

## **Colony PCR**

### colony preparation:

pick a colony and RE-suspend in 5µL DDW.

boil for 5minutes at 99°C and immediately chill on ice. (PCR program: colony\_prep)

use the 5µL as template for PCR.

### **Colony PCR for standard protocol**

Reaction mixture:

	µL
KAPA HiFi buffer	5
dNTPs	0.75
FWD primer	0.75
REV primer	0.75
Template	5
KAPA HiFi DNA polymerase	0.5
DDW	12.25
Total	25

Stage	Temp (°C)	time	
Total Denaturation	95°C	5min	
Denaturation	98°C	20sec	X 25
Annealing	55°C	30sec	
Polymerization	72°C	1.5min	
Final polymerization	72°C	5min	
Cool down	4°C	--	