SureVector Cloning Kits
Yeast Expression Vector Assembly

Product Guide

For Research Use Only. Not for use in diagnostic procedures.

Agilent Technologies

SureVector Cloning for Yeast Expression

Agilent's SureVector cloning products allow you to quickly and easily create custom vectors. Each vector consists of 6–7 assembled modules, one of which is your gene-of-interest (or a SureVector control gene). SureVector kits provide fragments for each module position. Select your fragments and add them to the assembly reaction to create your fully customized vector. You can include a module for a N- or C-terminal tag, or leave your gene-of-interest without a tag. See Figure 1 for a depiction of the module and assembly options.

This product guide describes the SureVector kits that can be used to create yeast expression vectors and provides guidelines on selecting module options to include in the assembly mixture. For instructions on setting up the SureVector assembly reactions and transforming the assembled vectors into *E. coli*, see the *SureVector Cloning Kits Protocol*, available online at http://www.chem.agilent.com/Library/usermanuals/Public/G7514-90000.pdf.

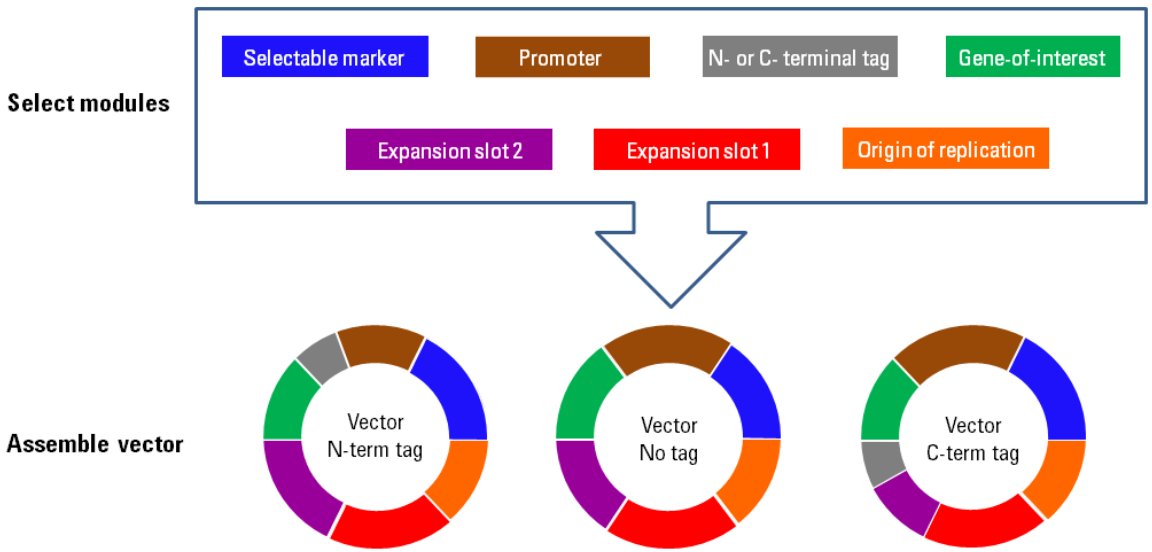

Figure 1 Module selection and vector assembly options

SureVector Kit Information

Agilent offers a variety of SureVector cloning kits (see Table 1). All of the kits include functional modules, and some of the kits also include the necessary assembly reagents. The SureVector Core Kit includes competent cells for transformation, or you can purchase competent cells separately.

NOTE Each cloning reaction requires the SureVector assembly reagents (i.e. SureVector Enzyme Mix, 10× SureVector Buffer, dNTP Mix, Dpn I, and 5× SureSolution), modules, and competent cells. Make sure that you have SureVector kits containing all of these necessary components.

Ordering information

Table 1 Agilent SureVector Core Kit and SureVector Yeast Expansion Kits

Product Name	Quantity	Agilent Part Number
SureVector kits that include functional modules, assembly reagents, and competent cells		
SureVector Core Kit	15 cloning reactions	G7514A
SureVector kits that include functional modules only (no assembly reagents or competent cells)		
SureVector Yeast N-terminal Expansion Kit	15 cloning reactions	G7517A
SureVector Yeast C-terminal Expansion Kit	15 cloning reactions	G7517B
Competent Cell Kit for use in SureVector transformations		
Agilent XL1-Blue Supercompetent Cells Kit	20 transformations	200236

Kit storage

Competent cells Upon receipt, immediately place the XL1-Blue Supercompetent Cells at the bottom of a –80°C freezer directly from the dry ice shipping container. Do not store the cells in liquid nitrogen.

All other SureVector components Store at –20°C upon receipt.

Online SureVector design tool

The online SureVector design site allows you to design your custom vector using on online tool. To access the design site, visit www.agilent.com/genomics/surevector and click **Create Your Map**. When finished designing your vector, click **Recommended Products** to see a list of the SureVector kits that you will need to assemble the vector with your chosen modules.

SureVector Modules

This section describes the modules (1 through 7) that are required to build a complete SureVector yeast vector, and the options available for each module. See Table 1 on page 3 for a list of SureVector part numbers.

