferase family 10 (fucosyltransferase)	12	9	3.57	1.37E-03
PF00679	Elongation factor G C-terminus	5	5	1.49	2.31E-03
PF00752	XPG N-terminal domain	5	5	1.49	2.31E-03
PF02463	RecF/RecN/SMC N terminal domain	5	5	1.49	2.31E-03
PF08389	Exportin 1-like protein	5	5	1.49	2.31E-03
PF13589	Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase	5	5	1.49	2.31E-03
PF00929	Exonuclease	7	6	2.08	3.38E-03
PF01926	50S ribosome-binding GTPase	11	8	3.27	3.47E-03

<b>PF03144</b>	Elongation factor Tu domain 2	11	8	3.27	3.47E-03
<b>PF00076</b>	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	84	35	24.97	5.80E-03
<b>PF14259</b>	RNA recognition motif (a.k.a. RRM, RBD, or RNP domain)	29	15	8.62	6.87E-03
<b>PF00136</b>	DNA polymerase family B	4	4	1.19	7.79E-03
<b>PF00383</b>	Cytidine and deoxycytidylate deaminase zinc-binding region	4	4	1.19	7.79E-03
<b>PF00488</b>	MutS domain V	4	4	1.19	7.79E-03
<b>PF00867</b>	XPG I-region	4	4	1.19	7.79E-03
<b>PF02984</b>	Cyclin, C-terminal domain	4	4	1.19	7.79E-03
<b>PF03104</b>	DNA polymerase family B, exonuclease domain	4	4	1.19	7.79E-03
<b>PF06470</b>	SMC proteins Flexible Hinge Domain	4	4	1.19	7.79E-03
<b>PF08542</b>	Replication factor C C-terminal domain	4	4	1.19	7.79E-03
<b>PF13959</b>	Domain of unknown function (DUF4217)	4	4	1.19	7.79E-03
<b>PF01336</b>	OB-fold nucleic acid binding domain	8	6	2.38	9.51E-03
<b>PF01398</b>	JAB1/Mov34/MPN/PAD-1 ubiquitin protease	8	6	2.38	9.51E-03
<b>PF00133</b>	tRNA synthetases class I (I, L, M and V)	6	5	1.78	9.76E-03
<b>PF05383</b>	La domain	6	5	1.78	9.76E-03
<b>PF08264</b>	Anticodon-binding domain of tRNA	6	5	1.78	9.76E-03

**Appendix C.14: Expanded list of Pfam domains enriched amongst the genes up-regulated in the ovaries of females from mixed sex infections compared to whole females.** The column “Total Genes” provides the number of genes encoding a given Pfam domain. “DEGs” provides the number of differentially expressed genes with that domain; “Expected” provides the number of differentially expressed genes with a given domain that is expected by chance.

GO ID	Term	Annotated	Significant	Expected	p-value
GO:0007018	Microtubule-based movement	60	24	5.52	1.20E-10
GO:0006941	Striated muscle contraction	145	32	13.33	1.80E-06
GO:0007283	Spermatogenesis	10	7	0.92	5.00E-06
GO:0030030	Cell projection organization	9	5	0.83	7.00E-05
GO:0006183	GTP biosynthetic process	6	4	0.55	9.10E-04
GO:0006228	UTP biosynthetic process	6	4	0.55	9.10E-04
GO:0006241	CTP biosynthetic process	6	4	0.55	9.10E-04
GO:0000226	Microtubule cytoskeleton organization	17	6	1.56	1.09E-03
GO:0009809	Lignin biosynthetic process	4	3	0.37	2.88E-03
GO:0006955	Immune response	11	4	1.01	6.69E-03
GO:0007275	Multicellular organismal development	286	37	26.29	8.21E-03
GO:0007602	Phototransduction	6	3	0.55	1.25E-02
GO:0006278	RNA-dependent DNA replication	32	7	2.94	2.35E-02
GO:0042026	Protein refolding	8	3	0.74	3.04E-02
GO:0006334	Nucleosome assembly	34	7	3.13	3.21E-02
GO:0015074	DNA integration	28	6	2.57	3.85E-02
GO:0018298	Protein-chromophore linkage	9	3	0.83	4.26E-02
GO:0006108	Malate metabolic process	4	2	0.37	4.46E-02
GO:0009073	Aromatic amino acid family biosynthetic ...	4	2	0.37	4.46E-02
GO:0006563	L-serine metabolic process	4	2	0.37	4.46E-02

