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USER MANUAL

DriverMap™ Single-cell AIR TCR-BCR Chain Pairing Assay (Human, Mouse, Hybrid Human-Mouse) - V2

v1 — Last update: 9 September 2026

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1. DriverMap™ Single-cell AIR TCR-BCR Chain Pairing Assay – V2

The set of DriverMap™ Single-cell Adaptive Immune Receptor (scAIR) Chain Profiling Assays enables identification of TCR/BCR paired full-length (V(D)J) immune receptor sequences of the single T or B cells sorted in a 96-well plate. This assay allows a cost-effective strategy for scAIR repertoire analysis of hundreds of sorted single cells, selected based on the expression of specific markers (e.g., activated, dysfunctional, memory, etc.), or after labeling with specific antigen or MHC peptide complexes. Furthermore, the same scAIR assay could be used to profile chain-pairing information for approximately 10,000 T or B cells deposited in bulk in a 96-well plate (100 cells per well). The paired full-length receptor sequence information is necessary to clone and express functional TCRs, BCRs, and antibodies for follow-up studies of receptor-antigen interaction and the development of cell-based or antibody-based therapies.

The DriverMap scAIR Chain Pairing Assays are designed to amplify in a multiplex RT-PCR reaction full-length (CDR1-CDR2-CDR3) regions for human, mouse or human-mouse hybrids of TCR or BCR chains, from T or B cells sorted or deposited in 96-well plates (s) and are available in several formats:

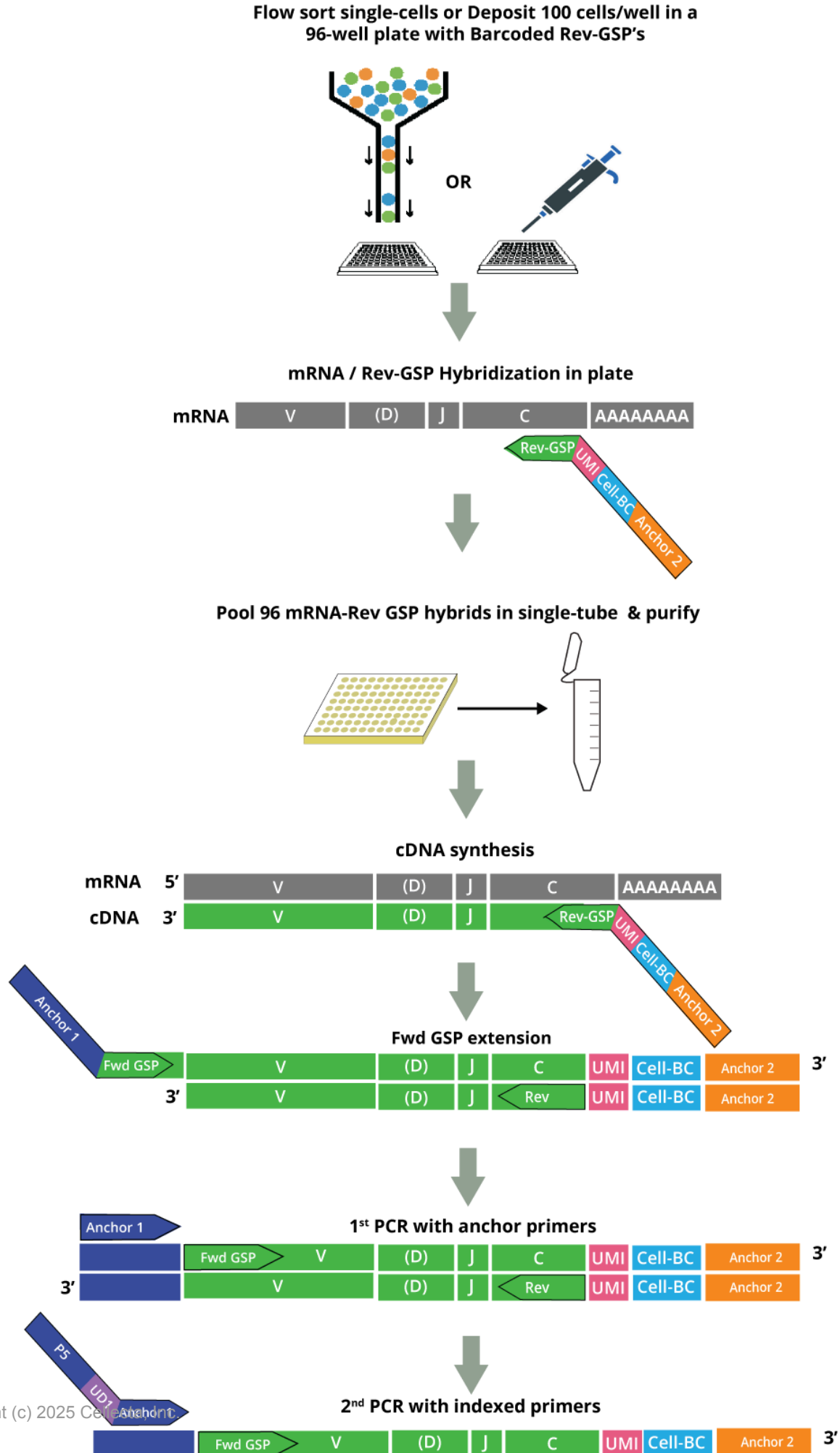
- **The scAIR TCR Chain Pairing Assay (Human/Mouse)** allows analysis of alpha-beta (TRB-TRA) chains of TCRs in sorted human or mouse T cells.
- **The scAIR BCR Chain Pairing Assay (Human/Mouse)** allows analysis of all isotypes of heavy-light (IGH, IGK, IGL) chains of BCRs in sorted human or mouse B cells.
- **The scAIR TCR Chain Pairing Assay (Hybrid, Human-Mouse)** allows analysis of human alpha-beta (TRB-TRA) V(D)J regions fused with mouse C region of hybrid TCRs in sorted mouse T cells.
- **The scAIR BCR Chain Pairing Assay (Hybrid, Human-Mouse)** allows analysis of human heavy-light (IGH, IGK, IGL) V(D)J regions fused with different isotypes of mouse C region of hybrid BCRs in sorted mouse B cells.

The amplified immune receptor cDNA products for all assays contain Validator Barcodes (VBCs), cell/well-specific barcodes, and P5 & P7 indexes compatible with Illumina's Next-Generation Sequencing (NGS) to analyze paired immune receptor chain RNA sequences at single-cell resolution.

Key Features of the DriverMap scAIR Chain Pairing Assay:

• The reverse gene-specific primers (Rev GSPs) for immune receptors are designed with 10-nt cell/well-specific barcodes to pool cells from each plate in a single test tube immediately after the initial Rev GSP-RNA hybridization step. This protocol design significantly simplifies the workflow, allows us to process several plates simultaneously, and reduces library preparation time for follow-up reverse transcription and PCR steps.
• The reverse GSPs are also designed with VBCs, which facilitate the accurate quantification of the copy number of cDNA molecules used in amplification steps and the selection of correct immune receptor chains from mutated sequences generated at amplification and NGS steps.
• The scAIR Chain Pairing assay works with FACS-sorted cells or bulk cells deposited in a 96-well plate without needing special microfluidic equipment or complex split-pool barcoding workflows.
• The scAIR Chain Pairing employs several mechanisms to minimize off-target and primer dimer background product amplification.
• The kit contains control CD3 T cell, CD19 B cell, or PBMC RNA that can be run in parallel as a positive control. DriverMap Spike-in Controls can also be purchased to supplement the single-cell experiment as additional controls to assess assay sensitivity.
• The assay is not only designed to analyze single cells, but it can also be loaded with T or B cell bulk cells (e.g., 100 cells per well). The chain pairing information for medium-high abundant clonotypes is deconvoluted by statistical analysis of the co-appearance of clonotype-specific chain pairing information from each well.
• NGS data can be analyzed with open-source or MiXCR, Platforma, and RSEM software packages available online.

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Fig. 1. Outline of DriverMap scAIR Chain Pairing assays for paired TCR/BCR chain analysis using combination of forward gene-specific primers (Fwd GSPs) designed for FR1 region and barcoded reverse gene-specific primers (Rev GSPs) designed for C region of immune receptor genes. TCR/BCR Rev-GSPs have 96 cell/well-specific barcodes (Cell-BC) and four validator barcodes (VBCs) to facilitate quantitative analysis of paired receptor chains. Anchor 1 and Anchor 2 are sequences of universal primers used in first amplification step. P5-UD1-Anchor1 and P7-UD2-Anchor2 are primers with Illumina UD indexes used in second PCR step.

Related Products and Services:

- DriverMap™ AIR TCR/BCR Profiling Kit (Bulk) (human/mouse RNA/DNA)
- DriverMap™ EXP Human Genome-wide 19K Profiling Kit
- DriverMap™ Spike-in Controls
- DriverMap™ scAIR Immunophenotyping Assays (with immune markers)

Visit our website for scAIR Custom Services from Cellecta.

Please read the entire user manual before proceeding with your experiment.

Click the ? Download as PDF link located at the bottom of the left menu to download the PDF version of this user manual.

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2. DriverMap™ scAIR TCR/BCR Chain Pairing Kit Components

The DriverMap™ scAIR TCR Kit contains enough reagents to prepare NGS samples starting from eight 96-well plates (768 cells) with sorted single T/B cells.

Last modified: 7 July 2025

2.1. scAIR BCR Chain Pairing Kit Components (Human/ Mouse)

Box 1: Kit Components

Component	Description	Cap Color	Conc	Total Volume
Forward GSP BCR primer mix (human/ mouse)	Mix of forward gene-specific primers to amplify CDR1-2-3 region of IGH, IGK, and IGL	n/a	20X	40 µl
scAIR BCR Positive Control RNA	<.Total RNA isolated frm normal donor PBMC or mouse spleen	n/a	20 pg/µl (human) or 50 pg/µl (mouse)	100 µl
SPRI bead Dilution Buffer	Buffer to dilute SPRIselect beads two-fold prior to use	n/a	1X	3 ml
Water	Milli-Q purified water to prepare reaction mixes	White	NA	2 ml
dNTP mix	Mix of dATP, dGTP, dCTP and dTTP, 10mM each	White	20X to 50X	80 µl
RT-EXT Buffer	cDNA synthesis and Forward gene-specific primer extension reaction buffer	Green	5X	250 µl
Reverse Transcriptase	Multiplex-optimized M-MLV Reverse Transcriptase (RT)	Green	20X	25 µl
Hot-Start RT Aptamer	Aptamer which inactivates RT at room temperature but loses inhibition activity at 50°C	Green	20X	25 µl
PCR Buffer	Reaction Buffer for 1st and 2nd PCR steps	Red	5X	600 µl
DNA polymerase	Multiplex-optimized thermostable hot-start DNA polymerase	Red	100X	35 µl
Anchor Primer Mix	Forward and Reverse Anchor-specific primers (AP1 and AP2) for 1st PCR	Red	10X	250 µl
Primer Removal Enzyme	Enzyme mix for primer removal	Orange	25X	50 µl
Forward SeqDNA NGS Primer	Forward SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqDNA NGS Primer	Reverse SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Forward SeqIND NGS Primer	Forward SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl

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Reverse SeqIND NGS Primer	Reverse SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl

Box 2: Kit Components

Component	Description	Conc	Total Volume
scAIR-BCR Rev GSP Master Plate (human/ mouse)	96-well plate, each well contains 50 µl of hybridization buffer and a mix of barcoded (well-specific and VBC barcodes) reverse gene-specific primers (Rev GSPs) designed for the C region of IGH, IGK, and IGL mRNAs	1X	50 µl/ well in a 96-well plate

Extra Materials Required: (Sold Separately as Catalog # DM-NGS-SetA-B2, DM-NGS-SetB-B2, DM-NGS-SetC-B2, DM-NGS-SetD-B2)

Component	Description	Conc	Total Volume
Dual Index Primer Set Plate	96 Dual DNA/RNA UD Indexes primer pairs, pre-aliquoted; one plate sufficient to process 96 plates	10X	20 µl/well

Kit contents should be stored at -20°C. Shelf life is 1 year from the date of receipt.

Last modified: 10 June 2026

2.2. scAIR TCR Chain Pairing Kit Components (Human/ Mouse)

Box 1: Kit Components

Component	Description	Cap Color	Conc	Total Volume
Forward GSP TCR primer mix (human/ mouse)	Mix of forward gene-specific primers to amplify the CDR1-2-3 region of TRA and TRB	n/a	20X	40 µl
scAIR TCR Positive Control RNA (human/ mouse)	Total RNA from CD3+ T cells isolated from normal donor PBMC or mouse spleen (Strain CD1)	White	20 pg/µl (human) or 50 pg/µl (mouse)	100 µl
SPRI bead Dilution Buffer	Buffer to dilute SPRIselect beads two-fold prior to use	n/a	1X	3 ml
Water	Milli-Q purified water to prepare reaction mixes	White	NA	2 ml
dNTP mix	Mix of dATP, dGTP, dCTP and dTTP, 10mM each	White	20X to 50X	80 µl
RT-EXT Buffer	cDNA synthesis and Forward gene-specific primer extension reaction buffer	Green	5X	250 µl

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Reverse Transcriptase	Multiplex-optimized M-MLV Reverse Transcriptase (RT)	Green	20X	25 µl
Hot-Start RT Aptamer	Aptamer which inactivates RT at room temperature but loses inhibition activity at 50°C	Green	20X	25 µl
PCR Buffer	Reaction Buffer for 1st and 2nd PCR steps	Red	5X	600 µl
DNA polymerase	Multiplex-optimized thermostable hot-start DNA polymerase	Red	100X	35 µl
Anchor Primer Mix	Forward and Reverse Anchor-specific primers (AP1 and AP2) for 1st PCR	Red	10X	250 µl
Primer Removal Enzyme	Enzyme mix for primer removal	Orange	25X	50 µl
Forward SeqDNA NGS Primer	Forward SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqDNA NGS Primer	Reverse SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Forward SeqIND NGS Primer	Forward SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqIND NGS Primer	Reverse SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl

Box 2: Kit Components

Component	Description	Conc	Total Volume
scAIR TCR-Rev GSP Master Plate (human/ mouse)	96-well plate, each well contains 50µl of hybridization buffer and a mix of barcoded (with well-specific barcodes and VBC barcodes), reverse gene-specific primers designed for the C region of TRA and TRB mRNAs	1X	50 µl/ well in a 96-well plate

Extra Materials Required: (Sold Separately as Catalog # DM-NGS-SetA-B2, DM-NGS-SetB-B2, DM-NGS-SetC-B2, DM-NGS-SetD-B2)

Component	Description	Conc	Total Volume
Dual Index Primer Set Plate	96 Dual DNA/RNA UD Indexes primer pairs, pre-aliquoted, one plate sufficient to process 96 plates	10X	20 µl/well

Kit contents should be stored at -20°C. Shelf life is 1 year from the date of receipt.

