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Salinan Kertas Kerja yang telah dibentangkan di "International Congress of Immunology", 1-6 November, New Delhi, India.

Merujuk kepada perkara di atas, saya dengan ini melampirkan sesalinan kertas kerja yang saya bentangkan di Congress tersebut untuk simpanan dan rujukan pihak puan.

Sekian, terima kasih.

Yang benar,

.....

Prof. Madya Norazmi Mohd. Nor
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THE USE OF ASSEMBLY PCR FOR CLONING OF A MALARIAL EPITOPE INTO *Mycobacterium smegmatis* - IMPORTANCE OF OVERCOMING CODON BIAS

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Mycobacterium bovis bacille Calmette Geurin (BCG) has been suggested to be an attractive vehicle for the delivery of various vaccine candidates, including those for malaria. However, despite possessing strong immunoadjuvant properties, recombinant BCG containing these malarial epitopes fail to elicit significant specific immunogenicity in mice. One possibility for this may be the codon bias between these organisms: mycobacteria is G:C rich while plasmodia is A:T rich. To test this hypothesis, we cloned the candidate malarial epitope, the C-terminus of *Plasmodium falciparum* merozoite surface protein-1 (MSP-1C) using assembly PCR into *Mycobacterium smegmatis*. This technique involved the generation of the full 300bp fragment from 18, overlapping 40bp oligonucleotides designed in favour of mycobacteria codon usage. The fragment was cloned into the *M. leprae* 18kDa gene driven by the mycobacterial hsp60 promoter in a plasmid designated pUS937. The whole hsp60/18kDa/MSP-1C cassette was then cloned into a mycobacterial replicative plasmid, pUS972, to produce pUS1754. A clone (pUS1758) containing the 'native' homologue of MSP-1C generated by PCR of genomic *P. falciparum* DNA was also constructed. Western blot analyses using an antibody against the 18kDa epitope showed that recombinants containing pUS1754 had approximately 100-fold higher expression levels than those containing pUS1758. Two additional clones containing the 'native' MSP-1C also showed low level expression as compared to the clone containing pUS1754. Thus overcoming codon bias for selected vaccine candidates may be important in increasing the level of their expression and hence could improve the immunogenecity of such epitopes cloned into mycobacteria.

INTRODUCTION

Mycobacterium bovis bacille Calmette Guerin (BCG) is currently used as a live vaccine against tuberculosis.

Although its efficacy in protecting against tuberculosis is controversial, it is an attractive vehicle for the delivery of various candidate vaccine epitopes including those for malaria (1,2,3).

This is because BCG is;

- safe
- cheap and easy to mass produce
- provides long term protection
- stimulates the cellular immune response strongly

However, early studies showed that malarial epitopes cloned into BCG were poorly immunogenic (1,2).

This may be due to low expression of such epitopes because of codon bias.

Mycobacteria is G:C rich (~ 70%), while

Plasmodia is A:T rich (~70%)

METHODS

To test this hypothesis, we;

- performed assembly PCR ( ): a two-step PCR on equimolar concentrations of several oligonucleotides to produce a DNA fragment coding for the *Plasmodium falciparum* merozoite surface protein-1 C terminus (MSP-1_{19}) with mycobacterium-preferred codon usage

- cloned the product into a plasmid vector, pUS937 ( ), at the *Bgl II* site within the *M. leprae* 18kDa gene driven by the hsp60 promoter; resulting in the expression of a fusion protein

- re-cloned the hsp60/18kDa+MSP-1_{19} cassette from pUS937-derived constructs into a shuttle vector, pUS972 ( ) to produce pUS1765

- re-cloned the same cassette ( ) but
  a) without MSP-1_{19} - pUS1763
  b) containing the native MSP-1_{19} - pUS1758

- transformed the fast growing mycobacterium, *M. smegmatis* with 100ng plasmid DNA by electroporation

- performed Western blotting using an antibody against the *M. leprae* 18kDa epitope.

