Appendices

Appendix I – List of *Plasmo*GEM IDs for each gene and corresponding annotation.

Gene ID	*Plasmo*GEM ID	Gene name
p230p-tag	PbGEM-226060	p230p-tag
PBANKA_051490	PbGEM-015545	28 kDa ookinete surface protein (P28)
PBANKA_051500	PbGEM-015561	25 kDa ookinete surface antigen precursor (P25)
PBANKA_103780	PbGEM-097822	secreted ookinete adhesive protein (SOAP)
PBANKA_103440	PbGEM-039254	plasmepsin IV (PM4)
PBANKA_110420	PbGEM-122074	3-methyl-2-oxobutanoate dehydrogenase (lipoamide), putative
PBANKA_140160	PbGEM-062476	methyl transferase-like protein, putative
PBANKA_020580	PbGEM-082161	serine/threonine protein kinase, putative (IK2)
PBANKA_030850	PbGEM-009884	protein kinase, putative (TKL1)
PBANKA_031030	PbGEM-072470	protein kinase 7 (PK7)
PBANKA_031140	PbGEM-111762	serine/threonine protein kinase, putative
PBANKA_031420	PbGEM-010677	calcium dependent protein kinase 1 (CDPK1)
PBANKA_040110	PbGEM-084034	serine/threonine protein kinase, putative (SRPK1)
PBANKA_040820	PbGEM-111826	calcium dependent protein kinase 3 (CDPK3)
PBANKA_040940	PbGEM-111754	protein kinase, putative (PKRP)
PBANKA_041040	PbGEM-111698	glycogen synthase kinase 3 (GSK3)
PBANKA_052140	PbGEM-072474	RIO-like serine/threonine kinase, putative
PBANKA_061520	PbGEM-087803	calcium dependent protein kinase 4 (CDPK4)
PBANKA_061670	PbGEM-111690	NIMA related kinase 4 (NEK4)
PBANKA_071730	PbGEM-072538	cdc2-related protein kinase 3 (CRK3)
PBANKA_080560	PbGEM-072522	O-sialoglycoprotein endopeptidase, putative
PBANKA_080800	PbGEM-111786	cdc2-related protein kinase 4 (CRK4)
PBANKA_082710	PbGEM-072502	protein kinase, putative
PBANKA_083560	PbGEM-028140	cAMP-dependent protein kinase catalytic subunit (PKAc)
PBANKA_090110	PbGEM-111746	protein kinase, putative
PBANKA_090380	PbGEM-111794	serine/threonine protein kinase, putative
PBANKA_091210	PbGEM-111738	casein kinase 1 (CK1)
PBANKA_092520	PbGEM-093973	calcium-dependent protein kinase 7 (CDPK7)
PBANKA_092550	PbGEM-111850	calcium dependent protein kinase 6 (CDPK6)
PBANKA_093300	PbGEM-072518	serine/threonine protein kinase, puative
PBANKA_093370	PbGEM-111778	mitogen-activated protein kinase 2 (MAP2)
PBANKA_101330	PbGEM-036210	mitogen-activated protein kinase 1 (MAP1)
PBANKA_101980	PbGEM-111858	serine/threonine protein kinase, putative
PBANKA_112270	PbGEM-111714	protein kinase, putative (TKL4)
PBANKA_112690	PbGEM-099789	protein kinase PK4 (PK4)
PBANKA_122500	PbGEM-111674	serine/threonine protein kinase, FIKK family
PBANKA_130520	PbGEM-053796	serine/threonine protein kinase, putative
PBANKA_130690	PbGEM-104812	serine/threonine protein kinase, putative (SRPK2)
PBANKA_130920	PbGEM-104970	serine/threonine kinase-1, putative
PBANKA_131800	PbGEM-105530	serine/threonine protein kinase, putative
PBANKA_135150	PbGEM-111682	calcium dependent protein kinase 5 (CDPK5)
PBANKA_135260	PbGEM-111802	serine/threonine protein kinase, putative
PBANKA_136210	PbGEM-108848	protein kinase, putative
PBANKA_141450	PbGEM-111842	protein kinase, putative
PBANKA_142160	PbGEM-065291	calcium/calmodulin-dependent protein kinase, putative
PBANKA_144560	PbGEM-111706	protein kinase, putative
PBANKA_146050	PbGEM-072542	serine/threonine protein kinase, putative

Appendix II – Primers used to genotype cloned mutants.

