



Preparing bacterial cultures for DNA extraction and PCR

How to grow, check and harvest a pure bacterial culture so the DNA is clean and the PCR or sequencing works first time.

Good culture preparation is the first step to clean DNA and successful PCR, Sanger sequencing or whole genome sequencing.

Goal	Why it matters
A pure, healthy culture	Prevents mixed templates and background noise
Harvest in late-log phase	High cell density gives enough DNA
Avoid overgrowth	Old cultures contain degraded DNA and inhibitors
Aseptic technique	Contamination can ruin PCR and sequencing

Step 1. Start from a single colony

- Streak the strain on a suitable agar plate (for example LB or nutrient agar) and incubate overnight at the right temperature (usually 37 °C).
- Pick one well-isolated colony with a sterile loop or tip. Do not pick from areas where colonies touch.

Step 2. Grow a liquid culture

- Medium: LB, TSB or another medium suitable for the species; 3–10 mL in a sterile tube or flask.
- 30–37 °C, shaking at 180–220 rpm, for 12–18 hours (overnight). Slow growers such as *Bacillus* or *Staphylococcus* may need longer.

Step 3. Check growth

OD600	Growth phase	Good for DNA?
0.3–0.6	Early log	No
0.6–1.0	Late log	Yes
Above 1.5	Stationary	No: more dead cells and degraded DNA

Step 4. Harvest the cells

1. Transfer 1.5–2 mL of culture to a microcentrifuge tube.
2. Spin at 6,000–10,000 × g for 5–10 minutes and remove all the supernatant.
3. Optional: wash the pellet once with 1× PBS or TE to remove medium.

Step 5. Extract DNA or send the pellet

Use the pellet for DNA extraction, or send it to us as agreed in your quote. For a quick colony PCR, use a fresh colony or a boiled lysate.

Common problems

Problem	Cause	Solution
Other bacteria present	Mixed colony or unsterile tools	Single colonies, aseptic technique
Low DNA yield	Weak growth or early harvest	Longer incubation or larger volume
Degraded DNA	Overgrown culture, poor storage	Harvest in log phase; do not leave cultures at room temperature
PCR inhibition	Medium carry-over (salts, proteins, antibiotics)	Wash the pellet; dilute crude lysate

Checklist

- ✓ Single colony from a fresh plate
- ✓ Grown in sterile broth under the right conditions
- ✓ Harvested at OD600 about 0.6–1.0
- ✓ Pellet collected (and washed if needed)
- ✓ Tube labelled with your sample ID

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