APPENDICES

Appendix A

Preparation of some reagents and buffers

1. Preparation of media for *E.coli*

1.1. LB (Luria-Bertani) medium (1L)

Bacto-tryptone	10 g
Bacto-yeast extract	5 g
NaCl	10 g

Stir until the ingredients have dissolved. Adjust the final volume to 1 L with distilled water (For LB plate agar, add 15 g for Bacto-agar per liter). Autoclave at 15 psi (120°C for 20 min)

2. Preparation of solutions and buffers for *P. berghei* culture

2.1. Complete culture medium

Culture medium: RPMI1640 with L-glutamine and 4.95 g HEPES per liter culture medium

Preparation of medium:

- dissolve 10.41 g RPMI 1640 medium in 1 L water (add powder slowly under continuous stirring)
- add 2 g $NaHCO_3$ and 4.95 g HEPES
- add 50.000 I.U. Neomycin (stock-solution of 10.000 I.U. /ml; Gibco)
- sterilize by filtration through a 0.2 µm sieve

- store at -20°C

- immediately prior to use, fetal bovine serum is added at a final concentration of 25% (v/v) to give complete culture medium

2.2. Nycodenz stock solution

- dissolve 138 g Nycodenz-powder in 500 ml buffered medium (see below) (density 1.15 g/ml at 20°C)

- autoclave for 20 min at 120°C and store at 4°C

Buffered medium:

- 5 mM Tris/HCl 605.7 mg/l, pH 7.5
- 3 mM KCl 223.7 mg/l
- 0.3 mM Ca Na_2EDTA 112.3 mg/l

2.3. Pyrimethamine-solution for intraperitoneal injection

Dissolve pyrimethamine in DMSO to a desirable stock solution. Dilute the stock solution 20 times with PBS to 5%DMSO for avoiding DMSO toxicity in mice.

2.4. *P. berghei* freezing solution

30% glycerol in PBS. Autoclave

2.5. Parasite lysis buffer

10 mM Tris pH8.0, 0.4 M NaCl, 1 mM EDTA, 1%SDS

2.6. 0.2% Saponin

Dissolve 0.2 g saponin in 100 ml RPMI-Hepes, filter sterilise and store at 4°C

3. Preparation of solutions for Southern blot analysis

3.1. 10X Maleic Acid Buffer

For 500ml:

58 g Maleic Acid (final conc. 10M)

43.8 g NaCl (final conc. 1.5M)

adjust the pH to 7.5 with NaOH (pH changes quickly)

Autoclave

3.2. 1X Maleic Acid Buffer

Dilute the 10 x Maleic Acid Buffer in DDW

3.3. 20X SSC

For 1 liter

175.3 g NaCl (final conc. 3M)

88.2 g Sodium Citrate-$2H_2O$ (final conc. 0.3M)

3.4. Washing Buffer

1 x Maleic Acid, 0.3% Tween 20

3.5. Denharts Hybridization Solution

6X SSC, 5X Denharts, 0.1%SDS

3.6. 10X Blocking Solution

Dissolve Blocking Reagent 10% (w/v). (bottle 4 of DIG kit) in 1X Maleic Acid Buffer at 65°C. Place on stirrer and mix. Autoclave and store at 4°C.

3.7. 1X Blocking Solution

Dilute the 10X Blocking Solution with 1XMaleic Acid. Store at 4°C

3.8. Detection Buffer

0.1 M Tris HCl, 0.1 M NaCl, pH 9.5

3.9. CSPD (vial 5) 100X

Thaw 100X CSPD (vial 5 from DIG kit) when the kit arrives and make 20 µl aliquots. Freeze these aliquots. Avoid repeated freeze/thaw cycles. Twenty microliter diluted 1:100 with Detection Buffer make up 2 ml (enough for the chemiluminescent detection of a 10 × 10 cm memebrane).

Appendix B

ED_{50} of pyrimethamine against transgenic parasites

Table S-1 Mean ED_{50} of pyrimethamine against PbGFP, *PbPf*S108N, *PbPf*DHFR3m1 and *PbPf*DHFR3m2 transgenic parasites.

Parasite line	ED_{50} value (mg/kg)			Mean of ED_{50} ± SD.
	Exp. I	Exp.	Exp.	
PbGFP	0.01	0.02	0.02	0.02 ± 0.01
*PbPf*S108N	1.10	0.84	1.30	1.08 ± 0.23
*PbPf*DHFR3m1	2.35	1.52	0.96	1.61 ± 0.70
*PbPf*DHFR3m2	1.53	0.83	0.87	1.07 ± 0.39

Appendix C

Point mutations of DHFR enzymes that confer resistance to antifolate drugs

Table S-2 Point mutations of DHFR enzymes that confer resistance to antifolate drugs found in transgenic parasites *PbPf*DHFR3m1 and *PbPf*DHFR3m2 and in previously reported non-*Plasmodium* system and field isolates.

Parasite line	Mutations of DHFR	References
*PbPf*DHFR3m1 (M55I+S108N+S189C)	M55 corresponds to F31of *h*DHFR. F31A, F31G, F31S	(Chunduru et al., 1994)
	F31P	(Volpato et al., 2007)
	F31R	(Patel et al., 1997)
	N51I+S108N+S189R	(Ferlan et al., 2001)
*PbPf*DHFR3m2 (C50Y+S108N+F116S)	C50R+N51I+S108N+ DHPS mutation (A437G+K540E+A581G) C50R+N51I+S108N+I164L+ BR+DHPS mutation (A437G+K540E+A581G)	(Vasconcelos et al., 2000)
	N51I+C59R+S108N+C50S	(Hankins et al., 2001)
	C50Y, C50N, C50S	(Japrung et al., 2007)

Table S-2 Point mutations of DHFR enzymes that confer resistance to antifolate drugs found in transgenic parasites *PbPf*DHFR3m1 and *PbPf*DHFR3m2 and in previously reported non-*Plasmodium* system and field isolates (continued).

Parasite line	Mutations of DHFR	References
	C50R, C50R+C59R	(Chusacultanachai et al., 2002)

BR: Bolivia repeat, a five amino acid repeat insertion between codons 30 and 31.

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