TPRV +ve CSF Sample DNA libraries working outline

TPRV\_CSF\_Batch\_05

NextSeq Batch 18\_R

# Sample Summary

**NOTE: The NextSeq Batch: 18 failed due to an unknown reason. We then reloaded the same library in another cartridge and the batch was denoted as NextSeq Batch: 18\_R.**

**NextSeq Batch: 18\_R**

**Loading Date: 2-Feb-2023**

A total of 12 samples (+ 2 EC) were extracted in this batch.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Set no** | **Batch Sl** | **Specimen\_ID** | **CSF\_Tetra\_Seq ID** | **CSFColDt** | **Sample Vol** | **PBS** | Remark |
| Set-1 | 1 | 3010008785 | CSF\_0078\_TP4 | Nov-22 | 200 |  | CMOSH |
| Set-1 | 2 | 3010008924 | CSF\_0079\_TP4 | 16-Nov-22 | 200 |  | CMOSH |
| Set-1 | 3 | 3020004887 | CSF\_0080\_TP4 | 4-Apr-22 | 160 | 40 | CMOSH |
| Set-1 | 4 | 3010008978 | CSF\_0081\_TP4 | 16-Nov-22 | 110 | 90 | CMOSH |
| Set-1 | EC |  | TPRV\_CSF\_Batch05\_EC1 |  |  |  |  |
| Set-2 | 5 | 10606 | CSF\_0082\_TP4 | 13-Apr-09 | 200 |  | OLD |
| Set-2 | 6 | 10815 | CSF\_0083\_TP4 | 17-Aug-09 | 200 |  | OLD |
| Set-2 | 7 | 11274 | CSF\_0084\_TP4 | 1-Aug-10 | 200 |  | OLD |
| Set-2 | 8 | 11531 | CSF\_0085\_TP4 | 17-Feb-11 | 165 | 35 | OLD |
| Set-2 | 9 | 10475 | CSF\_0086\_TP4 | 3-Jan-09 | 200 |  | OLD |
| Set-2 | 10 | 10771 | CSF\_0087\_TP4 | 29-Jul-09 | 185 | 15 | OLD |
| Set-2 | 11 | 10562 | CSF\_0088\_TP4 | 18-Mar-09 | 94 | 106 | OLD |
| Set-2 | 12 | 10836 | CSF\_0089\_TP4 | 6-Sep-09 | 200 |  | OLD |
| Set-2 | EC2 |  | TPRV\_CSF\_Batch05\_EC2 |  |  |  |  |

# Set: 01

## Date: 16-Jan-2023

**Lab person: Anisur**

### Step 01: Extraction

**Extraction Protocol: QIAamp DNA mini kit (Protocol: DNA Purification from Blood or Body Fluids)**

We did spin the serum samples first at 14000 **rpm for 10 mins** before starting the extraction.

A total of 4 **samples + 1 EC** were extracted for DNA.

* Elution = 25ul AE Buffer
* Incubation for 5 min at room temp
* Double Elution
* **Checkpoint: After Extraction, the products were stored in –80C.**

## Date: 17-Jan-2023

**Lab person: Anisur**

### Step 02: Multiplex PCR

We used 10uM diluted TPRV specific **pool-1** and **pool-2** PCR primer for this step.

After DNA extraction, Multiplex PCR was done using pool-1 and pool-2 primers. **NEBNext® Ultra™ II Q5® Master Mix (M0544)** was used for amplification.

* The PCR and library prep protocol was adapted from the [ARTIC-NEB: SARS-CoV-2 Library Prep V.4](https://www.protocols.io/view/artic-neb-sars-cov-2-library-prep-bp2l6n69rgqe/v4) protocol.
* As nanodrop results of this batch was quite low for all samples upon discussion with Tanmoy vhai, we did not do qubit analysis at this step.

Our target was to take around 22 ng DNA input (as per Biohub recommendation). Though protocol suggests picking **1.25 ul** for DNA (**+ 3 ul of water**), as the DNA concentration of most of the samples was quite low, we took full **4.25 ul** volume of DNA.

