CHAPTER 3

RESULTS

1. Extraction of DNA from *P. falciparum*

The DNA used in this study came from erythrocytic forms of *P. falciparum* isolate K1 cultured *in vitro*, using phenol-chloroform extraction method. The extracted DNA dissolved in TE buffer was analysed by measuring it's absorption spectrum from 190 to 400 nm, using TE solution as a blank. DNA concentration was determined by measuring the absorbance at 260 nm, and assuming that 1 OD_{260} unit was equivalent to 50 µg DNA/ml. The ratio of A_{260} : A_{280} was in the range of 1.8-1.9. A typical spectrum of plasmodial DNA is shown in figure 4. When the extracted DNA was analysed by agarose gel electrophoresis, it consisted of fragment greater than 23.5 Kb (figure 5). The extracted DNA was digested with Bam HI, Hind III, Sal I and EcoRI and analysed on 0.7% agarose gels. All digested DNA samples except that digested with Sal I showed a smeared background with discrete bands superimposed, indicating that *P. falciparum* had a repetitive DNA components. Hind III (5'...AAGCTT...3') and EcoRI (5'...GAATTC...3'), AT-rich recognition enzymes, generated smaller size fragments than Bam HI (5'...GGATCC...3'). The enzyme Sal I which recognized GC rich nucleotide sequences could digest K1 DNA to a limited extent (Figure 5).

2. Pattern of restriction enzyme digested DNA used for cloning

DNA of *P. falciparum* isolate K1 was digested with EcoR I* and pUN 121 plasmid vector was cut with enzyme EcoR I. Restriction enzyme

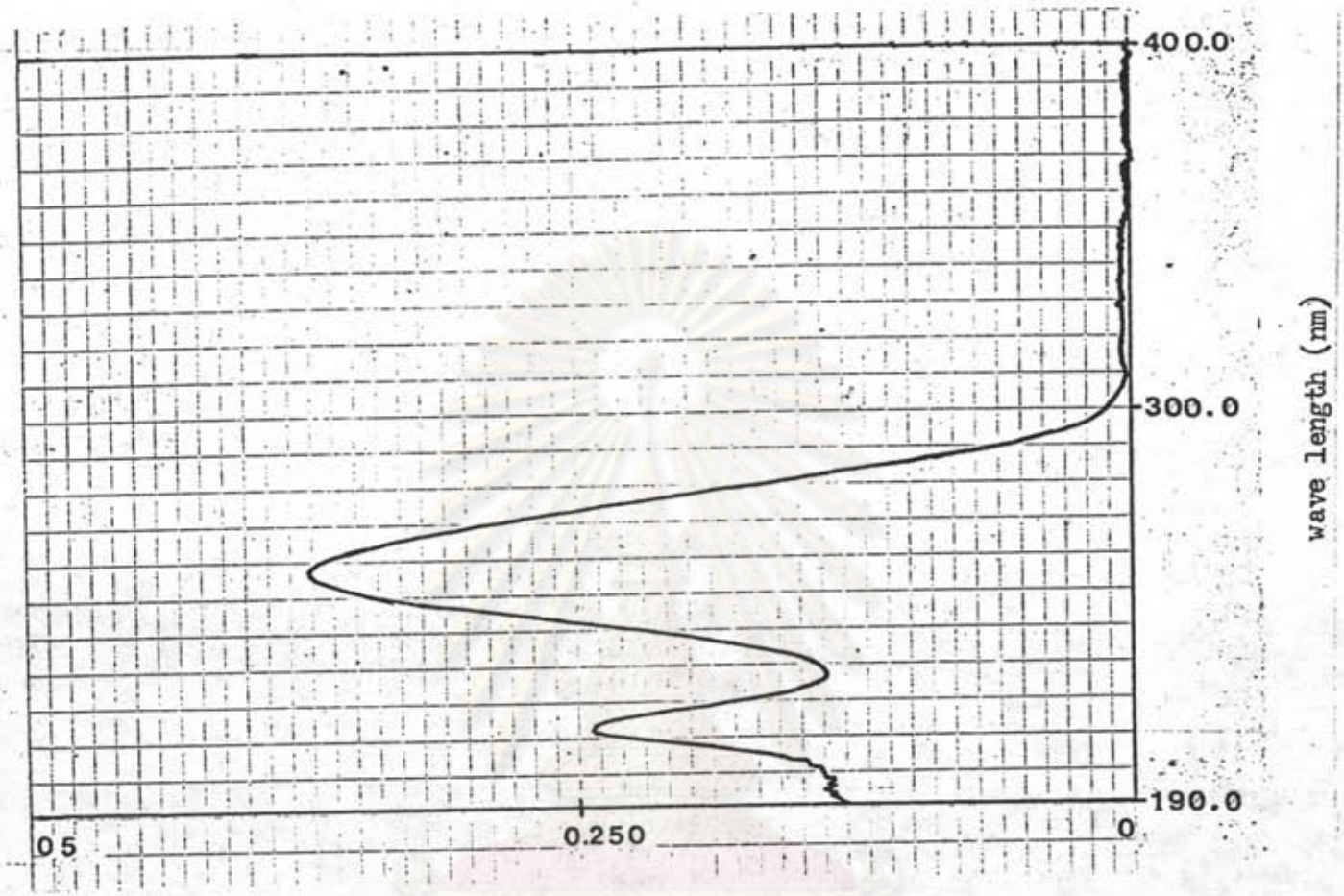


Fig. 4 The absorption spectrum of extracted *P. falciparum* strain K1 DNA. The spectra was obtained by a Shimadsu UV-visible Recording Spectrophotometer

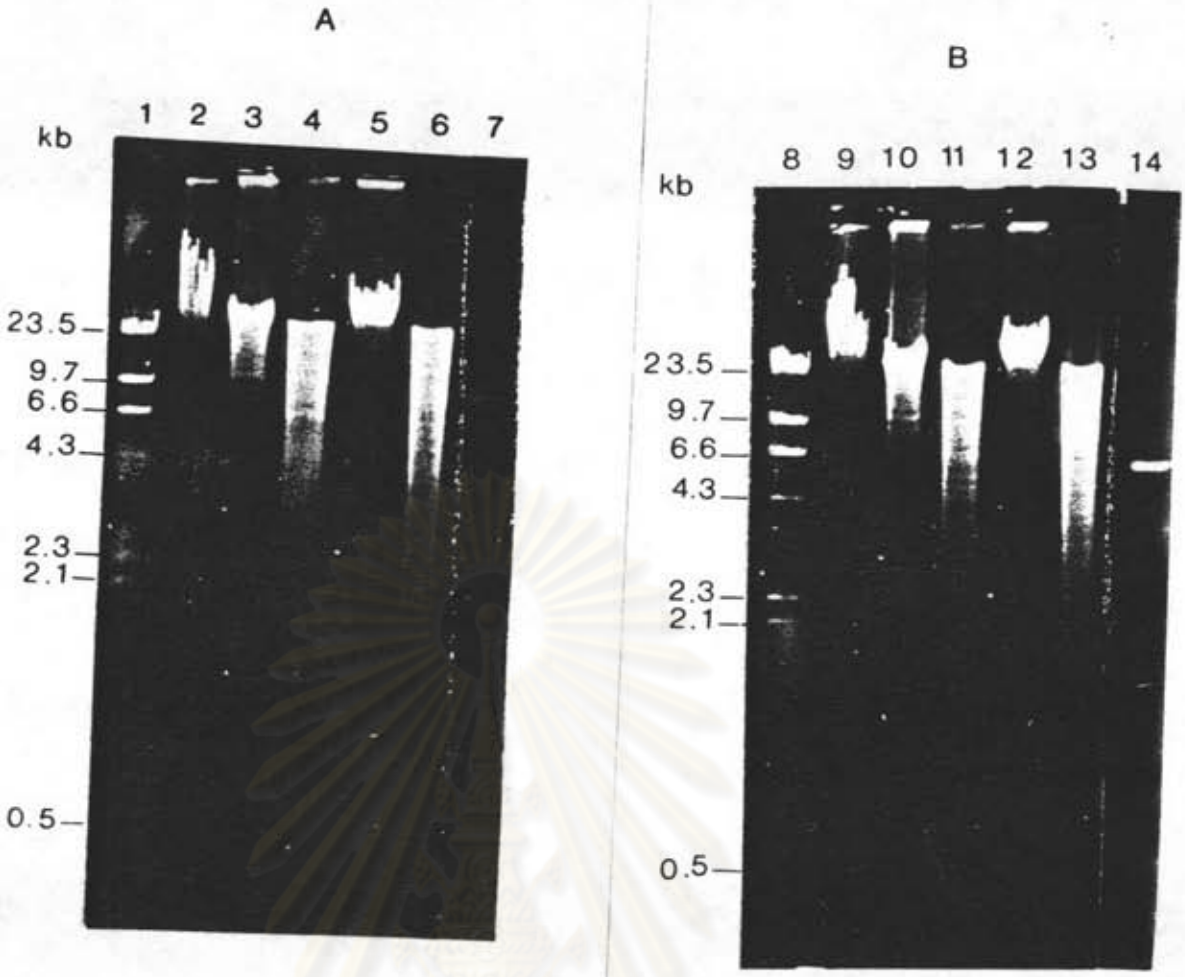


Fig. 5 Gel analysis of *P. falciparum* DNA after digestion with restriction enzymes. Two micrograms of DNA was used in each lane. DNA was electrophoresed on 0.7% agarose gel at 50V, 6hr.

The gel contained in

lane 1, 8 : the size marker, λ DNA digested with Hind III

lane 2, 9 : K1 DNA uncut

lane 3, 10: K1 DNA digested with Bam HI

lane 4, 11: K1 DNA digested with Hind III

lane 5, 12: K1 DNA digested with Sal I

lane 6, 13: K1 DNA digested with Eco RI

lane 7 : Bam HI digested pUNK1-45

lane 14 : Bam HI digested pUNK1-34

digested DNA was characterized by electrophoresis in 0.7% agarose gel. The plasmodial DNA digested with EcoRI* (figure 6 ; lane 7, 9) showed more intense staining regions in the size range of 2.1 kb to less than 0.5 Kb than DNA that was digested with EcoRI (figure 6, lane 6, 8). However in one preparation (figure 6, lane 5), there were no differences between DNA digested under conditions to generate EcoRI* and that for normal EcoRI cut. The sample in lane 5 was not used in further experiments. λDNA digested with EcoRI and EcoRI* were used as control digestion conditions.

3. Ligation of digested DNA with vector.

The digested K1 DNA samples (figure 6, lane 7 and 9) and pUN 121 vector digested with EcoRI were ligated with T4 DNA ligase. The recombinant DNA ligation product and pUN 121 self ligation product were analysed on 0.7% agarose gel (figure 7). EcoRI digested plasmid pUN 121 exhibited single band of 4.4 kb. Self ligated pUN 121 products consisted of 3 bands, that of linear dimer (light band between 9.7 and 6.6 Kb), circular dimer (band between 23.5 and 9.7 Kb) and polymeric forms (band larger than 23.5 kb). The ligation product of pUN 121 Eco RI and K1 EcoRI* (figure 7 lane 4, 5, 6) showed smear patterns of fragments larger than 4.3 kb, indicating that ligation between the fragments occured.