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2.3. scAIR BCR Chain Pairing Kit (Hybrid, Human-Mouse)

Box 1: Kit Components

Component	Description	Cap Color	Conc	Total Volume
Forward GSP BCR primer mix (Hybrid)	Mix of forward gene-specific primers to amplify the CDR1-2-3 region of human IGH, IGK, and IGL	n/a	20X	40 µl
scAIR BCR Positive Control RNA (mouse)	Total RNA isolated from mouse spleen (strain CD1)	n/a	50 pg/µl	100 µl
SPRI bead Dilution Buffer	Buffer to dilute SPRIselect beads two-fold prior to use	n/a	1X	3 ml
Water	Milli-Q purified water to prepare reaction mixes	White	NA	2 ml
dNTP mix	Mix of dATP, dGTP, dCTP and dTTP, 10mM each	White	20X to 50X	80 µl
RT-EXT Buffer	cDNA synthesis and Forward gene-specific primer extension reaction buffer	Green	5X	250 µl
Reverse Transcriptase	Multiplex-optimized M-MLV Reverse Transcriptase (RT)	Green	20X	25 µl
Hot-Start RT Aptamer	Aptamer which inactivates RT at room temperature but loses inhibition activity at 50°C	Green	20X	25 µl
PCR Buffer	Reaction Buffer for 1st and 2nd PCR steps	Red	5X	600 µl
DNA polymerase	Multiplex-optimized thermostable hot-start DNA polymerase	Red	100X	35 µl
Anchor Primer Mix	Forward and Reverse Anchor-specific primers (AP1 and AP2) for 1st PCR	Red	10X	250 µl
Primer Removal Enzyme	Enzyme mix for primer removal	Orange	25X	50 µl
Forward SeqDNA NGS Primer	Forward SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqDNA NGS Primer	Reverse SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Forward SeqIND NGS Primer	Forward SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqIND NGS Primer	Reverse SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl

Box 2: Kit Components

Component	Description	Conc	Total Volume
scAIR-BCR Rev GSP Master Plate (Hybrid)	96-well plate, each well contains 50 µl of hybridization buffer and a mix of barcoded (well-specific and VBC barcodes) reverse gene-specific primers (Rev GSPs) designed for the C region of mouse IGH, IGK, and IGL mRNAs	1X	50 µl/ well in a 96-well plate

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Extra Materials Required: (Sold Separately as Catalog # DM-NGS-SetA-B2, DM-NGS-SetB-B2, DM-NGS-SetC-B2, DM-NGS-SetD-B2)

Component	Description	Conc	Total Volume
Dual Index Primer Set Plate	96 Dual DNA/RNA UD Indexes primer pairs, pre-aliquoted; one plate is sufficient to process 96 plates	10X	20 µl/well

Kit contents should be stored at -20°C. Shelf life is 1 year from the date of receipt.

Last modified: 10 June 2026

2.4. scAIR TCR Chain Pairing Kit (Hybrid, Human-Mouse)

Box 1: Kit Components

Component	Description	Cap Color	Conc	Total Volume
Forward GSP TCR primer mix (Hybrid)	Mix of forward gene-specific primers to amplify the CDR1-2-3 region of human TRA and TRB gene	n/a	20X	40 µl
scAIR TCR Positive Control RNA (mouse)	Total RNA isolated from mouse spleen (strain CD1)	n/a	50 pg/µl	100 µl
SPRI bead Dilution Buffer	Buffer to dilute SPRIselect beads two-fold prior to use	n/a	1X	3 ml
Water	Milli-Q purified water to prepare reaction mixes	White	NA	2 ml
dNTP mix	Mix of dATP, dGTP, dCTP and dTTP, 10mM each	White	20X to 50X	80 µl
RT-EXT Buffer	cDNA synthesis and Forward gene-specific primer extension reaction buffer	Green	5X	250 µl
Reverse Transcriptase	Multiplex-optimized M-MLV Reverse Transcriptase (RT)	Green	20X	25 µl
Hot-Start RT Aptamer	Aptamer which inactivates RT at room temperature but loses inhibition activity at 50°C	Green	20X	25 µl
PCR Buffer	Reaction Buffer for 1st and 2nd PCR steps	Red	5X	600 µl
DNA polymerase	Multiplex-optimized thermostable hot-start DNA polymerase	Red	100X	35 µl
Anchor Primer Mix	Forward and Reverse Anchor-specific primers (AP1 and AP2) for 1st PCR	Red	10X	250 µl
Primer Removal Enzyme	Enzyme mix for primer removal	Orange	25X	50 µl
Forward SeqDNA NGS Primer	Forward SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqDNA NGS Primer	Reverse SeqDNA NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Forward SeqIND NGS Primer	Forward SeqIND NGS Primer for cDNA sequencing	Blue	100 µM	50 µl
Reverse SeqIND NGS	Reverse SeqIND NGS Primer for cDNA sequencing	Blue	100	50 µl

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Primer			µM	

Box 2: Kit Components

Component	Description	Conc	Total Volume
scAIR-TCR Rev GSP Master Plate (Hybrid)	96-well plate, each well contains 50 µl of hybridization buffer and a mix of barcoded (well-specific and VBC barcodes) reverse gene-specific primers (Rev GSPs) designed for the C region of mouse TRA and TRB gene	1X	50 µl/ well in a 96-well plate

Extra Materials Required: (Sold Separately as Catalog # DM-NGS-SetA-B2, DM-NGS-SetB-B2, DM-NGS-SetC-B2, DM-NGS-SetD-B2)

Component	Description	Conc	Total Volume
Dual Index Primer Set Plate	96 Dual DNA/RNA UD Indexes primer pairs, pre-aliquoted; one plate is sufficient to process 96 plates	10X	20 µl/well

Kit contents should be stored at -20°C. Shelf life is 1 year from the date of receipt.

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3. Additional Materials Required

Depending on the type of samples and procedural options followed in the protocol, several of the following items will be required to run the assay.

Additional Reagents and Apparatus for Hybridization, cDNA Synthesis, Amplification, and Amplified Libraries QC

Name	Recommended Manufacturer	Catalog #	Purpose
SPRIselect, 5 ml (Magnetic Beads)	Beckman Coulter	B23317	RNA-RevGSP hybrid and PCR product purification
Dynabeads® MPC®-S (Magnetic Particle Concentrator) for 1.5 ml Test Tubes	Thermo Fisher	A13346	RNA-RevGSP hybrid product purification
Catalyst 96 slotted ring magnetic plate	ALPAQUA	A000550	RNA-RevGSP hybrid product purification in 96-well plate
96-well PCR plates	Thermo Fisher	A43673	RT-PCR reactions
Clear Adhesive Film for 96-well plates	Bio-Rad	MSB1001	PCR plate seal
8-channel pipettor	various	various	To pipette 8-rows across when setting up reactions
High Sensitivity DNA Kit for Bioanalyzer	Agilent	5067-4626	PCR product QC/ quantification
High Sensitivity NGS Fragment Analysis Kit (1 bp – 6000 bp) for Fragment Analyzer	Agilent	DNF-474-1000	PCR product QC/ quantification
Qubit® dsDNA HS (High Sensitivity) Assay Kit	Thermo Fisher	Q32851	Quantify Indexed Libraries for NGS
NextSeq® 1000/2000 XLeap-SBS P1 Reagent Kit (600 cycles)	Illumina	20100981	Next-Gen Sequencing
NextSeq® 1000/2000 XLeap-SBS Read Primer Kit	Illumina	20112859	Next-Gen Sequencing with custom primers
MiSeq Reagent Kit v3 (600 cycles)	Illumina	MS-102-3003	Next-Gen Sequencing
MiSeq i100 Series 25M Reagent Kit (600 cycles)	Illumina	20115696	Next-Gen Sequencing

Recommended Instrumentation

This protocol is optimized for the instruments specified below. Some modifications and optimization to the protocol may be necessary if using different models or instruments from other manufacturers.

Description	Model	Manufacturer
Spectrophotometer (NanoDrop or equivalent)	NanoDrop® ND-2000	Thermo Fisher
Thermal Cycler (Veriti 96-Well or equivalent)	Veriti® 96-Well	Thermo Fisher
Instrument or Agarose Gel System to analyze DNA fragments or Slab Gel Electrophoresis System to analyze amplified DNA products	2100 Bioanalyzer®	Agilent
	Fragment Analyzer™	Agilent
	Agarose gel electrophoresis/imaging system	many suppliers
DNA Quantification	Qubit 3.0 Fluorometer	Thermo Fisher

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Next-Generation Sequencer	NextSeq 2000, MiSeq	Illumina
Thermomixer R	Thermomixer R or MixPlate with lock for vortexing PCR plates	Eppendorf/Fisher Scientific
FisherBrand Mini Plate Spinner Centrifuge	Centrifugation of plate to collect droplets from walls	Fisher Scientific

Other than the specific reagents and instruments listed above, the protocol assumes the user has access to standard materials (e.g., polypropylene tubes, pipette tips), equipment (tabletop centrifuges, pipettes), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

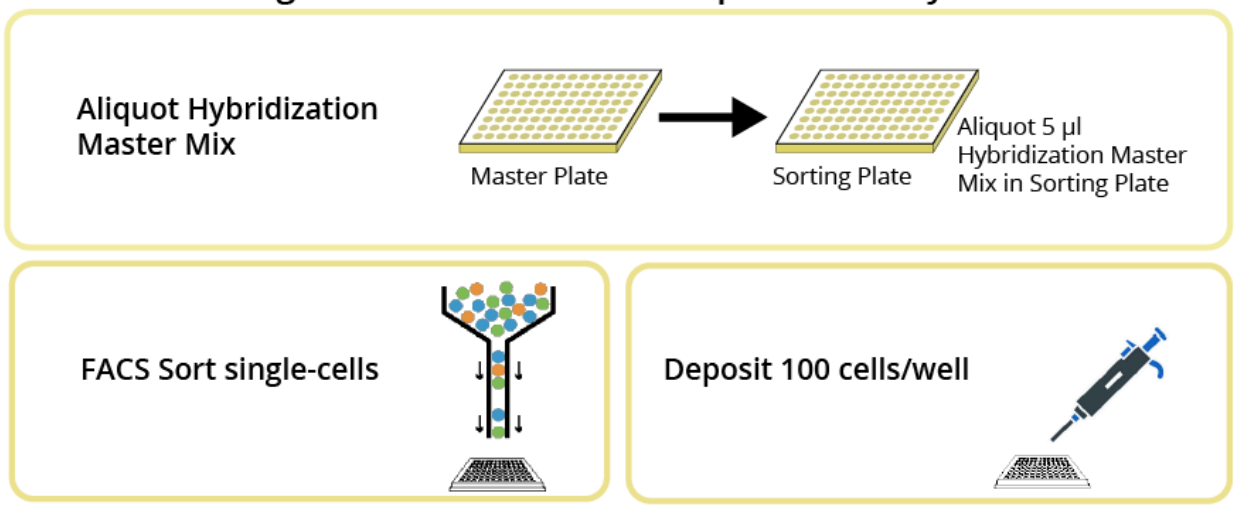
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4. General Procedure

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Fig 2. Overview of DriverMap scAIR Assay



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4.1. Workflow Outline

1. From the provided Master Plate, aliquot Hybridization master mix (5 µl/well) containing a mix of barcoded Reverse GSPs in 1-8 of 96-well plates.
2. For transferring cells into plates, you have two options: (1) Flow sort T or B cells in a 96-well plate(s) with aliquoted hybridization buffer for single cells per well or 100 cells/well. (2) Deposit a pool of pure T, B cells, or PBMC by adjusting the concentration of the pool to 100 cells/µl and measuring by a hemocytometer.
3. After hybridization of cellular mRNA with Rev GSPs, the 96 mRNA-Rev GSP hybrids (each with a cell/well-specific barcode) are pooled together and purified by SPRIselect magnetic beads.
4. Pooled, purified mRNA-Rev GSP hybrids are eluted from SPRIselect beads, and cDNA is synthesized by Reverse transcriptase from Rev GSPs.
5. Sense DNA strand (a complementary strand of cDNA product) is synthesized by DNA polymerase using a mix of Forward (Fwd) GSPs. Fwd GSPs and Rev GSPs are designed to cover both full-length (CDR1-2-3) immune receptor chains and 100-250 bp gene-specific cDNA fragments of T/B cell marker genes (for DriverMap scAIR Immunophenotyping Assay).
6. Fwd GSP-extended DNAs are amplified using universal anchor primers (AP1 and AP2). The Second round of nested PCR with Illumina's UD dual-indexed anchor primers allows combining different NGS libraries together (e.g., from two to eight 96-well plates) and adds P5 and P7 sequences necessary for Illumina's Paired-End 600-nt sequencing on NextSeq 2000/MiSeq instruments.

Last modified: 11 June 2026

4.2. Experiment Planning Guidelines

The scAIR assay protocol requires as input a suspension of viable single or a pool of T/B cells sorted or deposited into 96-well sorting plates with 5µl of Hybridization Master Mix aliquoted by the customer from the provided Master Plate. Specific protocols for isolation and Flow sorting of T/B cells are dependent on specific research needs and are outside of the scope of the current protocol. As a rule, T/B cells could be isolated from PBMC or dissociated tissue samples using standard protocols developed and validated for scRNA-seq technologies. As described in the protocol below, T or B cells can be deposited in the plate wells as single cells or a pool of T or B cells (approximately 100 cells/well for bulk chain pairing assay). To generate high-quality scAIR data, it is important to:

- Use T/B cells with at least 70% viability. Dying/non-viable cells release "contaminating" RNA in sorting media, which generates background in scAIR data. Furthermore, dead cells usually generate cell aggregates. Consider dead cell removal (e.g., by magnetic microbeads) for low-viability cell samples.
- Reduce cell stress at straining, filtering, thawing, centrifugation, washing, and resuspension steps (pipetting).
- Use optimal media for purification and sorting T/B cells, e.g., 1X PBS with 1% BSA for cell washing and resuspension.
- For cell sorting to reduce cell shear stress, use a 70-100 µm nozzle or consider using a low-pressure cell sorter. Add live/dead cell marker before sorting.
- Sort T/B cells directly in hybridization buffer to reduce contact of cells with the well/tube walls.
- For deposition of pool T or B cells, wash the cells in 1X PBS with 0.1% BSA, adjust the concentration of cells to 100 cells/ µl and measure by a hemocytometer, deposit 1 µl of cells per side wall, and briefly centrifuge the plate to mix the cells with hybridization buffer.

For detailed protocols related to Flow sorting of lymphocyte cells and cell fractions, please refer to: Guidelines for the use of flow cytometry and cell sorting in immunological studies

For a detailed overview of experimental strategies for designing AIR profiling experiments, please refer to the DriverMap AIR

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Technology Guide.

