

TOPO® Cloning reaction

Introduction

Use the following procedure to perform the TOPO® Cloning reaction. Set up the TOPO® Cloning reaction using the reagents in the order shown. All the information about the reaction available from http://tools.lifetechnologies.com/content/sfs/manuals/zeroblunttopo_man.pdf

Materials

- › Invitrogen Zero Blunt TOPO®-kit
 - › Salt solution
 - › pCR™II-Blunt-TOPO
- › Fresh PCR product
- › Sterilized water
- › Microcentrifuge tube
- ›

Procedure

1. Make a cloning reaction mix. Set up the TOPO® Cloning reaction using the reagents in the order shown:

Fresh PCR product: 0,5-4 ul
Salt solution: 1 ul
Water: add to a total volume of 5 ul
pCR™II-Blunt-TOPO: 1 ul
Total Volume: 6 ul

2. Mix the reaction gently and incubate for 5 minutes at room temperature (22°C–23°C).

Note: For most applications, 5 minutes will yield plenty of colonies for analysis. Depending on your needs, the length of the TOPO®-cloning reaction can be varied from 30 seconds to 30 minutes. For routine subcloning of PCR products, 30 seconds may be sufficient. For large PCR products (greater than 1 kb) or if you are TOPO®-cloning a pool of PCR products, increasing the reaction time will yield more colonies.

3. Place the reaction on ice and proceed to transformation.

Note: You may store the TOPO® Cloning reaction at –20°C overnight