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# Identification of protein-protein interactions between plasmodium falciparum and the human erythrocyte membrane protein 4.1

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# APPENDIX

## A-1 Reagents and standard laboratory methods

### A-1.1 Laemmli SDS-PAGE

The Laemmli gel (Laemmli, 1970) consists of a 4% stacking gel which is layered on top of a 12% resolving gel. Stock solutions are indicated in the reagent column below and final gel composition in the resolving and stacking columns.

| Reagent | Resolving gel | Stacking gel |
|---|---|---|
| 30% acrylamide (w/v) | 12ml (4ml) | 1.3ml (0.65ml) |
| 1% bis-acrylamide (w/v) | 3.2ml (1.1ml) | 1ml (0.5ml) |
| 4X resolving buffer (1.5M Tris-HCl, pH 8.8) | 7.5ml (2.5ml) | - |
| 4X stacking buffer (0.5M Tris-HCl, pH 6.8) | - | 2.5ml (1.3ml) |
| 10% SDS (w/v) | 160µl (55µl) | 20µl (10µl) |
| Water | 7ml (2.3ml) | 4.9ml (2.5ml) |
| 10% (w/v) ammonium persulphate (fresh) | 200µl (65µl) | 200µl (100µl) |
| TEMED | 15µl (5µl) | 7.5µl (4µl) |

The values indicate the volumes used preparing Sturdier vertical slab gels, while those in parentheses indicate the composition of Mighty Small Dual Tray gels.

Gels cast in a Mighty Small Dual tray were electrophoresed in chilled Laemmli running buffer (25mM Tris, 191mM glycine, 0.1% SDS) for 120 minutes at 30mA with cooling at 4$^{o}$C. Larger Sturdier vertical slab gels were electrophoresed at 75V overnight at room temperature.

### A-1.2 Fairbanks SDS-PAGE

This system (Fairbanks et al., 1971) was set up in a Hoefer Sturdier vertical slab gel unit (SE 400) and comprised an exponential acrylamide gradient ranging from 3.5-17%. The gradient was established using a Hoefer SG series gradient maker. A plunger was inserted into the right hand chamber holding 17% acrylamide, which prevented the contents from mixing with the 3.5% acrylamide in the left chamber. The gradient maker was connected to a peristaltic pump and the gel poured at a rate of 2.5ml/minute. The Fairbanks gel comprises the following components:

| Reagent | 3.5 % acrylamide | 17% acrylamide |
|---|---|---|
| 40% acrylamide, 1% bis (w/v) | 3ml | 3.4ml |
| 10X Fairbanks gel buffer | 3.4ml | 800μl |
| 10% SDS (w/v) | 680μl | 160μl |
| 25% glycerol (w/v) | - | 2ml |
| 10% (w/v) ammonium persulphate (fresh) | 400μl | 20μl |
| Water | 26.9ml | 1.62ml |
| TEMED | 11μl | - |
| 0.5% TEMED (w/v) | - | 500μl |

Gels were electrophoresed at 45V in 1X Fairbanks buffer (40mM Tris, 20mM sodium acetate, 2mM EDTA, glacial acetic acid to pH 7.4) containing 0.1% SDS overnight at room temperature.

### A-1.3 Preparation of samples for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

One hundred and fifty μl aliquots of membrane and protein extracts were solubilised in Eppendorf tubes containing 40μl 5X suspension solution (50mM Tris-HCl, pH 8.0, 5mM EDTA, 5% SDS, 25% sucrose), 5μl sucrose loading dye (2.5% sucrose, 0.05% bromophenol blue) and 2% final volume of β-mercaptoethanol. The contents were boiled for 1 minute and stored at -20$^{o}$C.

### A-1.4 Silver staining of SDS-PAGE gels

This highly sensitive method relies on the ability of amino acids to bind silver ions (Wilson, 1994). Following SDS-PAGE, proteins were fixed with 10% acetic acid/ 10% methanol for 30 minutes. The gel was cleared of fixing solution by washing in several changes of MilliQ water including an overnight wash. The next day, the gel was soaked for 30 minutes in MilliQ water containing DTT (0.005g/ml) and then in 0.1% silver staining solution (0.05g silver nitrate in 50ml water) for 30 minutes. To remove excess silver, the gel was rinsed briefly in MilliQ water followed by developing solution (3% sodium carbonate, 0.01% formaldehyde) for 1 minute. Fresh developer was added and closely monitored for the development of protein bands. The reaction was stopped with copious amounts of MilliQ water.

### A-1.5 Protein transfer and immunoblot

Polyacrylamide gels were placed in Coomassie Blue stain (250ml methanol, 100ml acetic acid, 0.5g Coomassie brilliant blue R-250 in a final volume of 1000ml MilliQ water) and destained thoroughly with 10% acetic acid until the background was clear. To remove the acetic acid, they were washed in several changes of MilliQ water including an overnight wash. Gels were incubated in a SDS buffer (25mM Tris, 192mM glycine, 1%

SDS) for 1 hr at 4$^{o}$C. Gels not stained with Coomassie blue were soaked for 10 minutes at room temperature in transfer buffer. A piece of Hybond-C nitrocellulose membrane and two pieces of filter paper were cut to the same size of the gel and equilibrated in transblot buffer (25mM Tris, 192mM glycine, 20% methanol) for 30 minutes. A piece of filter paper was placed on one of the sponges of the Hoefer wet blot cassette, followed by the gel, nitrocellulose membrane and second filter paper. A glass rod was used to remove air bubbles trapped between the layers. The cassette was closed and placed in the transfer tank with the membrane facing the anode. Proteins were transferred in pre-cooled transblot buffer with gentle stirring at 50V for two hrs at 8$^{o}$C.

Following the transfer, the blot was rinsed in Tris buffered saline (TBS: 0.05M Tris-HCl, 0.9% NaCl, pH 7.5) and then incubated in 3% bovine serum albumin (BSA) in TBS for 1 hr at room temperature. The blot was incubated overnight with primary antibody diluted in TBS, 1% BSA, and washed three times in TBS, 0.05% Tween 20 for five minutes with vigorous shaking. The membrane was subsequently incubated for 1 hr with a secondary antibody, which was also diluted in TBS, 1% BSA. The blot was washed as before and protein bands visualised using 4-chloro-1-naphthol for unstained gels or 1, 3, diaminobenzidine for stained gels. 15 mg 4-chloro-1-naphthol was dissolved in 5ml methanol and added to 25ml TBS, whereas 5mg diaminobenzidine was dissolved for 30 minutes in 25ml TBS in the dark with rapid stirring. The colour reaction was initiated by adding 12.5μl 30% hydrogen peroxide to the different substrate solutions and these were used to cover the blot. The reaction was stopped by rinsing the membrane in water.

