Thermal Cycler Protocol Download Guide for 10x Genomics Workflows

Introduction

10x Genomics workflows require compatible thermal cyclers to be programmed to execute various steps. This Technical Note provides guidance for downloading protocols that can be used to program Bio-Rad C1000 and Eppendorf Mastercycler X50s thermal cyclers. These protocols are available for direct download from the 10x Genomics Support website.

Bio-Rad and Eppendorf were not involved in the development of these protocols. This document is not an endorsement of these instruments.

All thermal cycler protocols were manually programmed according to their respective 10x Genomics product-specific User Guides, resulting in instrument-generated protocol files. These files can be loaded onto their respective thermal cyclers allowing for execution of each protocol without the need to manually enter individual steps. Ramp rates for the provided Eppendorf Mastercycler X50s protocols have been adjusted to 3°C/sec heating and 2°C/sec cooling, as required in the 10x Genomics product-specific User Guides.

Use a PC with Windows OS to download the desired zip files. Unzip and move extracted files to a FAT32-formatted USB drive, then upload and save to the instrument.

⚠️ *For Eppendorf Mastercycler X50s file transfer, move the folder named "Eppendorf" directly to the USB drive for upload. Eppendorf X50s instrument files must be in a top-level folder named "Eppendorf" to be recognized by the instrument for upload.*

Protocol files for Chromium workflows are listed in Tables 2-6, protocol files for Visium workflows are listed in Tables 7-11, and protocol files for Xenium workflows are listed in Table 12. Protocol files for discontinued products can be found in the Appendix. Some thermal cycler files may be used for multiple protocol steps.

Table 1: Understanding file names

Refer to the following table to understand protocol naming and when a file may be used for multiple protocol steps.

Example file name: 3'v3.1_SI-PCR_54C_12X	
3'v3.1	Product
SI-PCR = Sample Index PCR	Protocol step
54C = Annealing temperature of 54°C	Key Feature
12X = 12 total cycles*	Cycle number/time**

* Provided thermal cycler protocols are programmed with the final number of total cycles as stated in the file name. Instruments may handle repeated steps differently. Users should familiarize themselves with instrument software and refer to instrument User Guides for guidance on programming total cycle number.

** Refer to the product-specific User Guide for guidance on appropriate cycle number/time.

Before running a downloaded protocol, refer to the most current version of the product-specific User Guide to confirm the protocol steps and to modify the number of cycles or incubation time as required. Cycle number and incubation time may require adjustment depending on library, cell or tissue type, cell loading, and/or input mass. Edit and save the updated protocol with a new name to prevent confusion.

Table 2: Chromium Next GEM Single Cell Gene Expression 3' v3.1

Dual Index
Throughput: Standard, High
Product Extensions: Cell Surface Protein, Cell Multiplexing, CRISPR Screening

Protocol Step(s)	Protocol File Name	Variable Cycle Number
GEM-RT Incubation	3'v3.1_GEM-RT	
cDNA Amplification	3'v3.1_cDNA_Amp_63C_12X	Yes, refer to User Guide for cycle number
Fragmentation, End Repair, and A-tailing	3'v3.1_FRAG_ER-AT	
Adaptor Ligation	3'v3.1_Ad_Ligation	
Sample Index PCR for: • Gene Expression • Cell Surface Protein • Cell Multiplexing • CRISPR Guide RNA	3'v3.1_SI-PCR_54C_12X	Yes, refer to User Guide for cycle number
CRISPR Guide RNA: Feature PCR	3'v3.1_Feat_CRISPR-PCR_58C_dual	

Table 3: Chromium Next GEM Single Cell Immune Profiling v2

Throughput: Standard, High
Product Extensions: Cell Surface Protein, Immune Repertoire Profiling, Barcode Enabled Antigen Mapping, and CRISPR Screening

Protocol Step(s)	Protocol File Name	Variable Cycle Number
GEM-RT Incubation	5'v2_GEM-RT	
cDNA Amplification	5'v2_cDNA_Amp_63C_13X	Yes, refer to User Guide for cycle number
Enriched Library Construction: V(D)J Amplification 1 V(D)J Amplification 2	5'v2_VDJ_Amp_62C_12X	Yes, refer to User Guide for cycle number
V(D)J Library Construction: Fragmentation, End Repair, and A-tailing	5'v2_Frag_ER-AT_2min	
Gene Expression (GEX) Library Construction: Fragmentation, End Repair, and A-tailing	5'v2_Frag_ER-AT_5min	
Adaptor Ligation: V(D)J Library Construction Gene Expression (GEX) Library Construction	5'v2_Ad_Ligation	
Sample Index PCR for: • Gene Expression • V(D)J Library Construction • Cell Surface Protein • Barcode Enabled Antigen Mapping	5'v2_SI-PCR_54C_16X	Yes, refer to User Guide for cycle number
CRISPR Guide RNA Library Construction: • Feature PCR • Sample Index PCR	5'v2_Feat_CRISPR_PCR_62C	