Primer name	Sequence	Target/Comment
arg00059	GAATCACAATTGACCAGG	
arg00060	CAGTAAATTGCTATGATAAATC	
arg00080	GACTTCTGTAGCCATGATAGC	hdhfr::yfcu
arg00081	CGCCACACTACATGGTGAG	hdhfr::yfcu
arg00082	GAGCTCATAGTTATTGCTATTGC	
arg00084	AAAGAATTCTGATGGTTTACAATCACC	RNApol II
arg00085	AAAGCGGCCGCTTTCTTCCTGCATCTCCTC	RNApol II
arg00087	GTGCATTAACAGTTAGAAGAGG	
arg00089	CAGAAACACAATGTTGAAATTC	
arg00094	GTTTCGATTGCACGGACTTTG	
arg00102	GTGTAGAAGTAAATTCATACCC	
arg00214	GAACGGCACTGGTCAACTTG	
arg00215	TCATTCTTCGAAAACGATCT	
arg00216	CGGGGCCCTTATGCATAATC	
arg00218	CTTTGGTGACAGATACTACTG	
arg00255	GGGGGTTTGTGTGGAGGCG	
arg00331	TTGAACATTTGCGCATATATTGG	
arg00332	GTGCCAAATTATTATGGTATACC	
arg00367	CTCCAGCATATACTTGCATAG	
arg00368	CTTCACCAAATGAACCCTTTC	
arg00369	GATTGGGAGATGTTAGTTCTG	
arg00370	GGTTATGAGAAGTTAAACTACG	
arg00371	CCGGTATTTATCATCCAAGAG	
arg00372	CATTTGTTCATGCAATCATTCG	
arg00373	CCCACTACCTAATTTCCGAAC	
arg00374	GCATCATTGACACGAACTCG	
arg00375	GTGAAGAGAGGCTAAGGAGG	
arg00381	TGCACTTCACCAAAAGCGCCA	
arg00382	TCAACGGGAGGTAGCTCCAA	
arg00383	CCACGATCATTTAGAAAACACG	
arg00384	GCTCATATGCATTATACGCTTC	
arg00385	TACACTTTGAGGTTAATGTGC	
arg00386	GGTTATCTATACATTTATTTGTG	
arg00387	CTCTTAAAACTTGGGGGTAGG	
arg00388	GCAACAAGAAAGGAAGCATAAC	
arg00389	CTCCCTTCAAATTTATGCTGAC	
arg00390	GGGAAGTAACCTAATTTGCTG	
arg00396	GGCATGCCGTATTTTCCATG	
arg00397	ATGCATATTCTTTTGTTGCAGC	
arg00398	CACCCATCCAAACATATAAAAG	
arg00399	CTAGTAATAGTCAGTCTGGGG	

Appendix II – (cont) - Primers used to genotype cloned mutants.

Primer name	Sequence	Target/Comment
arg00400	TCCAAAGCGTTATGCCAAGTGT	
arg00401	GCAACTCGTTATATATTTCCG	
arg00402	CTTTTCCAGAACTAACTACTCC	
arg00403	CTTTTCTTGTAACACCCTCAG	
arg00404	TGGAACTACAAAATAGATTTCG	
arg00405	GCTCAAGCAACAGCAGGAC	
arg00406	ATCCAAATATGGTATTTTGAGC	
arg00407	CTGGAGATTCGTTTTGTTCAC	
arg00408	TTGTGTTTGCCGCATGTTGC	
arg00409	GATATTCCTAAAGATCTATCTG	
arg00410	ACGACAATGTGCATGCCTCA	
arg00416	CGTACTTGAATAGCTGTCTAC	
arg00417	CCCCAATATAATGAATATTCTG	
arg00418	GAGCATTCCGCAAAGTATGTC	
arg00419	GGGAACACATCCTTTTAGTTC	
arg00420	AGCCATTACCCGTTGTTTCG	
arg00421	GTGCCAAATTATTATGGTATAC	
arg00422	CCAGAATAATTTTGTTAGAATATAG	
arg00423	GTACAGTTTGTGGTATATATTCC	
arg00424	GGTGATAATAATGCATGCCAAG	
arg00425	GGTTCTATCTGTTTCATGTAC	
arg00426	CCTCAGAAAATGAATGGCAG	
arg00427	GGTGTAATCAAAGTAAAAGTGC	
arg00428	CTCCACCTTCATAAAATTCGG	
arg00429	CATAAAAGATTAAATCGTGCTCG	
arg00430	CAATTATGTGTAGAAAGAAACC	
arg00431	CCCTTTCCTAGTGGTTCTTC	
arg00432	GCCAATGTCCAAATAAATGTATC	
arg00433	CTATGCAAGTATATGCTGGAG	
arg00434	GGGGCAAGAAATGTCTACAC	
arg00435	CGCCACCTTCACATAATTCC	
arg00436	GATTTCCGTCAAATGTATTGGG	
arg00437	CCATCTGTTACTTTCATAGGG	

