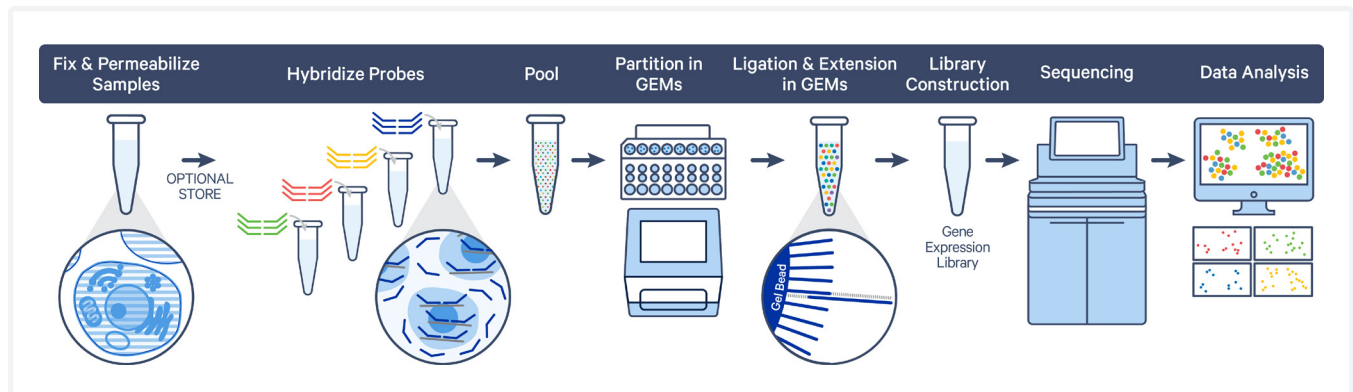


# Chromium Fixed RNA Profiling – Protocol Planner

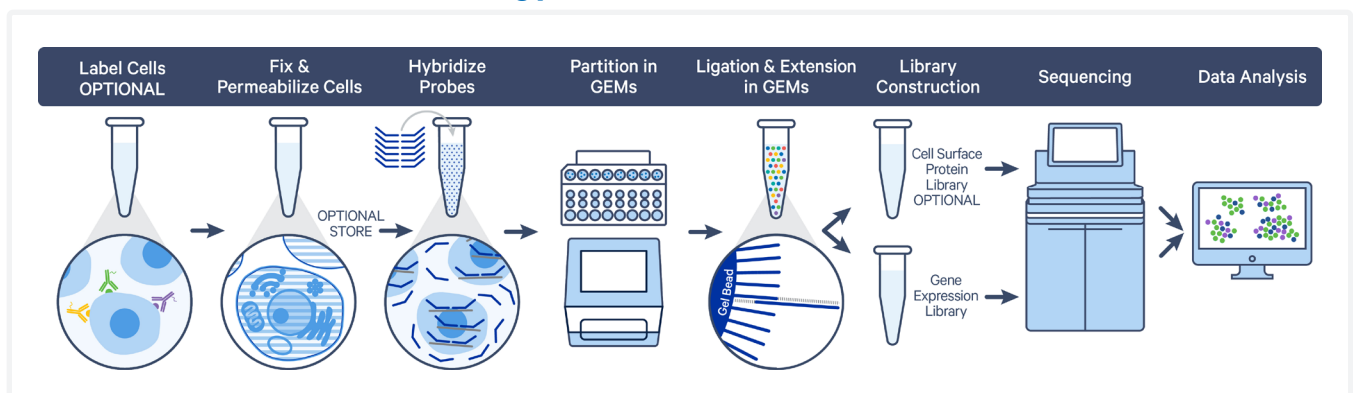
## Introduction

Chromium Fixed RNA Profiling offers comprehensive scalable solutions to measure gene expression in single cell and nuclei suspensions that are fixed with formaldehyde. This document provides workflow overview and additional equipment, kits, and reagents for the Chromium Fixed RNA Profiling protocols. The 10x Genomics Chromium Fixed RNA Profiling Reagent Kits are not listed in this document. An overview of the Chromium Fixed RNA Profiling data analysis is also included in this document.

