Large (Klenow) Fragment Blunting (M0210)

New England Biolabs

Abstract

Protocol for blunting ends by 3' overhang removal and fill-in of 3' recessed (5' overhang) ends using DNA Polymerase I, Large (Klenow) Fragment (M0210)

Citation: New England Biolabs Large (Klenow) Fragment Blunting (M0210). **protocols.io** dx.doi.org/10.17504/protocols.io.cpgvjv
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Materials

DNA Polymerase I Klenow Fragment - 200 units M0210S by New England Biolabs

Protocol

Step 1.
DNA should be dissolved in 1x NEBuffer or T4 DNA Ligase Reaction buffer supplemented with 33 µM each dNTP.

ANNOTATIONS
Alexander Chamessian 18 Dec 2015
I went right into a BstAPI digest that was done in Cutsmart. NEB says that DNA Poly Klenow has 100% activity in Cutsmart buffer.

Step 2.
Add 1 unit of Klenow per microgram DNA.

REAGENTS
DNA Polymerase I Klenow Fragment - 200 units M0210S by New England Biolabs

Step 3.
Incubate for 15 minutes at 25°C.

DURATION
00:15:00

Step 4.
Stop reaction by adding EDTA to a final concentration of 10 mM.

ANNOTATIONS
Alexander Chamessian 18 Dec 2015
Rather than EDTA, I just went for the heat inactivation, using 80C instead.

Step 5.
Heat for 20 minutes at 75°C.

DURATION

00:20:00