



Thunder**Bolts**<sup>TM</sup>

## RainDance ThunderBolts<sup>TM</sup>

### Myeloid Panel Manual

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**RainDance Technologies, Inc.**  
**Effective Date: April 15, 2015**

# RainDance ThunderBolts™ Myeloid Panel Manual

*The RainDance ThunderBolts™ Myeloid Panel and RainDrop® Digital PCR System are for Research Use Only. Not for use in diagnostic procedures.*

## Introduction

The **RainDance ThunderBolts Myeloid Panel** is a next-generation sequencing (NGS) panel that enables customers to profile a wide range of sample types with high accuracy, simple workflow, fast turnaround time, and only 10 ng of amplifiable DNA. The ThunderBolts Myeloid Panel uses single molecule PCR to target 49 known Myeloid cancer genes including tumor suppressors, mutation hotspots and drug resistance markers. The panel runs on the compact, deployable RainDrop® digital PCR System Source Instrument and is optimized for subsequent sequencing on the Illumina HiSeq® and MiSeq® systems. The streamlined workflow features the RainDance DirectSeq™ method that integrates Illumina NGS adaptors and eliminates separate library preparation steps.

## Disclaimer

The ThunderBolts Myeloid Panel may not perform as intended if the user fails to follow the applicable instructions provided by RainDance and RainDance will not provide warranty service or support to a user who does not follow the applicable instructions provided by RainDance.

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## ThunderBolts Myeloid Panel Assay Protocol

This procedure will provide a detailed workflow for preparing your DNA sample for sequencing on the Illumina HiSeq and MiSeq Systems. It will also provide reference to a workflow for analyzing the sequencing data. It is assumed that the user knows how to operate the Illumina systems and obtain valid sequencing data.

### RainDance Materials Needed

- RainDrop System Source Instrument (p/n 20-04401)
- One of the following:
  - ThunderBolts Myeloid Panel Pack, 48 sample (p/n 20-07216)
  - ThunderBolts Myeloid Panel Pack, 24 sample (p/n 20-07218)
  - ThunderBolts Myeloid Panel Pack (Germany-version), 48 sample (p/n 20-07219)
- Each of the Myeloid Panel Packs contains the following:
  - ThunderBolts Myeloid Panel Reagent Pack (p/n 20-07211, p/n 20-07212, or p/n 20-07213)
    - ThunderBolts Myeloid Panel Library Set 1
    - ThunderBolts Myeloid Panel Library Set 2
  - ThunderBolts Assay Consumable Pack (p/n 20-7205 or p/n 20-7207)
    - RainDance RainDrop System Source Chip
    - RainDance Droplet Destabilizer
    - RainDance 25X Drop Stabilizer
    - Carrier Oil

### Storing and Handling ThunderBolts Myeloid Panel Set 1 and Set 2, 20x Primer Library Stocks (Established Panels)

- Established ThunderBolts Set 1 and Set 2 Primer Libraries are provided as 20x formulations from RainDance Technologies.
- Each tube of 20x primer library stock contains a minimum volume of 113  $\mu$ l or 57  $\mu$ l, the equivalent amount for 54 x 40  $\mu$ l or 27 x 40  $\mu$ l ThunderBolts Myeloid Panel reactions, respectively.
- If you intend to use the entire ThunderBolts Myeloid Panel Set 1 and Set 2 material within one week, the tubes should be thawed, centrifuged to settle condensation, vortexed on high for 10 seconds and centrifuged again to settle contents. The tubes can then be stored at 4°C for up to one week.
- If you intend to use smaller portions of the ThunderBolts Myeloid Panel Set 1 and Set 2 material over an extended period of time, it is recommended that upon receipt of the 20x primer library stocks, the tubes are thawed, centrifuged to settle condensation, vortexed on high for 10 seconds, centrifuged again to settle contents, and then aliquoted into up to six tubes per set with each tube containing 18  $\mu$ l (9 reactions worth of material) of the ThunderBolts Myeloid Panel Libraries. The tubes should then be stored at -20°C until ready to use.