### A-1.6 Incomplete RPMI culture medium

Sixteen grams RPMI 1640 medium (+ 25mM HEPES, + L Glutamine, - sodium bicarbonate) (Gibco$^{TM}$), 4.0g glucose, 44mg hypoxanthine and

50μg/ml gentamycin in 1000ml MilliQ water. The medium was sterilised by filtration through 0.22μm filter units with the aid of a peristaltic pump and stored at 4$^{o}$C.

### A-1.7 Complete RPMI culture medium

Human AB plasma was obtained from the South African Blood Transfusion Service (Johannesburg General Hospital, South Africa) and inactivated at 56$^{o}$C for 2 hrs. The plasma was centrifuged at 1 100 *g* for 15 minutes and the supernatant stored at -70$^{o}$C. 20% complete culture medium was prepared by supplementing 40ml incomplete RPMI medium with 10ml inactivated plasma as well as 2.1ml sterile 5% sodium bicarbonate. A 10% complete solution comprised 90ml incomplete RPMI medium, 10ml AB plasma and 4.2ml of sodium bicarbonate. The different media were stored at 4$^{o}$C and used within 4 days.

### A-1.8 Microscopic analysis of stained blood smears

An aliquot of *P. falciparum* culture was removed from a tissue flask using a sterile glass Pasteur pipette and a smear prepared on a glass slide. The Rapid Haematology Staining Kit was used for fixing and staining. Alternatively, the smear was fixed for 10 seconds in methanol and stained for 15 minutes in 20% Giemsa solution. The solution was prepared by diluting Giemsa stain 1:5 in a phosphate buffer containing 3.5g $KH_2PO_4$ and 14.5g $Na_2HPO_4.2H_2O$ per 1000ml MilliQ water. Excess stain was removed by washing the slides under running water. At least three fields were examined on the slide with a Zeiss Axiostar microscope under oil immersion at 1000X magnification. The parasitaemia was calculated as an average percentage of infected erythrocytes in the total number of erythrocytes.

### A-1.9 Phenol-chloroform extraction and ethanol precipitation of nucleic acids

This protocol describes a general method for the purification of small volumes of nucleic acid solutions in 1.5ml Eppendorf tubes (Moore and Dowhan, 2004). DNA and RNA were extracted from solution using either buffered phenol pH 8.0 or phenol pH 4.2 respectively.

Solutions were adjusted to an appropriate volume with TE buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA) followed by the addition of an equal volume of 1:1 phenol/chloroform. The contents of the tube were mixed thoroughly and micro-centrifuged for one minute to separate the aqueous and organic phase. The top phase containing the DNA or RNA was transferred to a new tube and 100μl TE buffer added to the remaining organic phase to improve recovery. This was centrifuged and the supernatant pooled with that from the first extraction. An equal volume of chloroform was then added to remove any residual phenol and centrifuged briefly. One tenth volume of 3M sodium acetate pH 5.2 and two and a half volumes ice-cold 100% ethanol were added to the supernatant and precipitated on ice water for 2hrs or overnight at -20$^{o}$C. The precipitate was pelleted at top speed for 30 minutes in a Sorvall RMC14 refrigerated micro-centrifuge and the pellet washed in 70% ethanol to remove salt and small organic molecules. The pellet was air-dried and dissolved in an appropriate volume of nuclease-free water. DNA and RNA were stored at -20$^{o}$C and -70$^{o}$C respectively.

### A-1.10 DNA sequencing and preparation of acrylamide gels for electrophoresis

*DNA sequencing:* PCR products were prepared for sequencing using a PCR product pre-sequencing kit. 5μl reactions were mixed with 1U ExoSAP and incubated at 37$^{o}$C for 15 minutes, after which the enzymes were inactivated at 80$^{o}$C for the same amount of time. Exonuclease I (Exo)

is responsible for removing any residual single stranded primers, whereas Shrimp Alkaline Phosphatase (SAP) dephosphorylates remaining dNTP from the PCR reaction.

The DNA sequence and reading frame of *P. falciparum* inserts were determined using the Sequenase® 2.0 sequencing kit as per manufacturer's instructions. In brief, 2.5-4pmol T7SelectUP primer was added to the pre-treated PCR products, boiled for three minutes to denature the DNA template and then snap-cooled in ice water to promote primer annealing. A cocktail containing 1μl Sequenase buffer, 0.5μl 0.1M DTT, 1μl 1:5 diluted dNTP, 2.5μCi [$\alpha^{32}$P] dATP (800Ci/mmol, 10μCi/μl) and 1μl 1:7 diluted Sequenase DNA polymerase was prepared and added to the PCR/primer samples. The mixture was placed at room temperature for 2 minutes, followed by transferring 1.8μl aliquots to four tubes each containing 1.3μl ddNTP. Samples were incubated at 37$^{o}$C for 5 minutes and the reaction stopped with 2μl stop solution (25mM EDTA pH 7.5, 95% deionised formamide, 0.1% bromophenol blue, 0.1% xylene cyanol FF). The sequencing reactions were resolved on 8% acrylamide gels and DNA visualised by exposure to X-ray film (2.5.1.3).

*Preparation of acrylamide gels and DNA electrophoresis:* Glass sequencing plates (30cm x 40cm) were washed with Extran and rinsed with MilliQ water followed by ethanol. The plates were assembled with 0.4mm spacers and two 19 well combs in an EZCast gel casting boot. An 8% denaturing acrylamide gel (total volume: 60ml) was prepared as follows from stock solutions indicated in the reagent column:

| Reagent | Gel composition |
|---|---|
| 40% acrylamide (w/v) | 12ml |
| 2% bis-acrylamide (w/v) | 12ml |
| Urea | 24g |
| 10X TBE | 6ml |
| Water | 11.2ml |
| 10% (w/v) ammonium persulphate (fresh) | 480µl |
| TEMED | 12µl |

A sequencing gel was assembled in a BRL S2 electrophoresis model. The gel was heated at 60W for 1 hr in 1X TBE running buffer (10X TBE: 0.9M Tris, 0.9M boric acid, 2mM EDTA), samples loaded and then electrophoresed at the same power for 2 hrs. The gel was removed from the plates and exposed to X-ray film overnight in a cassette at -70$^{o}$C. The film was developed with a Kodak processor in the casualty ward of the Johannesburg General Hospital, South Africa.

