PRODUCT INFORMATIOIN

DNA blunting and Ligation

Product Code: BS243-BS244

Storage:

Store at -20^{o}C. Avoid frequent thawing as this diminishes the quality of the kit.

Description and Notes:

1. Construction of new hybrid DNA molecules involves such standard procedures as DNA treatment with appropriate restriction endonuc-leases and subsequent ligation. Subcloning is a simple matter when cohesive ends at the termini of target fragment are either identical, or compatible with those of the new vector. In many cases, however, termini of the target fragment and the vector are incompatible. Hence, it is necessary to obtain blunt-ended DNA that can be ligated with any other blunt-ended DNA. There are several ways of such conversion.

2. Our kit is based on the polymerase activity of the Klenow DNA Polymerase that allows the filling-in of incompatible recessed 3'-termini. For the restriction fragments obtained after digestion with two different endonucleases it is possible to repair only one end selectively. This is performed by cleaving DNA with the restriction enzyme I, filling-in the ends with Klenow DNA Polymerase of *E.* coli DNA polymerase I, heat inactivation of polymerase at 75^{o}C for 10min prior to digestion with the restriction enzyme II. Cleavage with the enzyme II can be done in the same reaction buffer if buffers for both restriction enzymes are compatible.

3. T4 DNA ligase joins efficiently DNA molecules with blunt or cohesive ends. Components of this kit allow carrying out the ligation reaction in a short time (1 hour). Inclusion of PEG into

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

ligation buffer accelerates the rate of blunt-ended DNA ligation.

4. During ligation reaction, both fragment and vector DNA have the capacity to recircularise and to form tandem oligomer. Therefore it is necessary to adjust carefully the concentration and ratio of insert/vector termini in the ligation reaction in order to optimise the yield of “correct” ligation products.

5. The estimation of concentration of linear DNA (3' or 5') ends:
   pmol ends = pmol DNA×[(number of cuts x 2) + 2]
   1µg of 1000 bp DNA = 3.04pmol ends;
   1µg of linear pUC18/19 DNA = 1.14pmol ends;
   1µg of linear pBR322 DNA = 0.7pmol ends;
   1µg of linear SV40 DNA = 0.58pmol ends;
   1µg of linearϕX174 DNA = 0.56pmol ends;
   1µg of linear M13mp18/19 DNA = 0.42pmol ends
   1µg of lambda phage DNA = 0.06pmol ends.

6. Ligation reaction mixture should contain an equal or higher (up to 3 fold) concentration of insert DNA termini compared to vector DNA. Reasonable number of useful recombinants will be obtained when the ratio of vector DNA to insert DNA termini is < 1.

7. Recircularisation of vector DNA can be minimized by removal of the 5'-phosphates from both termini of linear DNA with Bacterial Alkaline Phosphatase or Calf Alkaline Phosphatase. Digested with several restriction endonucleases and dephosphorylated pUC19 DNA cloning vectors are available from Fermentas.

8. After the recessed 3'-termini has been filled by Klenow DNA Polymerase, DNA fragments should be purified from unincorporated dNTPs by chromatography, ethanol precipitation or other methods. Bacteriophage T4 DNA ligase is not significantly inhibited by the presence of dNTPs, in order to maximize the yield of the recombinant DNA molecules DNA should be purified.

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

9. In the case when DNA fragments are purified by gel electrophoresis, agarose of high quality is preferential, since some products may contain DNA ligase inhibitors that are difficult to remove. Addition of BSA to the ligation mix to the final concentration of 500µg/ml is helpful in such cases.

Kit Components:

Components	BS243, 10 Reactions	BS244, 20 Reactions
Klenow Fragment (3U/µl)	15µl	30µl
10×Klenow Fragment Reaction Buffer	100µl	200µl
dNTP Mix (0.5mmol/L)	20µl	40µl
T4 DNA Ligase (2U/µl)	20µl	40µl
10×Ligation Buffer	100µl	200µl
PEG 4000 Solution (50%)	300µl	600µl
Sterilized ddH_2O	1.5ml	1.5ml

Procedures:

Filling-in Recessed 3'-Termini

1. In a microcentrifuge tube prepare 10~15µl of digested DNA (0.1~ 4µg) in water.

2. Add the following components to the same tube:

10×Klenow fragment reaction buffer	2µl
dNTP mix	2µl
Sample DNA	50ng/5µl
Sterilized ddH_2O up to	20µl
Klenow fragment (3U)	1µl
Final volume	30µl

Vortex the tube and spin down in a microcentrifuge for 3~5sec.

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

3. Incubate the mixture for 10min at 37℃.

4. Stop the reaction by heating at 75℃ for 10min.

DNA Insert Ligation into Plasmid Vector

1. In a microcentrifuge tube prepare 5~10µl mix in water (or TE buffer pH7.8) of digested, if preferred blunt ended, dephospho-rylated and purified plasmid (400~800ng) and foreign DNA to be inserted.

2. Add the following components to the same tube:

10×ligation buffer	2µl
PEG 4000 solution (for blunt ends only)	2µl
T4 DNA ligase	1µl
Sterilized ddH_2O up to	20µl

Vortex the tube and spin down in a microcentrifuge for 3~5s.

3. Incubate the mixture for 1 hour at 22℃.

4. Use the mixture for transformation.

Notes:

1. Klenow DNA Polymerase of *E.coli* DNA polymerase I works in any buffer from Five Buffer Plus System used for digestion with restriction endonucleases. If buffers for restriction enzymes and Klenow DNA Polymerase are incompatible, the DNA should be digested with the restriction enzyme and subsequently repurified by phenol extraction, ethanol precipitation and resuspended in sterilized ddH_2O or TE buffer prior to the treatment with Klenow DNA Polymerase.

2. You can directly use the digested plasmid or DNA fragment solution for ligation if you perform experiments in any buffer from Five Buffer Plus System.

3. Ligation reaction for plasmid the size of pUC18 should contain between 15µg and 30µg of vector/ml and for a plasmid the

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

size of pBR322 - between 20µg and 40µg of vector/ml. Ligation reaction mixture should contain an equal or higher (up to 3-fold) concentration of foreign DNA termini than of vector DNA.

4. If the ligation reaction was not complete or if it is essential to obtain the maximum yield of useful recombinants, prolong the reaction time (overnight).

5. The resultant ligation reaction mixture can be used directly for bacterial transformation according calcium chloride method. The excess of the ligation mixture in regard to competent cells may decrease the transformation efficiency. For the electro-transformation it is necessary to inactivate T4 DNA ligase by chloroform extraction and ethanol (optional) precipitation.

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Please visit www.biobasic.com

A world Leader in Serving Science

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com