# <u>Appendix</u>

Strain	Freezing	Cultured?	Phenotyped	Sequenced	RNA yield	Gametocytogenesis
	date				(ng)	
278	02/05/2008	Grew	Yes	Yes	3472	Yes
788	28/09/2008	Grew	Yes	Yes	2052	No
3106	27/04/2008	Grew	Yes	Yes	1870	No
3135	04/04/2007	Grew	Yes	Yes	6612	No
3541	07/01/2006	Grew	Yes	Yes	3420	No
3769	20/12/2004	Grew	Yes	Yes	2348	No
5802	15/03/2004	Grew	Yes	Yes	2192	Yes
5809	10/03/2004	Grew	Yes	Yes	3421	Yes
5814	19/03/2004	Grew	Yes	Yes	2751	Yes
6390	24/02/2007	Grew	Yes	Yes	4018	Yes
9050	04/02/2007	Grew	No	Yes	N.e.	Yes
5188	23/02/2004	Lysed on thawing	No	No	N.e.	No
93	31/10/2007	Did not grow	No	No	N.e.	No
229	28/09/2008	Did not grow	No	No	N.e.	No
254	No date	Did not grow	No	No	N.e.	No
255	24/04/2006	Did not grow	No	No	N.e.	No
303	15/03/2004	Did not grow	No	No	N.e.	No
527	19/04/2002	Did not grow	No	No	N.e.	No
806	21/07/2008	Did not grow	No	No	N.e.	No
808	21/06/2008	Did not grow	No	No	N.e.	No
1546	19/08/2007	Did not grow	No	No	N.e.	No
1551	17/07/2007	Did not grow	No	No	N.e.	No
1975	19/07/2007	Did not grow	No	No	N.e.	No
2215	13/05/2006	Did not grow	No	No	N.e.	No
2790	26/03/2006	Did not grow	No	No	N.e.	No
3265	19/07/2007	Did not grow	No	No	N.e.	No
3307	12/06/2007	Did not grow	No	No	N.e.	No
3329	25/07/2007	Did not grow	No	No	N.e.	No
3626C	28/05/2004	Did not grow	No	No	N.e.	No
4333D	07/11/2006	Did not grow	No	No	N.e.	No
4382	15/03/2007	Did not grow	No	No	N.e.	No
4406	29/07/2007	Did not grow	No	No	N.e.	No
4406	10/08/2007	Did not grow	No	No	N.e.	No
4416	08/10/2007	Did not grow	No	No	N.e.	No
4418	28/10/2007	Did not grow	No	No	N.e.	No
5777	24/02/2004	Did not grow	No	No	N.e.	No
5803	15/03/2004	Died @ 3 weeks	No	No	N.e.	Yes
5849C	12/05/2004	Did not grow	No	No	N.e.	No
6164z	27/04/2006	Did not grow	No	No	N.e.	No
6184z	27/04/2006	Did not grow	No	No	N.e.	No
6215z	24/05/2006	Did not grow	No	No	N.e.	No
8033	25/07/2007	Did not grow	No	No	N.e.	No
8033	17/07/2007	Did not grow	No	No	N.e.	No
9051	07/02/2007	Did not grow	No	No	N.e.	No
9704z	25/03/2006	Did not grow	No	No	N.e.	No
5762	16/02/2004	Died @ 2 weeks	No	No	No	Ves

## Table A1. Summary of all field isolates received from Peru.

 Table A1. Summary of all field isolates received from Peru. N.e. = RNA not extracted.



**Fig. A1. Parasitaemia of each phenotyping invasion assay, without using post-invasion trypsin treatment.** The bars represent the mean parasitaemia calculated from two experiments of three replicates. Error bars are SEMs. These profiles are quantitatively similar to those in Results Fig. 3.3, due to post-invasion trypsin treatment having very little impact on true parasitaemia.



**Fig. A2.** Invasion phenotypes of Peruvian field isolates, without using post-invasion trypsin treatment. Error bars are SEMs. These profiles are quantitatively similar to those in Results Fig. 3.4, due to post-invasion trypsin treatment having very little impact on true parasitaemia.

