Digestion BclI

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Abstract

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Protocol

Step 1.
Add:
- nuclease-free water qsp50 µL > 43µL
- 10X Buffer G 5 µL
- 1µg DNA (1µg/µL)
- 1 µL BclI 10U/µL > 10U/1µg DNA

Mix gently and spin down for a few seconds.

**Star Activity : An excess of BclI (20 U/µg DNA x 1 hour) may result in star activity

Step 2.
Incubate at 55°C for 1-16 hours**.
It's possible to use extended digestion for 16 hours by adding 0.5U for 1 µg of DNA in 50 µL

DURATION
01:30:00

Step 3.
- Inactivated at 80°C in 20 min.
> For only 10 U of enzyme

or - adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration

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.