

Protocol for iGEM Prefix and Suffix Restriction

Protocol code: Rest

Materials

- High Fidelity Enzymes
- DNA
- 10X CutSmart Buffer
- Nuclease Free Water
- 0.2mL tubes

Procedure

1. Prepare reactions as described in the following tables

UPSTREAM PART

Reagent	1 Reaction (25µL)
DNA	500ng
10X CutSmart Buffer	2.5µL
SpeI-HF	0.5µL
EcoRI-HF	0.5µL
Nuclease Free Water	up to 25µL

DOWNSTREAM PART

Reagent	1 Reaction (25µL)
DNA	500ng
10X CutSmart Buffer	2.5µL
PstI-HF	0.5µL
XbaI-HF	0.5µL
Nuclease Free Water	up to 25µL

VECTOR

Reagent	1 Reaction (25µL)
DNA	500ng
10X CutSmart Buffer	2.5µL
PstI-HF	0.5µL
EcoRI-HF	0.5µL

Nuclease Free Water	up to 25µL
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2. Incubate at 37°C for 5-15 minutes.
3. Inactivate at 80°C for 20 minutes.
4. Store at -20°C.