### A-1.11 Genomic DNA extraction from human white blood cells

Venous blood was collected in an acid citrate dextrose tube and centrifuged in a Jouan BR3.11 at 1 100 *g* for 10 minutes at 4$^{o}$C. A 100µl aliquot of the buffy coat was removed and the contaminating erythrocytes lysed with 400µl 0.17M ammonium chloride (Talmud et al., 1991). The mixture was incubated at room temperature for 20 minutes and centrifuged in a Sorvall RMC14 microcentrifuge at top speed for 1 minute. The supernatant was discarded and the white blood cell pellet washed three times with 0.9% saline. The pellet was resuspended in 100µl 0.05M

sodium hydroxide and boiled for 10 minutes to release the DNA. The solution was neutralised with 12.5μl 1M Tris-HCl pH 8.0.

### A-1.12 Plasmid DNA purification: alkaline lysis method

The underlying principle of alkaline lysis involves the selective denaturation of high molecular weight bacterial DNA. The method exploits both size and conformational differences between chromosomes and plasmids (Engebrecht et al., 2004).

Overnight *E. coli* cultures were pelleted at 15 000 *g* in a Sorvall RMC14 microcentrifuge at 4$^{o}$C for 1 minute. The cells were resuspended in 100μl ice-cold lysis buffer (25mM Tris-HCl pH 8.0, 10mM EDTA, 50mM D(+) glucose) and incubated at room temperature for 5 minutes. 200μl fresh 0.2M sodium hydroxide, 1% SDS solution was added to denature the genomic DNA. This was followed by the addition of 50μl ice-cold potassium acetate (pH 4.8). Nuclear material and cellular proteins were allowed to precipitate on ice for 15 minutes, and then centrifuged for five minutes at 15 000 *g* to clarify. The supernatant was collected and plasmid DNA purified by phenol-chloroform extraction and ethanol precipitation (A-1.9).

The DNA precipitate was resuspended in 100μl TE buffer containing 0.5μg DNase-free RNase (500μg/ml). The sample was incubated at 37$^{o}$C for 30 minutes to degrade contaminating RNA, after which 40μl 5M potassium acetate and 260μl water were added. DNA was extracted with phenol-chloroform, precipitated with ethanol and the purified plasmid resuspended in 50μl TE buffer for storage at -20$^{o}$C.

### A-1.13 SOC medium

SOB medium was prepared by combining 20g tryptone, 5g yeast extract and 0.5g sodium chloride in a final volume of 1000ml MilliQ water. The mixture was sterilised by autoclaving at $120^{o}C$, $15lb/in^{2}$. Prior to use, sterile filtered 1M $MgCl_2$ and 1M $MgSO_4$ were added to a final concentration of 10mM. SOC medium was prepared immediately before use by adding 2ml sterile 20% (w/v) glucose solution to 98ml SOB medium.

## A-2 Sequences and vector maps

### A-2.1 Two-base anchored primers

5’ TTT TTT TTT TTT VN 3’

V = A, G and C

N = A, G, C, and T

### A-2.2 Spectrin and band 3 primers for size standards

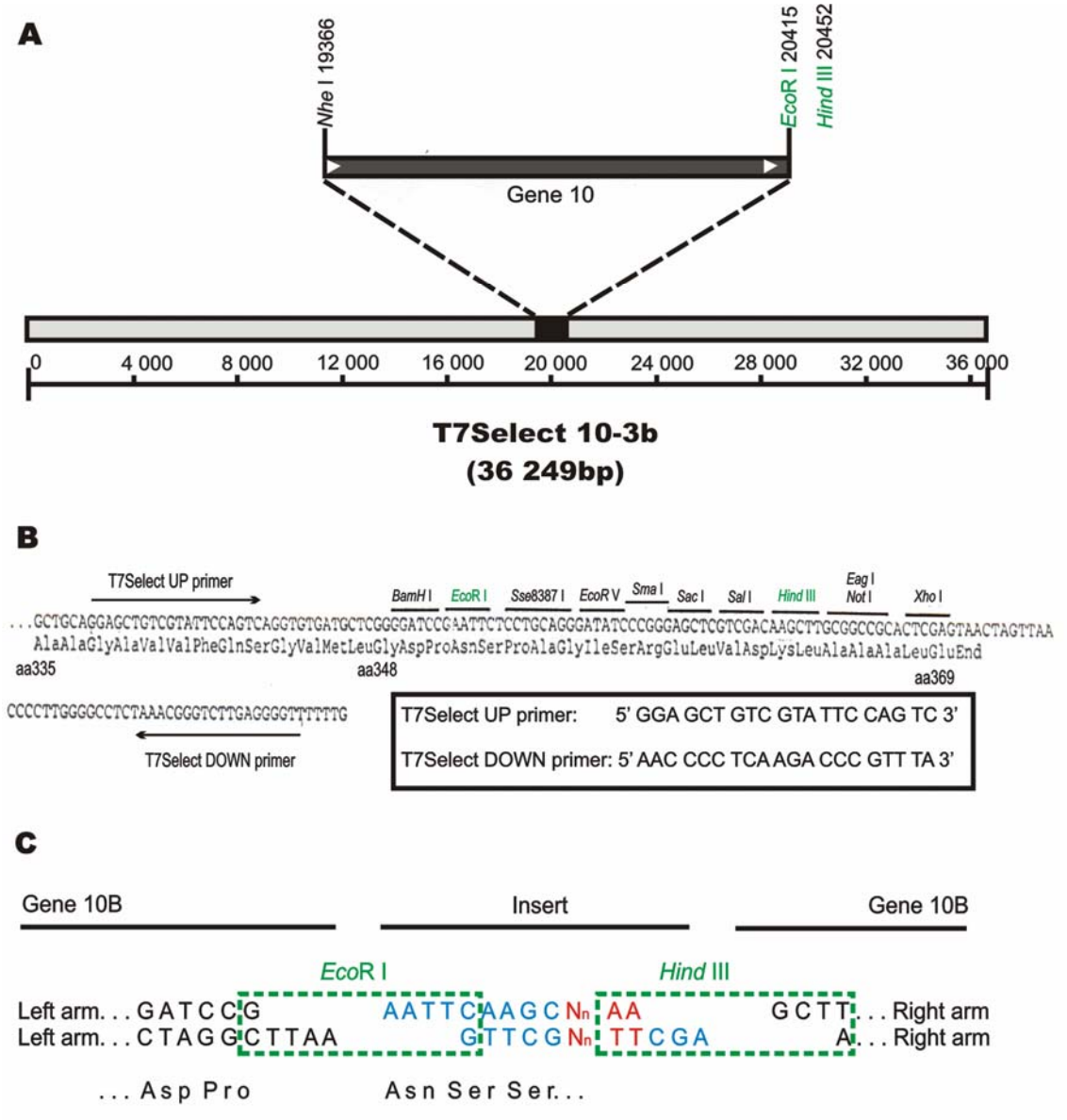
| Primer sequence (5’-3’) | Exons amplified | Product size |
|---|---|---|
| **F1:** TAGGGTCTGCTCTGAGGCAAT<br>**R1:** CACATATAAGCGGGGCAACAT | 2 | 347bp |
| **F2:** GTGTTTTATTCCCAGCCCCA<br>**R2:** CCCCCTTTACAGGTGAGCAA | 18 and 19 | 722bp |

Primers F1 and R1 are specific for sequences in exon 2 of human erythrocyte $\alpha$-spectrin, whereas F2 and R2 anneal to sequences in exons 18 and 19 of band 3. **F**, forward primer and **R**, reverse primer.

