

Reagent Handling

Still unsure after reading this and the 10x protocol? Ask us! We're here to help.

Template Switch Oligo B

If no liquid is present and only a dried pellet is visible, resuspend according to the current 10x protocol. If liquid is already present, it has been resuspended by a previous user—**do not resuspend again**. If no pellet or liquid can be seen discard and resuspend a new tube.

DynaBeads

Located in the **cold room**. Vortex thoroughly before use to ensure the beads are fully resuspended.

Cleanup Buffer

Thaw using the **thermomixer, not a water bath: 65°C, 2,000 rpm for 10 minutes**. Tape down the tube before shaking.

Cleanup Reagents

Buffer EB, 10% Tween, 80% ethanol, and SPRIselect Beads are located at the **customer bench** and should be kept at **ambient temperature**.

Library Amp Mix

Use **only for the Sample Index PCR step**. Do not use for the cDNA amplification step.

Protocol FAQ

PART 1 — Cell Loading & RT Incubation

Gasket and Chip

Gaskets and chips are **single-use only**. Use a new gasket and chip for each run.

10x Labels/Identifiers

Before starting, record all 10x labels/identifiers from the **chip and Gel Bead cartridge**. Keep these records with your run information.

This information may be needed for troubleshooting, including in the event of a clog.

Cell Concentration and Target Recovery

10x Genomics recommends a cell concentration of **1,300–1,600 cells/μL** for loading (see 10x protocol, page 34).

Our core generally recommends targeting a **maximum cell recovery of 20,000 cells**. A lower cell recovery means fewer cells share the sequencing reads, which may result in greater sequencing depth per cell if the same number of reads is generated.

If you need suggestions for your specific experiment, **consult Kristen before starting**.

Chip Loading

Pipette **slowly and carefully** to avoid introducing bubbles.

GEM Transfer

When transferring GEMs, take a **picture of the emulsion while it is inside the multichannel pipette tips**. Keep the picture with your run records for troubleshooting if needed.

PART 2 — cDNA Cleanup

DynaBeads Cleanup Mix

After adding the DynaBeads Cleanup Mix, the strip tube will be **very close to full**. Take extra care to avoid **overflowing or cross-contaminating adjacent tubes**.

Do not cap the tubes while they are full.

Preserve Removed Supernatant

Save **all supernatant removed during the cleanup**. Keep the supernatant until **cDNA QC results have been received**.

Supernatant Removal — Step 2.1 H

Remove **all supernatant from the tube, including the white interface layer**.

PART 3 — Sample Index PCR

PCR Cycle Number

The **PCR cycle number is determined based on the cDNA TapeStation QC results**.

Use the **Region Table** from the TapeStation results we sent you to obtain the cDNA concentration in **pg/μL**.

The cDNA input volume is always a quarter (**10 μL**).

cDNA Input Calculation

We do not dilute cDNA for tapestation unless specified otherwise.

Convert the concentration to ng/μL:

$$\text{pg/}\mu\text{L} \div 1,000 = \text{ng/}\mu\text{L}$$

Then calculate the total cDNA input:

$$\text{ng/}\mu\text{L} \times 10 \mu\text{L} = \text{cDNA input (ng)}$$

Use the calculated **cDNA input amount** to determine the appropriate Sample Index PCR cycle number according to the 10x protocol.