

COLONY PCR

MATERIAL:

- Colonies on Plate
- Dynazymes (Finzymes)
- Primers (10 μ M) for amplification of fragment

PROCEDURE:

- Dip each colony into 20 μ l ddH₂O and pipet up and down.
- Mix primers, dNTP, 10x reaction buffer, polymerase, bacterial solution and ddH₂O for each reaction according to scheme.

Start PCR-program (see example)

| | | |
|-------|----------|-------------|
| 94 °C | 5 min | |
| 94 °C | 30 sec | } 30 cycles |
| 56 °C | 1 min | |
| 72 °C | 2 min | |
| 72 °C | 10 min | |
| 4 °C | ∞ | |

SCHEME:

1.0 μ l dNTP (2 mM) \rightarrow "C"
2.0 μ l 10x reaction buffer \rightarrow "P"
0.5 μ l Forward primer (10 μ M)
0.5 μ l Reverse primer (10 μ M)
1.0 μ l Bacterial solution
0.2 μ l Polymerase \rightarrow "TagTaq"
14.8 μ l ddH₂O

Σ 20.0 μ l