### A-2.3 T7Select 10-3b vector map and cloning cassette

The T7Select vector is a linear double stranded DNA sequence consisting of 36 249bp (**A**). The vector is provided as *Eco*R I/*Hind* III digested arms ready for directional cloning of appropriate inserts. To provide compatibility, inserts are digested with *EcoR* I and *Hind* III and then cloned in a multiple cloning cassette in frame with gene 10B directly after amino acid 348 (**B**). The first codon is GAT (aspartic acid) followed by CCG (proline) (**C**). Sequences in blue are provided by appended *Eco*R I/*Hind* III directional cloning linkers, whereas the nucleotides in red are provided by parasite inserts ($N_n$ = *P. falciparum* cDNA sequence). The adenosine and thymine residues (red) are provided by the 3’ end of the parasite cDNA strand.

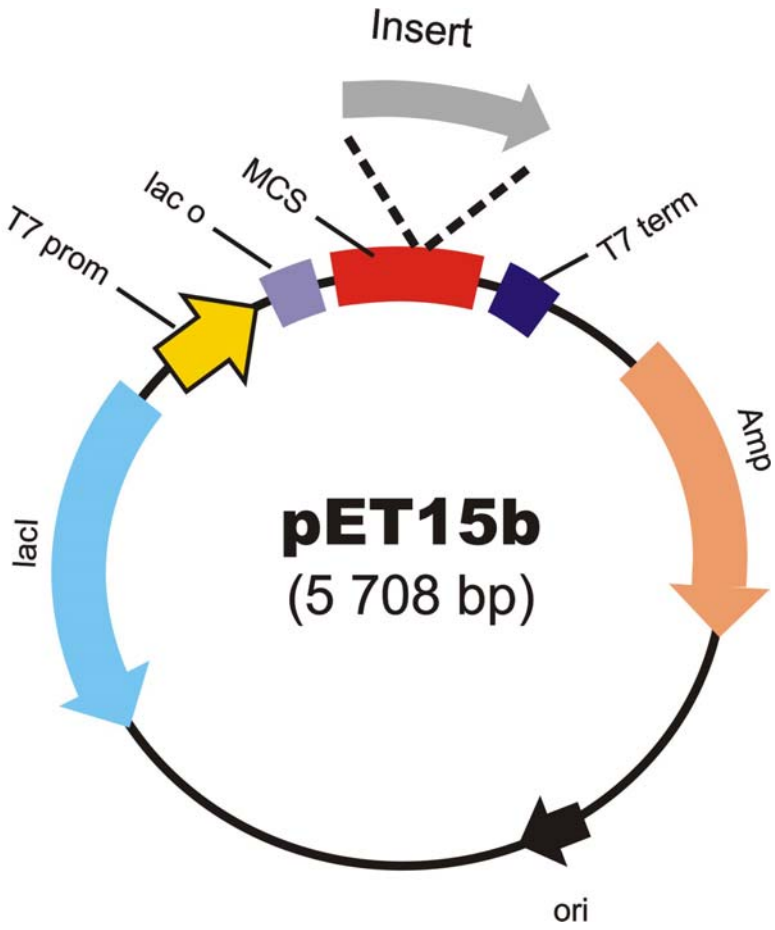
Ligation of insert and vector reconstitutes the *Eco*R I and *Hind* III restriction endonuclease recognition sequences.



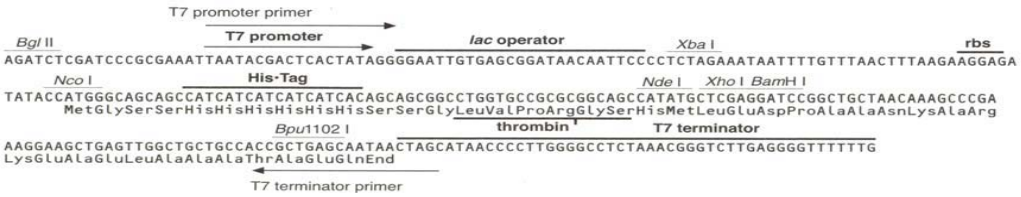

### A-2.4 pET15b vector map and cloning cassette

The pET vector is a powerful system for cloning and expressing recombinant proteins in *E. coli*. Protein expression is driven by a T7 promoter (T7 prom), which contains a downstream *lac* operator (*lac* o) sequence. pET15b houses a multiple cloning site (MCS) or cassette, T7 terminator sequence (T7 term), *lac*I repressor sequence and ampicillin

resistance gene (Amp). The origin of replication (ori) is also indicated in the vector map below.

