

Making Megaprimers

Materials

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

Protocol

1. Prepare PCR mix
2. Add PCR mix to tubes with template and primers
3. Place PCR tubes in PCR machine
4. Set PCR conditions + run

PCR Recipe

Tube 1	X1
Template	1uL
5' primer	1uL
3' primer	1uL
dNTP	1uL
dH ₂ O	21uL

5x Buffer	10uL
dH ₂ O	14uL
Phusion enzyme	0.5uL
Total Volume	50uL

PCR Conditions (30x runs)

98°C	1:00 min
98°C	0:30 min
48°C à 54 °C	0:30 min
72°C	2:00 min
72°C	5:00 min
12°C	HOLD