

Sep 20, 2019

## Restriction Digest

DOI

[dx.doi.org/10.17504/protocols.io.7h6hj9e](https://dx.doi.org/10.17504/protocols.io.7h6hj9e)

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2019 iGEM NEFU\_China

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**DOI:** [dx.doi.org/10.17504/protocols.io.7h6hj9e](https://dx.doi.org/10.17504/protocols.io.7h6hj9e)

**Protocol Citation:** 宏亮 董 2019. Restriction Digest. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7h6hj9e>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** September 20, 2019

**Last Modified:** September 20, 2019

**Protocol Integer ID:** 27934

**Keywords:** Digest, Restriction enzyme, Single digested, Double digested

## Abstract

This protocol includes three different methods of digestion.



## Materials

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 D6492 (OMEGA bio-tek)



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).

## DpnI digested

16 Add the following reagents to a tube.



<b>Total</b>	<b>10ul</b>	<b>30ul</b>	<b>50ul</b>
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).