



Bacterial DNA for whole genome sequencing

How to grow, harvest and extract high-molecular-weight bacterial DNA for Illumina or long-read whole genome sequencing, with quality targets and troubleshooting.

Whole genome sequencing needs pure, intact, high-molecular-weight DNA free from RNA, protein and inhibitors. This matters for short reads (Illumina) and even more for long reads (Nanopore, PacBio).

1. Grow the culture

- Inoculate 5–10 mL broth (LB, TSB or another suitable medium) with one fresh, single colony.
- Incubate at the right temperature (for example 37 °C) with shaking at 200 rpm for 12–18 hours, to mid- or late-log phase (OD600 about 0.6–1.0). Avoid overgrowth.

2. Harvest the cells

- Centrifuge at 6,000–10,000 × g for 5–10 minutes and discard the supernatant.
- Recommended: resuspend in 1× PBS or TE, spin again and discard, to remove medium that may contain inhibitors.

3. Extract the DNA

Platform	DNA needed	Suggested method
Illumina (short reads)	Clean, moderate size	Silica column kits (for example Qiagen, Promega)
Nanopore / PacBio (long reads)	Very pure, above 30 kb	Phenol–chloroform or HMW kits (MagAttract HMW, Nanobind)

1. Gram-positive bacteria: lysozyme (20 mg/mL) at 37 °C for 30–60 minutes.
2. Proteinase K + SDS to lyse cells and digest proteins; add RNase A.
3. Purify by phenol–chloroform or magnetic beads.
4. Ethanol-precipitate to concentrate if needed. Do not vortex; pipette gently.

4. Check quality before sending

Check	Tool	Target
Concentration	Qubit (dsDNA assay)	At least 50 ng/μL and 1 μg in total
Purity	NanoDrop	A260/280 1.8–2.0; A260/230 at least 2.0
Integrity	0.8% agarose gel or TapeStation	One high-molecular-weight band, little smearing

Troubleshooting

Problem	Cause	Solution
Degraded DNA	Harsh pipetting, old culture, nucleases	No vortexing, fresh culture, work on ice
Low yield	Poor lysis, little biomass	More culture; lysozyme for Gram-positives
RNA contamination	No RNase step	Add RNase A
Protein or phenol carry-over	Incomplete clean-up	Extra ethanol wash or column clean-up
Inhibitors	Medium carry-over, polysaccharides	Wash the pellet; bead clean-up

You can also send a pure culture or cell pellet and we will extract the DNA. A contaminated culture gives a contaminated genome, so always start from a single, verified colony.

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