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## Restriction Digest

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**Protocol status:** Working

We use this protocol and it's working

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## Abstract

This protocol includes three different methods of digestion.

## Materials

Restriction enzyme

Buffer 2.1/3.1/CutSmart

## Troubleshooting

## Safety warnings

 Please wear gloves for the experiment.



## Single digested

- 1 Add the following reagents to a tube.

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

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