**Appendix C.15: Expanded list of GO terms enriched amongst the genes up-regulated in testes compared to ovaries.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in testes compared to ovaries; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

GO ID	Term	Annotated	Significant	Expected	p-value
GO:0006364	rRNA processing	108	18	6.14	3.10E-05
GO:0042254	ribosome biogenesis	137	26	7.79	1.10E-04
GO:0009086	methionine biosynthetic process	5	3	0.28	1.67E-03
GO:0006468	protein phosphorylation	337	30	19.16	8.79E-03
GO:0001682	tRNA 5'-leader removal	3	2	0.17	9.30E-03
GO:0006996	organelle organization	328	32	18.65	1.07E-02
GO:0007420	brain development	4	2	0.23	1.79E-02
GO:0006338	chromatin remodeling	4	2	0.23	1.79E-02
GO:0006333	chromatin assembly or disassembly	63	7	3.58	2.21E-02
GO:0007275	multicellular organismal development	286	26	16.26	3.23E-02
GO:0007219	Notch signaling pathway	22	4	1.25	3.33E-02
GO:0051028	mRNA transport	84	10	4.78	3.37E-02
GO:0007050	cell cycle arrest	6	2	0.34	4.15E-02
GO:0016485	protein processing	15	3	0.85	4.98E-02

**Appendix C.16: Expanded list of GO terms enriched in genes up-regulated in ovaries compared to testes.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in ovaries compared to the testes; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

Gene ID	Description	Average Gene Count	Fold Change	Adjusted p-value
Smp_075370	Uncharacterised protein	2546.97	281.45	3.20E-161
Smp_123830	Collagen alpha (xi) chain	12391.26	247.02	1.23E-204
Smp_214190	Calpain	7468.55	223.17	1.28E-194
Smp_161790	Uncharacterised protein	2177.89	170.65	8.69E-168
Smp_130280	Neurogenic locus notch protein	2542.52	165.81	2.54E-157
Smp_084090	Uncharacterised protein	2213.81	158.02	1.07E-155
Smp_018250	Troponin i	12126.92	129.72	2.78E-215
Smp_045200	Tegument-allergen-like protein	7170.98	107.84	6.62E-286
Smp_144910	Collagen type I:II:III:V:XI alpha	9835.41	105.69	2.09E-187
Smp_006860	PDZ and LIM domain protein Zasp	3301.43	72.02	1.59E-193
Smp_074560	Uncharacterised protein	2562.52	70.69	9.58E-160
Smp_144280	Tensin	3376.96	67.30	4.93E-190
Smp_156960	Nardilysin (M16 family)	4146.65	66.18	1.13E-187
Smp_020070	Uncharacterised protein	6841.37	65.49	4.86E-219
Smp_045550	Annexin	3514.23	49.80	5.55E-182
Smp_196250	Supervillin	4252.86	47.39	2.39E-194
Smp_179810	Oncosphere protein tso22e	17872.05	44.39	0
Smp_085540	Myosin heavy chain, putative	77964.61	43.45	1.65E-185
Smp_086330	Myophilin	5101.28	37.76	2.37E-184
Smp_171780	SPARC protein	2760.33	31.76	7.19E-189
Smp_049270	Major egg antigen (p40)	6759.56	31.12	2.09E-227
Smp_037230	Fimbrin	7826.62	24.88	2.47E-215
Smp_087250	Muscle LIM protein	2504.41	24.05	9.54E-158
Smp_044010	Tropomyosin	17835.33	17.14	2.26E-159
Smp_123300	Follistatin	99.62	9.67	4.29E-11

**Appendix C.17: Genes up-regulated in whole males from mixed sex infections compared to testes.** Average read counts are a measure of absolute expression across all samples, and fold changes provided a measure of gene expression in whole males relative to testes. The *p*-value has been adjusted for multiple hypothesis testing.