Table 4: Chromium Next GEM Single Cell ATAC v2

Protocol Step(s)	Protocol File Name	Variable Cycle Number
Isothermal Incubation	ATACv2_Isothermal_Inc_30min	
GEM Incubation	ATACv2_GEM_Inc	
Sample Index PCR	ATACv2_SI-PCR_67C_9X	Yes, refer to User Guide for cycle number

Table 5: Chromium Next GEM Single Cell Multiome ATAC + Gene Expression

Protocol Step(s)	Protocol File Name	Variable Cycle Number
Isothermal Incubation	Multiome_Isothermal_Inc	
GEM Incubation	Multiome_GEM_Inc	
Pre-Amplification PCR	Multiome_PreAmp_PCR_63C	
ATAC: Sample Index PCR	Multiome_ATAC_SI-PCR_67C_9X	Yes, refer to User Guide for cycle number
Gene Expression: cDNA Amplification	Multiome_cDNA_Amp_9X	Yes, refer to User Guide for cycle number
Gene Expression: Fragmentation, End Repair, and A-tailing	Multiome_FRAG_ER-AT	
Gene Expression: Adaptor Ligation	Multiome_Ligation	
Gene Expression: Sample Index PCR	Multiome_GEX_SI-PCR_54C_14X	Yes, refer to User Guide for cycle number

Table 6: Chromium Fixed RNA Profiling

Singleplex, Multiplex
Extensions: Cell Surface Protein

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Probe Hybridization	Fixed_RNA_Probe_Hyb	Yes, refer to User Guide for incubation time
GEM Incubation	Fixed_RNA_GEM_Inc	
Pre-Amplification PCR	Fixed_RNA_PreAmp_PCR_63C	
Sample Index PCR for: • Gene Expression • Cell Surface Protein	Fixed_RNA_SI-PCR_54C_12X	Yes, refer to User Guide for cycle number

Table 7: Visium Spatial Tissue Optimization (Fresh Frozen)

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Tissue Permeabilization	Visium_TO_Perm	Yes, refer to User Guide for incubation time
Fluorescent cDNA synthesis	Visium_TO_cDNA_synth	
Tissue Removal	Visium_TO_Tissue_removal	

Table 8: Visium Spatial Gene Expression (Fresh Frozen)

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Tissue Permeabilization	Visium_FF_Perm	Yes, refer to User Guide for incubation time
Reverse Transcription	Visium_FF_RT	
Second Strand Synthesis	Visium_FF_SS_synth	
cDNA Amplification	Visium_FF_cDNA_Amp_63C_12X	Yes, refer to User Guide for cycle number
Fragmentation, End Repair, and A-tailing	Visium_FF_Frag_ER-AT	
Adaptor Ligation	Visium_FF_Ad_Lig	
Sample Index PCR	Visium_FF_SI-PCR_67C_10X	Yes, refer to User Guide for cycle number

Table 9: Visium Spatial Gene Expression (FFPE)

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Probe Hybridization	Visium_FFPE_Hyb_16hr	Yes, refer to User Guide for incubation time
Probe Ligation	Visium_FFPE_Lig	
Post Ligation Wash	Visium_FFPE_Post_Lig_Wash	
RNA Digestion and Probe Release	Visium_FFPE_RNA_Digest_Probe_Release	
Probe Extension	Visium_FFPE_Ext	
Sample Index PCR	Visium_FFPE_SI-PCR_63C_19X	Yes, refer to User Guide for cycle number

Visium Protocols

Table 10: Visium CytAssist Spatial Gene Expression (FFPE, Fresh Frozen, Fixed Frozen)

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Decrosslinking: • FFPE Tissue	CytAssist_FFPE_Decrosslinking	
Decrosslinking: • Fixed Frozen (FxF) Tissue	CytAssist_FxF_Decrosslinking	
H&E Destaining	CytAssist_Destaining	
Probe Hybridization	CytAssist_Hyb_16hr	Yes, refer to User Guide for incubation time
Probe Ligation	CytAssist_Ligation	
Post Ligation Wash	CytAssist_Post_Lig_Wash	
Probe Extension	CytAssist_Extension	
Pre-Amplification	CytAssist_PreAmp	
Sample Index PCR	CytAssist_SI-PCR_63C_14X	Yes, refer to User Guide for cycle number

Table 11: Visium CytAssist Spatial Gene and Protein Expression (FFPE)

