



SureVector Cloning Kits ***E. coli* Expression Vector Assembly**

Product Guide

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

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Agilent Technologies

SureVector Cloning for *Escherichia coli* 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 *E. coli* 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>.

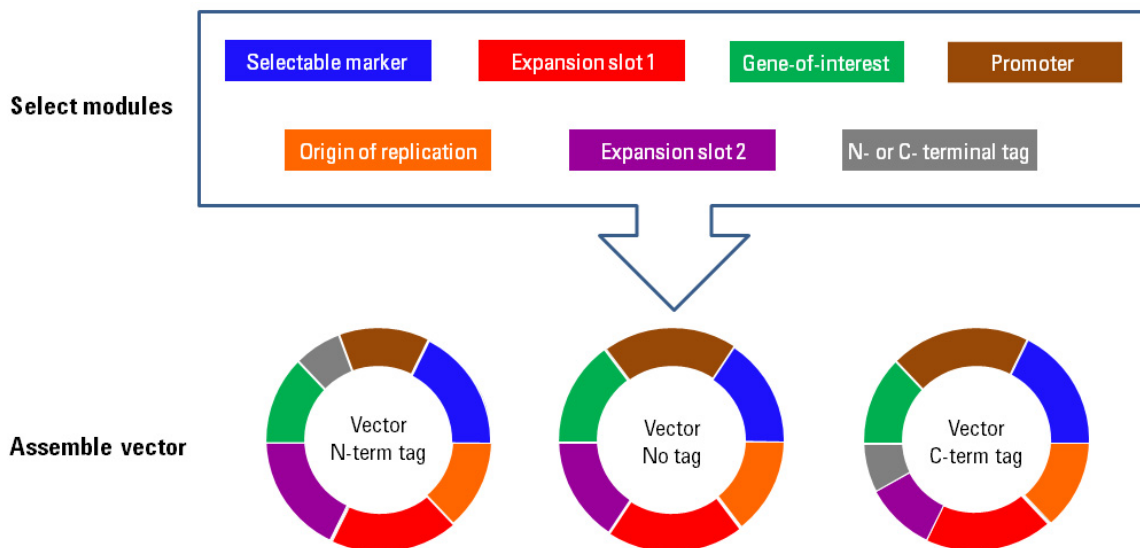


Figure 1 Module selection and vector assembly options

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 *E. coli* 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 and assembly reagents (no competent cells)		
SureVector <i>E. coli</i> Selection Kit	5 cloning reactions	G7518A
SureVector <i>E. coli</i> N-terminal Promoter Kit	5 cloning reactions	G7518B
SureVector <i>E. coli</i> C-terminal Promoter Kit	5 cloning reactions	G7518C
SureVector <i>E. coli</i> N-terminal Tag Kit	5 cloning reactions	G7518D
SureVector <i>E. coli</i> C-terminal Tag Kit	5 cloning reactions	G7518E
SureVector kits that include functional modules only (no assembly reagents or competent cells)		
SureVector <i>E. coli</i> N-terminal Expansion Kit	15 cloning reactions	G7515A
SureVector <i>E. coli</i> C-terminal Expansion Kit	15 cloning reactions	G7515B
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 *E. coli* vector, and the options available for each module. See [Table 1](#) on page 3 for a list of SureVector part numbers.

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 LacI Repressor	Expression of <i>lacI</i> in <i>E. coli</i>	G7514A, G7518A, G7518B, G7518C, G7518D, G7518E

Module 5: Promoters

Select 1 per reaction

Module name	Description	SureVector kits containing the module
SureVector T7-HIS6 <i>E. coli</i> Promoter*	Bacteriophage T7 promoter fused to HIS6 tag	G7514A, G7518A, G7518B, G7518D
SureVector T7 <i>E. coli</i> Promoter	Bacteriophage T7 promoter	G7515A, G7515B, G7518B, G7518C, G7518D, G7518E,
SureVector Tac <i>E. coli</i> Promoter	Tac promoter	G7515B, G7518B, G7518C, G7515A
SureVector Rhamnose <i>E. coli</i> Promoter	Rhamnose Promoter	G7515A, G7515B, G7518C

* If using the SureVector T7-HIS6 *E. coli* 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 CBP <i>E. coli</i> Expression Tag (N-term)	Calmodulin binding protein tag (N-terminal)	G7515A
SureVector GST <i>E. coli</i> Expression Tag (N-term)	Glutathione-S-transferase binding protein tag (N-terminal)	G7515A, G7518D

Module 6: Tags

Select 0–1 per reaction

Module name	Description	SureVector kits containing the module
SureVector HIS6 <i>E. coli</i> Expression Tag (N-term)	Hexa Histidine affinity tag (N-terminal)	G7515A, G7518B
SureVector His-DsbA <i>E. coli</i> Expression Tag (N-term)	Hexa Histidine tagged disulfide isomerase solubility tag (N-terminal)	G7515A
SureVector MBP <i>E. coli</i> Expression Tag (N-term)	Maltose binding protein tag (N-terminal)	G7515A, G7518D
SureVector SBP <i>E. coli</i> Expression Tag (N-term)	Streptavidin binding protein tag (N-terminal)	G7515A, G7518D
C-Terminal Tags		
SureVector CBP Expression Tag (C-term)	Calmodulin binding protein tag (C-terminal)	G7515B, G7518E
SureVector Myc Expression Tag (C-term)	c-Myc epitope tag (C-terminal)	G7515B
SureVector HA Expression Tag (C-term)	Hemagglutinin epitope tag (C-terminal)	G7515B
SureVector HIS6 Expression Tag (C-term)	Hexa Histidine affinity tag (C-terminal)	G7515B, G7518C
SureVector SBP Expression Tag (C-term)	Streptavidin binding protein tag (C-terminal)	G7515B, G7518E
SureVector Thioredoxin Expression Tag (C-term)	Thioredoxin solubility tag (C-terminal)	G7515B, G7518E

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 <i>lacZα</i> in <i>E. coli</i> ; compatible with N-terminal SureVector tags and with SureVector T7-HIS6 <i>E. coli</i> promoter	G7515A, G7518B, G7518D
SureVector LacZ <i>E. coli</i> Control (C-term)	Constitutive expression of <i>lacZα</i> in <i>E. coli</i> ; compatible with C-terminal SureVector tags	G7515B, G7518C, G7518E

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 *E. coli*.

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