A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-13; Unit I)

Expression Vectors

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Expression vector

- Special type of cloning vector that have the transcription and translation signals necessary for regulation of expression of cloned genes
- Expression of foreign gene in expression vector can be achieved by use of
- A. promoters
- B. Expression cassette

Promoters

- *E.coli* promoter for faithful expression in *E.coli*. Example is ptac
- P*tac* (tac promoter/operator) hybrid promoter composed of -35 bp of tryp promoter and the -10 bp region of UV5 promoter/O
- IPTG inducible
- Example is pGEX series, pET series etc.

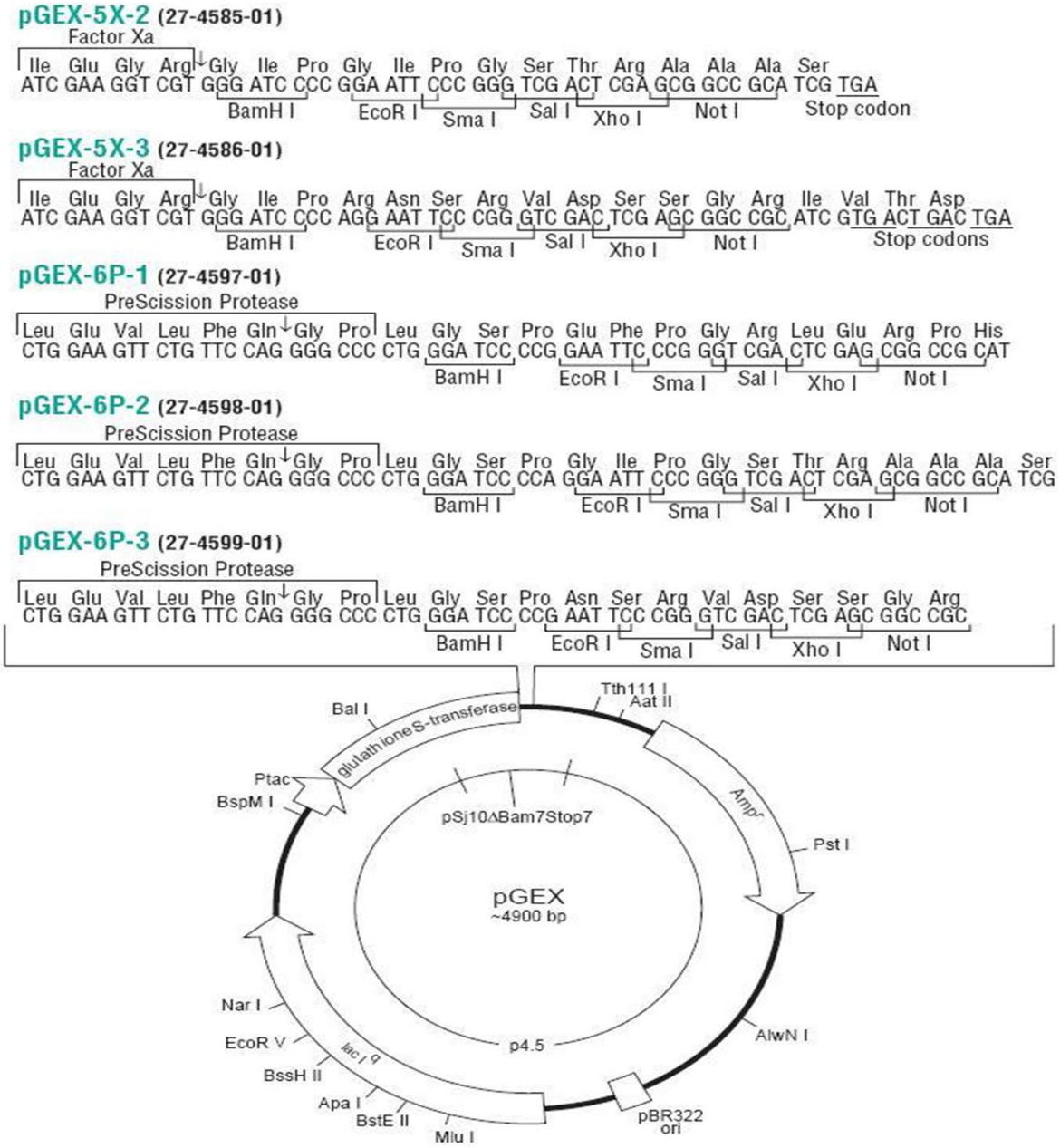
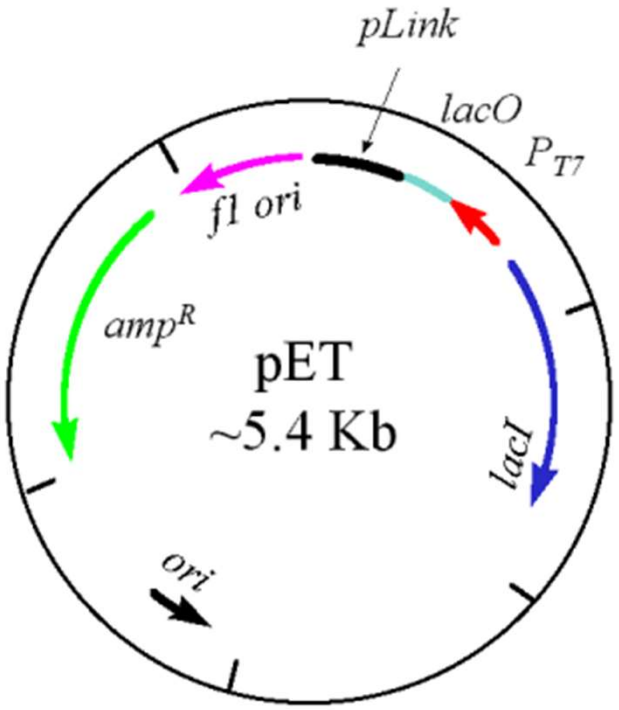


Fig 2. Map of the glutathione S-transferase fusion vectors showing the reading frames and main features. See Appendix 2 for the control regions of the ten vectors.

