

pSH18-34

pSH18-34 Technical Information

Introduction

pSH18-34 (Catalog no. V611-20) is a 10.5 kb *lacZ* reporter plasmid designed for use with the Hybrid Hunter™ System. The plasmid can be transformed into appropriate yeast strains to generate a reporter strain for detection of LexA-mediated protein-protein interactions.

Shipping/Storage

20 µg of plasmid DNA, lyophilized in TE, is supplied. Lyophilized plasmid is shipped at room temperature and should be stored at -20°C.

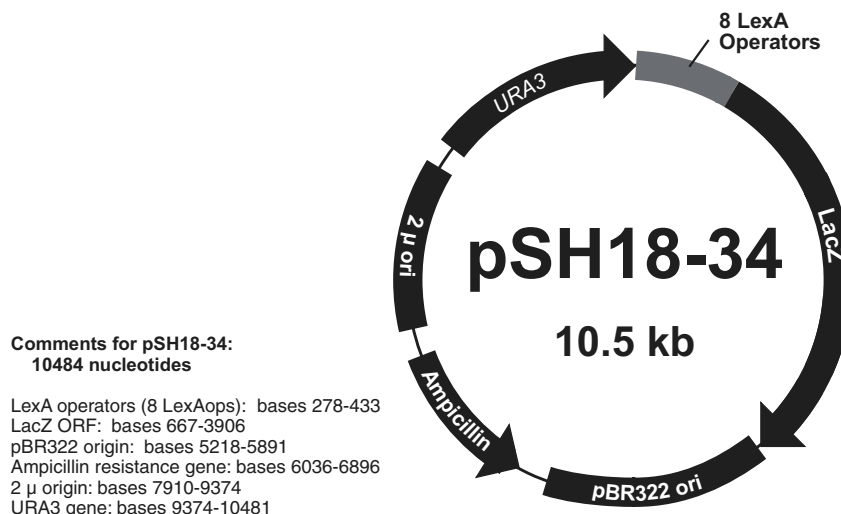
Features of pSH18-34

The table below summarizes the features of pSH18-34 (10484 bp). All features have been functionally tested.

Feature	Benefit
<i>LexA</i> operators (8 <i>LexA</i> ops)	Allows transcriptional activation of the <i>lacZ</i> gene by LexA-containing bait plasmids
<i>lacZ</i> gene	Reporter gene to allow selection of positive protein-protein interactions by assaying for β-galactosidase activity
pBR322 origin	Allows replication and maintenance of the plasmid in <i>E. coli</i>
Ampicillin resistance gene	Selection of vector in <i>E. coli</i>
2 µ origin	Maintenance and high-copy replication in yeast
<i>URA3</i> gene	Auxotrophic selection of the plasmid in Ura ⁻ yeast hosts (e.g. L40, EGY48, or EGY191)

Map of pSH18-34

A map of pSH18-34 is provided below. The complete sequence of pSH18-34 is available for downloading from our web site (<http://www.invitrogen.com>) or from Technical Service (see the other side).



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pSH18-34 Technical Information, continued

Propagation and Maintenance

To propagate pSH18-34, follow the steps outlined below.

1. Resuspend pSH18-34 in 20 μ l sterile water to prepare a 1 μ g/ μ l stock solution.
2. Dilute a portion of the stock solution and transform a *recA*, *endA* *E. coli* strain like TOP10, TOP10F', DH5 α , or equivalent. Use 10 ng of plasmid for transformation of *E. coli*.
3. Select for transformants on LB plates containing 50 to 100 μ g/ml ampicillin.
4. Store the stock solution at -20°C when finished.
5. Be sure to prepare a glycerol stock of each strain containing plasmid for long-term storage.



To transform pSH18-34 into a yeast host strain, the user should be familiar with basic yeast molecular biology and microbiological techniques including preparation of YC minimal medium and YPD medium. Please refer to Current Protocols in Molecular Biology, *Saccharomyces cerevisiae*, pp. 13.01 to 13.2.12 (Ausubel *et al.*, 1994) for information on preparing yeast media and handling yeast. Recipes for preparation of YC and YPD media are also provided in the Hybrid Hunter™ System manual.

Yeast Transformation

You may use any suitable small-scale yeast transformation protocol of your choice to transform pSH18-34 into a yeast host strain. Select for transformants on uracil-deficient YC medium. Alternatively, Invitrogen offers the *S. c.* EasyComp™ Kit (Catalog no. K5050-01) for quick and easy preparation of competent yeast cells that can be used immediately or stored frozen for future use. Transformation efficiency is guaranteed at $>10^3$ transformants per μ g DNA. For more information, please call Technical Service (see below).

β -galactosidase Assay

Once you have created the *lacZ* reporter strain, you may use this strain to detect LexA-mediated protein-protein interactions with the Hybrid Hunter™ System. Positive interactions can be verified by assaying for β -galactosidase activity. We recommend using a β -galactosidase filter assay to assess activity. Please refer to the Hybrid Hunter™ System manual or Current Protocols in Molecular Biology, Section 20 for a detailed protocol.

References

The pSH18-34 plasmid was constructed as described in Estojak, *et.al.*, 1995. For more information about the generation of this vector, please refer to the following references:
Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience), Section 20.
Estojak, J., Brent, R., and Golemis, E. A. (1995). Correlation of Two-Hybrid Affinity Data with In Vitro Measurements. *Mol. Cell. Biol.* 15, 5820-5829.

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