

Restriction Digest of DNA

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Abstract

Protocol adapted to match the one followed by Northeastern_Boston

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Protocol

Step 1.

Select restriction enzymes to digest your plasmid.

Step 2.

Determine an appropriate reaction buffer by reading the instructions for your enzyme.

Step 3.

In a 1.5mL tube combine the following: DNA (all amounts are for a typical reaction; your amount may vary depending on the enzymes).

Typical mixture for single digest:

| Component | Volume |
|---------------------|----------|
| Nuclease-free Water | 16 ul |
| 10X Buffer EcoRI | 2 ul |
| DNA (0.5/1 ug/ul) | 1 ul |
| EcoRI | 0.5-2 ul |

Typical Mixture for Double Digest:

| Component | Volume |
|---------------------|--------|
| Nuclease-free Water | 15 ul |
| 10X Buffer 0 | 2 ul |
| DNA (0.5-1 ug/ul) | 1 ul |
| EcoRI | 1 ul |
| PstI | 1 ul |

AMOUNT

1 µg Additional info:

Step 4.

Mix gently by pipetting.

Step 5.

Incubate tube at appropriate temperature (usually 37°C) for 1 hour.

 DURATION

01:00:00