4. Transformation

The ligated products of EcoRI*-digested Plasmodial DNA and EcoRI-digested pUN 121 were transformed into *E. coli* by DMSO method. DNA library in *E. coli* of approximately 20,000 colonies were obtained on tetracycline plates. This library was large enough to ensure that more than 99% of Plasmodial genomic DNA were represented. This was based on calculation using the formula $N = \frac{\ln(1-P)}{\ln(1-f)}$, where P = 0.99,

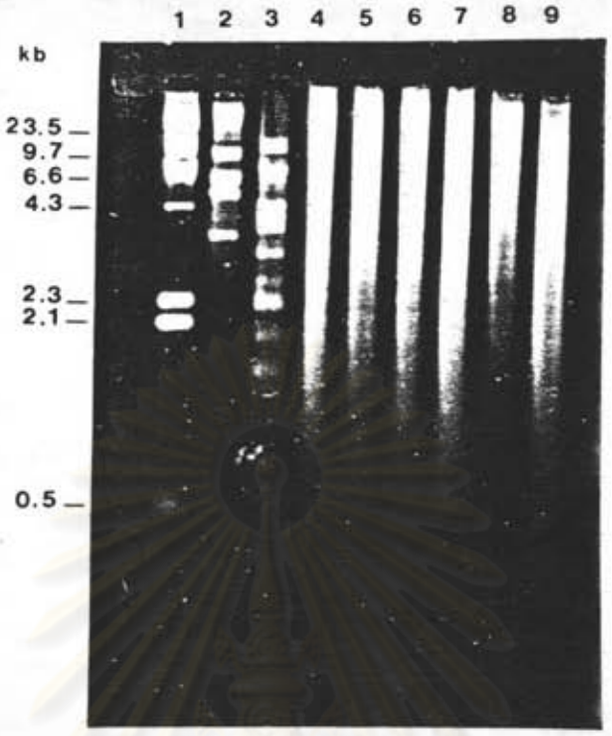


Fig. 6 Gel analysis of *P. falciparum* DNA after digestion with restriction endonucleases, EcoRI and Eco RI*. Two microgram of parasite DNA was used in each lane. DNA was electrophoresed on 0.7% agarose gel at 80V, 3hr. The gel contained in

lane 1 : the size marker, λ DNA digested with Hind III

lane 2 : λ DNA digested with EcoRI

lane 3 : λ DNA digested with EcoRI*

lane 4, 6, 8 : K1 DNA digested with EcoRI*

lane 5, 7, 9 : K1 DNA digested with EcoRI*

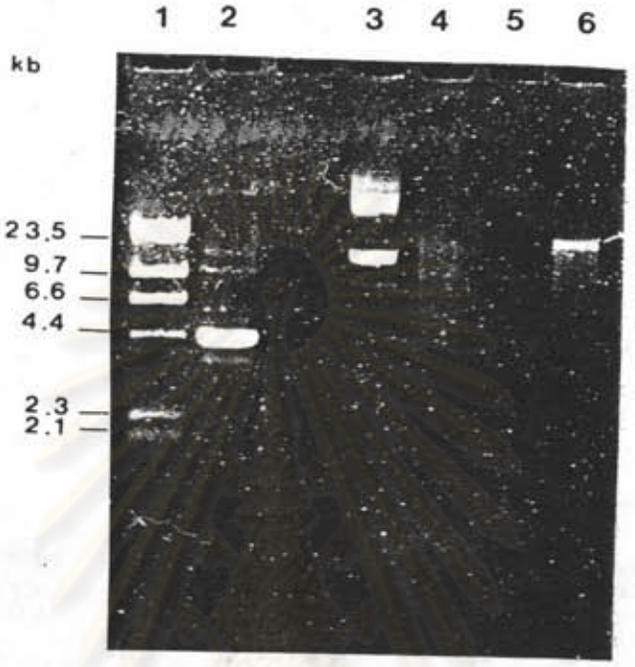


Fig. 7 Ethidium bromide staining of ligated product.

lane 1 : the size marker, λDNA digested with Hind III

lane 2 : Eco RI digested pUN 121

lane 3 : ligated pUN 121

lane 4, 5, 6 : ligated pUN 121 Eco RI-K1 Eco RI*

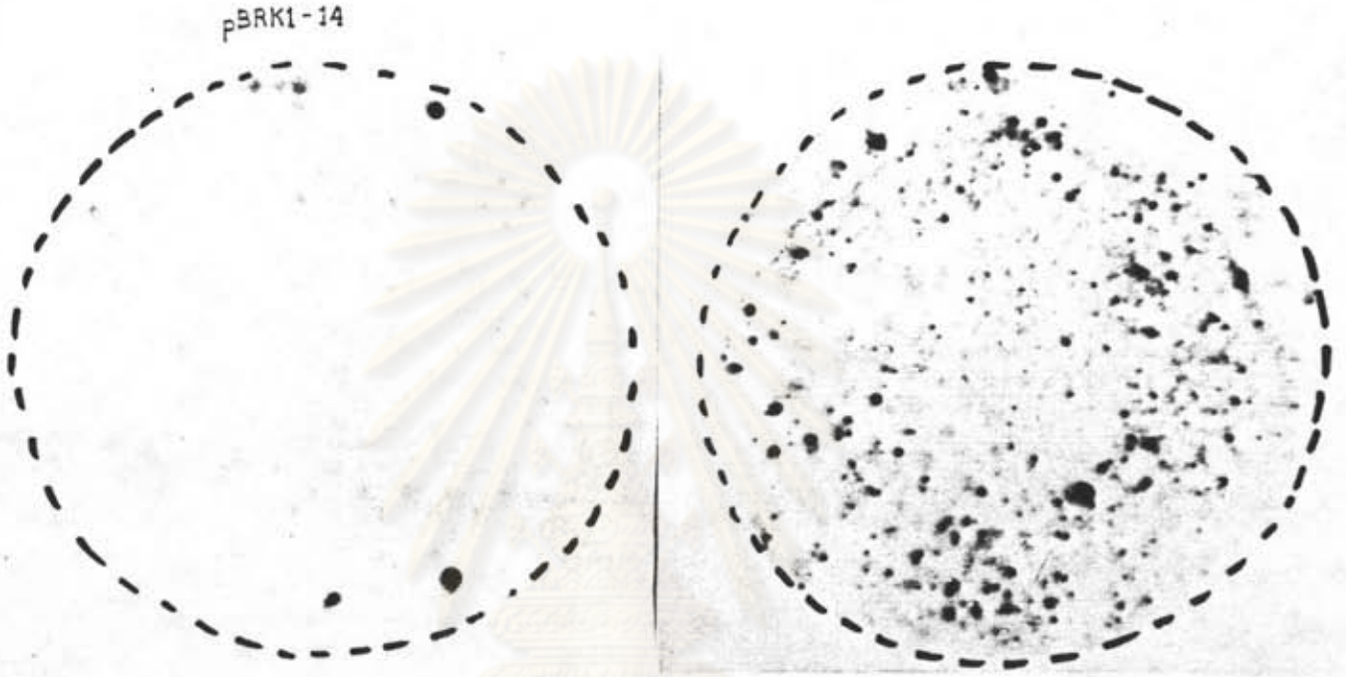


Fig. 8 Autoradiograms of colony hybridization of 20000colonies with total genomic DNA of K1 probe. The specific activity of radioactive probe was 4×10^7 cpm/µg and 10^6 cpm/ml of radioactive probe were added per filter. Colonies with strong intensity signal in autoradiograms were selected.

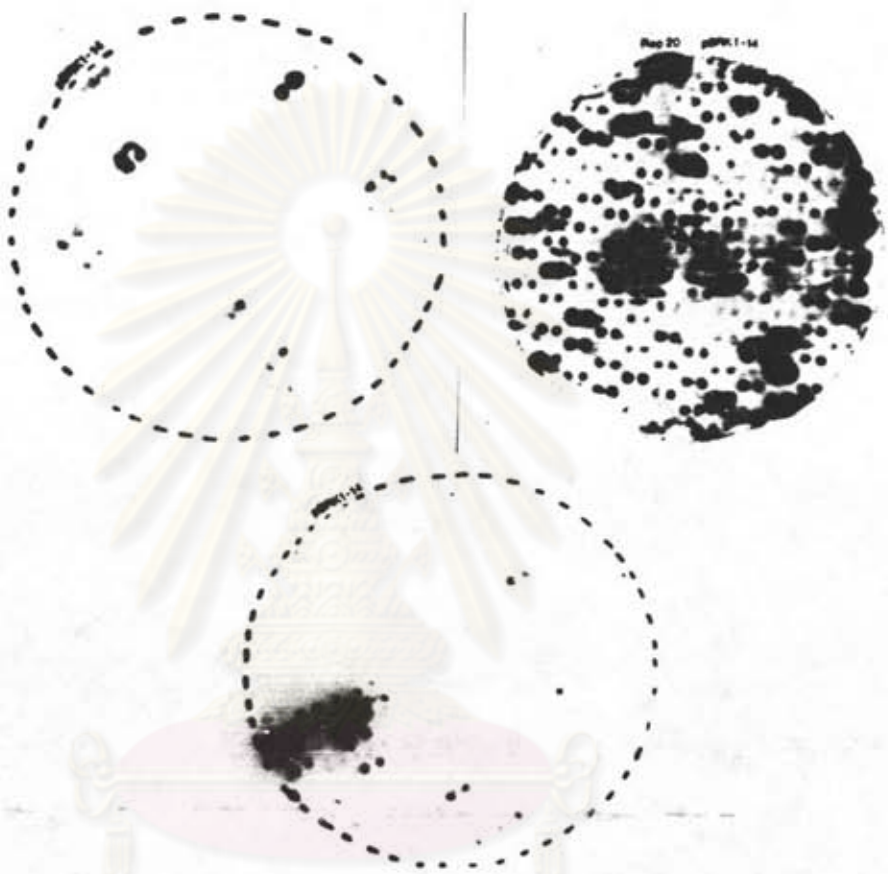


Fig. 9 Autoradiograms of hybridization of 400 selected colonies with total genomic DNA of K1 probe. Duplicate colonies were grown on nitrocellulose plates.

f = size of *P. falciparum* DNA piece (4 kb)/ *P. falciparum* DNA genomic size (10^7 b). The transformation efficiency (number of colonies per µg of plasmid DNA) of recombinant DNA was $6x10^4$ colonies/µg and transformation efficiency of control pUN 121 was $1x10^7$ colonies/µg.

5. Colony hybridization

Twenty-thousand transformants on tetracycline plates were transfered to nitrocellulose filters and hybridized with K1 genomic DNA. About 400 colonies produced intense signals (figure 8) with the probe.

These 400 colonies were picked up and duplicates were grown on nitrocellulose plates. These plates were then re-hybridized with radiolabelled K1 genomic DNA and the signals compared with colonies containing Rep 20 and pBRK1-14 (figure 9). Now only fifty-three colonies gave signals which were stronger than that of pBRK1-14 and similar in intensity to Rep 20.

6. Characterization of recombinant plasmids

The recombinant plasmids from these 53 colonies were extracted by the rapid alkaline method and the presence of plasmids checked on 0.7% agarose gels (data not shown). These recombinant plasmids were given the nomenclature pUNK1-1, pUNK1-2, etc. The extracted recombinant plasmids pUNK1-1 to pUNK1-53 were then cut with BamHI. All digested plasmids were electrophoresed on 0.7% agarose gel (figure 10a-f). From the data, it was clear that each clone contain an insert of different size. The size of the recombinant plasmids and inserts were estimated from a calibration curve of mobility versus molecular weight (table 3).