Comparison	Fold Change	Adjusted p-value
Expression in ovaries from single sex infections compared to ovaries from mixed sex infections	9.07	8.58E-12
Expression in females from single sex infections compared to females from mixed sex infections	12.34	9.46E-31

**Appendix C.18: Expression of the gene encoding follistatin (Smp\_122300) in female worms and their ovaries.** The first column specifies the samples in which expression of Smp\_122300 was compared. The fold change provides a measure of gene expression in the first sample compared to the second. Smp\_122300 expression was up regulated in the single sex sample in both cases. The *p*-value has been adjusted for multiple hypothesis testing.

GO term	Description	Total Genes	DEGs	Expected	p-value
GO:0006281	DNA repair	122	45	14.67	2.50E-10
GO:0006270	DNA replication initiation	15	12	1.8	2.70E-09
GO:0007049	Cell cycle	207	71	24.89	8.70E-08
GO:0006260	DNA replication	161	57	19.36	1.50E-07
GO:0007067	Mitotic nuclear division	88	33	10.58	1.30E-06
GO:0007126	Meiotic nuclear division	8	6	0.96	6.70E-05
GO:0006941	Striated muscle contraction	145	34	17.44	7.50E-05
GO:0006364	rRNA processing	108	26	12.99	3.30E-04
GO:0006298	Mismatch repair	11	6	1.32	8.00E-04
GO:0007059	Chromosome segregation	18	12	2.16	9.00E-04
GO:0009086	Methionine biosynthetic process	5	4	0.6	9.40E-04
GO:0042254	Ribosome biogenesis	137	36	16.48	1.19E-03
GO:0051276	Chromosome organization	168	39	20.2	1.61E-03
GO:0007064	Mitotic sister chromatid cohesion	3	3	0.36	1.73E-03
GO:0007018	Microtubule-based movement	60	15	7.22	4.00E-03
GO:0006268	DNA unwinding involved in DNA replication	11	5	1.32	6.15E-03
GO:0008285	Negative regulation of cell proliferation	5	3	0.6	0.014
GO:0006547	Histidine metabolic process	6	3	0.72	0.014
GO:0000184	Nuclear-transcribed mRNA catabolic process	39	10	4.69	0.015
GO:0006310	DNA recombination	39	10	4.69	0.015
GO:0042592	Homeostatic process	58	5	6.98	0.015
GO:0016568	Chromatin modification	111	23	13.35	0.015
GO:0007076	Mitotic chromosome condensation	9	4	1.08	0.016
GO:0006333	Chromatin assembly or disassembly	63	12	7.58	0.018
GO:0006261	DNA-dependent DNA replication	42	22	5.05	0.018
GO:0006269	DNA replication, synthesis of RNA primer	6	3	0.72	0.026
GO:0030212	Hyaluronan metabolic process	6	3	0.72	0.026
GO:0006435	Threonyl-tRNA aminoacylation	6	3	0.72	0.026
GO:0008283	Cell proliferation	11	6	1.32	0.040
GO:0031570	DNA integrity checkpoint	3	2	0.36	0.040
GO:0001682	tRNA 5'-leader removal	3	2	0.36	0.040
GO:0006529	Asparagine biosynthetic process	3	2	0.36	0.040
GO:0006486	Protein glycosylation	58	11	6.98	0.043

**Appendix C.19: Expanded list of GO terms enriched amongst the gene up regulated in the ovaries of females from mixed sex infections compared to those from single sex infections.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in ovaries; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