Appendix III – Primers used for genotyping of the STM screen

Gene ID	Gene specific primer	Sequence	Pairs with
p230p-tag	arg00448	GGAACAATATGGCTGTTCAATG	arg00218
PBANKA_051490	arg00447	GGATTCCGTGAATGATCCCC	arg00216
PBANKA_051500	arg00449	TGTTCCCGTTGTAACAGTGCA	arg00216
PBANKA_103780	arg00446	TTTCCCACTGCGTACCCTTT	arg00218
PBANKA_103440	arg00452	AGACAAACTTTGCCCACAACA	arg00216
PBANKA_110420	arg00451	AAAGCCAGAAACGACATGAA	arg00218
PBANKA_140160	arg00450	CATGGCTATGACCGACAGAG	arg00218
PBANKA_020580	arg00471	CGAAGCGCTTTACCATGTGGGC	arg00218
PBANKA_030850	arg00472	AGTGCATACGCTTCATGACGCT	arg00216
PBANKA_031030	arg00473	AACCGAAGTGCTCTTTGCGA	arg00216
PBANKA_031140	arg00474	TGCTACCTTACGCATTGGACA	arg00218
PBANKA_031420	arg00371	CCGGTATTTATCATCCAAGAG	arg00216
PBANKA_040110	arg00475	TGATGCGGATTTGTGTGTGT	arg00218
PBANKA_040820	arg00386	GGTTATCTATACATTTATTTGTG	arg00218
PBANKA_040940	arg00476	AGCAATGATGTAGGATGTGCA	arg00216
PBANKA_041040	arg00470	AGCGAGTTCCCGTGCACTCA	arg00218
PBANKA_052140	arg00477	TCCAAAGCGTTATGCCAAGTGT	arg00218
PBANKA_061520	arg00255	GGGGGTTTGTGTGGAGGCG	arg00216
PBANKA_061670	arg00234	GCACACTCACCTGAAATGTCG	arg00216
PBANKA_071730	arg00478	TGGTTCAATTGTTGAGCAAAGTCCT	arg00218
PBANKA_080560	arg00479	TCGGAAAAACCTTGAAAGCGCT	arg00218
PBANKA_080800	arg00480	TCGCTAGTTATATATGCTCACGCT	arg00216
PBANKA_082710	arg00481	ACATTACCAGCAGTTGCCCA	arg00216
PBANKA_083560	arg00482	TCAAGTGAAACGGAATAGAAGCGA	arg00218
PBANKA_090110	arg00483	TCAGAAAGGTATACGTCAACGGT	arg00216
PBANKA_090380	arg00484	AGCTTGTATGTCGATTCGAGA	arg00218
PBANKA_091210	arg00485	ACGATGTGTGCAGCAGGTCT	arg00216
PBANKA_092520	arg00486	TGTCTCCCTAAAAGGCATGTGCA	arg00218
PBANKA_092550	arg00381	TGCACTTCACCAAAAGCGCCA	arg00218
PBANKA_093300	arg00487	AGCAGTGCACACAAAAGAAGA	arg00218
PBANKA_093370	arg00256	ACCATGAGTGCATGCATAGGA	arg00216
PBANKA_101330	arg00453	CGCGTGGAAAACTGTGGGC	arg00216
PBANKA_101980	arg00488	TGCCCGGAATGCACATATGTTGC	arg00216
PBANKA_112270	arg00489	TGGGGAGTACCTTGCCCATGCA	arg00216
PBANKA_112690	arg00490	AGTATTGCCCCATCCATTGCT	arg00218
PBANKA_122500	arg00491	TGTCTGACTCTCCATGGTGTCCCA	arg00218
PBANKA_130520	arg00456	GAGTACCTGTTGGTCACGC	arg00216
PBANKA_130690	arg00225	TGCCCTTTTGATGCCAAGACG	arg00216
PBANKA_130920	arg00492	TCACGCATCGGGGATTTGTCA	arg00216
PBANKA_131800	arg00493	ACGGAGCACAATGTATGCCATGGA	arg00218
PBANKA_135150	arg00494	TCGACGGTACTGTCTGACTGGTCA	arg00216
PBANKA_135260	arg00259	CTGGCGCACGGCAAAACCC	arg00218
PBANKA_136210	arg00262	ACGACAATGTGCATGCCTCA	arg00218
PBANKA_141450	arg00272	CCACAAAGCAATTCCGGTGC	arg00218
PBANKA_142160	arg00454	TCTAAATCGCGGCTTTCACA	arg00218
PBANKA_144560	arg00495	TGCTCAAGCAACAGCAGGACA	arg00216
PBANKA_146050	arg00173	CCTGGAATTGTTTCCCCACAC	arg00218

Appendix IV – Primers used for barcode sequencing.

