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# A versatile expression vector system for mammalian cell factories


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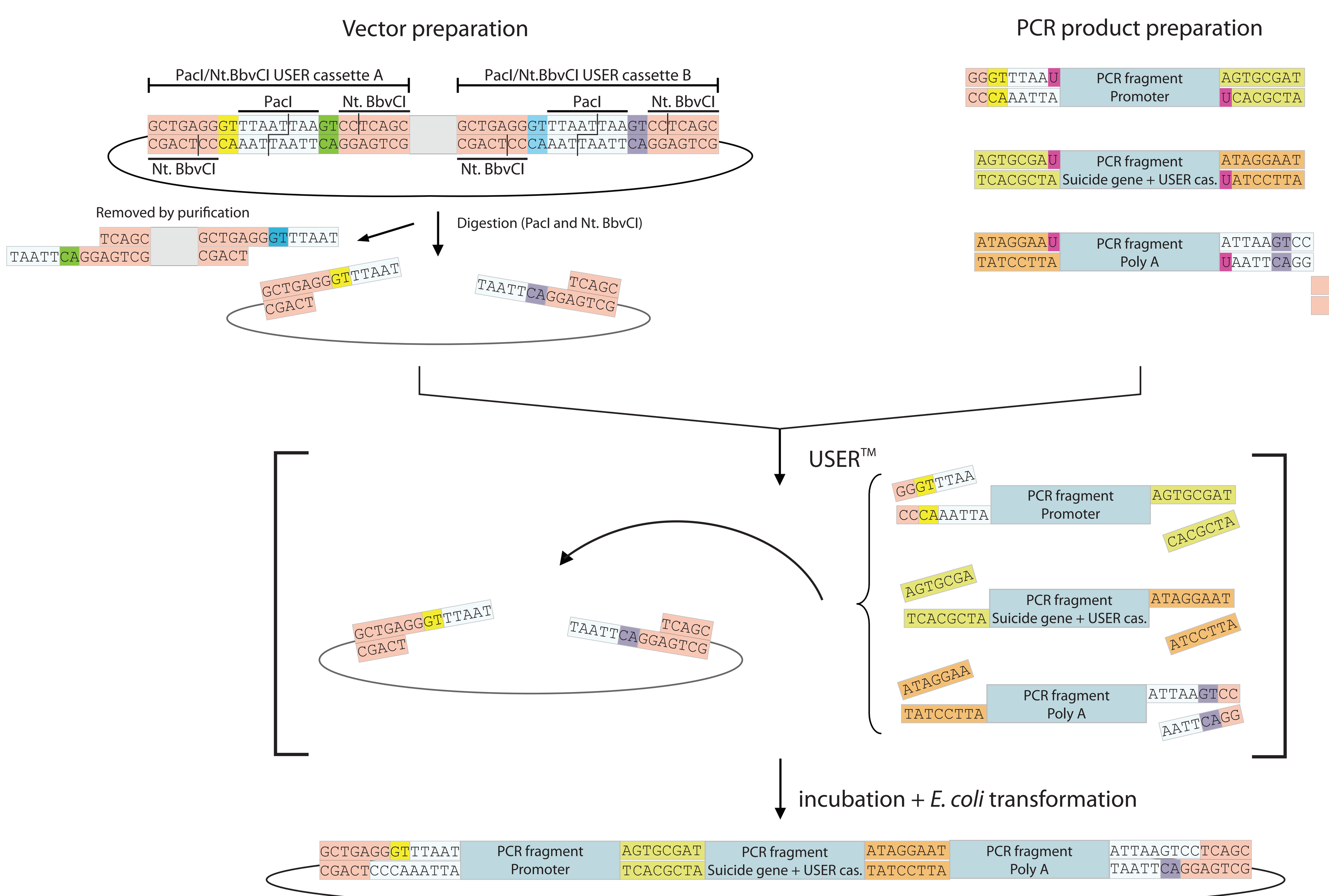
## Aim

The development of the field of mammalian cell factories requests fast and high-throughput methods which means high need for simpler and more efficient cloning techniques. This project applies the ligation-free USER™ (uracil-specific excision reagent) cloning technique to construct mammalian expression vectors with maximum flexibility.