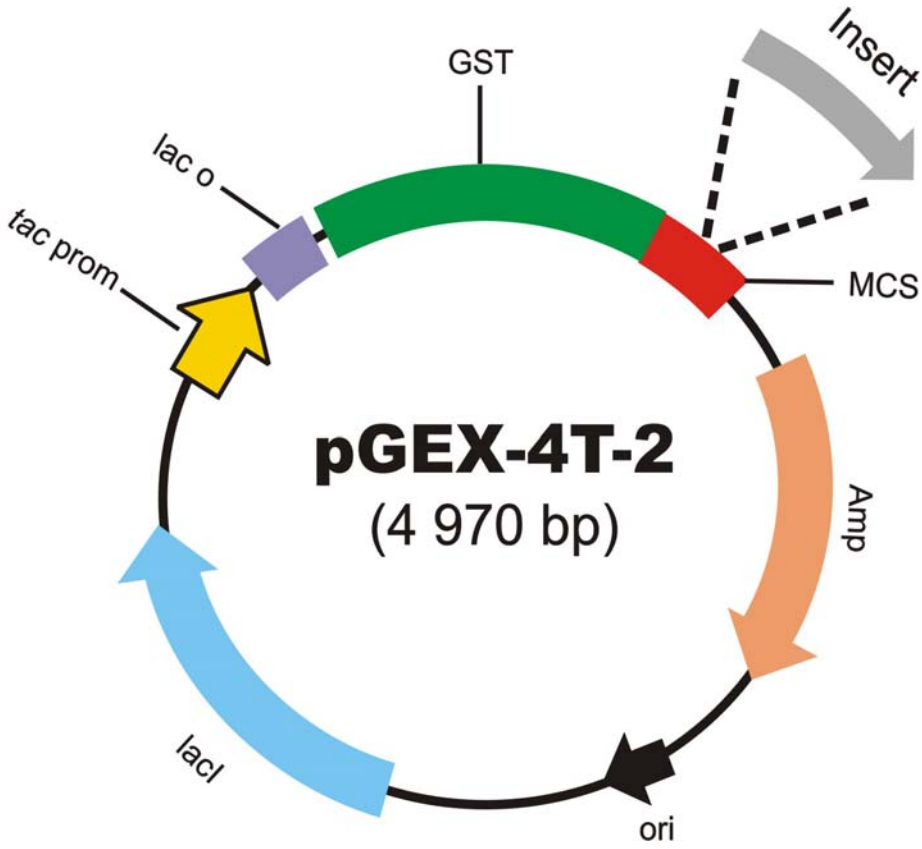



Vector sequence incorporating the T7 promoter, 5' His-tag, *Nde* I and *Bam*H I cloning site and T7 terminator is indicated below:

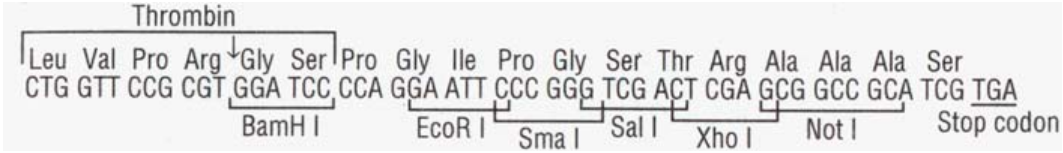


### A-2.5 pGEX-4T-2 vector map and cloning cassette

The pGEX-4T-2 vector offers a *tac* promoter (*tac* prom) for chemically inducible, high level expression of recombinant proteins. A *lac* operator sequence (*lac* o) is situated downstream of the promoter followed by a region encoding GST. A multiple cloning site (MCS: nucleotides 930-967) or cassette contains six restriction sites, which facilitates the unidirectional cloning of appropriate inserts. A thrombin site is located immediately after the GST region and allows cleavage of fusion proteins with thrombin. The β-lactamase gene region (Amp), *lac* I repressor region and origin of plasmid replication (ori) is indicated.



The thrombin recognition sequence and *Bam*H I/*Xho* I cloning sites used in this study are indicated below:




5’ pGEX-4T-2 sequencing primer: GGGCTGGCAAGCCACGTTTGGTG

3’ pGEX-4T-2 sequencing primer: CCTCTGACACATGCAGCTCCCGT

### A-2.6 PCR primers for amplification of 4.1R domain sequences

| Domain | PCR primer (5’ – 3’) | RE |
|---|---|---|
| 10kDa | **F**: CATG*GGATCC* TGGAAGAAAAAGAGAGAAAG | *Bam*H I |
| | **R**: CATG*CTCGAG*TCA GGGTGAGTGAGTGGATAAG | *Xho* I |
| 16kDa | **F**: CATG*GGATCC* TTTCGATACAGTGGCCGGACT | *Bam*H I |
| | **R**: CATG*CTCGAG*TCA TGCTTCTGTGGGCTCTGGCT | *Xho* I |
| 22kDa | **F**: CATGGGATCC TTCCGAACTCTTAACATCAATGGGCAAA | *Bam*H I |
| | **R**: CATGCTCGAGTCA CTCATCAGCAATCTCGGTCTCC | *Xho* I |
| 30kDa | **F**: CATGGAATTC ATGCACTGCAAGGTTTCTTTGT | *Eco*R I |
| | **R**: CATGCTCGAGTCA TTTGGATCCTAGCGCAAG | *Xho* I |

Forward (F) and reverse (R) primer sequences were designed for amplifying the different human 4.1R domain sequences. Endonuclease recognition sequences are underlined and the corresponding restriction enzyme (RE) indicated. Nucleotides highlighted in yellow were additional bases required for efficient digestion, while those highlighted in blue indicate a stop codon.

### A-2.7 Spectrin/4.1R♠ and 4.1R♣ specific *P. falciparum* binding peptides and corresponding nucleotide sequences

The letter N in the DNA sequence represents any of the four nucleotides due to unclear sequencing data. Nucleotides in green represent portions of the cDNA inserts that were sequenced and submitted to PlasmoDB for bioinformatic analyses. The sizes of the interacting peptides and corresponding nucleotide sequences were estimated by agarose gel electrophoresis of the cDNA inserts (Figure 19).

*PFB0150c*♣ *(clone 1: putative protein kinase)*

NSLQCDLYFLDDNKEKDVSKKRKAQLKDEY (30 amino acids)

AATTCTTTACAATGTGATTTATATTTTTTGGATGACAACAAAGAAAAAG
ATGTTAGTAAGAAAAGAAAAGCTCAATTGAAGGATGAATAT

*PF07_0128*♣ *(clone 2: EBA-175)*

CENEISVKYCDHMIHEEIPLKTCTKEKTRN (30 amino acids)

TGTNANAACGAAATTTCTGTAAAATATTGTGACCATATGATTCATGAAG
AAATCCCATTAAAAACATGCACTAAAGAAAAAACAAGAAAT

*PFA0125c*♠♣ *(clone 3: EBA-181)*

NSVVDRATDSMNLDPEKVHNENMSDPNTNT (30 amino acids)

AATTCTGTTGTAGATAGAGCAACAGATAGTATGAATTTAGATCCTGAA
AAGGTTCATAATGAAAATATGAGTGATCCAAATACAAATACT

*PF14_0201*♠♣ *(clone 4: Hypothetical protein)*

EELQQNDEDAESLTKENSKSEEQENEDSTDAEAIDKEEVETEEKGKDEQ
KKDEQKEQDEE (60 amino acids)

NAAGAATTACAACAAAATGATGAAGATGCTGAATCTCTAACAAAGGAA
AATTCAAAATCGGAAGAACAAGAAAATGAAGATTCAACAGATGCTGAA
GCTATTGACAAAGAAGAAGTAGAAACAGAAGAAAAAGGAAAAGATGA
ACAAAAAAAAAGACGAACAAAAAGAACAAGATGAAGAA

*PFE0570w*♣ *(clone 5: Hypothetical protein)*

TNYYNMREKNIYNILCNNDSNNYVLFNSNEKYSMNNKISNSILSNMIMNK
QDNNNININQNNNNNNNNNMNEGGSETLYSSFTKEIEKLKKEVRKCEES
Y (100 amino acids)