**Master mix and template calculation:**

|  |  |  |
| --- | --- | --- |
|  | 0.5 rxn | 0.5 rxn |
| **Component** | **Pool 1 (ul)** | **Pool 2 (ul)** |
| NEBNext Ultra II Q5 Hot Start 2x master mix | 6.25 | 6.25 |
| Primer pool | 2 | 2 |
| Water | 0 | 0 |
| Template | 4.25 | 4.25 |
| Total | 12.5uL | 12.5uL |

**PCR profile:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Temp** | **Time** | **Cycles** |
| Heat Activation | 98 °C | 30s | 1 |
| Denaturation | 98 °C | 15s | 30 |
| Annealing | 64 °C | 05 min |
| Hold | 4 °C | ∞ |  |

**Lid: 105°C**

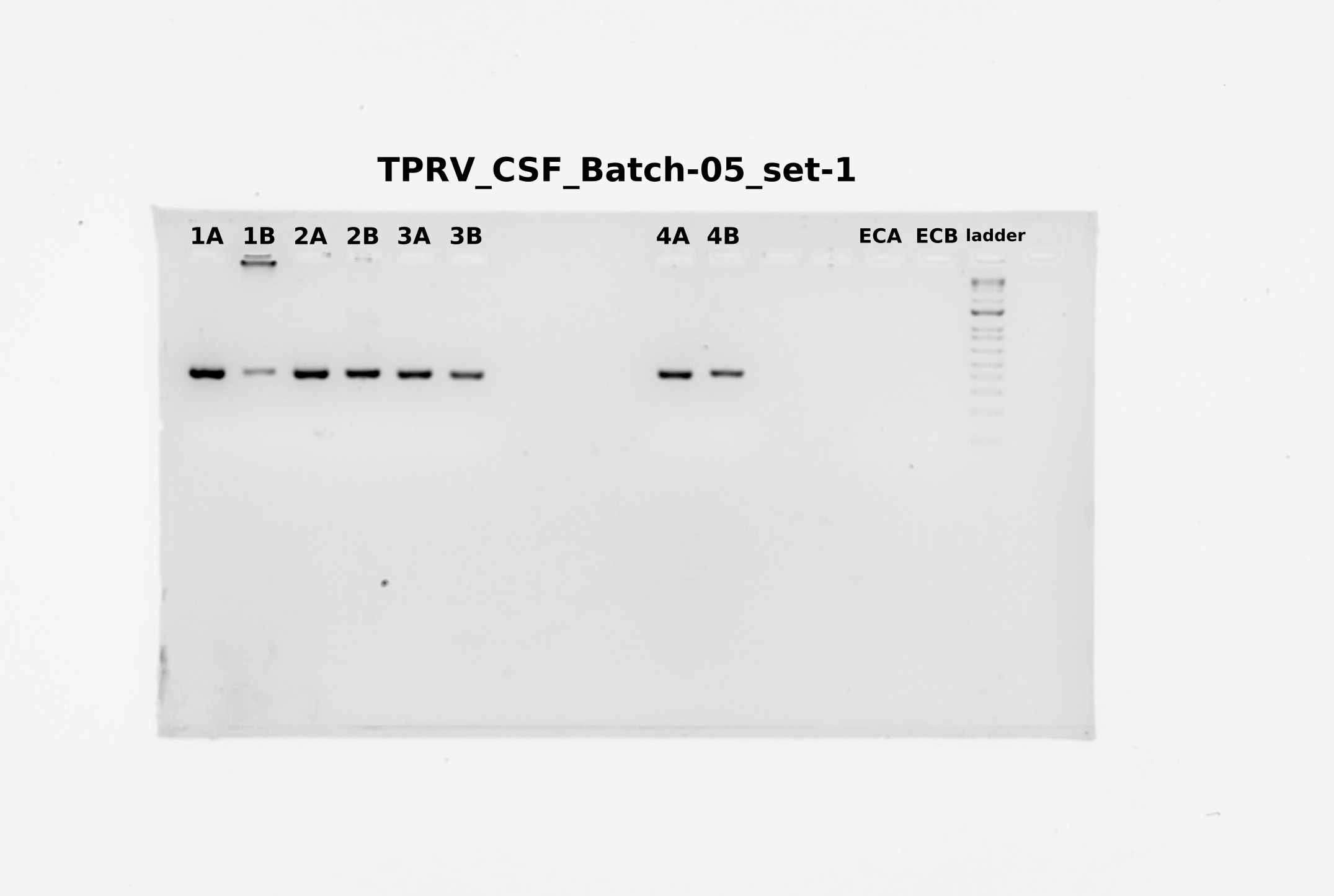
* **Checkpoint: After PCR, the products were stored in –80C.**

## Date: 18-Jan-2023

**Lab person: Anisur**

### Step 03: Gel Electrophoresis

* 1.5% gel
* 100 V
* 400 mA
* 55 mins



After getting the gel image, we decided to select all samples for library prep.