Phage promoters

- P_L regulated CI repressor
- $T_{7,}$ SP6 promoters for high level transcription
- Example is T_7 expression vector is pET series vector
- a *lacI* gene which codes for the *lac* repressor protein
- a T7 promoter which is specific to only T7 RNA polymerase (not bacterial RNA polymerase) and also does not occur anywhere in the prokaryotic genome
- a *lac* operator which can block transcription
- a polylinker
- an f1 origin of replication (so that a single-stranded plasmid can be produced when co-infected with M13 helper phage)
- an ampicillin resistance gene
- a ColE1 origin of replication



Expression vector for eukaryotic cells

- Promoter from prokaryotic genes express poorly in eukaryotes.
- Plant specific and animal specific promoter
- **Nopaline synthase (nos) promoter** from T-DNA
- 200 bp long
- Contains several DNA sequence motif which direct the expression of linked gene
- Promoter is active in basal region of plant
- One duplication, increased gene expression thrice

35s RNA promoter of CaMV

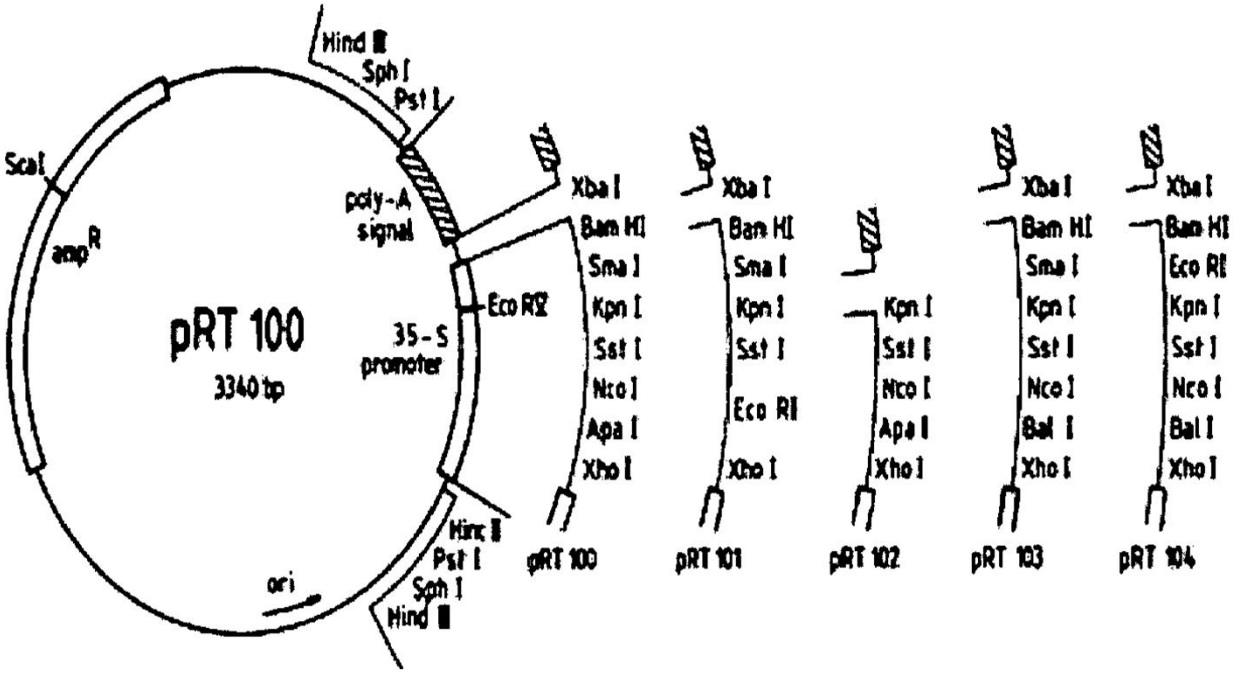
- ~343 bp long
- Cauliflower mosaic virus
- Para retrovirus contain DNA not RNA
- Contain strong transcriptional promoter
- On duplication result in 10 fold increase in expression
- Mainly in leaf

Expression cassettes

- Cassette means a device containing film such as magnetic tape for insertion into equipment like tape recorder, VCR etc
- Expression cassette is combination of DNA sequences, which allow faith full expression of the cloned gene
- Insertion of foreign gene/DNA fragment after the promoter for expression
- Ex pRT plasmids have been derived from pUC 18/19

pRT series vector

- Series of pRT100 differ to each other in polylinker sequence
- Each flanked by 35s promoter on one end
- A polyA sequence at other end
- Ampicillin resistant gene marker present



Acknowledgement and Suggested Readings:

1. Gene Cloning and DNA Analysis: An Introduction; Sixth Edition ; T. A. Brown; Wiley – Blackwell Publications
2. Principles of Gene Manipulation; Sixth Edition; Sandy B Primrose, Richard M Twyman and Robert W. Old; Wiley – Blackwell Publications
3. Biotechnology: Applying the Genetic Revolution; David P. Clark and Nanette J. Pazdernik; Academic Press (Elsevier)

Thanks