Primer name	Sequence
arg00444	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGTAATTCGTGCGCGTCAG
arg00445	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTTCAATTTCGATGGGTAC
PE1.0	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T 1)
iPCRindex1	CAAGCAGAAGACGGCATACGAGATTGCTAATCACTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex2	CAAGCAGAAGACGGCATACGAGATTAGGGGGATTCGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex3	CAAGCAGAAGACGGCATACGAGATAGTTTCCCAGGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex4	CAAGCAGAAGACGGCATACGAGATCCTGGGAGGTAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex5	CAAGCAGAAGACGGCATACGAGATATACCACAAATGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex6	CAAGCAGAAGACGGCATACGAGATGATCTCTCGGGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex7	CAAGCAGAAGACGGCATACGAGATACCCTATACTCGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex8	CAAGCAGAAGACGGCATACGAGATCTCAATTAAGAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex9	CAAGCAGAAGACGGCATACGAGATCGACAGAACGTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex10	CAAGCAGAAGACGGCATACGAGATTCGCCATTATGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex11	CAAGCAGAAGACGGCATACGAGATATGTTCCGGCCGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex12	CAAGCAGAAGACGGCATACGAGATTCTTGAAGTGAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex13	CAAGCAGAAGACGGCATACGAGATGAAGGCCAGCTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex14	CAAGCAGAAGACGGCATACGAGATCCAATGTGCAGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex15	CAAGCAGAAGACGGCATACGAGATATCGAAGGACCGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex16	CAAGCAGAAGACGGCATACGAGATTCGGGTGCGAAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex17	CAAGCAGAAGACGGCATACGAGATGTAATTTACGGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex18	CAAGCAGAAGACGGCATACGAGATATATCGACTACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex19	CAAGCAGAAGACGGCATACGAGATTGATTCTTACAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex20	CAAGCAGAAGACGGCATACGAGATACGGCGGGCCTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex21	CAAGCAGAAGACGGCATACGAGATCTTGCGTGGAGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex22	CAAGCAGAAGACGGCATACGAGATTAATCAAAGACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex23	CAAGCAGAAGACGGCATACGAGATGGCGGGCTCTAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex24	CAAGCAGAAGACGGCATACGAGATCCTCCATTTCTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex25	CAAGCAGAAGACGGCATACGAGATAACCAGCGCTGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex26	CAAGCAGAAGACGGCATACGAGATTATTCGTCAACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex27	CAAGCAGAAGACGGCATACGAGATGCGCTGATGCAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex28	CAAGCAGAAGACGGCATACGAGATCTCATATGGCTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex29	CAAGCAGAAGACGGCATACGAGATACAGGGGCAGGGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex30	CAAGCAGAAGACGGCATACGAGATGGTTTTATACCGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex31	CAAGCAGAAGACGGCATACGAGATGCATGACTTTAGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRindex32	CAAGCAGAAGACGGCATACGAGATTTCTGAGTTCTGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T

Appendix V – Targetability and fitness measurements for ePKs.

