pBluescript II RI Predigested Vector

INSTRUCTION MANUAL

Catalog #212250

Revision A

For In Vitro Use Only

212250-12

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pBluescript II RI Predigested Vector

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MATERIALS PROVIDED

Materials provided	Concentration	Quantity
pBluescript II RI predigested vector[a,b]	20 ng/µl	55 µl (1100 ng)
Test insert (kanamycin-resistance gene; 1.4 kb)	10 ng/µl	3 µl (30 ng)

[a] The pBluescript II RI predigested vector is digested with *Eco*R I and CIAP-treated.

[b] The pBluescript II RI predigested vector is derived from pBluescript II SK (+).

STORAGE CONDITIONS

All components: –20°C

ADDITIONAL MATERIALS REQUIRED

High efficiency competent cells (≥5 × 10^9 cfu/µg DNA for preparing libraries)
LB-ampicillin agar plates[§]
LB-kanamycin agar plates[§]
Isopropyl-β-D-thio-galactopyranoside (IPTG)
5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal)

[§] See *Preparation of Media and Reagents*.

Revision A

INTRODUCTION

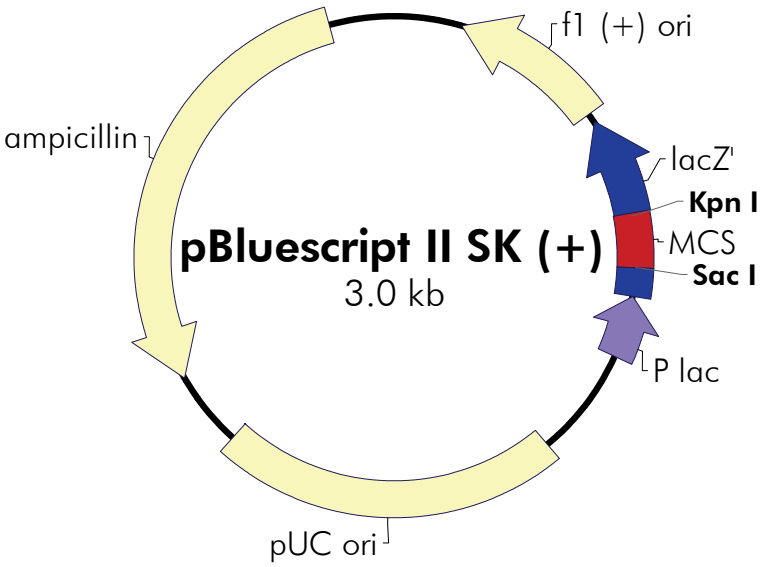
The pBluescript II RI predigested vector is a high-copy-number pUC-based plasmid with ampicillin resistance and the convenience of blue-white color screening. The vector supplied is predigested with *Eco*R I restriction enzyme and then is CIAP-treated to reduce the background of nonrecombinants. The multiple cloning site (MCS) contains unique restriction enzyme recognition sites organized with alternating 5´- and 3´-overhangs to allow serial exonuclease III/mung bean nuclease deletions. T3 and T7 RNA polymerase promoters flank the polylinkers for *in vitro* RNA synthesis. The choice of promoter used to initiate transcription determines which strand of the DNA insert will be transcribed. *Bss*H II sites flank the T3 and T7 promoters. This rare six-base restriction enzyme allows the insert plus the RNA promoters to be excised and used for gene mapping.

Expression in the pBluescript II RI vector is driven by the *lac* promoter, which is repressed in the presence of the LacI protein and is inducible by isopropyl-β-D-thio-galactopyranoside (IPTG). In bacteria expressing the *lac*ZΔM15 mutation and *lacI*, such as Stratagene XL10-Gold cells, colonies containing vector without insert will be blue in the presence of 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-gal) and IPTG. Ampicillin-resistant colonies containing vector with insert will be white and can express the inserted gene as a fusion protein. The MCS and T7 and T3 RNA polymerase promoter sequences are present in the N-terminal portion of a *lac*Z gene fragment. There are 36 amino acids of the *lacZ* protein from the methionine (Met) sequence to the *Eco*R I site. There are a total of 131 amino acids of the *lacZ* protein present, but this coding sequence is interrupted by the large MCS.

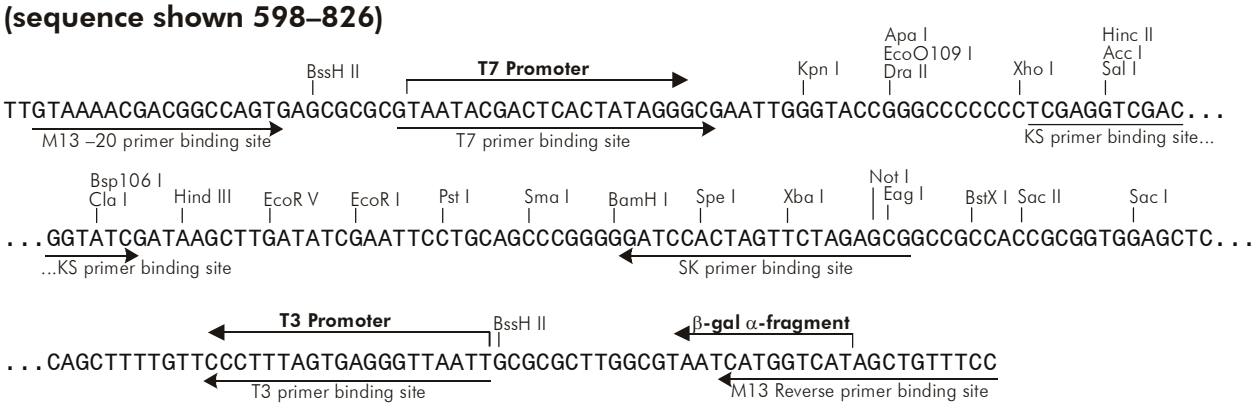
The pBluescript II SK (+) vector can be rescued as single-stranded DNA (ss-DNA). pBluescript II phagemids contain a 454-bp filamentous f1 phage intergenic region (M13 related), which includes the 307-bp origin of replication. The (+) orientation of the f1 intergenic region allows the rescue of *lacZ* sense ss-DNA by a helper phage. This ss-DNA can be used for dideoxynucleotide sequencing (Sanger method) or site-directed mutagenesis.

