



Lab protocol: bacterial DNA extraction, 16S PCR and gel check

A simple phenol–chloroform DNA extraction, 16S rRNA PCR (27F/1492R) and agarose gel protocol for labs that prepare their own DNA or PCR products before sending them to us.

Use this protocol if you prefer to send extracted DNA or PCR products. If you do not have these facilities, send a pure culture or pellet instead and we will do it for you.

1. Genomic DNA extraction

1. Inoculate a single colony into 2 mL nutrient broth and grow overnight at 37 °C.
2. Transfer 1.5 mL of culture to a microcentrifuge tube and centrifuge at 13,000 rpm for 10 minutes.
3. Add 350 µL lysis buffer and 20 µL proteinase K (20 mg/mL); incubate at 65 °C for 30 minutes.
4. Cool to room temperature, add 3 µL RNase A and incubate at 37 °C for 10 minutes.
5. Add an equal volume (about 370 µL) of phenol:chloroform:isoamyl alcohol (25:24:1) and mix well.
6. Centrifuge at 13,000–14,000 rpm for 10 minutes and transfer the upper (aqueous) layer, about 200 µL, to a fresh tube without touching the interface.
7. Add an equal volume of chloroform:isoamyl alcohol (24:1), mix, and centrifuge again at 13,000–14,000 rpm for 10 minutes.
8. Transfer the upper layer (about 150 µL) to a new tube and add 800 µL ice-cold absolute ethanol.
9. Mix by inverting and keep at –20 °C for 1 hour; centrifuge at 13,000 rpm for 10 minutes.
10. Discard the liquid; add 1 mL chilled 70% ethanol with 20 µL 7.5 M ammonium acetate; keep at –20 °C for 30 minutes.
11. Centrifuge at 13,000 rpm for 10 minutes, remove the liquid and air-dry the pellet.
12. Dissolve the DNA in 50 µL TE buffer (pH 7.6) at 37 °C for 10 minutes.

Lysis buffer (200 mL)

Ingredient	Final concentration	Amount
Tris-HCl, pH 8.0	100 mM	3.02 g
EDTA, pH 8.0	20 mM	1.49 g
NaCl	100 mM	1.17 g
SDS	1% (w/v)	2.0 g
Distilled water		to 200 mL

Dissolve Tris, EDTA and NaCl in about 150 mL water, add SDS slowly to avoid foaming, adjust to pH 8.0 and make up to 200 mL. Sterilise (for example by autoclaving at 121 °C for 15 minutes) and store at room temperature.

2. 16S rRNA PCR (25 µL)

Component	Volume	Note
2× PCR master mix	12.5 µL	Taq, buffer, dNTPs
Primer 27F (10 µM)	0.5 µL	0.2 µM final
Primer 1492R (10 µM)	0.5 µL	0.2 µM final
Template DNA	1.0 µL	About 10–100 ng
Nuclease-free water	10.5 µL	To 25 µL

Always include a no-template control.

Step	Temperature	Time	Cycles
Initial denaturation	94 °C	3 min	1
Denaturation	94 °C	30 s	30
Annealing	55 °C (optimise 50–58 °C)	30 s	
Extension	72 °C	90 s	
Final extension	72 °C	5 min	1
Hold	4 °C	∞	

Expected product: a single band of about 1,500 bp. Too much template can inhibit the reaction; for difficult templates, heat the DNA at 95 °C for 5 minutes and chill on ice before use.

3. Agarose gel check

1. Dissolve 0.5 g agarose in 50 mL 1× TAE or TBE (1% gel) by heating; cool to about 60 °C, add the DNA stain and pour with the comb in place.
2. Mix 5 µL PCR product with 1 µL 6× loading dye; load next to a 1 kb ladder.
3. Run at 90–120 V for 20–30 minutes and view under UV or blue light.

Use 1% agarose for 500–1,500 bp products and 1.5–2% for products under 500 bp. Ethidium bromide is mutagenic: wear gloves, or use a safer stain such as SYBR Safe.

4. Sending to BD Genomes

Send unpurified PCR products at 10–20 ng/µL, 20 µL per reaction, with at least 10 µL of each primer (universal 16S and ITS primers are not needed). PCR products travel safely at room temperature. Sequencing is done by dye-terminator cycle sequencing and capillary electrophoresis at our partner laboratory (Sanger et al., 1977).

Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977;74(12):5463–5467.

Questions before sending samples? WhatsApp / phone +880 1308-222824 · bdgenome@yahoo.com · bdgenomes.com/guides/bacterial-dna-protocol/