### Step 04: Pool and SPRI (Magnetic Bead Purification) Clean

* Used SPRI bead **1x ratio** of beads-to-total volume of sample.
* Elution: 36ul from 40ul water.

### Step 05: Normalization

After measuring the DNA concentration by Qubit, we normalized the volumes to take **10-100ng** DNA input in **13ul** volume.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Batch Sl** | **Specimen ID** | **DNA (100-fold) quantification with Qubit** | **DNA (10-fold) quantification with Qubit** | **Calculated DNA conc (Raw) ul** | **Need for normalization (8ng/ul; 13 ul; total 104ng DNA)** | **Water (to make 13 ul)** | **Pick from** |
| 1 | 3010008785 | 0.66 | 6.6 | 66 | 1.58 | 11.42 | Raw |
| 2 | 3010008924 | 0.642 | 6.42 | 64.2 | 1.62 | 11.38 | Raw |
| 3 | 3020004887 | 0.488 | 4.88 | 48.8 | 2.13 | 10.87 | Raw |
| 4 | 3010008978 | 0.142 | 1.42 | 14.2 | 7.32 | 5.68 | Raw |
| EC |  |  |  | too low | 1 | 12 | Raw |

### Step 06: Fragmentation

|  |  |  |
| --- | --- | --- |
| **Reagent** | **0.5x rxn** | **Aliquot** |
| Normalized DNA (10-100ng) | 13 |  |
| (Yellow) NEBNext Ultra II FS Reaction Buffer | 3.5 | 4.5ul |
| (Yellow) NEBNext Ultra II FS Enzyme mix | 1 |
| Total volume | 17.5 |  |

**PCR profile:**

|  |  |  |
| --- | --- | --- |
| **Step** | **Temp** | **Time** |
| Fragmentation | 37°C | 5 min |
| Enzyme Deactivate | 65°C | 30 min |
| Hold | 4 °C | **∞** |
| **Lid: 75°C** | | |

### Step 07: Adapter Ligation

|  |  |  |
| --- | --- | --- |
| **Reagent** | **0.5x rxn** | **Aliquot** |
| FS Reaction Mixture | 17.5 |  |
| (Red) NEBNext Ultra II Ligation Master Mix | 15 | 15.5ul |
| (Red) NEBNext Ligation Enhancer | 0.5 |
| (Red) NEBNext Adaptor for Illumina (1:100 dilution)\* | 1.25 | Add separately |
| Total volume | 34.25 |  |

**\*This Master Mix is very thick, taking all volume altogether results in loss of volume. So, take to total volume in small aliquots.**

**\*\*Add adapter separately from the master mix to avoid adapter dimers.**

**PCR Profile:**

|  |  |  |
| --- | --- | --- |
| **Steps** | **Temp** | **Time** |
| Step 1 | 20°C | 15 min |
| Hold | 4 °C | **∞** |
| **Lid: Heat off** | | |

### Step 08: SPRI (Magnetic Bead Purification) Clean

* Used SPRI bead **0.9x ratio** of beads-to-total volume of sample.

|  |  |
| --- | --- |
| Bead Volume | 30.83ul |
| Elution | 7.5ul from 9ul water |

### ***Step 09: PCR Enrichment / Barcoding***

|  |  |  |
| --- | --- | --- |
| **Reagent** | **0.5x rxn** | Aliquot |
| Purified, adaptor-ligated cDNA | 7.5 |  |
| (white) USER Enzyme (Cat no. M5505L, 250uL) | 1.5 | **14** |
| (blue) NEBNext Ultra II Q5 master mix | 12.5 |
| 5uM i7 barcoded primer (NEB index primer/TruSeq/or similar) | 5\* |  |
| 5uM i5 barcoded primer (NEB Universal primer/TruSeq/or similar) |  |
| Total volume | **26.5** |  |

