Supercoiling DNA Using DNA Gyrase (*E. coli*) (NEB #M7636)

Materials

- 5X Gyrase Reaction Buffer
- DNA Gyrase (*E. coli*)
- Nuclease-free water

Materials Required but not Supplied

DNA Gyrase (*E. coli*)
- Nuclease-free Water (NEB #B1500)

Overview

This protocol describes the method to convert relaxed DNA into its supercoiled form using DNA Gyrase (*E. coli*) (NEB #M7636).

Protocol

Reaction Setup

1. Set up the following reaction on ice (note: enzyme should be added last):

COMPONENTS	30 µl REACTION
DNA (relaxed, circular)	500 ng – 2.5 µg
5X Gyrase Reaction Buffer	6 µl (1X)
DNA Gyrase (*E. coli*)	0.5 µl
Nuclease-free Water	Up to 30 µl

2. Incubate reaction at 37°C for 45 minutes.

3. Inactivate by heating at 65°C for 20 minutes if needed.