Digestion NotI

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Abstract

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Protocol

Step 1.
Add:
- nuclease-free water qsp 50 µL > 47 µL
- 5µL 10X Buffer O
- 1µL DNA (1 µg/µL)
- 1 µL NotI 1 (10U/µL) *

• Mix gently and spin down for a few seconds.

Step 2.
Incubate at 37°C for 1-16 hours. The digestion reaction may be scaled either up or down
> It's possible to process extended incubation by adding 0.25U/µg of DNA in 50 µL of reaction volume.

DURATION
01:30:00

Step 3.
incubation at 80°C for 20 min.

Step 4.

1. Measure the volume of the DNA sample.
> 50 µL

2. Add 1/10 volume of sodium acetate, pH 5.2, (final concentration of 0.3 M) - These amounts assume that the DNA is in TE only; if DNA is in a solution containing salt, adjust salt accordingly to achieve the correct final concentration.
> 5 µL

3. Mix well.

4. Add 2 to 2.5 volumes of cold 100% ethanol (calculated after salt addition).
> 110 (2V)

5. Mix well.

6. Place on ice or at -20 degrees C for >20 minutes.

7. Spin a maximum speed in a microfuge 10-15 min.

8. Carefully decant supernatant.

9. Add 1 ml 70% ethanol. Mix. Spin briefly. Carefully decant supernatant.

10. Air dry or briefly vacuum dry pellet.

11. Resuspend pellet in the appropriate volume of TE or water.