Last modified: 9 July 2025

4.3. General Procedural Guidelines

- Keep enzyme components on the ice during use. All other components, including primers, may be placed at room temperature. Thaw components, gently vortex, and spin down before use. Please be sure to dissolve any precipitate if visible.
- Use Good Laboratory Practices to minimize cross-contamination of products. If possible, store all AIR kit components and perform the first part of the procedure (from cell sorting, RNA-Rev GSP hybridization, cDNA synthesis through PCR setup) in a “pre-PCR” room or at least in a PCR clean location set aside for RNA work. Use a set of equipment, pipettes, test tubes, other consumables, and all general reagents dedicated to work involving RNA. After running the RT and cDNA synthesis, run the PCR amplification reactions and analyze, purify, and store PCR products in a separate lab or area designated for PCR using a set of equipment, consumables, and reagents dedicated to working with PCR products. Always change pipette tips for adding components to new samples.
- To minimize the hands-on time and mistakes in liquid deposition in sorting/hybridization 96-well plate, we recommend running the assay using an 8-channel (or 12-channel) pipette. For aliquoting 5µl of Hybridization Master Mix from the provided Master plate in a new plate(s), use a new tip for each well for the Master plate to avoid cross-contamination between different barcoded RevGSPs.
- For bulk chain pairing assay, use a multichannel pipette to deposit 1 µl of the pool of T/B cells on the side wall of each well (to avoid cross-contamination between different wells) and briefly centrifuge the plate to mix the cells with hybridization buffer.
- For efficient binding of RNA-Rev GSPs hybrid to SPRIselect beads, it is important to uniformly mix beads with hybridization solution, which could be achieved by mixing the plate content in an Eppendorf Thermomixer R or MixMate shaker. For all enzymatic (extension) and PCR steps, we recommend using 0.2-ml or 0.5-ml test tubes.
- Do not reuse clear adhesive films to seal 96-well plates.
- SPRIselect beads are usually stored at +4°C and need to be adjusted to room temperature prior to use. Resuspend the beads using a shaker and dilute 4x using the provided SPRI bead dilution buffer. Dilution of SPRIselect beads with the provided dilution buffer allows for a reduced amount of beads used to purify RNA-RevGSP hybrids and a reduced volume of elution buffer for the reverse transcription step.
- Pipette viscous enzyme solutions slowly. After adding them to the reaction mix, ensure complete mixing by vortexing or pipetting up and down several times. Vortexing in an Eppendorf plate shaker is a preferable choice for 96-well plates, as pipetting in small well volumes is more time-consuming and could generate air bubbles.
- For several steps in the procedure, we recommend making a Master Mix by combining adequate amounts of the key reagents into a single tube and then aliquoting the appropriate portion from this combined mix into each sample. This approach helps ensure a consistent amount of reagent is added to all samples. For these Master Mix reactions, first calculate the volume of each component you need for all the reactions you are running, and pool the appropriate volumes of reagents together into a single tube. It is not necessary to add additional reagents to cover pipet variance, as the protocol below includes approximately 10% of extra reagents. Mix the pooled reagents, then pipette the required portion into each sample. If you are using a multichannel pipette, aliquot Master Mix in a tube strip (or reservoir) first, then pipette from this strip into each sample.
- For all scAIR protocol incubation steps in the thermocycler machine use a heated lid set up at standard 105°C.

Last modified: 7 July 2025

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5. Sorting or Depositing T/B Cells

The scAIR Rev GSP Master Plates provided in the kit contain 50 µl per well of pre-aliquoted Hybridization buffer containing a mix of barcoded Reverse TCR (designed for alpha and beta) or BCR (designed for H, K, and L chains) C-region primers. The scAIR Rev GSP Master Plates contain enough reagents to prepare up to eight 96-well sorting PCR plates.

Last modified: 7 July 2025

5.1. Protocol for Sorting or Depositing T/B Cells

1. Before use, thaw the provided Master Plates with Hybridization Master Mixes, vortex the plate in an Eppendorf plate shaker, and centrifuge the plate with a benchtop mini plate-spinning centrifuge or any bucket centrifuge (e.g., 2,000 rpm, 1 min) to collect droplets to the bottom of the wells.
2. Remove the seal from the Master plate and transfer 5 µl of Hybridization Master Mix from each well of the Master plate into 1 to 8 new 96-well PCR plate(s) compatible with a cell sorter. Importantly, use a new tip for each well of the Master plate to avoid cross-contamination of barcoded reverse GSPs between different wells. A Master plate with wells of unused Hybridization Master Mix can be stored at +4°C for up to 6 months.
3. Load the cells into each well. For this step, there are two options depending on the application: (a) Sort cells by flow or (b) pipette a defined number of cells into each well.
   (a) For sorting by flow cytometry: Load the 96-well sorting PCR plate with 5 µl of pre-aliquoted Hybridization Master Mix into the motorized stage holder. Follow the single-cell sorting protocol as per the specific instrument to sort single cells for experimental samples. We recommend including a control well for your plate by sorting 100 cells in one of the wells of this plate. This control well containing cells before sorting, can be used as a positive control to ensure cellular RNA integrity after sorting and for troubleshooting purposes.
   (b) For deposition of a pool of T or B cells: Wash and adjust the concentration of the cells in 1xPBS buffer to 100 cells/µl using a hemocytometer. Deposit 1 µl on the side wall of each well in the plate with 5 µl of pre-aliquoted Hybridization Master Mix.
4. Immediately after sorting or deposition, seal the plate, and use a benchtop mini plate-spinning centrifuge (or any other centrifuge) to spin the plate for 10-15 secs. This will propel any drops with cells that are on the side walls of the wells down into the buffer. You can proceed with library prep immediately or freeze and store the plate(s) at -80°C prior to use for the scAIR profiling experiment. To reduce sequencing cost, up to 8 plates (e.g., from different experiments) could be run together for the follow-up scAIR-NGS protocol.

Last modified: 9 July 2025

5.2. Positive Control Experiment

Prior to running experimental samples, we strongly recommend that you run a positive control experiment with a set of T/B cells (e.g., from any non-experimental PBMC sample) with the positive control RNA included in the kit. The positive control experiment is designed to troubleshoot potential problems in the sorting step, to check the stability of RNA in sorted cells, and to measure the sensitivity of the scAIR assay protocol.

To run the positive control experiment, take one of the PCR plates with pre-aliquoted 5 µl of Hybridization Master Mixes (prepared in Step 5.1), and deposit the following:
1. 100 presorted T/B cells into 24 wells (e.g., A1-H3). Dilute presorted cells (e.g., PBMC) in 1xPBS to a concentration of approximately 4 T/B cells/µl based on the estimated percent of T/B cells in the presort sample and aliquot 1 µl per well.
2. 100 T/B cells sorted into 24 wells (four cells per well, e.g., A4-H6).
3. 25 T/B cells sorted into 24 wells (one cell per well, e.g., A7-H9).
4. Aliquot 1 µl of scAIR positive control RNA provided in the kit in 24 wells (e.g., A10-H12).

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Freeze and store the positive control plate at -80°C before use for the scAIR profiling experiment. For scAIR profiling using a positive control plate:
1. Follow the standard protocol described below (Step 6) but run each subset of presorted, sorted, and positive control RNA as a separate sample by combining together hybridization reactions (after adding SPRIselect beads, step 6.1.5) from each subset of 24 wells.
2. Analyze yield of amplified cDNA products after 2nd amplification step (with indexed primers) as described in Section 7.1. If analysis of the amplified indexed libraries reveals amplification of expected PCR products (Fig.3), you could proceed with scAIR profiling of your experimental samples.
3. If you can see amplification products in presorted and positive control RNA samples, but not in sorted cells, it could indicate problems with FACS gating, sorting, or degradation of RNA in sorted cells. Based on our experience, degradation of RNA in sorted cells is a common problem that could be solved by using the Cell Sorter Cleaning Best Practices as described in the paper. If no amplification products are seen in sorted cells, but amplification products are present in control RNA and pre-sorted cells, please test the presence of RNAse in the fluid collected after sorting by using the RNaseAlert™ Lab Test Kit.

Last modified: 8 January 2026

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6. DriverMap™ scAIR Protocol

Last modified: 15 May 2025

6.1. Hybridization with Rev-GSP Primers

In this step, T/B cells sorted or deposited in the 96-well PCR plate(s) are incubated in a hybridization buffer containing barcoded Rev GSPs at high temperatures. In this hybridization step, mRNA-Rev GSP hybrids are generated, and 96 barcoded mRNA-Rev GSP hybrids are combined and purified with SPRIselect magnetic beads. If you run more than one 96-well plate with sorted single T/ B cells, each plate gives one pooled reaction tube; i.e., don't combine pooled samples from different plates. The protocol below specifies the volume of reagents for processing one 96-well plate with sorted single T/B cells or cell pools deposited in wells. The same protocol could be used for processing the plate with a Positive Control RNA sample or a Positive Control RNA sample spiked with TCR/BCR synthetic controls (in each well). The scAIR kit provides enough reagents to process up to 8 Pooled samples (each from a 96-well plate). In the current and all follow-up steps, proportionally increase the volume of reagents to assemble Master mixes if you are using more than one plate.

Please note that the proposed volumes of Master Mixes for all steps already include a 10-20% excess of reagents, e.g., it is not necessary to increase the volume of reagents to prepare Master Mixes.

Note: Hybridization, cDNA synthesis, and Forward Primer Extension steps should be set up in a pre-PCR room.

1. Thaw the plate with sorted cells (stored at -80°C, Section 5.1) and briefly centrifuge the plate to collect droplets. Load the plate in the thermocycler (or in an air incubator), and run the following program to hybridize mRNAs with Reverse GSPs:

Temperature	Time
60°C	60 min
25°C	∞

2. During Hybridization (Step 1), prepare Diluted SPRIselect beads in the reservoir designed for an 8-channel pipette, mix well the viscous beads and dilution buffer, and keep it at room temperature.

Reagents	Volume per plate, µl
SPRIselect Beads	150
SPRI bead Dilution Buffer, 1X	450
Total	600

3. Add 5 µl (1x volume) of Diluted SPRIselect Beads (prepared in Step 2) into each plate well using an 8-channel pipette, seal the plate, briefly centrifuge the plate, and mix SPRIselect beads and solution in wells using an Eppendorf plate shaker. Importantly, you need to optimize the speed of the plate shaker (approximately 1,400-1,800 rpm) to completely mix viscous SPRIselect beads with a solution in each well. Check visually that each well contains a homogeneous brown color solution in the whole volume.

4. Shake the plate in the Eppendorf plate shaker at 1400 rpm for 10 minutes at room temperature.

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5. Using an 8-channel pipette, combine the contents of all wells of the 96-well plate together in a reservoir designed for an 8-channel pipette. Transfer the whole volume of the pooled sample into a 1.5 ml test tube. Pipette slowly and try to collect all viscous solutions from wells and reservoirs. Place the 1.5 ml test tube with the pooled sample in a magnetic separation device designed for 1.5 ml test tubes for approximately 5 min, until the liquid appears completely clear. Carefully remove and discard the clear supernatant from the test tube without disturbing the magnetic bead pellet.

✻ Note: If you run several 96-well plates, each 96-well plate's content needs to be pooled separately, as each plate has the same set of 96-well-specific indexes in Rev GSPs.

6. Add 1 ml of freshly prepared 80% ethanol to the pooled sample test tube without removing the test tube from the Magnetic Stand, wait for 2 minutes, carefully remove, and discard the supernatant without disturbing the bead pellet.

7. Repeat 80% ethanol washing in Step 6.

8. Briefly centrifuge the pooled sample test tube at low speed and place the test tube in the Magnetic Stand. Use a 200-µl pipette to remove the residual ethanol droplets from the bottom of the test tube and air-dry the beads at room temperature for approximately 5 minutes until the disappearance of ethanol droplets on the test tube surface and the magnetic bead pellet appears dry and no longer shines.

- Note: During the process of drying the magnetic beads, prepare the Reverse Transcriptase Master Mix as described in the next section.

! Immediately proceed to the next step (cDNA Synthesis)

Last modified: 11 June 2026

6.2. cDNA Synthesis

In this step, the purified mRNA-Reverse GSP hybrids eluted from SPRIselect beads are extended by Reverse Transcriptase to generate cDNA (antisense strand of mRNA). The protocol below specifies volumes of reagents and Master Mixes for one pooled sample purified from one 96-well plate.

1. Prepare the Reverse Transcriptase Buffer Master Mix as follows:

RT Buffer Master Mix Component	Volume per sample, µl
RT-EXT Buffer, 5X	6
dNTP Mix	1.5
Water	19.5
Hot-start RT Aptamer, 20X	1.5
Reverse Transcriptase, 20X	1.5
Total	30

2. Gently vortex Master Mix and spin down briefly to collect droplets. Add 28 µl of the RT Master Mix into pooled sample 2 ml test tube with magnetic beads (from Step 6.1) and gently resuspend SPRIselect beads attached to the tube

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surface by pipetting. Avoid the formation of bubbles. Briefly centrifuge the tube at low speed and place the tube in a Magnetic Stand for 1-2 minutes until the supernatant is clear. Transfer the whole volume ~25 µl of clear supernatant without beads from the tube to the new 0.2-0.5 ml test tube compatible with the used thermocycler. If the supernatant contains a significant number of bubbles, briefly centrifuge the test tube(s) before loading into the thermocycler

3. Load the test tube in the thermal cycler and start running the following program (use standard 105°C heating lid temperature):

Temperature	Time
50°C	30 min
72°C	10 min
4°C	∞

4. Add 1 µl of the Primer Removal Enzyme to the sample, mix contents by pipetting 3 times, spin down to collect droplets, load the sample test tube in the thermal cycler, and run the following program:

Temperature	Time
37°C	20 min
95°C	5 min
4°C	∞

Stopping Point in procedure: cDNA samples may be stored at 4°C overnight or at –20°C for longer periods.

Last modified: 11 June 2026

6.3. Forward GSP Extension

In this step, the pool of Forward Gene-Specific Primers with adjoining Anchor 1 sequences (Fig.1) is annealed to cDNA template generated in previous step, extended by DNA polymerase and generates sense strands of the target amplicons flanked from both sides by Anchor 1 and Anchor 2 sequences. The protocol below specifies volumes of reagents and Master Mixes for one pooled cDNA sample.

1. Prepare the Forward GS Primer Extension Master Mix as follows:

Forward GS Primer Extension Master Mix Component	Volume per sample, µl
RT-EXT Buffer, 5X	6
Water	20.4
Forward GSP TCR or BCR primers, 20X	3
DNA Polymerase, 100X	0.6
Total	30

2. Gently vortex Master Mix and spin down briefly to collect droplets. Spin down the sample test tube (from step 6.2), and add 25 µl of the Forward GS Primer Extension Master Mix:

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Component	Volume, µl
cDNA (from the step above)	26
Forward GS Primer Extension Master Mix	25
Total	51

3. Mix contents by pipetting three times, briefly spin down the tube, load the tube in the thermal cycler, and run the following program:

Temperature	Time
98°C	1 min
68°C	10 min
4°C	∞

4. Add 2 µl of the Primer Removal Enzyme to the sample, mix contents by pipetting 3 times, spin down to collect droplets, load the sample test tube in the thermal cycler, and run the following program:

Temperature	Time
37°C	30 min
95°C	5 min
4°C	∞

! Immediately proceed to the next step (PCR with Anchor Primers)

Last modified: 13 August 2025

6.4. First PCR with Anchor Primers

This step utilizes universal Anchor PCR primers to amplify the target DNA fragments flanked with the Anchor 1 and Anchor 2 sequences generated during the previous Forward Gene-Specific Primer Extension step. The protocol below specified volumes of reagents and Master Mixes for one pooled DNA sample.