**\*Barcode was added separately to each sample.**

**Barcode Layout:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Set no** | **Batch Sl** | **Specimen\_ID** | **CSF\_Tetra\_Seq ID** | **Barcode layout** | **Barcode plate** |
| Set-1 | 1 | 3010008785 | CSF\_0078\_TP4 | A10 | IDT-15156265 |
| Set-1 | 2 | 3010008924 | CSF\_0079\_TP4 | D10 | IDT-15156265 |
| Set-1 | 3 | 3020004887 | CSF\_0080\_TP4 | E10 | IDT-15156265 |
| Set-1 | 4 | 3010008978 | CSF\_0081\_TP4 | F10 | IDT-15156265 |
| Set-1 | EC |  | TPRV\_CSF\_Batch05\_EC1 | G10 | IDT-15156265 |

**PCR Profile:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Steps** | **Temp** | **Time** | **Cycle** |
| Step 1 | 37°C | 15 min | 1 |
| Step 2 | 98°C | 30 sec | 1 |
| Step 3 | 98°C | 30 sec | 12 |
| 65°C | 75 sec |
| Step 4 | 65°C | 5 min | 1 |
| Hold | 4 °C | **∞** |  |
| **Lid: 105°C** | | | |

### ***Step 10: 0.8x SPRI (Magnetic Bead Purification) Clean***

* Used SPRI bead **0.8x ratio** of beads-to-total volume of sample.

|  |  |
| --- | --- |
| Bead Volume | 21.2ul |
| Elution | 25ul from 27ul water |

### ***Step 11: 0.75x SPRI (Magnetic Bead Purification) Clean***

* Used SPRI bead **0.75x ratio** of beads-to-total volume of sample.

|  |  |
| --- | --- |
| Bead Volume | 18.75ul |
| Elution | 15ul from 17ul water |

* **Checkpoint: Barcoded purified products were stored in -80°C.**

# Set: 02

## Date: 22-Jan-2023

**Lab person: Anisur**

### Step 01: Extraction

**Extraction Protocol: QIAamp DNA mini kit (Protocol: DNA Purification from Blood or Body Fluids)**

We did spin the serum samples first at 14000 **rpm for 10 mins** before starting the extraction.

A total of 8 **samples + 1 EC** were extracted for DNA.

* Elution = 25ul AE Buffer
* Incubation for 5 min at room temp
* Double Elution
* **Checkpoint: After Extraction, the products were stored in –80C.**

### Step 02: Multiplex PCR

We used 10uM diluted TPRV specific **pool-1** and **pool-2** PCR primer for this step.

After DNA extraction, Multiplex PCR was done using pool-1 and pool-2 primers. **NEBNext® Ultra™ II Q5® Master Mix (M0544)** was used for amplification.

* The PCR and library prep protocol was adapted from the [ARTIC-NEB: SARS-CoV-2 Library Prep V.4](https://www.protocols.io/view/artic-neb-sars-cov-2-library-prep-bp2l6n69rgqe/v4) protocol.
* As nanodrop results of this batch was quite low for all samples upon discussion with Tanmoy vhai, we did not do qubit analysis at this step.

Our target was to take around 22 ng DNA input (as per Biohub recommendation). Though protocol suggests picking **1.25 ul** for DNA (**+ 3 ul of water**), as the DNA concentration of most of the samples was quite low, we took full **4.25 ul** volume of DNA.

**Master mix and template calculation:**

|  |  |  |
| --- | --- | --- |
|  | 0.5 rxn | 0.5 rxn |
| **Component** | **Pool 1 (ul)** | **Pool 2 (ul)** |
| NEBNext Ultra II Q5 Hot Start 2x master mix | 6.25 | 6.25 |
| Primer pool | 2 | 2 |
| Water | 0 | 0 |
| Template | 4.25 | 4.25 |
| Total | 12.5uL | 12.5uL |

**PCR profile:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | **Temp** | **Time** | **Cycles** |
| Heat Activation | 98 °C | 30s | 1 |
| Denaturation | 98 °C | 15s | 30 |
| Annealing | 64 °C | 05 min |
| Hold | 4 °C | **∞** |  |
| **Lid: 105°C** | | | |

* **Checkpoint: After PCR, the products were stored in –80C.**

## Date: 23-Jan-2023

**Lab person: Anisur**

### Step 03: Gel Electrophoresis

Upon discussion with Tanmoy vhai, we did not do gel electrophoresis for these samples and decided to select all samples for library prep.