## Cloning strategy

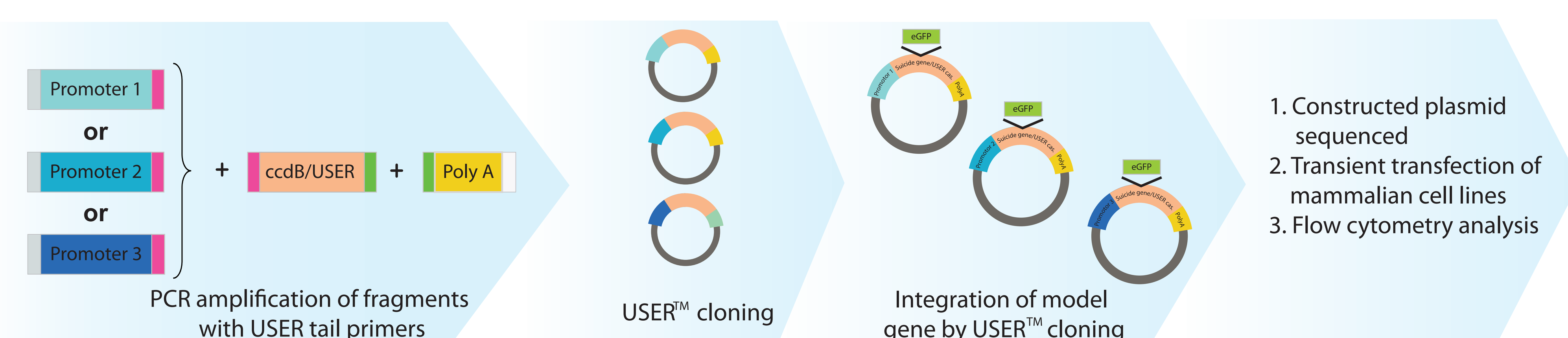
The USER™ cloning is a ligation independent cloning technique. The method applies long complementary overhangs to anneal to each other to form a stable hybridisation product that can be used to transform *E. coli* without prior ligation. The overhangs on the PCR products are custom-made and their generation is restriction site-independent. Assembly of complex DNA constructs made of up several fragments can easily be fused seamlessly together. The method provides optimal flexibility. The selected cloning strategy is given in the figure below (1, 2).




The cloning strategy for preparing a basic transient expression vector with a promoter, a USER cassette for gene insertion and a polyA tail.

## Analysis of promotor expression vectors

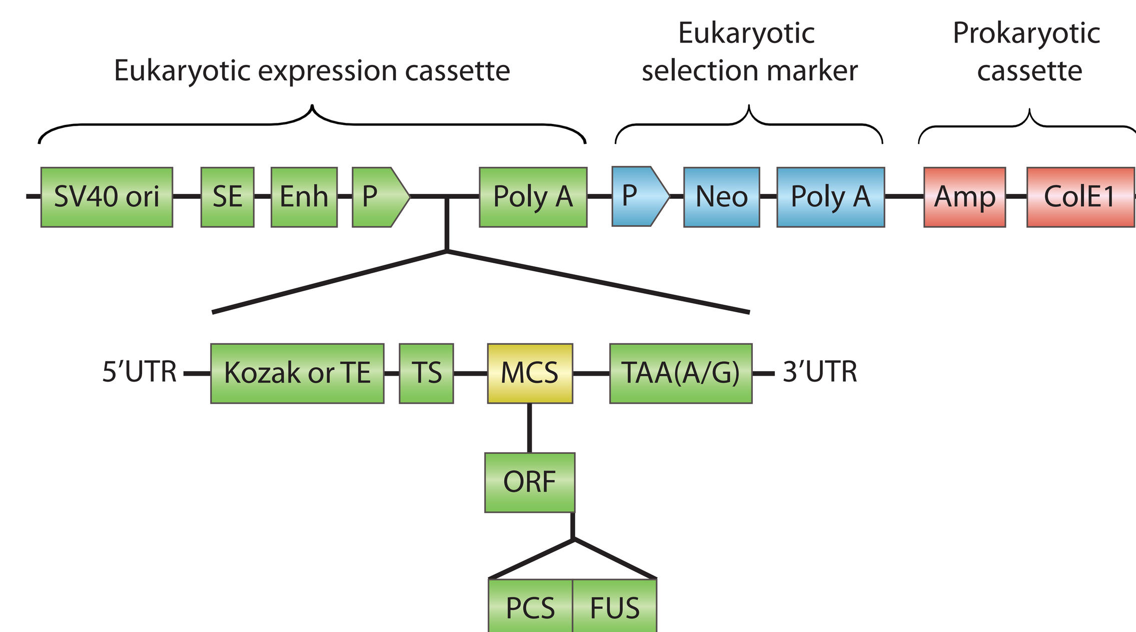
A vector libary of promotors with different expression levels is constructed by using the same primer tails at the different promotors. The functionality of the constructed vectors will be tested in CHO and HEK293 cell lines by insertion of eGFP as model protein.




Strategy for preparing a basic transient expression vectors with promoter of different expression levels. The eGFP is used as model gene for analysis of expression

## Mammalian expression vector parts

Traditionally mammalian expression vectors contain elements for expression in mammalian cells, selection in mammalian cells and their replication and selection in bacteria. Basic and optional vector parts which in principle easily could be inserted by USER™ are illustrated below.




Basic and optional components of a mammalian expression vector; Eukaryotic origin of replication (e.g. SV40 ori), structural elements (SE), enhancer (Enh), promoter (P), translational enhancer (TE), targeting signal (TS), Multiple cloning site (MCS) termination signal (TAA(A/G), open reading frame (ORF), protease cleavage site (PCS), fusion protein or tag (FUS), selection marker (e.g. Neo), polyadenylation (polyA). Selection marker (Amp) and replication origin (ColE1) for *E. coli* (3).

## Perspectives

The simplicity and flexibility of the USER™ cloning technique facilitate the adaption of the vector system into high-throughput cloning. Especially, fusion proteins and site-directed mutations are easily prepared. In the future, the system is planned to include site-specific integration and knockout cassettes.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This project is funded by the Lundbeck Foundation. Thanks to Dorte Marie Koefoed Holm for assistance and providing the AsiSi/Nb.BtsI cassette with the suicide gene-USER cassette. Thanks to the Danish Cancer Society for providing template plasmids and the HEK293 cell line.


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