Gene	Annotation	Chromosome	Start	Finish	Size (aa)	SNP Position	3D7 Reference base	SNP base	Reads	Reference AA	Mutated AA	AA Position
MSP-1	PFI1475w	MAL9	1201801	1206964	1720	1202181	G	т	190	S	I	127
						1202806	Т	А	4	D	Е	335
						1202850	А	G	1	Ν	S	350
						1202853	С	А	4	т	К	351
						1203239	Α	С	63	К	Q	480
						1203377	Α	G	49	Ν	D	526
						1205108	С	т	69	н	Y	1103
						1205322	т	С	92	V	А	1174
MSP-10	PFF0995c	MAL6	851374	852952	525	851416	Α	G	23	F	L	513
Pf92	PF13_0338	MAL13	2564890	2567281	796	2565982	А	С	66	К	т	364
						2566523	G	А	94	E	к	545
						2567063	Α	С	65	т	Р	725
RAMA	MAL7P1.208	MAL7	394420	396777	785	395539	G	С	129	E	Q	374
						396574	Α	G	73	К	E	719
						396583	G	А	75	D	Ν	722
EBA-140	MAL13P1.60	MAL13	89421	93455	1210	93122	G	А	71	L	F	112
EBA-175	MAL7P1.176	MAL7	1413430	1418305	1462	1414265	Α	G	83	К	E	279
MTRAP	PF10_0281	MAL10	1181002	1182499	498	1181681	т	С	66	Y	н	227
MSP3	PF10_0345	MAL10	1404191	1405256	354	1404394	т	С	116	L	S	68
MSP7	PF13_0197	MAL13	1419283	1420339	351	1419437	т	А	88	К	Ν	301
						1419488	А	С	106	D	E	284
Pf41	PFD0240c	MAL4	273708	274845	378	274612	С	А	61	К	Ν	78
RAP2	PFE0080c	MAL5	84040	85237	398	85096	т	G	61	Ν	н	48
RhopH2	PFI1445w	MAL9	1175192	1180752	1378	1175691	Α	G	60	I	V	167
						1175895	G	А	54	А	т	235
Rh1	PFD0110w	MAL4	144097	153112	2971	144766	G	А	38	E	к	191
						145010	т	А	22	I	к	272
						145012	А	G	26	К	E	273
						146968	С	т	35	L	F	925
						147183	А	т	36	L	F	996
						148354	G	А	41	E	К	1387
						149371	G	А	29	E	К	1726

### Table A2. SNPs present in Peruvian isolate 6390.

Gene	Annotation	Chromosome	Start	Finish	Size (aa)	SNP Position	3D7 Reference base	SNP base	Reads	Reference AA	Mutated AA	AA Position
						149930	т	С	29	V	А	1912
Rh5	PFD1145c	MAL4	1086801	1088589	526	1087775	С	т	64	С	Y	203
PTRAMP	PFL0870w	MAL12	703887	704946	352	704481	т	А	57	Ν	К	198
TLP	PFF0800w	MAL6	684738	688854	1371	685237	G	А	48	V	I	167
						687145	А	т	80	S	С	803
PF10_0323	PF10_0323	MAL10	1335219	1336287	355	1335366	т	А	83	Ν	К	49
PFL2505c	PFL2505c	MAL12	2114613	2122473	2215	2115215	G	С	37	т	R	2016
						2115685	А	т	57	Ν	К	1859
						2116716	G	С	47	Q	E	1516
						2117920	т	А	50	К	Ν	1114
RON2	PF14_0495	MAL14	2134233	2140803	2189	2138624	А	С	114	E	D	1464
						2138627	А	G	109	Ν	S	1465
GAMA	PF08_008	MAL8	1238356	1240573	738	1239800	С	т	53	Μ	I	258
						1239889	т	С	69	т	А	229
						1240374	т	А	53	Ν	I	67
PTRAMP	MAL12P1.174	MAL12	703887	704946	281	704481	т	А	57	Ν	К	198
PFL0865W	MAL12P1.173	MAL12	700523	702204	309	701619	G	т	25	E	D	289
PF10_0350	PF10_0350	MAL10	1420529	1422668	712	1421655	А	G	26	т	А	376
MSP3.8	PF10_0355	MAL10	1432494	1434783	762	1432628	А	т	50	E	D	45
						1432629	А	т	46	E	D	45
						1432699	А	G	47	К	E	69
						1433171	А	A/C	28/24	К	т	226
						1433179	G	G/A	52/28	D	К	229
						1433181	т	T/A	51/31	D	К	229
						1433190	А	A/T	58/24	К	Ν	232
						1433204	А	A/C	80/26	Y	S	237
						1433209	т	T/G	84/22	S	G	239
						1433210	С	C/G	86/21	S	G	239
						1433211	А	A/T	95/14	S	G	239
						1433242	А	A/G	91/25	N	G	250
						1433243	А	A/G	89/26	N	G	250
						1433251	т	T/A	77/41	L	т	253
						1433252	т	T/C	, · - 79/46	L	т	253
						1433264	А	A/T	88/77	н	L	257
								,				-