ACCAATTATTACAATATGAGAGAAAAGAATATATATAATATATTATGTA
ATAATGATAGTAATAATTATGTTCTATTTAATAGTAATGAAAAATATTCT
ATGAATAATAAGATTAGTAATTCAATATTGAGTAATATGATAATGAATA
AACAAGATAATAATAATATAAATATTAATCAAAATAATAATAATAATAAT
AATAATAATATGAATGAAGGAGGAAGCGAAACATTATATAGCAGCTTT
ACTAAAGAAATTGAAAAGTTAAAAAAGGAAGTTAGGAAGTGTGAAGAA
AGTTAT

*PFA0420w*♣ *(clone 6: Hypothetical protein)*

EEVQGEEAQGEEVQGEEVQGEEAHEEPANDEPA (33 amino acids)

NAAGAGGTACAAGGAGAAGAGGCACATGAAGAACCAGCAAATGACG
AACCAGCAAATGACGAACCAGCAAATGACGAGCCAGCAAATGAAGAG
CCAGCA

*PF11_0191*♣ *(clone 7: Hypothetical protein)*

KKKKNKNKKNKNKRKHDEDQHSEHSHQDQD (30 amino acids)

AAAAAAAAAAAAAATAAAAATAAAAAAAATAAAAATAAACGAAAACATG
ATGAAGACCAACACAGTGAACACAGTCACCAAGATCAAGAT

*MAL6P1.48*♣ *(clone 8: Hypothetical protein)*

NSKKKLLNNNINNNINTKKSGWDINHNNNE (30 amino acids)

AATTCAAAAAAAAAATTATTAAATAATAATATCAATAATAATATTAATAC
TAAAAAATCTGGATGGGATATCAATCATAATAATAATGAA

*MAL13P1.278*♠ *(Serine/ Threonine protein kinase)*

NSIYEKVNIDNDKVKKKNLHSINDKKIKIN (30 amino acids)

AATTCTATATATGAAAAGNTTAATATAGATAATGATAAGGTAAAAAAGA
AAAACTTACATTCAATAAATGATAAGAAAATTAAAATAAAT

*PFI1570c*♠ *(Putative aminopeptidase)*

NSQNKRDDQMCHSFNDKDVSNHNLDKNTIE (30 amino acids)

AATTCACAAAATAAAAGAGATGATCAGATGTGCCATTCGTTTAATGAT
AAAGATGTTAGTAATCATAATTTAGATAAAAATACAATTGAA

### A-2.8 Secondary structure of *P. falciparum* filament repeat motifs

SSPro software (http://www.igb.uci.edu/servers/psss.html) was used for predicting secondary structure of myosin and neurofilament-like motifs in *P. falciparum* 4.1R binding domains (Table 9).

H: alpha-Helix

C: coil

E: Beta sheet

The amino acids in pink and underlined represent 4.1R specific residues

*PFA0125c (clone 3: EBA-181)*

Myosin-like motif- amino acid position: 716-1235

QLDKLFKERCSCMDTQVLEVKNKEMLSIDSNSEDATDISEKNGEEELYVNHNSVSVASGNKE
CC HHHHHH H HCCC CC EEEE ECC CH E EEECCCC CCCCHHCHC CCCE EEE EE CCC EEE EC CCC C
IEKSKDEKQPEKEAKQTNGTLTVRTDKDSDRNKGKDTATDTKNSPENLKVQEHGTNGETIKE
HHCCCC CCCCHH HHH HCC CEEEE EE CCC C CCC C CCC CCCCCC CCCC CCEE EE CC CC CCEECC
EPPKLPESSETLQSQEQLEAEAQKQKQEEEPKKKQEEEPKKKQEEEQKREQEQKQEQEEE
CCC CCCCC HHHHH HHH HHHHHH HH HH H HCCC CCC CCCCCCC CH HHH HHH HH HHH HH HHHH
EQKQEEEQQIQDQSQSGLDQSSKVGVASEQNEISSGQEQNVKSSSPEVVPQETTSENGSS
H HH HHHH HHHHH HC CCC CCC CCC EE EECC C CCCCCC C CCCCC CC CCCE CCCC CCCC CCC C
QDTKISSTEPNENSVVDRATDSMNLDPEKVHNENMSDPNTNTEPDASLKDDKKEVDDAKKE
C CCEECCCCC CCC CEEC CCCC C CC CCC CHCCC CC CCCC C CCCCC CCCCC CC HHHH HHHHHH
LQSTVSRIESNEQDVQSTPPEDTPTVEGKVGDKAEMLTSPHATDNSESESGLNPTDDIKTTD
HH HHHHHHCCCCCC C CCCCC CCCCC EC CC CCC H HHHHCCCC CCC CC CCC CCCC CCCCCCCCC
GVVKEQEILGGGESATETSKSNLEKPKDVEPSHEISEPVLSGTTGKEESELLKSKSIETKGET
CC EEH EEHCCC CC CCCC CCCCCCC CCC CCCC CCCCCCCCCCCCC CCCH HHHHH CCCCECCCCC
DPRSNDQEDATDDVVENSRDDNNSLSNSVDNQSNVLNREDPIASETEVVSEPEDSSRIITTE
CC C CCC CCH CCCC HECC CC CC CC CCCC CCCC C CCCCC CCC CCCCCEEE ECCCCCCCEEEEEE
VPSTTVKPPDEKRSEEVGEKEAKEIKVE
CCC CCCCCCC CCC CHH H CHHH HHEEECC

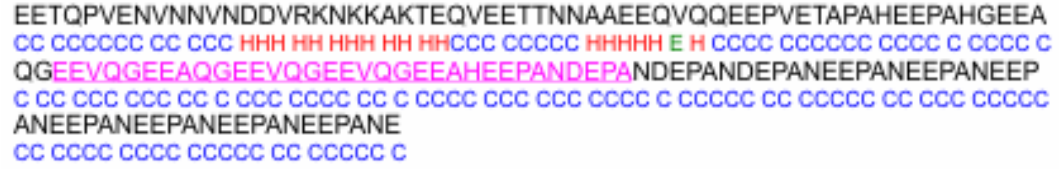
*PF14_0201 (clone 4: Hypothetical protein)*

Myosin-like motif- amino acid position: 405-944



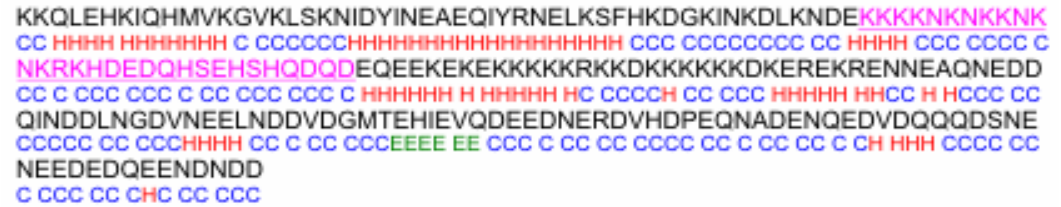

*PFA0420w (clone 6: Hypothetical protein)*

Neurofilament-like motif- amino acid position: 38-165




*PF11_0191 (clone 7: Hypothetical protein)*

Myosin-like motif- amino acid position: 400-594



## A-2.9 *P. falciparum* peptide sequences selected for cloning into protein expression vectors

Primers utilised for the PCR amplification of annotated protein regions are shown in the 5'-3' orientation. Underlined nucleotides in the primers represent restriction endonuclease recognition sequences. Stop codons are highlighted in yellow. The 4.1R binding sequence in each annotated region is shown in pink and underlined.