1. Prepare the Anchor PCR Master Mix as shown below:

Anchor PCR Master Mix Component	Volume per sample, µl
PCR Buffer, 5X	11
dNTP Mix	1.1
Anchor Primer Mix, 10X	11
Water	31.3
DNA Polymerase	0.6
Total	55

2. Gently vortex Master Mix and spin down briefly to collect droplets. Add 50 µl of Anchor PCR Master Mix to each sample test tube from Step 6.3:

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Component	Volume per sample, µl
Forward GS Primer Extension DNA (after primer removal step)	53
Anchor PCR Master Mix (prepared above)	50
Total	103

3. Mix content by pipetting 3 times and spin down to collect droplets.

✻ Note: Take the sample test tube from the PCR-free room and perform all follow-up Amplification, Purification, and NGS steps in the location dedicated to PCR work. Never bring amplified library products back to the pre-PCR room.

4. Load the plate in the thermal cycler in a location dedicated to PCR work. Run the following program using the recommended number* of PCR cycles:

Temperature	Time	Cycles
98°C	30 sec	1
98°C	20 sec	20-26* Note below
65°C	10 sec	
72°C	45 sec	
72°C	30 sec	1
4°C	∞	1

✻ Note: For a sorted single-cell sample, run 26 cycles. For the bulk chain pairing assay with deposited 100 cells/well, run 20 cycles. For the scAIR Positive Control RNA sample, run 24 cycles for the TCR assay and 21 cycles for the BCR assay. For the scAIR Positive Control RNA sample, run 22 cycles. The number of cycles in the 1st PCR in many cases needs to be optimized due to differences in transcriptional activity of sorted cells. E.g., activated cells are more transcriptionally active than naïve/dysfunctional cells. For plasma B cells, we recommend using 20 cycles, and for other B cells, 25 cycles. For TCR, we recommend 27 cycles. Differences in amplification efficiency between different samples could also be adjusted in the 2nd PCR step (see below).

! Stopping Point in procedure: PCR products may be stored at 4°C for a week.

Last modified: 13 August 2025

6.5. Second PCR with Indexed Primers

This step adds a dual unique DNA/RNA UDP index combination to each 1st PCR Product generated in the previous step, as well as universal flanking P5 and P7 sequences needed for cluster formation on the Illumina NGS flow cell (Outline).

The Dual Index Primer Set Plate is sold separately and contains a unique combination of Forward and Reverse DNA/RNA UDP index primers in each of 96 wells. Each well contains 20 µL of unique index primer combination (10X concentration), which is enough to run x4 single-cell plates (5 µl per 50 µl reaction). One unique index primer combination should be used for

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each sample (triplicate samples have different indexes). For a small number of samples (e.g., 2-3 plates with sorted cells), consider running the 2nd PCR step in triplicate using different index primers to avoid low-complexity index sequencing in the NGS step. Always record the well coordinates of index primers used for each sample to avoid problems in the NGS sequence deconvolution step. Prior to use, thaw the Dual Index Primer Set Plate, briefly centrifuge the plate to collect droplets at the bottom of the wells, remove the seal slowly (to prevent cross-contamination), and aliquot 5 µl for each sample (45 µl) as described below. Seal the plate with a new sealing film and store at +4 °C (for immediate use) or at -20 °C (for prolonged storage). See Appendix. List of Dual DNA/RNA UDP Indexes for the sequences of each Forward and Reverse Index combination.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

Arrangement of the index pairs in the supplied Index Primer Plate.

1. Prepare enough of the 2nd PCR Master Mix, following the formulation below for all samples and controls, plus 5% extra volume of all components:

PCR Master Mix Component	Volume per sample, µl
PCR Buffer, 5X	30
dNTP Mix	3.3
Water	108
DNA Polymerase, 100X	1.7
Total	145

*Note: If you are using a multichannel pipette, aliquot the master mix in a tube strip to minimize pipetting steps.

2. Gently vortex the 2nd PCR Master Mix, then briefly spin down to collect droplets. Remove the plate seal from the Index Primer Master Plate.

3. Aliquot 45 µl of the 2nd PCR Master Mix into selected wells of the new 96-well plate and add 5 µl of unique index primer combination in each well from the Dual Index Primer Set Plate. For each sample aliquot 2nd PCR Mix in 3 different wells (e.g. A1, A2, A3 for sample 1 and A4, A5, A6 for sample 2). To avoid index-to-index contamination, add index primers from the Index Primer Plate using a new tip for each well. Seal the Index Primer Plate with a new sealing film and store at +4 °C for up to 3 months for future use.

4. Spin down, then remove the seal from the 1st PCR Plate. Transfer 2 µl of the 1st PCR products to each of the three Index Primer PCR reactions allocated for each sample (e.g., aliquot sample 1 in wells A1, A2, A3 and sample 2 in

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wells A4, A5, A6) prepared in step 3. We recommend that you immediately record the Sample name and well number/ index number (e.g., Sample 1 in wells A1, A2, A3 and sample 2 in wells A4, A5, A6) for all the samples, including the positive control. This will help minimize mistakes in the NGS deconvolution step.

Component	Volume per sample, µl
Anchored PCR Product (from the step above)	2
Index PCR Master Mix (above)	50
Total	52

4. Seal the plate (or portion of the plate) with new adhesive film, and spin it down to collect droplets. Load the plate in the thermal cycler, and run the following program:

Temperature	Time	Cycles
98°C	30 sec	1
98°C	20 sec	8
65°C	10 sec	
72°C	45 sec	
72°C	30 sec	1
4°C	∞	1

! Stopping Point in procedure: PCR products may be stored at 4°C for a week.

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7. NGS Prep for Sequencing

The product from the Second PCR with Indexed Primers step contains both P5 and P7 sequences and dual UD Indexes designed for Next-Gen Sequencing (NGS) on Illumina instruments. The protocol in this section provides the instructions to analyze and adjust the amount of each Amplified Indexed Library to obtain similar reads for each sample, to combine and clean up samples before loading onto the instrument, and guidelines for sequencing and data analysis.

Last modified: 25 March 2025

7.1. QC, Quantify and Combine Samples for NGS

In this step, the yield of products from the Index Primer PCR Reaction—the Amplified Indexed Libraries of transcripts from each sorted plate sample—is analyzed, measured, adjusted if necessary, and then pooled in equimolar amounts for sequencing.

Perform QC and Quantify Amplified Indexed Libraries

1. Analyze the Amplified Indexed Libraries using one of the following methods:

    - **Standard Method:** Separate 5 µl of Amplified Indexed Libraries on a 3% agarose-TAE gel and analyze the size distribution of NGS probes by UV transilluminator. See Fig. 3 below for the expected results of the amplified library generated from scAIR positive control RNA (1 plate) after 2nd PCR step with indexed primers.

    - **Alternative Method:** Analyze 1 µl of each of the Amplified Indexed Libraries on either an Agilent Bioanalyzer with the Agilent High Sensitivity DNA Kit (Cat.# 5067-4626), Tape Station or Fragment Analyzer using the High Sensitivity NGS Analysis Kit (Cat.# DNF-473-1000) using the manufacturer's protocol. Fig. 4 below shows the results of the analysis of amplified 2nd PCR products using the Fragment Analyzer. For the human and mouse scAIR Chain Pairing assay, CDR1-2-3 TCR and BCR amplicons have a size of approximately 500 bp. Depending on the composition of TCR/BCR clonotypes, amplified CDR1-2-3 products could appear as a few bright bands (low diversity samples) or as a broader peak around 500-600bp (high diversity samples).

> ✻ Note: In some cases, excess PCR primers compromise resolution and complicate data analysis with the BioAnalyzer or Fragment Analyzer systems. For such cases, we recommend running a conventional gel to look at the samples.

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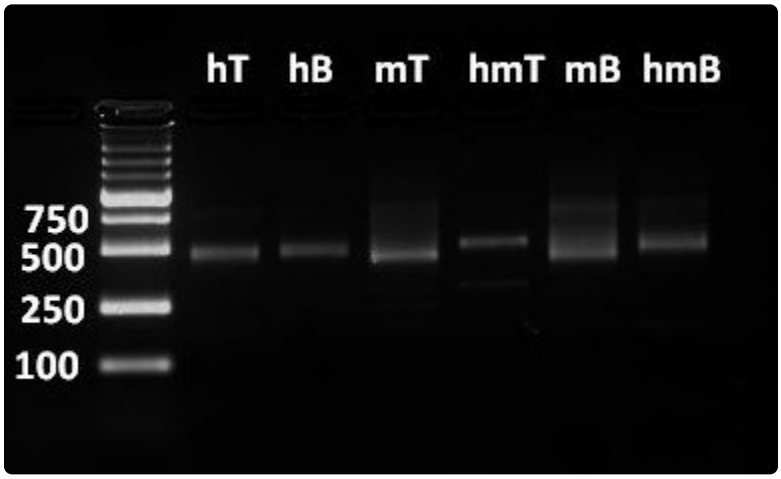
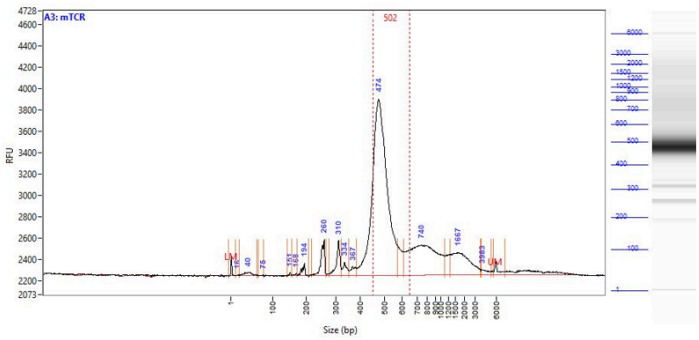
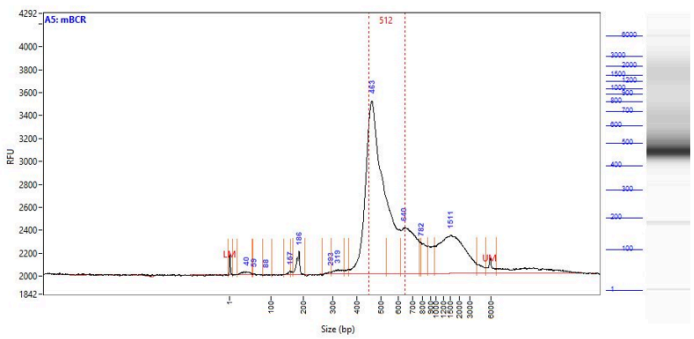


Fig 3. Gel electrophoresis of Amplified Indexed Libraries. Gel image of amplicons after Index Primer PCR Reaction of scAIR chain pairing assays generated from positive control RNAs for TCR-Human (hT), BCR-Human (hB), TCR-Mouse (mT), TCR-Hybrid, human-mouse (hmT), BCR-Mouse (mB) and BCR-Hybrid human-mouse (hmB), and scAIR-BCR-Mark30 (B) libraries. For TCR-Hybrid and BCR-Hybrid samples amplified products are generated from human Forward FR1primers which are conservative between human and mouse FR1 sequences.

2. Adjust the Second PCR cycle number (optional step)
   The yield of amplified products after 2nd amplification step significantly depends on the transcriptional activity of sorted cells. For example, activated T or B cells are more transcriptionally active than naïve cells. Amplified products generated from Positive Control RNA (included in the kit) could be a useful reference for the analysis of PCR products and adjustment/troubleshooting of the scAIR protocol. If the analysis of amplified products didn't reveal any visible yield of expected PCR products, run the sample with the low yield of amplified indexed library for an additional 3-5 PCR cycles and repeat the analysis. Optimize the number of cycles in 2nd PCR step to get visible PCR products in all experimental samples (generated from different plates). It is not necessary to achieve the same yield of PCR products for different amplified indexed libraries; differences in the yield between samples will be adjusted when the samples are combined in the next step.

   - **Quantify amplified indexed libraries using Fragment Analyzer/Bioanalyzer:** Analyze yields of the Amplified Indexed Libraries using Fragment Analyzer/Bioanalyzer software and combine different Amplified Indexed Library products (from all replicate samples) together in equal amounts using quantitation gates set up between 450-700 bp. Please refer to Section 8 for recommendations on how many samples (replicates) can be combined together for different NGS flow cells. If PCR primers complicate data analysis, we recommend removing PCR primers by incubation amplified products for each sample with Primer Removal Enzyme (see step below) and repeat analysis/quantification of Amplified Indexed Libraries in Bioanalyzer or Fragment Analyzer.





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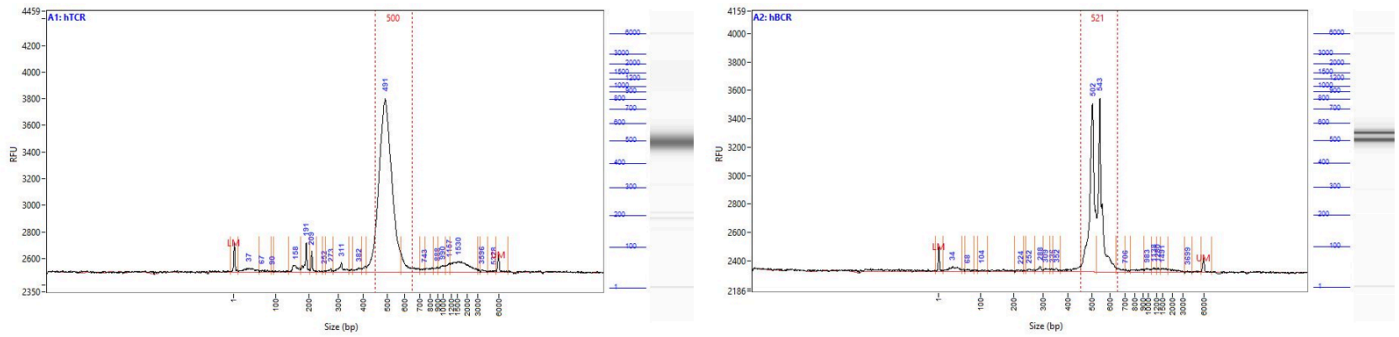


Fig 4. Fragment Analyzer Image of Amplified Indexed Libraries of scAIR chain pairing assays generated from positive control RNAs for TCR-Human (mT), BCR-Human (hB), TCR-Mouse (mT), and BCR-Mouse (mB) assays.