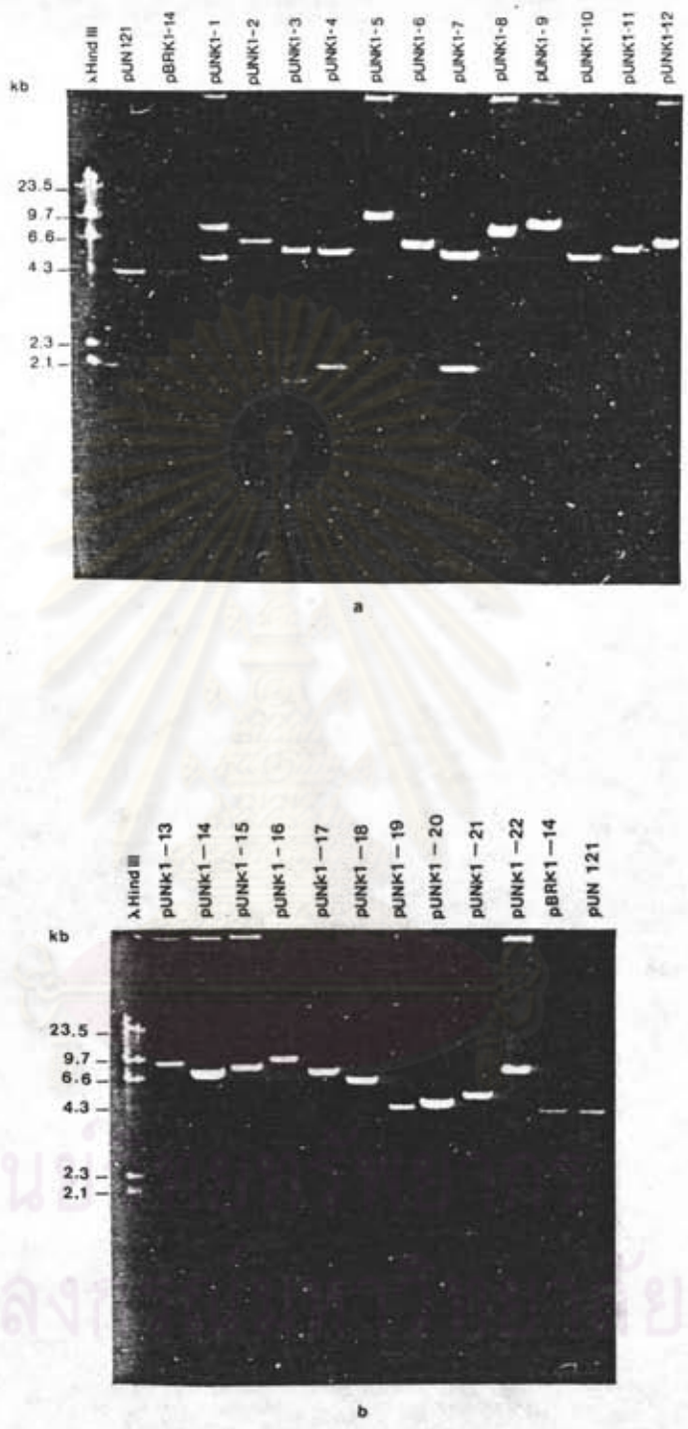
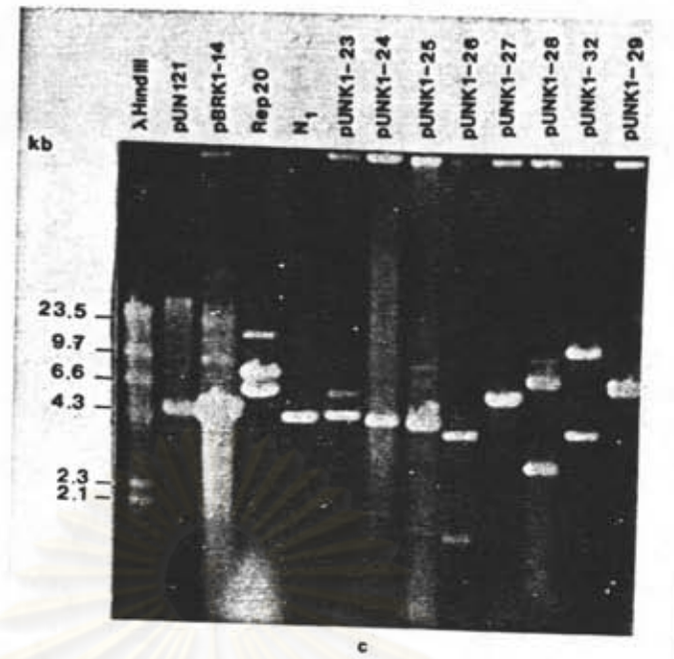


Fig. 10 a, b Ethidium bromide staining pattern of the digested plasmids on 0.7% agarose gel. pUN 121 and pBRK1-14 were used as control.



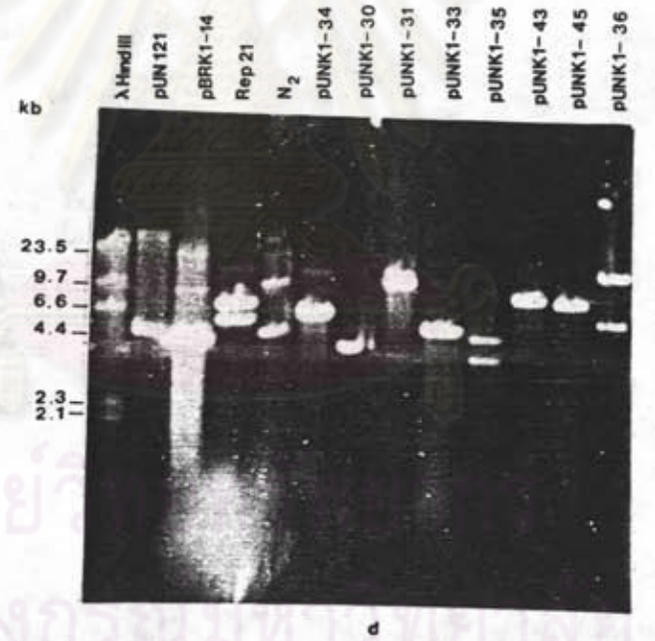
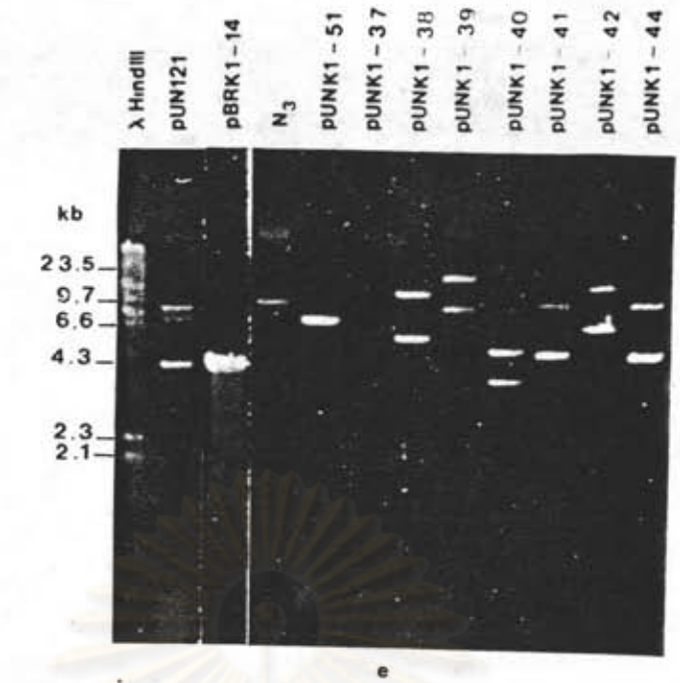
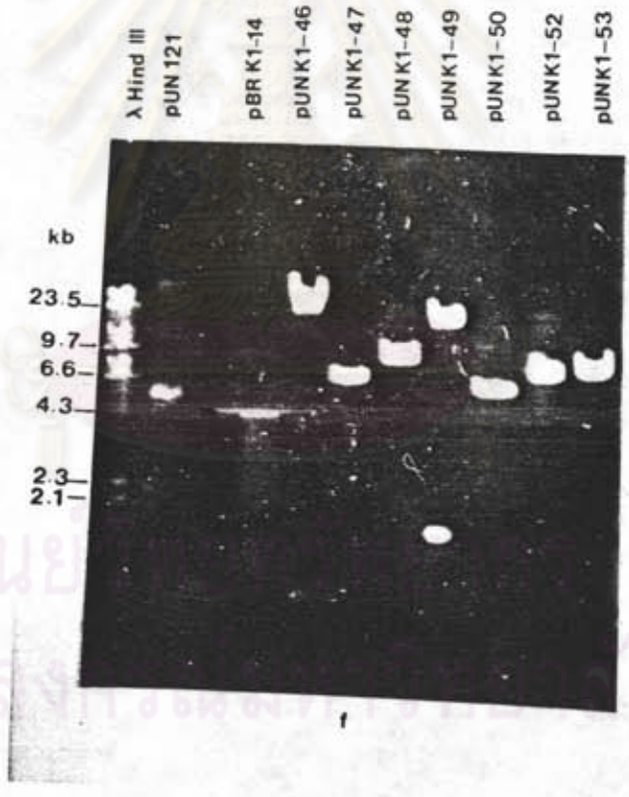


Fig. 10 c, d Ethidium bromide staining pattern of the digested plasmids on 0.7% agarose gel. pUN 121, pBRK1-14 and Rep 20 were used as positive control, N_1 and N_2 the weak intensity clone from double colony hybridization, as negative control.



e



f

Fig. 10 e, f Ethidium bromide staining pattern of the digested plasmids on 0.7% agarose gel. pUN 121, pBRK1-14 as positive contral, N_3 the weak intensity colony from double colony hybridization, as negative control.

