Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	028
Title	Platform Expression Vectors in Use at CESG
Research Unit	Cloning
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Name	Expression System	Origin of Replication	Cloning Method	Lethal Cassette	N-terminal His Tag	Other Tag(s)	Vector Derived Cleavage Site	Selectable Marker	Notes
pVP13	T5 (LacIq)	colE1	Gateway	*ccdB*-CAT	His6	MBP	-	Amp-Cm	*E. coli* expression
pVP16	T5 (LacIq)	colE1	Gateway	*ccdB*-CAT	His8	MBP	-	Amp-Cm	*E. coli* expression
pVP33K	T5 (LacIq)	colE1	FlexiVector	Bar-CAT	His8	MBP/TetraCys	HRV 3CP/TEV	Kan-Cm	*E. coli* expression
pVP33A	T5 (LacIq)	colE1	FlexiVector	Bar-CAT	His8	MBP/TetraCys	HRV 3CP/TEV	Amp-Cm	*E. coli* expression
pVP56A	T5 (LacIq)	colE1	FlexiVector	Bar-CAT	His8	MBP	HRV 3CP/TEV	Amp-Cm	*E. coli* expression
pVP56K	T5 (LacIq)	colE1	FlexiVector	Bar-CAT	His8	MBP	HRV 3CP/TEV	Kan-Cm	*E. coli* expression
pVP65K	T5 (LacI)	colE1	FlexiVector	Bar-CAT	His8	MBP	TVMV	Kan-Cm	*E. coli* expression/ *in vivo* cleavage
pVP68K	T5 (LacI)	colE1	FlexiVector	Bar-CAT	His8	MBP	HRV 3CP	Kan-Cm	*E. coli* expression
pVP80A	T5 (LacI)	colE1	FlexiVector	*sacB*-CAT	His8	MBP	HRV 3CP	Amp-Cm	*E. coli* expression
pVP80K	T5 (LacI)	colE1	FlexiVector	*sacB*-CAT	His8	MBP	HRV 3CP	Kan-Cm	*E. coli* expression
pVP81K	T5 (LacI)	colE1	FlexiVector	*sacB*-CAT	His8	MBP	TVMV	Kan-Cm	*E. coli* expression/ *in vivo* cleavage
pVP87K	T5 (LacI)	colE1	FlexiVector	*sacB*-CAT	His8	periplasmic MBP	TVMV	Kan-Cm	*E. coli* expression/periplamic targetting
pVP91A	T5 (LacI)	colE1	FlexiVector	*sacB*-CAT	His8	-	-	Amp-Cm	*E. coli* expression
pCDF91S	T5 (LacI)	CloDF13	FlexiVector	*sacB*-CAT	His8	-	-	Strep-Cm	*E. coli* expression/co-expression
pEU-His-Flexi	SP6	pMB1	FlexiVector	Bar-CAT	His6	-	-	Amp-Cm	cell free expression
pEU-GST-Flexi	SP6	pMB1	FlexiVector	Bar-CAT	-	GST	-	Amp-Cm	cell free expression
pEU-HSBC	SP6	pMB1	FlexiVector	*sacB*-CAT	His6	-	-	Amp-Cm	cell free expression

Summary

CESG has developed over 70 specialized for use in *E coli*, mycobacterium, yeast, insect cells and wheat germ cell free expression. The vectors shown in the table are the most frequently used in our current protein production platform. Our vectors are modular by design, with easily interchangeable promoters, selectable markers, and amino-terminal tags. Most of these vectors use the restriction/ligation base Flexi®Vector cloning system developed by Promega (Madison, WI). We have changed the lethal gene in some of our Flexi®Vectors to the *sacB* gene so we no longer require the proprietary and difficult to use BR610 strain needed to propagate *E coli* containing Barnase. The *sacB* gene is lethal only in the presence of sucrose and thus can be propagated in any commonly used *E. coli* strains in medium lacking sucrose. Most of these vectors are designed such that the TEV protease cleavage site is incorporated into the insert so that the tags and the amino acids derived from the 5' cloning site are removed. However, some of our vectors have protease cleavage sites designed into the vector. In addition to the vector derived cleavage sites, two vectors, pVP65K and pVP81K, express the corresponding protease which cleaves the MBP-fusion protein *in vivo*, librating the His tagged target protein. The pVP87K vector has the periplasmic form of MBP that targets the fusion protein to the periplasm or membrane proteins to the periplasmic membrane. The pCDF91S vector has a compatible origin of replication with the colE1 origin that's in most of

our other vectors allowing for the maintenance of two different vectors in the same cell for protein-protein complex studies. All vectors shown here are available through the PSI-MR or are in the process of submission.	
Acquiring the Technology	Contact Brian Fox bgfox@biochem.wisc.edu.
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