Gene	Annotation	Chromosome	Start	Finish	Size (aa)	SNP Position	3D7 Reference base	SNP base	Reads	Reference AA	Mutated AA	AA Position
		_			· /	1433274	A	A/G	92/96	I	М	260
						1433276	G	G/A	90/98	R	к	261
						1433281	А	A/G	88/108	S	G	263
						1433305	А	A/G	82/104	I	V	271
						1433308	А	A/C	97/64	R	Q	272
						1433309	G	G/A	87/90	R	Q	272
						1433314	G	G/A	87/103	D	N	274
						1433325	G	G/A	82/98	М	I	277
						1433343	А	A/T	71/92	к	Ν	283
						1433344	G	G/A	70/86	E	к	284
						1433351	Т	T/A	69/98	I	к	286
						1433365	А	A/G	54/89	К	E	291
						1433374	А	A/G	48/80	к	G	294
						1433375	А	A/G	46/70	К	G	294
						1433378	Т	T/A	43/60	I. I.	к	295
						1433379	Т	T/A	54/41	l. I	К	295
						1433380	Т	T/C	47/68	Y	Q	296
						1433382	Т	T/A	55/57	Y	Q	296
						1433383	А	A/T	58/64	Ν	Y	297
						1433386	G	G/A	56/62	E	К	298
						1433392	А	A/G	59/69	N	V	300
						1433393	А	A/T	68/55	Ν	V	300
						1433395	G	G/A	66/82	D	N	301
						1433398	А	A/G	75/83	К	D	302
						1433400	А	A/T	74/83	к	D	302
						1433669	Т	С	7	М	т	392
						1433754	Т	G	9	Ν	К	420
						1433797	G	А	10	E	N	435
						1433799	А	т	18	E	N	435
MAL4P1.41b	PFD0207c	MAL4	240477	243386	639	240827	А	т	38	F	Y	565
RON4	PF11_0168	MAL11	602981	606917	1201	606433	А	т	58	I	Ν	162
RON5	MAL8P1.73	MAL8	806922	814591	1156	812208	G	т	24	F	1	288

 RON5
 MAL8P1.73
 MAL8
 806922
 814591
 1156
 812208
 G
 T
 24
 F
 L
 288

 Table A2. SNPs present in Peruvian isolate 6390. In red: SNPs called on fewer than 10 reads. In green: 2 SNPs correspond to one amino acid change. In blue: 3 SNPs correspond to one amino acid change.
 In blue: 3 SNPs

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**Fig. 2.1. Enzyme treatments in tubes and the 96-well plate set up.** Tubes: sRBC: stained untreated RBC; A: untreated (identical to Tube sRBC); B: neuraminidase treated; C: low trypsin treated; D: high trypsin treated; E: neuraminidase and high trypsin treated; F: chymotrypsin treated. 96-well plate: grey wells contain PBS; blue wells contain no parasites, only sRBC and unstained RBC (a control for flow analysis); white wells contain a 1:1 mix of sRBC and pRBC. Only the top three rows are trypsin treated post-invasion. P35.

