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

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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

Protocol materials

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

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)**

	A	B	C
	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**
	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.