## Workflow Overview – Chromium Fixed RNA Profiling for Multiplexed Samples



## Workflow Overview – Chromium Fixed RNA Profiling for Singleplexed Samples with Feature Barcode technology for Cell Surface Protein



## Document Resources – Chromium Fixed RNA Profiling for Multiplexed Samples

### Sample Fixation

Fix single cell and nuclei suspensions.

Demonstrated Protocol CG000478



### Library Construction

Construct Fixed RNA – Gene Expression libraries.

User Guide CG000527

## Document Resources – Chromium Fixed RNA Profiling for Singleplexed Samples with Feature Barcode technology for Cell Surface Protein

### Optional Cell Surface Protein Labeling

Label samples with antibody-oligonucleotide conjugates.

Demonstrated Protocol CG000529



### Sample Fixation

Fix single cell and nuclei suspensions.

Demonstrated Protocol CG000478



### Library Construction

Construct Fixed RNA – Gene Expression & Cell Surface Protein libraries.

User Guide CG000477

## Kits, Reagents & Equipment – Sample Preparation

The items in the tables below have been validated by 10x Genomics and are highly recommended for the Chromium Fixed RNA Profiling. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc.

### For Optional Cell Surface Protein Labeling for Chromium Fixed RNA Profiling for Singleplexed Samples with Feature Barcode technology (CG000529)

| Vendor                   | Item  | Part Number |
|--------------------------|---|-------------|
| BioLegend                | Human TruStain FcX (Fc Receptor Blocking Solution)                                | 422301      |
|                          | True-Stain Monocyte Blocker   | 426101      |
|                          | TotalSeq™-B Antibody-Oligonucleotide Conjugates                                   | -           |
|                          | Cell Staining Buffer  | 420201      |
| Thermo Fisher Scientific | UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)                                    | AM2616      |
|                          | Trypan Blue Stain (0.4%)  | T10282      |
|                          | Fetal Bovine Serum, qualified, heat inactivated                                   | 16140071    |
| Millipore Sigma          | Bovine Serum Albumin In DPBS (10%)<br><i>Alternative to Thermo Fisher product</i> | A1595       |
| Miltenyi Biotec          | MACS BSA Stock Solution<br><i>Alternative to Thermo Fisher product</i>            | 130-091-376 |
| Corning                  | Phosphate-Buffered Saline, 1X without Calcium and Magnesium                       | 21-040-CV   |
| VWR                      | Fetal Bovine Serum (FBS)<br><i>Alternative to Thermo Fisher product</i>           | 97068-085   |
| <b>Equipment</b>         |   |             |
| Thermo Fisher Scientific | Countess II FL Automated Cell Counter   | AMQAF1000   |
|                          | Countess II FL Automated Cell Counting Chamber Slides                             | C10228      |