### Required Supplies

- P2, P10, P20, P200, and P1000 pipettes
- 1-20  $\mu$ l and 20-200  $\mu$ l 12-channel multi-channel pipettes
- Sterile aerosol resistant filtered pipette tips

- Vortex mixer
  - Mini-microcentrifuge for both microtubes and 8-strip 0.2 ml PCR tubes
  - 8-strip 0.2 mL PCR tubes & caps (Axygen #PCR-0208-CP-C)
  - DynaMag-96 magnetic plate (Life Technologies #12331D)
  - A thermal cycler with the following specifications, which allows for proper thermal cycling of the emulsion. If you use a thermal cycler that does not meet these specifications, data may be compromised.
    - Thermal cycler block format should accommodate standard 0.2 ml tubes
    - Adjustable-height heated lids are recommended
    - The surface of the heated lid should be hard and free of texture
    - Adjustable ramp speed
- Note:* Centrifugal thermal cyclers are not recommended.

## Required Reagents

- TaqMan® Genotyping Master Mix (Life Technologies #4371355)
- Platinum Taq DNA Polymerase High Fidelity (Life Technologies #11304-029)
- 50 mM MgSO<sub>4</sub> (included in Life Technologies #11304-029)
- 40 mM combined (10 mM/ea.) dNTP Solution Mix (New England Biolabs #N0447L)
- Oligonucleotide PCR primers (Integrated DNA Technologies)
- DNase/RNase-free water (molecular biology grade)
- Trizma Hydrochloride Buffer Solution, pH 8.0 (Sigma #T2694-1L)
- Absolute Ethanol (Sigma E7023-500ML)
- DMSO (Sigma #D8418-100ML)
- Betaine Anhydrous (Sigma #B2629-100G)
- SPRIselect Reagent Kit (Beckman Coulter #B23318)

## Droplet Generation of ThunderBolts Myeloid Panel Reactions

1. Set up 1st PCR reactions.

**NOTE:** for our current primer libraries, there are 2 companion sets, Myeloid Set 1 and Set 2; for multiple sample setup, make sure to create two Master Mixes, one containing Set 1 Primers and the second containing Set 2 Primers:

ThunderBolts Myeloid Panel 1 <sup>st</sup> PCR Reactions	1x40 µl rxn
2x Genotyping Master Mix	20
25x (12.5%) Droplet Stabilizer	1.6
ThunderBolts Myeloid Set 1 or Set 2 Primers (20x)	2
DNA (5 ng – 75 ng)	up to 14.4 µl
H <sub>2</sub> O	variable
<b>TOTAL:</b>	<b>40 µl final volume</b>

2. Assemble one premix for ThunderBolts Myeloid Set 1 Primer Libraries and one premix for ThunderBolts Myeloid Set 2 Primer Libraries.