### Step 04: Pool and SPRI (Magnetic Bead Purification) Clean

* Used SPRI bead **1x ratio** of beads-to-total volume of sample.
* Elution: 36ul from 40ul water.

### Step 05: Normalization

After measuring the DNA concentration by Qubit, we normalized the volumes to take **10-100ng** DNA input in **13ul** volume.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Batch Sl** | **Specimen ID** | **DNA (100-fold) quantification with Qubit** | **DNA (10-fold) quantification with Qubit** | **Calculated DNA conc (Raw) ul** | **Need for normalization (8ng/ul; 13 ul; total 104ng DNA)** | **Water (to make 13 ul)** | **Pick from** |
| 5 | 10606 | 0.296 | 2.96 | 29.6 | 3.51 | 9.49 | Raw |
| 6 | 10815 | 0.592 | 5.92 | 59.2 | 1.76 | 11.24 | Raw |
| 7 | 11274 | 1.19 | 11.9 | 119 | 8.74 | 4.26 | 10F |
| 8 | 11531 | 0.486 | 4.86 | 48.6 | 2.14 | 10.86 | Raw |
| 9 | 10475 | 0.442 | 4.42 | 44.2 | 2.35 | 10.65 | Raw |
| 10 | 10771 | 0.902 | 9.02 | 90.2 | 1.15 | 11.85 | Raw |
| 11 | 10562 | 0.422 | 4.22 | 42.2 | 2.46 | 10.54 | Raw |
| 12 | 10836 | 1.09 | 10.9 | 109 | 9.54 | 3.46 | 10F |
| EC2 |  |  |  | 0.038 | 1.00 | 12.00 | Raw |

### Step 06: Fragmentation

|  |  |  |
| --- | --- | --- |
| **Reagent** | **0.5x rxn** | **Aliquot** |
| Normalized DNA (10-100ng) | 13 |  |
| (Yellow) NEBNext Ultra II FS Reaction Buffer | 3.5 | 4.5ul |
| (Yellow) NEBNext Ultra II FS Enzyme mix | 1 |
| Total volume | 17.5 |  |

**PCR profile:**

|  |  |  |
| --- | --- | --- |
| **Step** | **Temp** | **Time** |
| Fragmentation | 37°C | 5 min |
| Enzyme Deactivate | 65°C | 30 min |
| Hold | 4 °C | **∞** |
| **Lid: 75°C** | | |

### Step 07: Adapter Ligation

|  |  |  |
| --- | --- | --- |
| **Reagent** | **0.5x rxn** | **Aliquot** |
| FS Reaction Mixture | 17.5 |  |
| (Red) NEBNext Ultra II Ligation Master Mix | 15 | 15.5ul |
| (Red) NEBNext Ligation Enhancer | 0.5 |
| (Red) NEBNext Adaptor for Illumina (1:100 dilution)\* | 1.25 | Add separately |
| Total volume | 34.25 |  |

**\*This Master Mix is very thick, taking all volume altogether results in loss of volume. So, take to total volume in small aliquots.**

**\*\*Add adapter separately from the master mix to avoid adapter dimers.**

**PCR Profile:**

|  |  |  |
| --- | --- | --- |
| **Steps** | **Temp** | **Time** |
| Step 1 | 20°C | 15 min |
| Hold | 4 °C | **∞** |
| **Lid: Heat off** | | |

### Step 08: SPRI (Magnetic Bead Purification) Clean

* Used SPRI bead **0.9x ratio** of beads-to-total volume of sample.

|  |  |
| --- | --- |
| Bead Volume | 30.83ul |
| Elution | 7.5ul from 9ul water |

## Date: 24-Jan-2023

**Lab person: Anisur**

### ***Step 09: PCR Enrichment / Barcoding***

|  |  |  |
| --- | --- | --- |
| **Reagent** | **0.5x rxn** | Aliquot |
| Purified, adaptor-ligated cDNA | 7.5 |  |
| (white) USER Enzyme (Cat no. M5505L, 250uL) | 1.5 | **14** |
| (blue) NEBNext Ultra II Q5 master mix | 12.5 |
| 5uM i7 barcoded primer (NEB index primer/TruSeq/or similar) | 5\* |  |
| 5uM i5 barcoded primer (NEB Universal primer/TruSeq/or similar) |  |
| Total volume | **26.5** |  |

