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Restriction Digest

¹
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1 *Works for me* dx.doi.org/10.17504/protocols.io.7h6hj9e

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ABSTRACT

This protocol includes three different methods of digestion.

MATERIALS TEXT

Restriction enzyme

Buffer 2.1/3.1/CutSmart

SAFETY WARNINGS

Please wear gloves for the experiment.

Single digested

- 1 Add the following reagents to a tube.

Total	10ul	30ul	50ul
DNA	0.4ug	1.2ug	2ug
Enzyme	0.2ul	0.6ul	1ul
Buffer(10*)	1ul	3ul	5ul
ddwater Fill the rest with water			

- 2 Pipette up and down thoroughly.
- 3 Put at 37°C for about 2 hours (according to different enzymes).

Single digested+CIP

- 4 Add the following reagents to a tube.

Total	10ul	30ul	50ul
DNA	0.4ug	1.2ug	2ug
Enzyme	0.2ul	0.6ul	1ul
Buffer(10*)	1ul	3ul	5ul
ddWater	Fill the rest with water	Fill the rest with water	Fill the rest with water

- 5 Pipette up and down thoroughly.
- 6 Put at 37°C for about 2 hours (according to different enzymes).
- 7 Put at 65°C for about 20 min.(according to different enzymes)
- 8 Purification of enzyme digestion products with Cycle Pure Kit D6492ΩOMEGA bio-tekΩ
- 9 DNA product concentrations were measured using Nano

- 10 Add the following reagents to a tube:

Total	10ul	30ul	50ul
DNA product	0.4ug	1.2ug	2ug
CIP(NEB)	0.2ul	0.6ul	1ul
Cutsmart(10*)	1ul	3ul	5ul
ddWater	Fill the rest with water	Fill the rest with water	Fill the rest with water

- 11 Pipette up and down thoroughly.
- 12 Put at 37°C for about 2 hours (according to different enzymes).

Double digested

- 13 Add the following reagents to a tube.

Total	10ul	30ul	50ul
DNA	0.4ug	1.2ug	2ug
Enzyme 1	0.2ul	0.6ul	1ul
Enzyme 2	0.2ul	0.6ul	1ul
Buffer(10*)	1ul	3ul	5ul
ddwater	Fill the rest with water	Fill the rest with water	Fill the rest with water

- 14 Pipette up and down thoroughly.
- 15 Put at 37°C for about 2 hours (according to different enzymes).

16 Add the following reagents to a tube.

Total	10ul	30ul	50ul
DNA product	0.4ug	1.2ug	2ug
DpnI(NEB)	0.2ul	0.6ul	1ul
Cutsmart(10*)	1ul	3ul	5ul
ddwater	Fill the rest with water	Fill the rest with water	Fill the rest with water

17 Pipette up and down thoroughly.

18 Put at 37°C for about 2 hours (according to different enzymes).



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