SureVector Cloning Kits

Protocol

Version C0, September 2015

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

Agilent Technologies

Notices

Manual Part Number

G7514-90000

Edition

Version C0, September 2015

Agilent Technologies, Inc.
5301 Stevens Creek Rd
Santa Clara, CA 95051 USA

Technical Support

For technical product support, contact Agilent at (800) 227-9770 or techservices@agilent.com

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In this Guide...

This document describes how to use the SureVector cloning kits to create custom vectors.

1 Before You Begin

This chapter provides important information on getting started with SureVector cloning.

2 Protocol

This chapter provides guidelines and instructions on how to perform the SureVector cloning protocol.

3 Troubleshooting

This chapter contains suggestions for troubleshooting your SureVector cloning.

4 Reference Information

This chapter provides recipe information for preparation of the bacterial growth media.

1
Before You Begin

This chapter provides important information on getting started with SureVector cloning.

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.

Overview of SureVector cloning

Agilent's SureVector cloning protocol creates custom cloning vectors using a unique enzyme mix that allows a gene-of-interest to be cloned into a fully customizable vector backbone. The SureVector cloning kits (described on page 10) contain various DNA modules that each serve a specific functional purpose in the resulting vector. The modules include selectable markers, origins of replication, expansion elements, transcriptional promoters, and N- or C-terminal tags to be fused with the gene-of-interest (see Figure 1 on page 8). You choose which modules to include, then combine them in a single tube, along with the necessary assembly reagents, for assembly into a custom vector. You then combine the assembly reaction with XL1-Blue Supercompetent cells to transform the vector into *Escherichia coli*.

For assistance in designing your custom vector using the SureVector cloning kits, visit Agilent's online SureVector design site. Go to www.agilent.com/genomics/surevector and click **Create Your Map**.

If you are designing an *E. coli* expression vector See the *E. coli* Expression Vector Assembly Product Guide for information on the module options in the SureVector *E. coli* expansion kits, promoter kits, and tag kits. This product guide is available at: http://www.agilent.com/cs/library/usermanuals/Public/G7514-90001.pdf.

If you are designing a mammalian expression vector See the Mammalian Expression Vector Assembly Product Guide for information on the module options in the SureVector mammalian expansion kits. This product guide is available at: http://www.agilent.com/cs/library/usermanuals/Public/G7514-90002.pdf.

If you are designing a yeast expression vector See the Yeast Expression Vector Assembly Product Guide for information on the module options in the SureVector yeast expansion kits. This product guide is available at: http://www.agilent.com/cs/library/usermanuals/Public/G7514-90003.pdf.