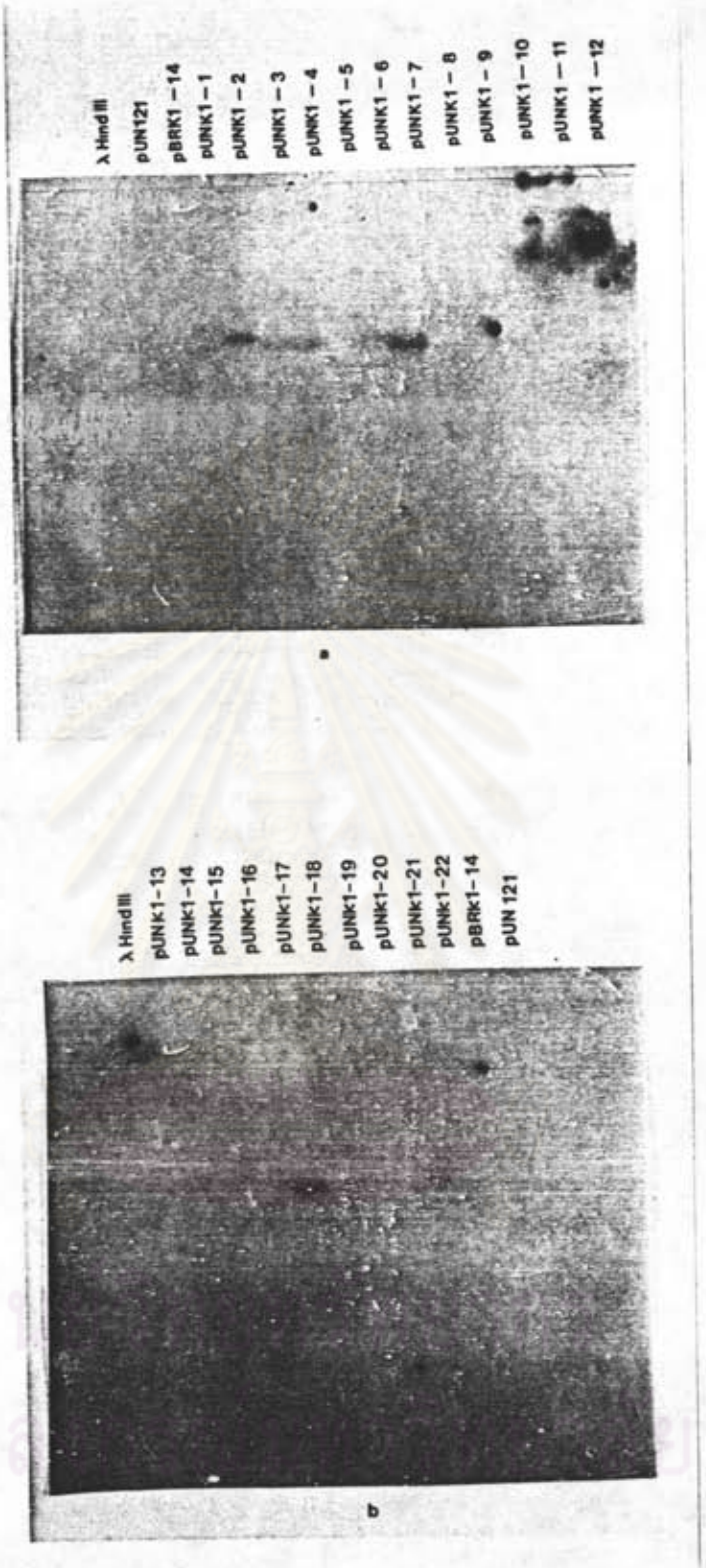


Fig. 11 a, b Autoradiograms of Southern blot hybridization with K1 genomic DNA probe. The specific activity of probe was 2×10^7 cpm/μg

Fig. 11 c, d Autoradiograms of Southern blot hybridization with K1 genomic DNA probe. The specific activity of probe was 2×10^7 cpm/µg

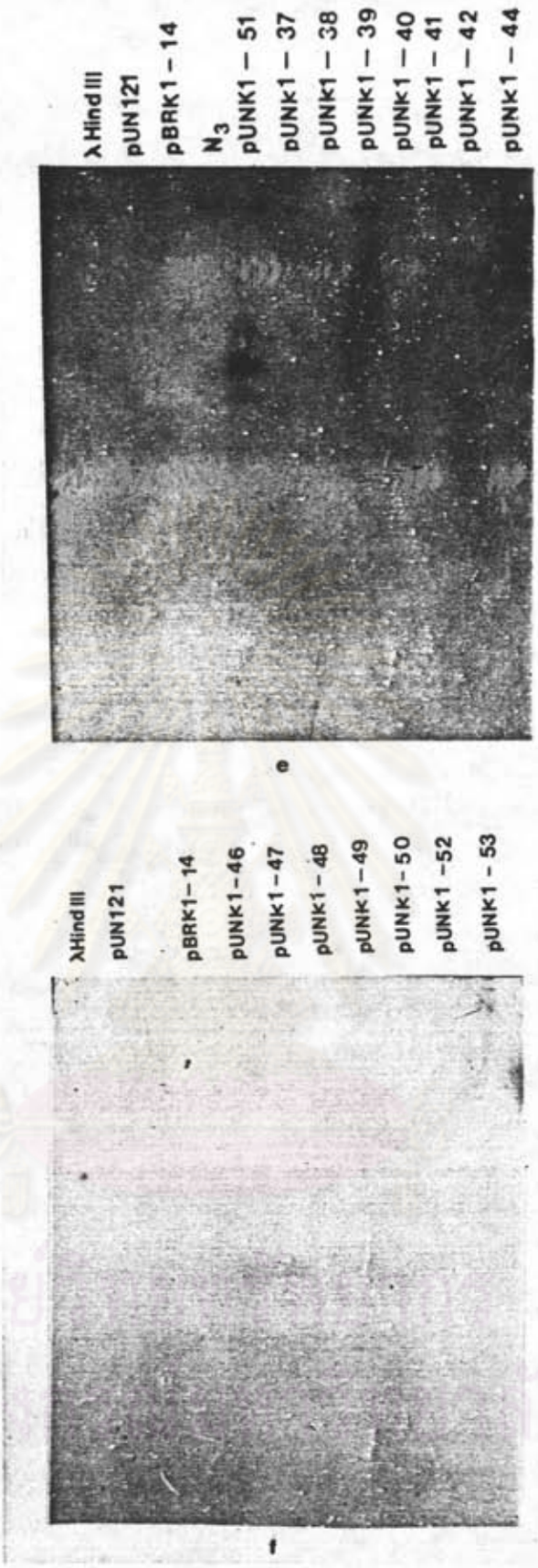


Fig. 11 e, f Autoradiograms of Southern blot hybridization with K1 genomic DNA probe. The specific activity of probe was 2×10^7 cpm/µg

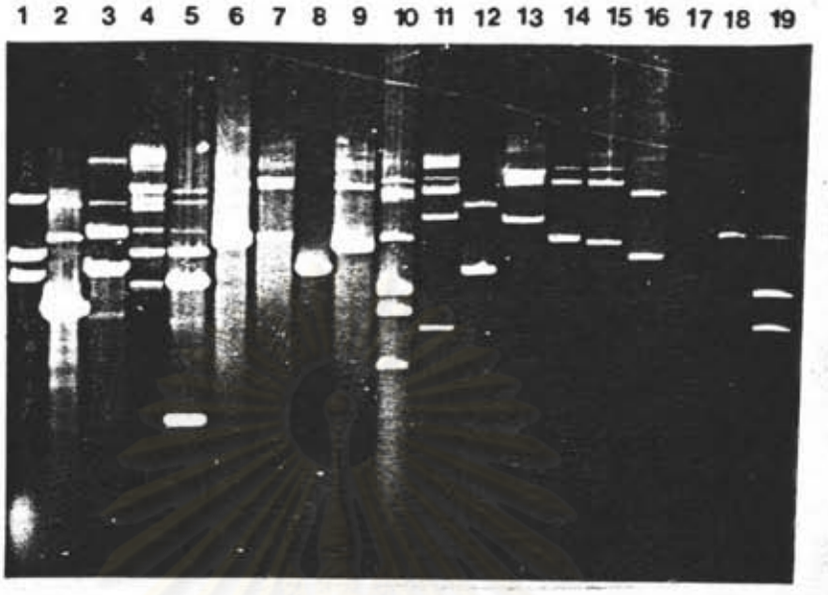


Fig. 12 Ethidium bromide staining pattern of digested plasmids which give strong signal in figure 11 comparing with plasmid pBRK1-14 and Rep-20

lane 1 Rep 20 digested with Hind III
lane 2 pBRK1-14
lane 3 pUNK1-2
lane 4 pUNK1-4
lane 5 pUNK1-7
lane 6 pUNK1-8
lane 7 pUNK1-16
lane 8 pUNK1-18
lane 9 pUNK1-22
lane 10 pUNK1-25
lane 11 pUNK1-32
lane 12 pUNK1-34
lane 13 pUNK1-31
lane 14 pUNK1-43
lane 15 pUNK1-45
lane 16 pUNK1-51
lane 17 pUNK1-37
lane 18 pUNK1-38
lane 19 pUNK1-39

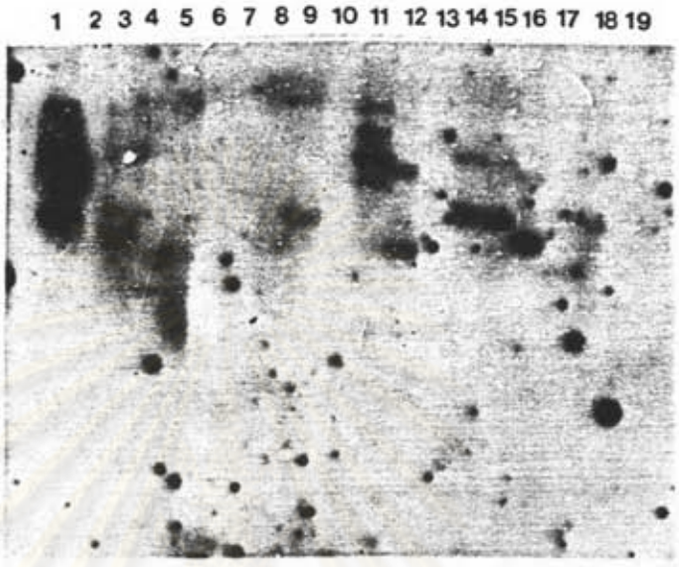


Fig. 13 Autoradiograms of Southern blot hybridization of figure 12 with *P. falciparum* K1 genomic DNA probe. The specific activity of probe was 3×10^7 cpm/µg

Table 3 Size of recombinant plasmids

Recombinant plasmids	size (kb)	insert size (kb)
pUNK1-1	12.7	8,3
pUNK1-2	6.4	2.0
pUNK1-3	7.6	3.2
pUNK1-4	7.8	3.4
pUNK1-5	8.1	3.7
pUNK1-6	6.2	1.8
pUNK1-7	7.0	2.6
pUNK1-8	7.0	2.6
pUNK1-9	7.4	3.0
pUNK1-10	5.5	1.1
pUNK1-11	6.2	1.8
pUNK1-12	6.4	2.0
pUNK1-13	7.8	3.4
pUNK1-14	6.6	2.2
pUNK1-15	7.3	2.9
pUNK1-16	7.8	3.4
pUNK1-17	6.9	2.5
pUNK1-18	6.4	2.0
pUNK1-19	4.9	0.5
pUNK1-20	5.0	0.6
pUNK1-21	5.5	1.1
pUNK1-22	7.3	2.9
pUNK1-23	5.1	0.7
pUNK1-24	5.0	0.6
pUNK1-25	5.0	0.6

Table 3

Recombinant plasmids	size (kb)	insert size (kb)
pUNK1-26	6.5	8.1
pUNK1-27	6.2	1.8
pUNK1-28	10.7	6.3
pUNK1-29	6.8	2.4
pUNK1-30	4.6	0.2
pUNK1-31	9.5	5.1
pUNK1-32	14.0	9.6
pUNK1-33	5.7	1.3
pUNK1-34	6.5	2.1
pUNK1-35	10.0	5.6
pUNK1-36	18.6	14.2
pUNK1-37	9.8	5.4
pUNK1-38	15.0	10.6
pUNK1-39	19.8	15.4
pUNK1-40	9.0	4.6
pUNK1-41	13.6	9.2
pUNK1-42	16.8	12.4
pUNK1-43	8.5	4.1
pUNK1-44	13.5	9.1
pUNK1-45	8.3	3.9
pUNK1-46	12.5	8.1
pUNK1-47	6.4	2.0
pUNK1-48	7.7	3.3
pUNK1-49	12.2	7.8
pUNK1-50	6.1	1.7
pUNK1-51	6.8	2.4
pUNK1-52	7.1	2.7
pUNK1-53	7.5	3.1