3. Each premix should contain enough reagents for the number of reactions to be performed plus one additional reaction for overage.
4. Each premix should contain all components listed above in Step 1 minus DNA which will be added on an individual reaction basis.
5. Once premixes for Set 1 and Set 2 Primer Libraries (minus DNA) have been assembled, vortex tubes for 10 seconds and centrifuge briefly to settle contents.
6. Dispense the appropriate volume of premix (minus DNA) into the wells of a fresh 0.2 ml PCR tube strip.
7. Add half of the intended DNA sample to the appropriate well of the PCR tube strip containing the Set 1 Master Mix, and add the other half of the intended DNA sample to the corresponding well of the PCR tube strip containing the Set 2 Master Mix (40 µl final volume per well/reaction).  
**NOTE:** The amount of input DNA that is added to each of the Set 1 and Set 2 Master Mixes must be in the range of 5 ng – 75 ng for a total input range combined for Set 1 and Set 2 of 10 ng – 150 ng.
8. Cap the strip, vortex for 5 seconds and centrifuge to settle contents.
9. Generate emulsions (please refer to RainDrop System Operator's Manual LCN 50-04333, Chapter 5, "Performing a Source Run").
10. Launch the ICS software for the Source instrument and log in.
11. Click **Setup a Run**, Select the **Run Info** tab, and click **Open Door**.
12. Transfer the reactions from the PCR tube strip in Step 8 into the corresponding wells of a RainDrop System Source chip (wells A to H from the strip tubes into wells A to H of the Source Chip) Please refer to RainDrop System Operator's Manual LCN 50-04333, Page 2-7.
13. Place a fresh 0.2 ml PCR tube strip (for emulsion collection) into the PCR strip nest of the RainDrop System Source instrument, scan the Source chip in the **Run Data** tab and align the RainDrop System Source chip over the emulsion collection PCR tube strip using the chip alignment pins (please refer to RainDrop System Operator's Manual, LCN 50-04333, Page 2-4/Figure 2-3).
14. Gently press the Source chip down to secure in place while threading the two alignment pins on either side.
15. Close the lid of the Source instrument, check the Status Indicators in the ICS user interface to ensure readiness of the instrument, and complete sample information input in the **Run Data** tab.
16. Click **Start Run**.
17. At the completion of the run, lift the door to its full open position, remove and discard the used chip into a solid chemical waste container, remove the strip containing the collected emulsions, position strip on a 96-well PCR rack, cap securely and place strip in thermal cycler.

## 1<sup>st</sup> PCR: Droplet Thermal Cycling and Amplification

1. Thermal cycling using the following parameters (3.5 hours):

### ThunderBolts Myeloid Panel 1<sup>st</sup> PCR Thermal Cycling

STEP 1: 94°C/2 min  
 STEP 2: RAMP 1°C/sec to 94°C  
 STEP 3: 94°C/30 sec  
 STEP 4: RAMP 1°C/sec to 54°C  
 STEP 5: 54°C/30 sec  
 STEP 6: RAMP 1°C/sec to 68°C  
 STEP 7: 68°C/1 min  
 STEP 8: GOTO STEP 2, 54x (TOTAL = 55 cycles)  
 STEP 9: 68°C/10 min  
 STEP 10: HOLD AT 12°C

## 1<sup>st</sup> PCR SPRIselect™ Bead Purification

1. To break the emulsions, add 50 µL of Droplet Destabilizer per emulsion, recap securely with a fresh strip cap, vortex for 30 seconds on high, and centrifuge for 2 minutes on a bench top minifuge to separate the aqueous and oil phases (2000 x g, 6000 rpm).
2. Using a P200 multi-pipette, remove all but 5-10 µL of the oil phase from the bottom of the PCR strip tubes (oil phase is below the aqueous phase).

**STOPPING POINT:** Once droplet emulsions have been destabilized and oil is removed, the aqueous reactions remaining in the PCR strips may be capped and stored at -20°C for up to 7 days until ready to proceed to the next step.

1. 40 µL of each 1<sup>st</sup> PCR reaction usually yields ~33 µL of aqueous volume following emulsion de-stabilization. The actual amount of aqueous volume recovered from each individual source should be confirmed during the first ThunderBolts Myeloid Panel run and used as the standard going forward.
2. Fully re-suspend SPRIselect™ beads by inversion.
3. Aliquot 29.7 µL of SPRIselect beads into each of the aqueous volumes. This corresponds to a 0.9x bead-to-reaction volume ratio for the 1<sup>st</sup> PCR reactions.
4. Pipet the full volume of beads plus aqueous sample 10 times to mix fully and until the slurry appears uniform in density. Let sit at room temperature for 5 minutes.
5. After 5 minutes, firmly position the strip (or plate) onto a 96-well magnet plate (DynaMag-96 Side magnet, Life Technologies #12331D), and let rest for 2-3 minutes or until pellet is formed and the supernatant is clear.
6. Remove supernatants using a multi-channel pipette; moving vertically downward slowly making sure not to disturb the pellets.
7. When removing supernatants, if the beads are also getting sucked up in the pipet tips, re-deposit into the well, wait 30 seconds or until bead pellet has re-formed and the supernatant is clear and re-pipette supernatant.
8. With the strip still on the magnet, wash wells with 180 µL/well of freshly-made 85% ETOH by pipetting 5 times.
9. Let rest for 1 minute, and if supernatant is clear, slowly and carefully remove all the ETOH.