3. Remove excess primers from the pooled Amplified Indexed Library by adding 2 µl of Primer Removal Enzyme to the combined sample (e.g., 100 µl), then incubate at 37°C for 30 minutes.

> ! Stopping Point in procedure: PCR products may be stored at 4°C overnight or at -20°C for a few weeks.

Last modified: 11 June 2026

7.2. Purification & Quantification of Amplified Indexed Library

The purpose of this step is to remove any residual primers and reagents from the pooled Amplified Indexed Library so that the preparations are ready for NGS.

1. Add 1.5x volume of SPRIselect beads (adjusted to room temperature) to pooled Amplified Indexed Library, mix in an Eppendorf tube, and pipet up and down 5 times to thoroughly mix the bead suspension with the pooled Amplified Indexed Library.

2. Incubate the mixture for 5 minutes at room temperature.

3. Place the tube in the Magnetic Stand for 1 minute or until the solution is clear. Carefully remove and discard the supernatant without disturbing the pellet.

4. Add 500 µl of freshly prepared 80% ethanol to the tube.

5. Place the tube in the Magnetic Stand for 2 minutes or until the solution is clear. Carefully remove and discard the supernatant without disturbing the pellet.

6. Repeat steps 4 and 5 for a second wash.

7. Briefly centrifuge tubes at low speed and place the tubes in the Magnetic Stand. Use a 200-µl pipette to remove the residual ethanol droplets from the tube and air-dry the beads at room temperature for 2 minutes.

8. Add 45 µl of TE buffer to the pellet to disperse the beads and let stand for 1 minute.

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9. Place the tube on the Magnetic Stand for 1 minute. Transfer 40 µl of the supernatant to a new Eppendorf tube.

10. Measure the concentration of pooled Amplified Indexed Library sample using the Qubit® dsDNA High Sensitivity Assay or equivalent.

11. Dilute the pooled Amplified Indexed Library sample to 2.6 ng/µl, which corresponds to a concentration of 10nM for the following NGS step.

> ! Stopping Point in procedure: PCR products may be stored at 4°C overnight or at -20°C for a few days.

Last modified: 7 July 2025

7.3. Next Generation Sequencing of Pooled Amplified Indexed Libraries

Pooling of Amplified indexed libraries

For the DriverMap scAIR TCR/BCR Chain Pairing Assays, which generates a low complexity pool of amplified products for TCR/BCR genes for 96 single cells (per plate), sequencing depth is not a critical issue for most Illumina's NGS kits. Generally, we recommend using low-output 600-cycle Paired-End NGS reagent kits and aim for 5 million reads per sample or 50 million reads for eight pooled Amplified Indexed Library samples. Under these NGS sequencing depth conditions, you could generate at least 20 reads per number of molecules (for TCR or BCR chains), an optimal condition for downstream NGS data analysis. Please refer to the table below for guidelines on how many samples may be combined for different instruments.

Instrument	Reads per flow cell	Number of pooled Amplified Library samples (each sample is triplicate) per flow cell
MiSeq	15 million (V2 Reagent Kit)	3
MiSeq	25 million (V3 Reagent Kit)	5
NextSeq 2000	100 million (P1 flow cell)	8

Sequencing on NextSeq 1000/2000. The instructions below are provided for sequencing of pooled Amplified Indexed Libraries using the NextSeq 1000/2000 XLEAP-SBS P1 reagent kit (600 cycles) (Cat.# 20100981) and NextSeq 1000/2000 XLEAP-SBS Read Primer Kit (Cat.# 20112859).

> ✻ For full-length immune receptor sequencing, a 600-cycle kit is required, which is available only on the NextSeq 1000/2000 P1 Reagents, P2 Reagents, MiSeq v2, v3, or MiSeq i100 25M Reagent Kit.

Sequencing of Pooled Amplified Indexed Libraries on NextSeq 1000/2000

The instructions below are provided for sequencing of pooled Amplified Indexed Libraries using the NextSeq 1000/2000 XLEAP-SBS P1 reagent kit (600 cycles) Cat.# 20100981, and NextSeq 1000/2000 XLEAP-SBS Read Primer Kit (Cat.#

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20112859).

Follow the standard Illumina protocol for the XLEAP-SBS kit on how to prepare XLEAP-SBS consumables, dilute libraries, and set up a sequencing run:
https://support-docs.illumina.com/IN/NextSeq10002000/Content/IN/NextSeq2000_1000/Protocol_seq_xleap.htm

Modifications of the standard Illumina protocol, specific for the use of sc-AIR assay custom sequencing primers are the following:

1. Preparation of diluted pooled Amplified Indexed Library. Dilute purified pooled Amplified Indexed Library (from Section 7.2) to 1,000 pM loading concentration by mixing 21.6 µl of RSB buffer with 2.4 µl of purified pooled Indexed Library (10 nM). Add 2 µl of PhiX Control (1 nM concentration) to the diluted indexed library.

2. Preparation of diluted Custom AIR Sequencing Primers. Custom AIR sequencing primers provided in the kit need to be diluted using HT1 buffer (from NextSeq 1000/2000 XLEAP-SBS Read Primer Kit),

    - Prepare Custom DNA Read primer mix: combine 1.8 µl of Forward SeqDNA primer (100 µM) and 1.8 µl of Reverse SeqDNA primer (100 µM) with 600 µl of HT1 buffer.

    - Prepare Custom Index primer mix: Combine 3.6 µl of Forward SeqIND primer (100 µM) and 3.6 µl of Reverse SeqIND primer (100 µM) with 600 µl of HT1 buffer.



3. Before loading the diluted Amplified Indexed Library and Custom Read and Custom Index primer mixes, make sure that the reagent cartridge is thawed and inspected before proceeding. Invert the cartridge several times to ensure complete reagent mixing. You will not be able to do this once the indexed library and primers are loaded.

4. Transfer 600 µl of the custom read primer into the “Custom 1” well and 600 µl of the custom index primer into the “Custom 2” well.

5. Load 26 µl of diluted Amplified Indexed Library in the Library port well.

6. Log in to BaseSpace to run monitoring and select manual setup mode. When in manual setup mode, enter the read lengths, then specify the custom primer wells from the drop-down under each read to the correct well.

Program	Cycles

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Custom 1:	309
Custom 2:	10
Custom 2:	10
Custom 1:	309

Note: The program has a total of 638 cycles. There are enough reagents in the 600-cycle kit to run at least 638 cycles.

7. This protocol will direct the instrument to perform NGS using the custom primers, not the default stock material inside the cartridge. Once that is complete, you can follow the standard protocol to start the run: https://support-docs.illumina.com/IN/NextSeq10002000/Content/IN/NextSeq2000_1000/Protocol_seq_xleap.htm

Last modified: 15 May 2025

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8. Data Processing and Analysis

The protocol in this section describes the procedure to extract T- and B- Cell receptor repertoire from NGS data generated from the Cellecta DriverMap AIR profiling kit using MiXCR or Platforma computational tool.

MiXCR is the leading software used for immune repertoire data analysis. It is free for academic and non-profit use (see License). For commercial use, a license can be requested at MiLaboratories-Licensing Information or by email to licensing@milaboratories.com. Please refer to Appendix C for installing instructions.

There are also other open-source bioinformatic tools/frameworks used to analyze immune repertoires. Please refer to these papers for a comparison of these computational tools:

- Rigorous benchmarking of T-cell receptor repertoire profiling methods for cancer RNA sequencing
- Benchmarking immunoinformatic tools for the analysis of antibody repertoire sequences

Tools	Published year	Aligner	Website
TRUST4	2008	IMGT	TRUST4 Github
IMGT/HighV-QUEST	2012	GPA (NW)	IMGT
IgBLAST	2013	BLAST	NCBI
IMSEQ	2015	SCF matching	IMTools
LymAnalyzer	2015	Fast-tag	LymAnalyzer

Mac OS and Linux OS are recommended for data processing and analysis.

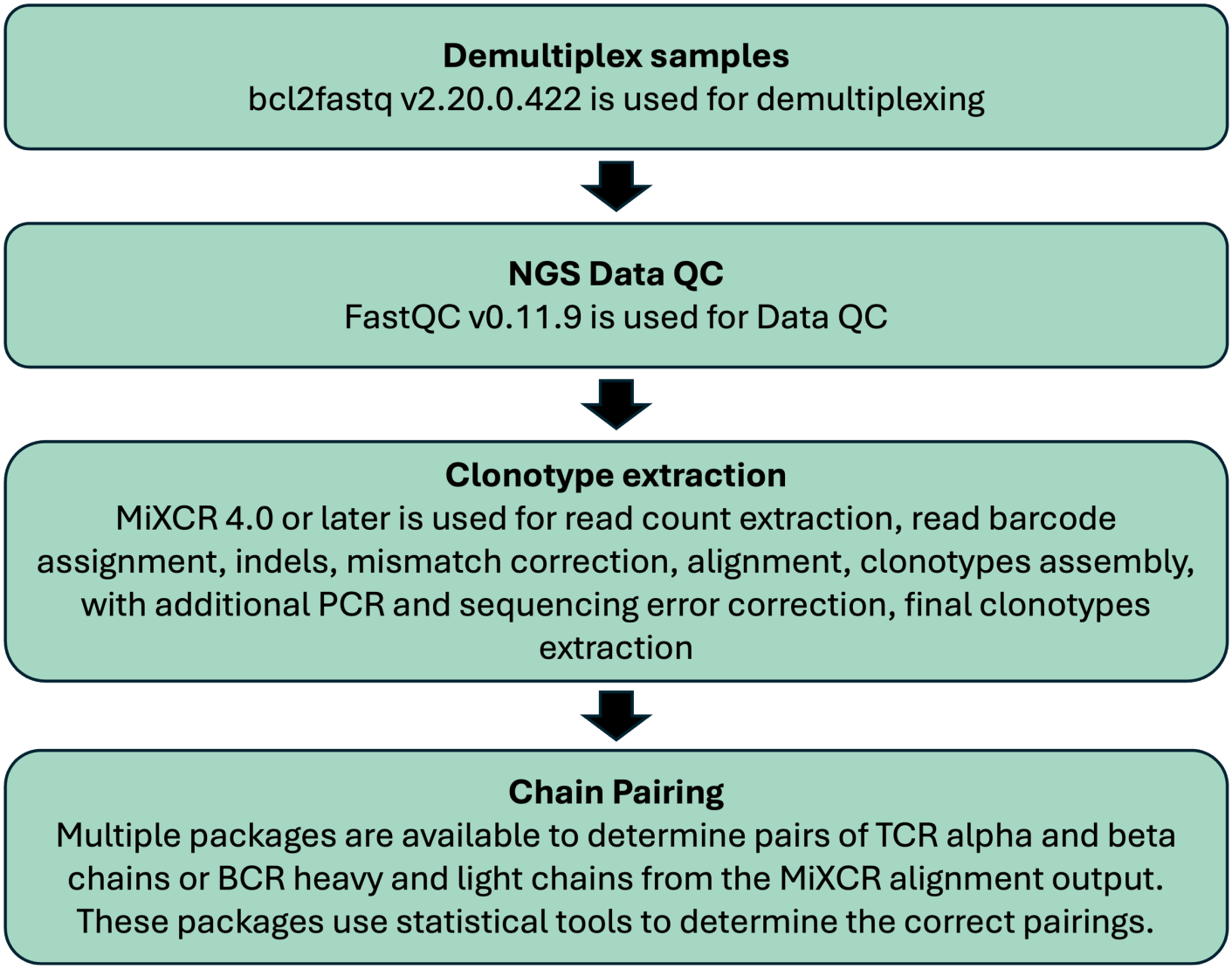
> Cellecta does not own any of these software packages and is not responsible for maintaining them. In case you don't have the license, Cellecta also provides custom data analysis services using the MiXCR software package. Please email tech@cellecta.com for more information.

Last modified: 15 May 2025

8.1. Extract T- and B- Cell Receptor Chain Pairing Workflow

The following bioinformatics workflow is recommended for the DriverMap scAIR assay:

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8.2. Convert Sequencing Data to FASTQ Format

bcl2fastq2 converter is used to demultiplex sequencing data and convert base call (BCL) files to FASTQ files. The user manual can be downloaded from the following link:Illumina Documentation

Refer to Appendix D or download Example sample sheet *(Right-click link and select Save Link As… to save .txt file)*

This step can be overlooked if demultiplexed fastq files were generated from NGS sequencing core.

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8.3. NGS Data QC

FastQC provides a modular set of analyses that you can use to give a quick impression of whether your data has any

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problems of which you should be aware before doing any further analysis. The user manual can be found at the following link:FastQC Guide

Last modified: 24 February 2025

8.4. Alignment and clonotypes assembly using MiXCR

Clonotype extraction can be performed using MiXCR. The pipeline includes alignment of raw sequencing reads using align, error correction using correctAndSortTags, assembly of aligned sequences into clonotypes using assemble, and exporting the resulting clonotypes into tab-delimited files using exportClones. The reads can be aligned with MiXCR using the following command:

```
mixcr -Xmx40g analyze local:cellecta-new-singleCell-4xVBC6 \
--use-local-temp \
--export-productive-clones-only \
--tag-pattern $TAG_PATTERN \
--rigid-left-alignment-boundary \
--floating-right-alignment-boundary C \
--assemble-clonotypes-by {CDR1Begin:FR4End} \
$READ_1 \
$READ_2 \
$OUTPUT_NAME
```

where:

```
$READ_1, $READ_2 = paired end read file names (e.g. sample_a_R1.fastq.gz, sample_a_R2.fastq.g
z)
$OUTPUT_NAME = name of output files (e.g. sample_a)
```

The $TAG_PATTERN is always:

```
'^(R1:*)\^(MIWELL:N{10})(MIVBC:GCATCA)(R2:*)|^(MIWELL:N{10})(MIVBC:AGTCGT)(R2:*)|^(MIWELL:N{1
0})(MIVBC:TCGCAT)(R2:*)|^(MIWELL:N{10})(MIVBC:AGCGTA)(R2:*)'
```

where MIWELL specifies the well-barcode and MIVBC specifies the VBC.

The preset can be accessed by saving the file cellecta-new-singleCell-4xVBC6.yaml in the directory of the MiXCR script.

More details can be found on the MiXCR official website: Cellecta AIR Assay Guide.

Last modified: 15 May 2025

8.5. Identification of Chain Pairs

Multiple open-source software are available to identify pairings for TCR alpha and beta chains or BCR heavy and light chains from bulk loading of 96-well plates. The following tools have been published and are available online:

Once alignment has been completed, the exported clonotype tables from MIXCR are used as input to the tools indicated above. The installation and analysis procedures can be found on each software's GitHub page. Note that the operating

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system and package installation requirements can differ for each software.