RESULTS

A previous study showed that clones of *M. smegmatis* carrying the native MSP-1_{19} epitope resulted in

- delayed growth of the transformants (6 days)
- a reduced transformation efficiency by at least 10-fold from expected levels
- very low expression when reacted with the anti-18kDa antibody in Western blotting ( )

Using assembly PCR, it was observed that the

- growth of transformants improved to within the expected time (within 4 days)
- transformation efficiency was as expected for *M. smegmatis* (10^4 cfu/μg DNA)
- level of expression of the fusion protein was increased by at least 100-fold in clones containing the synthetic MSP-1_{19} as compared to those containing the native MSP-1_{19} ( )

CONCLUSIONS

Bias towards mycobacterium codon usage for the cloning of A:T rich gene fragments like those for malaria may be an important consideration for the cloning of such epitopes into BCG.

The use of assembly PCR is a very attractive tool for cloning synthetic DNA fragments, and is probably the best method for cloning composite candidate molecules coded for by genes located in different regions such as those for the previously described synthetic malarial vaccine, Spf66.

REFERENCES

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2. Matsumoto *et al.*, 1996, *Vaccine* 14; 54.
3. Norazmi and Dale, 1997, *Biotech. Lett.* 19: 1135.

ACKNOWLEDGEMENTS

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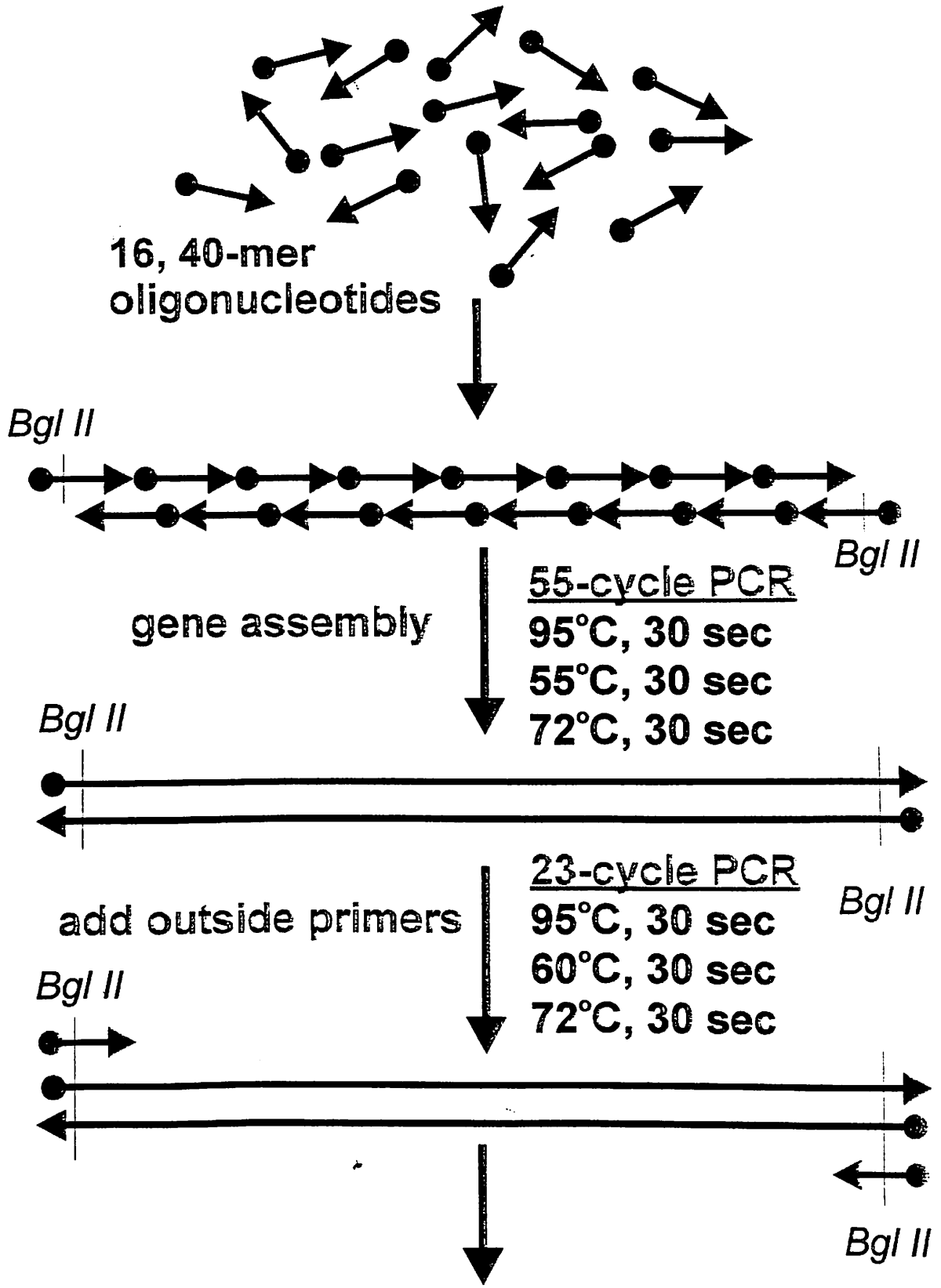

