

Mutagenesis PCR

Materials

1. PCR machine
2. PCR + 1.5mL tubes
3. PCR mix
4. Pipets

Protocol

1. Add all the necessary agents
2. Insert into PCR machine, set conditions and run

Recipe

5x Phusion Buffer	5
Template (100ng)	1.5
Primers (6uM)	1.5
Ampligase (5U/uL)	1.5
NAD+ (50mM)	0.25
Phusion	0.25
dNTP (10mM)	0.5
DMSO	0.75
dH ₂ O	11.75
Total	25 uL

PCR Conditions

98°C	30 sec
98°C	15 sec
55°C	1:00 min
72°C	3:00 min
72°C	10:00 min
12°C	HOLD