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APPENDIX I

LIST OF EQUIPMENT AND CHEMICALS

1. Equipment for cultivation and sample preparation

   - Filtering apparatus to sterile RPMI medium and $NaHCO_3$
     - One-arm flask 1000 ml.
     - Filtering apparatus
     - Millipore filter type GS with pore size 22 um.
     - Whatman No. 3 filter paper
     - Silicone rubber tubing
     - Air pump
   - Laminar-flow hood
   - Autoclave
   - Hot air oven
   - Incubator maintained at 37 $^{\circ}$ c
   - Refrigerator 4°c for storing solutions, with freezing compartment for storage of serum
   - Centrifuge
   - Superspeed refrigerated centrifuge
   - Microscope with 100 x oil immerslon objective lens
   - Water bath with shaker
   - pH meter
   - Balance

- Magnetic stirrer
- Hot plate
- Multiblock heater
- Hand counter for counting parasites
- Liquid nitrogen (-178 $^{\circ}$ c) refrigerator
- Dessicator and lid with stopcock
- Stopcock grease
- White candles
- Alcohol lamp
- Cotton for plugging pipettes
- Tissue culture dishes (35 x 10 and 60 x 15 mm)
- Graduated cylinder (100, 1000 ml)
- Beakers (250 and 1000 ml)
- Flasks (250 ml)
- Medium bottles
- Syringe (1 ml)
- Glass Pasteur pipettes
- Graduated pipettes (5, 10, and 100 ml)
- Bulbs for Pasteur pipettes
- Sterilizing canisters for graduated and Pasteur pipettes
- Pipette aid
- Automatic adjustable micropipettes (100 ml) and tips
- Glass test tubes with screw caps
- Glass centrifuge tubes

- Polypropylene graduated conical tubes with caps (15 ml)
- Round-bottom plastic tubes (15 and 50 ml)
- Racks
- Volumetric flask
- Insert moulds (5x10x2 mm)
- Bent-tip pipettes
- Ampoules with screw caps
- Glass microscopic slides (2.5 x 7.5 cm)
- Stopwatch
- Mortar
- Wax paper
- Thermometer
- Spoon
- Spatula
- Parafilm
- Sealing tape
- Self-adhesive labels
- Aluminum foil

2. Pulse Field Gradient Gel Electrophoresis apparatus

2.1 Pulsaphore ™ System (LKB 2015)

- Electrophoresis unit with interlock lid
- Control unit
- Power supply (2301 Macrodrive 1, LKB)
- Cooling system (2219 Multitemp II Thermostatic Circulator, LKB)

- Hexagonal array electrode
- Gel supporting tray (30 x 30 cm)
- Gel casting frame (15 x 15 cm)
- 16-well combs (5 x 10 x 2 mm wells)
- Levelling kit including horizontal table and spirit level
- Gel knife
- Silicone rubber tubing for draining buffer
- Cold room for maintaining solution at 4 °c
- Dark room
- UV.transilluminator (Macro Vue, LKB 2011)
- Mask
- Gloves
- Plastic boxes for containing stained and destained gels
- Polaroid film type 665
- Handy polaroid FCR-10
- Black and white film (Kodak ectapan 125)
- Camera with red filter

2.2 CHEF™-DR II System (Bio-Rad)

- Gel chamber with hexagonal electrode
- Drive module
- Pulsewave 760 switcher
- Model 200/2.0 power supply
- Variable speed pump
- Casting stand (5.5 inches x 5 inches)

- Comb holder
- 10, 15, 20 well comb (1.5 mm thick)
- 12-inch Tygon tubing

3. Densitometer (Ultrascan XL densitometer, LKB)
4. Spectrophotometer (Perkin-Elmer Lambda spectrophotometer)
5. Materials for hybridisation
   - Microcentifuge tubes
   - Sephadex G-50
   - Hybond-N membrane (Amersham)
   - Hybond-C membrane (Amersham)
   - Filter membrane
   - Random primer labelling kit (Stratagene)
   - Hybridiser (HB-1, Techne corporation) with hybridisation tube complete with end cap
   - Plastic bag
   - Ice
   - X-ray film (Dupont)
   - Intensifying screen (Dupont)
   - Freezer -70 °c
6. Liquid scintillation counter (LS 7000, Beckman)
7. Chemicals used in this study are in the analytical grade and listed in alphabetical order as follows:-
   - Absolute ethanol
   - Absolute glycerol
   - Absolute methanol