Figure 1:
Assembly PCR procedure

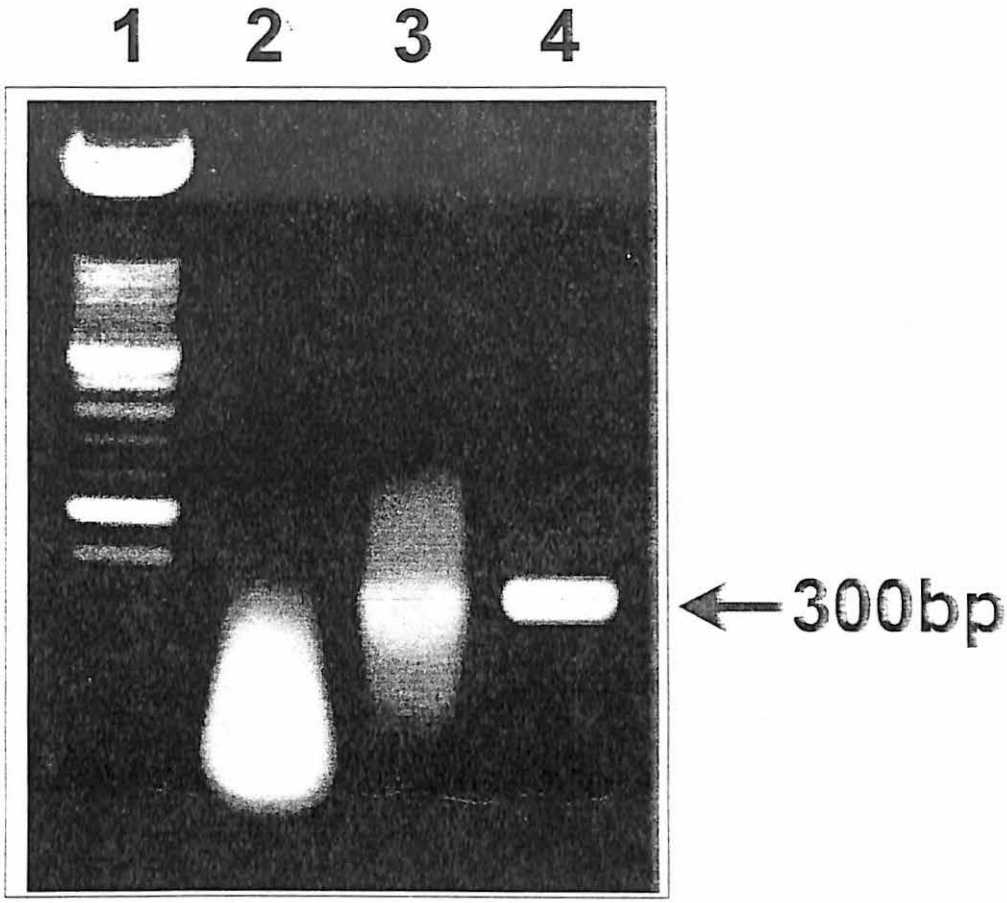

Figure 2:
Generation of synthetic MSP-1_{19} using assembly PCR

1 = 100bp size markers
2 - equimolar mixture of 16 oligos
3 - first stage assembly PCR
4 - second stage assembly PCR showing expected sized product (arrow)