#### Table 3.1. The 11 isolates that were cultured successfully. P46.

**Fig. 3.1. Dot plot of an invasion assay.** The plot indicates invasion of strain 3D7 into untreated erythrocytes. The x-axis is DDAO-SE cell labelling and the y-axis is Hoechst 33342 parasite DNA labelling. The four populations (clockwise from bottom right) are: target uninfected cells, donor uninfected cells, donor infected cells and target infected cells. Target infected cells (top right), is the population that varies with enzyme treatment and determines the phenotype. P48.

**Fig. 3.2. Confocal fluorescence microscopy of erythrocytes from the same well as the dot plot above.** The four panels are of the same field with different fluorescence excitation. Clockwise (from bottom right): All fields merged; brightfield; violet laser – Hoechst 33342 parasite labelling; red laser – DDAO-SE cell labelling. Blue arrow: infected, donor erythrocyte. Yellow arrow: uninfected, target erythrocyte. Green arrow: infected, target erythrocyte. Pink arrow: uninfected, donor erythrocyte. P48.

**Fig. 3.3. Parasitaemia bar graphs of each phenotyping invasion assay.** The bars represent the mean parasitaemia calculated from two experiments of three replicates. Error bars are SEMs. P49.

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**Fig. 3.8.** Parasite Multiplication Rates (PMRs) found in Peruvian field strains and three lab strains, **Dd2, 3D7 and HB3.** PMRs fall into the same clusters as seen in invasion profiles with isolates that have Type I invasion profiles also having PMRs of above 6, while Type II invasion profiles correlate to a PMR below 4. P55.

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Fig. 3.10. Invasion efficiencies into enzyme treated erythrocytes and PMR of Peruvian field isolates. Spearman's rank correlation coefficient ( $r_s$ ) and a two-tailed *P*-value are given for each plot. Correlation with a *P*-value of <0.05 was considered significant. P58.

**Fig. 3.11. The MapSeq interface.** Genotyping view allows the user to select samples and genome region and identifies the SNPs present with respect to the 3D7 reference sequence. The "compare groups" function creates a list of mutations that are present across a cohort of samples. Analysing populations uses principal component analysis (PCA) to compare different samples. P59.

**Fig. 3.12.** The LookSeq genome browser: a short segment of chromosome 1 of Peruvian isolate **3135.** Base number is plotted on the x-axis, and paired read length is on the y-axis. Reads are in blue, the darker blue indicates greater coverage of that area. The grey strips indicate the sequence between paired reads. Red areas signify SNPs called with respect to the 3D7 reference sequence (far right). On the left is an insertion / deletion characterised by the gap in reads and the increase in paired read length either side. P59.

**Fig. 3.13. PCA plot of all sequenced** *Plasmodium* **samples.** Peru strains are purple hexagons, red circles are lab strains and blue squares are field isolates from Ghana. The plot is generated from PCA analysis of all SNPs found in the genome, with the minimum of ten reads for a SNP call to be made. P60.

**Fig. 3.14. Peruvian isolates with type one invasion profiles are closely related to Dd2.** This is a magnified view of the group on the extreme left of Fig. 3.13. As can be seen from the scales, there is virtually no variation between these isolates. Peruvian isolates are represented as purple hexagons, while Dd2 is a red circle. P61.

**Table 3.3. Fragment sizes of polymorphic loci from** *msp1* **and** *msp2.* Highlighted in red are fragment sizes from Peruvian isolates that had loci that were of very similar size to lab isolates Dd2 or W2. P62.

