

λSCREEN: A High Efficiency cDNA Cloning Vector for Protein Expression, Purification, and Subtractive Probe Synthesis

Robert Mierendorf and Robert Novy — Novagen, Inc.

The pioneering work of several groups during the 1970's and early 1980's (1-6) led to the development of the bacteriophage λ cloning system, which has played a critical role in molecular biology. Over the last decade a number of popular vectors have emerged, which allow the isolation of genes based on screening with nucleic acid probes and on the expression of polypeptides that can be detected by ligand binding or other functional assays. Other features, such as additional cloning sites and simple conversion to plasmid subclones, have been incorporated more recently to facilitate manipulation and analysis of recombinants.

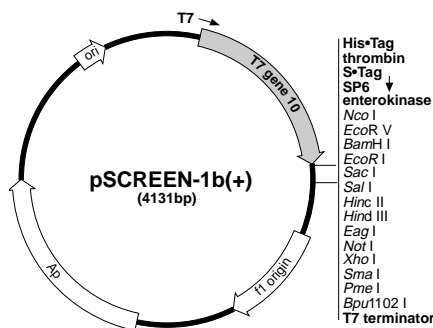
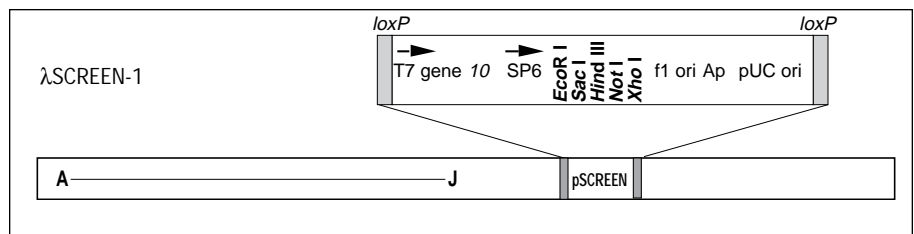
While current vectors have proved useful, none has effectively exploited newly developed approaches for protein expression and purification. In addition, many current commercial vectors are not well-suited for the synthesis of full-length subtractive probes, since they either lack conveniently placed phage promoters or downstream rare restriction sites. Novagen originally offered two cDNA cloning vectors, λEXlox[®] and λSHlox[®], which were designed for T7-driven expression and probe synthesis, respectively (7). We have now developed a unique vector, λSCREEN-1, which combines and expands the features of both original vectors. This upgraded vector fulfills the most stringent criteria for ease-of-use, powerful expression with many purification options, and potential for RNA synthesis for subtractive hybridization or *in vitro* translation analysis.

Vector Construction and Features

The base vector used to construct λSCREEN-1 was λlox, a derivative of λNM1150 (7). λNM1150 carries the *b538* deletion, in which *att* and *int* regions are missing (resulting in an obligatory lytic phage), has the 434 immunity region, and is *red⁻* (recombination defective) (5). This vector backbone produces high-titers on

gene silence until introduced into pET System hosts, ensuring clone stability

- Fusion proteins contain N-terminal 260aa T7•Tag[®], His•Tag[®] and S•Tag[™] sequences, allowing rapid affinity purification and detection
- Optional removal of T7•Tag + His•Tag with thrombin, or T7•Tag, His•Tag and S•Tag with enterokinase



- f1 origin allows single stranded DNA production from plasmid subclones
- SP6 promoter proximal to cloning sites allows unfused RNA synthesis
- Downstream *Pme* I site among rarest in cDNA, allowing full-length probe synthesis (8)

a variety of host strains (typically > 10¹¹ pfu/ml liquid culture), and produces superior DNA yields to many other vectors. The pSCREEN-1 vector was constructed, sequenced and functionally verified while in plasmid form. The pSCREEN plasmid was inserted into the λlox vector after removal of *Xho* I sites in the phage backbone to produce λSCREEN-1. The finished vector was reverified by restriction mapping and PCR analysis, and tested for expression using plaque lift assays in parallel with λEXlox. Maps of λSCREEN-1 and pSCREEN-1, which is the plasmid produced by *cre-loxP* mediated subcloning, are shown above.

The λSCREEN-1 vector system has the following features:

- Unique cloning sites for *Eco*R I, *Sac* I, *Hind* III, *Not* I and *Xho* I; compatible with many directional cloning strategies
- Ability to express cloned sequences as fusion proteins under T7 promoter control for expression screening
- Easy conversion to plasmid subclones by *cre-loxP* site-specific recombination *in vivo*
- pSCREEN-1 subclones maintain target

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