- Agarose (type II Medium EEO, Sigma)
- Agarose (type VII low gelling temperature, Sigma)
- Agarose (low melt preparative grade, Bio-Rad)
- Agarose (molecular biology certified, Bio-Rad)
- [α-^{32}P] dCTP
- Boric acid
- β-tubulin probe
- DHFR probe
- Disodium hydrogen phosphate (Na_2HPO_4)
- DNA size standard (Bio-Rad 170-3605)
- Double distilled water
- D-sorbitol
- Ethidium bromide
- Ethylene diamine tetraacetic acid (EDTA)
- Garamycin
- Giemsa's powder
- Heparin
- HEPES buffer (N-2-Hydroexylthylpiperazine-N'-2-ethanesulfonic acid)
- Hydrogen chloride (HCl conc.)
- Liquid scintillation fluid
- 2-mercaptoethanol
- N-lauroylsarcosine (Sigma)
- Potassium dihydrogen phosphate (KH_2PO_4)
- Proteinase K (BRL)

- Saponin
- Siliga gel
- Sodium citrate
- Sodium chloride (NaCl)
- Sodium dihydrogen phosphate (NaH_2PO_4)
- Sodium dodecyl sulfate
- Sodium hydrogen carbonate ($NaHCO_3$) powder
- Sodium hydroxide pellet (NaOH)
- Sodium pyrophosphate
- Tri-sodium citrate
- Tris (hydroxymethyl) aminomethane (Fluka)
- Tris - HCl (Fluka)
- Zymolyase 60,000

APPENDIX II

PREPARATION OF SOLUTIONS

1. Working culture media

RPMI 1640 powder	10.40	gm
HEPES buffer	5.94	gm
Garamycin	1.00	ml

This medium was made up to 960 ml with double distilled water and stirred until dissolved. HEPES was added to final concentration of 25 mM with pH of 6.75and garamycin was added to that of 40 µg to inhibit bacterial contamination. Sterilized by 0.45µm millipore filtration,the medium was then dispensed aseptically in 100 ml aliquotsintosterile bottles and stored at 4°c. This had a shelf life of 1 month.

Before utilization, 4ml of 5% $NaHCO_3$ was added into the 100 ml RPMI medium. It was referred to "incomplete medium" with a final concentration of 0.2% (w/v) $NaHCO_3$. After mixing for a few seconds,the yellow-hued medium turned orange indicating the shift in pH from 6.75 to 7.4. For cultivation, incomplete medium was supplemented which 10% human serum. It was then called "complete medium."

If both of these media turned red denoting a shift to an alkaline pH due to the loss of CO_2 upon storage, they would not be used.

2. Sodium hydrogen carbonate (5% w/v)

Dissolved 50 gm of $NaHCO_3$ in 1000 ml double distilled water. This solution was also sterilized by millipore filtration, dispensed in aliquots and stored at 4°c.

3. D-sorbitol (5% w/v)

Dissolved 5 gm D-sorbitol in 100 ml double distilled water. This solution was then passed through all of the procedures as described for 5% $NaHCO_3$.

Note:- The synchrony of the culture was lost by the third intraerythrocytic cycle after sorbitol treatment.

4. Human sera

Sera used to supplement in the cultured medium were obtained from any group of clotted human blood. 300 ml of blood drawn from each donor into sterilized bottles were allowed to clot at 4°c for 3-4 days (longer than this advice resulted in haemolysis). Erythrocytes were eliminated; sera were then transferred into sterilized glass test tubes and heat inactivated in a periodic-shaking water bath at 56°c for 30 min. After labelled and cooled to room temperature, tubes containing sera were stored in a freezing compartment of refrigerator. They were melted in a room temperature before used.

5. Human erythrocytes

300 ml of human blood group O, as a source of erythrocytes, were bled from a donor into a standard blood-collection bag containing citrate-phosphate-dextrose solution (CPD) as an anticoagulant. These uninfected erythrocytes were used as the host cells for malaria parasites. Prior to adding these cells to cultures, 5 ml of them were washed three times with 5 ml of incomplete media (as described above) by centrifugation at 1,5000 rpm for 10 min; the plasma and the leucocytes in buffy coat on the top of packed cells were eliminated before each resuspension. Finally, complete media were added to make a final suspension to 50% haematocrit.