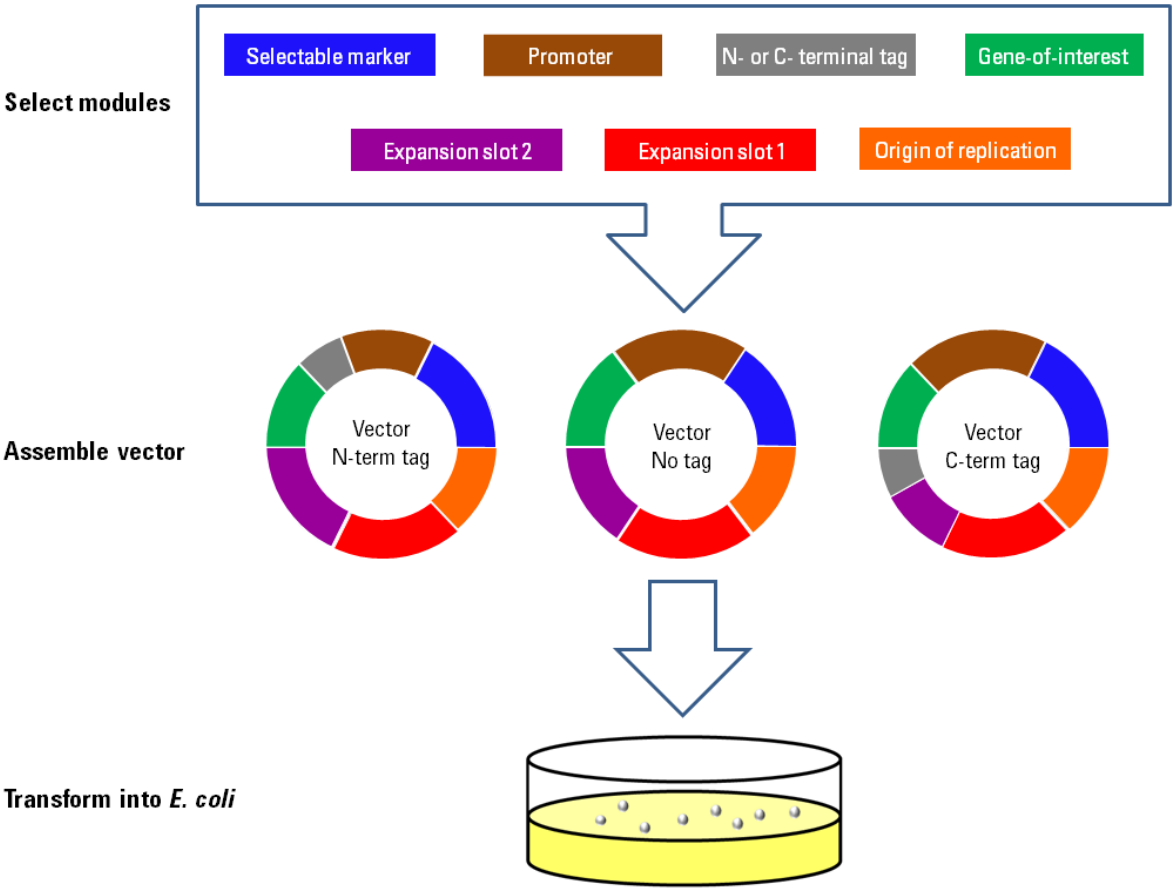

Figure 1 SureVector cloning overview

Required reagents and equipment

Table 1 contains the list of reagents and equipment that are required for the protocol.

Table 1 Required Equipment and Reagents

Equipment or reagent
Agilent SureVector cloning kits containing assembly reagents, modules, and competent cells; see "SureVector cloning kits" on page 10 for information
Prepared gene-of-interest DNA fragment, see "Gene-of-interest insert" on page 15 for information
Agilent SureCycler 8800 thermal cycler, or other programmable thermal cycler
0.2-mL thin-wall PCR tubes, or other tubes suitable for your thermal cycler
Heat block set to 37°C (or thermal cycler set to 37°C)
Water bath set to 42°C
Shaking incubator set to 37°C
NZY medium, see "Preparation of media" on page 30 for recipe
LB-agar plates with the appropriate antibiotic, X-gal, and IPTG (if using), see "Preparation of media" on page 30 for recipe
LB-agar plates with ampicillin (for pUC18 control transformations), see "Preparation of media" on page 30 for recipe
14-mL BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059)
DNase-free dH_20

SureVector cloning kits

Agilent offers a variety of SureVector cloning kits (listed below). 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.

Table 2 Agilent SureVector Cloning 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 *E. coli* Selection Kit	5 cloning reactions	G7518A
SureVector *E. coli* N-terminal Promoter Kit	5 cloning reactions	G7518B
SureVector *E. coli* C-terminal Promoter Kit	5 cloning reactions	G7518C
SureVector *E. coli* N-terminal Tag Kit	5 cloning reactions	G7518D
SureVector *E. coli* C-terminal Tag Kit	5 cloning reactions	G7518E
SureVector kits that include functional modules only (no assembly reagents or competent cells)		
SureVector *E. coli* N-terminal Expansion Kit	15 cloning reactions	G7515A
SureVector *E. coli* C-terminal Expansion Kit	15 cloning reactions	G7515B
SureVector Mammalian N-terminal Expansion Kit	15 cloning reactions	G7516A
SureVector Mammalian C-terminal Expansion Kit	15 cloning reactions	G7516B
SureVector Yeast N-terminal Expansion Kit	15 cloning reactions	G7517A
SureVector Yeast C-terminal Expansion Kit	15 cloning reactions	G7517B

Table 2 Agilent SureVector Cloning Kits

Product Name	Quantity	Agilent Part Number
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.

SureVector module selection

Table 3 lists the modules (1 through 7) that are required to build a complete SureVector cloning vector. The table also lists the options for each module that are included in the SureVector Core Kit (p/n G7514A). Additional module options are available in the other SureVector kits. See Table 2 on page 10 for a list of SureVector kits currently available from Agilent.

