

Restriction Digest

Introduction

Restriction digestion of Insert fragments and Plasmids.

Materials

- 10x NEB buffer
- Restriction enzymes
- for plasmids: cip Phosphatase
- DNA-sample

Procedure

1. Pipette following table, add enzymes at last.

Total volume in μl	50			
Buffer concentration	10	fold		
Volume [μL]				
	Plasmid with 1 Enzyme	Plasmid with 2 Enzymes	Insert with 1 Enzyme	Insert with 2 Enzymes
DNA-sample	42.5	41	43.5	42
Buffer	5	5	5	5
Enzyme 1	1.5	1.5	1.5	1.5
Enzyme 2	0	1.5	0	1.5
Phosphatase cip	1	1	0	0

2. Incubate at 37°C for 4 hours or more

3. Stop the reaction with heat, 80°C for 20 min, or by directly adding loading dye and load onto the gel.