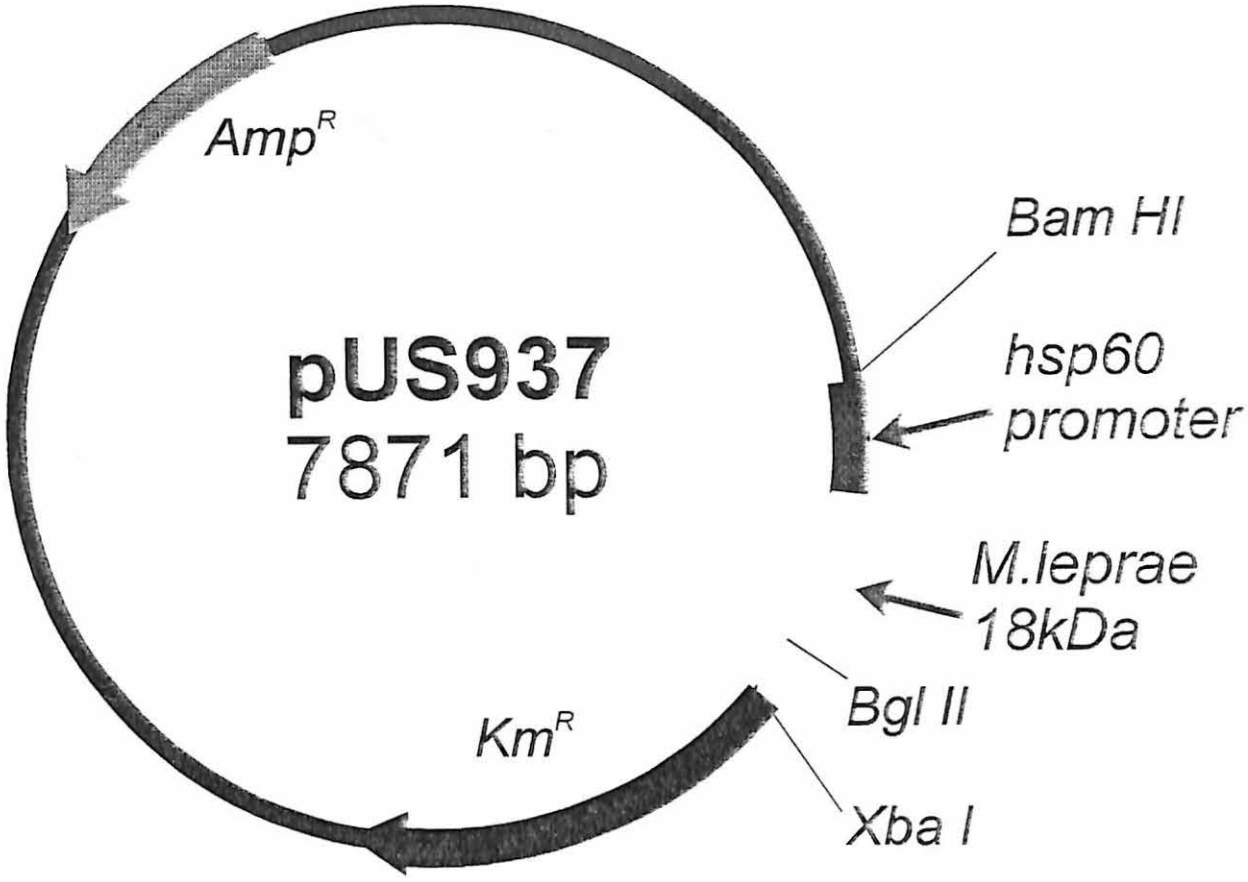

Figure 3:
Plasmid vector used for initial cloning

Synthetic or native MSP-1$_{19}$ was cloned into the Bgl II site within the **M. leprae** *18kDa gene resulting in the expression of a fusion protein*