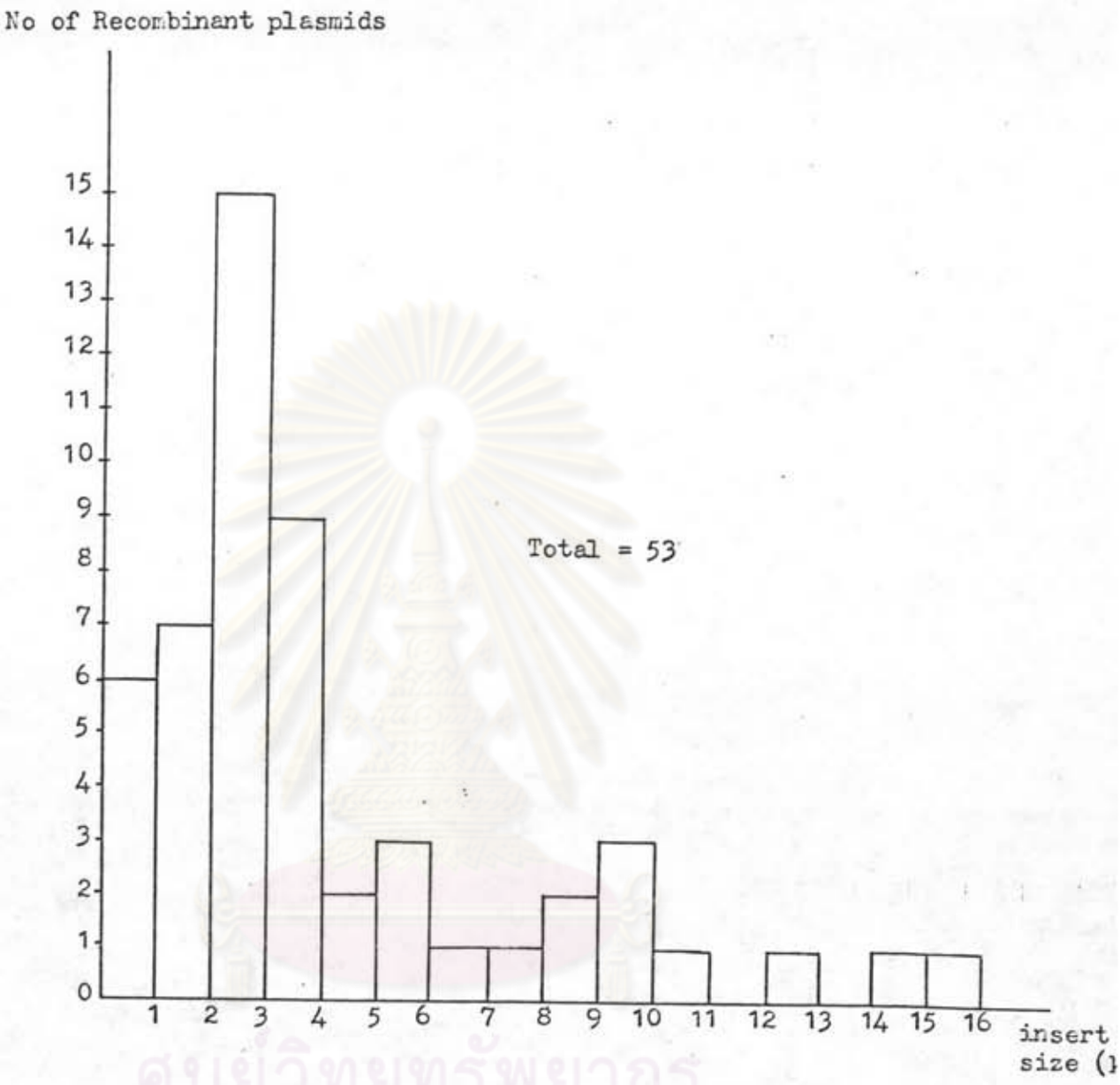


Fig. 14 Histogram show the frequency of insert size of 53 recombinant plasmids

Seventy percent of 53 recombinant plasmids contained insert size of 0.2-4.8 kb (figure 14). The smallest insert was 0.2 kb (pUNK1-30) and the largest was 15.4 kb (pUNK1-39). In order to identify the fragments that specifically contain repeated elements, the gels were blotted and hybridized with total K1 genomic DNA (figure 11 a-f). The recombinant plasmids pUNK1-2,-4,-7 (figure 11a), pUNK1-18,-22(figure 11b), pUNK1-32 (figure 11C), pUNK1-34,-43,-45 (figure 11d), pUNK1-51,-39 (figure 11e) were selected based on their strong intensities compared to pBRK1-14. The recombinant plasmids from figure11f were not selected, because of they did not show any intense bands.

All selected recombinant plasmid were digested with BamHI and Southern blot hybridized again with K1 genomic DNA probe (figure 13). The recombinant plasmids pUNK1-8,-16, -13, -25, -31, -37 and -38 from figure 13a-e were used as negative control. The plasmid pUNK1-32, -34, -43, -45, and -51 were selected based on similar intensity of their bands to Rep. 20.

7. Comparison of sensitivity of recombinant plasmids

Purified plasmids (10 and 2 ng) of pUNK1-32, -34, -43, -45, -51, pBRK1-14 and Rep 20 were dotted on nylon membrane and hybridized with K1 genomic DNA probe. The data showed that pUNK1-34 and pUNK1-45 had stronger intensity spots than pUNK1-32, -43 and -51. However, all spots were weaker than those of Rep 20. The plasmid pBRK1-14 failed to show any intensity (figure 15). K1 genomic DNA cross hybridized with 5 ng of DNA of *P. cynomolgi*, *P. knowlesi*, *P. chabaudi*, *P. vivax* and man, but not of *An. dirus*. Fifty and five ng of purified pUNK 1-32, -34, -43, -45 and -51 did not hybridized with mixed DNA of *P. vivax*, *P. chabaudi*, *P. knowlesi*, *P. cynomolgi*, *An. dirus* and human. One hundred ng of each

DNA were used as probe in dot blot hybridization experiment (figure 16). The positive control was 5-1 ng of the DNA which was used as the probe.

8, Detecting parasites in blood stage

E. coli clones containing pUNK1-32, -34, and -45 were grown and their plasmids extracted. They were used as probes to test to detect 20 µl of malaria infected human blood. The filter was also spotted with parasite and human DNA.

After 48 hours of exposure, experiments using serialy diluted parasites showed that pUNK1-32 could detect $5x10^4$ parasites, equivalent to 0.05% parasitemia (5 parasites in 10^4 red blood cells) based on $5x10^9$ red blood cell/ml blood (figure 17). The pUNK1-32 probe was able to detect 25 ng of *P. falciparum* DNA and 12.5 pg of pUNK1-32.

Probes pUNK1-34 and pUNK1-45 could detect $5x10^3$ parasite, equivalent to 0.005% parasitemia (figure 18-19). The pUNK1-34 could detect 10 ng of *P. falciparum* DNA and 12.5 pg of plasmid pUNK1-34. On the other hand *P. flaciparum* DNA gave a strong intense band with pUNK1 -45 probe, which could detect 6.25 pg plasmid pUNK1-45. The specific activities of the two probes were similar.

Regarding specificity of the recombinant probes, pUNK1-32 did not cross-hybridize with 100 ng human DNA, nor with 100 ng of *P. chabaudi, P. knowlesi, P. cynomolgi* DNA (spotted as a mixture of 300 ng) (figure 17). The pUNK1-34 did not cross-hybridization with 100 ng DNA of human and *P. knowlesi* and *P. cynomolgi* (spotted as a mixture for 200 ng) but did cross-react with 100 ng DNA of *P. chabaudi* (figure 18). The pUNK1-45 probe did not cross hybridize with 100 ng of human DNA, 100 ng of *P. chabaudi, P. knowlesi,* and *P. cynomolgi* (spotted as a mixture of 300 ng) nor with and 100 ng of *An. dirus* A, B,C, D (figure 19).

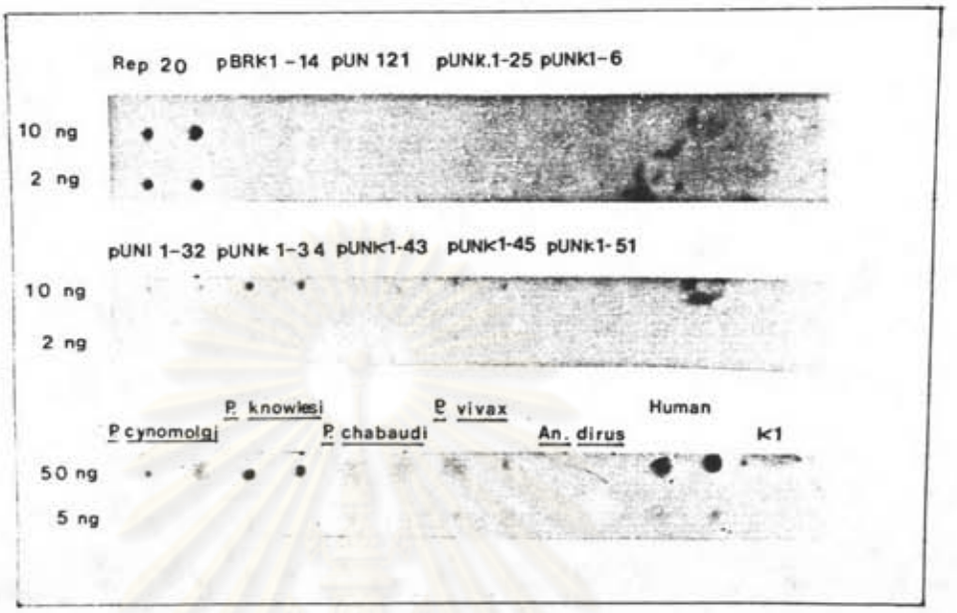


Fig. 15 Autoradiogram of dot blot hybridization of recombinant plasmid pUNK1-32, pUNK1,34, pUNK1-43, pUNK1-45, and pUNK1-51 with *P. falciparum* K1 genomic DNA probe. The specific activity of probe was $3x10^7$ cpm/µg. 1ng and 100 pg of K1 DNA as positive control. pUNK1-25 and pUNK1-6 were negative controls, other plasmodial DNA. *An. dirus* DNA, and human DNA were dotted for test hybridized with K1 DNA.

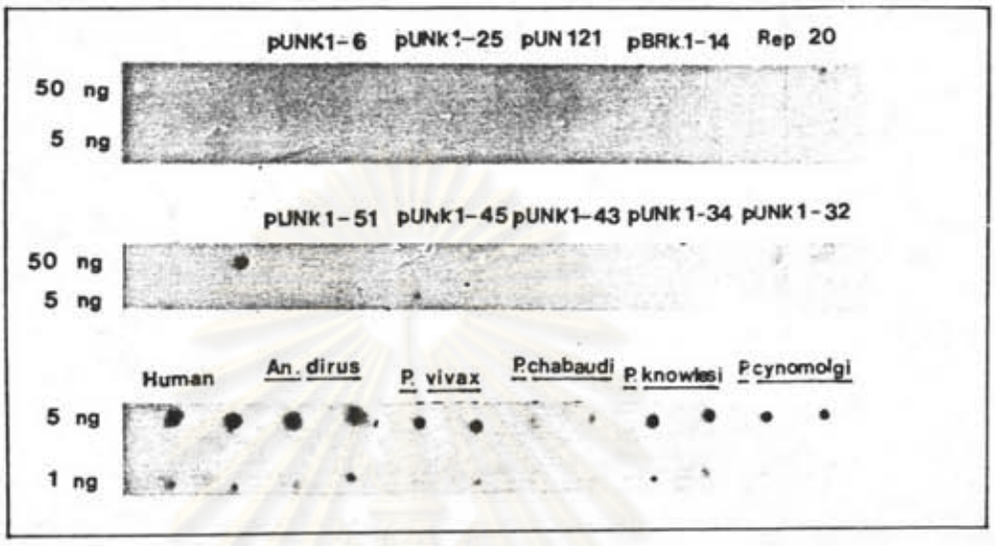


Fig. 16 Autoradiogram of dot blot hybridization of recombinant plasmids pUNK1-32, pUNK1-34, pUNK1-43, pUNK1-45, pUNK1-51 with others plasmodial DNA, *An. dirus* DNA and human mixed probes. The spacific activity of probes were $2x10^7$ cpm/µg. pUNK1-6 and pUNK1-25 were negative controls, other plasmodial DNA, *An. dirus* DNA and human DNA were dotted as positive control.