*PF07_0128 (GST-EBA-175)*

1282-HGNRQDRGGNSGNVLNMRSNNNNFNNIPSRYNLYDKKLDLDLYENRNDSTTKE LIKKLAEINKCENEISVKYCDHMIHEEIPLKTCTKEKTRNLCCAVSDYCMSYFTYDSEEYY NCTKREFDDPSYTCFRKEAFSSM-1423

Forward primer: CATGGGATCCATTAATCATAGTCATCATGGAAAC

*Bam*H I

Reverse Primer: CATGCTCGAGTCACATACTTGAAAAAGCCTCC

*Xho* I

*PFA0125c (6His- EBA-181)*

945-PEVVPQETTSENGSSQDTKISSTEPNENSVVDRATDSMNLDPEKVHNENMSDPN TNTEPDASLKDDKKEVDDAKKELQSTVSRIESNEQDVQSTPPEDTPTVEGKVGDKAEML TSPHATDNSESESGLNPTDDIKTTDGVVKEQEILGGGESA-1097

Forward primer: CATGCATGCATATGCCTGAAGTAGTTCCACAAGAA

*Nde* I

Reverse primer: CATGGGATCCTTATGCACTTTCACCTCCCCC

*Bam*H I

*PF11_0191* *(6His- Hypothetical protein)*

408-QHMVKGVKLSKNIDYINEAEQIYRNELKSFHKDGKINKDLKNDEKKKKNKNKKNKNK
RKHDEDQHSEHSHQDQDEQEEKEKEKKKKKRKKDKKKKKKDKEREKRENNEAQNEDD
QINDDLNGDVNEELNDDVDGMTEHIEVQDEEDNERDVHDPEQNADENQEDVDQQQDS
NENEEDEDQE-588

Forward primer: CATGCATGCATATGCAACACATGGTTAAGGGTGTC

*Nde* I

Reverse primer: CATGGGATCCTTATTCTTGATCTTCGTCCTC

*Bam*H I

*PF14_0201* *(6His- Hypothetical protein)*

735-SDDATHKETQEKSDQEPSQNIQEDNSDEKHAENEENVEQIETDSNVSEEANDEN
KDNMQTTTDEGTEELQQNDEDAESLTKENSKSEEQENEDSTDAEAIDKEEVETEEKGK
DEQKKDEQKEQDEEEDGEKENKHKSSETTNETVTDIEENKNEVKGEEHLQGSEQSIEA
SESSQKDETKETEDKEEYVNANDDESSEEDTTPNETNKTDNGSSFFFAMSNAL-957

Forward primer: CATGCATGCATATGAGCGACGATGCAACTCACAAA

*Nde* I

Reverse primer: CATGGGATCCTTAGAGTGCATTACTCATAGCAAA

*Bam*H I

*PFA0420w* *(6His-Hypothetical protein)*

25-PDLVTDENEKTNVEETQPVENVNNVNDDVRKNKKAKTEQVEETTNNAAEEQVQQEE
PVETAPAHEEPAHGEEAQGEEVQGEEAQGEEVQGEEVQGEEAHEEPANDEPANDEPA
NDEPANEEPANEEPANEEPANEEPANEEPANEEPANEEPANE-179

Forward primer: CATGCATGCATATGCCAGACCTTGTAACCGATG

*Nde* I

Reverse primer: CATGGGATCCTTATTCATTTGCTGGTTCTT

*Bam*H I

## A-3 List of suppliers of chemicals and equipment

| | |
|---|---|
| ACD vacutainer tubes | Becton Dickinson, UK |
| Acetone | Merck, USA |
| Acrylamide | BDH, UK |
| Agar | Oxoid, UK |
| Agarose | FMC, USA |
| Ammonium chloride | Sigma, USA |
| Ammonium persulphate | Stratagene, USA |
| Ampicillin | Sigma, USA |
| AMV reverse transcriptase | Promega, USA |
| β-mercaptoethanol | Merck, USA |
| Bactotryptone | Oxoid, UK |
| Beckman DU-65 spectrophotometer | Beckman, USA |
| Beckman J2-21 centrifuge and JA-17 rotor | Beckman, USA |
| Biotin-NHS ester | Roche, Germany |
| Bis-acrylamide | BDH, UK |
| BL21-CodonPlus® (DE3) RIL competent cells | Stratagene, USA |
| Blue/orange loading dye | Promega, USA |
| Bovine serum albumin | Pierce, USA |
| BRL model S2 electrophoresis apparatus | BRL, USA |
| Bromophenol blue | BDH, UK |
| BugBuster® HT | Novagen, USA |
| Chloramphenicol | Sigma, USA |
| 4-chloro-1-naphtol | Sigma, USA |
| Calf intestinal phosphatase | Roche, Germany |
| Chloroform | SMM Chemicals, RSA |
| Conical centrifuge tubes | Nunc, Denmark |
| Coomassie brilliant blue R-250 | BDH, UK |
| Coomassie® Plus Protein Assay reagent | Pierce, USA |
| Cryotubes | Nunc, Denmark |
| DC power supply PS 500X | Hoefer, USA |
| DEAE cellulose anion exchange matrix | Pharmacia, Sweden |
| DEPC | Sigma, USA |
| DH5α competent cells (subcloning efficiency) | GibcoBRL, USA |
| Dialysis snakeskin tubing | Pierce, USA |
| 1, 3, diaminobenzidine | Sigma, USA |
| Dithiothreitol | Roche, Germany |
| DMSO | BDH, UK |
| 100bp DNA ladder | Promega, USA |
| DNA mass ladder | GibcoBRL, USA |
| DNA MassRuler™ | Fermentas, Canada |
| Dynal® mRNA Direct Kit | Dynal, Norway |

