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Title:	Vectors with a flexible multiple cloning site and modular epitope tags for gene expression studies in <i>Schizosaccharomyces pombe</i>
Authors:	Sajeevan, Aiswarya (/jspui/browse?type=author&value=Sajeevan%2C+Aiswarya) Pandian, Rakesh (/jspui/browse?type=author&value=Pandian%2C+Rakesh) Mishra, Shravan Kumar (/jspui/browse?type=author&value=Mishra%2C+Shravan+Kumar)
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Abstract:	The fission yeast <i>Schizosaccharomyces pombe</i> is a widely used eukaryotic model organism for cell biology, genetics and metabolomics studies. <i>S. pombe</i> -specific autonomously replicating shuttle vectors are frequently used for gene expression studies because such vectors can stably replicate, and cells harbouring them can be easily selected. Vectors have been designed with varying promoter strengths and epitope tags, yet higher flexibility and diversity further augmenting their application is desired. Here, we have prepared a set of twenty vectors with auxotrophic markers for leucine and uracil by modifying the widely used pREP plasmid with a thiamine-repressible nmt1 (no message in thiamine) promoter. The vectors offer distinct benefits, including an improved multiple cloning site (MCS) with unique cutting sites for more restriction enzymes and a set of N- and C-terminal 3xMYC, 3xFLAG and EGFP epitope tags for visualization and purification of expressed proteins. Further, different constitutive and thiamine-repressible promoters are used for varying a gene's expression, as verified by western blot detection of vector-borne fusion proteins in <i>S. pombe</i> . Importantly, DNA sequences encoding various elements including the epitope tags are kept modular with respect to their flanking restriction sites. Vectors reported here enhance the repertoire of tools currently available for molecular studies in <i>S. pombe</i> .
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