10. Visually inspect bottom of wells to ensure that no ETOH is left behind. If there is, re-place the tubes on the magnet, wait 15 seconds and remove remaining ETOH.
11. Remove strip from the magnet and let sit at room temperature for 5 minutes maximum to dry bead pellets.
12. Add 20  $\mu$ l of 10 mM Tris-HCl pH 8.0 per well with a multi-channel pipette; scraping the pellets off the sides of the wells by flushing down with the Tris and vigorously pipette 10 times to re-suspend pellets.
13. Let the strip sit off magnet at room temperature for 2 minutes, then re-place on the magnet for 2 minutes to separate beads.
14. With a multi-channel pipette, collect 17  $\mu$ l of eluent from each tube and deposit in a fresh 8-well PCR tube strip(s).

**STOPPING POINT:** Once the bead-purified, eluted 1<sup>st</sup> PCR reactions have been collected, the PCR strips may be capped and stored at -20°C for up to 7 days until ready to proceed to the next step.

## 2<sup>nd</sup> PCR: Addition of Adaptor and Index Sequences

1. Set up 2<sup>nd</sup> bulk PCR reactions with appropriate Illumina bar-coded primers.  
**NOTE:** Please ensure that the same Illumina barcoded 2<sup>nd</sup> PCR primers are used per matched pair of 1<sup>st</sup> PCR template DNA samples, as described in Step 1:

ThunderBolts Myeloid Panel 2 <sup>nd</sup> PCR Reactions	1x25 $\mu$ l rxn	
10x Platinum Taq Pol HiFi Buffer	3.25	
50 mM MgSO <sub>4</sub>	0.875	
dNTP mix (10 mM.ea.)	1.125	
4M Betaine	2.50	
DMSO	1.25	
10x Barcoded Illumina primers (5 $\mu$ M)	2.50	2.5 $\mu$ l/rxn
Platinum Taq Pol HiFi (5 units/ $\mu$ l)	0.50	
1st PCR Template DNA	13.0	13 $\mu$ l/rxn
TOTAL:	25 $\mu$ l	
ASSEMBLE PREMIX WITH ALL COMPONENTS MINUS PRIMERS AND DNA		
ADD PRIMERS IN PRE-PCR WORK SPACE; ADD 1st PCR DNA IN POST-PCR WORK SPACE		

## 2<sup>nd</sup> PCR: Bulk Thermal Cycling and Amplification

1. Thermal cycling using the following parameters (50 minutes):

### ThunderBolts Myeloid Panel 2<sup>nd</sup> PCR Thermal Cycling

STEP 1: 94°C/2 min  
 STEP 2: RAMP 1°C/sec to 94°C  
 STEP 3: 94°C/30 sec  
 STEP 4 : RAMP 1°C/sec to 56°C  
 STEP 5: 56°C/30 sec  
 STEP 6: RAMP 1°C/sec to 68°C  
 STEP 7: 68°C/1 min  
 STEP 8: GOTO STEP 2, 9x (TOTAL = 10 cycles)  
 STEP 9: 68°C/10 min  
 STEP 10: HOLD AT 12°C

**STOPPING POINT:** Once the bulk 2<sup>nd</sup> PCR reactions have finished thermal cycling, the reactions may be capped and stored at -20°C for up to 7 days until ready to proceed to the next step.