Gene ID	Description
<b>Smp_130980</b>	Cyclin k
<b>Smp_163380</b>	Cyclin dependent kinase 2
<b>Smp_076560</b>	Cyclin t1
<b>Smp_124130</b>	Cyclin dependent kinase 1
<b>Smp_073340</b>	Cyclin dependent kinase 5
<b>Smp_152760</b>	Cyclin protein FAM58A
<b>Smp_137370</b>	Cyclin dependent kinase 16
<b>Smp_090980</b>	Cyclin dependent kinase 20
<b>Smp_172050</b>	Cyclin y
<b>Smp_103950</b>	Cyclin dependent kinase 10
<b>Smp_172700</b>	Cyclin dependent kinase 6
<b>Smp_127600</b>	Cyclin y
<b>Smp_176620</b>	Cyclin dependent kinase 11
<b>Smp_129360</b>	Cyclin G2
<b>Smp_150040</b>	Cyclin dependent kinase 7
<b>Smp_153920</b>	Cyclin G2
<b>Smp_003000</b>	Cyclin dependent kinase 9
<b>Smp_041820</b>	Cyclin dependent kinase 16
<b>Smp_102650</b>	Cyclin dependent kinase
<b>Smp_157240</b>	Cyclin dependent kinase 1
<b>Smp_156990</b>	Cyclin dependent kinase 19
<b>Smp_080730</b>	Cyclin dependent kinase 1
<b>Smp_094760</b>	Cyclin dependent kinase 9
<b>Smp_047190</b>	Cyclin g associated kinase
<b>Smp_039670</b>	Cyclin L1
<b>Smp_212930</b>	Cyclin h
<b>Smp_155330</b>	Cyclin dependent kinase 14
<b>Smp_167410</b>	Cell division cycle protein 123
<b>Smp_004060</b>	Cell division cycle 20
<b>Smp_134020</b>	Cell division cycle protein 27
<b>Smp_070690</b>	Cell division cycle 16
<b>Smp_208070</b>	CDC16 cell division cycle 16
<b>Smp_007510</b>	Cell division cycle 2 protein kinase 5
<b>Smp_177120</b>	Cell division cycle 7 protein
<b>Smp_129320</b>	Cell division cycle 5 protein
<b>Smp_193440</b>	Cell division cycle and apoptosis regulator
<b>Smp_132020</b>	Cell division cycle 20 protein 1 cofactor
<b>Smp_037400</b>	Cell division cycle protein 23

## Appendix C.20: Description of cyclins other cell cycle-related genes.



Gene ID	Description
Smp_161920	Actin
Smp_175590	FGFR-A
Smp_157300	FGFR-B
Smp_142050	ERK1
Smp_144590	Ras-GAP
Smp_104110	Rho
Smp_137610	FAK
Smp_161230	Ras-GEF/Sos
Smp_035190	Ras-GEF/Sos
Smp_125360	Csk
Smp_003230	Grb2
Smp_068720	Diaphanous
Smp_176990	Raf
Smp_199010	Ras-GEF/Sos
Smp_046600	Actin
Smp_177870	Ras-GEF/Sos
Smp_183710	Actin
Smp_080120	TbRII
Smp_073470	Nuclear receptor
Smp_153500	Tyrosine Kinase
Smp_062950	Growth factor receptor-bound protein
Smp_179910	Ras
Smp_173940	Frizzled
Smp_072660	Ras-GAP
Smp_144390	Type IIb activin receptor (SmRK2)
Smp_164680	Ras-GAP
Smp_047900	ERK2
Smp_009760	14-3-3
Smp_093700	PMRC1
Smp_136300	TK5
Smp_085910	Smad 2
Smp_097730	SRF
Smp_019790	SmRTK1
Smp_049760	TbRI
Smp_079230	SmFKBP12
Smp_168370	Vav2
Smp_033950	Smad 4
Smp_041500	MEK
Smp_196040	Shc
Smp_169020	Ras-GAP
Smp_146810	Diaphanous 2