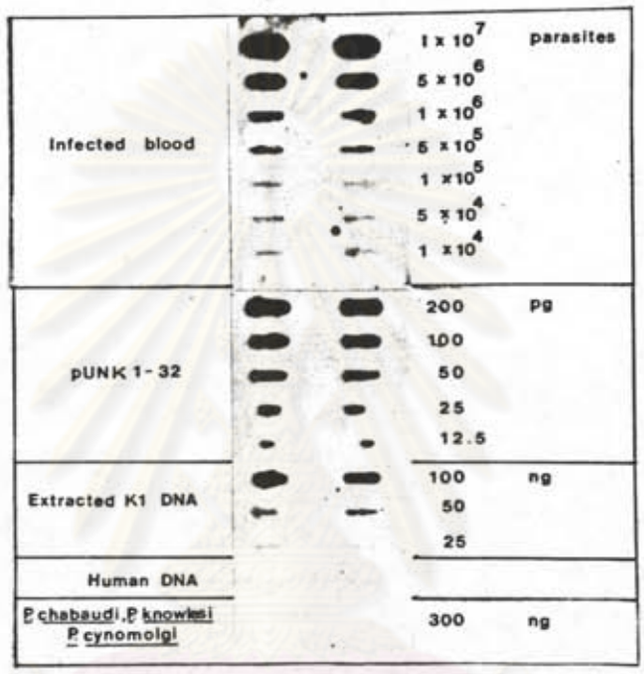


Fig. 17 Autoradiograms of slot blot hybridization of infected blood with pUNK1-32 probe. pUNK1-32 and extracted K1 DNA were bloted as positive control. The specific activity of probe was about 5×10^7 cpm/µg

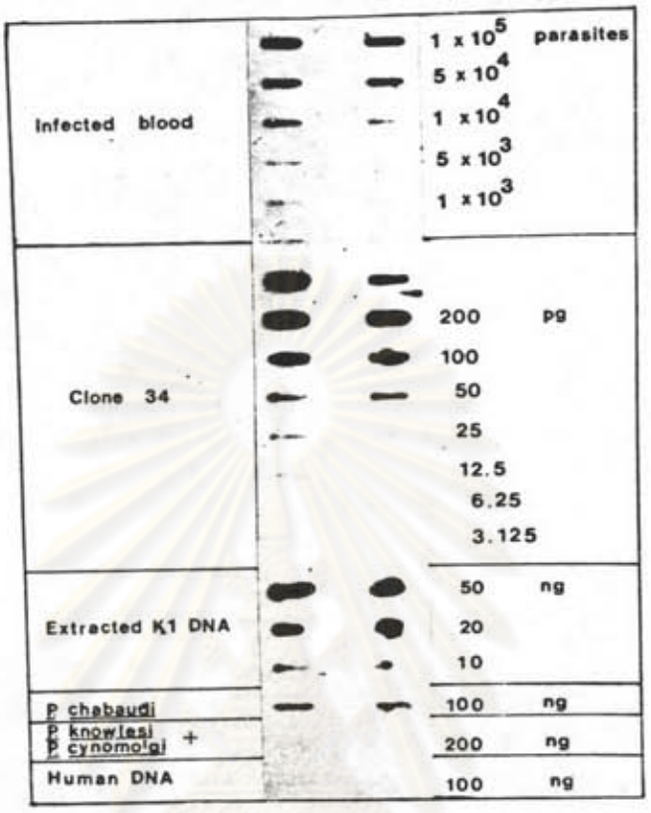


Fig. 18 Autoradiograms of slot blot hybridization of infected blood with pUNK1-34 probe. pUNK1-34 and extracted K1 genomic DNA were bloted as positive control. The specific activity of probe was 5.3×10^7 cpm/µg

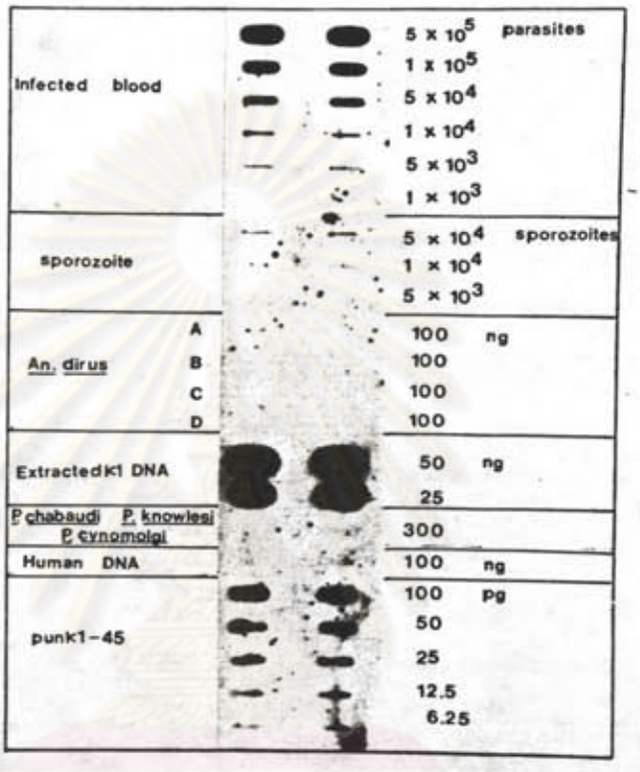


Fig. 19 Autoradiogram of Southern blot hybridization of infected blood and sporozoites which were removed from mosquito's glands, with pUNK1-45 probe, pUNK1-45 and extracted K1 genomic DNA were blotted as positive control. The specific activity of probe was about 3×10^7 cpm/µg.

9. Detection of oocysts and sporozoites in infected mosquitoes

pUNK1-34 was used as a probe to detect sporozoites which had been removed from the salivary glands of infected mosquitoes. Dot blot hybridization showed that pUNK1-34 could detect 1,000 sporozoites. The sensitivity of the probe was at the level of 3pg of pUNK1-34 (figure 20).

When pUNK1-45 was used as probe in dot blot hybridization reaction, it could detect 1,000-5,000 sporozoites (figure 19 and 21). In addition, pUNK1-45 was used to detect oocysts and sporozoites in homogenized mosquitoes at 8-9 days after ingesting infectious *P. falciparum* gametocystes. The oocysts in a quarter of abdomen and sporozoites in a head-thorax part could be detected. By day 15-23 post-ingestion of *P. falciparum* gametocysts, oocysts were detected in half of *An. dirus* abdomen and sporozoites were detected in a quarter of head-thorax part. Sensitivity of the probe was at the level of 6.2 pg of plasmid, 0.1 ng of extracted K1 DNA. By using 2 *An. dirus* mosquitoes as negative control, there was no cross-hybridization between pUNK1-45 probe and *An. dirus* DNA in 48 hours exposure.

In order to confirm the hybridization reaction between pUNK1-45 and the parasite in infected mosquitoes, pUNK1-45 was used as probe to detect sporozoites in each infected mosquito's salivary gland. The results (figure 22) indicated that pUNK1-45 was able to detect sporozoites in 1/10 of a gland in 5 out of 7 samples of infected mosquito. The sensitivity of the probe was at the level of 3 pg of plasmid pUNK1-45 and 0.1 ng of extracted K1 genomic DNA.

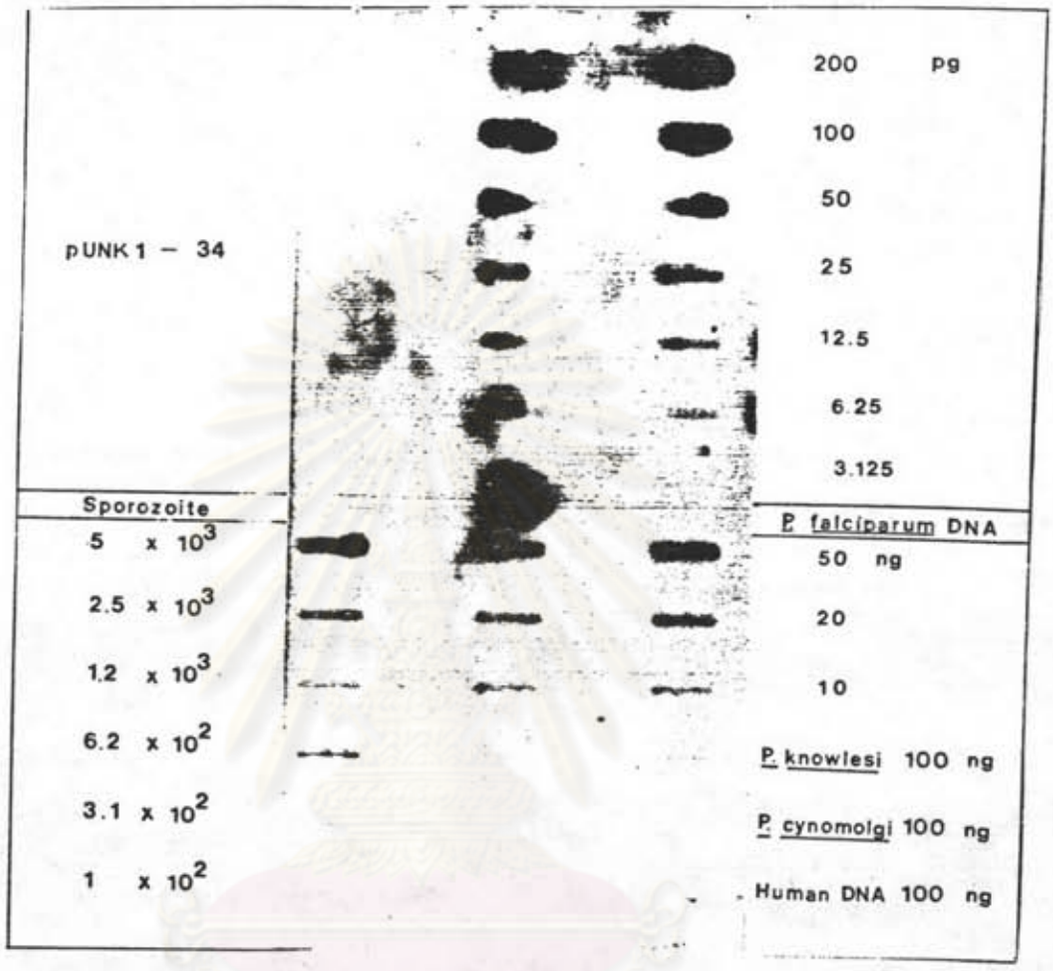


Fig. 20 Autoradiogram of slot blot hybridization of sperozoites with pUNK1-34 probe. pUNK1-34 and extracted K1-DNA were blotted as positive control. The specific activity of probe was 3.7×10^7 cpm/µg.