Table 3 SureVector modules and module options in the **SureVector Core Kit**

Modules	Options in the SureVector Core Kit	
	Module name	**Description**
Module 1: Bacterial selectable marker – Include one per reaction	SureVector Amp^R Selectable Marker	Ampicillin selection in *E. coli*
	SureVector Kan^R Selectable Marker	Kanamycin selection in *E. coli*
	SureVector Chl^R Selectable Marker	Chloramphenicol selection in *E. coli*

Table 3 SureVector modules and module options in the **SureVector Core Kit**

Modules	Options in the SureVector Core Kit	
	Module name	**Description**
Module 2: Bacterial origin of replication – Include one per reaction	SureVector pUC Origin	*E. coli* origin of replication (100–200 copies/cell)
	SureVector p15a Origin	*E. coli* origin of replication (10–12 copies/cell)
	SureVector pBR322 Origin	*E. coli* origin of replication (10–20 copies/cell)
Module 3: XP1 expansion site module – Include one per reaction	SureVector XP1 Linker	Linker for expansion site 1
	SureVector yARS	Yeast autonomous replication sequence in *S. cerevisiae*
Module 4: XP2 expansion site module – Include one per reaction	SureVector XP2 Linker	Linker for expansion site 2
	SureVector Neo^R Mammalian Selectable Marker	Neomycin selection in mammalian cells
	SureVector LEU2 Yeast Selectable Marker	Leucine auxotroph selection in *S. cerevisiae*
	SureVector LacI Repressor	Expression of *lacI* in *E. coli*
Modules 5 and 6: Promoters and Tags – Include one fused **promoter-tag** per reaction*	SureVector T7-HIS6 *E. coli* Promoter	Bacteriophage T7 promoter fused to HIS6 tag
	SureVector CMV-HIS6 Mammalian Promoter	Mammalian CMV promoter fused to HIS6 tag
	SureVector GAL1-HIS6 Yeast Promoter	*S. cerevisiae* GAL1 promoter fused to HIS6 tag
Module 7: Gene-of-interest or control insert – Include one per reaction	SureVector LacZ Control	Constitutive expression of *lacZ*α in *E. coli*

* In the SureVector Core Kit, the options for the promoter and tag modules (modules 5 and 6) are fused together. Other SureVector kits provide individual promoters and tags which must be added separately.

2
Protocol

This chapter provides guidelines and instructions on how to perform the SureVector cloning protocol.

Preprotocol considerations

Control reactions

Agilent recommends assembling positive and negative control vectors.

The assembly reaction for the positive control vector contains a SureVector LacZ Control insert in place of the gene-of-interest DNA insert. This positive control reaction can help you identify potential problems with your gene-of-interest fragment. On the transformation plate, XL1-Blue colonies that include the SureVector LacZ Control in the assembled vector are blue in color due to the presence of IPTG and X-gal in the plates.*

NOTE

Make sure that you select the appropriate SureVector LacZ Control insert so that the 5′ and 3′ ends of the insert are compatible with the adjacent SureVector modules. Selection is based on the expression system (*E. coli*, mammalian, or yeast) and the tag used in the vector (N- or C-terminal). Currently, none of the SureVector kits offer a LacZ Control insert that is suitable for vectors that do not include either a N- or C-terminal tag. If you are assembling a vector without any tag, you will not be able to assemble a positive control vector containing a LacZ Control insert.

The assembly reaction for the negative control vector contains water in place of the gene-of-interest insert (module 7 in Table 3).

* The LacZα gene in the SureVector LacZ Control is constitutively expressed in *E. coli* while the genomic copy of the LacZ-omega gene requires the presence of an inducer, e.g. IPTG. If you intend to use blue-white color screening to identify positive transformants in the positive control assembly reactions, Agilent recommends including IPTG in the plating media because it intensifies the blue color. The exceptions to this recommendation are vectors that include the SureVector Tac *E. coli* Promoter, which could express the gene-of-interest in the presence of IPTG. See "Preparation of media" on page 30 for media preparation instructions.