#### Appendix C.21: Descriptions of genes of the MAPK signalling pathway.

Pathway	Description	Total Genes	DEGs	Expected	p-value
<b>smm03010</b>	Ribosome	110	91	55.90	7.52E-13
<b>smm03008</b>	Ribosome biogenesis in eukaryotes	62	57	31.51	1.80E-12
<b>smm03050</b>	Proteasome	32	29	16.26	1.52E-06
<b>smm03013</b>	RNA transport	103	73	52.34	1.12E-05
<b>smm00190</b>	Oxidative phosphorylation	69	52	35.06	1.38E-05
<b>smm03020</b>	RNA polymerase	24	20	12.20	7.79E-04
<b>smm03060</b>	Protein export	19	15	9.66	8.63E-03
<b>smm00970</b>	Aminoacyl-tRNA biosynthesis	34	23	17.28	0.020
<b>smm04141</b>	Protein processing in endoplasmic reticulum	89	51	45.23	0.040
<b>smm00240</b>	Pyrimidine metabolism	59	35	29.98	0.044

**Appendix C.22: Complete list of KEGG pathways enriched amongst the genes up-regulated in ovaries of females mixed sex infections compared to those of single sex infections.** The column “Total Genes” provides the number of genes in the KEGG pathway. “DEGs” provides the number differentially expressed genes in that pathway; “Expected Genes” provides the number of differentially expressed genes in a given pathway that is expected by chance

Domain	Description	Total Genes	DEGs	Expected	p-value
PF00227	Proteasome subunit	14	14	4.62	1.781E-07
PF00400	WD domain, G-beta repeat	141	72	46.55	3.273E-06
PF00270	DEAD/DEAH box helicase	55	33	18.16	2.375E-05
PF03357	Snf7	9	9	2.97	4.612E-05
PF00179	Ubiquitin-conjugating enzyme	26	18	8.58	1.341E-04
PF01398	JAB1/Mov34/MPN/PAD-1 ubiquitin protease	8	8	2.64	1.400E-04
PF14259	RNA recognition motif (a.k.a. RRM, RBD, or RNP domain)	29	19	9.57	2.547E-04
PF10584	Proteasome subunit A N-terminal signature	7	7	2.31	4.249E-04
PF01399	PCI domain	14	11	4.62	5.494E-04
PF03144	Elongation factor Tu domain 2	11	9	3.63	1.141E-03
PF00009	Elongation factor Tu GTP binding domain	17	12	5.61	1.384E-03
PF00153	Mitochondrial carrier protein	26	16	8.58	1.896E-03
PF00085	Thioredoxin	16	11	5.28	2.966E-03
PF00467	KOW motif	5	5	1.65	3.910E-03
PF00679	Elongation factor G C-terminus	5	5	1.65	3.910E-03
PF05193	Peptidase M16 inactive domain	5	5	1.65	3.910E-03
PF08389	Exportin 1-like protein	5	5	1.65	3.910E-03
PF00271	Helicase conserved C-terminal domain	75	35	24.76	4.477E-03
PF00753	Metallo-beta-lactamase superfamily	7	6	2.31	6.050E-03
PF13893	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	13	9	4.29	6.669E-03
PF00118	TCP-1/cpn60 chaperonin family	11	8	3.63	6.965E-03
PF01926	50S ribosome-binding GTPase	11	8	3.63	6.965E-03
PF00076	RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	84	37	27.73	9.403E-03
PF00137	ATP synthase subunit C	4	4	1.32	0.012
PF00383	Cytidine and deoxycytidylate deaminase zinc-binding region	4	4	1.32	0.012
PF00579	tRNA synthetases class I (W and Y)	4	4	1.32	0.012
PF00613	Phosphoinositide 3-kinase family, accessory domain (PIK domain)	4	4	1.32	0.012
PF01237	Oxysterol-binding protein	4	4	1.32	0.012
PF03129	Anticodon binding domain	4	4	1.32	0.012
PF01423	LSM domain	14	9	4.62	0.013
PF00443	Ubiquitin carboxyl-terminal hydrolase	21	12	6.93	0.013
PF00133	tRNA synthetases class I (I, L, M and V)	6	5	1.98	0.016
PF08264	Anticodon-binding domain of tRNA	6	5	1.98	0.016
PF00675	Insulinase (Peptidase family M16)	8	6	2.64	0.016

