CELLECTA

USER MANUAL

DriverMap™ AIR DNA CDR3 TCR-BCR Profiling Kit (Human/Mouse) - V2.1

v1 — Last update: 2 November 2026

Cellecta, Inc.

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1. DriverMap™ AIR DNA Profiling Assay (human/mouse)

The DriverMap™ AIR TCR/BCR DNA Profiling Kits combine multiplexed PCR amplification with the depth and precision of Next-Generation Sequencing (NGS) to quantitatively measure TCR or BCR rearranged gene copy number (corresponding to a number of T and B cells) and analyze the CDR3 sequences of immune receptor genes. AIR TCR and AIR BCR Kits allow simultaneous profiling of CDR3 repertoire for all four TCR chains (TRA, TRB, TRD, TRG) and all three BCR chains (IGH, IGK, and IGL), respectively, in separate reactions.

Main advantages of DriverMap™ AIR DNA Assay:

- An easy-to-run, one-tube, single-day assay without intermediate purification step allows unbiased, comprehensive profiling of CDR3 regions for all seven TCR/BCR chains from genomic DNA isolated from whole blood, PBMC, tissue, or FFPE.
- The assay provides robust, quantitative, and reproducible measurements of the DNA clonotype copy number over as much as 4-orders of magnitude differences in representation level.
- The DriverMap protocol employs several mechanisms to minimize the amplification of off-target and primer dimer background products.
- A redundant set of experimentally validated forward primers (designed for variable FR3 region) and reverse primers (designed for the J region) are designed for functional TCR and BCR genes with the exclusion of non-functional pseudogenes and ORFs (as defined by IMGT database https://www.imgt.org/IMGTrepertoire/).
- The indexing primers are designed with Illumina's DNA 10-n UD Indexes to minimize NGS index-swapping background issues, and amplified products are compatible with 300-n paired-end NGS sequencing using Illumina's NextSeq/NovaSeq instruments.
- The reverse gene-specific primers are designed with Validator Barcodes (VBCs) to facilitate normalization and quantification of the copy number of TCR/BCR DNA molecules used in amplification steps and detection of low abundance clonotypes above background level.
- The concentration of reverse gene-specific primers is optimized to provide balanced amplification efficiency of all chains for TCR (TRA, TRB, TRD, and TRG) or BCR (IGH, IGK, and IGL) reactions.
- NGS data can be analyzed with the MiXCR software package available online. Cellecta also provides custom MiXCR data analysis services on request.

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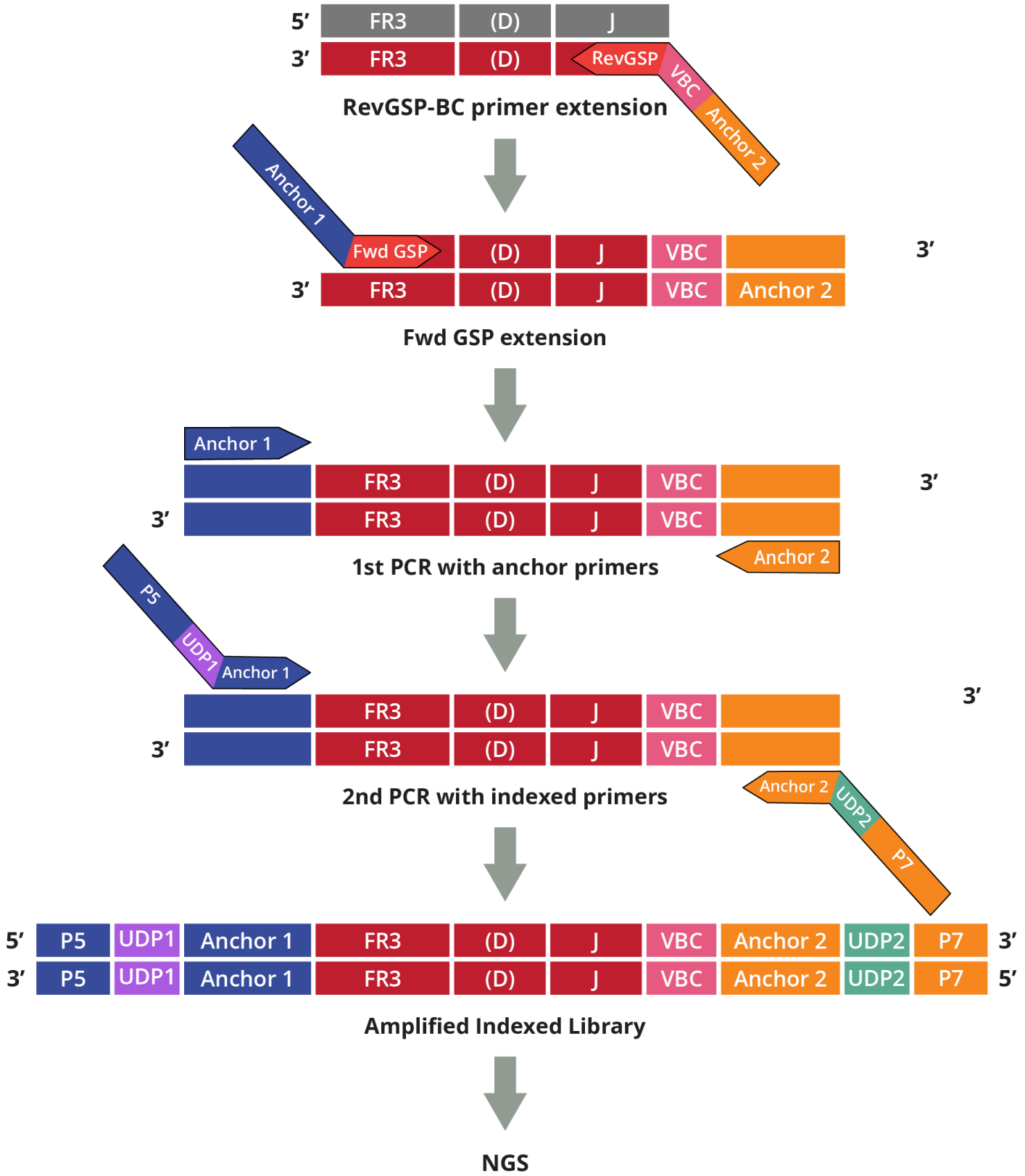


Fig.1. Outline of AIR DNA multiplex-PCR technology. Step1: Reverse gene-specific primers (GSP) with eight VBCs targeting TCR or BCR J-region isoforms (in separate reactions) are annealed to single, complementary DNA strand (top strand) and extended with DNA polymerase. Step 2: Forward GSPs are annealed to extended DNA template and after extension by DNA polymerase generate CDR3 regions flanking from both sides by universal anchor sequences (AP1 and AP2). Step 3: In the first PCR step, anchored universal primers (AP1 and AP2) amplify CDR3 DNA fragments. Step 4: The second PCR step amplifies CDR3 fragments using indexed primers. The indexed amplified products (separately for TCR and BCR) are combined in equal amounts and analyzed by

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NGS.

Additional Profiling Assays Compatible with the AIR DNA Assay:

- **DriverMap™ AIR TCR-BCR Profiling Kit (human RNA)** is designed to generate comprehensive AIR CDR3 repertoire profiling of all seven TCR (TRA, TRB, TRD, and TRG) and BCR (IGH, IGK, and IGL) chains from RNA samples. AIR RNA assay provides higher sensitivity in detecting CDR3 clonotypes than AIR DNA assay, especially from small biological samples. Both AIR RNA and AIR DNA assay could be effectively used together to identify antigen-induced clonotypes. For more details, please refer to AIR Technical Guide.
- **DriverMap™ EXP Genome-Wide Profiling Kit** is a convenient single-tube multiplex RT-PCR-NGS protocol that robustly measures the expression level of all 19,000 human protein-coding genes starting from a small amount of total RNA (down to single-cell level). Combined EXP and AIR profiling can be used for comprehensive phenotypic characterization of RNA samples and unbiased discovery of novel biomarkers associated with specific CDR3 clonotypes.

Related Products and Services:
For more detailed recommendations for your experimental design, please refer to the AIR Technical Guide. For more information on Cellecta's DriverMap kits and services, please visit the DriverMap Adaptive Immune Receptor (AIR) Profiling Service webpage.

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

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

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2. DriverMap™ AIR TCR-BCR Kit Components

DriverMap™ AIR TCR/BCR DNA Profiling Kit contains enough reagents to prepare NGS samples starting from genomic DNA purified from blood, tissue, cells, or other biological samples, including Positive Control DNA. AIR TCR and AIR BCR kits have the same common reagent set but different TCR-specific or BCR-specific primer sets.

