# Colony PCR

Protocol:

1. Add 20 µl of sterilized distillated water into each PCR tube.
2. Pick the colonies with sterilized pipette tips and transfer them to the PCR tubes respectively.
3. Conduct PCR according to the following PCR profile for preparing **DNA template** by lysing the bacterial cells:

| | |
|---|---|
| 98°C | 5 minutes |
| 12°C | ∞ |

4. Mix the following reagents as a **master mix**:

| | Volume (µL) (For 1 set) | Volume (µL) (For 12 sets) |
|---|---|---|
| Takara Ex Taq (5 units/µL) | 0.1 | 1.2 |
| 10X Taq Buffer | 2 | 24 |
| dNTP (2.5 mM) | 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 DNA template into 19 µl of master mix in each PCR tube.
6. Conduct PCR according to 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 | ∞ | |