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**Protocol status:** Working
We use this protocol and it's working

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# Economic and easy bacterial and yeast colony PCR with 2x premix Gotaq-Green (Promega) or DreamTaq-Green (Thermo Fisher)

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ABSTRACT

Slightly modified method Gotaq Promega or DreamTaq (Thermo), economical, quick and ready-to-load. Perfect for yeast genotyping and screening

## 1 Bacterial colony PCR

GoTaq Green Master Mix **Promega Catalog #M7122**

PCR reactions using ProFlex PCR system, Applied Biosystems. Thermal Cycler

### 1.1

| | A | B | C |
|---|---|---|---|
| | **Reagent Name** | **Initial Conc.** | **Volume (µL)** |
| | gotaq green 2x | 2X | 5 |
| | oligo 1 | 10uM | 0.6 |
| | oligo 2 | 10 uM | 0.6 |
| | cell suspension in water | cells suspension | 0.8 |
| | ddH2O | N/A | 3 |
| | **TOTAL Volume:** | **10 uL** | |
| | | | |

| | A | B | C | D |
|---|---|---|---|---|
| | | | | |
| | **Number of Cycles** | **Step Name** | **Temperature** | **Duration** |
| | 1 | Initial Denaturation | 98oC | 10min |
| | 30 | Denaturation | 98oC | 10 sec |
| | | Annealing | 50-55oC | 30 sec |
| | | 1min (depends on the length) | 72oC | 1min per 1kb |
| | 1 | Hold | 4-10oC | hold |

Note

Maximum (good quality) PCR product up to ~3kb. The best yield is 0.2kb-2kb

Note

pick a colony from agar plate and swirl in sterile water 50-100 µL . From liquid culture, dilute saturated culture 5-10 µL in sterile 50-100 µL water.
This is a source for reaction (taking 0.8 µL ).

Keep the rest in ice, for re-culture of positively verified clones.

**1.2** Load on 1-1.2% agarose gel 8-10 µL and visualise under GelDoc or another UV source.

## 2 Yeast colony PCR (S.cerevisiae, Pichia, Candida)

**2.1** Alkaline yeast cells lysis treatment. Pick a yeast colony from agar plate and swirl in sterile 20mM NaOH 50-100 µL . 15m

Optionally, a liquid culture or a agar-plate pick colony resusupend in the sterile water or YPD, take small volume (~20uL) and mix with the same volume of 40mM NaOH.

20mM NaOH-cells resuspension incubate 95 °C 00:10:00 and immediately transfer to ice/ 4 °C for at least 00:05:00

**2.2**

| | A | B | C |
|---|---|---|---|
| | **Reagent Name** | **Initial Conc.** | **Volume (µL)** |
| | gotaq green 2x | 2X | 5 |
| | oligo 1 | 10uM | 0.6 |
| | oligo 2 | 10 uM | 0.6 |
| | cell suspension in 20mM | yeast suspension | 0.8 |
| | ddH2O | N/A | 3 |
| | **TOTAL Volume:** | **10 uL** | |
| | | | |

| | A | B | C | D |
|---|---|---|---|---|
| | A | B | C | D |
| | **Number of Cycles** | **Step Name** | **Temperature** | **Duration** |

| | A | B | C | D |
|---|---|---|---|---|
| | 1 | Initial Denaturation | 98oC | 2min |
| | 30-33 | Denaturation | 98oC | 10 sec |
| | | Annealing | 52-55oC | 1min 30sec |
| | | 1min (depends on the length) | 72oC | 1min per 1kb |
| | 1 | Hold | 4-10oC | hold |

**Note**

Maximum PCR product up to ~3kb. The best yield is 0.2kb-2kb

**Note**

Important - annealing 1min30sec

**2.3** Load on 1-1.2% agarose gel 8-10 µL and visualise under GelDoc or another UV source.