Colony PCR GoTaq® G2 DNA Polymerase

- First alternative:
  - ◊ Pick one colony with a sterile tip and elute in 100 µL ddH_2O or medium
  - ◊ Store the colony at 4 °C while colony PCR is running
- Second alternative:
  - ◊ Pick one colony with a sterile tip and streak cells at marked position on a new plate
  - ◊ Put tip in PCR tube already containing the reaction mixture
- For both alternatives continue as follows (one reaction mix):
  - ◊ 5 µL 5x Green or Colorless GoTaq® Reaction Buffer
  - ◊ 0.5 µL PCR Nucleotide Mix (10 mM each)
  - ◊ 0.25 µL upstream primer (0.1 – 1.0 µM)
  - ◊ 0.25 µL downstream primer (0.1 – 1.0 µM)
  - ◊ 0.25 µL GoTaq® G2 DNA Polymerase (Promega)
  - ◊ 1 µL template DNA
  - ◊ 17.75 µL ddH_2O
- PCR program:

	Steps	**°C**	**m:s**
	Cell lysis and denaturation	95.0	3:00
30 – 35 x	Denaturation	95.0	0:30
	Primer annealing	annealing temperature	0:30
	Extension	72.0	1 min/kb
	Final extension	72.0	5:00
	Hold	8.0	PAUSE

From: Promega