*This list may not include some standard laboratory equipment.*

**For Cells & Nuclei Fixation (CG000478)**

| Vendor   | Item  | Part Number   |            |
|--|---|---|------------|
| <b>For Cell Thaw &amp; Sample Fixation</b>       |   |   |            |
| 10x Genomics                                     | Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit  | 1000414   |            |
| Millipore Sigma                                  | Bovine Serum Albumin In DPBS (10%)<br><i>Alternative to Thermo Fisher product</i>                                       | A1595   |            |
| Thermo Fisher Scientific                         | UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)  | AM2616  |            |
|  | Formaldehyde (37% by Weight/Molecular Biology), Fisher BioReagents  | BP531-25  |            |
|  | Nuclease-free Water (not DEPC-Treated)  | AM9937  |            |
| Miltenyi Biotec                                  | MACS BSA Stock Solution<br><i>Alternative to Thermo Fisher product</i>  | 130-091-376   |            |
| Corning  | Phosphate-Buffered Saline, 1X <i>without Calcium and Magnesium</i>  | 21-040-CV   |            |
|  | *Corning RPMI 1640 1X with L-Glutamine  | 10-040-CM   |            |
| VWR  | *Seradigm Premium Grade Fetal Bovine Serum (FBS)  | 97068-085   |            |
|  | <i>*Only needed for cell thawing.</i>   |   |            |
| <b>For Sample Filtration</b>                     |   |   |            |
| Sysmex   | Sterile Single-Pack CellTrics Filters   | 04-004-2326   |            |
| Miltenyi Biotec                                  | Pre-Separation Filters (30 µm)  | 130-041-407   |            |
|  | <i>Choose either Sysmex or Miltenyi Biotec filter.</i>  |   |            |
| <b>For Cell Counting</b>                         |   |   |            |
| Nexcelom Biosciences                             | ViaStain AOPI Staining Solution   | CS2-0106-5mL  |            |
|  | †Cellaca MX High-throughput Automated Cell Counter  | MX-112-0127   |            |
| Thermo Fisher Scientific                         | †Countess II FL Automated Cell Counter  | Choose Ethidium Homodimer-1, Trypan Blue, or AO/PI Staining Solution based on the presence of debris in the sample. | AMAQAF1000 |
|  | Countess II FL Automated Cell Counting Chamber Slides   |   | C10228     |
|  | Trypan Blue Stain (0.4%)  |   | T10282     |
|  | Ethidium Homodimer-1  |   | E1169      |
|  | †Choose either Countess or Cellaca.   |   |            |
| <b>For Storage &amp; Post-Storage Processing</b> |   |   |            |
| Acros Organics                                   | Glycerol, 99.5%, for molecular biology, DNase, RNase and Protease free<br><i>Alternative to Millipore Sigma product</i> | 327255000   |            |
| Millipore Sigma                                  | Glycerol for molecular biology, ≥99.0% <i>Alternative to Acros Organics product</i>                                     | G5516-100ML   |            |
|  | Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free  | 126615  |            |
|  | Protector RNase Inhibitor   | 3335402001  |            |
| <b>Additional Materials</b>                      |   |   |            |
| Eppendorf  | DNA LoBind Tubes 2.0 ml   | 022431048   |            |
|  | ThermoMixer C   | 5382000023  |            |

*This list may not include some standard laboratory equipment.*

## Kits, Reagents & Equipment – Library Construction (CG000477 & CG000527)

The items in the table below have been validated by 10x Genomics and are highly recommended for the Chromium Fixed RNA Profiling. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment such as water baths, centrifuges, vortex mixers, pH meters, freezers etc

