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λ-Terminase

Cat. Nos. LT4450 and LT44200

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1. Introduction

λ-Terminase is an endonuclease that recognizes a sequence of about 100 bp at the cos region of the λ genome. It cleaves in this region to generate termini with complementary 12-base, 5′ overhangs.[1] The size of cos-site containing BAC, fosmid, and cosmid clones is readily determined by linearizing the DNA with λ-Terminase and separating the DNA by pulse-field gel electrophoresis. Lambda Terminase can also be used for chromosomal mapping and for generating restriction maps of (large insert clones).

This enzyme is a heteromeric protein possessing several activities including DNA binding, nicking of the DNA at the cos site, and dissociation of the cohesive ends. ATP serves as a required cofactor for λ-Terminase activity. While only the binding of ATP is necessary for cos site nicking, ATP hydrolysis is necessary for cohesive end dissociation.[1]

Epicentre's λ-Terminase is highly purified to provide a reproducible level of cleavage activity. However, under certain conditions, the hydrolysis of ATP during the cos-site nicking step contributes to non-specific nicking of the DNA.[1] Epicentre's revised protocol incorporates a new maximum efficiency (ME) buffer which minimizes this lack in specificity. The ATP analog AMPPNP can also be used in place of ATP to minimize non-specific nicking.

λ-Terminase is available in 50- and 200-Unit sizes at a concentration of 2 U/µl. 10X ME Buffer, 10X TA Buffer, and 10 mM ATP Solutions are also provided with the enzyme.

2. Applications

- Rapid sizing of BAC, fosmid, or cosmid clones.
- Generation of restriction maps of BAC, fosmid, or cosmid clone inserts (Fig. 1).[2]
- Specific cleavage of chromosomal DNA for physical mapping.[3]

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

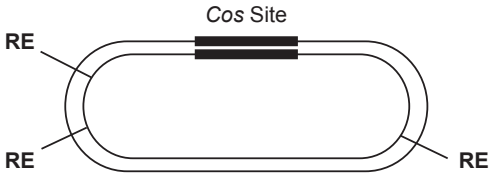
Storage Buffer: λ-Terminase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1% Triton® X-100.

Unit Definition: One unit linearizes a minimum of 70% of one microgram of the cosmid DNA vector pHC79 in 30 min at room temperature under standard assay conditions.

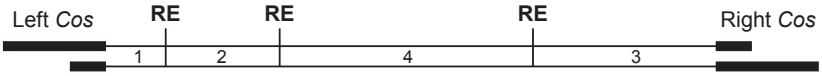
10X ME Buffer: 500 mM Tris-HCl (pH 8.0) and 50 mM $MgCl_2$.

10X TA Buffer: 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT.

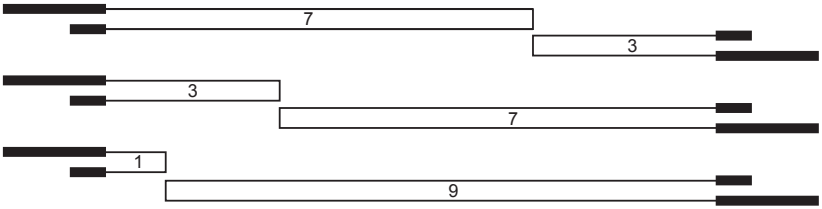
Contaminating Activity Assays: λ-Terminase is free of detectable exo- and endonuclease, and RNase activities except for the inherent endonucleolytic property of the enzyme.



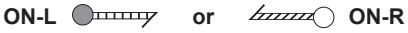
1. Cleavage with λ-Terminase

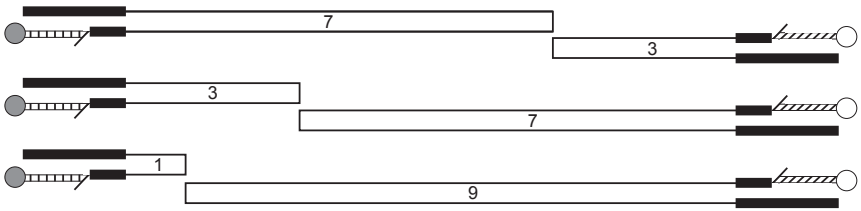


2. Partial Restriction Digest



3. Hybridization with a labeled *Cos*-site-specific Oligonucleotide





4. Separate products by electrophoresis

5. Visualize using autoradiography

Figure 1. Cosmid Insert Restriction Mapping.

4. Related Products

The following products are also available:

- CopyControl™ BAC Cloning Kits
- CopyControl™ Fosmid Library Production Kits
- MaxPlax™ Lambda DNA Packaging Extract
- GELase™ Agarose Gel-Digesting Preparation

The cosmid or fosmid clone is linearized by digestion with λ-Terminase resulting in the formation of unique 12-base, 5′ overhangs at the end of each molecule (step 1). The linearized clone is then digested under conditions resulting in partial restriction products with the enzyme whose recognition sites are to be mapped (step 2). Possible fragment lengths are indicated (in kilobases) on the diagram. A population of molecules each with a "left" or "right" *cos* site end and a restriction site end is generated. A labeled *cos*-site-specific oligonucleotide is then hybridized to the digestion products (step 3). The oligonucleotide (ON-L or ON-R) will be complementary to its specific overhang ("left" or "right") thus labeling only those molecules with the appropriate *cos* site end. Oligonucleotides can be radio- or fluorescently-labeled. The reaction products are then separated by gel electrophoresis and visualized using autoradiography. The size of each resultant labeled fragment will indicate the mapping location of a restriction site from the position of the label (*cos* site) in the original clone. Map positions can be verified by use of the other *cos*-site-specific oligonucleotide in a subsequent hybridization. In a similar manner, restriction maps of λ-clone inserts can be generated by beginning the analysis at step 2 of this protocol since such clones will already be in a linear form with *cos* site termini.