				Day 5 p. t.			Day 6 p. t.			Day 7 p. t.			Day 8 p. t.				
P. berghei **gene ID**	**Gene name**	**Tewari *et al.* 2010**	**This study**	**Fitness**	**SD**	**p**	**Fitness**	**SD**	**p**	**Fitness**	**SD**	**p**	**Fitness**	**SD**	**p**	**Average fitness, days 5-8**	**Assessment**
PBANKA_135150	*cdpk5*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_092520	*cdpk7*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_031140		Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_083560	*pka*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_090380		Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_091210	*ck1*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_071730	*crk-3*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_130920	*lammer/(CLK1)*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_093300	*prk4*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_080800	*crk-4*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_090110		Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_093860	*ck2*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_101090	*tkl5*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_144300	*nek-1*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_135090	*pk6*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_080560		Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_122500	*fikk*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_100820	*pkg*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_112690	*pk4*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential
PBANKA_052140	*rio2*	Possibly essential	Possibly essential	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	**NA**	NA	NA	NA	Possibly essential†□
PBANKA_040110	*srpk*	KO confirmed	Targetable	**0.59**	*0.18*	2.5E-02	**0.65**	*0.24*	1.3E-01	**0.80**	*0.08*	7.8E-02	**0.83**	*0.15*	2.1E-01	0.72	Confirmed KO
PBANKA_040820	*cdpk3*	KO confirmed	Targetable	**1.00**	*0.09*	1.0E+00	**0.97**	*0.05*	2.2E-01	**1.01**	*0.07*	7.6E-01	**1.04**	*0.06*	2.9E-01	1.01	Confirmed KO
PBANKA_040940	*pkrp*	KO confirmed	Targetable	**0.98**	*0.21*	9.2E-01	**1.03**	*0.09*	7.4E-01	**0.98**	*0.09*	9.0E-01	**1.02**	*0.05*	8.5E-01	1.00	Confirmed KO
PBANKA_061520	*cdpk4*	KO confirmed	Targetable	**0.94**	*0.13*	5.8E-01	**0.89**	*0.10*	4.1E-02	**0.98**	*0.07*	7.4E-01	**0.81**	*0.08*	1.4E-02	0.90	Confirmed KO
PBANKA_061670	*nek-4*	KO confirmed	Targetable	**0.87**	*0.19*	2.0E-01	**1.00**	*0.16*	9.7E-01	**1.02**	*0.11*	7.4E-01	**1.02**	*0.16*	8.5E-01	0.98	Confirmed KO
PBANKA_082710		KO confirmed	Targetable	**0.38**	*0.22*	2.5E-03	**0.65**	*0.06*	1.7E-04	**0.91**	*0.08*	2.9E-02	**1.03**	*0.38*	8.4E-01	0.74	Confirmed KO
PBANKA_092550	*cdpk6*	KO confirmed	Targetable	**0.86**	*0.12*	8.9E-02	**0.90**	*0.05*	8.7E-03	**0.79**	*0.10*	8.1E-03	**0.97**	*0.12*	6.8E-01	0.88	Confirmed KO
PBANKA_093370	*map-2*	KO confirmed	Targetable	**0.99**	*0.11*	9.5E-01	**0.95**	*0.06*	1.8E-01	**1.05**	*0.06*	2.1E-01	**1.03**	*0.05*	4.2E-01	1.00	Confirmed KO
PBANKA_101330	*map-1*	KO confirmed	Targetable	**1.04**	*0.11*	5.8E-01	**0.92**	*0.04*	4.1E-03	**1.06**	*0.04*	4.1E-02	**1.04**	*0.04*	8.5E-02	1.02	Confirmed KO
PBANKA_101980	*cdlk*	KO confirmed	Targetable	**0.20**	*0.02*	5.0E-06	**0.53**	*0.13*	1.7E-02	**0.62**	*0.42*	4.2E-01	**1.13**	*0.19*	8.0E-01	0.62	Confirmed KO
PBANKA_112270	*tkl4*	KO confirmed	Targetable	**0.46**	*0.33*	7.8E-02	**0.28**	*0.11*	2.9E-03	**0.55**	*0.16*	3.0E-02	**0.69**	*0.11*	6.2E-02	0.49	Confirmed KO
PBANKA_130690	*srpk2*	KO confirmed	Targetable	**0.83**	*0.32*	5.3E-01	**1.00**	*0.09*	9.7E-01	**1.01**	*0.14*	9.5E-01	**1.05**	*0.09*	3.9E-01	0.97	Confirmed KO
PBANKA_131800	*kin*	KO confirmed	Targetable	**1.08**	*0.29*	6.9E-01	**0.99**	*0.10*	1.9E-01	**0.97**	*0.07*	2.0E-01	**1.00**	*0.11*	8.4E-01	1.01	Confirmed KO
PBANKA_135260		KO confirmed	Targetable	**0.88**	*0.27*	5.8E-01	**0.98**	*0.07*	5.6E-01	**1.00**	*0.09*	9.5E-01	**1.03**	*0.04*	3.9E-01	0.97	Confirmed KO
PBANKA_146050	*gak*	KO confirmed	Targetable	**0.83**	*0.12*	8.9E-02	**0.74**	*0.09*	2.6E-03	**0.83**	*0.05*	1.4E-04	**1.17**	*0.11*	8.5E-02	0.89	Confirmed KO
PBANKA_030850	*tkl1*	Possibly essential	Targetable	**0.86**	*0.28*	5.3E-01	**0.96**	*0.21*	8.4E-01	**1.14**	*0.18*	1.7E-01	**1.04**	*0.06*	3.9E-01	1.00	New KO
PBANKA_031420	*cdpk1*	Possibly essential	Targetable §	**0.95**	*0.14*	5.5E-01	**0.89**	*0.05*	1.4E-03	**1.10**	*0.05*	2.7E-03	**1.00**	*0.06*	9.5E-01	0.98	New KO Ω
PBANKA_041040	*gsk-3*	Possibly essential	Targetable	**0.99**	*0.03*	8.2E-01	**1.00**	*0.04*	9.3E-01	**1.05**	*0.05*	5.6E-01	**1.01**	*0.03*	8.5E-01	1.01	New KO Ω
PBANKA_082960		Possibly essential	Targetable	**1.03**	*0.04*	7.5E-01	**1.02**	*0.08*	7.5E-01	**1.01**	*0.06*	7.5E-01	**1.05**	*0.02*	2.5E-02	1.03	New KO ◊
PBANKA_130520		Possibly essential	Targetable	**1.02**	*0.12*	9.5E-01	**0.98**	*0.13*	8.4E-01	**1.01**	*0.10*	8.3E-01	**0.99**	*0.08*	9.5E-01	1.00	New KO *
PBANKA_136210	*tkl3*	Possibly essential	Targetable	**1.05**	*0.22*	7.7E-01	**0.99**	*0.09*	9.6E-01	**1.06**	*0.14*	4.6E-01	**0.92**	*0.09*	2.0E-01	1.01	New KO *◊
PBANKA_141450		Possibly essential	Targetable	**0.91**	*0.33*	7.3E-01	**1.00**	*0.16*	9.7E-01	**0.94**	*0.07*	1.7E-01	**0.99**	*0.10*	8.5E-01	0.96	New KO *
PBANKA_142160		Possibly essential	Targetable	**1.01**	*0.13*	1.0E+00	**0.93**	*0.07*	3.4E-02	**1.10**	*0.16*	2.4E-01	**1.01**	*0.12*	9.5E-01	1.01	New KO *
PBANKA_144560	*rio1*	Possibly essential	Targetable	**0.23**	*0.00*	2.0E-03	**0.67**	*0.04*	1.1E-02	**0.91**	*0.08*	2.7E-01	**0.94**	*0.01*	5.9E-02	0.69	New KO ◊†
PBANKA_020580	*eik2; uis1*	KO confirmed	No integration	**NA**	*NA*	NA	**NA**	*NA*	NA	**NA**	*NA*	NA	**NA**	*NA*	NA	NA	False negative
PBANKA_031030	*pk7*	KO confirmed	No integration	**NA**	*NA*	NA	**NA**	*NA*	NA	**NA**	*NA*	NA	**NA**	*NA*	NA	NA	False negative

