Dpn1 digestion of PCR fragments

Introduction

DpnI digestion is performed to remove template DNA from PCR amplified product prior to transformation.

DpnI (e.g. #R0176S from BioLabs, 20,000 U/ml) cleaves methylated sites from *in vivo* double stranded DNA. Reaction volume of the DpnI digestion can be scaled in proportion to the amount of the PCR amplified backbone needed for subsequent Assembly.

DpnI is Time Saver qualified (i.e. 5-10 minutes should technically be enough) but it is good practice to incubate samples for at least 1 hour at 37°C to ensure full digestion.

Optionally, an enzyme deactivation step can follow digestion, with incubation at 80°C for 20 min.

Digestion products can be purified according to section 5.1 of the NucleoSpin. Gel and PCR Clean-up kit manual (Macherey-Nagel, 2017).

Source: https://international.neb.com/products/r0176-dpni#Product%20Information

Reccommendations (in case we need to do troubleshooting):

DpnI

- Keep on ice when not in the freezer
- check expiration date
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units for genomic DNA in a 1 hour digest.
- The enzymes should be the last component added to reaction
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts. Extra wash steps during purification are recommended.

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of 1 µg of substrate
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt) as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol) can be problematic in smaller reaction volumes. The following guidelines can be used for techniques that require smaller reaction volumes.

Stopping a Reaction (when further manipulation of the DNA is required)

- Heat inactivation can be used
- Remove enzyme by using a spin column (NEB #T1030) or phenol/chloroform extraction

Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Control DNA (DNA with multiple known sites for the enzyme, e.g. lambda or adenovirus-2 DNA) with restriction enzyme to test enzyme viability
- If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA or phenol) is present, the control DNA will not cut after mixing.

Source: https://international.neb.com/protocols/2012/12/07/optimizing-restriction-endonuclease-reactions

Materials

›

- PCR reaction products (in PCR tubes, following a finished run)
- Ice bucket + ice
- DpnI enzyme (keep on ice when not in freezer: Exposure to temperatures above -20°C should be minimized whenever possible)
- 37°C heating block
- optional: 80°C heating block
- piette + tips
- microcentrifuge

Procedure

DpnI digestion

1. Run PCR to amplify PCR fragment
2. Add 0.5 uL of DpnI (buffer from PCR reaction is enough, no need for cutsmart).
3. Incubate for 1-2h at 37 degrees celcius
4. Run a gel purification
5. Run the USER cloning protocol