**Appendix C.23: Expanded list of Pfam domains enriched amongst the genes up-regulated in ovaries of females from mixed sex infections compared to those of single sex infections.** The column “Total Genes” provides the number of genes encoding a given Pfam domain. “DEGs” provides the number of differentially expressed genes with that domain; “Expected” provides the number of differentially expressed genes with a given domain that is expected by chance.

Gene ID	Description	Fold Change	Adjusted p-value
Smp_210790	Transmembrane BAX inhibitor motif containing	1.18	1.05E-01
Smp_136730	Cathepsin D	1.66	2.73E-02
Smp_179800	p21 activated protein kinase 1 Dpak1	2.01	1.09E-13
Smp_043360	sBH3-1	4.49	3.77E-04
Smp_207000	Anamorsin homologue	1.84	4.99E-08
Smp_028500	Caspase 3	1.35	2.69E-02
Smp_197180	Apoptosis inhibitor 5	1.51	2.51E-09
Smp_022110	p21 activated protein kinase interacting protein	1.95	1.50E-28
Smp_084610	FAS associated factor 2	2.01	5.71E-29
Smp_196040	Uncharacterised protein	2.67	3.71E-48
Smp_002410	14-3-3 epsilon 2	1.85	6.72E-42
Smp_137540	Apoptosis inducing factor 1, mitochondrial	2.29	5.03E-29
Smp_009760	14-3-3 protein homolog 1	1.45	1.12E-11
Smp_044000	Bax inhibitor 1	1.34	5.66E-08
Smp_172010	Caspase 7	0.95	9.41E-01
Smp_077540	FAS associated factor 1	1.20	2.46E-02
Smp_032000	Caspase	0.97	8.35E-01
Smp_148130	FAS binding factor	0.32	1.23E-39
Smp_213250	Bcl 2 ous antagonist:killer	0.85	6.12E-03
Smp_213730	FAS death assoicated protein	0.83	5.69E-03
Smp_013040	Cathepsin D	0.77	9.99E-08
Smp_034840	14-3-3 epsilon	0.81	1.08E-04
Smp_072180	Apoptosis regulator BAX	0.70	1.87E-05
Smp_141270	Caspase 8	0.82	6.50E-03
Smp_095190	Bcl 2 ous antagonist:killer	0.53	2.40E-05
Smp_129670	Apoptosis stimulating of P53	0.97	7.51E-01
Smp_041630	BCL2 apoptosis inhibitor domain containing protein	0.86	8.99E-02
Smp_168470	BCL2 apoptosis inhibitor domain containing protein	0.76	9.36E-03

**Appendix C.24: Expression of putative apoptosis related genes in the ovaries of females from single sex infections, compared to ovaries from mixed sex infections.** The fold change provides a measure of expression in the ovaries of ovaries from single sex infections compared to those from mixed sex infecitons (That means fold changes greater than 1 indicate an up regulation in the ovaries from single sex infections). The *p*-value has been adjusted for multiple hypothesis testing.