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 (modules 5 and 6 in Table 3). The overlap sequences for each promoter/tag option are provided for *E. coli*, mammalian, and yeast expression vectors (see Table 4 through Table 6 on page 16). 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 4 ***E. coli* Expression Vectors** – Overlap sequences for gene-of-interest PCR primers

For vectors with a promoter-tag fusion module	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′ CCTTGTTTAACTTTAAGAAGGAGATATACAT 3′
	Downstream primer	5′ ACTTCCACCGCCTCCAGAACCTCCGCCACC 3′
For vectors with a promoter module and no tag	Upstream primer	5′ CCTTGTTTAACTTTAAGAAGGAGATATACAT 3′
	Downstream primer	5′ CTCGAGGAGATATTGTACACTAAACCAAATG 3′

Table 5 **Mammalian Expression Vectors** – Overlap sequences for gene-of-interest PCR primers

For vectors with a promoter-tag fusion module	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′ CCTTGTTTAAACTTTAAGAGGAGGGCCACC 3′
	Downstream primer	5′ ACTTCCACCGCCTCCAGAACCTCCGCCACC 3′
For vectors with a promoter module and no tag	Upstream primer	5′ CCTTGTTTAAACTTTAAGAGGAGGGCCACC 3′
	Downstream primer	5′ CTCGAGGAGATATTGTACACTAAACCAAATG 3′

Table 6 **Yeast Expression Vectors** - Overlap sequences for gene-of-interest PCR primers

For vectors with a promoter-tag fusion module	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′

The SureVector cloning protocol works well with gene-of-interest inserts up to 3 kb. For inserts >3 kb, assembly efficiency may not be optimal, and you may need to screen a greater number of colonies on the transformation plate to identify one that contains the correct vector.

Your gene-of-interest DNA insert needs to be purified and stored in low TE buffer (5 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA), or dH_20, at a concentration of 0.05 pmol/µL. Agilent recommends Herculase II Fusion DNA Polymerase (Agilent p/n 600675) for PCR amplification of the gene-of-interest insert and the StrataPrep DNA Gel Extraction Kit (Agilent p/n 400766) for purification of the insert.

NOTE Purification of the PCR-amplified gene-of-interest insert is necessary to reduce carry over of the parental DNA into the assembly reactions.

For quality purposes, run a sample of your insert on an agarose gel to make sure that only one band is present and that the band is the expected size.

Cycling conditions

Assembly of the modules into a complete vector occurs during the thermal cycling step of the protocol. The optimal thermal cycling conditions for your assemblies depend on the modules you selected and the features of your gene-of-interest insert (e.g., size and GC content).

The assembly protocol includes four different recommendations for the thermal cycling program (see Table 8 through Table 11 on pages 20 and 21). Each program is optimized for a different type of assembly reaction based on the modules included in the assembly. Select the program appropriate for your assembly reactions. Table 7 on page 18 summarizes the four programs.

Table 7 Summary of thermal cycling programs

Thermal Cycling Program	Description
Program A See Table 8 on page 20	For assemblies that only contain modules from the SureVector Core Kit (p/n G7514A) or SureVector *E. coli* Selection Kit (p/n G7518A)
Program B See Table 9 on page 21	For assemblies containing promoters or selectable markers from the SureVector *E. coli* Expansion Kits and/or SureVector *E. coli* Promoter Kits and/or SureVector *E. coli* Tag Kits (p/n G7515A, G7515B, G7518B, G7518C, G7518D, G7518E)
Program C See Table 10 on page 21	For assemblies containing promoters or selectable markers from the SureVector Mammalian Expansion Kits (p/n G7516A, G7516B)
Program D See Table 11 on page 21	For assemblies containing promoters or selectable markers from the SureVector Yeast Expansion Kits (p/n G7517A, G7517B)

Although thermal cycling programs A–D were developed using a variety of gene-of-interest inserts, the features of your specific gene-of-interest insert may impact the optimal cycling conditions for your assemblies. If you find that the cycling conditions require further optimization, Agilent recommends that you first try adjusting the temperature and duration of the annealing step in segment 2. This step plays a critical role in assembling the individual modules into a complete vector.

Protocol

Prepare the SureVector reagents

dNTP Mix

The dNTP Mix is one of the assembly reagents included with certain SureVector kits. See Table 2 on page 10 for a list of SureVector kits that include assembly reagents.

- After the initial thawing of the dNTP Mix, aliquot the mixture into single-use volumes and store the aliquots at –20°C to avoid multiple freeze-thaw cycles.
- Use the dNTP Mix that is provided with a SureVector kit. Do not use other sources of dNTPs.

SureSolution

A 5× stock of SureSolution is one of the assembly reagents included with certain SureVector kits. See Table 2 on page 10 for a list of SureVector kits that include assembly reagents. After the initial thawing of the 5× SureSolution, dilute the 5× stock to 1× using the instructions below.