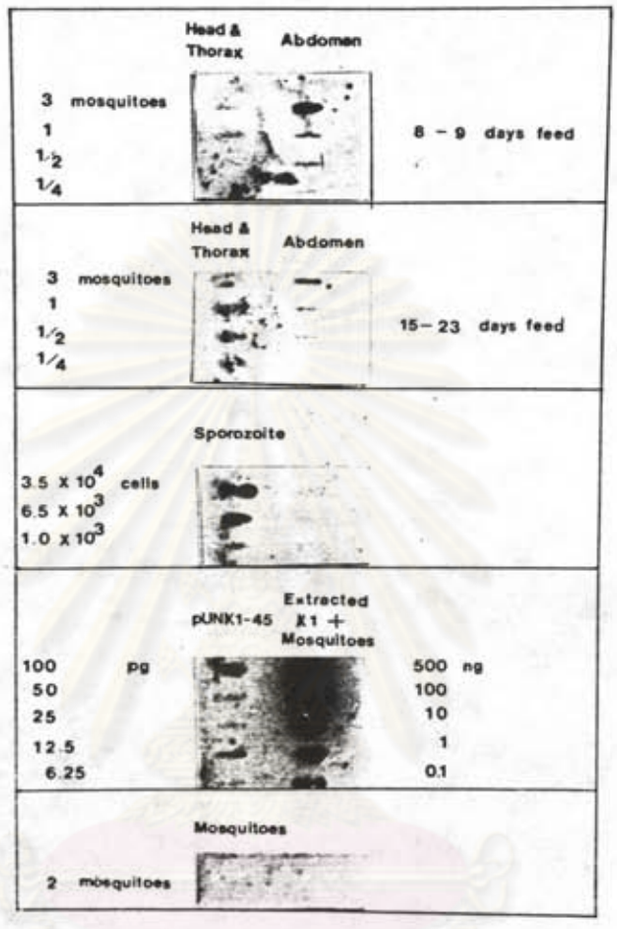


Fig. 21 Autoradiogram of slot blot hybridization of sporozoites and oocysts in homogenized infected mosquito, with pUNK1-45 probe. pUNK1-45 and extracted K1 genomic DNA were blotted as positive control. The negative control was two *An. dirus* DNA. The specific activity of probe was 3.2×10^7 cpm/µg .

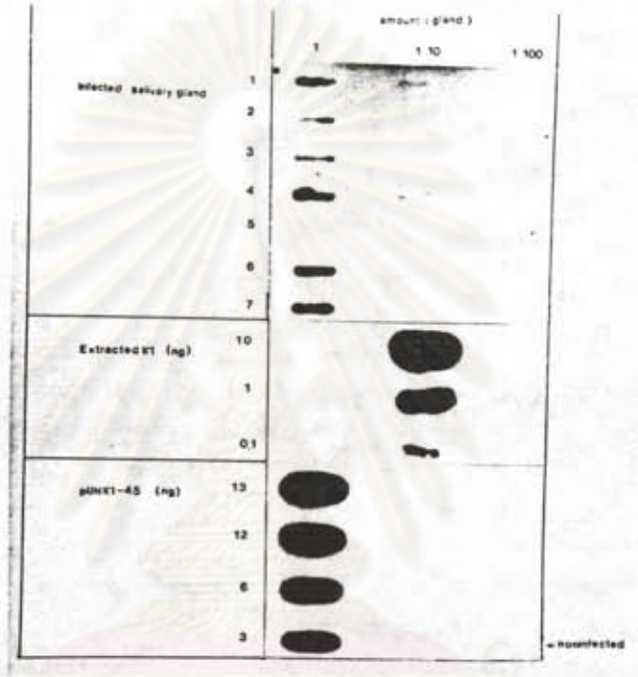


Fig. 22 Autoradiogram of slot blot hybridization of infected mosquito's salivary gland with pUNK1-45 probe. pUNK1-45 and extracted K1 genomic DNA were blotted as positive control, the negative control was noninfected mosquite.

10. Southern hybridization of *P. falciparum* DNA with recombinant probes.

P. falciparum isolate K1 was digested with restriction enzymes Bam HI, Hind III, EcoR I and Sal I. The digested DNA was then separated on 0.7 % agarose gel (figure 5) and blot transfered onto nylon membrane filter. The gel shown in figure 5A was hybridized with pUNK1-45 probe and the gel in figure 5B was hybridized with pUNK1-34 probe.

P. falciparum K1 DNA digested with Bam HI EcoRI and SalI and hybridized with pUNK1-34 probe showed a smeared hybridization pattern ranging in size from 6.6 Kb to 50 Kb (figure 23, lanes 10, 12 and 13). However for Hind III digestion, a single band of 5 kb was observed. The incomplete digested pUNK1-34 was used as positive control.

Using pUNK1-45 as probe, the hybridization patterns obtained were smeared (figure 24). Bam HI digested fragments showed a broad band of 23.5 kb. Three bands of 12, 15 and 20 kb were observed for Hind III fragments. The Sal I digested fragments showed smeared band of about 50 kb. The hybridized band of EcoRI digested fragments showed one strong signal of about 23.5 kb and a weak one signal about 7 kb.

The incomplete digested pUNK1-45 was used as positive control. The 9.7 and 0.5 kb of λHind III were hybridized with vector part (C1 gene) of the recombinant probes.

11. Restriction map of recombinant plasmids

The recombinant plasmids pUNK1-34 and pUNK1-45 were digested with a number of restriction enzymes. The insert of pUNK1-34 had

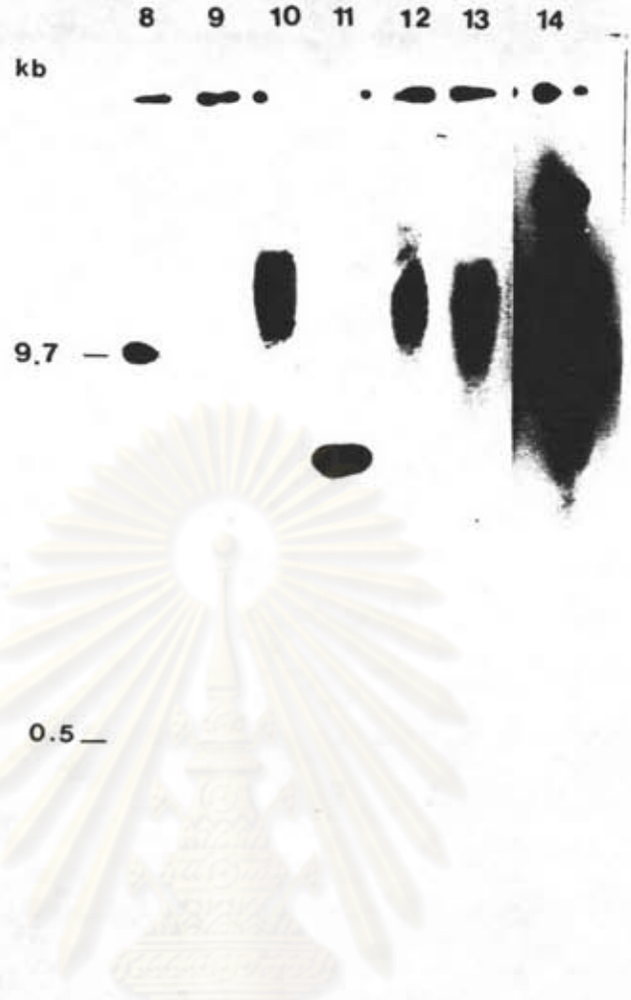


Fig. 23 Autoradiogram of Southern blot of *P. falciparum* K1 DNA digested with various enzymes, the gel was showed in figure 5B. The filter was hybridized to pUNK1-34 probe The specific activity of probe was 5.4×10^7 cpm/µg. The filter was exposed to x-ray film at -70°C for 5 hours

lane 8 : λHind III marker

lane 9 : uncut K1 genomic DNA

lane 10 : K1 DNA digested with Bam H1

lane 11 : K1 DNA digested with HindIII

lane 12 : K1 DNA digested with Sal I

lane 13 : K1 DNA digested with EcoRI

lane 14 : pUNK1-34 digested with BamHI

Fig. 24 Autoradiogram of Southern of *P. falciparum* K1 DNA digested with BamHI, Hind III, EcoRI and SalI. The gel was showed in figure 5A. The filter was hybridized to pUNK1-45 probe. The specific activity of probe was 4.8×10^7 cpm/µg. The filter was exposed to x-ray film at -70°C for 5hours.

lane 1 : λHindIII marker

lane 2 : uncut K1 genomic DNA

lane 3 : K1 DNA digested with Bam HI

lane 4 : K1 DNA digested with HindIII

lane 5 : K1 DNA digested with Sal I

lane 6 : K1 DNA digested with EcoRI

lane 7 : pUNK1-45 digested with Bam HI

Acc I, Cla I and Pvu II sites. Acc I could cut the vector at Sal I site, but there were no Cla I and Pvu II site in the vector (figure 25). In addition, there were no sites for Bam HI, Hind III, Sal I or Pst I in inserted DNA and EcoRI could not remove inserted DNA from the recombinant (data not shown). From analysis of the restriction size fragments it was possible to generate a restriction map for pUNK1-34 as shown in figure 27. AccI and ClaI sites were proximal to the EcoRI* cloning sites, and Pvu II site was located between AccI and Cla I sites.

The pUNK1-45 had Nde I, Kpn I and Cla I sites (figure 26) in the inserted DNA. There were no sites of these enzymes in the vector (data not shown). The inserted DNA had no site for Bam HI, Hind III, Sal I and Pst I (data not shown). The restriction map of pUNK1-45 is shown in figure 28. Nde I and Cla I sites were proximal to the EcoRI* cloning sites, and Kpn I site was located between Nde I and Cla I sites.

Fig. 25. Gel analysis of recombinant plasmid pUNK1-34 digestion with endonuclease. DNA was electrophoresed through 0.7% agarose gel stained in 2.5 µg/ml ethidium bromide. The gel contained in

