Colony PCR

The standard *Taq* DNA polymerase chain reaction protocol and guidelines are largely based on NEB recommendations for NEB *Taq* [2], and Thermo recommendations for DreamTaq Green 2× PCR master mix [3]; optimization information (additives, troubleshooting protocols) is from elsewhere.
Colony PCR was first described by Saris *et al*., 1990 [1].
Genotyping PCRs are PCRs intending to produce products whose presence and size are used to infer information about the genotype of the sample. A colony PCR is a genotyping PCR that uses cells as the template. *Taq* DNA polymerase is far, far more error prone than high-fidelity polymerases used to prepare DNAs for assembly/cloning (*e.g.* 5000× less than Q5). However, *Taq* is quite more economical to use, and for genotyping PCRs, sequence fidelity is generally not needed, as PCR products are not intended for downstream use. Additionally, *Taq* polymerase is robust enough to handle a sizeable amount of bacterial colonies or suspensions being added directly to the PCR unpurified, simplifying genotyping. Colonies can be resuspended in a single PCR to use it as a template, followed by inoculating broth or agar with the residual cells on the tip; or colonies can first be resuspended in liquid and a portion of the resuspension used in a PCR (required for using one colony/suspension in multiple PCRs).

PCR master mixes combine the enzyme, buffer, and dNTPs at a 2× concentration, so that only primers, template, and remaining water need be added. Some mixes are formulated for GC-rich templates. Some enzymes or PCR mixes have a blend of DNA polymerases for robustness, *e.g.* NEB One*Taq*, which NEB has compared with other manufacturers' products against high-GC templates. Some polymerases or mixes have hot-start capability, which allow room-temp reaction setup without worry of nonspecific amplification, through an aptamer keeping the polymerase inactivated until the reaction is heated. Some mixes (ones with a color) already have a density reagent and tracking dyes as found in gel loading dyes, and can thus be loaded directly into a gel after thermocycling, *e.g.* NEB "quick-load" products or ones with colors in their name.
–Shyam Bhakta

Reaction Setup

Component	10 µL Rxn	25 µL Rxn	Final Conc
Nuclease-free water	to 10 µL	to 25 µL	–
10× Standard *Taq* Reaction Buffer *or* 10× ThermoPol™ Reaction Buffer[1]	1 µL	2.5 µL	1×. Mix with water before adding enzyme.
Taq DNA polymerase	0.0625 µL	0.125 µL	0.5%$_{V/V}$ with 5 U/µL enzyme. 0.025 U/µL rxn.
10 mM dNTPs	0.2 µL	0.5 µL	200 µM
10 µM forward primer *or 100 µM*	0.2 µL *or 0.02 µL*	0.5 µL *or 0.05 µL*	0.2 µM each
10 µM reverse primer *or 100 µM*			
Template DNA/cells	variable 1 µL cells	variable 2.5 µL cells	10%$_{V/V}$ cell suspension 1 pg–1 ng plasmid/viral DNA 1–1000 ng genomic DNA

[1]ThermoPol Buffer contains a nonionic detergent to increase enzyme stability during longer incubations.

1. *Cell template preparation from colonies, method 1:*
   Colony PCRs are more reproducible when colonies are first resuspended in liquid and a portion is used as a template in one or more PCRs, leaving the remainder for later inoculation.
   a. Aliquot 10–20 µL PBS or growth medium (no antibiotics necessary) into as many 250 µL thermocycler tubes/strips as unique colonies you desire to run reactions on. Label with colony IDs.
   b. Using pipette tips, pick colonies into these tubes, wiping them on the inside of the tube into the liquid to dislodge the cells, and pipetting the cells to disperse them.
   c. You may optionally remove a small amount of cell suspension for later liquid or solid culturing, especially if wanting to pre-lyse the cells. A few µL may be multichannel pipetted onto an agar dish and grown for later use of isolates of desired genotypes. Similarly, a few µL may be used to immediately inoculate liquid cultures for further experiments or DNA purification, accelerating growth by the 2 hr required for colony PCR and gel electrophoresis.
   d. Pre-lysis of cells may improve reliability of colony PCRs by removing cell solids and extracting DNA into the liquid, though *Taq* polymerase is quite robust to cell templates, so it is not necessary. But to do this,

Thermocycling (*Taq*)

	Step	Temperature	Time	Notes
	Lid preheating	105°C		
	Initial Denaturation	95°C	1–3 min	1 min for purified plasmid /linear/bacterial DNA. 3 min for complex templates, or to reliably lyse cells. 4–5 min for high-GC.
25–35 cycles	Denaturation	95°C	15 s	15–30 s.
	Annealing	*50–68°C	**30 s**	15–60 s. *Find T_{anl} on Tm calculator
30–35 genomic	Extension	68°C	1 min /kb	
	Final Extension	68°C	5 min	Holding temp is unnecessary and bad for thermocycler (ref).

Note: When needing to destroy non-synthetic (non-amplicon) DNA, DpnI will not work in standard *Taq* buffer, requiring gel/column purification before digesting. DpnI does have full activity in ThermoPol reaction buffer, however.