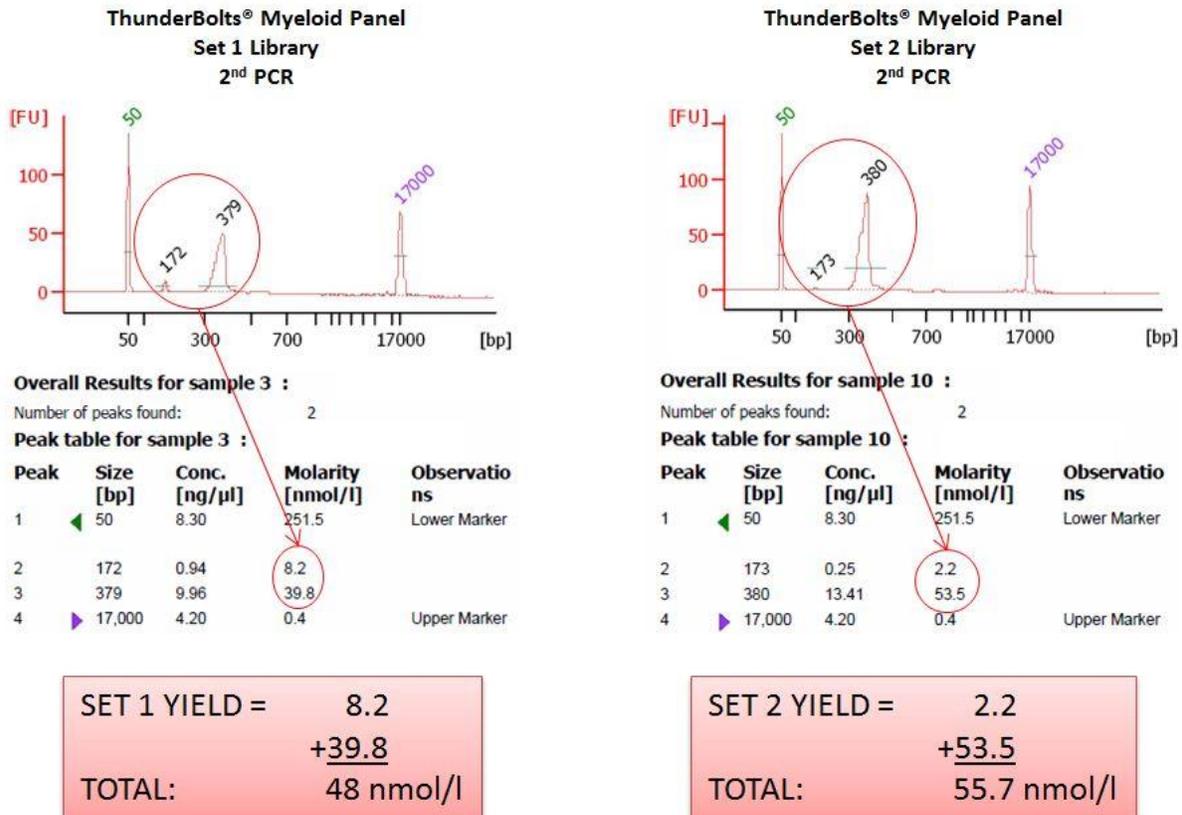
## 2<sup>nd</sup> PCR SPRIselect Bead Purification

