

1

Purify and Fragment mRNA

Date/Time: \_\_\_\_\_

Operator: \_\_\_\_\_

- Vortex RPB tube
- Thermal cycle RBP plate (mRNA Denaturation program)
- Incubate RBP plate on bench (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Incubate RBP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Incubate RBP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Centrifuge ELB tube (600 xg, 5 s)
- Thermal cycle RBP plate (mRNA Elution 1 program)
- Centrifuge BBB tube (600 xg, 5 s)
- Incubate RBP plate on bench (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Incubate RBP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Incubate RBP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Thermal cycle RBP plate  
(Elution 2 – Frag – Prime program)

BBB Reagent: \_\_\_\_\_

BWB Reagent: \_\_\_\_\_

ELB Reagent: \_\_\_\_\_

FPF Reagent: \_\_\_\_\_

RPB Reagent: \_\_\_\_\_

RBP Plate Barcode: \_\_\_\_\_

2

Synthesize First Strand cDNA

Date/Time: \_\_\_\_\_

Operator: \_\_\_\_\_

- Incubate RBP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

- Centrifuge FSA tube (600 xg, 5 s)
- Centrifuge SuperScript II+FSA tube (briefly)
- Centrifuge CDP plate (briefly)
- Thermal cycle CDP plate (Synthesize 1<sup>st</sup> Strand)

CDP Reagent: \_\_\_\_\_

FSA Reagent: \_\_\_\_\_

CDP Plate Barcode: \_\_\_\_\_

Project: \_\_\_\_\_

Batch: \_\_\_\_\_

Date: \_\_\_\_\_

### 3 Synthesize Second Strand cDNA

Date/Time: \_\_\_\_\_

CTE Reagent (optional): \_\_\_\_\_

Operator: \_\_\_\_\_

RSB Reagent: \_\_\_\_\_

Centrifuge CTE tube (600 xg, 5 s)

SMM Reagent: \_\_\_\_\_

Centrifuge SMM tube (600 xg, 5 s)

ALP Plate Barcode: \_\_\_\_\_

Incubate CDP plate (16°C, 60 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Let stand CDP plate (RT)

Vortex AMPure XP Beads

Incubate CDP plate on bench (RT, 15 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate CDP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate CDP plate on magnet (RT, 30 s)

Let stand CDP plate on magnet (RT, 15 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Centrifuge RSB tube (600 xg, 5 s)

Incubate CDP plate on bench (RT, 2 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate CDP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

## 4 Adenylate 3' Ends

Date/Time: \_\_\_\_\_ CTA Reagent (optional): \_\_\_\_\_

Operator: \_\_\_\_\_ ATL Reagent: \_\_\_\_\_

Centrifuge ALP plate (280 xg, 1 m) (if stored) RSB Reagent: \_\_\_\_\_

Centrifuge CTA tube (600 xg, 5 s)

Thermal cycle ALP plate (ATAIL70 program)

## 5 Ligate Adapters

Date/Time: \_\_\_\_\_ CTL Reagent (optional): \_\_\_\_\_

Operator: \_\_\_\_\_ LIG Reagent: \_\_\_\_\_

Centrifuge AR000X tubes (600 xg, 5 s) or RAP (280 xg, 1 m) RNA Adapter Plate \_\_\_\_\_ or

Centrifuge CTL, LIG, STL tubes (600 xg, 5 s)

RNA Adapter Indices:

Incubate ALP plate (30°C, 10 m):

AR001 \_\_\_\_\_ AR013 \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

AR002 \_\_\_\_\_ AR014 \_\_\_\_\_

Vortex AMPure XP Beads

AR003 \_\_\_\_\_ AR015 \_\_\_\_\_

Incubate ALP plate on bench (RT, 15 m):

AR004 \_\_\_\_\_ AR016 \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

AR005 \_\_\_\_\_ AR018 \_\_\_\_\_

Incubate ALP plate on magnet (RT, 5 m):

AR006 \_\_\_\_\_ AR019 \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

AR007 \_\_\_\_\_ AR020 \_\_\_\_\_

Incubate ALP plate on magnet (RT, 30 s)

AR008 \_\_\_\_\_ AR021 \_\_\_\_\_

Let stand ALP plate on magnet (RT, 15 m):

AR009 \_\_\_\_\_ AR022 \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

AR010 \_\_\_\_\_ AR023 \_\_\_\_\_

Incubate ALP plate on bench (RT, 2 m):

AR011 \_\_\_\_\_ AR025 \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

AR012 \_\_\_\_\_ AR027 \_\_\_\_\_

Incubate ALP plate on magnet (RT, 5 m):

RSB Reagent: \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

STL Reagent: \_\_\_\_\_

Vortex AMPure XP Beads

CAP Plate Barcode: \_\_\_\_\_

Incubate CAP plate on bench (RT, 15 m):

PCR Plate Barcode: \_\_\_\_\_

Start \_\_\_\_\_ Stop \_\_\_\_\_

RAP Plate Barcode: \_\_\_\_\_

Let stand CAP plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate CAP plate on magnet (RT, 30 s)

Let stand CAP plate on magnet (RT, 15 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate CAP plate on bench (RT, 2 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate CAP plate on magnet (RT, 5 m): Start \_\_\_\_\_ Stop \_\_\_\_\_

## 6 Enrich DNA Fragments

Date/Time: \_\_\_\_\_

PMM Reagent: \_\_\_\_\_

Operator: \_\_\_\_\_

PPC Reagent: \_\_\_\_\_

Centrifuge PMM and PPC tubes (600 xg, 5 s)

RSB Reagent: \_\_\_\_\_

Centrifuge PCR plate (280 xg, 1 m) (if stored)

TSP1 Plate Barcode: \_\_\_\_\_

Thermal cycle PCR plate (PCR program)

Vortex AMPure XP Beads

Incubate PCR plate on bench (RT, 15 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate PCR plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate PCR plate on magnet (RT, 30 s)

Let stand PCR plate on magnet (RT, 15 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate PCR plate on bench (RT, 2 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

Incubate PCR plate on magnet (RT, 5 m):

Start \_\_\_\_\_ Stop \_\_\_\_\_

## 7 Validate Library

Date/Time: \_\_\_\_\_

Validated by gel or  Agilent Bioanalyzer

Operator: \_\_\_\_\_

## 8 Normalize and Pool Libraries

Date/Time: \_\_\_\_\_

Single-Indexed  Dual-Indexed

Operator: \_\_\_\_\_

DCT Plate Barcode: \_\_\_\_\_

PDP Plate Barcode: \_\_\_\_\_