Cell Lysis for RNA Preparation from Single Cells v1201

Samples of a project, i.e. samples that should be compared to each other must contain similar (identical) amounts of RNA, which usually means similar (identical) numbers of cells.

Recommendation for cells purified by cell sorting:

We highly recommend sorting 1 to 10 cells into a drop of PBS and checking under the microscope that the sorted events truly are cells.

Check upfront the volume of liquid that will be added to the lysis buffer; this is dependent on the nozzle size and other parameters. If you plan to add between 5 and 10 µl, use 90 µl lysis buffer. For volumes <5 µl follow the description below.

Thaw lysis buffer at room temperature; once the last piece of ice is thawed, vortex shortly and store on ice. Prepare aliquots of 45 µl lysis buffer in 2 ml Eppendorf tubes according to the number of samples to be processed (16 samples maximum per batch). Freeze aliquots not needed on the same day.

Add cells in a maximum volume of 5 µl to the lysis buffer aliquots, flick twice with your fingers (do not vortex or flick too often) and bring to 65°C for 15 minutes. Bring cells as quickly as possible to 65°C after isolation.

Processing of samples after heat inactivation at 60°C

1 to 100 cells: Cell lysates have to be processed immediately (within 1 to 2 hours after lysis). Bring the lysates immediately at room temperature to IRB´s Functional Genomics Core. Samples must arrive before 10:00 AM.

Over 100 cells: Cells lysates can be stored overnight at 4°C. Transport them on wet ice to IRB´s Functional Genomics Core. Do not freeze!

Over 1,000 cells: Cells lysates can be frozen. Transport them on dry ice to IRB´s Functional Genomics Core.

Lysis buffer:

20 mM DTT, 10 mM Tris.HCl pH 7.4, 0.5% SDS, 0.5µg/µl proteinase K

Proteinase K digests itself; therefore, keep lysis buffer as shortly as possible at elevated temperature.