1. Fully re-suspend SPRIselect beads by inversion.
2. Aliquot 20 µl of SPRIselect beads into each of the aqueous volumes. This corresponds to a 0.8x bead-to-reaction volume ratio for the 2<sup>nd</sup> PCR reactions.
3. Add the aqueous sample to corresponding well containing beads and pipette 10 times to mix fully. Let sit at room temperature for 5 minutes.
4. After 5 minutes, firmly position the strip onto the 96-well magnet plate and let rest for 2-3 minutes or until the beads have formed a solid pellet and the supernatant is clear.
5. Remove supernatants using a multi-channel pipette by moving vertically downward slowly, making sure not to disturb the pellets.
6. When removing the supernatants, if beads are also getting sucked up in the pipette tips, re-deposit into the well, wait 30 seconds and re-pipette the supernatant.
7. With the strip still on magnet, wash the wells with 180 ul/well of freshly-made 85% ETOH by pipetting 5 times.
8. Let rest for 1 minute, and if the supernatant is clear, and slowly and carefully remove all the ETOH.
9. Visually inspect the bottom of the wells to ensure that no ETOH is left behind. If there is, re-place on the magnet, wait 15 seconds, and remove the remaining ETOH.
10. Remove the strip from the magnet and let rest at room temperature for 5 minutes to dry bead pellets.
11. Add 20 µl of 10 mM Tris-HCl pH 8.0 per well with a multi-channel pipette scraping the pellets off the sides of the wells by flushing down with the Tris and vigorously pipetting 10 times to re-suspend the pellets.
12. Let strip sit off the magnet at room temperature for 2 minutes, then re-place on the magnet for 2 minutes to separate the beads.
13. With a multi-channel pipette, collect 17 µl of eluent from each tube and deposit in a fresh 8-well PCR tube strip(s).

**STOPPING POINT:** Once the bead-purified 2<sup>nd</sup> PCR reactions have been collected, the PCR strips may be capped and stored at -20°C for up to 7 days until ready to proceed to the next step.

## DNA Quantification Prior To MiSeq Sequencing Run

1. Run 1  $\mu\text{l}$  of each reaction on Agilent Bioanalyzer 12k chip (40 min/12 samples).
2. NOTE: While RainDance Technologies uses Agilent Bioanalyzers for quantification of 2<sup>nd</sup> PCR amplicons, other technologies are also suitable for quantification (Caliper, Experion, etc.).
3. For each Agilent Bioanalyzer chromatogram, add up and record all product yields (expected for the target amplicons between 328 and 378 bp, as well as all the off-target non-specific products <320 bp and >390 bp) as a function of Molarity (nmol/L) for each sample.



4. Using the number for TOTAL nmol/L for each sample, aliquot 5  $\mu\text{l}$  of each sample into a new tube and dilute to 2 nmol/L (2 nM) using H<sub>2</sub>O as diluent. To calculate the amount of H<sub>2</sub>O necessary to achieve a 2 nM final concentration, use the formula: (Concentration) \* (Volume) = (Concentration)<sub>Final</sub> \* (Volume)<sub>Final</sub>
  - Example: For the sample illustrated above (“ThunderBolts Myeloid Panel Set 1”, left panel), the total concentration from the 2<sup>nd</sup> PCR reaction is 48 nM. To dilute 5  $\mu\text{L}$  of that sample to a final concentration of 2 nM:  
 $(48 \text{ nM}) * (5 \mu\text{L}) = (2 \text{ nM}) * (\text{Volume})_{\text{Final}}$ 
    - This means that for the sample above, (Volume)<sub>Final</sub> = 120  $\mu\text{L}$
    - The original volume of sample added from the 2<sup>nd</sup> PCR was 5  $\mu\text{L}$ . This means that 115  $\mu\text{L}$  of water should be combined with the 5  $\mu\text{L}$  of sample to produce the desired final volume of 120  $\mu\text{L}$
5. Prior to sequencing, combine equal volumes of each diluted sample in a fresh tube (aggregate pooled sample concentration = 2 nmol/L)

**STOPPING POINT:** Once the 2<sup>nd</sup> PCR reactions have been diluted to 2 nM and combined into a single 2 nM pool, the tube may be stored at 4°C for up to 3 days until ready to proceed to the next step.

## ThunderBolts Myeloid Panel Illumina MiSeq Protocol

This section contains the protocol used to run ThunderBolts Myeloid Panel amplicons on the Illumina MiSeq Instrument.

