Protocol

Self-circularization of Linear DNA

This protocol is for the Self-circularization of Linear DNA

1. Prepare the following reaction mixture:

Linear DNA	10-50 ng
10X T4 DNA Ligase buffer	5 µl
T4 DNA Ligase	5 u
Water, nuclease-free	to 50 µl
Total volume	**50 µl**

2. Mix thoroughly, spin briefly and incubate:
   - sticky-ends for 10 min at 20°C,
   - blunt-ends for 1 hour at 22°C.
3. Use up to 5 µl of the mixture for transformation of 50 µl chemically competent cells and 1-2 µl per 50 µl of electrocompetent cells.

Note

- The electrotransformation efficiency may be improved by:
  - heat inactivation of T4 DNA Ligase at 65°C for 10 min or at 70°C for 5 min,
  - purification of DNA, using GeneJET™ PCR Purification Kit (#K0701), or by chloroform extraction.
- The overall number of transformants may be increased by extending the reaction time to 1 hour.

IMPORTANT NOTES

- Polyethylene glycol (PEG) greatly increases the ligation efficiency of blunt-end DNA ligation. The recommended concentration of PEG 4000 in the ligation reaction mixture is 5% (w/v).
- Do not exceed the recommended amount of T4 DNA Ligase in the rection mixture.
- Binding of T4 DNA Ligase to DNA may result in a band shift in agarose gels. To avoid this, incubate samples with 6X Loading Dye & SDS Solution (#R1151) at 70°C for 5 min and chill on ice prior to loading.
- For efficient transformation, the volume of the ligation reaction mixture should not exceed 10% of the competent cell volume.

North America
Technical Services:
techservice.genomics@thermofisher.com
Customer Services:
customerservice.genomics@thermofisher.com
Tel 800 235 9880
Fax 800 292 6088

Europe and Asia
Technical Services:
techservice.emea.genomics@thermofisher.com
Customer Services:
customerservice.emea.genomics@thermofisher.com

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