| | |
|---|---|
| EDTA | Roche, Germany |
| EGTA | Merck, Germany |
| Eppendorf Mastercycler gradient thermal | Eppendorf, Germany |
| Eppendorf tubes | Eppendorf, Germany |
| Ethanol | BDH, UK |
| Ethidium bromide | Roche, Germany |
| Extran | Merck, Germany |
| EZCast Gel Casting Boot | GibcoBRL, USA |
| FastPlasmid™ Mini Kit | Eppendorf, Germany |
| Falcon tubes | Becton Dickinson, USA |
| Filter tips | QSP, USA |
| Filter paper | Whatman, USA |
| 0.22μm filters | Millipore, USA |
| Formaldehyde | Merck, USA |
| Gel Casting Tray | Hoefer, USA |
| Geldoc scanning system | Synoptics, UK |
| Gentamycin | Sigma, USA |
| Giemsa stain | Merck, Germany |
| Glacial acetic acid | Saarchem, RSA |
| D(+) Glucose | Saarchem, RSA |
| Glutathione, reduced | Sigma, USA |
| Glycerol | Sigma, USA |
| Glycine | BDH, UK |
| Goat anti-GST antibody | Amersham, UK |
| Goat anti-mouse peroxidase conjugated IgG | Sigma, USA |
| Goat anti-rabbit peroxidase conjugated IgG | Roche, Germany |
| Growth tubes | BD Biosciences, USA |
| Guanidium thiocyanate | Fluka, Switzerland |
| Heating block | Hagar, RSA |
| High Fidelity PCR Master Mix | Roche, Germany |
| His-Select™ magnetic agarose beads | Sigma, USA |
| Hoefer GS300 Scanning Densitometer | Hoefer, USA |
| Hoefer SG series gradient maker | Hoefer, USA |
| Hoefer Sturdier Vertical Slab Gel Unit (SE400) | Hoefer, USA |
| Hybond-C nitrocellulose membrane | Amersham, UK |
| Hydrochloric acid | Saarchem, RSA |
| Hydrogen peroxide | Merck, USA |
| Hypoxanthine | Sigma, USA |
| Imidazole | Sigma, USA |
| IPTG | Invitrogen, USA |
| Isopropanol | Saarchem, RSA |
| Jouan BR3.11 centrifuge | Jouan, France |

| | |
|---|---|
| Laminar flow hood | Labotec, RSA |
| Laurylsarcosine | Sigma, USA |
| LKB Redi fraction collector | Pharmacia, Sweden |
| MagneGST™ paramagnetic particles | Promega, USA |
| Magnesium chloride | Saarchem, RSA |
| Magnesium sulphate | Saarchem, RSA |
| Magnetic particle concentrator | Roche, Germany |
| Methanol | Saarchem, RSA |
| Mighty Small Dual Casting Tray | Hoefer, USA |
| Mineral oil | Sigma, USA |
| MiniElute™ Gel Extraction Kit | Qiagen, Germany |
| Mouse anti-His antibody | Amersham, UK |
| Oligonucleotide primers | Inqaba Biotech, RSA |
| Overnight Express™ Autoinduction system | Novagen, USA |
| Parafilm | Whatman, UK |
| Pefablock® SC | Roche, Germany |
| PCR Master Mix Kit | Roche, Germany |
| PCR product pre-sequencing kit | Amersham, UK |
| PCR tubes | QSP, USA |
| Peristaltic pump SJ-1211 | Atto Corporation, Japan |
| pET15b expression vector | Novagen, USA |
| pGEX-4T-2 expression vector | Amersham, UK |
| Phenol | ICN Biochemicals, USA |
| PMSF | Roche, Germany |
| Polycon A and Pre-fix X-ray solution | AXIM, RSA |
| Polyethylene glycol 20 000 | Fluka, Switzerland |
| Ponceau S | Sigma, USA |
| Potassium acetate | Saarchem, RSA |
| Potassium chloride | Saarchem, RSA |
| Potassium di-hydrogen orthophosphate | Saarchem, RSA |
| Promega Core® Buffer Systems | Promega, USA |
| Protease Inhibitor Cocktail Set III | Calbiochem, USA |
| Proteinase K | Roche, Germany |
| [$\alpha^{32}$P] dATP | Amersham, UK |
| QIAquick® PCR purification Kit | Qiagen, Germany |
| Rabbit anti-4.1R antibody | St. Elizabeth's Medical Centre, Boston, USA |
| Rabbit anti-goat peroxidase conjugated IgG | Sigma, USA |
| Rapid DNA Ligation Kit | Roche, Germany |
| Rapid Haematology Staining Kit | SAIMR, RSA |
| Restriction enzymes | Promega, USA |
| RNase-free DNase I | Roche, Germany |

| | |
|---|---|
| RNaseZap | Ambion, USA |
| RNasin® Ribonuclease Inhibitor | Promega, USA |
| Rosetta™ 2 (DE3) competent cells | Novagen, USA |
| RPMI culture medium | Invitrogen, USA |
| | |
| Saponin | USB, UK |
| SDS | BDH, UK |
| Sequenase® PCR product sequencing kit | Amersham, UK |
| Silver nitrate | Merck, USA |
| Slide-A-Lyzer dialysis (MINI) cassettes | Pierce, USA |
| Sodium acetate | BDH, UK |
| Sodium chloride | Saarchem, RSA |
| Sodium citrate | Saarchem, RSA |
| Sodium di-hydrogen orthophosphate | Saarchem, RSA |
| di-Sodium hydrogen orthophosphate | Saarchem, RSA |
| Sodium hydroxide | Saarchem, RSA |
| Sorvall RMC-14 centrifuge | Sorvall, USA |
| Streptavidin magnetic particles | Roche, Germany |
| Streptavidin peroxidase conjugate | Roche, Germany |
| Supersignal® West Pico Chemiluminescent Kit | Pierce, USA |
| | |
| TaKaRa Ex Taq | TaKaRa, Japan |
| TE Transphor Electrophoresis Unit | Hoefer, USA |
| TEMED | Promega, USA |
| Tris | BDH, UK |
| Tryptone | Sigma, USA |
| T7Select® OrientExpress cDNA Cloning system | Novagen, USA |
| T7Select® Phage Display system | Novagen, USA |
| Tween 20 | Merck, Germany |
| | |
| Urea | GibcoBRL, USA |
| UV Transilluminator | UVP, USA |
| | |
| X-ray film | Agfa, Germany |
| XL10-Gold β-mercaptoethanol mix | Stratagene, USA |
| | |
| Yeast Extract | Oxoid, UK |
| | |
| Zeiss Axiostar microscope | Zeiss, Germany |