**Fig. 3.15.** Dot plots from Peruvian phenotyping assays of the invasion of erythrocytes that have not been enzyme treated. Staining: DDAO-SE (x-axis), SYBR Green I (y-axis). (a) 788; (b) 3135; (c) 3769. 788 produced four well separated populations as expected, also seen in Fig. 3.1. 3135 and 3769 have an extra, intermediate population between events that are SYBR Green I positive (parasitised) and SYBR Green I negative (uninfected). P64.

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Fig. 3.21. Parasitaemia of a phenotyping invasion assay using Dd2, comparing replicates with and without post-invasion trypsin treatment. P71.

Fig. 3.22. Flow cytometry dot plots of forward scatter (x-axis) and side scatter (y-axis) from Peruvian isolate 3135, to compare the relative size and intracellular complexity of the intermediate population compared to the remainder of the sample. (a) The whole sample of 3135. (b) The intermediate population as a subset of the whole population in (a). P73.

Table 3.5. The elapsed time (hours) of the parasite intra-erythrocytic life cycle when measurements of the intermediate population were taken after initiating an invasion assay. It should be noted that the time of the life cycle is an estimate accurate  $\pm$  six hours. Where more than one parasite life cycle stage was seen in the culture, the predominating stage is listed first. P74.

**Fig. 3.23.** Variation of the intermediate population with life cycle. (a) Time course 1; (b) Time course 2; (c) Time course 3. The intermediate population (y-axis) is expressed as a percentage of the Hoechst 33342 events (the top two populations in Fig. 3.17). P75.

**Fig. 3.24. Antibody specificity controls.** (a) Uninfected erythrocytes: 1° anti-MSP-1 19 kDa and 2° antibody (negative control). (b) Peruvian isolate 3541 containing 2% schizonts: 2° antibody only (negative control). (c) Peruvian isolate 3541 containing 2% schizonts: 1° anti-MSP-1 19 kDa and 2° antibody (positive control). P76.

**Fig. 3.25. Peruvian isolate 3541 at a parasitaemia of 6% rings.** (a) 2° antibody only (negative control). (b) 1° anti-MSP-1 19 kDa antibody and 2° antibody (negative control). P77.

**Fig. 3.26.** Peruvian isolate 3769 at a parasitaemia of 6% rings. (a) 2° antibody only (negative control). (b) 1° anti-MSP-1 19 kDa antibody and 2° antibody. P77.

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**Table 3.6. Mycoplasma detection.** Readings A and B are bioluminescence values. (A) background reading; (B) mycoplasma detection reading. B:A ratio >1 indicates the presence of mycoplasma. P80.

Fig. 4.1. Invasion efficiencies (%) into neuraminidase treated (x-axis) and chymotrypsin treated (y-axis) erythrocytes. Spearman's rank correlation coefficient ( $r_s$ ) and a two-tailed *P*-value are given for each plot. Correlation with a *P*-value of <0.05 was considered significant. P84.

**Fig. 4.2. PMR (x-axis) and invasion efficiency (%) into chymotrypsin treated (y-axis) erythrocytes.** Spearman's rank correlation coefficient ( $r_s$ ) and a two-tailed *P*-value are given for each plot. Correlation with a *P*-value of <0.05 was considered significant. P84.

Table A1. Summary of all field isolates received from Peru. N.e. = RNA not extracted. P88.

**Fig. A1. Parasitaemia of each phenotyping invasion assay, without using post-invasion trypsin treatment.** The bars represent the mean parasitaemia calculated from two experiments of three replicates. Error bars are SEMs. These profiles are quantitatively similar to those in Results Fig. 3.3, due to post-invasion trypsin treatment having very little impact on true parasitaemia. P89.

**Fig. A2.** Invasion phenotypes of Peruvian field isolates, without using post-invasion trypsin treatment. Error bars are SEMs. These profiles are quantitatively similar to those in Results Fig. 3.4, due to post-invasion trypsin treatment having very little impact on true parasitaemia. P90.

**Table A2. SNPs present in Peruvian isolate 6390.** In red: SNPs called on fewer than 10 reads. In green: 2 SNPs correspond to one amino acid change. In blue: 3 SNPs correspond to one amino acid change. P91-93.