### RainDance ThunderBolts Myeloid Panel Illumina Sample Sheet Guide

The location of the Sample Sheet Repository can be found by opening Illumina Experiment Manager (IEM) on the MiSeq and choosing 'Folder Settings'. After making any alteration to the sample sheet template or saving a copy of a sample sheet, ensure that the file remains in the Comma Separated Value (.csv) file format.

### Optional

- ThunderBolts FASTQ Sample Sheet Template.csv

### Completing the Sample Sheet from the Provided Template

1. In column B, fill in the date in the format MM/DD/YYYY. Also in column B, the 'Investigator Name', 'Experiment Name', and 'Description' fields are optional.
2. [Reads] and [Settings] are pre-filled with standard values.
3. Replace the generic sample IDs in column A as desired, making sure to pair each sample with the correct index (listed in the column 'I7\_Index\_ID' as A001-A048). The sample sheet template contains the full list of 48 ThunderBolts-compatible 2<sup>nd</sup> PCR indexes as well as their 6-base sequences (see the Primer Reference List document for the full sequence of the forward and reverse 2<sup>nd</sup> PCR primers and the 48 index sequences).
4. The 'Sample\_ID', 'Sample\_Plate', 'Sample\_Project', and 'Description' fields are optional.
5. For any indexes not being used, delete the entire row from the sample sheet.
6. Save the sample sheet in the sample sheet repository maintaining the .csv file format.

**NOTE:** When naming the sample sheet, avoid using any periods/decimal points in the name, or the analysis will not start properly after the run.

### Completing the Sample Sheet Using Illumina Experiment Manager (IEM)

Sample sheets for analysis of samples processed with the ThunderBolts Myeloid Panel can also be produced using the Illumina Experiment Manager software; however, IEM only lists 24 of the 48 possible index options (to use one of the 24 unlisted indexes, either copy the index sequences from 'ThunderBolts FASTQ Sample Sheet Template.csv' or choose to view the sample sheet in Excel when prompted by IEM and enter the sample details and index sequences manually.

Use the following parameters to produce raw FASTQ files for analysis:

1. When creating the sample plate, select the **TruSeq LT** sample prep kit with **one** index read
2. When creating the sample sheet, select **MiSeq > Other > FASTQ Only** or **HiSeq > HiSeq FASTQ Only**
3. For Workflow Parameters select:
  - a. Sample Prep Kit: TruSeq LT
  - b. Index Reads: 1
  - c. Cycles Read 1: 250
  - d. Cycles Read 2: 250
4. **On the right-hand side of the workflow parameters screen, choose ONLY 'Custom Primer for**

Read 1' and 'Custom Primer for Read 2' - uncheck the boxes for all other available options

## RainDance ThunderBolts Myeloid Panel Illumina MiSeq Setup and Run

Under no circumstances should the custom sequencing primers be combined with the standard Illumina sequencing primers already present in sequencing cartridge wells 12 and 14. Combining the sequencing primers will result in extremely poor sequencing results.

**NOTE:** There is no need to use a PhiX spike-in for the sake of diversity, and a standard PhiX control will not work as intended due to the use of custom sequencing primers. A better choice for a sequencing quality control is a known good sample from a previous run (indexed with a barcode not being used in the current run).

Custom primer setup is now detailed in a separate Illumina document:

[http://supportres.illumina.com/documents/documentation/system\\_documentation/miseq/miseq\\_using\\_custom\\_primers\\_15041638\\_b.pdf](http://supportres.illumina.com/documents/documentation/system_documentation/miseq/miseq_using_custom_primers_15041638_b.pdf)

## Requirements

- RNase/DNase-free H<sub>2</sub>O
- 1N NaOH
- HT1 buffer (from box 1 of 2 of the MiSeq Reagent Kit)
- Custom Sequencing Primers 1 and 2 (see 'ThunderBolts Myeloid Panel Primer Reference List' for sequences)
- MiSeq® v2 or v3 Reagent Kit