Tools	Published year	Publication	Github
ALPHABETR	2017	Identifying T Cell Receptors from High-Throughput Sequencing: Dealing with Promiscuity in TCRα and TCRβ Pairing	link
MAD-HYPE	2019	A Bayesian framework for high-throughput T cell receptor pairing	link
T-SHELL	2024	TIRTL-seq: Deep, quantitative, and affordable paired TCR repertoire sequencing	link

Each of these software uses the co-occurrence of TCRα and TCRβ chains in 96-well plates. Frequentist or Bayesian statistics is used to pair the receptor chains confidently. Highly co-occurring chains are assigned the most likely true receptor pairs. Each of these software programs has its unique advantages:

- **ALPHABETR** can identify complex pairings that include TCRαs that have the same TCRβ chain or cells that contain one unique TCRβ and two TCRαs.
- **MAD-HYPE** uses Bayesian statistics to identify highly confident pairings.
- **T-SHELL** considers the usage of co-occurrence and the correlation of the read counts belonging to the receptor sequences.

Depending on the sample type and number of wells used for analysis, loading ~100 cells per well can yield somewhere between dozens to hundreds of pairings. Experiments that use more than 1 plate (i.e.,> 96 wells) for one sample source can even yield a greater number of pairings.

Note: The T-SHELL program allows for the loading of thousands of cells within each well. This allows for a yield of thousands of receptor pairs. However, this has not been tested with the DriverMap™ Single cell AIR TCR-BCR Chain Pairing Assay.

Last modified: 8 July 2025

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9. Appendices

Appendix A. Tips and Troubleshooting
Appendix B. Structure of Amplified Indexed Library
Appendix C. Obtaining and Installing Software for Data Analysis
Appendix D. List of Barcodes in the 96-well Plate

Last modified: 15 May 2025

9.1. Appendix A. Tips and Troubleshooting for scAIR Kit

Observation	Possible Cause	Recommended Action
The yield of PCR products for sorted T/B cells is low, but is good for the Positive Control RNA sample — or — Low yield of PCR products for both sorted cells and Positive Control RNA samples	Error in sorting cells, or cellular RNA is degraded	Optimize sorting/gating conditions, sort only viable cells using dead-cell staining protocol, and add RNAse inhibitor (e.g., 1µl/well) in Hybridization Master mix before sorting. Perform routine cleaning and maintenance of the sorter by following the guidelines and use RNaseAlert™ Lab Test Kit. Please refer to Section 5.2 for running the Positive Control Experiment before running experimental samples
	Low RNA content in sorted cells	Increase the number of amplification cycles in the First PCR with an Anchor primer or 2nd PCR with Indexed Primers.
	RT step is not optimal	Please check for procedural mistakes. Make master mixes where possible. For individual reaction setups, make sure that the correct volume of RT-EXT Buffer and RT enzyme is added into each reaction.
	PCR cycling conditions are not optimal	Increase PCR amplification cycles. Ensure proper dispensing and mixing of viscous components at each step.

Observation	Possible Cause	Recommended Action
Low Cluster Density	Inefficient purification from low molecular weight RNA products or low yield of correct target PCR products	Re-purify PCR products using different technology (e.g. QIAGEN QIAquick PCR Purification Kit), and/or increase the concentration of pooled Indexed Library products in the cluster generation step.

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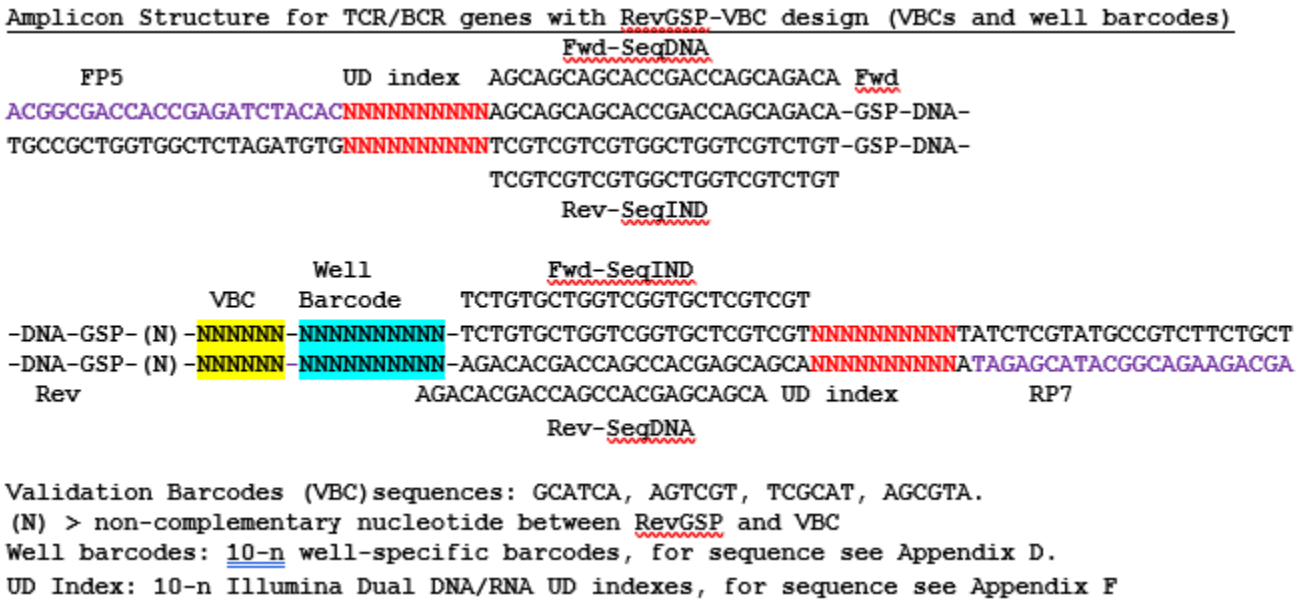
Observation	Possible Cause	Recommended Action
Yields of PCR products are significantly higher than Positive Control	RNA content in sorted cells is too high	Decrease number of PCR cycles in 1st or 2nd PCR steps
Low fluorescence readings on Illumina sequencer — or — Lower than expected number of on-target reads — or — High mutation rate in the Index reads	PCR primers from amplified indexed library quantification step (Quantify and Combine Samples for NGS) were not removed	Incubate Amplified Indexed Library with Primer Removal Buffer and Primer Removal Enzyme and repeat purification and quantification steps.
Lower than expected number of on-target reads	Yield of amplified products is too low or PCR cycle number too low	Increase the number of PCR cycles in the 1st PCR or 2nd PCR step.
	Amplified Indexed Library has off-target contaminations	Re-purify pooled Amplified Indexed Library in agarose gel (200bp-700bp products) and repeat quantification and NGS step.
Uneven representation of Indexed Libraries	Inaccurate Indexed Library quantification	Check that you correctly calculated the molar concentration of each indexed library sample.
	Inaccurate Indexed Library mixing	Re-quantify the indexed library samples and mix them in equimolar amounts.
Inconsistent library yields from replicate or similar sorted cell samples	Sample evaporation in thermal cycler or mistakes in adding correct volume of reagents in different wells	Seal 96-well plates well with an Adhesive Film Applicator and use a Compression Pad. Visually control the volume of reagents aliquoted in different wells/test tubes and similar volumes in wells/test tubes after incubation steps.

Last modified: 26 November 2025

9.2. Appendix B. Structure of Amplified Indexed Library

Amplicon Structure and position of NGS sequencing primers

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9.3. Appendix C. Obtaining and Installing Software for Data Analysis

MiXCR Installation on different operation systems (More details can be obtained from the following link: https://mixcr.readthedocs.io/en/master/install.html).

MiXCR installation on Mac OS / Linux using Homebrew

Homebrew is a simple package manager developed for Mac OS and also ported to Linux. To install MiXCR using Homebrew just type the following commands:

```
$ brew tap milaboratory/all
$ brew install mixcr
```

MiXCR Installation on Mac OS X / Linux / FreeBSD from zip distribution

- Check that you have Java 1.8+ installed on your system by typing in the command line:
  `$ java -version`
- Download latest binary distributaion of MiXCR from the release page on GitHub
- Unzip the archive
- Add extracted folder of MiXCR distribution to your PATH variable or add a symbolic link for MiXCR script to your bin/ folder (e.g. ~/bin/ in Ubuntu and many other popular Linux distributions)

MiXCR Installation on Windows

Currently, there is no execution script or installer for Windows. Still, MiXCR can easily be used by direct execution from the jar file.

- Check that you have Java 1.8+ installed on your system by typing in the command line:
  `$ java -version`

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- Download latest binary distribution of MiXCR from the release page on GitHub
- Unzip the archive
- Use mixcr.jar from the archive in the following way:

```
> java -Xmx4g -Xms3g -jar path_to_mixcr\jar\mixcr.jar ...
```

Bcl2fastq2 and FastQC Installation

Details on how to download, install, and execute bcl2fastq2 and FastQC can be obtained from the links below:
Download and install bcl2fastq2: https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software/downloads.html
Download FastQC: https://www.bioinformatics.babraham.ac.uk/projects/download.html#fastqc
Install FastQC: https://raw.githubusercontent.com/s-andrews/FastQC/master/INSTALL.txt

> Note: FastQC can also be installed and run as a “click-and-go” Desktop application on your Windows/Linux FastQC v0.11.9 (Win/Linux zip file) or MacOS personal computer FastQC v0.11.9 (Mac DMG image).

Last modified: 12 August 2024

9.4. Appendix D. List of Dual DNA/RNA UDI

Download NGS Example Sample Sheet for Set A, B, C and D *(Right-click link and select Save Link As… to save .txt file)*

Set A Unique Dual Index Kit (1-96)

Sample_ID	Sample_Name	I7_Index_ID	index	I5_Index_ID	index2
A1	1	UDP0001	GAACTGAGCG	UDP0001	CGCTCCACGA
A2	2	UDP0002	AGGTCAGATA	UDP0002	TATCTTGTAG
A3	3	UDP0003	CGTCTCATAT	UDP0003	AGCTACTATA
A4	4	UDP0004	ATTCCATAAG	UDP0004	CCACCAGGCA
A5	5	UDP0005	GACGAGATTA	UDP0005	AGGATAATGT
A6	6	UDP0006	AACATCGCGC	UDP0006	ACAAGTGGAC
A7	7	UDP0007	CTAGTGCTCT	UDP0007	TACTGTTCCA
A8	8	UDP0008	GATCAAGGCA	UDP0008	ATTAACAAGG
A9	9	UDP0009	GACTGAGTAG	UDP0009	CACTATCAAC
A10	10	UDP0010	AGTCAGACGA	UDP0010	TGTCGCTGGT
A11	11	UDP0011	CCGTATGTTC	UDP0011	ACAGTGTATG
A12	12	UDP0012	GAGTCATAGG	UDP0012	AGCGCCACAC
B1	13	UDP0013	CTTGCCATTA	UDP0013	CCTTCGTGAT
B2	14	UDP0014	GAAGCGGCAC	UDP0014	AGTAGAGCCG
B3	15	UDP0015	TCCATTGCCG	UDP0015	TCGTGCATTC
B4	16	UDP0016	CGGTTACGGC	UDP0016	CTATAGTCTT
B5	17	UDP0017	GAGAATGGTT	UDP0017	TTGCTGCCGA
B6	18	UDP0018	AGAGGCAACC	UDP0018	CCATCATTAG

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B7	19	UDP0019	CCATCATTAG	UDP0019	AGAGGCAACC
B8	20	UDP0020	GATAGGCCGA	UDP0020	GCCATGTGCG
B9	21	UDP0021	ATGGTTGACT	UDP0021	AGGACAGGCC
B10	22	UDP0022	TATTGCGCTC	UDP0022	CCTAACACAG
B11	23	UDP0023	ACGCCTTGTT	UDP0023	ACGTTCCTTA
B12	24	UDP0024	TTCTACATAC	UDP0024	TTACAGTTAG
C1	25	UDP0025	AACCATAGAA	UDP0025	CCATCTCGCC
C2	26	UDP0026	GGTTGCGAGG	UDP0026	TTGCTCTATT
C3	27	UDP0027	TAAGCATCCA	UDP0027	AATGGATTGA
C4	28	UDP0028	ACCACGACAT	UDP0028	CCGCATACGA
C5	29	UDP0029	GCCGCACTCT	UDP0029	CGAGGTCGGA
C6	30	UDP0030	CCACCAGGCA	UDP0030	ATTCCATAAG
C7	31	UDP0031	GTGACACGCA	UDP0031	GTCCGTAAGC
C8	32	UDP0032	ACAGTGTATG	UDP0032	CCGTATGTTC
C9	33	UDP0033	TGATTATACG	UDP0033	TGTAATCGAC
C10	34	UDP0034	CAGCCGCGTA	UDP0034	CACGGCTAGT
C11	35	UDP0035	GGTAACTCGC	UDP0035	TCACCAACTT
C12	36	UDP0036	ACCGGCCGTA	UDP0036	AATATTGCCA
D1	37	UDP0037	TGTAATCGAC	UDP0037	CGCGGTGATC
D2	38	UDP0038	GTGCAGACAG	UDP0038	ACGGATGGTA
D3	39	UDP0039	CAATCGGCTG	UDP0039	TTCCTACAGC
D4	40	UDP0040	TATGTAGTCA	UDP0040	CATTAGTGCG
D5	41	UDP0041	ACTCGGCAAT	UDP0041	TTCAGTTGTC
D6	42	UDP0042	GTCTAATGGC	UDP0042	CCTGACCACT
D7	43	UDP0043	CCATCTCGCC	UDP0043	AACCATAGAA
D8	44	UDP0044	CTGCGAGCCA	UDP0044	TGGCCGGATT
D9	45	UDP0045	CGTTATTCTA	UDP0045	AACCTTATGG
D10	46	UDP0046	AGATCCATTA	UDP0046	TGGTAGAGAT
D11	47	UDP0047	GTCCTGGATA	UDP0047	TTCGCCACCG
D12	48	UDP0048	CAGTGGCACT	UDP0048	CCTATTGTTA
E1	49	UDP0049	AGTGTTGCAC	UDP0049	CGTGTACCAG
E2	50	UDP0050	GACACCATGT	UDP0050	TACACGTTGA
E3	51	UDP0051	CCTGTCTGTC	UDP0051	TCACAACAGT
E4	52	UDP0052	TGATGTAAGA	UDP0052	AAGGACGCAC
E5	53	UDP0053	GGAATTGTAA	UDP0053	AGGATGTGCT
E6	54	UDP0054	GCATAAGCTT	UDP0054	TGCGACGGAA
E7	55	UDP0055	CTGAGGAATA	UDP0055	AGTGGTTAAG
E8	56	UDP0056	AACGCACGAG	UDP0056	TATCCGAGGC
E9	57	UDP0057	TCTATCCTAA	UDP0057	CCAGTCGACG
E10	58	UDP0058	CTCGCTTCGG	UDP0058	TTGACTAGTA