6. Cryoprotectant solution

Glycerol	70	ml
4.2% sorbitol in 0.9% NaCl	180	ml

7. Giemsa's staining solution

Giemsa powder	0.6	gm
Glycerol	50.0	ml
Absolute methanol	50.0	ml

A small amount of Giemsa powder and glycerol were gradually added and mingled in a mortar. The dye was ground thoroughly and then poured into a clean dark bottle. The

grinding was reiterated in this manner until all powder was given out. The Giemsa-glycerol blend was incubated in periodic-shaking water bath at 55-60 °c for 6-8 hrs, then cooled down to room temperature. 50 ml of methanol was added and the mixture was shaked resulting in homogeneity. The bottle was put aside at 37 °c for 2 weeks. After filtered, Giemsa's stain was kept in a lightproof bottle until used.

8. Phosphate-buffered saline (1x PBS)

$NaH_2PO_4 \ _2H_2O$	2.106	gm
(or NaH_2PO_4 anhydrous	1.620	gm)
Na_2HPO_4	8.733	gm
NaCl	4.500	gm

Made up to 1000 ml with distilled water and adjusted to pH 7.4 with concentrated HCL. Sterilized by autoclaving.

9. 0.5 M EDTA stock solution

Weighed out 186.1 gm disodium ethylene diamine tetracetate. $2H_2O$ in 800 ml distilled water. Swirled vigorously on a magnetic stirrer. Adjusted to pH 8.0 by adding approximately 20 gm of NaOH pellets. Made up the volume to 1000 ml prior to autoclaving.

10. Lysing solution

Dissolved 0.121 gm Tris in 0.5 M EDTA (as previously

mentioned). Made this solution up to 100 ml and then 1 gm of N-lauroylsarcosine was added. The final concentration was 100 ml of 1% N-lauroylsarcosine in 0.5 M EDTA and 0.1 M Tris.

11. Tris-borate EDTA stock solution (5x TBE)

Tris base	54.0	gm
Boric acid	27.5	gm
0.5 M EDTA (pH 8.9)	20.0	ml

After made up to 1,000 ml with distilled water, the solution was autoclaved. Before using as a PFGE buffer, this stock solution was diluted to a final 0.5x concentration.

12. Ethidium bromide stock solution

1 gm of ethidium bromide was added in 100 ml of autoclaved double-distilled water and agitated vigorously on a magnetic stirrer for several hours to ensure that it has already been dissolved. The solution was kept at 4 °c in a dark bottle or aluminum foil-wrapped container.

Warning : Gloves and mask should be worn during preparation because ethidium bromide was a powerful mutagen.

13. Prehybridizing solution

Solution was prepared from 6x SSC (20x stock solution), 0.2% SDS (10% stock solution), 0.1% Sodiumpyrophosphate, and 500 µg/ml Heparin.

Appendix III

DIVISION of BIOLOGICAL SCIENCES
Institute of Cell and Molecular Biology

The University of Edinburgh
Darwin Building
King's Buildings
Mayfield Road
Edinburgh EH9 3JR

Fax 031 668 3870
Telex 727442 (UNIVED G)
Telephone 031 650 1000
or direct dial 031 650 5368

November 22, 1991

Dr. Sodsri Thaithong
Biology 1 Building
Faculty of Science
Chulalongkorn University
Bangkok 10330
Thailand

Dear Sodsri

It was nice meeting you in Edinburgh last month. I hope you had an enjoyable stay.

I thought it necessary that I write to you as we recently obtained some information which explains Anchalee's lack of success in probing chromosome blots when she was in Edinburgh. I think we mentioned to you that we suspected that the nylon membranes we were using were the cause. Earlier this week a representative from Amersham, who market the nylon membranes, confirmed that the membranes did not work using the protocol followed by Anchalee. This, despite the fact that this protocol is given in the booklet supplied with their material. We are very annoyed with them about this and I am especially sorry for Anchalee as she worked very hard. Her lack of results is totally due to the Amersham material and I would ask that this be taken into account for her MSc exam, which I understand will be soon.

As Geoffrey has no doubt told you the revised manuscript has been sent to MBP. Now that this has been done I will write to CHEMAL about further funding for the project.

With best wishes

Rob

Robert Ridley

Biography

My name is Anchalee Punpuckdeekoon. I was born on July 17, 1966 in Bangkok, Thailand.

After earning a Bachelor Degree of Science from Chulalongkorn University, on April 14, 1987, I have concomitantly attended a Master's Degree in the same institution.