lane 1 : the size marker, λDNA digested with Hind III

lane 2 : pUN121 digested with AcoI

lane 3 : pUN121 digested with Bam HI

lane 4 : pUN121 digested with ClaI

lane 5 : pUN121 digested with HindIII

lane 6 : pUN121 digested with PvuII

lane 7 : pUNK1-34 digested with AcoI

lane 8 : pUNK1-34 digested with Bam HI

lane 9 : pUNK1-34 digested with Cla I

lane 10 : pUNK1-34 digested with Hind III

lane 11 : pUNK1-34 digested with PvuII

lane 12 : pUNK1-34 digested with Cla I, Hind I

lane 13 : pUNK1-34 digested with PvuII, Bam HI

lane 14 : pUNK1-34 digested with PvuII, Hind III

lane 15 : pUNK1-34 digested with AccI, Bam HI

lane 16 : pUNK1-34 digested with AccI, Hind III

lane 17 : pUNK1-34 digested with ClaI, Bam HI

lane 18 : pUNK1-34 digested with PvuII, Hind III

lane 19 : pUNK1-34 digested with ClaI, Hind III Bam HI

lane 20 : pUNK1-34 digested with AccI, Hind III

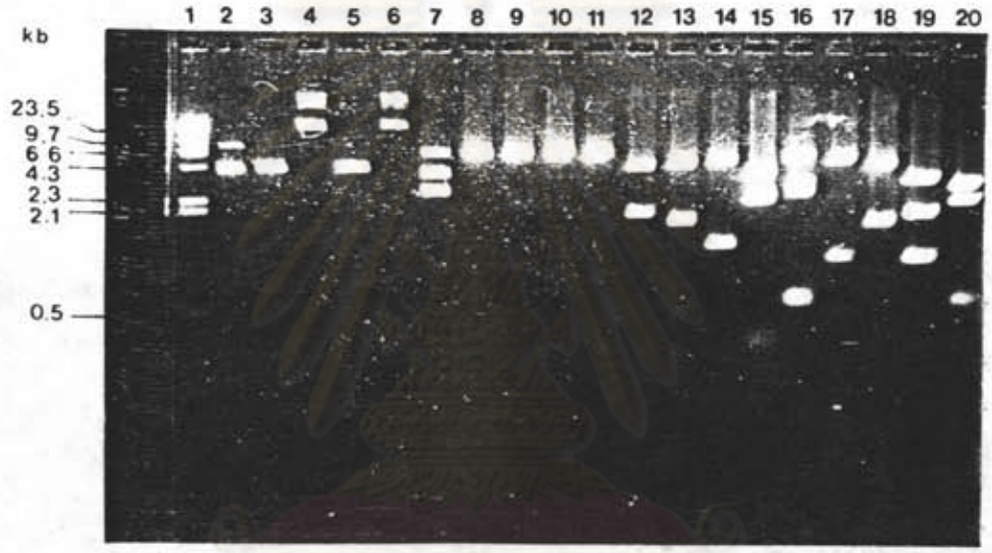


Fig. 26 Gel analysis of recombinant plasmid pUNK1-45 digestion with restriction endonuclease. DNA was electrophoresed through 0.7% agarcse gel and stained in 2.5 µg/ml ethidium bromide. The gel contained in

lane 1,9 : the size marker, λDNA digested with Hind III

lane 2 : recombinant plasmid pUNK1-45 uncut

lane 3 : recombinant plasmid pUNK1-45 digested with ClaI

lane 4 : recombinant plasmid pUNK1-45 digested with Cla I and Bam HI

lane 5 : recombinant plasmid pUNK1-45 digested with Cla I and Hind III

lane 6 : recombinant plasmid pUNK1-45 digested with Nde I

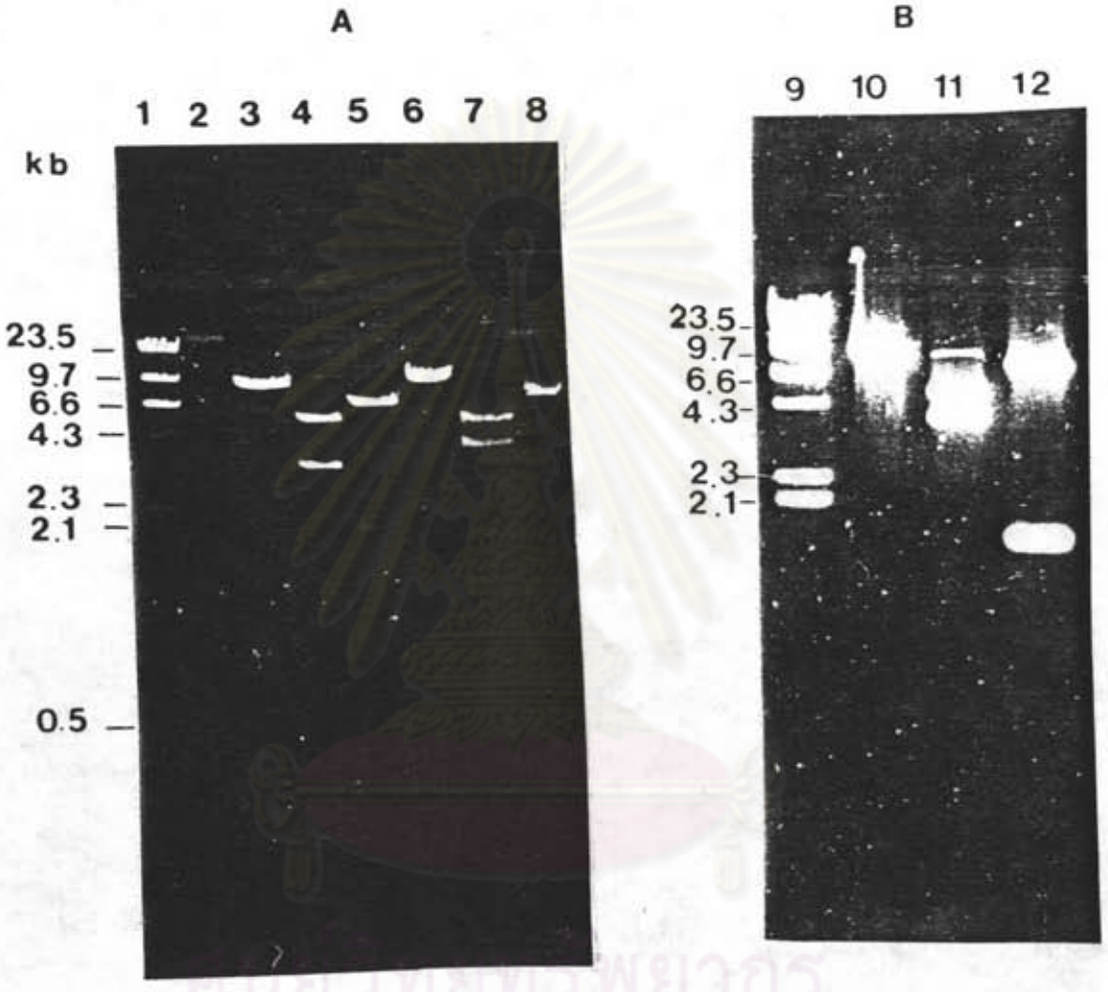
lane 7 : recombinant plasmid pUNK1-45 digested with Nde I and Bam HI

lane 8 : recombinant plasmid pUNK1-45 digested with Nde I and Hind III

lane 10 : recombinant plasmid pUNK1-45 digested with KpnI

lane 11 : recombinant plasmid pUNK1-45 digested with KpnI and Bam HI

lane 12 : recombinant plasmid pUNK1-45 digested with Kpn I and Hind III



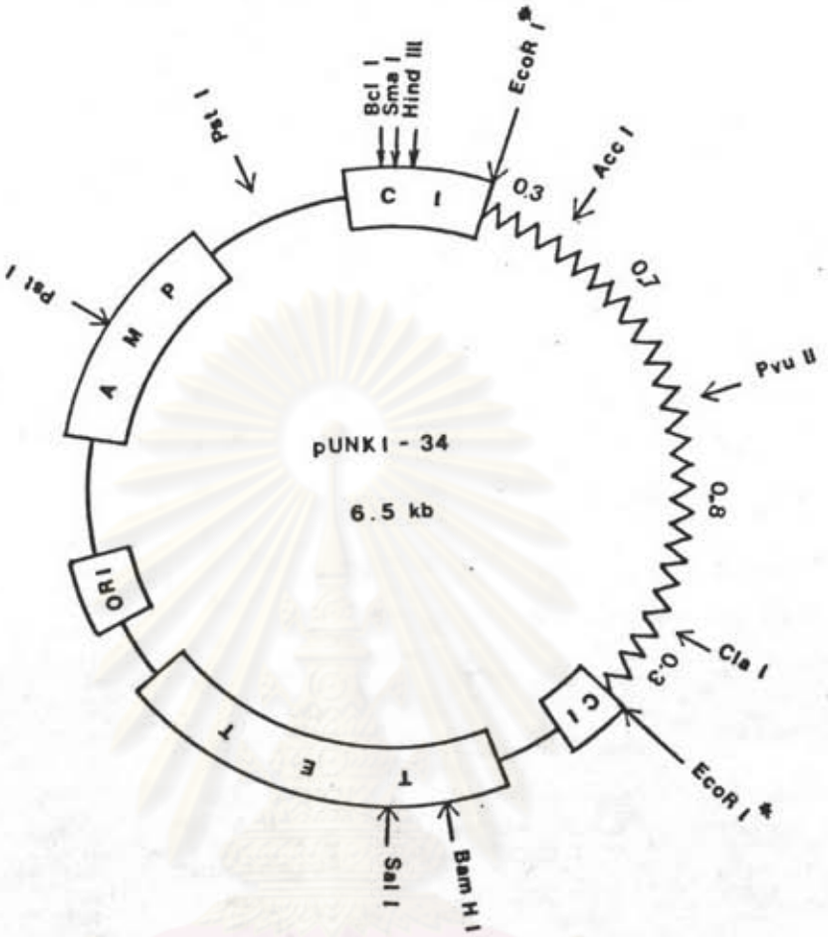


Fig. 27 A restriction map of pUNK1-34. The length of restriction fragment were estimate from molecular weight-mobility curve of λHind III fragment. It has EcoRI sites at its ends. The map shows AccI, PvuII and ClaI site.

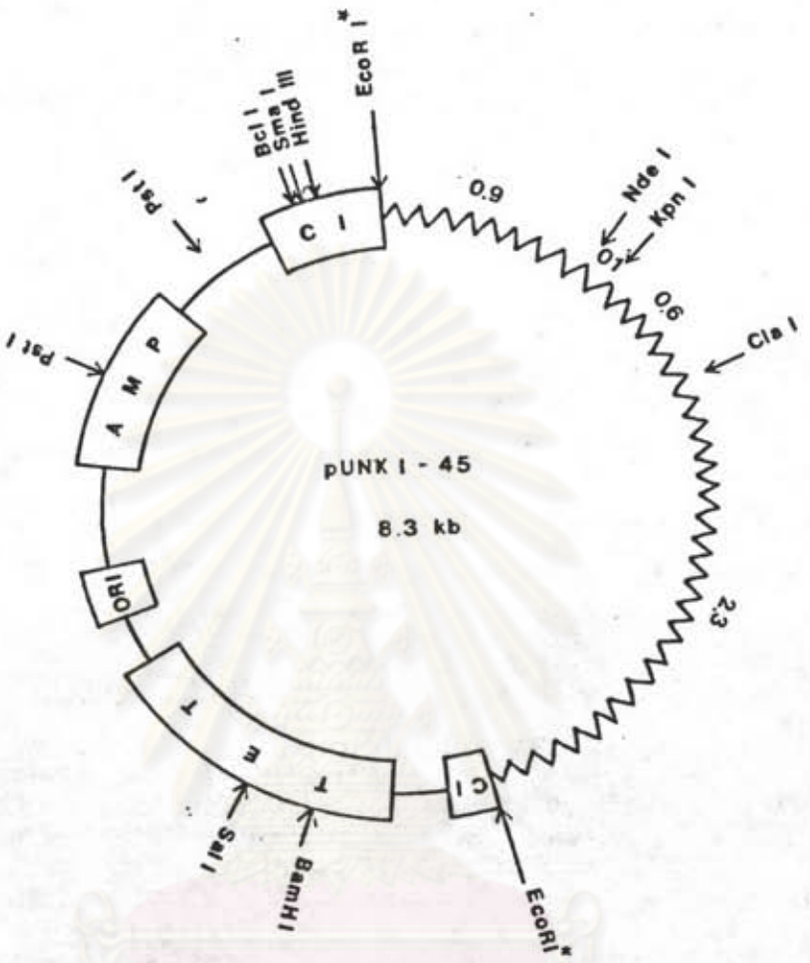


Fig. 28 A restriction map of pUNK1-45. The length of restriction fragment were estimate from molecular weight-mobility curve of λHind III fragment. It has EcoRI site at its ends. The map shows Nde I, KpnI and ClaI site.