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E11	59	UDP0059	CTGTTGGTCC	UDP0059	AACGGTCTAT
E12	60	UDP0060	TTACCTGGAA	UDP0060	CTGGAACTGT
F1	61	UDP0061	TGGCTAATCA	UDP0061	CTACATGCCT
F2	62	UDP0062	AACACTGTTA	UDP0062	TGAGACTTGC
F3	63	UDP0063	ATTGCGCGGT	UDP0063	GCGGAGCCAA
F4	64	UDP0064	TGGCGCGAAC	UDP0064	AGTATCAGTT
F5	65	UDP0065	TAATGTGTCT	UDP0065	TATGCCTTAC
F6	66	UDP0066	ATACCAACGC	UDP0066	CGCAGCAATT
F7	67	UDP0067	AGGATGTGCT	UDP0067	GGAATTGTAA
F8	68	UDP0068	CACGGAACAA	UDP0068	GTGCTAGGTT
F9	69	UDP0069	TGGAGTACTT	UDP0069	TCCACACAGA
F10	70	UDP0070	GTATTGACGT	UDP0070	TTGGAATTCC
F11	71	UDP0071	CTTGTACACC	UDP0071	AAGCGCGCTT
F12	72	UDP0072	ACACAGGTGG	UDP0072	ACAACGCTCA
G1	73	UDP0073	CCTGCGGAAC	UDP0073	AGCCTATGAT
G2	74	UDP0074	TTCATAAGGT	UDP0074	CCTTCTAACA
G3	75	UDP0075	CTCTGCAGCG	UDP0075	TACATCCATC
G4	76	UDP0076	CTGACTCTAC	UDP0076	TGACGGCCGT
G5	77	UDP0077	TCTGGTATCC	UDP0077	GTAAGCAACG
G6	78	UDP0078	CATTAGTGCG	UDP0078	TATGTAGTCA
G7	79	UDP0079	ACGGTCAGGA	UDP0079	AACGAGGCCG
G8	80	UDP0080	GGCAAGCCAG	UDP0080	CGGATGCTTG
G9	81	UDP0081	TGTCGCTGGT	UDP0081	AGTCAGACGA
G10	82	UDP0082	ACCGTTACAA	UDP0082	TCGCTATGAG
G11	83	UDP0083	TATGCCTTAC	UDP0083	TAATGTGTCT
G12	84	UDP0084	ACAAGTGGAC	UDP0084	AACATCGCGC
H1	85	UDP0085	TGGTACCTAA	UDP0085	AGTACTCATG
H2	86	UDP0086	TTGGAATTCC	UDP0086	GTATTGACGT
H3	87	UDP0087	CCTCTACATG	UDP0087	AGGAGGTATC
H4	88	UDP0088	GGAGCGTGTA	UDP0088	ACTTACGGAT
H5	89	UDP0089	GTCCGTAAGC	UDP0089	AAGATACACG
H6	90	UDP0090	ACTTCAAGCG	UDP0090	TTCATGGTTC
H7	91	UDP0091	TCAGAAGGCG	UDP0091	TATGATGGCC
H8	92	UDP0092	GCGTTGGTAT	UDP0092	GGAAGTATGT
H9	93	UDP0093	ACATATCCAG	UDP0093	ATTGCACATA
H10	94	UDP0094	TCATAGATTG	UDP0094	CACCTTAATC
H11	95	UDP0095	GTATTCCACC	UDP0095	TTGTCTACAT
H12	96	UDP0096	CCTCCGTCCA	UDP0096	CACCGATGTG

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Set B Unique Dual Index Kit (97-192)

Sample_ID	Sample_Name	I7_Index_ID	index	I5_Index_ID	index2
A1	97	UDP0097	TGCCGGTCAG	UDP0097	TTGTATCAGG
A2	98	UDP0098	CACTCAATTC	UDP0098	CACAACTTAA
A3	99	UDP0099	TCTCACACGC	UDP0099	TCACTGTCCG
A4	100	UDP0100	TCAATGGAGA	UDP0100	GTTGTAGTGC
A5	101	UDP0101	ATATGCATGT	UDP0101	CCAGGCACCA
A6	102	UDP0102	ATGGCGCCTG	UDP0102	AGGCCGTGGA
A7	103	UDP0103	TCCGTTATGT	UDP0103	TACACTACAA
A8	104	UDP0104	GGTCTATTAA	UDP0104	CGTGTCGTGG
A9	105	UDP0105	CAGCAATCGT	UDP0105	ATACATCACA
A10	106	UDP0106	TTCTGTAGAA	UDP0106	TATTGCGCTC
A11	107	UDP0107	GAACGCAATA	UDP0107	ACAGTAAGAT
A12	108	UDP0108	AGTACTCATG	UDP0108	ACCACGACAT
B1	109	UDP0109	GGTAGAATTA	UDP0109	TGATGGCTAC
B2	110	UDP0110	TAATTAGCGT	UDP0110	TTCTTAACCA
B3	111	UDP0111	ATTAACAAGG	UDP0111	ACGAACAACA
B4	112	UDP0112	TGATGGCTAC	UDP0112	ATGTTGTTGG
B5	113	UDP0113	GAATTACAAG	UDP0113	CTGAGCCGGT
B6	114	UDP0114	TAGAATTGGA	UDP0114	TCAGATTAAC
B7	115	UDP0115	AGGCAGCTCT	UDP0115	ACGTTAGCCG
B8	116	UDP0116	ATCGGCGAAG	UDP0116	AATTCTTGGA
B9	117	UDP0117	CCGTGACCGA	UDP0117	CAACGTTCGG
B10	118	UDP0118	ATACTTGTTC	UDP0118	TCGGCGGTTA
B11	119	UDP0119	TCCGCCAATT	UDP0119	CAGCACGGAG
B12	120	UDP0120	AGGACAGGCC	UDP0120	AGCTGGAATG
C1	121	UDP0121	AGAGAACCTA	UDP0121	TAGCATAACC
C2	122	UDP0122	GATATTGTGT	UDP0122	ACCGTGTGGT
C3	123	UDP0123	CGTACAGGAA	UDP0123	AGAGAACCTA
C4	124	UDP0124	CTGCGTTACC	UDP0124	TCGAGCCATA
C5	125	UDP0125	AGGCCGTGGA	UDP0125	AACGCACGAG
C6	126	UDP0126	AGGAGGTATC	UDP0126	TGCCAACTGG
C7	127	UDP0127	GCTGACGTTG	UDP0127	AATGCGAACA
C8	128	UDP0128	CTAATAACCG	UDP0128	CGATGCGGTT
C9	129	UDP0129	TCTAGGCGCG	UDP0129	TTAACCTTCG
C10	130	UDP0130	ATAGCCAAGA	UDP0130	CAGTGGCACT
C11	131	UDP0131	TTCGGTGTGA	UDP0131	ATACTTGTTC
C12	132	UDP0132	ATGTAACGTT	UDP0132	CAGCAATCGT
D1	133	UDP0133	AACGAGGCCG	UDP0133	ATCCAGGTAT

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D2	134	UDP0134	TGGTGTTATG	UDP0134	TAGAATTGGA
D3	135	UDP0135	TGGCCTCTGT	UDP0135	CGCTGTCTCA
D4	136	UDP0136	CCAGGCACCA	UDP0136	TAATTAGCGT
D5	137	UDP0137	CCGGTTCCTA	UDP0137	CTCGAATATA
D6	138	UDP0138	GGCCAATATT	UDP0138	TATCGGACCG
D7	139	UDP0139	GAATACCTAT	UDP0139	ACTCTATTGT
D8	140	UDP0140	TACGTGAAGG	UDP0140	CTAATAACCG
D9	141	UDP0141	CTTATTGGCC	UDP0141	ACTTGTTATC
D10	142	UDP0142	ACAACTACTG	UDP0142	TGTGATAACT
D11	143	UDP0143	GTTGGATGAA	UDP0143	TTACCTGGAA
D12	144	UDP0144	AATCCAATTG	UDP0144	CCTCTACATG
E1	145	UDP0145	TATGATGGCC	UDP0145	TATGACAATC
E2	146	UDP0146	CGCAGCAATT	UDP0146	ATAGCGGAAT
E3	147	UDP0147	ACGTTCCTTA	UDP0147	CACAGCGGTC
E4	148	UDP0148	CCGCGTATAG	UDP0148	CCGGTTCCTA
E5	149	UDP0149	GATTCTGAAT	UDP0149	GTCCACCGCT
E6	150	UDP0150	TAGAGAATAC	UDP0150	CGAATCTATA
E7	151	UDP0151	TTGTATCAGG	UDP0151	TGGCCTCTGT
E8	152	UDP0152	CACAGCGGTC	UDP0152	CAATAGGAAT
E9	153	UDP0153	CCACGCTGAA	UDP0153	CTGAGGAATA
E10	154	UDP0154	GTTCGGAGTT	UDP0154	TCAGAAGGCG
E11	155	UDP0155	ATAGCGGAAT	UDP0155	TACTCTGCGC
E12	156	UDP0156	GCAATATTCA	UDP0156	AATTGGCGCC
F1	157	UDP0157	CTAGATTGCG	UDP0157	CGCCATATCT
F2	158	UDP0158	CGATGCGGTT	UDP0158	ACCAAGCAGG
F3	159	UDP0159	TCCGGACTAG	UDP0159	ATTGTTCGTC
F4	160	UDP0160	GTGACGGAGC	UDP0160	TGGACCGCCA
F5	161	UDP0161	AATTCCATCT	UDP0161	GTAACTGAAG
F6	162	UDP0162	TTAACGGTGT	UDP0162	ACGGTCAGGA
F7	163	UDP0163	ACTTGTTATC	UDP0163	TCTAGGCGCG
F8	164	UDP0164	CGTGTACCAG	UDP0164	AACTTATCCT
F9	165	UDP0165	TTAACCTTCG	UDP0165	TGTCTGGCCT
F10	166	UDP0166	CATATGCGAT	UDP0166	CCGTTCAAGG
F11	167	UDP0167	AGCCTATGAT	UDP0167	GTAGGTGGTG
F12	168	UDP0168	TATGACAATC	UDP0168	ATACAAGCAA
G1	169	UDP0169	ATGTTGTTGG	UDP0169	TCATAGATTG
G2	170	UDP0170	GCACCACCAA	UDP0170	ATCAGTACCA
G3	171	UDP0171	AGGCGTTCGC	UDP0171	GTTGGATGAA
G4	172	UDP0172	CCTCCGGTTG	UDP0172	TGGTGTTATG
G5	173	UDP0173	GTCCACCGCT	UDP0173	GCTAATAGGA

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G6	174	UDP0174	ATTGTTCGTC	UDP0174	AATCTAGAGA
G7	175	UDP0175	GGACCAGTGG	UDP0175	TAGGCTCGCG
G8	176	UDP0176	CCTTCTAACA	UDP0176	AGAGCTTATC
G9	177	UDP0177	CTCGAATATA	UDP0177	TCGACATCTC
G10	178	UDP0178	GATCGTCGCG	UDP0178	ACATATCCAG
G11	179	UDP0179	TATCCGAGGC	UDP0179	CTTATTGGCC
G12	180	UDP0180	CGCTGTCTCA	UDP0180	GGTGAGTAAT
H1	181	UDP0181	AATGCGAACA	UDP0181	TCCGCCAATT
H2	182	UDP0182	AATTCTTGGA	UDP0182	AAGTTGACAA
H3	183	UDP0183	TTCCTACAGC	UDP0183	AGAATTCGCC
H4	184	UDP0184	ATCCAGGTAT	UDP0184	GCTGACGTTG
H5	185	UDP0185	ACGGTCCAAC	UDP0185	TGATGTAAGA
H6	186	UDP0186	GTAACTTGGT	UDP0186	AGGTATGGCG
H7	187	UDP0187	AGCGCCACAC	UDP0187	AAGACATTAG
H8	188	UDP0188	TGCTACTGCC	UDP0188	CCTCCGGTTG
H9	189	UDP0189	CAACACCGCA	UDP0189	TGCTACTGCC
H10	190	UDP0190	CACCTTAATC	UDP0190	TCTATCCTAA
H11	191	UDP0191	TTGAATGTTG	UDP0191	CTAGATTGCG
H12	192	UDP0192	CCGGTAACAC	UDP0192	AGTACAACTC

Set C Unique Dual Index Kit (193-288)

Sample_ID	Sample_Name	I7_Index_ID	index	I5_Index_ID	index2
A1	193	UDP0193	TCTCATGATA	UDP0193	TCCACGTGTT
A2	194	UDP0194	CGAGGCCAAG	UDP0194	CCGGTAACAC
A3	195	UDP0195	TTCACGAGAC	UDP0195	GTAACAATCT
A4	196	UDP0196	GCGTGGATGG	UDP0196	CATTGGTCAA
A5	197	UDP0197	TCCTGGTTGT	UDP0197	TGCCGGTCAG
A6	198	UDP0198	TAATTCTGCT	UDP0198	ATTGATGAGA
A7	199	UDP0199	CGCACGACTG	UDP0199	CTGTTGGTCC
A8	200	UDP0200	GAGGTTAGAC	UDP0200	GCAATACATT
A9	201	UDP0201	AACCGAGTTC	UDP0201	TCCAGAGATC
A10	202	UDP0202	TGTGATAACT	UDP0202	ATGGCGCCTG
A11	203	UDP0203	AGTATGCTAC	UDP0203	GGTCTATTAA
A12	204	UDP0204	GTAACTGAAG	UDP0204	TCGCGACTCC
B1	205	UDP0205	TCCTCGGACT	UDP0205	CTCTGGCGTT
B2	206	UDP0206	CTGGAACTGT	UDP0206	GTTAATTACG
B3	207	UDP0207	GAATATGCGG	UDP0207	TCAGTCTCGT
B4	208	UDP0208	GATCGGATAA	UDP0208	CGGCCGATAC
B5	209	UDP0209	GCTAGACTAT	UDP0209	ATGTCGTATT
B6	210	UDP0210	AGCTACTATA	UDP0210	GCCATATAAC