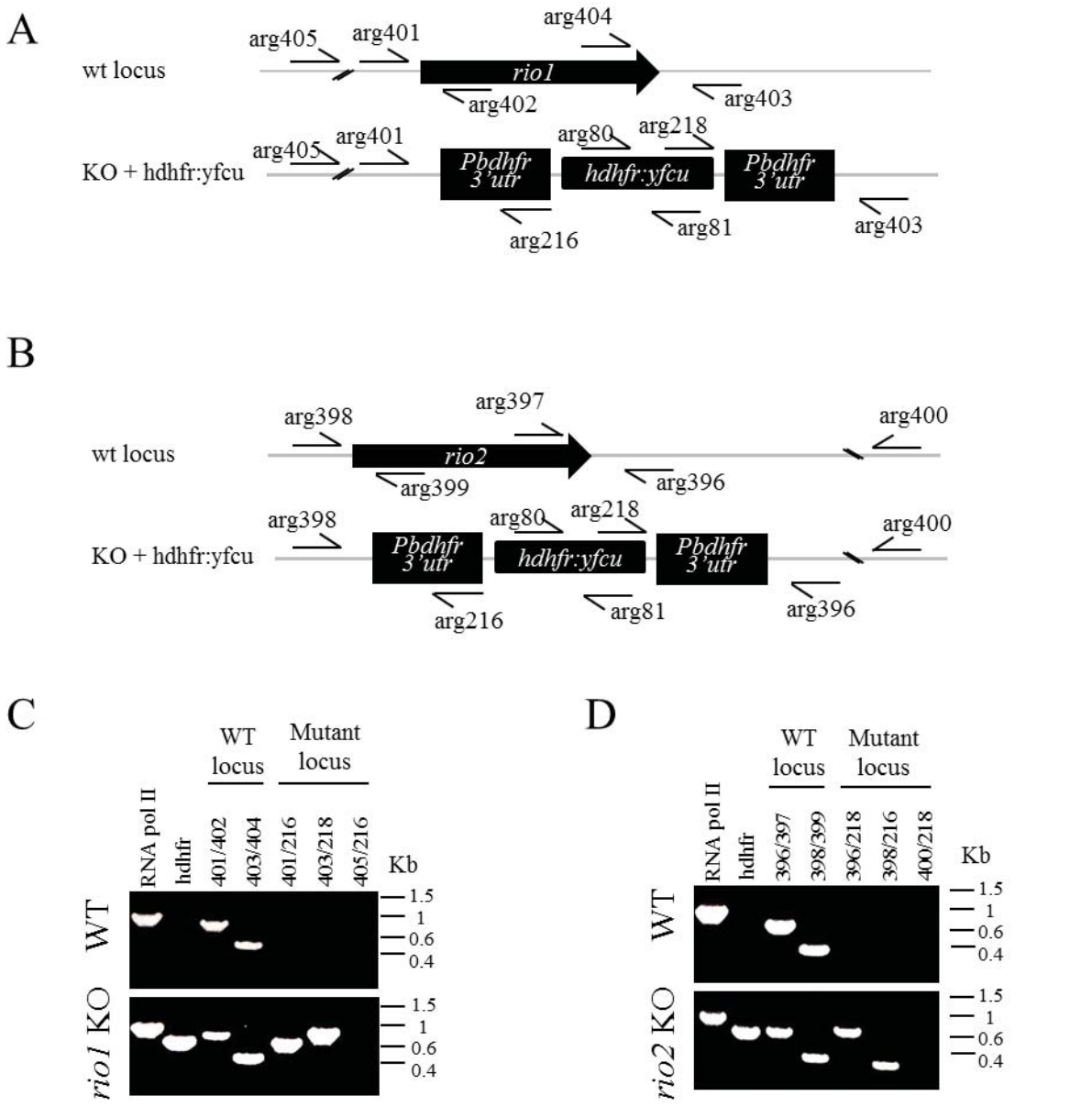
Appendix V – (cont) Targetability and fitness measurements for ePKs.

				Day 5 p. t.			Day 6 p. t.			Day 7 p. t.			Day 8 p. t.				
***P. berghei* gene ID**	**Gene name**	**Tewari *et al.* 2010**	**This study**	**Fitness**	**SD**	**p**	**Fitness**	**SD**	**p**	**Fitness**	**SD**	**p**	**Fitness**	**SD**	**p**	**Average fitness, days 5-8**	**Assessment**
PBANKA_051490	*p28*		Targetable	**0.92**	*0.15*	5.3E-01	**0.98**	*0.10*	9.2E-01	**0.98**	*0.05*	7.4E-01	**1.01**	*0.06*	8.5E-01	0.97	Normal growth reference
PBANKA_051500	*p25*		Targetable	**1.01**	*0.12*	9.8E-01	**1.03**	*0.02*	1.0E-01	**1.04**	*0.07*	2.4E-01	**0.98**	*0.04*	3.9E-01	1.02	Normal growth reference
PBANKA_103780	*soap*		Targetable	**1.00**	*0.08*	1.0E+00	**0.95**	*0.07*	1.1E-01	**1.06**	*0.05*	1.1E-01	**1.03**	*0.05*	3.8E-01	1.01	Normal growth reference
PBANKA_030600	*p230p*		Taggable	**1.07**	*0.12*	5.8E-01	**1.04**	*0.07*	4.7E-01	**0.91**	*0.03*	8.1E-03	**0.98**	*0.09*	8.5E-01	1.00	Normal growth reference
PBANKA_140160			Targetable	**0.51**	*0.12*	1.1E-04	**0.46**	*0.06*	1.3E-05	**0.58**	*0.07*	2.1E-05	**0.87**	*0.07*	1.4E-02	0.60	Attenuated reference
PBANKA_110420	*bckdh e1b*		Targetable	**0.74**	*0.12*	6.9E-03	**0.58**	*0.07*	1.3E-05	**0.67**	*0.05*	2.8E-06	**0.61**	*0.06*	6.6E-05	0.65	Attenuated reference
PBANKA_103440	*pm4*		Targetable	**0.79**	*0.20*	8.9E-02	**0.73**	*0.05*	1.3E-05	**0.71**	*0.12*	4.3E-03	**0.70**	*0.08*	2.2E-03	0.73	Attenuated reference