1. Transfer 100 µL of the 5× SureSolution to a DNase-free 1.5-mL tube.
2. Add 400 µL of DNase-free dH_20 directly to the tube to dilute the SureSolution to a 1× concentration. Mix well by vortexing.
3. Aliquot the 1× SureSolution into single-use volumes and store the aliquots at –20°C to avoid multiple freeze-thaw cycles.

   Use the 1× SureSolution in the assembly protocol.

Assemble the vectors

1 Thaw the gene-of-interest DNA insert and the needed SureVector assembly reagents and modules on ice.

NOTE

Each assembly reaction needs to include one SureVector module from each of the functional groups (bacterial selectable markers, bacterial origins of replication, XP1 expansion site modules, XP2 expansion site modules, promoters, and tags). See Table 3 on page 11 for a list of modules in each functional group.

2 Program the thermal cycler with the appropriate program for your assembly reactions, then pre-warm the thermal block to 95°C. The four different thermal cycling programs are described in Table 8 through Table 11. Use the program that is optimized for your assemblies.

See "Cycling conditions" on page 17 for further information on selecting a thermal cycling program.

Table 8 Cycling Program A - For assemblies that only contain modules from the **SureVector Core Kit** or **SureVector *E. coli* Selection Kit** (p/n G7514A, G7518A)

Segment	Cycles	Temperature	Duration
1	1	95°C	1 minute
2	8	95°C	20 seconds
		60°C	20 seconds
		68°C	1 minute
3	1	68°C	1 minute
4	1	4°C	2 minutes

Table 9 Cycling Program B - For assemblies containing promoters or selectable markers from the **SureVector *E. coli* Expansion Kits** and/or **SureVector *E. coli* Promoter Kits** and/or **SureVector *E. coli* Tag Kits** (p/n G7515A, G7515B, G7518B, G7518C, G7518D, G7518E)

Segment	Cycles	Temperature	Duration
1	1	95°C	1 minute
2	8	95°C	20 seconds
		55°C	90 seconds
		68°C	1 minute
3	1	68°C	1 minute
4	1	4°C	2 minutes

Table 10 Cycling Program C - For assemblies containing promoters or selectable markers from the **SureVector Mammalian Expansion Kits** (p/n G7516A, G7516B)

Segment	Cycles	Temperature	Duration
1	1	95°C	1 minute
2	8	98°C	30 seconds
		62°C	1 minute
		68°C	1 minute
3	1	68°C	1 minute
4	1	4°C	2 minutes

Table 11 Cycling Program D - For assemblies containing promoters or selectable markers from the **SureVector Yeast Expansion Kits** (p/n G7517A, G7517B)

Segment	Cycles	Temperature	Duration
1	1	95°C	30 seconds
2	8	95°C	20 seconds
		55°C	90 seconds
		68°C	1 minute
3	1	68°C	1 minute
4	1	4°C	2 minutes

3 Using the volumes listed in Table 12, prepare the assembly reactions in tubes that are suitable for your thermal cycler (e.g. 0.2-mL thin-wall tubes). Mix each reaction gently by pipetting up and down or tapping the tube.

Table 12 Reagent volumes for assembly reactions

Reagent	Gene-of-interest reaction	Negative control reaction	Positive control reaction
10× SureVector Buffer	2 µL	2 µL	2 µL
DNase-free dH_20*	0–2 µL	2–4 µL	0–2 µL
Bacterial selectable marker	2 µL	2 µL	2 µL
Bacterial origin of replication	2 µL	2 µL	2 µL
XP1 expansion site module	2 µL	2 µL	2 µL
XP2 expansion site module	2 µL	2 µL	2 µL
Promoter-tag fusion module, or promoter module	2 µL	2 µL	2 µL
Tag module (if using)	2 µL	2 µL	2 µL
Gene-of-interest insert (0.05 pmol/µL stock)	2 µL	—	—
SureVector LacZ Control (N-term OR C-term)	—	—	2 µL
dNTP Mix	1 µL	1 µL	1 µL
SureSolution (diluted to 1×)	2 µL	2 µL	2 µL
SureVector Enzyme Mix	1 µL	1 µL	1 µL
Total volume	20 µL	20 µL	20 µL

* The volume of water varies depending on whether or not the assembly reaction includes a separate tag module. Add a sufficient volume of water so that the final reaction has a total volume of 20 µL.