| Supplier                   | Description   | Part Number   |             |
|----------------------------|---|---|-------------|
| <b>Plastics</b>            |   |   |             |
| Eppendorf                  | DNA LoBind Tubes, 1.5 ml  | 022431021   |             |
|                            | DNA LoBind Tubes, 5.0 ml  | 0030108310  |             |
|                            | PCR Tubes 0.2 ml 8-tube strips  | Choose either Eppendorf, USA Scientific or Thermo Fisher Scientific PCR 8-tube strips.                              |             |
| USA Scientific             | TempAssure PCR 8-tube strip   |   | 1402-4700   |
| Thermo Fisher Scientific   | MicroAmp 8-Tube Strip, 0.2 ml   | N8010580  |             |
|                            | MicroAmp 8-Cap Strip, clear   | N8010535  |             |
| Corning                    | Corning Centrifuge Tubes with CentriStar Cap (15 ml), sterile   | 430790  |             |
|                            | Self-Standing Polypropylene Centrifuge Tubes (50 ml), sterile   | 430921  |             |
| Sysmex                     | Sterile single-pack CellTrics filters   | Choose either Sysmex or Miltenyi Biotec filter.   |             |
| Miltenyi Biotec            | Pre-Separation Filters (30 µm) <i>Alternative to Sysmex product</i>   |   | 130-041-407 |
| <b>Kits &amp; Reagents</b> |   |   |             |
| Thermo Fisher Scientific   | Nuclease-free Water   | AM9937  |             |
|                            | Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)  | 12090-015   |             |
|                            | Tween 20 Surfact-Amps Detergent Solution (10% solution)   | 28320   |             |
| Beckman Coulter            | SPRIselect Reagent Kit  | B23318  |             |
| Millipore Sigma            | Ethyl Alcohol, Pure (200 Proof, anhydrous)  | E7023-500ML   |             |
|                            | Glycerol for Molecular biology, ≥99.0%  | G5516   |             |
| Acros Organics             | Glycerol, 99.5%, for molecular biology, DNase, RNase and Protease free<br><i>Alternative to Millipore Sigma product</i> | 327255000   |             |
| Qiagen                     | Qiagen Buffer EB  | 19086   |             |
| Ricca Chemical Company     | Glycerin (glycerol), 50% (v/v) Aqueous Solution   | 3290-32   |             |
| <b>Cell Counting</b>       |   |   |             |
| Thermo Fisher Scientific   | Ethidium Homodimer-1  | E1169   |             |
|                            | Trypan Blue Stain (0.4%)  | Choose Ethidium Homodimer-1, Trypan Blue, or AO/PI Staining Solution based on the presence of debris in the sample. |             |
|                            | Countess II FL Automated Cell Counter   |   | T10282      |
|                            | Countess II FL Automated Cell Counting Chamber Slides   |   | AMAQAF1000  |
|                            | C10228  |   |             |
| Nexcelom Biosciences       | Cellaca MX High-throughput Automated Cell Counter   | MX-112-0127   |             |
|                            | ViaStain AO/PI Staining Solution  | CS2-0106-5mL  |             |

| Equipment                        |  |  |                         |
|----------------------------------|--|--|-------------------------|
| VWR                              | VWR Mini Centrifuge <i>Or any equivalent mini centrifuge</i>     | 76269-064  |                         |
|                                  | Divided Polystyrene Reservoirs                                   | 41428-958  |                         |
| Eppendorf                        | Eppendorf ThermoMixer C  | 5382000023   |                         |
|                                  | Eppendorf ThermoTop with condens.protect technology              | 5308000003   |                         |
|                                  | Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessels | 5360000038   |                         |
|                                  | Eppendorf SmartBlock 2.0 ml, thermoblock for 24 reaction vessels | 5362000035   |                         |
| Quantification & Quality Control |  |  |                         |
| Agilent                          | 2100 Bioanalyzer Laptop Bundle                                   | G2953CA  |                         |
|                                  | High Sensitivity DNA Kit   | Choose Bioanalyzer, TapeStation or LabChip based on availability & preference. | 5067-4626               |
|                                  | 4200 TapeStation   |  | G2991AA                 |
|                                  | High Sensitivity D5000: ScreenTape/ Reagents                     |  | 5067-5592/<br>5067-5593 |
|                                  | High Sensitivity D1000: ScreenTape/ Reagents                     |  | 5067-5584/<br>5067-5585 |
| PerkinElmer                      | LabChip GX Touch HT Nucleic Acid Analyzer                        |  | CLS137031               |
|                                  | DNA High Sensitivity Reagent Kit                                 | CLS760672  |                         |
| KAPA Biosystems                  | KAPA Library Quantification Kit for Illumina Platforms           | KK4824   |                         |