Box 1: Kit Components

Component	Description	Cap Color	Concentration	Volume (24 rxn) *	Volume (96 rxn)
Reverse TCR-J primer mix (human/mouse)	Mix of reverse gene-specific primers for J-region of TRA, TRB, TRG and TRD	n/a	20X	80 µl	280 µl
Reverse BCR-J primer mix (human/mouse)	Mix of reverse gene-specific primers for J-region of IGH, IGL and IGK	n/a	20X	80 µl	280 µl
Forward TCR-FR3 primer mix (human/mouse)	Mix of forward gene-specific primers for FR3-region of TRA, TRB, TRG and TRD	n/a	20X	80 µl	280 µl
Forward BCR-FR3 primer mix (human/mouse)	Mix of forward gene-specific primers for FR3-region of IGH, IGL and IGK	n/a	20X	80 µl	280 µl
Positive Control DNA (human/mouse)	Total PBMC DNA	white	250 ng/µl	20 µl	40 µl
Water	MilliQ purified water for reaction mixes	white	NA	3 ml	12 ml
dNTP Mix	Mix of dATP, dGTP, dCTP ,and dTTP	white	10mM each	100 µl	400 µl
EXT Buffer	Buffer for Reverse and forward gene-specific primer extension steps, 5X	green	4X	400 µl	1.6 ml
PCR Buffer	Reaction Buffer for 1st and 2nd PCR steps, 5X	red	5X	600 µl	2×1.2 ml
DNA Polymerase	Multiplex-optimized thermostable hot-start DNA polymerase	red	100X	60 µl	200 µl
Anchor Primer Mix	Forward/Reverse Anchor-specific Primers for 1st PCR, 10X	red	10X	300 µl	1.2 ml
Primer Removal Enzyme	Enzyme Mix for primer removal	orange	25-50X	140 µl	560 µl
Forward SeqDNA NGS Primer	Forward NGS Primer for DNA sequencing	blue	100 µM	30 µl	90 µl
Reverse SeqDNA NGS Primer	Reverse NGS Primer for DNA sequencing	blue	100 µM	30 µl	90 µl
Forward SeqIND NGS Primer	Forward NGS Primer for Index sequencing	blue	100 µM	30 µl	90 µl
Reverse SeqIND NGS Primer	Reverse NGS Primer for Index sequencing	blue	100 µM	30 µl	90 µl

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

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B2, DM-NGS-SetC-B2, DM-NGS-SetD-B2)

Component	Description	Conc	Volume (24 reactions) *	Volume (96 reactions)
Dual Index Primer Set Plate	96 Dual DNA/RNA UD Indexes primer pairs, pre-aliquoted, for 4 reactions in each well	10X	20 µl/well	20 µl/well

Kit contents should be stored at -20°C. Shelf life is 1 year.

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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.

For General DNA Preparation

Name	Recommended Manufacturer	Catalog #	Purpose
DNeasy Blood & Tissue Kit	QIAGEN	69506	DNA isolation from PBMC, T/B cells or tissues
Tempus Blood RNA tubes/Spin RNA isolation kit	Thermo Fisher	4342792/ 4380204	DNA and RNA isolation from whole blood
PAXgene Blood DNA kit/tubes	Qiagen	761133/ 761165	DNA isolation from whole blood
All Prep DNA/RNA FFPE kit	Qiagen	80234	DNA and RNA isolation from FFPE tissues

Additional Reagents for cDNA Synthesis, Amplification and Amplified Libraries QC

Name	Recommended Manufacturer	Catalog #	Purpose
Agencourt® AMPure® XP (Magnetic Beads)	Beckman-Coulter	A63881	RNA-RevGSP hybrid and PCR product purification
Dynabeads® MPC®-S (Magnetic Particle Concentrator) for 1.5 ml Test Tubes Magnetic Stand-96 for 96-well PCR plates	Thermo Fisher	A13346/ AM10027	RNA-RevGSP hybrid and PCR product purification
96-well PCR plates	Thermo-Fisher	A43673	RT-PCR reactions
Clear Adhesive Film for 96-well plates	Bio-Rad	MSB1001	PCR plate seal
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® 500/550 High Output Kit v2.5 (300 cycles) /NovaSeq	Illumina	20024908	NGS for CDR3 only
NextSeq 1000/2000 XLeap-SBS P1 Reagent Kit (300 cycles)	Illumina	20050264	NGS for CDR3 only
NextSeq 1000/2000 XLeap-SBS P2 Reagent Kit (300 cycles)	Illumina	20046813	NGS for CDR3 only
NextSeq 1000/2000 XLeap-SBS P3 Reagent Kit (300 cycles)	Illumina	20040561	NGS for CDR3 only

Recommended Instrumentation

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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 Gel System to Analyze DNA Fragmentation	2100 Bioanalyzer®	Agilent
	Fragment Analyzer™	Agilent
	Standard gel imaging system	many suppliers
DNA Quantification	Qubit 3.0 Fluorometer	Thermo Fisher
Next-Generation Sequencer	NextSeq, NovaSeq	Illumina
Thermomixer R (for microsampling AIR profiling)	Thermomixer R with heat block for 1.5-ml test tubes	Eppendorf/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 Procedural Recommendations

- Keep enzyme components on ice during use. All other components, including primer pools, 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, perform the first part of the procedure (from primer extension through first PCR setup) in a location set aside for pre-PCR work, and use a set of equipment, pipettes, test tubes, and other consumables dedicated for work with RNA and DNA samples purified from biological samples. Then run the PCR amplification reactions in a separate lab or area designated for PCR. Always change pipette tips for adding components to new samples.

- For a small number of samples (1-12), we recommend using 0.2-ml, 0.5-ml test tubes or tube strips to run the assay. To minimize the hands-on time and mistakes in a liquid deposition for large numbers of samples (e.g., 24-96), we recommend running the assay in a 96-well plate using an 8-channel (or 12-channel) pipette. After adding all the necessary reagents, seal the plate with Clear Adhesive Film and use a Compression Pad to minimize the evaporation from experimental samples.

- Do not reuse clear adhesive films to seal 96-well plates.

- Pipet viscous enzyme solutions slowly. After adding them to the reaction mix, ensure complete mixing by vortexing or pipetting up and down several times.

- 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 each 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. Then, add 5% to each volume to cover pipetting variance, and pool the appropriate volumes of reagents together into a single tube or well. Mix the pooled reagents, then pipette the required portion into each sample. If you use a multichannel pipette, aliquot Master Mix in a tube strip first, then pipette from this strip into each sample.

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5. Sample Preparation

Depending on the sample amount, either genomic DNA or isolated immune cells (i.e., DirectCell Protocol) can be used as input for the DriverMap AIR TCR/BCR DNA Assay.

- For bulk tissue, blood, or cell samples with more than ~50,000 cells, follow the recommendations of the General DNA Preparation Protocol
- For biological samples with a small number of cells—less than ~50,000 cells (e.g., sorted immune cells) or blood and tissue micro samples—we recommend using the immune cells directly as input. Follow the guidelines in the DirectCell Protocol below (Section 5.2).

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5.1. General DNA Preparation Protocol

Follow the guidelines below to isolate and quantify DNA for larger bulk tissue, blood, or cell samples. For comprehensive AIR repertoire analysis, including identification of antigen-activated clonotypes and expression profiling, consider purifying both RNA and DNA from the same biological samples.

Use the appropriate method to isolate genomic DNA. We recommend the following DNA isolation kits.

- **Whole blood:** We recommend using the Tempus (Thermo-Fisher) or PAXgene (QIAGEN) for DNA isolation. Based on our experience, the collection of 3 ml of whole blood in Tempus RNA Blood Tubes followed by RNA isolation using Tempus Spin RNA Isolation kit and DNA from the supernatant using ethanol precipitation after the first centrifugation step provides the most simple and reliable protocol for purification of both RNA and DNA from whole blood (Ferrante J.A, et al. A novel technique for isolating DNA from Tempus blood RNA tubes after RNA isolation. BMS Res, Notes (2018) 11:53).
- **PBMC, buffy coat, or fresh/frozen tissue:** for more than 50,000 cells, we recommend using the QIAGEN All Prep DNA/RNA micro/mini kit for both DNA and RNA purification, DNeasy Blood & Tissue kit for DNA purification and RNAeasy Micro/Mini Plus Kit for total RNA isolation. For tissue samples with low content of immune cells, please consider purifying the lymphocyte cell fraction before DNA isolation to increase the sensitivity of AIR DNA repertoire profiling. Please use water to elute DNA (avoid using TE buffer with a high ~1 mM concentration of EDTA).
- **FFPE tissue:** We recommend using the Qiagen All Prep DNA/RNA FFPE kit. With FFPE, the yield and quality of isolated DNA/RNA can vary significantly, depending on the age and method used to fix the sample.

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5.2. DirectCell™ Protocol

For a small number of cells, such as immune cell fractions (e.g., 500-50,000 cells), and tissue samples (less than ~1 mg), we recommend FACS sorting or magnetic-bead isolation without any DNA purification step. Using sorted cells directly increases the sensitivity of CDR3 clonotype detection as compared to DNA isolated from small biological samples. Refer to the guidelines in this section for preparation of these samples.

To use small samples directly in the assay, follow the procedure below:

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1. Collect cells in 1xPBS or any commonly used buffer without magnesium or EDTA. Transfer the cells into 1.5-ml or 0.5-ml test tubes, spin down at low speed (e.g., 500g for 5 min), and remove excess supernatant from the cell pellet, keeping the residual volume in the test tube 10 µl (to prevent cell loss due to complete removal of supernatant).
   OR
   Prepare dissociated tissue samples (e.g., by collagenase treatment). Adjust the volume of each sample to 10 µl. Depending on your cell collection method, this may require spinning down the cells and removing excess supernatant over 10 µl.

2. Follow the standard AIR protocol for primer extension and follow-up step using cell suspension instead of DNA.

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5.3. General Notes

AIR profiling of TCR and/or BCR chains:

- We recommend performing AIR DNA profiling **separately** for TCR (TRA, TRB, TRD, and TRG) and BCR (IGH, IGK, and IGL) chains, considering significant differences in the representation of T and B cells, which are present in many biological samples. E.g., In whole blood and PBMC samples, the number of T cells is 3-5-fold more than B cells. After the amplification step, which usually requires different cycle numbers for TCR and BCR chains, the amplified indexed products could be mixed together at equal amounts for the NGS step.

