

Protocol for Asymmetrical PCR

Prepare the reaction system as the following:

Component	volume
template	1uL
prime F	0.5uL(5pmol)
prime R	0.5μL
2×Tag	5μL
ddH ₂ O	3μL

React under different prime concentration ratio (F/R) with either plasmid or product after digestion as template:

1:1 20:1 50:1 1:0 1:1 (without template)

The reaction condition settings are as following:

94 °C 90s (94 °C 20s 40 °C 20s 72 °C 20s)
Cycles: 40

72 °C 10min 4 °C ∞