4 Load the reactions into the thermal cycler and run the thermal cycling program.

5 At the conclusion of the program, transfer the reactions to ice.

6 Add 1 µL of *Dpn* I to each reaction then transfer to a 37°C heat block or thermal cycler pre-heated to 37°C. Incubate the reactions at 37°C for 5 minutes then transfer to ice.

Use the *Dpn* I enzyme that is provided with a SureVector kit. Do not use other sources of *Dpn* I. See Table 2 on page 10 for a list of SureVector kits that include assembly reagents.

At this point, you can proceed directly to "Perform the transformations", below, or store the assembly reactions at –20°C until needed.

Perform the transformations

NOTE The transformation protocol provided here is appropriate for use with Agilent XL-1 Blue Supercompetent Cells (provided with the SureVector Core Kit and sold separately as p/n 200236). If you are using another competent cell line, follow the manufacturer's instructions for transformation.

1 Label the appropriate number of 14-mL polypropylene round-bottom tubes, then chill the tubes on ice. You will need one tube for each assembly reaction (including positive and negative controls) *plus* one additional tube for the pUC18 transformation control reaction.

2 Preheat NZY medium to 42°C. (See "Preparation of media" on page 30 for instructions on NZY medium preparation.)

3 Thaw the XL1-Blue Supercompetent Cells on ice. Once thawed, gently mix the cells, then aliquot 50 µL to each pre-chilled polypropylene tube, keeping the tubes on ice. (Each provided tube of XL1-Blue Supercompetent Cells contains 200 µL.)

4 Add 0.8 µL of β-Mercaptoethanol to each aliquot of cells. Swirl the tubes gently to mix.

Use the β-Mercaptoethanol provided with the XL1-Blue Supercompetent Cells Kit.

5 Incubate the cell samples on ice for 10 minutes, swirling gently every 2 minutes.

6 Add 1 µL of the appropriate assembly reaction to each cell sample. For the cell sample for the pUC18 transformation control, add 1 µL of the pUC18 Control Plasmid. Swirl the tubes gently to mix.

   The pUC18 Control Plasmid is included with the XL1-Blue Supercompetent Cells Kit.

7 Incubate the cell samples on ice for 30 minutes.

8 Heat-pulse the cell samples in a 42°C water bath for 45 seconds. The 45-second duration is critical.

9 Incubate the cell samples on ice for 2 minutes.

10 Add 450 µL of pre-warmed NZY medium to each cell sample. Incubate the samples at 37°C for 1 hour with shaking at 225–250 rpm.

11 Spread samples of each transformation onto the appropriate LB-agar plates. (See "Preparation of media" on page 30 for instructions on plate preparation.)

   - For transformations with an assembly reaction (including positive and negative controls), spread the following volumes onto LB-agar plates that contain the appropriate antibiotic.
     - 10 µL
     - 50 µL
     - 200 µL
   - For the pUC18 transformation, spread 2.5 µL onto an LB-agar plate containing ampicillin.

12 Incubate the plates at 37°C overnight (18–20 hours).

   You can now analyze the transformants or store the plates at 4°C until needed.

Analyze transformants

1 Count or estimate the number of colonies on each transformation plate. The expected number of colonies for each plate is listed in Table 13. Note that the number of colonies varies based on the volume of transformed cells that you spread on the plate.

Table 13 Expected number of colonies per plate for each vector

Vector		Expected # of colonies
Assembled vector	Gene-of-interest	100–300 (white)
	Positive control	100–300 (blue) <5–15 (white)
	Negative control	<10 (white)
pUC18 control vector		~100 (white)

More important than the total number of colonies on each plate is the difference in colony counts between the plates. For a successful vector assembly, expect to have 5× as many colonies on the gene-of-interest and positive control plates as on the negative control plates.

2 Verify the composition of your assembled gene-of-interest vector using restriction analysis, sequencing, or another method of DNA analysis.

3 Troubleshooting

This chapter contains suggestions for troubleshooting your SureVector cloning.