NOTE Your selections for the promoter module and tag module determine the overlap sequences needed for your gene-of-interest insert. See Table 2 on page 10 for sequence information.

Module 1: Selectable Markers

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector Amp^R Selectable Marker	Ampicillin selection in *E. coli*	G7514A, G7518A, G7518B, G7518C, G7518D, G7518E
SureVector Kan^R Selectable Marker	Kanamycin selection in *E. coli*	G7514A, G7518A
SureVector Chl^R Selectable Marker	Chloramphenicol selection in *E. coli*	G7514A, G7518A

Module 2: Bacterial Origins of Replication

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector pUC Origin	*E. coli* origin of replication (100–200 copies/cell)	G7514A, G7518A, G7518B, G7518C, G7518D, G7518E
SureVector p15a Origin	*E. coli* origin of replication (10–12 copies/cell)	G7514A
SureVector pBR322 Origin	*E. coli* origin of replication (10–20 copies/cell)	G7514A

Module 3: XP1 Expansion Site Modules

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector XP1 Linker	Linker for expansion site 1	G7514A, G7518A, G7518B, G7518C, G7518D, G7518E
SureVector yARS	Yeast autonomous replication sequence in *S. cerevisiae*	G7514A

Module 4: XP2 Expansion Site Modules

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector XP2 Linker	Linker for expansion site 2	G7514A
SureVector LEU2 Yeast Selectable Marker	Leucine auxotroph selection in *S. cerevisiae*	G7514A
SureVector URA3 Yeast Selectable Marker	Uracil auxotroph selection in *S. cerevisiae*	G7517A, G7517B
SureVector HIS3 Yeast Selectable Marker	Histidine auxotroph selection in *S. cerevisiae*	G7517A, G7517B
SureVector Hygromycin Yeast Selectable Marker	Hygromycin selection in *S. cerevisiae*	G7517A, G7517B

Module 5: Promoters

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector GAL1-HIS6 Yeast Promoter*	*S. cerevisiae* GAL1 promoter fused to HIS6 tag	G7514A
SureVector GAL 1 Yeast Promoter	*S. cerevisiae* GAL1 promoter	G7517A, G7517B
SureVector CUP1 Yeast Promoter	*S. cerevisiae* CUP1 promoter	G7517A, G7517B
SureVector ADH1 Yeast Promoter	*S. cerevisiae* ADH1 promoter	G7517A, G7517B

* If using the SureVector GAL1-HIS6 Yeast Promoter, do not include a tag module in your vector assembly. This promoter includes a HIS6 tag that is expressed as an N-terminal tag during protein expression.

Module 6: Tags

Select 0–1 per reaction

Module name	Description	SureVector kits containing the module
N-Terminal Tags		
SureVector FLAG® Yeast Expression Tag (N-term)	FLAG epitope tag (N-terminal); suitable for yeast expression vectors	G7517A
SureVector GFP Yeast Expression Tag (N-term)	Green Fluorescent Protein tag (N-terminal); suitable for yeast expression vectors	G7517A
SureVector HA Yeast Expression Tag (N-term)	Hemagglutinin tag (N-terminal); suitable for yeast expression vectors	G7517A
SureVector c-Myc Yeast Expression Tag (N-term)	c-Myc tag (N-terminal); suitable for yeast expression vectors	G7517A
SureVector HIS6 Yeast Expression Tag (N-term)	HIS6 tag (N-terminal); suitable for yeast expression vectors	G7517A
SureVector SBP Yeast Expression Tag (N-term)	Streptavidin Binding Protein tag (N-terminal); suitable for yeast expression vectors	G7517A
C-Terminal Tags		
SureVector FLAG® Expression Tag (C-term)	FLAG epitope tag (C-terminal); suitable for yeast or mammalian expression vectors	G7517B
SureVector GFP Expression Tag (C-term)	Green Fluorescent Protein tag (C-terminal); suitable for yeast or mammalian expression vectors	G7517B
SureVector HA Expression Tag (C-term)	Hemagglutinin tag (C-terminal); suitable for yeast, *E. coli*, or mammalian expression vectors	G7517B
SureVector c-Myc Expression Tag (C-term)	c-Myc tag (C-terminal); suitable for yeast, *E. coli*, or mammalian expression vectors	G7517B
SureVector HIS6 Expression Tag (C-term)	HIS6 tag (C-terminal); suitable for yeast, *E. coli*, or mammalian expression vectors	G7517B
SureVector SBP Expression Tag (C-term)	Streptavidin Binding Protein tag (C-terminal); suitable for yeast, *E. coli*, or mammalian expression vectors	G7517B

Module 7: Gene-of-interest or control insert

Use your **gene-of-interest** insert OR select 1 **LacZ Control** per reaction

Module name	Description	SureVector kits containing the module
Gene-of-interest	Purified gene-of-interest DNA insert	provided by user
SureVector LacZ Control (N-term)	Constitutive expression of *lacZ*α in *E. coli*; compatible with yeast and *E.coli* N-terminal SureVector tags	G7517A, G7514A
SureVector LacZ Yeast Control (C-term)	Constitutive expression of *lacZ*α in *E. coli*; compatible with yeast C-terminal SureVector tags	G7517B

Gene-of-Interest Insert

Your gene-of-interest DNA insert needs to have 5' and 3' ends that overlap the 5' and 3' ends of the adjacent SureVector modules. The easiest way to accomplish this is to PCR-amplify your gene-of-interest insert with PCR primers that include the appropriate overlap sequences. This method is illustrated in Figure 2.