5. Oligonucleotide Sequences

ON-R: 5′ - **GGG CGG CGA CCT -3′**

ON-L: 5′ - **AGG TCG CCG CCC -3′**

Sample Protocols for Clone Mapping

A. Digestion with λ-Terminase

1a. **For Cosmid and Fosmid Clones.** Combine the following reaction components on ice in the order given:

x	µl	sterile water
5	µl	10X ME Buffer
5	µl	10 mM ATP Solution
1	µg	DNA
0.5	µl	λ-Terminase (1 U)
50	µl	Total reaction volume

1b. **For BAC Clones.** Combine the following reaction components on ice in the order given:

x	µl	sterile water
2	µl	10X ME Buffer
2	µl	10 mM ATP Solution
150	ng	DNA
0.15	U	λ-Terminase (Dilute in 1X ME Buffer)
20	µl	Total reaction volume

2. Incubate at room temperature for 30 minutes.

B. Partial Restriction Enzyme Digestion

1. Cosmid and fosmid DNA may be partially restricted after the λ-Terminase digestion described above. Use 0.1 U of enzyme for 60 minutes or 1.0 U for 10 minutes.

   - or -

2. Cosmid and fosmid DNA may be partially restricted simultaneously during the λ-Terminase digestion by the addition of 0.1-1.0 U of restriction enzyme to the reaction. The choice of how much enzyme to add depends on the activity of the enzyme under the prescribed reaction conditions.

Notes:

- λ-Terminase should be heat inactivated at 70°C for 10 minutes before addition of the restriction enzyme.
- Restriction enzymes can be inactivated by heat treatment, addition of excess EDTA, or by organic extraction (see manufacturer's recommendations).
- The extent of completion of the restriction digest can be monitored by agarose gel electrophoresis of an aliquot of the reaction mixture.

C. Oligonucleotide 5′-end Labeling

1. Combine the following reaction components on ice in the order given:

x	µl	sterile water
2	µl	10X Polynucleotide Kinase (PNK) Reaction Buffer
5	µl	ON-R or ON-L oligonucleotide (5 pmol)
0.5	µl	γ-[^{32}P]-ATP (10-80 µCi), carrier-free
1	µl	T4 Polynucleotide Kinase (10 U)
20	µl	Total reaction volume

2. Incubate at 37°C for 30 minutes.
3. Heat inactivate PNK at 70°C for 5 minutes.

It is not necessary to remove unincorporated label from the completed reaction. Labeled oligonucleotides can be stored at –20°C between uses.

D. Oligonucleotide Annealing

1. Combine the following reaction components at room temperature in the order given:

x	µl	sterile water
0.2	µg	partially restricted DNA (λ-phage clone or λ-Terminase treated cosmid or fosmid clone)
1	µl	^{32}P-labeled ON-R or ON-L oligonucleotide from the above protocol
20	µl	Total reaction volume

2. Heat the tube to 70°C for 10 minutes.
3. Allow the tube to cool to room temperature on the bench top.
4. Add the agarose gel loading buffer of choice containing a tracking dye.

E. Labeled Fragment Visualization

1. Load 5-10 µl of the annealing reaction onto a 1% agarose gel and separate the labeled fragments by electrophoresis.
2. Dry down the gel and visualize products using autoradiography.

Exposure times will range from 2 hours to overnight depending on the specific activity of the ^{32}P label used and the amount of DNA labeled.

6. Technical Note

There are several different ways to produce a partial restriction enzyme digest. These techniques involve the use of sub-optimal reaction conditions for the restriction enzyme including the use of minimal amounts of enzyme, reduced digestion times, or altered reaction components. Due to the sensitivity of the restriction mapping procedure described above, it is usually unnecessary to "optimize" the partial restriction enzyme digestion step. Should this become necessary, a test digestion can be setup in order to define the conditions which yield the desired partial restriction results.

Digest 1 µg of DNA with the desired restriction enzyme under the manufacturer's recommended conditions. Remove aliquots (0.05-0.1 µg DNA) at 5 minute intervals directly into an agarose gel loading buffer. Separate products by agarose gel electrophoresis and visualize resultant fragments by fluorescent staining. From this data, determine which time point yields the desired distribution of partial restriction products, and incorporate these conditions after the digestion with λ-Terminase step.

7. References

1. Higgins, R. *et al.*, (1988) *Cell* **54**, 765.
2. Rackwits, H.R. *et al.*, (1985) *Gene* **40**, 259.
3. Wang, Y. and Wu, R. (1993) *Nucl. Acids Res*. **21**, 2143.

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