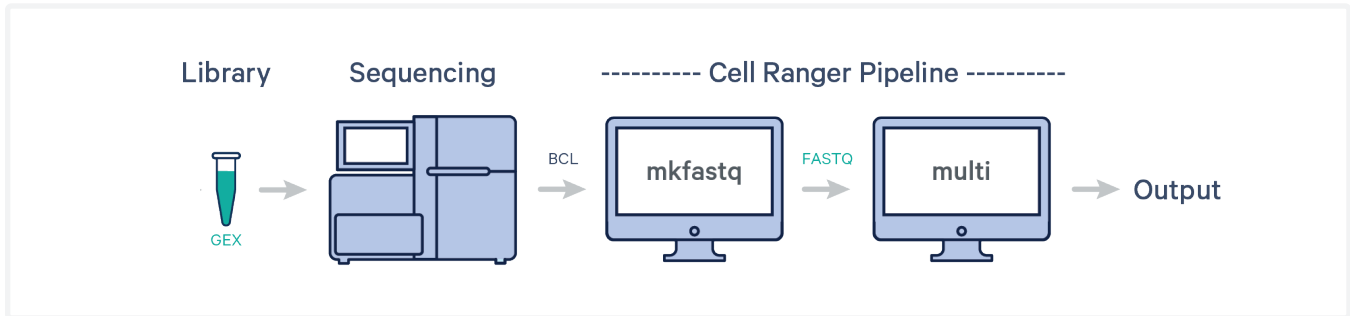
## Recommended Thermal Cyclers

| Supplier      | Description  | Part Number                 |
|---------------|--|-----------------------------|
| BioRad        | C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module                       | 1851197                     |
| Eppendorf     | Mastercycler X50s  | North America<br>6311000010 |
| Analytik Jena | Biometra TAdvanced 96 SG with 96-well block (silver, 0.2 mL) and gradient function | 846-x-070-241               |

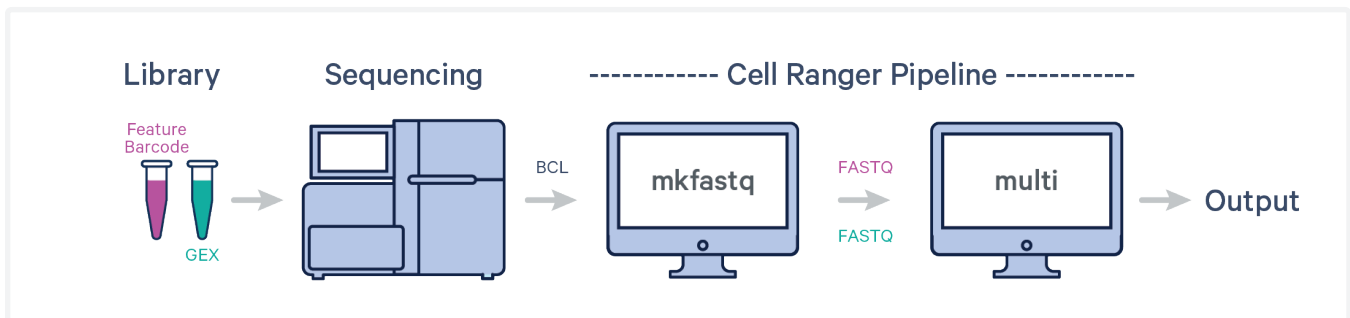
## Analysis of Chromium Fixed RNA Profiling Data

For detailed information on analysis of Chromium Fixed RNA Profiling data, refer to 10x Genomics Software Support site.

### Data Analysis Overview – Chromium Fixed RNA Profiling for Multiplexed Samples



### Data Analysis Overview – Chromium Fixed RNA Profiling for Singleplexed Samples with Feature Barcode technology for Cell Surface Protein



Key information that should be collected and provided to the data analyst/bioinformatician to ensure that parameters are set appropriately for Cell Ranger to analyze the data:

- Target number of cells to be recovered per sample
- The Probe Barcodes used for each sample in a multiplexing experiment
- Sample and library name to be appended to the FASTQ file name and the corresponding library type
- The sample indices used for each Chromium Fixed RNA Profiling – Gene Expression or Cell Surface Protein library.

## Document Revision Summary

|                        |   |
|------------------------|---|
| <b>Document Number</b> | CG000528  |
| <b>Title</b>           | Chromium Fixed RNA Profiling – Protocol Planner |
| <b>Revision</b>        | Rev A   |
| <b>Revision Date</b>   | May 2022  |

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