The appropriate overlap sequences for your gene-of-interest primers depends on which approach you are using for the promoter and tag modules. The overlap sequences for each promoter/tag option are listed in Table 2 on page 10. The overlaps are 30 nucleotides and the portion of the primer that compliments the gene-of-interest sequence needs to be 12–20 nucleotides. Thus, the resulting primers are typically 42–50 nucleotides long.

- **If you are using a promoter-tag fusion or an N-terminal tag:** In order for the gene-of-interest to be in the same reading frame as the tag, the first three nucleotides of the upstream primer that complement the gene-of-interest need to encode the first amino acid codon for the gene-of-interest. In the downstream primer, the region that complements the gene-of-interest needs to include a stop codon.
- **If you are using a C-terminal tag:** In order for the tag to be in the same reading frame as the gene-of-interest, the last three nucleotides of the downstream primer that complement the gene-of-interest need to encode the last amino acid codon for the gene-of-interest.

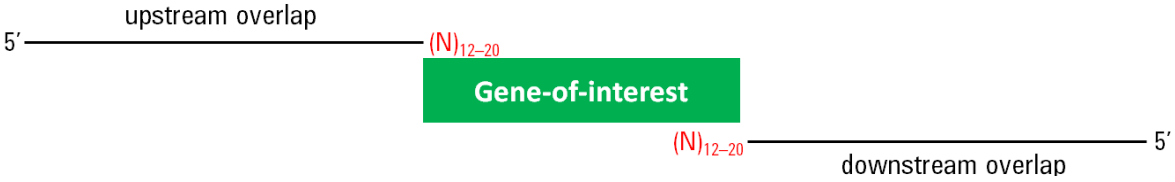

Figure 2 PCR method for adding overlap sequences to the 5′ and 3′ PCR primers

NOTE If desired, you can add sequences encoding a protease cleavage site in the upstream or downstream PCR primer to provide for cleavage between the translated tag and the gene-of-interest.

Table 2 **Yeast Expression Vectors** – Overlap sequences for gene-of-interest PCR primers

For vectors with a promoter-tag fusion module (i.e., the SureVector GAL1-HIS6 Yeast Promoter)	Upstream primer	5′ GGTGGCGGAGGTTCTGGAGGCGGTGGAAGT 3′
	Downstream primer	5′ CTCGAGGAGATATTGTACACTAAACCAAATG 3′
For vectors with a promoter module and an N-terminal tag module	Upstream primer	5′ GGTGGCGGAGGTTCTGGAGGCGGTGGAAGT 3′
	Downstream primer	5′ CTCGAGGAGATATTGTACACTAAACCAAATG 3′
For vectors with a promoter module and an C-terminal tag module	Upstream primer	5′ CTCTATACTTTAACGTCAAGGAGAAAAAACTATA 3′
	Downstream primer	5′ ACTTCCACCGCCTCCAGAACCTCCGCCACC 3′
For vectors with a promoter module and no tag	Upstream primer	5′ CTCTATACTTTAACGTCAAGGAGAAAAAACTATA 3′
	Downstream primer	5′ CTCGAGGAGATATTGTACACTAAACCAAATG 3′

For recommended cycling conditions for assembly of SureVector yeast expression vectors, see the SureVector Cloning Kits Protocol, available online at:
http://www.agilent.com/cs/library/usermanuals/Public/G7514-90000.pdf.

Endnotes

Agilent Technical Support

For technical product support, contact your local Agilent Support Services representative.

For US and Canada, call (800) 227-9770. For other countries, find your support center telephone numbers at www.agilent.com/chem/contactus.

Or send an e-mail to: techservices@agilent.com

Notices to Purchaser: Limited License

This product is provided under an agreement between Bio-Rad Laboratories and Agilent Technologies, Inc., and the manufacture, use, sale or import of this product is subject to US. Pat. No. 6,627,424 and EP Pat. No. 1 283 875 B1, owned by Bio-Rad Laboratories, Inc. Purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in PCR (but not real-time PCR) in the Research Field including all Applied Research Fields (including but not limited to forensics, animal testing, and food testing).

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 6,258,569, 6,171,785, 6,127,155, 6,030,787, 5,994,056, 5,876,930, 5,804,375, 5,789,224, 5,773,258 (claims 1 and 6 only), 5,723,591, 5,677,152 (claims 1 to 23 only), 5,618,711, 5,538,848, and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Trademarks

FLAG is a trademark and registered trademark of Sigma-Aldrich, Co., and its division Sigma-Aldrich Co. Newco Inc., in the US and elsewhere, and is used under license.

www.agilent.com

In this book

This book provides guidance on selecting SureVector kits and modules to create a custom vector for protein expression in *S. cerevisiae*.

Revision A0, September 2015

G7514-90003

Agilent Technologies