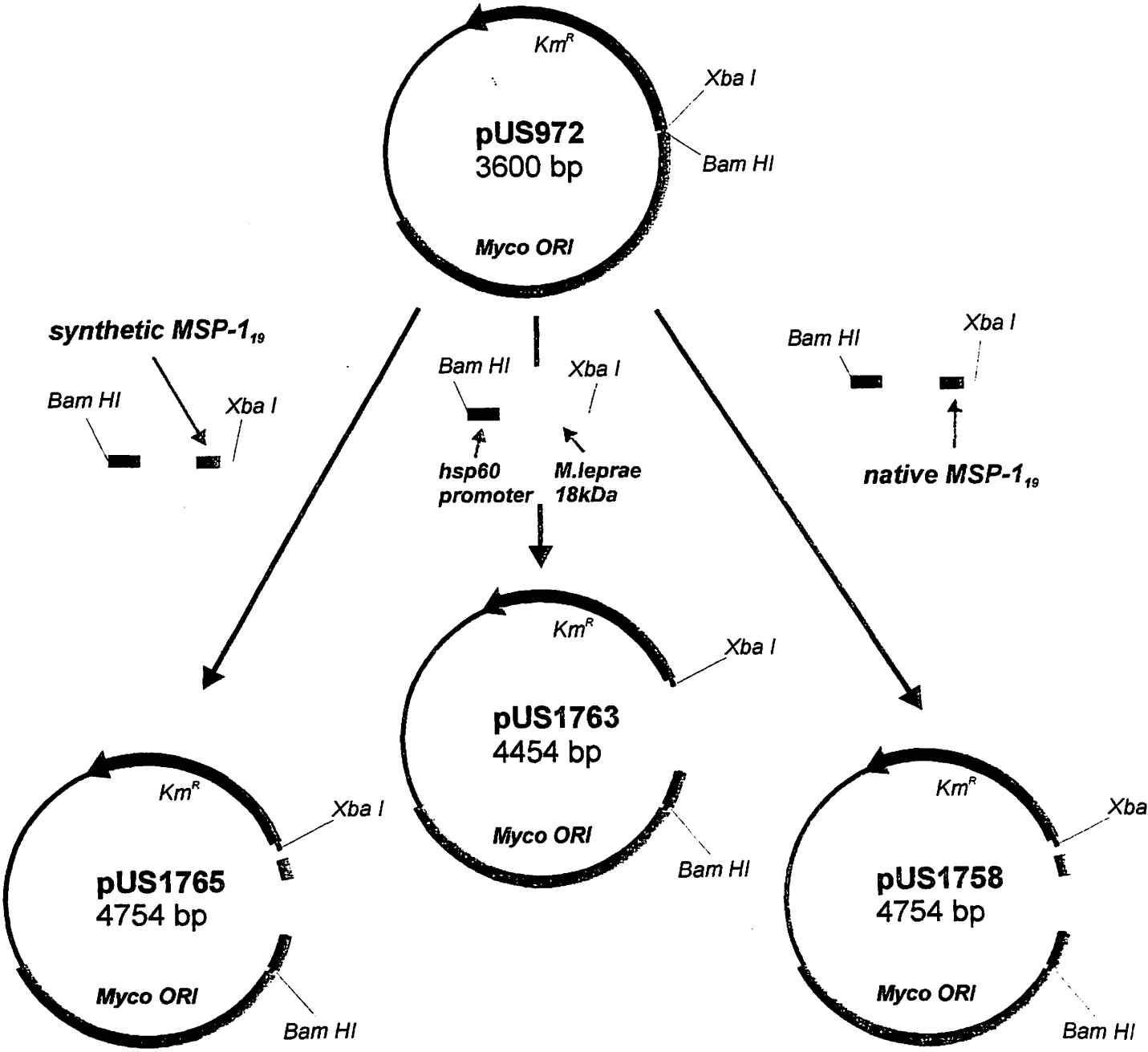

Figure 4:

