



Oct 24, 2020

Restriction Digest

Zhujun Wei¹¹2020 iGEM NEFU China**1** Works for me dx.doi.org/10.17504/protocols.io.bnwvmfe6

2020 iGEM NEFU China

 Zhujun Wei

ABSTRACT

This protocol includes three different methods of digestion.

DOI

dx.doi.org/10.17504/protocols.io.bnwvmfe6

PROTOCOL CITATION

Zhujun Wei 2020. Restriction Digest. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bnwvmfe6>

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Oct 24, 2020

LAST MODIFIED

Oct 24, 2020

PROTOCOL INTEGER ID

43701

MATERIALS TEXT

Restriction enzyme
Buffer 2.1/3.1/CutSmart

SAFETY WARNINGS

Please wear gloves for the experiment.

ABSTRACT

This protocol includes three different methods of digestion.

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).
- 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

Pipette up and down thoroughly.

11

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

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
Dpnl(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).