**\*Barcode was added separately to each sample.**

**Barcode Layout:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Set no** | **Batch Sl** | **Specimen\_ID** | **CSF\_Tetra\_Seq ID** | **Barcode layout** | **Barcode plate** |
| Set-2 | 5 | 10606 | CSF\_0082\_TP4 | B01 | IDT-1446944+945 |
| Set-2 | 6 | 10815 | CSF\_0083\_TP4 | C01 | IDT-1446944+945 |
| Set-2 | 7 | 11274 | CSF\_0084\_TP4 | D01 | IDT-1446944+945 |
| Set-2 | 8 | 11531 | CSF\_0085\_TP4 | E01 | IDT-1446944+945 |
| Set-2 | 9 | 10475 | CSF\_0086\_TP4 | F01 | IDT-1446944+945 |
| Set-2 | 10 | 10771 | CSF\_0087\_TP4 | A02 | IDT-1446944+945 |
| Set-2 | 11 | 10562 | CSF\_0088\_TP4 | G02 | IDT-1446944+945 |
| Set-2 | 12 | 10836 | CSF\_0089\_TP4 | H02 | IDT-1446944+945 |
| Set-2 | EC2 |  | TPRV\_CSF\_Batch05\_EC2 | B03 | IDT-1446944+945 |

**PCR Profile:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Steps** | **Temp** | **Time** | **Cycle** |
| Step 1 | 37°C | 15 min | 1 |
| Step 2 | 98°C | 30 sec | 1 |
| Step 3 | 98°C | 30 sec | 12 |
| 65°C | 75 sec |
| Step 4 | 65°C | 5 min | 1 |
| Hold | 4 °C | **∞** |  |
| **Lid: 105°C** | | | |

### ***Step 10: 0.8x SPRI (Magnetic Bead Purification) Clean***

* Used SPRI bead **0.8x ratio** of beads-to-total volume of sample.

|  |  |
| --- | --- |
| Bead Volume | 21.2ul |
| Elution | 25ul from 27ul water |

### ***Step 11: 0.75x SPRI (Magnetic Bead Purification) Clean***

* Used SPRI bead **0.75x ratio** of beads-to-total volume of sample.

|  |  |
| --- | --- |
| Bead Volume | 18.75ul |
| Elution | 15ul from 17ul water |

* **Checkpoint: Barcoded purified products were stored in -80°C.**

# Equi-conc

## Date: 26-Jan-2023

**Lab person: Anisur**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Set no** | **Batch Sl** | **Qubit (ng/uL) after 20-fold dilution** | **Qubit (ng/uL) after 10-fold dilution** | **Original Conc** | **Volume picked (Desired DNA input 7ng)** | **Decision for Pick** |
| Set-1 | 1 | 1.1 | 2.2 | 22 | 3.18 | 10F |
| 2 | 0.996 | 1.992 | 19.92 | 3.51 | 10F |
| 3 | 1.17 | 2.34 | 23.4 | 2.99 | 10F |
| 4 | 1.63 | 3.26 | 32.6 | 2.15 | 10F |
| EC | too low |  |  | 0.50 | 10F |
| Set-2 | 5 | 1.22 | 2.44 | 24.4 | 2.87 | 10F |
| 6 | 0.616 | 1.232 | 12.32 | 5.68 | 10F |
| 7 | 0.9 | 1.8 | 18 | 3.89 | 10F |
| 8 | 1.57 | 3.14 | 31.4 | 2.23 | 10F |
| 9 | 1.29 | 2.58 | 25.8 | 2.71 | 10F |
| 10 | 1.16 | 2.32 | 23.2 | 3.02 | 10F |
| 11 | 1.67 | 3.34 | 33.4 | 2.10 | 10F |
| 12 | 1.61 | 3.22 | 32.2 | 2.17 | 10F |
| EC2 | too low |  |  | 0.50 | 10F |

**Note\*\* we pooled all tetra libraries in a single tube.**

* Total Vol: 37.50 ul
* Bead Vol: 28.13 ul
* Elution : 20 ul
* Pool conc. : 0.29 ng/ul (10F)
* Pool conc. : 2.9 ng/ul (RAW)