Construction of shuttle plasmids for cloning into *M. smegmatis* for the expression of the

18kDa epitope (pUS1763)
18kDa:synthetic MSP-1 19 (pUS1765)
18kDa:native MSP-1 19 (pUS1758)

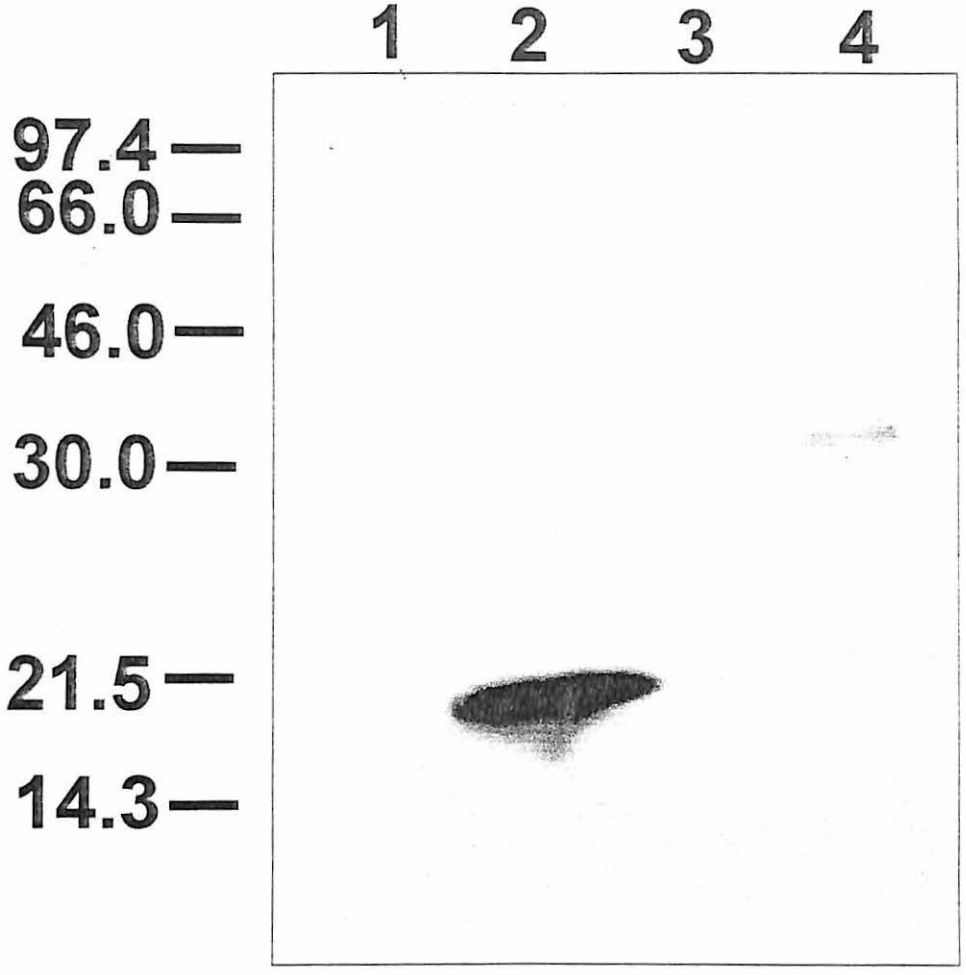

Figure 5:
Western blot analysis of
1) non-recombinant *M. smegmatis*
2) recombinant *M. smegmatis* expressing the 18kDa epitope only
(3&4) two recombinant *M. smegmatis* clones expressing the 18kDa:native MSP-1_{19} epitope