GO term	Description	Total Genes	DEGs	Expected	p-value
GO:0007155	Cell adhesion	226	91	34.46	3.80E-17
GO:0006278	RNA-dependent DNA replication	32	19	4.88	1.20E-08
GO:0030154	Cell differentiation	78	29	11.89	2.00E-06
GO:0006941	Striated muscle contraction	145	44	22.11	2.40E-06
GO:0007156	Homophilic cell adhesion	60	22	9.15	3.60E-05
GO:0007165	Signal transduction	581	128	88.6	4.00E-05
GO:0007169	Transmembrane receptor protein tyrosine kinase signalling pathway	31	14	4.73	6.90E-05
GO:0006813	Potassium ion transport	77	24	11.74	3.00E-04
GO:0006811	Ion transport	389	103	59.32	3.30E-04
GO:0015074	DNA integration	28	12	4.27	4.20E-04
GO:0006814	Sodium ion transport	64	20	9.76	9.20E-04
GO:0006816	Calcium ion transport	49	16	7.47	1.75E-03
GO:0007018	Microtubule-based movement	60	18	9.15	2.71E-03
GO:0006537	Glutamate biosynthetic process	3	3	0.46	3.54E-03
GO:0006508	Proteolysis	318	58	48.49	4.19E-03
GO:0007017	Microtubule-based process	101	32	15.4	5.89E-03
GO:0007283	Spermatogenesis	10	5	1.52	0.011
GO:0007601	visual perception	10	5	1.52	0.011
GO:0009073	Aromatic amino acid family biosynthetic process	4	3	0.61	0.013
GO:0009809	Lignin biosynthetic process	4	3	0.61	0.013
GO:0006879	Cellular iron ion homeostasis	7	4	1.07	0.013
GO:0006826	Iron ion transport	11	5	1.68	0.017
GO:0042278	Purine nucleoside metabolic process	128	21	19.52	0.023
GO:0007517	Muscle organ development	12	3	1.83	0.023
GO:0009060	Aerobic respiration	20	3	3.05	0.023
GO:0009154	Purine ribonucleotide catabolic process	44	5	6.71	0.023
GO:0007275	Multicellular organismal development	286	62	43.61	0.025
GO:0009165	Nucleotide biosynthetic process	115	18	17.54	0.026
GO:0006013	Mannose metabolic process	5	3	0.76	0.028
GO:0016055	Wnt signaling pathway	27	9	4.12	0.031
GO:0016042	Lipid catabolic process	26	8	3.96	0.031
GO:0006754	ATP biosynthetic process	50	8	7.62	0.032
GO:0006120	Mitochondrial electron transport, NADH to ubiquinone	9	4	1.37	0.036

**Appendix C.25: Expanded list of GO terms enriched amongst the genes up-regulated in ovaries of females single sex infections compared to those from mixed sex infections.** The column “Total Genes” provides the number of genes associated with a particular GO term. “DEGs” provides the number of genes associated with a given GO term that had significantly higher expression in ovaries of SS females; “Expected Genes” provides the number of differentially expressed genes associated with a given GO term that are expected by chance.

Pathway	Description	Total Genes	DEGs	Expected	p-value
<b>smm04142</b>	Lysosome	47	20	7.41	6.70E-06
<b>smm00330</b>	Arginine and proline metabolism	18	9	2.84	5.94E-04
<b>smm00760</b>	Nicotinate and nicotinamide metabolism	10	6	1.58	1.59E-03
<b>smm00511</b>	Other glycan degradation	8	5	1.26	3.21E-03
<b>smm04512</b>	ECM-receptor interaction	9	5	1.42	6.10E-03
<b>smm00052</b>	Galactose metabolism	14	6	2.21	0.012
<b>smm00600</b>	Sphingolipid metabolism	15	6	2.37	0.016
<b>smm02010</b>	ABC transporters	8	4	1.26	0.022
<b>smm04320</b>	Dorso-ventral axis formation	8	4	1.26	0.022
<b>smm04310</b>	Wnt signaling pathway	35	10	5.52	0.023
<b>smm00561</b>	Glycerolipid metabolism	17	6	2.68	0.029
<b>smm00500</b>	Starch and sucrose metabolism	14	5	2.21	0.041
<b>smm00062</b>	Fatty acid elongation	6	3	0.95	0.047
<b>smm00564</b>	Glycerophospholipid metabolism	35	9	5.52	0.049

**Appendix C.26: Complete list of KEGG pathways enriched amongst the genes up-regulated in ovaries of females from single sex infections compared to those of mixed sex infections.** The column “Total Genes” provides the number of genes in the KEGG pathway. “DEGs” provides the number differentially expressed genes in that pathway; “Expected Genes” provides the number of differentially expressed genes in a given pathway that is expected by chance.