Dream*Taq* master mix Thermocycling

	Step	Temperature	Time	Notes
	Lid preheating	105°C		
	Initial Denaturation, Enzyme Activation	95°C	1–3 min	1 min for purified plasmid/linear /bacterial DNA. 3 min for complex templates, or to reliably lyse cells.
25–35 cycles 40 cycles: <10	Denaturation	95°C	30 s	3–4 min for high-GC.
	Annealing	*50–68°C	30 s	T_m – 5°. *Find T_{anl}. Or use lower of OneTaq T_m s.

save some live cells for later growth (see previous step), before "boiling" the rest in a thermocycler at 98° C, 10 min and centrifuging down the cell debris to use the supernatant as the PCR template. Yeast or tougher bacteria may be boiled in 20 mM NaOH.

Cell template preparation from colonies, method 2:
An alternative when only needing one PCR per colony is to pick and resuspend a colony directly in a PCR and use the same tip to inoculate growth medium, by either swiping on agar or dipping and pipetting in broth.

2. For as many colony PCRs, assemble reaction components on ice/cold block in 250 µL thermocycler tubes or tube strips (unless using a hot-start polymerase, which doesn't require keeping cold).
    a. Enzyme must be added after at least buffer and water are mixed.
    b. Make master mixes when possible, as it reduces pipetting steps, reduces errors from pipetting small volumes, and maximizes component precision across reactions: combine all common components for the rea ctions together, and into PCR tubes, aliquot reaction volumes deficient by the volume of the variable component(s).
    e.g., if only the template varies across ten 10 µL reactions, reserving 1 µL for cell template: combine water, buffer, dNTPs, primers, any enhancers, and polymerase; mix and aliquot 9 µL of this master mix across ten tubes, before adding their unique cell templates. If using a 2× PCR master mix, combine it with water and primers before aliquotting. Cell suspensions may be multichannel-pipetted into PCRs for convenience.
    Note: Aliquots of 2× PCR master mixes can often be kept at 4°C for 1–3 months, depending on the product, so as to limit the master mix freeze-thaw cycles. NEB tested theirs for 15 freeze-thaw cycles.
    c. After adding the last component, mix reaction with pipette or by closing, flicking, and centrifuging tubes to recollect liquid at bottom.
3. Transfer the reactions to a thermocycler, optionally preheated to the denaturation temperature if not using a hot-start polymerase.
Pausing a program right after starting it will pause the protocol after the lid has finished preheating. Pausing the program right after the lid has preheated will hold the block at the step 1 denaturation temp.
4. Analyze PCRs using gel or capillary electrophoresis. As amplicon yields are ideally high, running smaller amounts of DNA than the entire reaction may give better DNA resolution.
Some PCR master mixes contain gel loading dye components and can be loaded directly.

Setup using a 2× PCR master mix

Component	10 µL Rxn	25 µL Rxn	Final Conc
2× PCR master mix	5 µL	12.5 µL	Contains polymerase, dNTPs, and buffer.
Nuclease-free water	5 µL – x	12.5 µL – x	x = primers + template
10 µM forward primer *or 100 µM*	0.1–1 µL *or 0.01-0.1 µL*	0.25–2.5 µL *or 0.025–0.25 µL*	0.1–1 µM each
10 µM reverse primer *or 100 µM*			
Template DNA /cells	variable, 1 µL cells	variable, 2.5 µL cells	10%$_{V/V}$ cell suspension 0.01–1 ng plasmid/viral DNA 100–1000 ng genomic DNA
template copies	Extension	72°C	1 min	1 min for 2 kb; for >2 kb add 1 min/kb. Reduce to 68° when >6 kb.
	Final Extension	72°C	5–15 min	Holding temp is unnecessary and bad for thermocycler (ref).

Troubleshooting

See Q5/Phusion PCR page's *PCR additives* and *Troubleshooting* sections, and first adapt the concepts of ramped or gradient T_{anl} while keeping other thermocycling parameters in accordance with manufacturer recommendations.

Andrew Hirning's protocol

1. Pipette 8 µL of LB into PCR tubes and 5 mL (+ antibiotics) into glass culture tubes.
2. Pick a colony off the agar plate and mix it into the LB in a PCR tube.
3. Transfer 5 µL of inoculated LB from PCR tube to overnight tube. Incubate tubes shaking.
4. Make the master mix (volumes given per reaction):
   17.5 µL water
   2.5 µL *Taq* Buffer
   0.5 µL *Taq* polymerase
   0.5 µL 10 mM dNTP
   0.5 µL 10 µM fwd primer
   0.5 µL 10 µM rev primer
5. Pipette 22 µL of the master mix into PCR tubes containing remaining 3 µL cell suspension (in LB).
6. Thermocycle appropriately and analyze on gel.

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1. Saris, Per EJ, Lars G. Paulin, and Mathias Uhlén. "Direct amplication of DNA from colonies of Bacillus subtilis and Escherichia coli by the polymerase chain reaction." *Journal of Microbiological Methods* 11.2 (1990): 121-126. https://doi.org/10.1016/0167-7012(90)90012-U
2. PCR Protocol for Taq DNA Polymerase | NEB
3. TFS-Assets/LSG/manuals/MAN0012704_DreamTaq_Green_PCR_MasterMix_K1081_UG.pdf (thermofisher.com)