The 18kDa:native MSP-1_{19} epitope was weakly expressed as compared to the 18kDa epitope

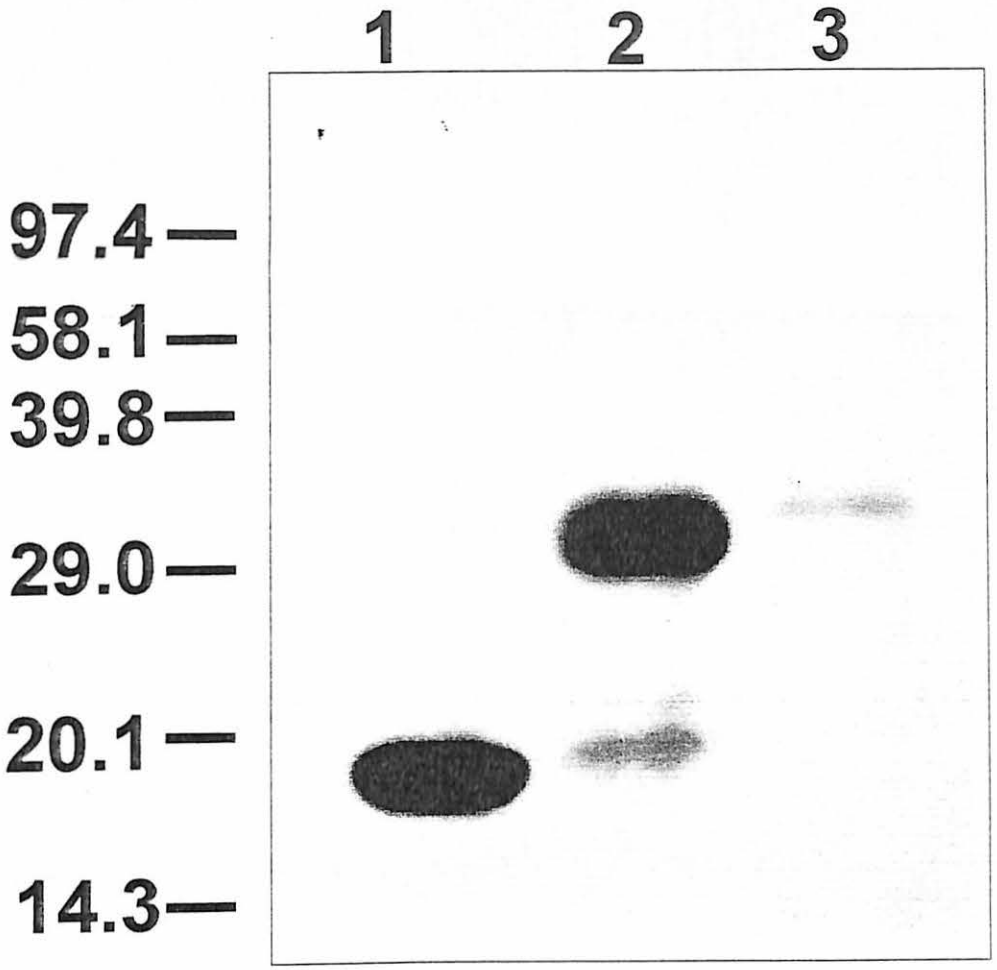

Figure 6:
Western blot analysis of recombinant *M. smegmatis* expressing the
(1) 18kDa epitope
(2) 18kDa:synthetic MSP-1_{19} epitope
(3) 18kDa:native MSP-1_{19} epitope

The 18kDa:synthetic MSP-1_{19} epitope was expressed at a similar level to the 18kDa epitope but at a much higher level to the 18kDa:native MSP-1_{19} epitope