



SureVector Cloning Kits Mammalian Expression Vector Assembly

Product Guide

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SureVector Cloning for Mammalian 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 mammalian 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.agilent.com/cs/library/usermanuals/Public/G7514-90000.pdf>.

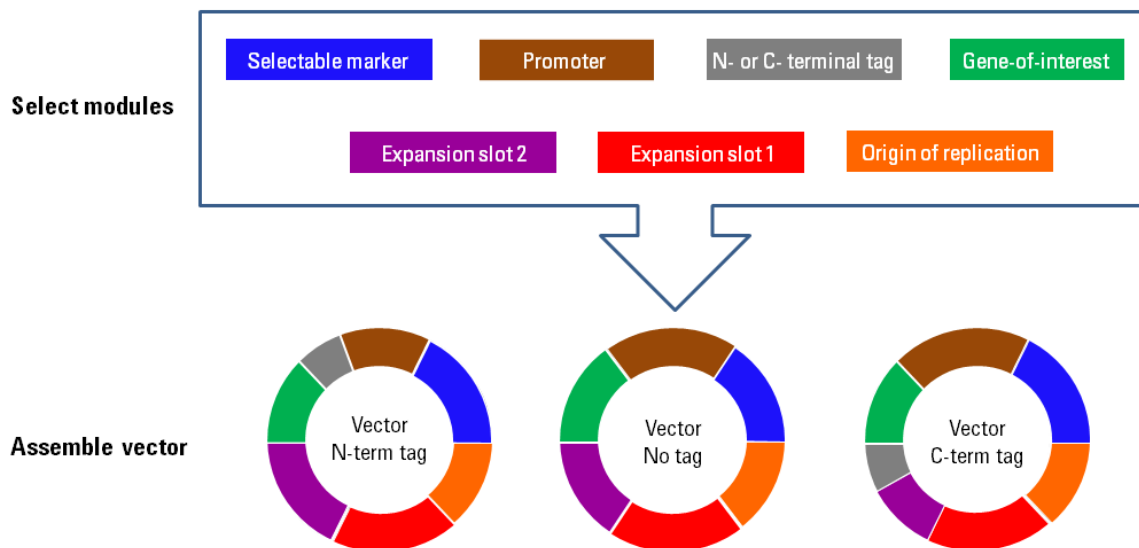


Figure 1 Module selection and vector assembly options

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 Mammalian 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 Mammalian N-terminal Expansion Kit	15 cloning reactions	G7516A
SureVector Mammalian C-terminal Expansion Kit	15 cloning reactions	G7516B
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 an 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 mammalian 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 <i>E. coli</i>	G7514A, G7518A, G7518B, G7518C, G7518D, G7518E
SureVector Kan ^R Selectable Marker	Kanamycin selection in <i>E. coli</i>	G7514A, G7518A
SureVector Chl ^R Selectable Marker	Chloramphenicol selection in <i>E. coli</i>	G7514A, G7518A

Module 2: Bacterial Origins of Replication

Select 1 per reaction

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

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 Neo ^R Mammalian Selectable Marker	Neomycin selection in mammalian cells	G7514A
SureVector Blastidicin Mamm Selectable Marker	Blasticidin selection in mammalian cells	G7516A, G7516B
SureVector Hygromycin Mamm Selectable Marker	Hygromycin selection in mammalian cells	G7516A, G7516B
SureVector Puromycin Mamm Selectable Marker	Puromycin selection in mammalian cells	G7516A, G7516B

Module 5: Promoters

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector CMV-HIS6 Mammalian Promoter*	Mammalian CMV promoter fused to HIS6 tag	G7514A
SureVector CMV Mamm Promoter	CMV mammalian promoter	G7516A, G7516B
SureVector EF1 α Mamm Promoter	EF1 α mammalian promoter	G7516A, G7516B
SureVector SV40 Mamm Promoter	SV40 mammalian promoter	G7516A, G7516B

* If using the SureVector CMV-HIS6 Mammalian 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 [®] Mamm Expression Tag (N-term)	FLAG epitope tag (N-terminal); suitable for mammalian expression vectors	G7516A
SureVector GFP Mamm Expression Tag (N-term)	Green Fluorescent Protein tag (N-terminal); suitable for mammalian expression vectors	G7516A
SureVector HA Mamm Expression Tag (N-term)	Hemagglutinin tag (N-terminal); suitable for mammalian expression vectors	G7516A
SureVector c-Myc Mamm Expression Tag (N-term)	c-Myc tag (N-terminal); suitable for mammalian expression vectors	G7516A
SureVector HIS6 Mamm Expression Tag (N-term)	HIS6 tag (N-terminal); suitable for mammalian expression vectors	G7516A
SureVector SBP Mamm Expression Tag (N-term)	Streptavidin Binding Protein tag (N-terminal); suitable for mammalian expression vectors	G7516A
C-Terminal Tags		
SureVector FLAG [®] Expression Tag (C-term)	FLAG epitope tag (C-terminal); suitable for mammalian or yeast expression vectors	G7516B
SureVector GFP Expression Tag (C-term)	Green Fluorescent Protein tag (C-terminal); suitable for mammalian or yeast expression vectors	G7516B
SureVector HA Expression Tag (C-term)	Hemagglutinin tag (C-terminal); suitable for mammalian, <i>E. coli</i> , or yeast expression vectors	G7516B
SureVector c-Myc Expression Tag (C-term)	c-Myc tag (C-terminal); suitable for mammalian, <i>E. coli</i> , or yeast expression vectors	G7516B
SureVector HIS6 Expression Tag (C-term)	HIS6 tag (C-terminal); suitable for mammalian, <i>E. coli</i> , or yeast expression vectors	G7516B
SureVector SBP Expression Tag (C-term)	Streptavidin Binding Protein tag (C-terminal); suitable for mammalian, <i>E. coli</i> , or yeast expression vectors	G7516B

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 Mamm Control (N-term)	Constitutive expression of <i>lacZα</i> in <i>E. coli</i> ; compatible with mammalian N-terminal SureVector tags	G7516A
SureVector LacZ Mamm Control (C-term)	Constitutive expression of <i>lacZα</i> in <i>E. coli</i> ; compatible with mammalian C-terminal SureVector tags	G7516B

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 complements 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 Mammalian Expression Vectors – Overlap sequences for gene-of-interest PCR primers

For vectors with a promoter-tag fusion module (i.e., the SureVector CMV-HIS6 Mammalian Promoter)	Upstream primer	5' GGTGGCGGAGGTTCTGGAGGCGGTGGAAGT 3'
	Downstream primer	5' CTCGAGGAGATATTGTACTAAACCAAATG 3'
For vectors with a promoter module and an N-terminal tag module	Upstream primer	5' GGTGGCGGAGGTTCTGGAGGCGGTGGAAGT 3'
	Downstream primer	5' CTCGAGGAGATATTGTACTAAACCAAATG 3'
For vectors with a promoter module and an C-terminal tag module	Upstream primer	5' CCTTGTTAACTTTAAGAGGAGGGCCACC 3'
	Downstream primer	5' ACTTCCACCGCTCCAGAACCTCCGCCACC 3'
For vectors with a promoter module and no tag	Upstream primer	5' CCTTGTTAACTTTAAGAGGAGGGCCACC 3'
	Downstream primer	5' CTCGAGGAGATATTGTACTAAACCAAATG 3'

For recommended cycling conditions for assembly of SureVector mammalian 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

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In this book

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

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