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Decrosslinking	CytAssist_FFPE_Decrosslinking	
H&E Destaining	CytAssist_Destaining	
Antibody Incubation	CytAssist_Antibody_Inc_16hr	Yes, refer to User Guide for incubation time
DNAse Treatment	CytAssist_DNAse_Treatment	
DNAse Inactivation	CytAssist_DNAse_Inactivation	
Probe Hybridization	CytAssist_Hyb_16hr	Yes, refer to User Guide for incubation time
Probe Ligation	CytAssist_Ligation	
Post Ligation Wash	CytAssist_Post_Lig_Wash	
Probe Extension	CytAssist_Extension	
Pre-Amplification	CytAssist_PreAmp	
Sample Index PCR	CytAssist_SI-PCR_63C_14X	Yes, refer to User Guide for cycle number

Visium Protocols

Table 12: Xenium In Situ Gene Expression (Fresh Frozen and FFPE)

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Xenium In Situ for FFPE only: • Decrosslinking	Xenium_FFPE_Decrosslink	
Probe Hybridization	Xenium_Probe_Hyb_16hr	Yes, refer to User Guide for incubation time
Post Hybridization Wash	Xenium_Post_Hyb_Wash	
Ligation	Xenium_Ligation	
Amplification	Xenium_Amplification	
Autofluorescence Quenching	Xenium_Quench	

Xenium Protocols

Appendix

Discontinued Products

Table 1: Chromium Next GEM Single Cell Gene Expression 3' v3.1 *(discontinued)*

Single Index
Throughput: Standard, Low, High
Product Extensions: Cell Surface Protein, Cell Multiplexing, CRISPR Screening

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
GEM-RT Incubation	3'v3.1_GEM-RT	
cDNA Amplification	3'v3.1_cDNA_Amp_63C_12X	Yes, refer to User Guide for cycle number
Fragmentation, End Repair, and A-tailing	3'v3.1_FRAG_ER-AT	
Adaptor Ligation	3'v3.1_Ad_Ligation	
Sample Index PCR for: • Gene Expression • Cell Surface Protein • Cell Multiplexing • CRISPR Guide RNA (single index)	3'v3.1_SI-PCR_54C_12X	Yes, refer to User Guide for cycle number
Single Index CRISPR Guide RNA: Feature PCR	3'v3.1_Feat_CRISPR-PCR_60C_single	

Table 2: Chromium Next GEM Single Cell V(D)J v1.1 *(discontinued)*

Product Extension: Cell Surface Protein

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
GEM-RT Incubation	5'v1.1_GEM-RT	
cDNA Amplification	5'v1.1_cDNA_Amp_67C_14X	Yes, refer to User Guide for cycle number
Enriched Library Construction: • Target Enrichment 1 • Target Enrichment 2	5'v1.1_Target_Enrich_67C_8X	Yes, refer to User Guide for cycler number
Enriched Library Construction: Fragmentation, End Repair, and A-tailing	5'v1.1_Frag_ER-AT_2min	
Gene Expression (GEX) Library Construction: Fragmentation, End Repair, and A-tailing	5'v1.1_Frag_ER-AT_5min	
Adaptor Ligation: • Enriched Library Construction • Gene Expression (GEX) Library Construction	5'v1.1_Ad_Ligation	
Sample Index PCR for: • Gene Expression • Enriched Library Construction • Cell Surface Protein	5'v1.1_SI-PCR_54C_16X	Yes, refer to User Guide for cycle number

Table 3: Chromium Next GEM Single Cell ATAC v1.1 *(discontinued)*

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Isothermal Incubation	ATACv1.1_Isothermal_Inc_60min	
GEM Incubation	ATAC_GEM_Inc	
Sample Index PCR	ATAC_SI-PCR_67C_9X	Yes, refer to User Guide for cycle number

Table 4: Targeted Gene Expression for Single Cell *(discontinued)*

Protocol Step(s)	Protocol File Name	Variable Cycle Number
Library Hybridization: • Pre-designed panels • Add-on Genes to pre-designed panels • Fully custom panels	Targeted_GEX_Library_Hyb	
Library Amplification	Targeted_GEX_Library_Amp_60C_10X	Yes, refer to User Guide for cycle number

Table 5: Targeted Gene Expression for Spatial (Fresh Frozen) *(discontinued)*

Protocol Step(s)	Protocol File Name	Variable Cycle Number/Incubation Time
Library Hybridization: • Pre-designed panels - Standard Panel • Add-on Genes to pre-designed panels	Targeted_GEX_Library_Hyb	
Library Hybridization - short cDNA protocol • Pre-designed panels • Add-on Genes to pre-designed panels	Targeted_GEX_Spatial_Library_Hyb_short_cDNA	Yes, refer to User Guide for incubation time
Library Amplification	Targeted_GEX_Library_Amp_60C_10X	Yes, refer to User Guide for cycle number

Document Revision Summary

Document Number	CG000648
Title	Thermal Cycler Protocol Download Guide
Revision	Rev B to Rev C
Revision Date	September 2023
Specific Changes	Updated protocol files for Chromium Fixed RNA Profiling (page 3).
General Changes	Updated for general minor consistency of format, language, and terms throughout.

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