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B7	211	UDP0211	CCACCGGAGT	UDP0211	CATGGCAGGC
B8	212	UDP0212	CTTACCGCAC	UDP0212	ATAGGTCTTA
B9	213	UDP0213	TTAGGATATC	UDP0213	CCATGGTATA
B10	214	UDP0214	TTATACGCGA	UDP0214	AACAGACGGC
B11	215	UDP0215	CGCTTAGAAT	UDP0215	TATCACTCTG
B12	216	UDP0216	CCGAAGCGCT	UDP0216	ATAGTTAGCA
C1	217	UDP0217	CACTATCAAC	UDP0217	CATTAACTGA
C2	218	UDP0218	TTGCTCTATT	UDP0218	TCAAGGTCAC
C3	219	UDP0219	TTACAGTTAG	UDP0219	ATATGCATGT
C4	220	UDP0220	CTAAGTACGC	UDP0220	TAGGTATGTT
C5	221	UDP0221	TAGTTCGGTA	UDP0221	CTACACATGG
C6	222	UDP0222	CTATTACTAC	UDP0222	GGAGAGACTC
C7	223	UDP0223	TAGCATAACC	UDP0223	TGCGCATAGC
C8	224	UDP0224	ACTCTATTGT	UDP0224	CATATGCGAT
C9	225	UDP0225	TAGTGGAAGC	UDP0225	TATAGGTACT
C10	226	UDP0226	CGCCATATCT	UDP0226	ATCTCCGGTC
C11	227	UDP0227	GCTTCATATT	UDP0227	AGGCTGAACG
C12	228	UDP0228	ACTAGCGCTA	UDP0228	GAGGAAGTAA
D1	229	UDP0229	GCTCTTAACT	UDP0229	GGTGGACGTG
D2	230	UDP0230	GTGGTATCTG	UDP0230	AGATAGTAGC
D3	231	UDP0231	TGACGGCCGT	UDP0231	ATGGTTGACT
D4	232	UDP0232	CAGTAATTAC	UDP0232	TACCGCCTCG
D5	233	UDP0233	TACAAGACTT	UDP0233	TGAACACCTG
D6	234	UDP0234	CTGTGGTGAC	UDP0234	CCTGTCTGTC
D7	235	UDP0235	CTCCACTAAT	UDP0235	AACAAGTACA
D8	236	UDP0236	ATAGTTAGCA	UDP0236	CTACTTAGAG
D9	237	UDP0237	ATAGGTCTTA	UDP0237	CTGTGGTGAC
D10	238	UDP0238	TTCTTAACCA	UDP0238	GGTATGTAGA
D11	239	UDP0239	AAGGAAGAGT	UDP0239	GCCTAACGTG
D12	240	UDP0240	GGAAGGAGAC	UDP0240	AGACTCACCA
E1	241	UDP0241	TGAACGCGGA	UDP0241	TCCTTCGAAG
E2	242	UDP0242	CCTGCAACCT	UDP0242	CTGACTCTAC
E3	243	UDP0243	TTCATGGTTC	UDP0243	TGACAATGTC
E4	244	UDP0244	ATCCTCTCAA	UDP0244	GCCTTGCGGA
E5	245	UDP0245	CACTAGACCA	UDP0245	ATAAGGCAGT
E6	246	UDP0246	ATTATCCACT	UDP0246	TACGTGCGTA
E7	247	UDP0247	ATGGCGTGCC	UDP0247	ACTTCAAGCG
E8	248	UDP0248	TCCAGAGATC	UDP0248	TGAAGTGCAG
E9	249	UDP0249	ATGTCCAGCA	UDP0249	TTGTCCGCTG
E10	250	UDP0250	CAACGTTCGG	UDP0250	ATGCGGATCC

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E11	251	UDP0251	GCGTATTAAT	UDP0251	CAACACCGCA
E12	252	UDP0252	CCGAATCTGG	UDP0252	CTTGATTCAT
F1	253	UDP0253	TCTCAATACC	UDP0253	CGCGAACGTC
F2	254	UDP0254	AAGCATCTTG	UDP0254	TTGTTGAATG
F3	255	UDP0255	TCAGTCTCGT	UDP0255	ATAATCCGTG
F4	256	UDP0256	TGCAAGATAA	UDP0256	CCGTCCTCAA
F5	257	UDP0257	GTAACAATCT	UDP0257	GTATACAGAG
F6	258	UDP0258	CCATGGTATA	UDP0258	CTCCGCGAGA
F7	259	UDP0259	TCATACCGTT	UDP0259	CTGCGTTACC
F8	260	UDP0260	GGCGCCATTG	UDP0260	ATTGCGCGGT
F9	261	UDP0261	AGCGAATTAG	UDP0261	TGTTCCGGCT
F10	262	UDP0262	TTAGACCATG	UDP0262	CTTCCTAGGA
F11	263	UDP0263	CACACAGTAT	UDP0263	TTAGGCTCAA
F12	264	UDP0264	TCTTGTCGGC	UDP0264	ACACAGGTGG
G1	265	UDP0265	TACCGCCTCG	UDP0265	GGTTGCGAGG
G2	266	UDP0266	CTGTTATATC	UDP0266	ACAGCTATAC
G3	267	UDP0267	TAACCGGCGA	UDP0267	CTAATGTAGC
G4	268	UDP0268	AAGAGAGTCT	UDP0268	AAGATTCGTA
G5	269	UDP0269	GTAGGCGAGC	UDP0269	TGCGCTCCTA
G6	270	UDP0270	AACTTATCCT	UDP0270	ACGCCAGTAC
G7	271	UDP0271	ATTATGTCTC	UDP0271	GCTCTTAACT
G8	272	UDP0272	TATAACAGCT	UDP0272	TTATACGCGA
G9	273	UDP0273	CCAATGATAC	UDP0273	CGGCACACTC
G10	274	UDP0274	GAGGCCTATT	UDP0274	TCCGGACTAG
G11	275	UDP0275	AGCTAAGCGG	UDP0275	GCGTATTAAT
G12	276	UDP0276	CTTCCTAGGA	UDP0276	ATACTCTAGG
H1	277	UDP0277	CGATCTGTGA	UDP0277	AGTCTTCCTA
H2	278	UDP0278	GTGGACAAGT	UDP0278	AAGGCCACGG
H3	279	UDP0279	AACAAGTACA	UDP0279	GGATATATCC
H4	280	UDP0280	AGATTAAGTG	UDP0280	CCAAGAGGTG
H5	281	UDP0281	TATCACTCTG	UDP0281	ATGTAACGTT
H6	282	UDP0282	AGAATTCGCC	UDP0282	GAGCTTGCCG
H7	283	UDP0283	CCTGACCACT	UDP0283	ATAGCCAAGA
H8	284	UDP0284	AGCTGGAATG	UDP0284	CGCATTCCGT
H9	285	UDP0285	TGATAACGAG	UDP0285	CCTGCGGAAC
H10	286	UDP0286	CATAGTAAGG	UDP0286	GTAACTTGGT
H11	287	UDP0287	ATTGGCTTCT	UDP0287	CTGCGAGCCA
H12	288	UDP0288	GTACCGATTA	UDP0288	AACGTTAGTT

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Set D Unique Dual Index Kit (289-384)

Sample_ID	Sample_Name	I7_Index_ID	index	I5_Index_ID	index2
A1	289	UDP0289	AGATAGTAGC	UDP0289	ATGGCGTGCC
A2	290	UDP0290	ATTAGAAGAC	UDP0290	TCCAGCCTGC
A3	291	UDP0291	GCTCGCACAT	UDP0291	ATTAAGCCAT
A4	292	UDP0292	AATATTGCCA	UDP0292	GGTGTCACCG
A5	293	UDP0293	TCGTGCATTC	UDP0293	ATACCAACGC
A6	294	UDP0294	AAGATACACG	UDP0294	TGTTAGCACA
A7	295	UDP0295	TGCAATGAAT	UDP0295	TTACTTCTGG
A8	296	UDP0296	CTATGAAGGA	UDP0296	CAGGTATAAG
A9	297	UDP0297	GAAGACTAGA	UDP0297	AAGTTCTAGT
A10	298	UDP0298	AGGAGTCGAG	UDP0298	GTAAGCCTAA
A11	299	UDP0299	TTCACTCACT	UDP0299	TCTCATGATA
A12	300	UDP0300	GGTCCGCTTC	UDP0300	CTTGTGTGAG
B1	301	UDP0301	TAGTGGCTTG	UDP0301	CCAAGTAACT
B2	302	UDP0302	ATTGAGGTCC	UDP0302	ATATAATCCG
B3	303	UDP0303	GGAGAGACTC	UDP0303	TCTGCTTCAA
B4	304	UDP0304	CCGCTCCGTT	UDP0304	CTTCGCCGTA
B5	305	UDP0305	ATACATCACA	UDP0305	TCAATGGAGA
B6	306	UDP0306	TAGGTATGTT	UDP0306	CTTGGTCTCG
B7	307	UDP0307	CACCTAGCAC	UDP0307	ATGTCCAGCA
B8	308	UDP0308	TTCAAGTATG	UDP0308	CGATACCATC
B9	309	UDP0309	TTAAGACAAG	UDP0309	CAATTAAGCC
B10	310	UDP0310	CACCTCTCTT	UDP0310	AGGAGTCGAG
B11	311	UDP0311	TTCTCGTGCA	UDP0311	CTCTGTGTAT
B12	312	UDP0312	GCTAGGAAGT	UDP0312	TCGTCCGAGA
C1	313	UDP0313	TTAATAGCAC	UDP0313	CAGACGTGGT
C2	314	UDP0314	CATTCACGCT	UDP0314	TGAGTACAAC
C3	315	UDP0315	GGCACTAAGG	UDP0315	GTTGACCTGA
C4	316	UDP0316	ATTCGGTACA	UDP0316	TCCTCGGACT
C5	317	UDP0317	ACTAATCTCC	UDP0317	AGATTAAGTG
C6	318	UDP0318	TGTGTTAGTA	UDP0318	TAACAGAGTA
C7	319	UDP0319	CAACGACCTA	UDP0319	ATCGAGTCGC
C8	320	UDP0320	CGGTCGGCAT	UDP0320	CCTTGCCTAG
C9	321	UDP0321	TCGACGCTAG	UDP0321	TTCGAAGAGG
C10	322	UDP0322	CTCGTAGGCA	UDP0322	AAGAGGATGA
C11	323	UDP0323	AAGTTCTAGT	UDP0323	TTATCTTACC
C12	324	UDP0324	CCAAGAGGTG	UDP0324	CTGGCTCGTT
D1	325	UDP0325	ATATCTGCTT	UDP0325	AGATTGTCTA

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D2	326	UDP0326	TGGATCTGGC	UDP0326	TTCAGCATTG
D3	327	UDP0327	TTGAATCCAA	UDP0327	ACACCGTGAC
D4	328	UDP0328	CACGGCTAGT	UDP0328	CTTGTACACC
D5	329	UDP0329	GAGCTTGCCG	UDP0329	CCTGCAACCT
D6	330	UDP0330	AGCTAGCTTC	UDP0330	CTCCGTATTA
D7	331	UDP0331	CAATCCTTGT	UDP0331	TGCGTCTTCG
D8	332	UDP0332	CACCTGTTGC	UDP0332	ATGTGTCAAT
D9	333	UDP0333	CGTCACCTTG	UDP0333	CAATCGGCTG
D10	334	UDP0334	AATGACTGGT	UDP0334	ACGCGTGAGA
D11	335	UDP0335	ATGATTCCGG	UDP0335	CACGTCAGAG
D12	336	UDP0336	TTAGGCTCAA	UDP0336	TTCCATTCGA
E1	337	UDP0337	TGTAAGGTGG	UDP0337	CCAAGGCCTT
E2	338	UDP0338	CAACTGCAAC	UDP0338	GTTGCGTTCA
E3	339	UDP0339	ACATGAGTGA	UDP0339	AGCTAAGCGG
E4	340	UDP0340	GCAACCAGTC	UDP0340	TTCCTCGGTG
E5	341	UDP0341	GAGCGACGAT	UDP0341	TGATTATACG
E6	342	UDP0342	CGAACGCACC	UDP0342	GTTCTGTCAT
E7	343	UDP0343	TCTTACGCCG	UDP0343	TGCAATGAAT
E8	344	UDP0344	AGCTGATGTC	UDP0344	CAGGACATGA
E9	345	UDP0345	CTGAATTAGT	UDP0345	CGATCGAATT
E10	346	UDP0346	TAAGGAGGAA	UDP0346	AATGTCGGAA
E11	347	UDP0347	AGCTTACACA	UDP0347	GGTCGTGCCA
E12	348	UDP0348	AACCAGCCAC	UDP0348	GTGCTGTGGC
F1	349	UDP0349	CTTAAGTCGA	UDP0349	ACAACTACTG
F2	350	UDP0350	GCCTAACGTG	UDP0350	CTTGAGAGCT
F3	351	UDP0351	ACTTACTTCA	UDP0351	TAATTCCAGA
F4	352	UDP0352	CGCATTCCGT	UDP0352	CTCCACTAAT
F5	353	UDP0353	GATATCACAC	UDP0353	ACATATAGTC
F6	354	UDP0354	AGCGCTGTGT	UDP0354	GTTCCGAACG
F7	355	UDP0355	TCACCGCGCT	UDP0355	CTAGTATCGA
F8	356	UDP0356	GATAGCCTTG	UDP0356	CATTGTGGTA
F9	357	UDP0357	CCTGGACGCA	UDP0357	TGGTATACCA
F10	358	UDP0358	TTACGCACCT	UDP0358	CAACGAGAGC
F11	359	UDP0359	TCGTTGCTGC	UDP0359	TTCACGAGAC
F12	360	UDP0360	CGACAAGGAT	UDP0360	AGGTGGCCTT
G1	361	UDP0361	GTGTACCTTC	UDP0361	TAGCTCACAG
G2	362	UDP0362	ACCTGGCCAA	UDP0362	CGATCTGTGA
G3	363	UDP0363	TGTCTGGCCT	UDP0363	ATTGGCTTCT
G4	364	UDP0364	AGTTAATGCT	UDP0364	CGGCTGCAGT
G5	365	UDP0365	GGTGAGTAAT	UDP0365	ACTAGATGTT

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G6	366	UDP0366	TACTCTGCGC	UDP0366	CATAGTAAGG
G7	367	UDP0367	AGGTATGGCG	UDP0367	GTCTCGCCAC
G8	368	UDP0368	TCCAGCCTGC	UDP0368	TGGATCTGGC
G9	369	UDP0369	GCCATATAAC	UDP0369	GATATTGTGT
G10	370	UDP0370	AGTGCGAGTG	UDP0370	ATTACCTCCA
G11	371	UDP0371	CTGAGCCGGT	UDP0371	TACGTGAAGG
G12	372	UDP0372	AACGGTCTAT	UDP0372	CCGCGTATAG
H1	373	UDP0373	GTTGCGTTCA	UDP0373	CAACTGCAAC
H2	374	UDP0374	CTTCAACCAC	UDP0374	AGGCGCATAA
H3	375	UDP0375	TCTATTCAGT	UDP0375	TGTACTGAGA
H4	376	UDP0376	CAAGACGTCC	UDP0376	TCCGTATACT
H5	377	UDP0377	TGAGTACAAC	UDP0377	GTCCAAGCGT
H6	378	UDP0378	CCGCGGTTCT	UDP0378	AATCTACTCC
H7	379	UDP0379	ATTGATACTG	UDP0379	GGAGCGTGTA
H8	380	UDP0380	GGATTATGGA	UDP0380	CTCTATCGGA
H9	381	UDP0381	TGGTTCTCAT	UDP0381	CGGCCTTGAG
H10	382	UDP0382	TCAACCACGA	UDP0382	TATGAACTTG
H11	383	UDP0383	TATGAACTTG	UDP0383	CCTAAGGATT
H12	384	UDP0384	AGTGGTTAAG	UDP0384	GTATTCCACC

Last modified: 4 September 2026

9.5. Appendix E. List of Barcodes in the 96-well Plate

List of barcodes in 96-well plate (Right-click link and select Save Link As…)

Last modified: 4 September 2026

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Last modified: 24 February 2025

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