

# 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 $\mu$ l REACTION
DNA (relaxed, circular)	500 ng – 2.5 $\mu$ g
5X Gyrase Reaction Buffer	6 $\mu$ l (1X)
DNA Gyrase ( <i>E. coli</i> )	0.5 $\mu$ l
Nuclease-free Water	Up to 30 $\mu$ l

2. Incubate reaction at 37°C for 45 minutes.
3. Inactivate by heating at 65°C for 20 minutes if needed.