Troubleshooting suggestions

Table 14 Troubleshooting suggestions based on potential observations

Observation	Possible Cause	Suggestion
Fewer than expected colonies on the gene-of-interest and positive control transformation plates	Inefficient transformation	Make sure you are using 14-mL BD Falcon polypropylene round-bottom tubes (BD Biosciences Catalog #352059) for the transformation protocol, since other tubes may be degraded by β-mercaptoethanol. In addition, the duration of the heat-pulse step has been optimized using these tubes.
		Make sure that the cells are heat-pulsed at 42°C for 45–50 seconds. Efficiency decreases sharply when cells are heat-pulsed for <45 seconds or for >60 seconds. Do not exceed 42°C.
		Agilent recommends XL1-Blue Supercompetent Cells for use in the SureVector transformation protocol. If you are experiencing inefficient transformation with another competent cell line, try the protocol with the Agilent XL1-Blue Supercompetent Cell Kit (p/n 200236).
Expected number of blue colonies on the positive control plate (with the SureVector LacZ control insert) but fewer than expected colonies on the gene-of-interest transformation plate	The gene-of-interest insert does not have compatible 5′ and 3′ ends	Review the information in "Gene-of-interest insert" on page 15.
	The gene-of-interest insert contains contaminants that interfere with assembly	Run the insert on an agarose gel then use the Agilent StrataPrep DNA Gel Extraction Kit (p/n 400766) to purify the insert. Following purification, run a sample of the insert on another agarose gel to verify that the insert is pure.
	Inefficient vector assembly of the gene-of-interest vector	Verify the concentration of your gene-of-interest insert. Make sure that the stock concentration of your gene-of-interest insert is 0.05 pmol/μL and that you are using 2 μL of the insert in the assembly reaction.
Excessive number of colonies on the negative control plate	The transformation reactions are contaminated	Start with fresh reagents and sterilized labware.

Table 14 Troubleshooting suggestions based on potential observations

Observation	Possible Cause	Suggestion
No blue colonies on the positive control plate (with the SureVector LacZ control insert)	Beta-galactosidase is not being expressed or is not functioning	Make sure that IPTG and X-gal were included in the plates. See "Preparation of media" on page 30.
		Even if the colonies do not turn blue, as long as the positive control plate has significantly more colonies than the negative control plate, the assembly was likely successful. If you continue to store the positive control plate at 4°C, you may see the blue color develop after several days.
		Make sure that your competent cells are capable of alpha-complementation. If unsure, use Agilent XL1-Blue Supercompetent Cells (p/n 200236).
Analysis of colonies from the gene-of-interest plate indicates internal deletions within the gene-of-interest	A high GC content in the gene-of-interest may promote formation of secondary structures that lead to internal deletions during the assembly thermal cycling program.	In the assembly thermal cycling program (see page 20 through page 21), increase the denaturation temperature from 95°C to 98°C to avoid secondary structure formation.

4
Reference Information

This chapter provides recipe information for preparation of the bacterial growth media.

Preparation of media

Use the recipes below to prepare the *E. coli* growth media needed for the transformation protocol.

NZY Broth (per liter)

In a clean flask combine:

- 10 g of NZ amine (casein hydrolysate)
- 5 g of yeast extract
- 5 g of NaCl
- 2 g of $MgSO_4 \cdot 7H_20$

Add deionized H_20 to a final volume of 1 liter then autoclave.

LB-agar plates for the pUC18 positive control transformations (per liter)

In a clean flask combine:

- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar

Add deionized H_20 to a final volume of 1 liter. Adjust pH to 7.0 with 5 N NaOH then autoclave.

When cooled to 55°C, add ampicillin to a final concentration of 100 µg/mL.

Pour into 100 × 15 mm petri dishes.

LB-agar plates for the assembly reaction transformations (per liter)

NOTE

If you want to use blue-white color screening to detect positive transformants on the plate containing the SureVector LacZ positive control insert, the plates need to include IPTG and X-gal.

You can also include IPTG and X-gal when plating transformations for your gene-of-interest assemblies, unless those assemblies use the SureVector Tac *E. coli* Promoter. With this promoter, IPTG may induce expression of the gene-of-interest, which could hinder colony growth.

In a clean flask combine:

- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar

Add deionized H_20 to a final volume of 1 liter. Adjust pH to 7.0 with 5 N NaOH then autoclave.

When cooled to 55°C, add:

- X-gal, if using (for a final concentration of 60 µg/mL)
- IPTG, if using (for a final concentration of 0.1 mM)
- Appropriate antibiotic (see table below for concentrations)

Antibiotic	Final concentration
Ampicillin	100 µg/mL
Kanamycin	50 µg/mL
Chloramphenicol	34 µg/mL

Pour into 100 × 15 mm petri dishes.

www.agilent.com

In this book

This document describes how to use the Agilent SureVector cloning kits to create custom cloning vectors that can be used in a variety of downstream applications.

Version C0, September 2015

G7514-90000

Agilent Technologies