Starting Amount of DNA:

- For quantitative AIR repertoire profiling, we recommend using 2 µg of DNA (from whole blood, PBMC, lymphoid tissues, or isolated immune cells). Using larger amounts of DNA, e.g., by running technical replicates (see below), can improve the detection of rare clonotypes but requires increasing the sequencing depth (and NGS cost). Using smaller amounts of DNA (i.e, 100 -500 ng) will reduce the number of clonotypes that can be reliably profiled. For example, running 0.5 µg of total whole blood/PBMC DNA instead of 2 µg will profile ~100-200 highly abundant clonotypes (i.e., clonotypes with more than 10 target CDR3 DNA molecules) as opposed to ~500 clonotypes.

- Some samples, such as FFPE blocks or tumor biopsies, contain impurities that inhibit the activity of DNA polymerase. To determine the optimal input amount for your samples, we recommend testing amplification efficiency using different starting amounts of DNA, e.g,. 0.5 µg, 1 µg, 2 µg, 5 µg. Alternatively, to get better quantitative data, we recommend running samples in triplicate, e.g., using 2×3=6 µg of DNA for each sample as mentioned below.

- To achieve the most comprehensive AIR repertoire profiling for tissue samples with low content of immune cells (e.g., cancer biopsies, non-lymphoid tissues), it is recommended to purify lymphocyte cells (e.g., by FACS or antibody-magnetic beads) before DNA isolation.

Replicates:

- For samples with enough DNA, we highly recommend running technical replicates. We recommend increasing the reaction volume and amount of DNA at primer extension steps (e.g., 3-fold) splitting the samples after the Forward Primer extension step, and running the first and second amplification steps in triplicate reactions for each sample. Using technical or amplification triplicates allows estimation of variability and expansion confident quantitation from ~100 clonotypes if a single replicate is used to ~500 clonotypes based on the statistical power gained with triplicate analyses (see also the AIR Technical Guide for more details).

- For purified immune cell samples, we do not recommend splitting the samples into triplicates since the number of cells

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is usually limited. In such cases, we recommend collecting multiple samples for each cell fraction to run the biological replicates in parallel.

Positive Control DNA isolated from a single PBMC donor is supplied with the kit. We recommend running at least one positive control sample in a similar amount to your experimental samples. This will help in troubleshooting, data analysis, and normalization across multiple sample runs.

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5.4. FFPE Guidelines

- We recommend using the Qiagen All Prep DNA/RNA FFPE kit. With FFPE the yield and quality of isolated DNA/RNA can vary significantly, depending on the age and method used to fix the sample.
- Certain impurities and high concentrations of FFPE-DNA can inhibit DNA polymerase activity for AIR-DNA profiling assay. To identify the optimal FFPE-DNA input amount, we suggest testing a range of DNA inputs (e.g., 0.5 µg, 1 µg, 2 µg, 5 µg) for at least one sample (e.g., sample with a high yield of purified DNA), in our experience 1-2 µg can be used for AIR-DNA assay without inhibition of DNA polymerase activity.
- For more information, please refer to our Technology Guide.

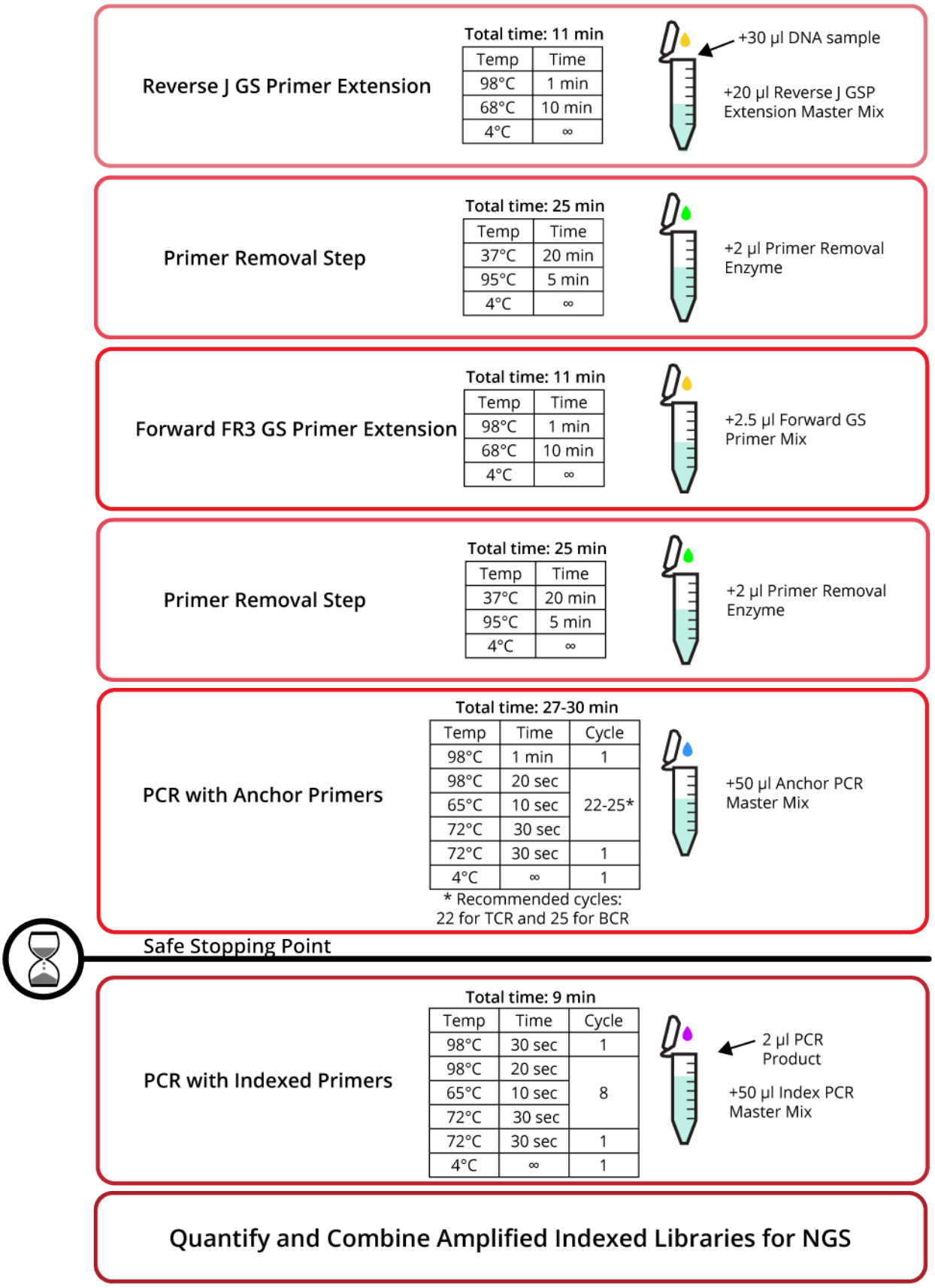
Protocol Optimization:

- Considering that FFPE samples usually have very different contents of T/B cells you could expect that only a fraction of samples will generate the expected correct TCR/BCR amplified products. Therefore, we recommended running 1st PCR for 26 cycles and using an aliquot from 1st PCR (2µl) to run 2nd PCR (50 ul) with index primers for 8 cycles. Analyze the yield of PCR products using a Bioanalyzer (or 3% agarose-TAE gel). Collect in the new test tubes 2nd PCR samples that demonstrate visible yield of correct TCR/BCR amplicons and run the rest of the samples (without visible amplified bands) for an additional 4 cycles and repeat Bioanalyzer analysis. Collect samples with amplified correct TCR/BCR amplicons and run an additional 4 cycles for samples without visible band. Based on our experience, the maximum number of PCR cycles in the 2nd PCR step could be 20 cycles. Using more than 20 cycles in the 2nd PCR, in general, is not recommended as 20 cycles could amplify PCR products from only a few template molecules. If you can’t see amplified products after 20 cycles (of 2nd PCR) it could be an indication that FFPE samples practically don’t have T/B cells or DNA is significantly damaged. In those cases, we recommend staining the slides to identify the content of T or B cells. Don’t over-cycle (using more cycles than necessary to amplify visible correct amplicons) the samples in 2nd PCR step. Don’t use more than 2ul of 1st PCR products in the 2nd PCR step (50ul), it could increase the amount of background, non-specific products.

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6. Outline of DriverMap™ AIR DNA Protocol



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6.1. Reverse J Gene-Specific (GS) Primers Extension

In this step, the pool of Reverse J GS primers with adjoining VBC's and AP2 sequences (see Outline of AIR Assay, Fig.1) is annealed to J regions (top, sense strand) of TCR or BCR genes, extended by DNA polymerase to generate Reverse GSP extended cDNA and purified from non-extended primers by nuclease treatment. The protocol assumes the reactions will be set off in a 96-well plate. For small numbers of samples, reactions can be done in tubes or strips also.

✻ Note: Reverse and Forward Primer Extension steps and assembly of 1st PCR reactions should be set up in a PCR-free room.

1. Prepare the Reverse J GS Primer Extension Master Mix as described below for all samples and controls (make 5% extra to account for pipetting error) and aliquot 20 µl in the wells of a 96-well plate:

Reverse J GS Primer Extension Master Mix Component	Volume per sample, µl
EXT Buffer, 4x	12.5
dNTP Mix	1.25
Reverse J Primer mix, 20x	2.5
Water	3
DNA polymerase	0.75
Total	20

✻ Note: If you decide to run biological or amplification triplicates, increase the number of wells and amount of DNA allocated for each sample, respectively.

2. Adjust the volume of each DNA Sample to 30 µl with water, as shown in the table.

Component	Volume per sample, µl
Genomic DNA (~0.5-2 µg)	1 – 30*
Water, to 30 µl final volume	0 – 29

✻ Note: For optimal input recommendations, please refer to Section 5.3 General Notes

3. Add 30 µl of DNA Sample to each well with pre-aliquoted 20 µl of Reverse J GS Primer Extension Master Mix.

Component	Volume, µl
DNA	30
Reverse J GS Primer Extension Master Mix	20
Total	50

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4. Mix contents by pipetting 3 times. Seal the plate with adhesive film, and spin down to collect droplets. Load the plate in the thermal cycler and run the following program:

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

5. Spin down the plate, remove the seal from the plate, then add 2 µl of the Primer Removal Enzyme to each reaction well of the plate. Mix contents by pipetting three times, seal the plate and spin down to collect droplets.

6. Load the plate in the thermal cycler, and run the following program:

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

! Immediately proceed to the next step (Forward FR3 Gene-Specific Primer Extension)

Last modified: 27 January 2025

6.2. Forward FR3 Gene-Specific (GS) Primer Extension

In this step, the pool of Forward FR3 GS Primers with adjoining Anchor 1 sequences (see Outline of AIR Assay, Fig.1) is annealed to Reverse GSP extended cDNA template generated in the previous step, extended by DNA polymerase, and generates the target CDR3 DNA region (top, sense strand) flanked from both sides by Anchor 1 and Anchor 2 sequences.

1. Spin down the plate from the previous step, remove the seal from the plate, then add 2.5 µl of the Forward FR3 GS Primer Mix to each reaction well of the plate:

Component	Volume, µl
Reverse J GS extension reaction	52
Forward FR3 GS Primer Mix	2.5
Total	54.5

2. Mix contents by pipetting 3 times, seal the plate with a new adhesive film, and spin down to collect droplets.

✻ Note: A new adhesive film is required to avoid cross-contamination.

3. Load the plate in the thermal cycler, and run the following program:

Temperature	Time

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98°C	1 min
68°C	10 min
4°C	∞

4. Spin down the plate, remove the seal from the plate, then add 2 µl of the Primer Removal Enzyme to each reaction well of the plate. Mix contents by pipetting 3 times, seal the plate, and spin down to collect droplets

5. Load the plate in the thermal cycler, and run the following program:

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

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

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6.3. First PCR with Anchor Primers

This step utilizes universal Anchor PCR primers to amplify the target CDR3 DNA fragments flanked with the universal Anchor 1 and Anchor 2 sequences generated during the previous **Forward FR3 Gene-Specific Primer Extension** step.

1. Prepare the 1st PCR Master Mix as shown below for all samples and controls, plus 5% extra volume of all components:

1st PCR Master Mix Component	Volume per sample, µl
PCR Buffer, 5X	10
dNTP Mix	1
Anchor Primer Mix, 10X	10
Water	28.5
DNA Polymerase	0.5
Total	50

2. Gently vortex Master Mix and spin down briefly to collect droplets. Spin down the Forward GS Primer Extension plate, remove the seal, then add 50 µl of 1st PCR Master Mix to each reaction well:

Component	Volume per sample, µl
Forward GS Primer Extension DNA (after primer removal step)	54
1st PCR Master Mix (prepared above)	50
Total	104

3. Mix content by pipetting three times. Seal the plate with new adhesive film and spin it to collect droplets.

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Note: Take the plate from the PCR-free room and perform all follow-up amplification, purification, and NGS steps in a location dedicated to PCR work. Do not bring amplified library products back to PCR-free 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	1 min	1
98°C	20 sec	22-25* Note below
65°C	10 sec	
72°C	30 sec	
72°C	30 sec	1
4°C	∞	1

Note: Below are some example guidelines based on input type. In many cases, the number of cycles needs to be optimized and adjusted based on specific cell types, quality of DNA, type of tissue sample and the content of T/B cells, etc.

- For samples that contain 2 µg of DNA isolated from lymphocyte-rich samples (e.g., whole blood, PBMC, and positive control PBMC DNA), we recommend starting with 22 PCR cycles for TCR or 25 PCR cycles for BCR. For similar samples with less DNA amount, you need to add extra cycles (e.g., if using 0.5 ug DNA, + 2 cycles).
- For purified immune cells (i.e, sorted T/B cells 2 ug of DNA), we recommend 22 cycles for TCR or BCR.
- For DNA samples with low content of immune cells (or in the DirectCell protocol), we recommend starting with 25 cycles for 250 ng of DNA (or 50,000 sorted cells) and adding more cycles if needed. For most samples, we recommend not exceeding 28 cycles to prevent non-specific PCR products.
- If looking for higher clonotype coverage, 5-10 µg DNA can be used by running replicates with 2.5 µg of DNA each with 28 PCR cycles.

Stopping Point in procedure: PCR products may be stored at 4°C overnight.

Last modified: 24 February 2026

6.4. 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 Index Primer Plate (sold separately) contains a unique combination of Forward and Reverse DNA/RNA UDP index primers in each of 96 wells for both 24 and 96 sample kits. Each well contains 20 µL of a unique index primer combination (10X concentration), which is enough to run x4 2nd PCR reactions (5 µl per 50 µl reaction). One unique index primer combination should be used for each sample (triplicate samples have different indexes). If you run a small number of samples (e.g., 2-3), 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 Index Primer Plate, briefly centrifuge the plate to collect droplets to 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 -20°C (for

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prolonged storage). See Appendix List of Dual DNA/RNA UDP Indexes for the sequences of each Forward and Reverse Index combination.

Please note, Index Primer Plates are sold as a separate product in four configurations (Set A, B, C and D). Catalog# DM-NGS-SetA-B2, DM-NGS-SetB-B2, DM-NGS-SetC-B2 and DM-NGS-SetD-B2. For more information, please visit the webpage. If you have an old kit with dried index plates, please follow the 2nd PCR Protocol here. All other library prep steps are the same.

	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:

2nd PCR Master Mix Component	Volume per sample, µl
PCR Buffer, 5X	10
dNTP Mix	1
Water	33.5
DNA Polymerase	0.5
Total	45

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, and spin down briefly to collect droplets. Remove the plate seal from the Index Primer Plate. Set up the Index Primer PCR Reactions as follows:

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 Index Primer Plateprovided in the kit. 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 Index Primer PCR reactions prepared in step 3. To avoid mistakes, ensure that samples in the 1st PCR plate are arranged in the same format as the Index PCR Primer pair mixes in the Index Primer PCR Reaction Plate (e.g. Sample 1A is aliquoted to well 1A). We recommend that you immediately record the Sample name and well number/index number

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(e.g., Sample 1 in well 1A) 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 (step above)	2
Index Primer PCR Reaction	50
Total	52

5. Seal the plate with new adhesive film and spin down to collect droplets.

6. 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	30 sec	
72°C	30 sec	1
4°C	∞	1

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

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7. NGS Prep and Sequencing

The product from the **PCR with Indexed Primers** step contains both P5 and P7 sequences and dual DNA/RNA UD Indexes for Next-Gen Sequencing (NGS) on Illumina instruments. The protocol in this section provides the instructions to normalize 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: 24 January 2025

7.1. 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 sample—is measured and then pooled in equimolar amounts for sequencing.

Quantify the 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 below for the expected results of amplified libraries generated from good-quality PBMC DNA samples. For both the AIR TCR and AIR BCR assays, the smear with several bright bands should be in the 200-300 bp range.

- 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), TapeStation or Fragment Analyzer using the High Sensitivity NGS Analysis Kit (Cat.# DNF-473-1000) using the manufacturer's protocol.

> ✻ 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 analyze the yield of amplified products.

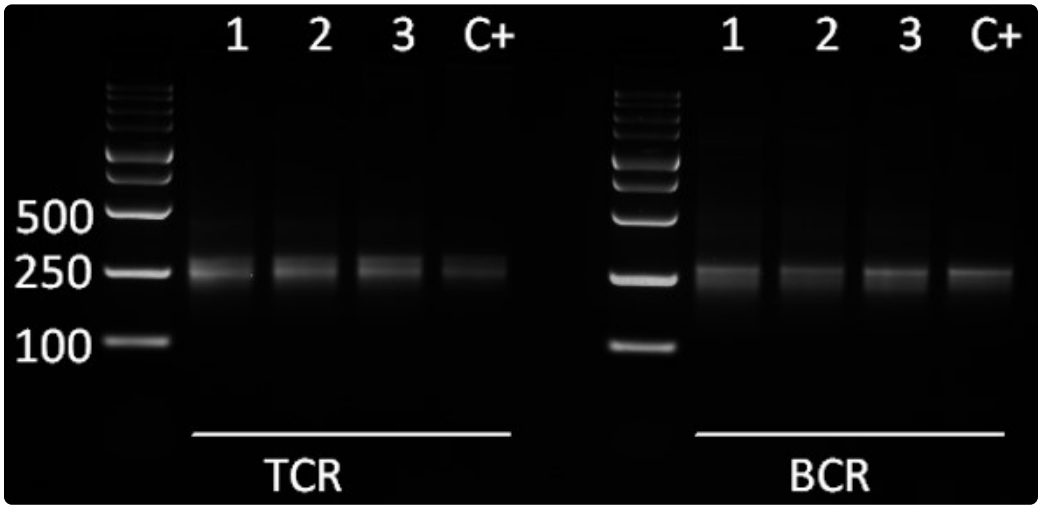


Fig 3: Gel electrophoresis analysis of amplified indexed libraries. Gel image of amplicons after second primer index PCR reaction. TCR/BCR: Lanes 1 (1.5 ug), Lane 2 (3 ug), Lane 3 (6 ug) of gDNA. Lane 4: Positive control DNA from AIR DNA kit (5 ug)

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2. Analyze yields of the Amplified Indexed Libraries. The yield should all be roughly the same for all experimental samples within +/- 2-3-fold levels and should be similar to the Positive Control PBMC DNA sample. A negative control sample should not generate any significant yield of amplified products. If some samples show significantly lower yield (e.g., >5-10-fold) of amplification products, it could be an indication of differences in the amount, quality of DNA or content of lymphocyte DNA used in AIR assay. For experimental DNA samples with significantly less yield of PCR products than other samples or the Positive Control DNA sample (i.e., >5-10-fold difference), you can rerun the Amplified Indexed Libraries in the thermal cycler (see Note below) for 2-5 additional cycles. After cycling, quantify the products again relative to the Positive Control DNA. Note: Do not include the Positive Control DNA sample in additional cycles. Remove the positive control sample from the plate and keep it as a reference to assess the quantity of your PCR samples.

> Note: It is not necessary to achieve the same yield of amplified products for all samples. Adjustments for small (2-5-fold) differences in the yield between samples will be made when the samples are combined for the sequencing step following this quantification procedure.

Remove Excess PCR Primers and Combine Samples

1. Remove excess primers from the completed PCR reactions by adding 1 µl of Primer Removal Enzyme to each of the Amplified Indexed Libraries and the Control sample, then incubate at 37°C for 30 minutes.

2. To ensure accurate quantification for sequencing, you need to quantify the yield of the Amplified Indexed Libraries. The preferred method is to analyze 2 µl of each of the Amplified Indexed Libraries using either an Agilent Bioanalyzer with the Agilent High Sensitivity DNA Kit (Cat.# 5067-4626), TapeStation, or Fragment Analyzer using the High Sensitivity NGS Analysis Kit (Cat.# DNF-473-1000) using the manufacturer's protocol. Quantifying the PCR products after the removal of PCR primers is more accurate than quantifying before the primer removal clean-up step.

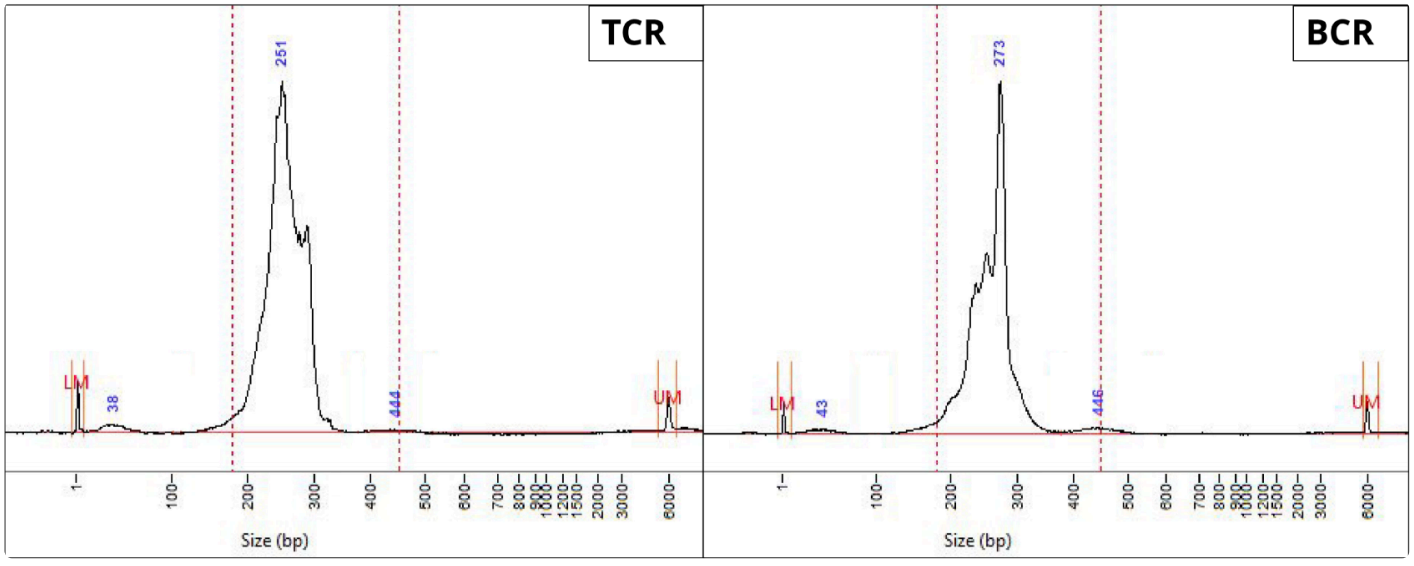


Fig 4: Size distribution of Amplified Indexed Library after Primer Removal step analyzed by fragment analyzer.

3. After primer removal and quantification, use the yield assessment of the Amplified Indexed Libraries as a basis to combine equimolar amounts of each of the Amplified Index Libraries into a single pool for NGS. For example, if the yield of Library 1 is twice that of Library 2, then mix 5 µl of Library 1 with 10 µl of Library 2. To minimize sample-to-sample sequencing variations, combine and load all experimental samples onto one NGS flow cell.

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For the DriverMap AIR Assay, which generates a complex pool of amplified TCR or BCR CDR3 regions (40K-100K from 5 µg of PBMC DNA), refer to the table below for guidelines on how many samples may be combined for different instruments and read depths. Generally, you should aim for 5-10 million reads per AIR sample (starting from 5 µg of PBMC DNA) or achieve at least an average of 20 reads per DNA molecule. For Illumina instruments, AIR profiling assays require the use of 300-n paired-end reagent kits.

Instrument	Reads per flow cell	Number of samples for multiplexing per flow cell	Reads per AIR sample
NextSeq 500/550, 300 cycles	120 million (medium throughput)	12-20	6-10M reads/ sample
NextSeq 500/550, 300 cycles	400 million (high throughput)	40-72	6-10M reads/ sample
NextSeq 2000, 300 cycles	100 million (P2 flow cell)	10-12	10M reads/sample
NextSeq 2000, 300 cycles	400 million (P2 flow cell)	40	10M reads/sample
NextSeq 2000, 300 cycles	1200 million (P3 flow cell)	96	10M reads/sample
NovaSeq Series, 300 cycles	6000 million (S2)	500	10M reads/sample

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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 Libraries so that the preparations are ready for NGS. To remove residual amount of genomic DNA we recommend using double (0.6V > 1.5V) AMPure XP purification strategy.

1. Add 0.6x volume of Agencourt® AMPure® XP Reagent (at room temperature) to pooled Amplified Indexed Libraries mix in an Eppendorf tube, and pipet up and down 5 times to thoroughly mix the bead suspension with the pooled Amplified Indexed Libraries.

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

3. Place the tube in the Magnetic Stand for 2 minutes or until the solution is clear. Carefully collect the supernatant without disturbing the bead pellet.

4. Add 0.9x volume of Agencourt® AMPure® XP Reagent (at room temperature) to pooled Amplified Indexed Libraries mix in an Eppendorf tube, and pipet up and down 5 times to thoroughly mix the bead suspension with the pooled Amplified Indexed Libraries.

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 bead pellet.

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

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7. 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.

8. Repeat steps 6 and 7 for a second wash.

9. Briefly centrifuge tubes at low speed and place the tube 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.

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

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

12. Measure the concentration of the pooled Amplified Indexed Library sample using the Qubit dsDNA High Sensitivity Assay.

13. Dilute the pooled Amplified Indexed Library to a concentration of 1.8 ng/µl, which corresponds to 10nM for the following NGS step.

Add 5-15% PhiX control based on the optimal loading requirements for your sequencer.

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

Last modified: 24 February 2026

7.3. Next Generation Sequencing (CDR3 ~ 300 bp)

The Amplified Index Libraries made from each total RNA sample should be run on an Illumina sequencer following the manufacturer's instructions. Generally, we recommend setting up the sequencing reactions to generate 5-10 million reads per sample (read depth per sample), including positive control samples. This depth works out to produce 50 reads, on average, per DNA molecule.

NextSeq 500/550

The procedure below is based on our experience with the NextSeq 500/550 using the 300-cycle NextSeq 500/550 High Output Kit v2.5 (Illumina Cat.#20024908).

1. Follow the standard Illumina procedures for Cluster Generation, starting with 10 nM of the purified PCR sample. We recommend adding 15% of PhiX to the library for NextSeq 500/550.

2. Add 6 µl of each of the custom sequencing primers into the appropriate wells of the Illumina reagent cartridge, as follows:
• Forward SeqDNA NGS Primer (Read 1 Sequencing Primer) into well #20
• Reverse SeqDNA NGS Primer (Read 2 Sequencing Primer) into well #21
• Forward SeqIND NGS Primer (Index 1 Sequencing Primer) into well #22
• Reverse SeqIND NGS Primer (Index 2 Sequencing Primer) into well #22

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*Note: We do not recommend adding primers to the “Custom” wells of the NextSeq 500/550 reagent cartridge. We add our in-house custom sequencing primers into the Illumina premixed primer wells to take advantage of the PhiX internal control. The addition of our in-house sequencing primers does not affect the performance of the flow cell.

3. Perform the NGS run using 300-cycle Paired End reads on the NextSeq 500/550. The optimal loading concentration for cluster generation of indexed libraries is approximately 1.8 pM for NextSeq 500/550. Use the following program for the sequence run:
h2. Program Setting:

Program	Custom Primer	Cycles
Read 1:	Forward SeqDNA	148
Index 1:	Forward SeqIND	10
Index 2:	Reverse SeqIND	10
Read 2:	Reverse SeqDNA	148

Note: The program has a total of 316 cycles. There are enough reagents in the 300-cycle kit (eg: Illumina Cat.# 20024908) to run at least 316 cycles

NextSeq 2000

The procedure below is based on our experience with the NextSeq 2000 using a 300-cycle P2 flow cell with XLeap-SBS chemistry (Illumina Cat.# 20046813). For other flow cells and instruments, please follow the manufacturer’s guidelines. To use custom sequencing primers on the NextSeq 1000/2000 instruments, you use both Illumina READ and AIR Custom Read sequencing primers in the custom primer wells. (see Illumina Guide: Prepare and Add Custom Primers). The Illumina READ primers (used to amplify PhiX) are available for purchase as a separate kit (item number 20112859 for XLEAP or 20046117 for Standard chemistry—see Consumable part numbers for the NextSeq 1000/2000). This kit also includes the needed HT1 buffer.

1. First, adjust each custom primer to 100uM concentration with the HT1 buffer. Before loading the sample and custom primer, invert the cartridge several times to ensure complete reagent mixing. You will not be able to do this once everything is loaded.

2. Mix 1.8uL of each Forward/Reverse SeqDNA NGS Primers into 600uL of Illumina VP21 or HP21 reagent—depending on the Illumina primer kit you are using (the VP21 and HP21 have the primers for PhiX). The final concentration of each primer will be 0.3 µM. Load the primer mixture into the Custom Well 1. This well is used for the READ cycles of the program.

3. Mix 3.6uL of each Forward/Reverse SeqIND Primer into 600uL of VP14 or BP14 reagent (final concentration of 0.6uM) and then load the mixture into the Custom Well 2. These primers are used during the INDEX cycles of the program.

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A. Well 1 B. Well 2

4. The optimal loading concentration for cluster generation of indexed libraries is approximately 800pM to a final volume of 24 µl, and add 5-10% PhiX Control.

5. When in manual setup mode, after selecting your read lengths (300-cycle Paired End reads), set the custom primer drop-down under each read to the correct well.

Program	Custom Primer	Cycles
Read 1:	Forward SeqDNA	159
Index 1:	Forward SeqIND	10
Index 2:	Reverse SeqIND	10
Read 2:	Reverse SeqDNA	159

Note: The program has a total of 338 cycles. There are enough reagents in the 300-cycle NextSeq XLEAP-SBS chemistry reagent kits (e.g., Illumina Cat.# 20046813) to run at least 338 cycles.

6. This will direct the instrument to pull the custom primer from these wells, not the stock material inside the cart. Once that is complete, you can follow the standard protocol to start the run.

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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.

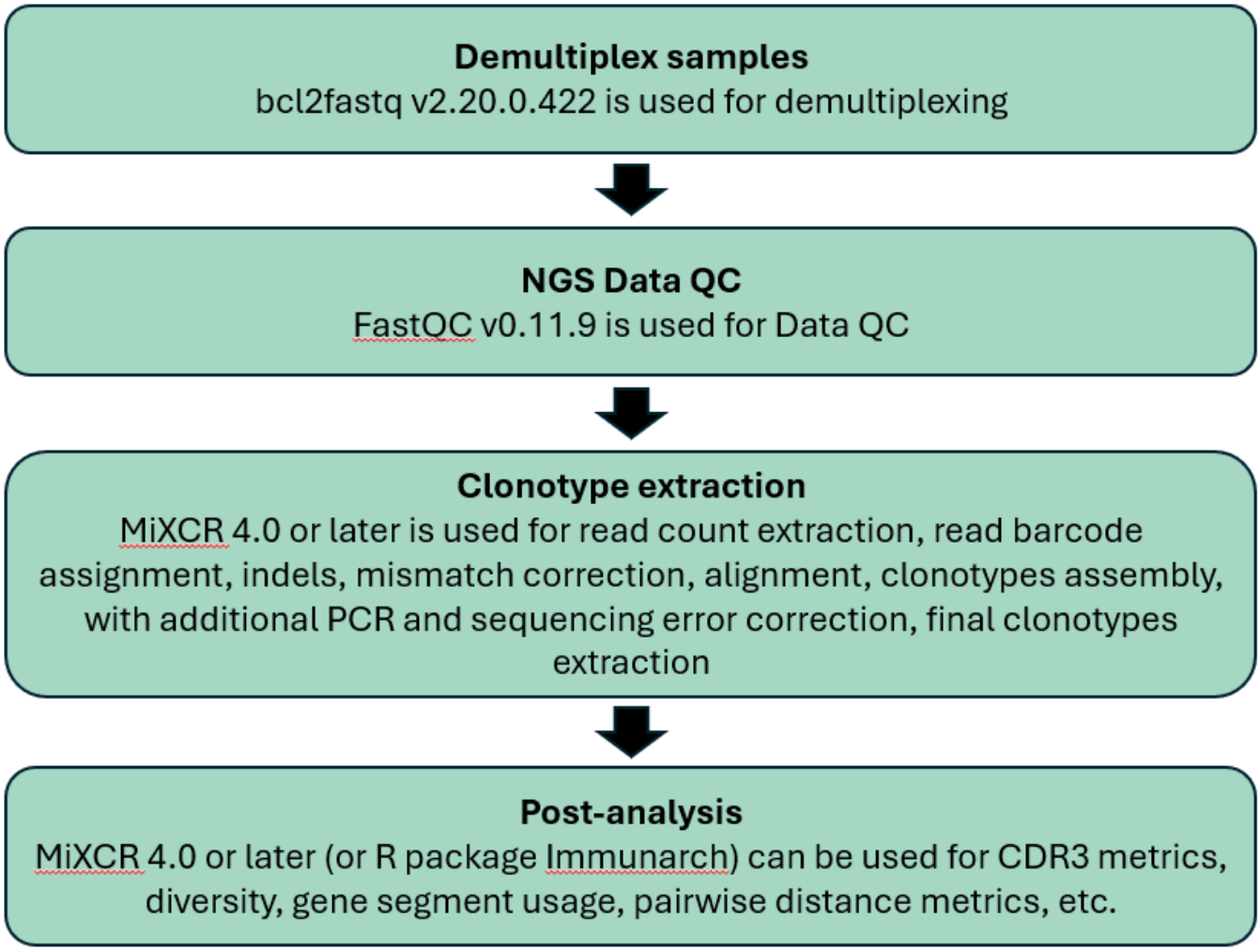
Cellecta does not own any of these software packages and is not responsible for maintaining them. In case you don't have the license, Cellecta also provides custom data analysis services using the MiXCR software package. Please email tech@cellecta.com for more information.

Last modified: 15 May 2025

8.1. Bioinformatic Workflow

The following bioinformatics workflow is recommended for DriverMap AIR 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.

Last modified: 26 November 2025

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

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link:FastQC Guide

Last modified: 24 February 2025

8.4. MiXCR Alignment and Clonotypes Assembly

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 file using exportClones.
The reads can be aligned with MiXCR using the following command:

```
mixcr analyze local:cellecta-new-bulk-kit \
--species $SPECIES \
--assemble-clonotypes-by $ASSEMBLY_TYPE \
$SPLIT_CLONES_CONFIG \
--$MOLECULE \
--tag-pattern $TAG_PATTERN \
$LEFT_BOUNDARY \
--floating-right-alignment-boundary $BOUNDARY \
--export-productive-clones-only \
$READ_1 \
$READ_2 \
$OUTPUT_NAME
```

where:

```
$SPECIES = hsa (human) or mmu (mouse)
$ASSEMBLY_TYPE = CDR3 (CDR3 amplicon) or {CDR1Begin:FR4End} (Full length amplicon)
$SPLIT_CLONES_CONFIG = --split-clones-by C (if working with B cells to distinguish isotypes)
or otherwise skip this option
$MOLECULE = rna (for RNA) or dna (for DNA)
$LEFT_BOUNDARY = --rigid-left-alignment-boundary (for RNA) or --floating-left-alignment-bound
ary (for DNA)
$BOUNDARY = J (for DNA) or C (for RNA)
$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:*)\^(MIVBC:GCATCAN)(R2:*)|^(MIVBC:AGTCGTN)(R2:*)|^(MIVBC:TCGCATCN)(R2:*)|^(MIVBC:AGCGTA
GN)(R2:*)|^(MIVBC:TACGACTN)(R2:*)|^(MIVBC:CTGATGAN)(R2:*)|^(MIVBC:CATAGCATN)(R2:*)|^(MIVBC:GT
AGGCTAN)(R2:*)'
```

is specified by the 8 validator barcodes.

The preset can be accessed by saving the file cellecta-new-bulk-kit.yaml in the directory of the MiXCR script that can be obtained here.

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

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8.5. Template Estimation with Validator Barcodes

We developed Validator barcodes (VBCs) as an alternative to UMIs. VBCs are short fragments from 6 – 8 nucleotides. These fragments are incorporated either during cDNA synthesis for RNA templates or barcode extension for DNA templates, both prior to PCR amplification. They assist in normalization strategy to estimate the number of molecular templates. For more information and reference datasets with VBC, please refer to the manuscript link

The number of original templates can be estimated from the read counts using the VBC. The code can be accessed from the Github Link. After MiXCR alignment, the template estimation program can be used directory on the MiXCR output. The two scripts below, which are used sequentially, use the VBC to (1) filter clonotypes based on read counts and (2) estimate the number of template nucleic acid molecules in the sample.

Barcode Hopping and VBC filtering

```
python3 run_filter_vbc.py $DIRECTORY $SAMPLE_NAME --mode $MODE
```

where:
$DIRECTORY = directory of mixcr folder containing clones tsv files
$SAMPLE_NAME = name of the current sample being analyzed (match with the MiXCR output prefix)
$MODE = ‘bulk’ or ‘single_cell’ (Default: ‘bulk’)

e.g. `python3 run_filter_vbc.py ~/Desktop/test_T/ test_T --mode bulk`

Template Molecule Estimation
python3 run_quantify.py $DIRECTORY $SAMPLE_NAME —mode $MODE

where:
$DIRECTORY = directory of mixcr folder containing clones tsv files
$SAMPLE_NAME = name of the current sample being analyzed (match with the MiXCR output prefix)
$MODE = ‘bulk’ or ‘single_cell’ (Default: ‘bulk’)

e.g. `python3 run_quantify.py ~/Desktop/test_T/ test_T --mode bulk`

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8.6. Advance Statistical Analysis

The downstream analysis also can be performed by MiXCR. MiXCR V4.0 and later provide new features to export results in tabular format and vector plots with various statistical comparisons. More details can also be found on MiXCR’s official website (https://docs.milaboratories.com/).

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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 Dual DNA/RNA UD Indexes

Last modified: 24 January 2025

9.1. Appendix A. Tips and Troubleshooting

Observation	Possible Cause	Recommended Action
The yield of PCR products for the DNA samples is low, but is good for the Positive Control DNA sample — or — Low yield of PCR products for both experimental and Positive Control DNA samples	Error in quantitation of input DNA, or DNA is degraded	Re-analyze the amount and quality of input DNA using Agilent Bioanalyzer, Agilent Fragment Analyzer, or Thermo Fisher NanoDrop.
	DNA input is too low	Add more DNA or increase the target amplification cycles (Refer to **PCR with Anchor Primers** for recommended cycle numbers based on input amounts).
	DNA contamination	Repurify DNA and elute with water, or reduce the amount of DNA in the reaction.
	Extension or PCR step is not optimal	Please check for procedural mistakes. Make master mixes where possible. For individual reaction setups, ensure that the correct volume of EXT Buffer, PCR Buffer, or dNTP is added to each reaction.
	PCR cycling conditions are not optimal	Increase PCR amplification cycles. Ensure proper dispensing and mixing of viscous components at each step. Avoid bubble formation.
	Non-uniform PCR amplification	Use only PCR plates compatible with your PCR machine; some plates don’t sit uniformly in the thermal block, causing non-uniform or failed amplification

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

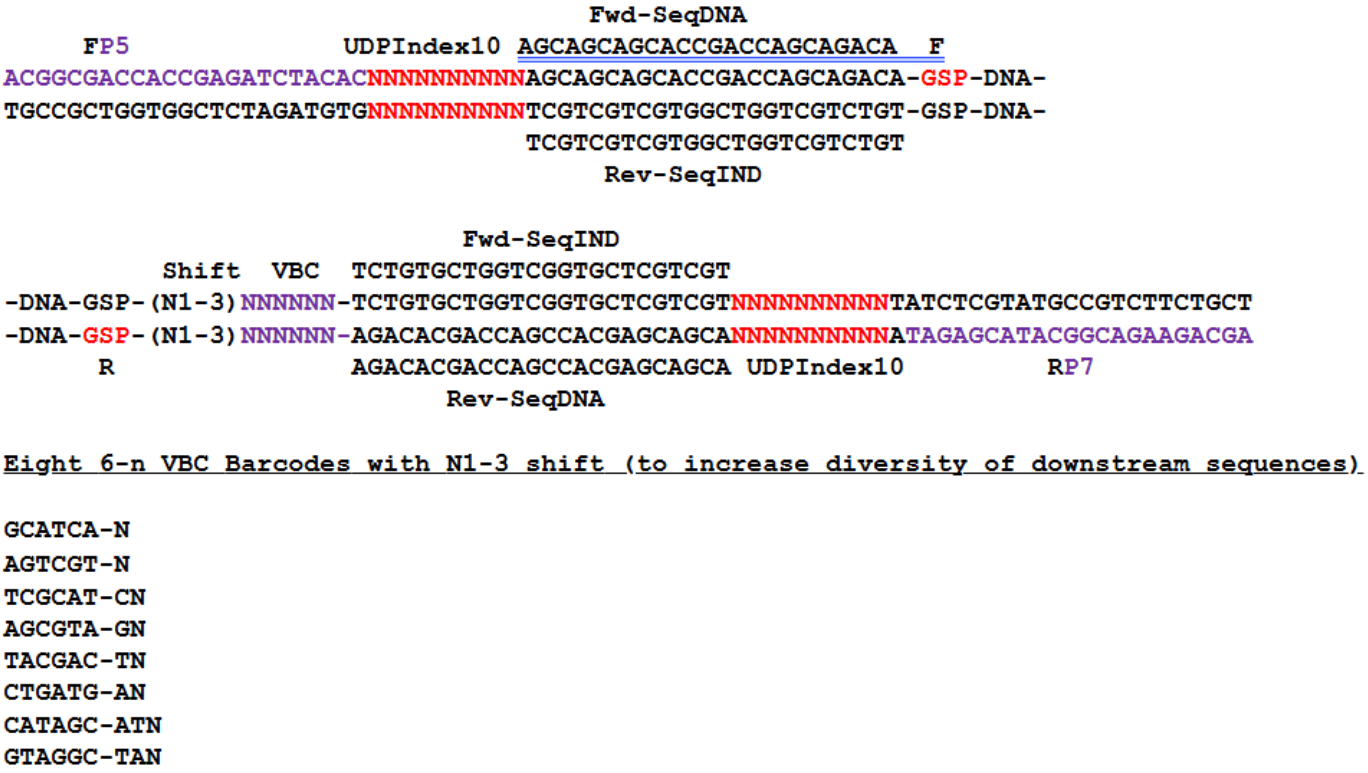
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Observation	Possible Cause	Recommended Action
Yields of PCR products are significantly higher than Positive Control	Incorrect quantitation of input DNA amount	Re-analyze the amount and quality of input DNA using Agilent Bioanalyzer, Agilent Fragment Analyzer, or Thermo Fisher NanoDrop.
	DNA input is too high	Add less DNA or decrease the number of PCR cycles in the **PCR with Indexed Primers** step.
Low fluorescence readings on the 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	Check that you added Primer Removal Master Mix in the **Quantify and Combine Samples for NGS** step. If it had been added, use a new vial of Primer Removal Reagent and repeat.
Lower than expected number of on-target reads	DNA input too low or PCR cycle number too low	Add more DNA or increase the number of PCR cycles in the target amplification step (**PCR with Anchor Primers**).
	DNA is degraded	Use the highest quality DNA possible. For degraded DNA, increase the number of PCR cycles.
Uneven representation of Indexed Libraries	Inaccurate Indexed Library quantification	Check that you correctly calculate 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 DNA samples	Sample evaporation in thermal cycler	Seal 96-well plates well with an Adhesive Film Applicator and use a Compression Pad. Fill empty wells with water to minimize evaporation from experimental samples. Use the same brand of PCR plates designed for your PCR machine; some plates don’t sit uniformly in the thermal block, causing non-uniform or failed amplification

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9.2. Appendix B. Structure of Amplified Indexed Library



Eight 6-n VBC Barcodes with N1-3 shift (to increase diversity of downstream sequences)

GCATCA-N
AGTCGT-N
TCGCAT-CN
AGCGTA-GN
TACGAC-TN
CTGATG-AN
CATAGC-ATN
GTAGGC-TAN

NGS primers for cDNA/DNA sequencing:
eSeqDNA-Fwd: AGCAGCAGCACCGACCAGCAGACA
eSeqDNA-Rev: ACGACGAGCACCGACCAGCACAGA

NGS primers for index sequencing:
eSeqIND-Fwd: TCTGTGCTGGTCGGTGCTCGTCGT
eSeqIND-Rev: TGTCTGCTGGTCGGTGCTGCTGCT

Reference Amplicon Data Sets for Download:

- DriverMap AIR BCR DNA CDR3 VBC-based
- DriverMap AIR BCR DNA CDR3 VBC-based
- DriverMap AIR TCR DNA CDR3 VBC-based
- DriverMap AIR TCR DNA CDR3 VBC-based
- DriverMap AIR BCR RNA CDR3 VBC-based
- DriverMap AIR BCR RNA CDR3 VBC-based
- DriverMap AIR TCR RNA CDR3 VBC-based
- DriverMap AIR TCR RNA CDR3 VBC-based
- DriverMap AIR BCR RNA CDR3 VBC-based
- DriverMap AIR TCR RNA CDR3 VBC-based
- DriverMap AIR BCR RNA Full Length VBC-based
- DriverMap AIR BCR RNA Full Length VBC-based
- DriverMap AIR TCR RNA Full Length VBC-based
- DriverMap AIR TCR RNA Full Length VBC-based

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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.com/mixcr/getting-started/installation/").

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`
- 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).

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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
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

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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
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

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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

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

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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
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

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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
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

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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
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

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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
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

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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

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

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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
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

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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
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

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H12	384	UDP0384	AGTGGTTAAG	UDP0384	GTATTCCACC

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10. Frequently Asked Questions

Please refer to the AIR Technology Guide for recommendations for experimental design, sample-input amounts, and assay design.

Assay:

Q1: What is included in the AIR profiling kit?
A1: The kit includes all the necessary reagents for reverse transcription, amplification, and primers for next-generation sequencing.

Q2: What is the difference between TCR/BCR CDR3 RNA kits for human and mouse?
A2: The TCR/BCR gene-specific primers for the human and mouse kits are different but all other reagents and protocols are the same.

Q3: Does the AIR profiling assay have Unique Molecular Identifiers (UMIs)?
A3: Cellecta's DriverMap AIR RNA and AIR DNA profiling assays do not have UMIs. The AIR RNA and AIR DNA profiling assays incorporate a redundant set of Validator Barcodes (VBCs) which enable easy identification of and correction for amplification and sequencing biases and error. The VBCs also provide a reference for normalization and copy number quantitation of amplified RNA or DNA molecules to facilitate detection of low-abundant clonotypes.

Q4: What is the difference between AIR RNA CDR3 and Full-length Receptor Kit?
A4: The CDR3 kit amplifies approximately 250-300 bp of the CDR3 region, whereas the full-length kit amplifies the full variable region ~600 bp (CDR1-CDR2-CDR3). For more information on sequencing instructions and applications for the two kits, please visit the AIR-RNA webpage to view the table Comparison of CDR3 vs Full-Length Variable Region Repertoire Profiling.

Q5: What sample types are compatible with the assay?
A5: Our assay is compatible with various sample types, including RNA or/and DNA samples isolated from whole blood, PBMC, immune cell fractions, tissue/cancer biopsies, or FFPE tissue samples. For more information on sample types and preparation guidelines, please refer to the Technology Guide: Applications of AIR profiling.

Q6: What is the recommended protocol for profiling a small number of cells, such as sorted cells or fresh frozen tissue?
A6: For a small number of cells (e.g., 5,000-50,000 cells), e.g., isolated by FACS, tissue or blood microsamples, we recommend using the Cellecta DirectCell™ Protocol which profiles directly from the cell lysate without the need to extract total RNA or DNA from the sample.

Q7: What is the optimal input amount for DNA as a starting material?
A7: To determine the optimal input amount for your samples, we recommend testing amplification efficiency using different starting amounts of DNA, e.g. 0.5 µg, 1 µg, 2 µg, 5 µg. Some samples such as FFPE blocks, or tumor biopsies contain impurities that inhibit the activity of DNA polymerase, so a maximum of 2 µg is recommended.

If you have enough input amount, we recommend running samples in triplicates, e.g. using 2×3=6 µg of DNA for each sample to get better quantitative data, as shown in Technology Guide Section 2.1, Fig. 9, which lists the number of clonotypes you can obtain from different amounts of input DNA material.

Q8: What is the better choice of starting material for FFPE samples, DNA or RNA?
A8: In our experience, DNA, due to its stability, generally provides more robust data with FFPE samples. However, RNA can lead to a more sensitive profiling of clonotypes provided that the RNA is not significantly degraded. For FFPE AIR-RNA

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profiling, we recommend using RNA with a RIN Score > 5, or RNA with at least 20% of fragments above 300-n. For best results, we recommend purifying both RNA and DNA from FFPE samples and running both AIR-RNA and AIR-DNA assays.

Q9: Can less than 500 ng of DNA be used for the AIR DNA Profiling Kit?
A9: As shown in Technology Guide Section 2.1, Fig. 9, profiling with 500 ng or lower is possible, but the number of detected clonotypes will be reduced. Furthermore, only top abundant clonotypes (with clonotype-specific cells numbering more than 10) could be quantitated.

Sequencing:

Q10: What is the recommended number of reads for the AIR protocol?
A10: For sequencing, we recommend around 5-10 million reads per sample, depending on the number of samples in one flow cell and the required sensitivity. More information about sequencing can be found in the User Manuals' NGS Prep and Sequencing Section.

Q11: How much PhiX is needed for the sequencing run?
A11: We recommend adding 15% of PhiX to the library (anywhere in the range 10-20% is acceptable).

Q12: Can AIR RNA and AIR DNA samples be sequenced together in the same lane?
A12: Yes, AIR-RNA and AIR-DNA samples can be combined for NGS, but due to differences in amplicon sizes (approximately 350 bp for RNA vs. approximately 250 bp for DNA), we suggest mixing AIR-RNA with AIR-DNA at a 1.5:1 ng ratio (e.g., 150 ng of AIR-RNA with 100 ng of AIR-DNA). Be sure to use the different indexing primers for RNA and DNA samples. NGS sequencing primers are the same for both AIR-RNA and AIR-DNA.

Q13: Which instruments can be used for CDR3 or full-length receptor sequencing?
A13: For most applications, we recommend using the Illumina NextSeq 2000 instrument with SBS-Leap sequencing chemistry kits due to low NGS cost and wide range of flow cells (between 100M to 1.8B reads for both 300-cycle and 600-cycle Paired-End reads). The NextSeq 500/550 could still could be used for CDR3 profiling, but the cost of sequencing is higher than when using the NextSeq 2000. The MiSeq instrument allows sequencing for the 600 cycles (full-length receptor sequence) but its scale is limited to 5-10 samples. Our kits use the reverse complement workflow, so for MiSeq, you would need to use the protocol with one index primer and the V2/V3 Kits. To optimize your sequencing protocol, please contact techsupport@illumina.com.

Q14: Can you sequence DriverMap AIR and DriverMap Human Genome-Wide Targeted RNA-Seq Expression (EXP) Profiling Kits in the same flow cell?
A14: Yes, the custom sequencing primers (Reverse and Forward SeqDNA and SeqIND primers) are the same in the DriverMap AIR and DriverMap Human Genome Wide Expression Profiling Kits. Please note that AIR and Expression profiling samples need to be amplified with different indexing primers to be sequenced in the same flow cell.

Q15: Can the libraries be sequenced on the MiSeq? Do you have any recommendations?
A15: Yes, the AIR library can be sequenced on the MiSeq instrument. Our kits use the reverse complement workflow, so only the V2/V3 kits are compatible with the MiSeq and you would need to use the protocol with one index primer. Attached is the Illumina Guide for Indexed Sequencing Overview. To optimize your sequencing protocol please contact techsupport@illumina.com.

Q16: Is it recommended to do sequencing on the NovaSeq 6000 or NovaSeq X Platform?
A16: As NGS with NovaSeq instruments provides a minimum of 8B reads per flow cell, NovaSeq instruments could be used for NGS analysis of AIR samples that are done on a large scale (i.e.> 500 samples).

Q17: Can sequencing be performed on NovaSeq 6000?

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A17: Yes, it is possible to use two custom indexing primers with the NovaSeq 6000 Reagent Kit v1.5, as the NovaSeq 6000 machine works with a mixture of Ind1 and Ind2 sequencing primers from one well. The recommended approach is to spike Cellecta primers in the wells with Illumina primers, as described in the Spiking custom primers into the Illumina sequencing primers | Illumina Knowledge. If you prefer not to spike in the primers, you can use the custom primers protocol described in the NovaSeq Series Custom Primers Guide (illumina.com).

We have a customer that used the SP flow cell and loaded 2nM with the protocol with 148-10-10 -148 cycles as follows:

Sequencing setup - NovaSeq 6000					
Kit version	**Primer name**	**Cartridge posititon**	**Total volume (mL)**	**Custom primer final conc. (uM)**	**Volume Primer (uL)**
S1 and S2 100/200/300 cycles	Read 1 (Seq DNA1)	24	4	0.3	12
S1 and S2 100/200/300 cycles	Read 2 (Seq DNA2)	13	2	0.3	6
S1 and S2 100/200/300 cycles	Index 1 (Seq Ind Fw)	23	5	0.3	15
S1 and S2 100/200/300 cycles	Index 2 (Seq Ind Rv)	23	5	0.3	15
Cellecta primers (uM):	**100**				

Please note that Cellecta has not internally validated this protocol, so to optimize your sequencing protocol please contact techsupport@illumina.com.

Q18: What are the recommended concentration and sequencing primer setup for NovaSeq 6000 kits?
A18: For NovaSeq 6000, we generally recommend starting at 100 pM (same as PhiX) if the library type loading concentration is unknown, then optimizing in 50-100 pM increments based on results. Here is Illumina resource for determining optimal loading based on PF and occupancy. To optimize your sequencing protocol please contact techsupport@illumina.com.

Q19: Can sequencing be performed on NovaSeq X?
A19: Yes, our kits use Dual DNA/RNA UDP Indexes and follow the reverse-complement workflow. For loading on the NovaSeq X, follow Illumina's protocol for NovaSeq X Custom Primers Protocol. To optimize your sequencing protocol please contact techsupport@illumina.com.

Data Analysis:

Q20: How can I analyze the data for TCR and BCR analysis?
A20: We recommend using the MiXCR software tool (MiLaboratories) for alignment and AIR repertoire data analysis. Here are the options for our customers:
- **Academic customers:** Academics may use the free, user-intuitive MiXCR software available for download on Platforma.bio.
- **Industry customers:** MiLaboratories offers a free trial period to test our kits on Platforma.bio or MiXCR command line version, starting with a 1-month free trial onwards. MiXCR packages include special presets for AIR kit analysis, the complete workflow can be set up with the name of the AIR Kit eg: MiXCR Guides Cellecta Kit
- **Cellecta bioinformatics services:** Additionally, Cellecta offers bioinformatics services, including running the MiXCR pipeline and providing output reports. Custom analysis can also be performed upon request. Here is an example report: 2024-02-13 Final Report. Request a quote or more information at quotes@cellecta.com.

Other bioinformatics tools/frameworks are also available to analyze immune repertoires as follows:

Tools	Published year	Aligner	Website
IMGT/HighV-QUEST	2012	GPA (NW)	https://www.imgt.org/IMGTindex/IMGTHighV-QUEST.php
IgBLAST	2013	BLAST	https://www.ncbi.nlm.nih.gov/igblast/
IMSEQ	2015	SCF matching	https://www.imtools.org/
LymAnalyzer	2015	Fast-tag	https://sourceforge.net/projects/lymanalyzer/

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Q21: How do I obtain a MiXCR license?
A21: For an academic license go to https://licensing.milaboratories.com/, for a business license email licensing@milaboratories.com and provide a Cellecta kit reference.

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11. Technical Support

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