## Prepare the DNA Samples and Custom Sequencing Primers (Standard MiSeq Protocol)

1. Prepare a fresh dilution of 0.2N NaOH (800 µL H<sub>2</sub>O + 200 µL 1N NaOH).
2. Using H<sub>2</sub>O, dilute a small portion of each purified 2<sup>nd</sup> PCR reaction to 2nM.
3. Combine equal portions of each diluted reaction to form the 2nM 'Library Pool' (Starting DNA concentration, 2nM).
4. Combine 5 µL of the 2nM library pool with 5 µL 0.2N NaOH (Resulting DNA concentration, 1nM).
5. Vortex briefly and spin down at a very low speed to collect (~280 x g).
6. Incubate for 5 minutes at room temperature.
7. Add 990 µL chilled HT1 buffer to the 20 µL of denatured DNA (Resulting DNA concentration, 10pM).
8. Vortex thoroughly and spin to collect.
9. Combine 480 µL of the 10pM library pool with 120 µL chilled HT1 buffer (Final DNA concentration, 8pM).
10. Vortex thoroughly and spin to collect.
11. Never mix sequencing primers 1 and 2 before or after dilution or while loading the MiSeq cartridge.
12. Using chilled HT1 buffer, prepare 600 µL of each sequencing primer at a final concentration of 0.5 µM.

## Load the Cartridge

1. Puncture the foil seals of wells 17, 18, and 20 using a separate, clean, empty 1 mL pipette tip for each well.
2. Load the full volume of the 8pM library pool (600  $\mu$ L) in well 17.
3. Load the full volume of 0.5  $\mu$ M Custom Sequencing Primer 1 (600  $\mu$ L) in well 18.
4. Load the full volume of 0.5  $\mu$ M Custom Sequencing Primer 2 (600  $\mu$ L) in well 20.

## ThunderBolts Myeloid Panel RainDance Sequencing Analysis Workflow

This section outlines the algorithms and parameters used by the RainDance Internal Sequencing Analysis Pipeline. It is meant to be a guide to other bioinformatics groups who may want to modify their internal pipeline or who would want to design a new pipeline specifically for the ThunderBolts Myeloid Panel.

### Assumptions

- No two primers start at the same genomic location
- No two primers have the same sequence
- R1 and R2 are both present in their respective fastq files, occur at the same line numbers in each file and are sorted by read name

### 1. Pre-processing of sequencing reads

- remove Illumina sequencing adapters
- remove target specific primers

### 2. Map processed reads to whole genome

- global alignment

### 3. Re-map un-mapped reads

- local alignment

### 4. Filter problematic read alignments

- primer dimers
- short reads
- single reads

### 5. Assign reads passing filters to amplicon

- count number of reads/amplicon

### 6. Soft-clip bases that overhang amplicon edges

### 7. Call variants with VarScan2

- depth  $\geq 100$
- base quality  $\geq 30$
- map quality  $\geq 20$
- no strand bias

## Disclaimers

The ThunderBolts Myeloid Panel may not perform as intended if the user fails to follow the applicable instructions provided by RainDance and RainDance will not provide warranty service or support to a user who does not follow the applicable instructions provided by RainDance.

The user acknowledges and agrees that consumables are for one-time use, for internal research purposes only, and are not for resale or other commercial distribution.

In no event shall RainDance be liable for any damages, whether in contract or tort (including, but not limited to, negligence) or under any other legal theory for consequential, special, exemplary, incidental or indirect damages, arising out of or in connection with the use or the inability to use the ThunderBolts Myeloid Panel or the results of using the ThunderBolts Myeloid Panel. The liability of RainDance in connection with the use of the ThunderBolts Myeloid Panel shall in no event exceed the amount of the invoice for the pertinent consumables.

## Contact Us

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*The RainDance ThunderBolts Myeloid Panel and the RainDrop System are for Research Use Only. Not for use in diagnostic procedures.*

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LCN 50-07660 Rev A