



Preparing RNA and cDNA for RNA-Seq

How to collect, preserve and extract intact, DNA-free RNA from cells and tissues, make cDNA, and meet the quality targets for RNA-Seq.

RNA breaks down quickly. Intact, clean RNA is essential for good cDNA and reliable transcriptome data.

Goal	Why
Intact RNA	Accurate, complete expression profiles
No DNA, protein or phenol	These inhibit reverse transcription and sequencing
RNase-free handling	Prevents degradation

1. Collect and preserve

- Use fresh samples, or snap-freeze in liquid nitrogen straight after harvest and store at -80°C .
- Or place tissue or cells in RNAlater (small pieces, fully covered).
- RNases are everywhere: use RNase-free tubes and tips, wear gloves and clean surfaces with an RNase decontaminant (for example RNaseZAP).

2. Lyse the cells

- **Bacteria and fungi:** lysozyme (Gram-positives) or bead-beating, in a guanidine-thiocyanate lysis buffer with β -mercaptoethanol.
- **Animal cells and tissues:** TRIzol or a kit lysis buffer; homogenise thoroughly (homogeniser, or mortar and pestle with liquid nitrogen).

3. Extract the RNA

Method	Pros	Cons
TRIzol (phenol–chloroform)	High yield	Toxic; risk of phenol carry-over
Spin columns (for example RNeasy, Zymo)	Clean and safe	Slightly lower yield; cost
Magnetic beads	Scalable	Set-up and cost

4. Remove DNA (essential)

Treat with RNase-free DNase I, on the column or in solution, then clean up again. Leftover genomic DNA distorts results.

5. Check quality

Check	Tool	Target
Purity	NanoDrop	A260/280 1.9–2.1; A260/230 above 2.0
Concentration	Qubit RNA assay	Enough for the planned work
Integrity	Bioanalyzer / TapeStation	RIN 7 or higher
Integrity (gel)	Denaturing agarose gel	Clear 28S and 18S bands, about 2:1

6. Make cDNA (if you send cDNA)

- For RNA-Seq we accept cDNA made with an **oligo(dT) primer**: at least 20 ng/μL and more than 30 μL per sample.
- Label tubes SampleID_Date, store at –80 °C and ship on ice.

7. Store

Keep RNA at –80 °C in RNase-free water or TE, in small aliquots to avoid freeze–thaw.

Troubleshooting

Problem	Cause	Solution
Degraded RNA	RNases, poor storage	Work on ice and quickly; RNase inhibitors
DNA contamination	Incomplete DNase step	Longer DNase treatment
Low yield	Poor lysis or elution	Better homogenisation; fresh reagents
Phenol carry-over	Incomplete TRIzol clean-up	Repeat chloroform step or column clean-up
Low RIN	Heat, repeated freeze–thaw	Keep on ice; aliquot immediately

Not sure you can extract RNA reliably? Send tissue in RNAlater instead and we will do the extraction and cDNA synthesis.

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