Domain	Description	Total Genes	DEGs	Expected	p-value
<b>PF00078</b>	Reverse transcriptase (RNA-dependent DNA polymerase)	24	21	6.67	1.480E-09
<b>PF00665</b>	Integrase core domain	13	13	3.61	5.693E-08
<b>PF00041</b>	Fibronectin type III domain	29	22	8.05	8.660E-08
<b>PF00077</b>	Retroviral aspartyl protease	14	13	3.89	5.767E-07
<b>PF13895</b>	Immunoglobulin domain	27	19	7.50	4.238E-06
<b>PF07679</b>	Immunoglobulin I-set domain	45	25	12.50	5.559E-05
<b>PF00011</b>	Hsp20/alpha crystallin family	11	9	3.06	2.792E-04
<b>PF09380</b>	FERM C-terminal PH-like domain	13	10	3.61	2.908E-04
<b>PF13499</b>	EF-hand domain pair	66	31	18.33	3.916E-04
<b>PF07686</b>	Immunoglobulin V-set domain	8	7	2.22	7.318E-04
<b>PF07645</b>	Calcium-binding EGF domain	7	6	1.94	2.310E-03
<b>PF13405</b>	EF-hand domain	12	8	3.33	4.741E-03
<b>PF00452</b>	Apoptosis regulator proteins, Bcl-2 family	4	4	1.11	5.938E-03
<b>PF01413</b>	C-terminal tandem repeated domain in type 4 procollagen	4	4	1.11	5.938E-03
<b>PF11901</b>	Protein of unknown function (DUF3421)	8	6	2.22	6.679E-03
<b>PF00008</b>	EGF-like domain	15	9	4.17	6.944E-03

<b>PF00595</b>	PDZ domain (Also known as DHR or GLGF)	54	23	15.00	7.106E-03
<b>PF01048</b>	Phosphorylase superfamily	6	5	1.67	7.141E-03
<b>PF01392</b>	Fz domain	6	5	1.67	7.141E-03
<b>PF13927</b>	Immunoglobulin domain	6	5	1.67	7.141E-03
<b>PF01221</b>	Dynein light chain type 1	31	15	8.61	7.360E-03
<b>PF00520</b>	Ion transport protein	43	19	11.94	8.619E-03
<b>PF09379</b>	FERM N-terminal domain	16	9	4.44	0.011
<b>PF00373</b>	FERM central domain	19	10	5.28	0.013
<b>PF00617</b>	RasGEF domain	9	6	2.50	0.014
<b>PF03133</b>	Tubulin-tyrosine ligase family	9	6	2.50	0.014
<b>PF07714</b>	Protein tyrosine kinase	46	19	12.78	0.017
<b>PF07690</b>	Major Facilitator Superfamily	43	18	11.94	0.017
<b>PF00754</b>	F5/8 type C domain	7	5	1.94	0.018
<b>PF00501</b>	AMP-binding enzyme	12	7	3.33	0.020
<b>PF01391</b>	Collagen triple helix repeat (20 copies)	12	7	3.33	0.020
<b>PF00036</b>	EF hand	3	3	0.83	0.021
<b>PF00992</b>	Troponin	3	3	0.83	0.021
<b>PF01596</b>	O-methyltransferase	3	3	0.83	0.021
<b>PF03188</b>	Eukaryotic cytochrome b561	3	3	0.83	0.021
<b>PF04434</b>	SWIM zinc finger	3	3	0.83	0.021
<b>PF04712</b>	Radial spokehead-like protein	3	3	0.83	0.021

**Appendix C.27: Expanded list of Pfam domains enriched amongst the genes up-regulated in ovaries of females from single sex infections compared to those of mixed sex infections.** The column “Total Genes” provides the number of genes encoding a given Pfam domain. “DEGs” provides the number of differentially expressed genes with that domain; “Expected” provides the number of differentially expressed genes with a given domain that is expected by chance.