Colony PCR analysis

Protocol:

1. Add 20 µL of sterilized distilled water into each PCR tube.
2. Pick colonies with a sterile pipette tip and resuspend a colony in a PCR tube.
3. Bacterial cells were lysed by heating at 98^{o}C for 5 minutes in a PCR machine (MJ Research).
4. A PCR master mix was prepared as shown in the following table for 12 PCR reactions:

	Volume (µL) (For 1 reaction)	Volume (µL) (For 12 reactions)
Takara Ex Taq polymerase (5 units/µL)	0.1	1.2
10X Taq Buffer	2	24
dNTP (2.5 mM each)	1.6	19.2
VF2 primer (10 mM)	2	24
VR primer (10 mM)	2	24
Sterilized distillated water	11.3	135.6
Total	19	228

5. Add 1 µL of heat-lyzed bacterial DNA template to 19 µL of master mix in each PCR tube.
6. PCR was performed using the following PCR profile:

98°C	3 minutes	
98°C	30 seconds	32 cycles
53°C	30 seconds	
72°C	1 minute	
72°C	10 minutes	
4°C	∞	