* PCR genotyping evidence for targetability but no independent clone generated.
§ This study and Jebiwott *et al*., 2013
† Confirmed by WGS of uncloned population
□ Confirmed by selection for target duplication
Ω Confirmed by genotyped clone
◊ Confirmed by PFGE analysis of uncloned population

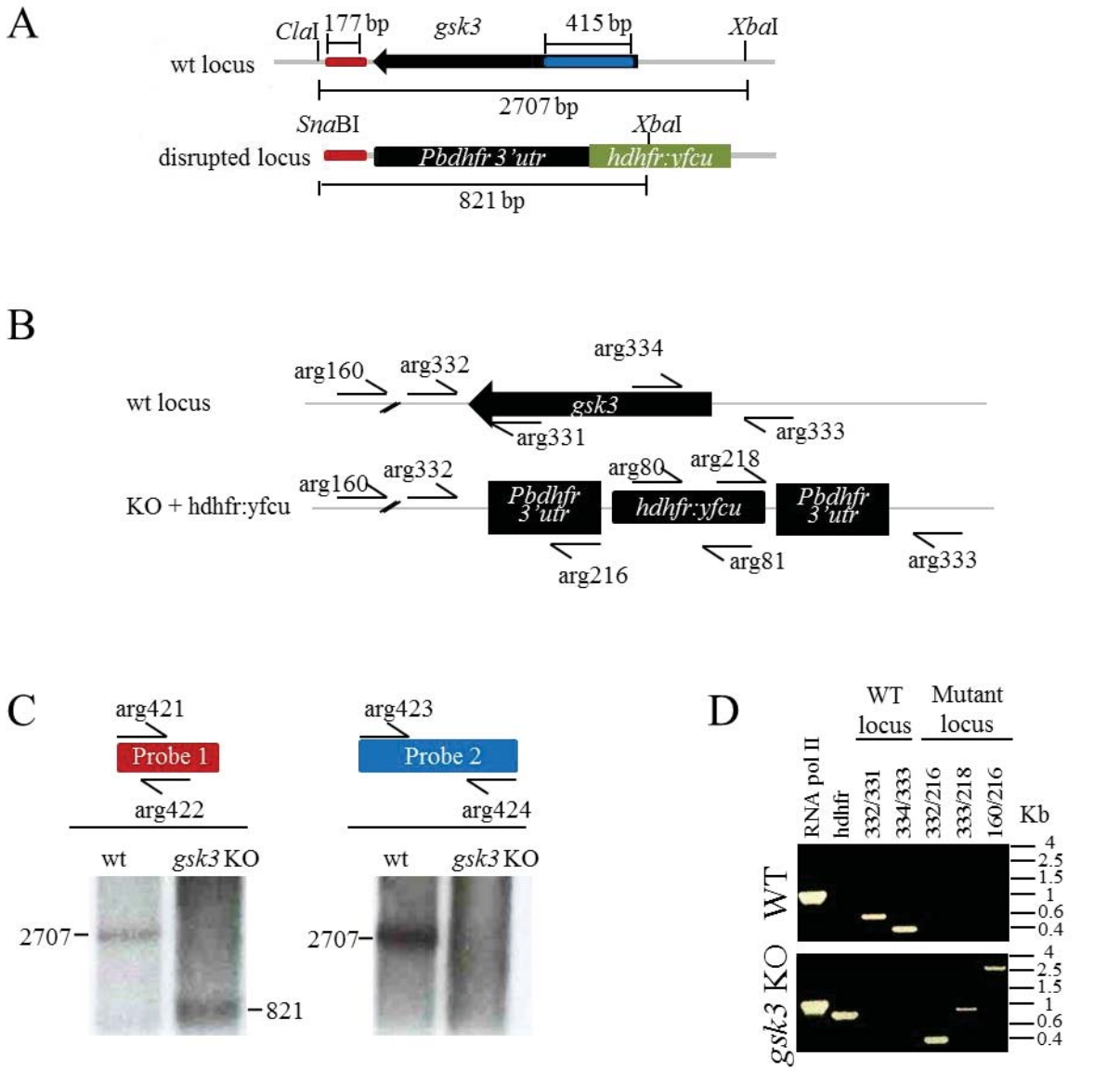
Targetability and fitness measurements for 46 eukaryotic protein kinase genes as determined by barcode sequencing, compared to data from a previous study by Tewari et al.[105], which used conventional gene targeting. p values are adjusted for multiple testing.

