sequence comprised of two 13 bp inverted repeats flanking an 8 bp spacer region which confers directionality (3). Recombination products depend on the location and relative orientation of the loxP sites. Two DNA species containing single loxP sites will be fused. DNA between directly repeated loxP sites will be excised in circular form while DNA between opposing loxP sites will be inverted with respect to external sequences.

Source: Purified from an E. coli strain carrying a plasmid encoding Cre Recombinase from bacteriophage P1 with additional N-terminal Ala and Gly residues (4).

Applications:
- Excision of DNA between two loxP sites
- Fusion of DNA molecules containing loxP sites
- Inversion of DNA between loxP sites

Supplied in: 15 mM Tris (pH 8.0), 250 mM NaCl, 0.3 mg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X Cre Recombinase Reaction Buffer, Control DNA (linearized pLox2+) (4).

Reaction Conditions: 1X Cre Recombinase Reaction Buffer. Incubate at 37°C.

1X Cre Recombinase Buffer:
33 mM NaCl 10 mM MgCl₂ 50 mM Tris-HCl (pH 7.5 @ 25°C)

Control DNA: Linearized pLox2+ is 3,625 bp in length, with a loxP site approximately 400 bp from each end. Between the loxP sites lie an origin of replication and an ampicillin-resistance gene. Recombination between these loxP sites produces a circular (2,787 bp), ampicillin-resistant plasmid (which migrates at approximately 1.7 kb on a 0.8% agarose gel) and one 838 bp DNA fragment.

Unit Definition: One unit is defined as the amount of enzyme necessary to produce maximal site-specific recombination of 0.25 µg pLox2+ control DNA in 30 minutes at 37°C in a total reaction volume of 50 µl. Maximal recombination is determined by agarose gel analysis and by transformation of reactions followed by selection on ampicillin plates.

Notes on Use:
- Incubation of the Cre Recombinase reaction mix at 70°C for 10 minutes is recommended before agarose gel analysis.
- Because the Cre Recombinase reaction is an equilibrium reaction, we observe 20–30% recombination on our loxP 2+ control substrate (Fig. 1). This modest yield produces a faint band on an ethidium bromide stained gel and the concomitant reduction in substrate staining intensity.

Quality Control Assays
16-Hour Incubation: Incubation of 10 units of Cre Recombinase with 1 µg of pX174 RF I DNA (Haelll digest) in 1X Cre Recombinase Reaction Buffer for 16 hours at 37°C resulted in no detectable exonuclease or endonuclease contamination.

Exonuclease Activity: Incubation of 10 units of enzyme with 1 µg sonicated [3H]DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Heat Inactivation: 40 units of enzyme were inactivated by incubation at 70°C for 10 minutes.

Notes on Use:
- Incubation of the Cre Recombinase reaction mix at 70°C for 10 minutes is recommended before agarose gel analysis.
- Because the Cre Recombinase reaction is an equilibrium reaction, we observe 20–30% recombination on our loxP 2+ control substrate (Fig. 1). This modest yield produces a faint band on an ethidium bromide stained gel and the concomitant reduction in substrate staining intensity.
• Longer incubation times will not improve recombination, and instead, will likely lead to higher molecular weight recombination products.

• Increasing the amount of Cre Recombinase in the reaction can inhibit recombination by forming loxP dependent Cre-DNA aggregates.

References: