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USER MANUAL

DriverMap™ EXP Human Genome-wide 19K Profiling Kit (hGW19K) - V3

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1. DriverMap™ EXP Human Genome-wide 19K Profiling Kit – V3

The DriverMap™ EXP Human Genome-wide 19K Profiling Kit (hGW19K) measures the expression level of almost 19,000 human protein-coding genes by combining highly multiplexed RT-PCR amplification with the depth and precision of Next-Generation Sequencing (NGS) quantitation. It is an easy-to-run, single-tube, single-day assay that can be run directly from total RNA isolated from cells, tissue, or blood, or directly from cellular extract using the DirectCell™ Protocol.

The development of the DriverMap™ EXP hGW19K Assay involved extensive optimization and experimental validation of tens of thousands of primer sets to identify a pool of primers that could be combined in a single multiplex RT-PCR reaction to amplify representative transcript sequences (i.e., conserved transcript regions common to different mRNA isoforms from the same gene) from each of ~19,000 protein-coding genes in the human genome. The amplified sequences are then quantified using NGS on the Illumina platform. The DriverMap™ EXP hGW19K Assay provides optimal quantitative expression data within a 10^4 – 10^5-fold dynamic range from 10-50 ng of total RNA and can be used effectively with as little as 1 ng of RNA. However, when using less than 10 ng of total RNA, the measurements for low abundant transcripts may be less reproducible.

Main advantages of DriverMap™ EXP hGW19K Assay:

- An easy-to-run, one-tube, single-day assay allows profiling of transcripts from total RNA isolated from whole blood, PBMC, tissue, or directly from a small number (e.g., 100-50,000) of purified cells without prior RNA isolation.
- The assay provides robust, quantitative, and reproducible measurements of the copy number of mRNAs over as many as 4-orders of magnitude differences in expression level.
- The assay provides increased sensitivity from a small amount of RNA (1-50 ng) using a set of highly experimentally-validated primers.
- The DriverMap EXP protocol employs several mechanisms to minimize amplification of off-target and primer dimer background products.
- No mRNA enrichment, rRNA, mitochondrial, beta-globin depletion, or other total RNA processing is required.
- The primers are designed using Illumina's DNA/RNA dual UD Indexes labeling strategy to minimize NGS index-swapping background issues, and amplified products are compatible with 100-150-cycle Paired-End NGS sequencing using Illumina's NextSeq/NovaSeq instruments.
- NGS data can be analyzed using the Salmon software package available online. Cellecta also provides custom data analysis services on request.

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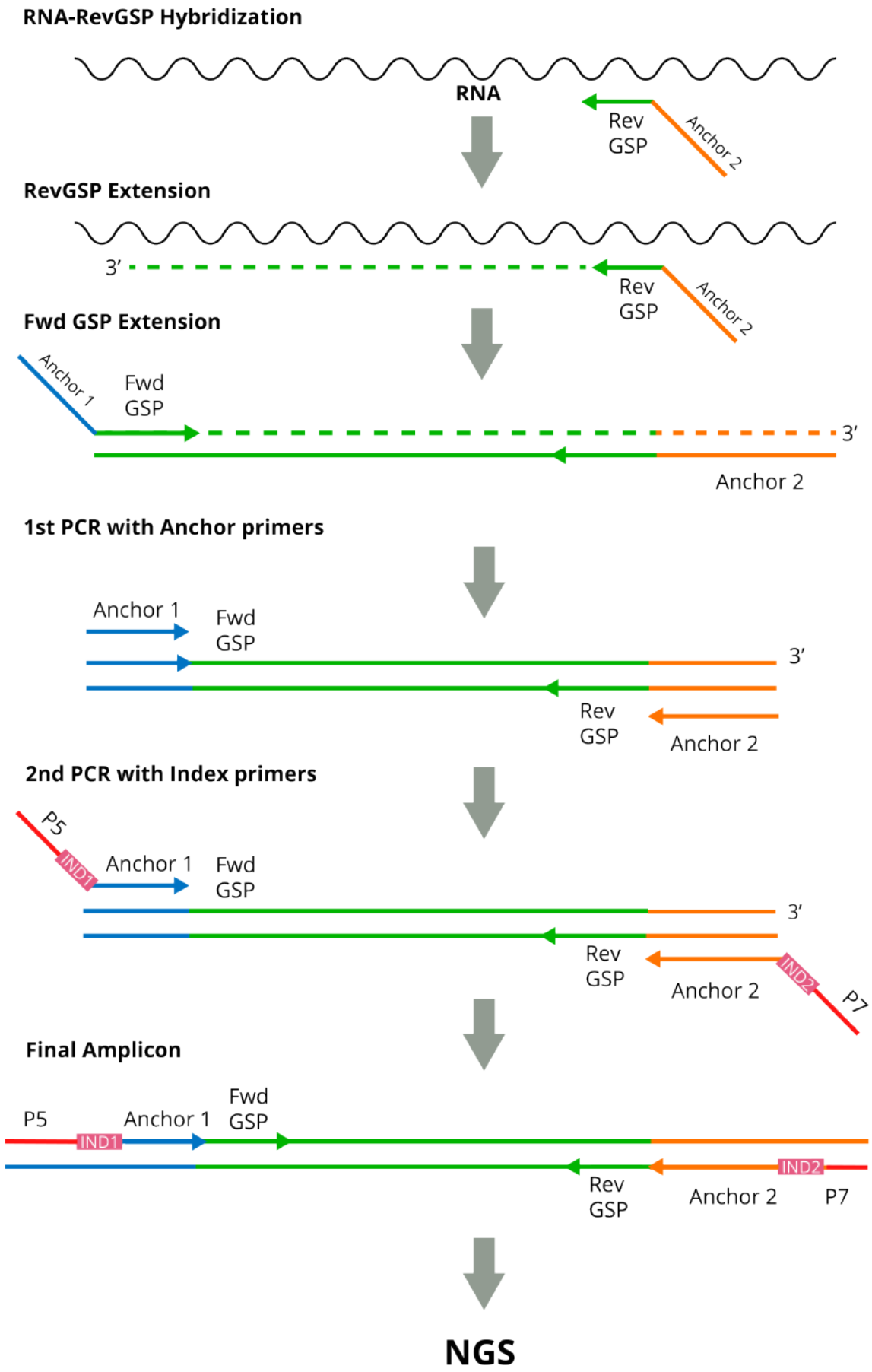


Fig. 1. Outline of DriverMap EXP RT-PCR-NGS assay for expression analysis of mRNA targets

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Related Products and Services:

- **DriverMap™ AIR TCR/BCR Profiling Kit (human/mouse RNA/DNA)** is designed to generate comprehensive AIR CDR3 repertoire profiling of all seven TCR (TRA, TRB, TRD, and TRG) and/or BCR (IGH, IGK, and IGL) chains from RNA or DNA samples. AIR RNA assay provides higher sensitivity in detecting full-length (CDR1-CDR2-CDR3) or 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 the AIR Technical Guide.

- **DriverMap™ EXP Human Genome-wide 19K Dried Blood Microsamples Profiling Kit** allows profiling from 30 µl of dried blood collected by Mitra® devices with VAMS® technology. Expression profiling in dried blood is a convenient strategy for longitudinal analysis of blood samples which could be collected directly by patients without going to a blood collection facility.

Cellecta also provides a DriverMap™ Expression Profiling Service. For more information, please visit our website: DriverMap EXP Service Webpage

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. Kit Components

This kit contains enough reagents for the preparation of 24 or 96 NGS samples starting from total RNA isolated from blood, tissue, cells, or other biological samples or cell extracts.

Box 1: Kit Components for DriverMap™ EXP hGW19K

Component	Description	Cap Color	Conc.	Volume (24 rxn)	Volume (96 rxn)
Forward hGW19K primer mix	Mix of forward gene-specific primers (Fwd GSP) for 19K human genes, 5X	n/a	5X	220 µl	880 µl
Reverse hGW19K primer mix	Mix of reverse gene-specific primers (Rev GSP) for 19K human genes, 5X	n/a	5X	110 µl	440 µl
Positive Control RNA	Total universal RNA (mix of total RNA isolated from several human tissues)	white	50 ng/ µl	5 µl	5 µl
Hybridization Buffer	Buffer for hybridization of Rev GSPs with mRNA, 4X	yellow	4X	1 ml	1 ml
Water	Milli-Q purified water for reaction mixes	white	NA	3 ml	12 ml
dNTP Mix	Mix of dATP, dGTP, dCTP and dTTP	white	10 mM	100 µl	400 µl
RT-EXT Buffer	cDNA synthesis and forward gene-specific primer extension reaction buffer, 5X	green	5X	250 µl	1 ml
Reverse Transcriptase	M-MLV Reverse Transcriptase (RT) optimized for multiplex RT-PCR assay	green	20X	30 µl	120 µl
Hot-start RT Aptamer	Aptamer which inactivates RT at room temperature but activates RT at 50°C	green	20X	30 µl	120 µl
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	50 µl	200 µl
Anchor Primer Mix	Forward/Reverse Anchor-specific Primers for 1st PCR, 10X	red	10X	300 µl	1.25 ml
Primer Removal Enzyme	Enzyme Mix for primer removal	orange	20-50X	80 µl	320 µl
Forward SeqDNA NGS Primer	Forward NGS Primer for cDNA sequencing	blue	100 µM	30 µl	90 µl
Reverse SeqDNA NGS Primer	Reverse NGS Primer for cDNA 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

Box 2: Kit Components (Sold Separately)

Component	Description	Conc	Volume (24	Volume (96

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			reactions) *	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 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.

For General RNA Preparation and QC

Name	Recommended Manufacturer	Catalog #	Purpose
RNAeasy Plus Micro/Mini Kit	QIAGEN	74034/74134	RNA isolation from PBMC, sorted cells or tissues
Tempus Blood RNA tubes/Spin RNA isolation kit	Thermo Fisher	4342792/ 4380204	RNA isolation from whole blood
RNA 6000 Pico Kit for 2100 Bioanalyzer	Agilent	5067-1511	RNA quality control calculation
HS RNA Analysis Kit for Fragment Analyzer	Agilent	DNF-472_0502	RNA quality control calculation

Additional Reagents 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 in 0.5-1.5-ml test tubes
Catalyst 96 slotted ring magnetic plate	Alpaqua	A000550	RNA-RevGSP hybrid product purification in 96-well plate
IGEPAL CA-630 (NP40)	Sigma	18896	Detergent additive for hybridization solution in DirectCell protocol
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 Mid Output Kit v2.5 (150 cycles)	Illumina	20024909	Next-Gen Sequencing
NextSeq® 500/550 High Output Kit v2.5 (150 cycles)	Illumina	20024908	Next-Gen Sequencing
NextSeq 1000/2000 XLeap-SBS P2 Reagent Kit (100 cycles)	Illumina	20100987	Next-Gen Sequencing
NextSeq 1000/2000 XLeap-SBS P4 Reagent Kit (100 cycles)	Illumina	20100994	Next-Gen Sequencing

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MiSeq Reagent Kit v3 (150 cycles)	Illumina	MS-102-3001	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
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 RNA purification, RNA-RevGSP hybridization, and cDNA synthesis through the first PCR setup) in a location set aside for RNA work, and use a set of equipment, pipettes, test tubes, and other consumables dedicated for work involving RNA. 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 or 0.5-ml test tubes or tube strips to run the assay. To minimize the hands-on time and mistakes in 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) pipet. After adding all the necessary reagents, seal the plate well with Clear Adhesive Film and use a Compression Pad to minimize evaporation from experimental samples.

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

- Pipette 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 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 are using a multichannel pipette, aliquot the Master Mix in a tube strip first, then pipette from this strip into each sample.

- For all incubation steps in the thermocycler machine use a heated lid set up at standard 105°C.

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5. Sample Preparation

Depending on the sample amount, either total RNA or isolated cells/tissue microsamples (i.e., DirectCell Protocol) can be used as input for the DriverMap EXP assay. No preliminary poly(A) selection or ribosomal/mitochondrial/globin RNA depletion is required:

- For bulk tissue, blood, or cell samples with more than ~10,000 cells, follow the recommendations of the General RNA Preparation Protocol below (Section 5.1).

- For biological samples with a small number of cells—less than ~10,000 cells (e.g., sorted immune cell fractions or cells grown in 96-well plates), or tissue microsamples—we recommend using the cells directly as input. Follow the guidelines in the DirectCell Protocol below (Section 5.2).

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5.1. General RNA Preparation Protocol

1. For larger bulk tissue, blood, or cell samples, follow the guidelines below to isolate, quantify, and QC total RNA. Generally, deep, quantitative expression profiling requires using 10-50 ng of total RNA isolated from whole blood, PBMC, tissue, or cell samples. Due to high sensitivity and specificity, the hGW19K assay can also be used for expression profiling from 1-5 ng RNA with reduced sensitivity and reproducibility in profiling of low abundant transcripts. Use the appropriate method to isolate total RNA. We recommend the following RNA isolation kits.
    - Whole blood: we recommend using the Tempus (Thermo Fisher) or PAXgene (QIAGEN) for total RNA isolation. Based on our experience, the collection of 3 ml of whole blood in Tempus RNA Blood Tubes followed by RNA isolation using the Tempus Spin RNA Isolation kit provides the most simple and reliable protocol for purification of high-quality (DNA-free) RNA from whole blood.
    - PBMC, buffy coat, or fresh/frozen tissue and cells: for more than 50,000 cells, we recommend using the QIAGEN RNeasy Micro/Mini Plus Kit for total RNA isolation.
    - FFPE tissue: we recommend using the Thermo Fisher RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Cat.# AM1975). With FFPE, the yield and quality of isolated RNA can vary significantly depending on the age and method used to fix the sample. It is critical to assess the quality of the RNA before proceeding with the DriverMap EXP protocol.

2. Ensure the extracted RNA is free of almost all DNA.
    - Small amounts of DNA (i.e, <5% genomic DNA contamination) do not interfere with the assay. With the Tempus and RNeasy Plus RNA Kits, a separate DNase treatment is not necessary. When using other RNA isolation protocols, a separate DNase treatment of RNA samples is recommended before starting the procedure. Follow the DNase treatment instructions in the manufacturer's RNA isolation kit manual. In the DirectCell protocol, DNase treatment is not required.

3. Quantify total RNA with the Thermo Fisher NanoDrop (or equivalent), and confirm the integrity of RNA in each sample before starting the assay by one of the following methods:
    - Determine the RIN number using the Agilent Bioanalyzer and Agilent RNA 6000 Pico Kit (Cat.# 5067-1511).
    - Determine the RIN number using the Agilent Fragment Analyzer and High Sensitivity RNA Analysis Kit (Cat.# DNF-472-1000).
    - Using a gel imager, calculate the 28S:18S rRNA ratio after running the RNA samples on an agarose gel.

The RIN number for your RNA samples should be no less than 5. If you are using FFPE RNA samples, you should additionally check the samples to ensure that a significant level (at least 20%) of RNA fragments is larger than 300 nt.

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5.2. DirectCell™ Protocol

For a small number of cells such as immune cell fractions or a small number of cells grown in 96-well plates (e.g. 500-50,000 cells), and tissue samples (up to ~1 mg), we recommend directly using cells in the DriverMap EXP Assay without an RNA purification step. Refer to the guidelines in this section for the preparation of these samples.

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

1. Purify cell fractions (e.g. by FACS) or prepare dissociated tissue samples (e.g., by collagenase treatment). Adjust the volume of each sample to a total of 12 µl. Depending on your cell collection method, this may require spinning down the cells and removing excess supernatant over 13 µl. Any type of cell collection buffer (e.g., 1x PBS or cell growth media) is compatible with the DirectCell protocol.

2. When you are ready to start the assay, prepare the Hybridization Buffer Master Mix as described in Step 1 of the Hybridization Procedure but add NP-40 to 0.1% to facilitate cell lysis, and then set up the Hybridization reaction with your sample as described.

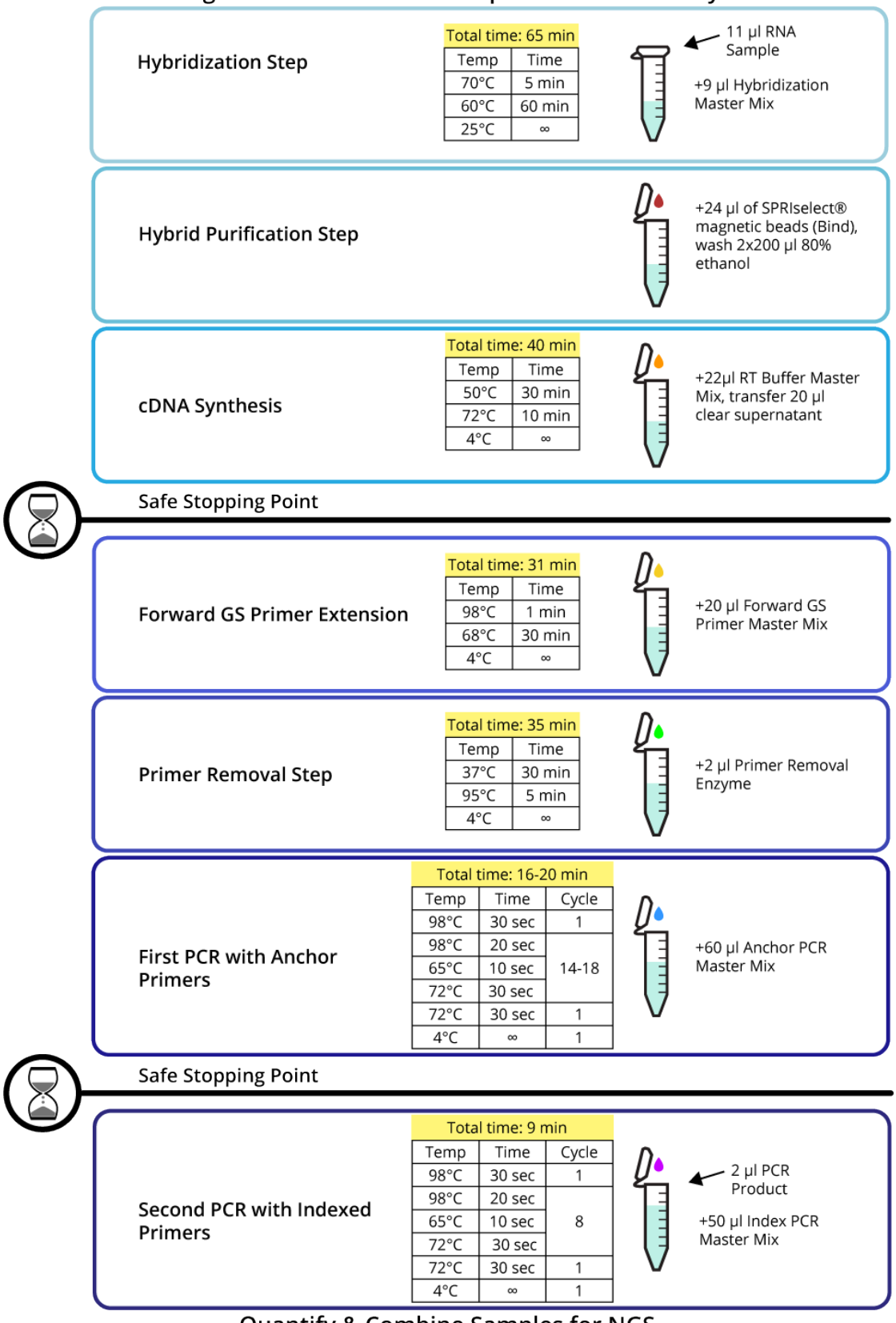
3. Follow the standard protocol for hybridization, SPRIselect magnetic bead purification of RNA-Rev GSP hybrids, cDNA synthesis, and amplification steps.

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6. Protocol Outline

Fig 2. Overview of DriverMap EXP hGW19K Assay



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6.1. Hybridization of mRNA with Reverse Gene-Specific Primers

In this step, Reverse hGW19K primers (Rev GSPs) are hybridized with target mRNAs, and mRNA-Rev GSP hybrids are purified from non-hybridized primers by SPRIselect magnetic beads. Both purified RNA and purified cells (Section 5.2 DirectCell protocol) can be used in this protocol. The protocol assumes that the reactions will be set in a 96-well PCR plate. For small numbers of samples, reactions can be done in tubes or strips.

Note: Hybridization, cDNA synthesis, and Forward Primer Extension steps should be set up in a pre-PCR room to avoid contamination with final PCR products.

1. Prepare a Hybridization Master Mix as described below for all samples and controls (make 5% extra to account for pipetting error) and aliquot 9 µl in the wells of a 96-well plate:

Hybridization Master Mix Component	Volume per sample, µl
Hybridization Buffer, 4X	5
Reverse hGW19K primer mix, 5X*	4
Total	9

Note: If you decide to run biological replicates, increase the number of tubes, volume, and amount of RNA allocated for each sample respectively.

2. Adjust the volume of each RNA Sample (e.g., 50 ng of RNA) to 11 µl with water as shown in the table or directly use 11 µl of RNA with low concentration (e.g., 5 ng/ µl).

Component	Volume per sample, µl
Total RNA (~50 ng)	1 – 11*
Water, to 11 µl final volume	0 – 10

Note: As a positive control, we recommend running 50 ng of positive control RNA included in the kit; and as a negative control, 11 µl of water.

3. Add 11 µl of RNA Sample to each well with the pre-aliquoted 9 µl of Hybridization Master Mix and mix contents using an Eppendorf plate shaker or by pipetting 3 times. Seal the plate with adhesive film and spin it down to collect droplets. Load the plate in the thermal cycler, and run the following program to hybridize mRNAs with Reverse hGW19K primers:

Temperature	Time
70°C	5 min

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60°C	60 min
25°C	∞

4. Add 24 µl (1.2x volume) of SPRIselect magnetic beads (adjusted to room temperature) to each reaction well, seal the plate, and mix SPRIselect beads and hybridization solution in wells using Eppendorf plate shaker. Importantly, you need to optimize the speed of the plate shaker (e.g., 16,500 rpm for 30-60 sec) to completely mix viscous SPRIselect beads with solution in each well. Check visually that each well contains a homogeneous brown color solution in the whole volume.

5. Incubate the mixture for 5-10 minutes at room temperature. While waiting, prepare the Reverse Transcriptase Master Mix based on the protocol in Step 6.2 and store on ice before use.

6. Place the plate in the Magnetic Stand for 96-well plates for 1-2 minutes or until the solution is clear. Carefully remove and discard the supernatant without disturbing the pellet.

7. Add 200 µl of freshly prepared 80% ethanol in each reaction well without removal of the plate from the Magnetic Stand, wait for 2 minutes, carefully remove, and discard the supernatant without disturbing the bead pellets.

8. Repeat 80% ethanol washing as in Step 7.

9. Briefly centrifuge the plate at low speed and place the plate in the Magnetic Stand. Use a 20-µl pipette to remove the residual ethanol droplets from reaction wells and air-dry the beads at room temperature for approximately 5 minutes. Visually check that no residual ethanol droplets are present in the wells. Avoid over-drying the SPRI beads.

! Immediately proceed to the next step (cDNA Synthesis)

Last modified: 4 September 2024

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).

1. Prepare the Reverse Transcriptase Master Mix as follows for each sample plus 5% extra volume of all components:

RT Buffer Master Mix Component	Volume per sample, µl
RT-EXT Buffer, 5x	4.4
dNTP Mix	1.1
Water	14.3
Hot-start RT Aptamer	1.1
Reverse Transcriptase	1.1
Total	22

2. Gently vortex Master Mix and spin down briefly to collect droplets. Add 22 µl of the Reverse Transcriptase Master Mix in each reaction well and resuspend SPRIselect beads attached to the well surface in the plate using an Eppendorf plate shaker (preferred choice) or by gentle pipetting without generating bubbles. Briefly centrifuge the plate at low

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speed and place the plate in a Magnetic Stand for 2 minutes. Transfer 20 µl of clear supernatant without beads from each reaction well to the new plate and seal the plate.

3. Load the plate in the thermal cycler (with heating block lid at standard 105°C) and start running the following program:

Temperature	Time
50°C	30 min
72°C	10 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: 19 July 2024

6.3. Forward Gene-Specific Primer Extension

In this step, the pool of Forward hGW19K Primers (Fwd GSPs) with adjoining Anchor 1 sequences generates sense strands of the target amplicons flanked from both sides by Anchor 1 and Anchor 2 sequences using the cDNA generated in the previous step as a template.

1. Prepare the Forward GSP Extension Master Mix as follows for all samples plus 5% extra volume of all components:

Forward GS Primer Extension Master Mix Component	Volume per sample, µl
RT-EXT Buffer, 5X	4
Water	7.4
Forward hGW19K Primer Mix, 5x	8
DNA Polymerase	0.6
Total	20

2. Gently vortex Master Mix and spin down briefly to collect droplets. Spin down the cDNA plate, remove the seal, then add 20 µl of the Forward GS Primer Extension Master Mix to each reaction well of the plate:

Component	Volume, µl
cDNA (previous step)	20
Forward GS Primer Extension Master Mix	20
Total	40

3. Mix contents by pipetting three 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.

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

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Temperature	Time
98°C	1 min
68°C	30 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 using an Eppendorf plate shaker or pipetting 3 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	30 min
95°C	5 min
4°C	∞

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

Last modified: 29 August 2024

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 GSP Extension step.

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

Anchor PCR Master Mix Component	Volume per sample, µl
PCR Buffer, 5X	12
dNTP Mix	1.2
Anchor Primer Mix, 10X	10
Water	36.2
DNA Polymerase	0.6
Total	60

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 60 µl of Anchor PCR Master Mix to each reaction well:

Component	Volume per sample, µl
Forward GS Primer Extension DNA (after primer removal step)	40
Anchor PCR Master Mix (prepared above)	60
Total	100

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3. Mix content by pipetting 3 times or by using an Eppendorf plate shaker. Seal the plate with new adhesive film and spin it down to collect droplets.

✻ Note: Take the plate 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 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	30 sec	1
98°C	20 sec	14-18* Note below
65°C	10 sec	
72°C	30 sec	
72°C	30 sec	1
4°C	∞	1

✻ Note: To avoid bias in gene expression levels by over-cycling samples, we recommend starting with 14 PCR cycles for samples that contained 50 ng of RNA or 10,000 cells with high content of polyA+ RNA for DirectCell protocol. For samples with less RNA or cells, you need to add extra cycles, e.g., 16 cycles for 10 ng RNA. The recommended number of cycles in many cases needs to be optimized and adjusted based on mRNA content in specific cell/tissue types, quality of RNAs, etc. To optimize the number of cycles in the First PCR, especially for RNA samples never used before, we recommend analyzing amplified cDNA products using 3% agarose-TAE gel electrophoresis or Bioanalyzer. For optimal cycle number, you should observe a weak smear of amplified cDNA products in the range of 200-400bp. Use a positive control RNA sample as a reference. If experimental samples do not generate visible amplification products, add 2-3 more amplification cycles, and analyze the samples again, as described above.

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

Last modified: 19 July 2024

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 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

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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 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

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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 (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 UDP 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: 19 July 2024

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 sample—are analyzed (adjusted if necessary), measured, and then pooled in equimolar amounts for sequencing.

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 below for the expected results of amplified libraries generated from experimental and positive control RNA samples. For the hGW19K assay, the smear with several bright bands is usually in the 180-450 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) or Fragment Analyzer using the High Sensitivity NGS Analysis Kit (Cat.# DNF-473-1000) using the manufacturer’s protocol.

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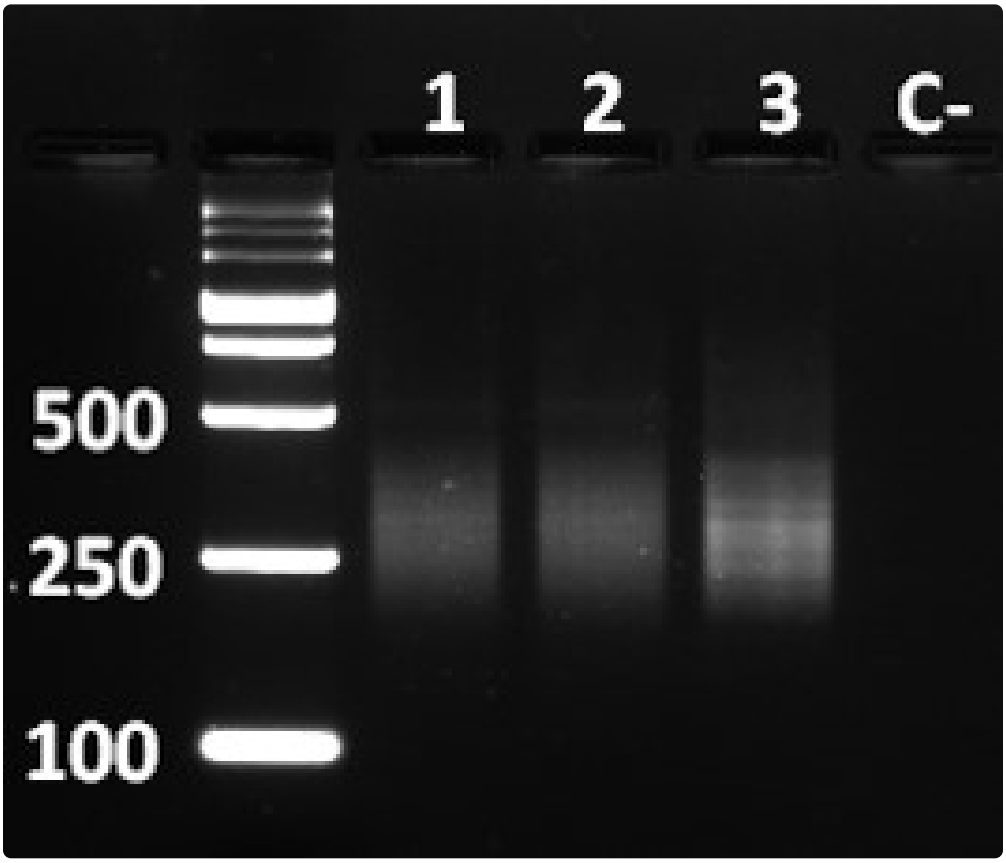


Fig. 3. Gel Electrophoresis of Amplified Indexed Libraries after Index Primer PCR Reaction using experimental RNA (1,2), positive control RNA (3), and negative control samples (C-).

2. Analyze yields of the Amplified Indexed Libraries. If amplified cDNA products are generated from samples with similar content of mRNA, the yield should all be roughly the same for all experimental samples within 2-3-fold levels. 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 mRNA content or quality of RNA used in the assay. If several or all the experimental RNA samples have significantly lower yield (not detected or very low intensity in comparison with positive control RNA), you can rerun these experimental RNA samples in the thermal cycler for 2-5 additional cycles. After cycling, quantify the products again relative to the Positive Control RNA. Adjustment for small differences (e.g. 2-5-fold) in the yield of amplified products between samples will be made when the amplified Indexed Libraries are quantitated (in Bioanalyzer) and combined for the sequencing step following this QC step.

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 Negative Control sample, then incubate at 37°C for 30 minutes. Do not inactivate the Primer Removal Enzyme by incubating the reaction at a high temperature, as it will melt the double-stranded PCR products and complicate the analysis of PCR products in the Bioanalyzer.

2. To ensure accurate quantification for sequencing, especially for samples with low yield of amplified products, you could consider repeating the quantification procedure of the Amplified Indexed Libraries. Quantifying the PCR products after the removal of PCR primers is more accurate than quantifying before the primer removal clean-up step. 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) or Fragment Analyzer using the High Sensitivity NGS Analysis Kit (Cat.# DNF-473-1000) using the manufacturer's protocol.

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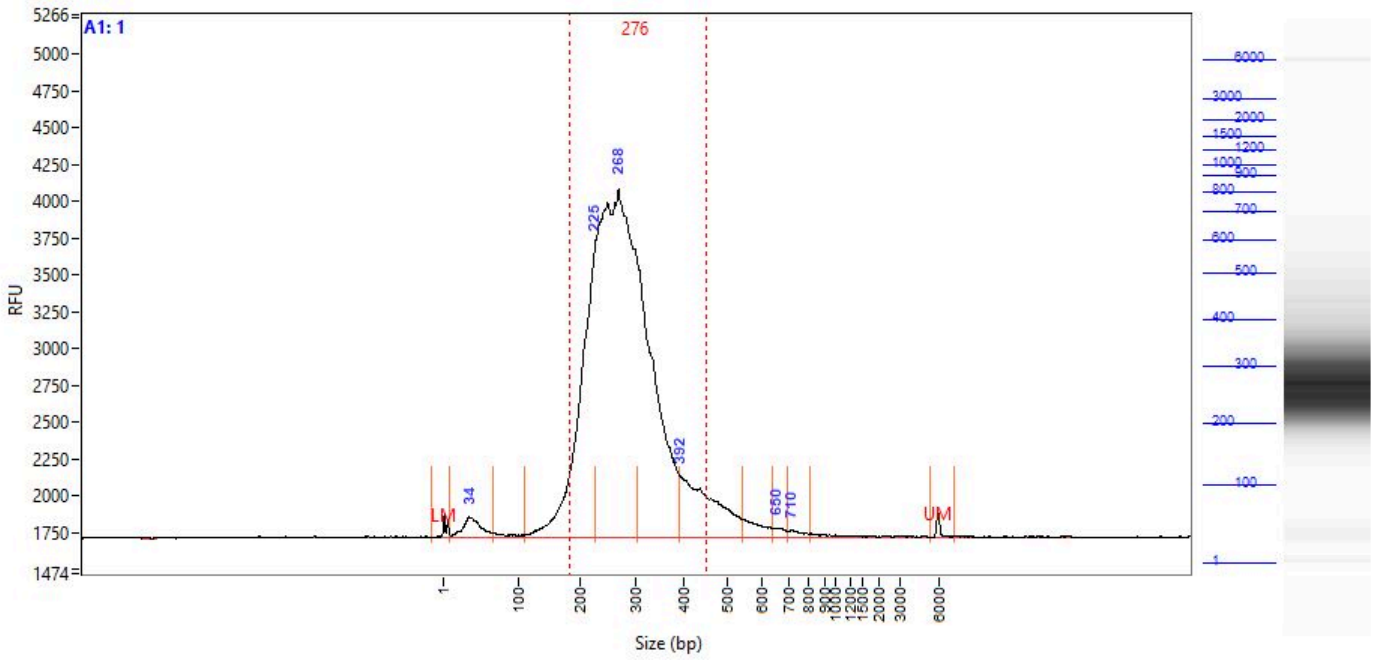


Fig. 4. Size distribution of Amplified Indexed Library after Primer Removal step analyzed by Fragment Analyzer.

3. Quantitate the yield of Amplified Indexed Libraries using smear analysis of amplified products in the region of 180-450bp (see Fig.4). 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 flow cell.

The DriverMap™ EXP kit generates approximately 10,000-15,000 amplified products, so MiSeq, NextSeq, and NovaSeq Illumina instruments can be used for NGS. We recommend using the NextSeq 2000 with the newest XLeap-SBS assay chemistry which provides the most cost-effective solution to sequence 10-1,000 samples in a single flow cell. MiSeq could be used to sequence a small number (5-10) of samples. The NovaSeq X instrument can be used for sequencing a large number of samples such as 384 (four 96 well plates) by combining with other NGS samples in one flow cell (with a minimum capacity of 8B reads per flow cell). Please refer to the table below for guidelines on how many samples may be combined for different instruments (MiSeq and NextSeq) and read depths. Generally, you should aim for approximately 5M reads per sample for regular and 10M reads for high-sensitivity NGS. For Illumina instruments, hGW19K assays require the use of 100-cycle or 150-cycle Paired-End reagent kits.

Instrument	Reads per flow cell	Number of samples for multiplexing per flow cell	Reads per sample
MiSeq Series	50 million (v3)	10	5M
NextSeq 500/550	120 million (Mid Output)	24	5M
NextSeq 500/550	400 million (High Output)	80	5M
NextSeq 2000	100 million (P1 flow cell)	20	5M
NextSeq 2000	400 million (P2 flow cell)	80	5M
NextSeq 2000	1.2 billion (P3 flow cell)	150-250	5M-10M
NextSeq 2000	1.8 billion (P4 flow cell)	192-384	5M-10M

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

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7.2. Purification 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.

1. Add 1.5x volume of SPRIselect magnetic beads (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 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 20-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.
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 Libraries sample using the Qubit® dsDNA High Sensitivity Assay.
11. Dilute the pooled Amplified Indexed Libraries probe sample to 1.6 ng/µl, which corresponds to a concentration of 10 nM for the following NGS step (for NextSeq).

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

Last modified: 30 August 2024

7.3. Next Generation Sequencing

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).

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Note: The procedure below is based on our experience with the NextSeq 500/550 using the 150-cycle NextSeq 500/550 Mid Output Kit v2.5 (Illumina Cat.# 20024909) and NextSeq 2000 using 100-cycle P2 flow cell with XLeap-SBS chemistry (Illumina Cat.# 20100987).

The multiplexing level may be modified to meet your experimental needs. For example, multiplex fewer samples together to generate more sequencing reads per sample (i.e., more depth) for increased and more sensitive detection of low abundant transcripts (e.g. cytokines) present across a broader dynamic range of abundance/expression levels, or, if you are mostly interested only in highly abundant/expressed transcripts, you can sequence more samples together which will be less expensive.

1. Follow the standard Illumina procedures for Cluster Generation starting with 10 nM of the purified PCR sample.

NextSeq 500/550
For the NextSeq 500/550, 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

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.

NextSeq 2000
For the NextSeq 2000, you must use the Custom wells of the reagent cartridge. HT1 buffer is required to mix with custom sequencing primers. 1.8 µl of each of the Read primers are mixed with 600 µl of HT1 buffer and loaded into Custom 1 well. 3.6 µl of each of the Index primers are mixed with 600 µl of HT1 buffer and loaded into the Custom 2 well.

- Forward SeqDNA NGS Primer (Read 1 Sequencing Primer) and
- Reverse SeqDNA NGS Primer (Read 2 Sequencing Primer) into Custom 1 well
- Forward SeqIND NGS Primer (Index 1 Sequencing Primer) and
- Reverse SeqIND NGS Primer (Index 2 Sequencing Primer) into Custom 2 well

2. Perform the NGS run using 150-cycle Paired End reads on the NextSeq 500/550 or 100-cycle Paired End reads on the NextSeq 2000 instrument. The optimal loading concentration for cluster generation of indexed libraries is approximately 1.8 pM for NextSeq 500/550 and 800pM for NextSeq 2000. Use the following program for the sequence run, and for the NextSeq 2000 be sure to also indicate the custom wells:

NextSeq 500/550

Program	Custom Primer	Cycles

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Read 1:	Forward SeqDNA	70
Index 1:	Forward SeqIND	10
Index 2:	Reverse SeqIND	10
Read 2:	Reverse SeqDNA	70

Note: The program has a total of 160 cycles. There are enough reagents in the 150-cycle kit (Illumina Cat.# 20024909) to run at least 160 cycles.

NextSeq 2000

Program	Custom Primer	Cycles	Custom Well
Read 1:	Forward SeqDNA	59	Custom 1
Index 1:	Forward SeqIND	10	Custom 2
Index 2:	Reverse SeqIND	10	Custom 2
Read 2:	Reverse SeqDNA	59	Custom 1

Note: The program has a total of 138 cycles. There are enough reagents in the 100-cycle P2 flow cell (Illumina Cat.# 20100987) to run at least 138 cycles.

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8. Data Processing and Analysis

This section provides information to process your data using the Salmon Software Package (available online). The process begins with extracting reads from FASTQ files generated by an Illumina sequencer. Before proceeding, ensure that your sample information is entered correctly into the Illumina SampleSheet. The SampleSheet file must include all indexing details and the sample names associated with your experiment.

Convert Sequencing Data to FASTQ Format

1. To convert Illumina BCL intensity reads into FASTQ files, use the following command line with bcl2fastq:

```
$ bcl2fastq -R /path/to/BCL_folder --sample-sheet /path/to/Samplesheet/SampleSheet.csv --ou
tput-dir /path/to/output_folder --create-fastq-for-index-reads --ignore-missing-bcls --mini
mum-trimmed-read-length 0 --mask-short-adapter-reads 0 --no-lane-split --use-bases-mas
k=Y*,I10,I10,Y*
```

After the conversion is complete, the specified output directory (/path/to/output_folder) will contain a set of demultiplexed FASTQ files. These files will be used as input for the Salmon software and should resemble the following:

```
filename_S0_I1_001.fastq.gz
filename_S0_I2_001.fastq.gz
filename_S0_R1_001.fastq.gz
filename_S0_R2_001.fastq.gz
```

Make sure to verify that the output files match your experimental setup before proceeding with Salmon analysis.

Last modified: 16 December 2024

8.1. Salmon Software Package

1. Getting Started with Salmon

2. Building an index using the Cellecta DriverMap EXP Assay V3 configuration file (Download link):

```
salmon index -t DM_V3_human.fa.gz -i DM_V3_human_index
```

3. Quantifying the samples:

```
salmon quant -i DM_V3_human_index -l A \
-1 data/sample_R1.fastq.gz \
-2 data/sample_R2.fastq.gz \
-p 8 --validateMappings -o quants/sample_quant
```

4. After quantification:
   Once you have your quantification results, you can use them for downstream analysis with differential expression tools like swish, DESeq2, edgeR, limma, or sleuth. Using the tximport package, you can import Salmon's transcript-level quantifications and optionally aggregate them to the gene level for gene-level differential expression analysis.

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You can read more about how to import Salmon's results into DESeq2 by reading the `tximport` section of the excellent DESeq2 vignette. For instructions on importing for use with edgeR or limma, see the tximport vignette. For using Salmon with swish for transcript-level analysis, see the swish vignette. For preparing Salmon output for use with sleuth, see the wasabi package.

Cellecta does not own the software package and is not responsible for maintaining them. Cellecta also provides custom data analysis services. Please email tech@cellecta.com for more information.

Last modified: 9 September 2024

8.2. DriverMap EXP hGW19k Amplicon Sequences

Download: Master Amplicon List

Note: For optimal assay performance, some housekeeping genes with very high expression levels and highly homologous sequences were excluded from the gene list, including globins, ribosomal proteins, and histones.

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9. Appendices

Appendix A. Tips and Troubleshooting
Appendix B. Structure of Amplified Indexed Library
Appendix C. List of Dual DNA/RNA UD Indexes

Last modified: 19 July 2024

9.1. Appendix A. Tips and Troubleshooting

Observation	Possible Cause	Recommended Action
The yield of PCR products for RNA samples is low but is good for Positive Control RNA sample — or — Low yield of PCR products for both experimental and Positive Control RNA samples	Error in quantitation of input RNA, or RNA is degraded	Re-analyze the amount and quality of input RNA using Agilent Bioanalyzer, Agilent Fragment Analyzer, or Thermo Fisher NanoDrop.
	RNA input is too low	Add more RNA, or increase target amplification cycles (Refer to **PCR with Anchor Primers** for cycle numbers recommended based on input amounts).
	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 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 RNA amount	Re-analyze the amount and quality of input RNA using Agilent Bioanalyzer, Agilent Fragment Analyzer, or Thermo Fisher NanoDrop.
	RNA input is too high	Add less RNA or decrease the number of PCR cycles in the **First PCR with Indexed Primers** step.
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 the amplified indexed library quantification step (**Quantify and Combine Samples for NGS**) were not removed	Add a Primer Removal Enzyme in the amplified indexed library and repeat Quantify and Combine Samples for the NGS step.
Lower than expected number of on-target reads	RNA input too low or PCR cycle number too low	Add more RNA or increase the number of PCR cycles in the target amplification step (**PCR with Anchor Primers**).
	RNA is degraded	Use the highest quality RNA possible. For degraded RNA, 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 RNA 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.

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9.2. Appendix B. Structure of Amplified Indexed Library



Last modified: 19 July 2024

9.3. Appendix C. List of Dual DNA/RNA UD Indexes

Download Example Sample Sheets for DriverMap

- for NextSeq 500
- for NextSeq 2000

Index Adapters Pooling Guide

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

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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
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

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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
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

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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
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

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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
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

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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
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

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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
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

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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
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

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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
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

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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
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

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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
H12	384	UDP0384	AGTGGTTAAG	UDP0384	GTATTCCACC

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