Appendix VI – Genotyping strategy for *rio1* and *rio2* KO mutants



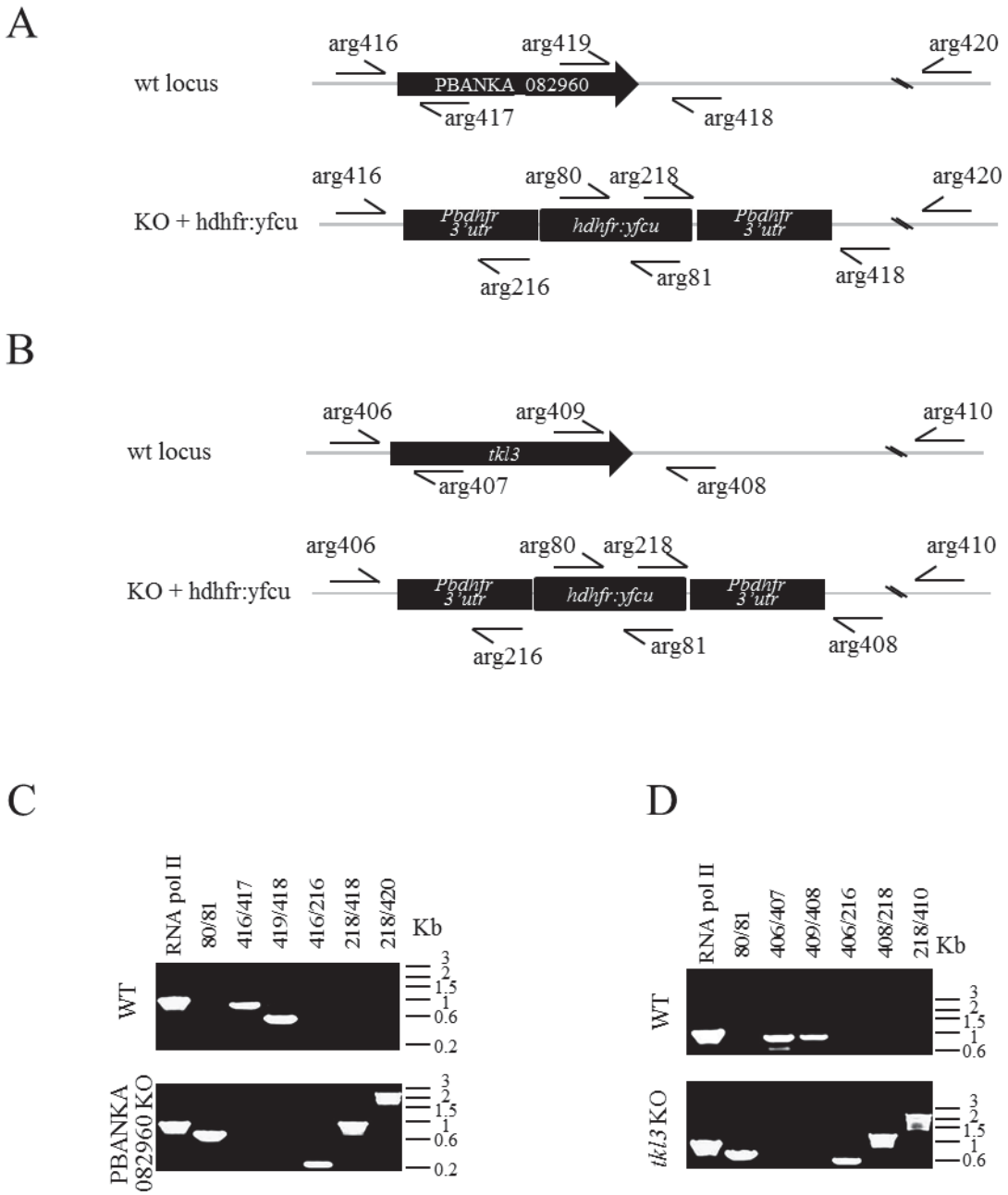
Genotyping of *rio* KO mutants.
(A,B) PCR genotyping strategy for *rio1* and *rio2* mutants, respectively; (C,D) PCR genotyping results showing the presence of both the KO vector and the WT gene in the target locus.

Appendix VII – Genotyping strategy for *gsk3* KO clones



Genotyping of *gsk3* KO clones.
(A) Southern blot strategy; (B) PCR genotyping strategy; (C) Southern blot results showing the correct diagnostic digestion patterns; (D) PCR genotyping results confirming *gsk3* gene deletion.

Appendix VIII – Genotyping strategy for the KO mutants of the PBANKA_082960 and *tkl3* genes.



Genotyping of the PBANKA_082960 and *tkl3* KO mutants.
(A,B) PCR genotyping strategy for the PBANKA_082960 and *tkl3* mutants, respectively; (C,D) PCR genotyping results showing the presence of the KO vector in the target locus and absence of WT contamination. This genotyping was performed in cloned mutants