Additional sequence and restriction site information for the pBluescript II SK (+) vector is available from the Stratagene website (*http://www.stratagene.com*).

pBluescript II SK (+) Phagemid



pBluescript II SK (+) Multiple Cloning Site Region (sequence shown 598–826)



Feature	Nucleotide Position
f1 (+) origin of ss-DNA replication	135–441
β-galactosidase α-fragment coding sequence (lacZ′)	460–816
multiple cloning site	653–760
T7 promoter transcription initiation site	643
T3 promoter transcription initiation site	774
lac promoter	817–938
pUC origin of replication	1158–1825
ampicillin resistance (*bla*) ORF	1976–2833

FIGURE 1 The pBluescript II SK (+) phagemid vector. The complete sequence and list of restriction sites are available at *www.stratagene.com*. The vector sequence is also available from the GenBank® database (accession #X52328). The vector supplied in this kit is predigested with *Eco*R I restriction enzyme.

CLONING PROTOCOL FOR pBLUESCRIPT II RI PREDIGESTED VECTOR

The pBluescript II RI vector is designed for the convenient insertion of DNA inserts compatible with the *Eco*R I cloning site. This vector features an MCS with nineteen unique, conveniently arranged restriction sites for subcloning the DNA sequence of interest.

Cloning Considerations

The DNA fragment to be inserted should have ends compatible with the *Eco*R I cohesive ends of the predigested pBluescript II RI vector.

The insert DNA should be suspended in a volume of TE buffer[§] that will allow the concentration of the insert DNA to be the same as the concentration of the vector DNA (0.02 µg/µl).

Ligating the Insert

For ligation, the ideal insert-to-vector molar ratio of DNA is variable; however, a reasonable starting point is a 1:1 insert-to-vector ratio. The ratio is calculated using the following equation:

$$X\ \mu g\ \text{of insert} = \frac{(\text{Number of base pairs of insert})\ (\ 0.02\ \mu g\ \text{of pBluescript vector})}{2961\ \text{bp of pBluescript vector}}$$

where *X* is the quantity of insert (in micrograms) required for a 1:1 insert-to-vector molar ratio. Multiply *X* by 2 to get the quantity of insert required for a 2:1 ratio, multiply X by 3 to get the quantity of insert required for a 3:1 ratio, etc.

1. Prepare one control and two experimental 5-µl ligation reactions by adding the following components to separate sterile 1.5-ml microcentrifuge tubes:

	Control	Experimental	
Ligation reaction components	**1[a]**	**2[b]**	**3[b]**
Prepared pBluescript II RI vector (0.02 µg/µl)	1.0 µl	1.0 µl	1.0 µl
Prepared insert (0.02 µg/µl)	0 µl	*X* µl	*X* µl
Test insert (0.01 µg/µl)	1.0 µl	0 µl	0 µl
rATP [10 mM (pH 7.0)]	0.5 µl	0.5 µl	0.5 µl
Ligase buffer (10×)[§]	0.5 µl	0.5 µl	0.5 µl
T4 DNA ligase (4 U/µl)	0.5 µl	0.5 µl	0.5 µl
Double-distilled water (ddH_2O) to 5 µl	1.5 µl	*Y* µl	*Y* µl

[a] This control verifies the ability of the vector to ligate the test insert.

[b] These experimental ligation reactions vary the insert-to-vector ratio. Expect a majority of the transformant colonies to represent recombinants.

2. Incubate the reactions overnight at 4°C.

[§] See *Preparation of Media and Reagents*

Transformation

1. Transform competent bacterial cells with 1–5 µl of the ligation reactions. See reference 1 for a transformation protocol.

   Note *We recommend using competent cells with transformation efficiencies ≥5 × 10^9 cfu/µg for preparing libraries.*

2. Plate the control-transformed cells on LB-kanamycin agar plates and the experimental-transformed cells on LB-ampicillin agar plates.

3. Incubate at 37°C overnight.

4. Pick 50 colonies, transfer them to an LB-ampicillin agar plate containing X-gal and IPTG, and incubate the plates at 37°C. White colonies contain the test insert.

Verifying the Presence of the Insert

Individual colonies can be examined to identify the plasmids with inserts and the insert size directly by PCR or by restriction analysis. The T3 and the T7 primers are recommended for PCR amplification and sequencing.

PREPARATION OF MEDIA AND REAGENTS

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes
(~25 ml/100-mm plate)

LB-Kanamycin Agar (per Liter)

Prepare 1 liter of LB agar
Autoclave
Cool to 55°C
Add 50 mg of filter-sterilized kanamycin
Pour into petri dishes (~25 ml/100-mm plate)

Ligase Buffer (10×)

500 mM Tris HCl (pH 7.5)
70 mM $MgCl_2$
10 mM dithiothreitol (DTT)

Note *rATP is added separately in the ligation reaction*

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

REFERENCES

1. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., *et al.* (1987). *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

ENDNOTES

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MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.