

The following points serve to highlight the main findings of this study:

1. A novel technique called bridge-overlap-extension PCR was developed for constructing chimeric genes. It is especially useful where the length of the shorter segment of the chimera is 90-300 bp.
2. A chimeric gene between the signal sequence of P-factor of *S. pombe* and mature SK was synthesized and expressed under the control of *nmt1* promoter. A level of expression of mature SK that was similar to that in *E. coli* and *P. pastoris* was achieved. The mature SK was secreted into periplasmic fraction. No glycosylation or significant degradation was observed.
2. A novel mutant of extracellular protease of *S. pombe*, *epp1* was isolated.
3. Expression of chimeric gene of SK in *epp1* mutant yielded better expression under stationary phase conditions with almost complete conversion into mature SK and full recovery of cellular SK, with no detectable degradation.
4. A promoter element was isolated by screening for DNA fragments that drive the expression of *gfp* reporter in *S. pombe* in response to temperature shift from 37°C to 25°C. Sequencing and activity of the new promoter revealed that this element corresponds to a small region of the *nmt1* promoter. Thus, we have uncovered a hitherto unknown function of a small region of the previously known *nmt1* promoter.
5. Expression of *gfp*,  $\beta$ -galactosidase and *cdc18* of *S. pombe* from the *nmt-185* promoter was compared to that with *nmt1*. The new promoter exhibits a 5-fold faster kinetics of induction achieving comparable levels of expression as with

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*nmt1* but in much shorter induction period. This new promoter should be of immense use as a research tool for expression of both homologous and heterologous genes in *S. pombe*.

7. A new vector incorporating the *nmt-185* promoter fragment and a multiple cloning sequence for insertion of gene of interest has been constructed. This vector may be useful for expression of proteins of commercial and pharmaceutical interest.