

1

Fragment DNA

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

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

Fragment DNA: Start \_\_\_\_\_ Stop: \_\_\_\_\_

Centrifuge Covaris tube (600 xg, 5 s)

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

CFP Plate Barcode: \_\_\_\_\_

DNA Plate Barcode: \_\_\_\_\_

IMP Plate Barcode: \_\_\_\_\_

2

Perform End Repair

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

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

Centrifuge CTE tube (600 xg, 5 s)

Shake IMP plate (1,800 rpm, 2 m)

Centrifuge IMP plate (280 xg, 1 m)

Incubate IMP plate (30°C, 30 m):

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

Vortex AMPure XP Beads

Shake IMP plate (1,800 rpm, 2 m)

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

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

Incubate IMP plate on magnet (RT, 15 m):

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

Incubate IMP plate on magnet (RT, 30 s)

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

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

Shake IMP plate (1,800 rpm, 2 m)

Centrifuge IMP plate (280 xg, 1 m)

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

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

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

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

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

ERP Reagent: \_\_\_\_\_

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

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

3

Adenylate 3' Ends

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

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

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

Shake ALP plate (1,800 rpm, 2 m)

Centrifuge ALP plate (280 xg, 1 m)

Incubate ALP plate (37°C, 30 m): Start \_\_\_\_\_ Stop \_\_\_\_\_

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

CTA Reagent (optional): \_\_\_\_\_

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

Project: \_\_\_\_\_  
Batch: \_\_\_\_\_  
Date: \_\_\_\_\_

# 4 Ligate Adapters

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

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

Centrifuge AD000X tubes (600 xg, 5 s) or DAP (280 xg, 1 m)

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

Shake ALP plate (1,800 rpm, 2 m)

Centrifuge ALP plate (280 xg, 1 m)

Incubate ALP plate (30°C, 10 m): Start \_\_\_\_\_ Stop \_\_\_\_\_

Shake ALP plate (1,800 rpm, 2 m)

Centrifuge ALP plate (280 xg, 1 m)

Vortex AMPure XP Beads

Shake ALP plate (1,800 rpm, 2 m)

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

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

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

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

Incubate ALP plate on magnet (RT, 30 s)

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

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

Shake ALP plate (1,800 rpm, 2 m)

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

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

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

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

Vortex AMPure XP Beads

Shake CAP plate (1,800 rpm, 2 m)

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

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

Incubate 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 \_\_\_\_\_

Shake CAP plate (1,800 rpm, 2 m)

Incubate CAP plate (RT, 2 m) Start \_\_\_\_\_ Stop \_\_\_\_\_

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

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

CTL Reagent (optional): \_\_\_\_\_

DNA Adapter Plate \_\_\_\_\_ or

DNA Adapter Indices:

AD001 \_\_\_\_\_ AD013 \_\_\_\_\_

AD002 \_\_\_\_\_ AD014 \_\_\_\_\_

AD003 \_\_\_\_\_ AD015 \_\_\_\_\_

AD004 \_\_\_\_\_ AD016 \_\_\_\_\_

AD005 \_\_\_\_\_ AD018 \_\_\_\_\_

AD006 \_\_\_\_\_ AD019 \_\_\_\_\_

AD007 \_\_\_\_\_ AD020 \_\_\_\_\_

AD008 \_\_\_\_\_ AD021 \_\_\_\_\_

AD009 \_\_\_\_\_ AD022 \_\_\_\_\_

AD010 \_\_\_\_\_ AD023 \_\_\_\_\_

AD011 \_\_\_\_\_ AD025 \_\_\_\_\_

AD012 \_\_\_\_\_ AD027 \_\_\_\_\_

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

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

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

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

DAP Plate Barcode: \_\_\_\_\_

PCR Plate Barcode (gel-free method only): \_\_\_\_\_

SSP Plate Barcode (gel method only): \_\_\_\_\_

**5 Purify Ligation Products (gel method only)**

Date/Time: \_\_\_\_\_ RSB Reagent: \_\_\_\_\_

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

- Centrifuge SSP plate (280 xg, 1 m) (if stored)
- Run gel (120 V, 120 m, constant): Start \_\_\_\_\_ Stop \_\_\_\_\_
- Incubate gel slices (RT) and vortex (every 2 m)

**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) CPP Plate Barcode: \_\_\_\_\_
- Shake PCR plate (1,600 rpm, 20 s) TSP1 Plate Barcode: \_\_\_\_\_

- Centrifuge PCR plate (280 xg, 1 m)
- Thermal cycle (PCR Program): Start \_\_\_\_\_ Stop: \_\_\_\_\_

 Vortex AMPure XP Beads Shake CPP plate (1,800 rpm, 2 m) Incubate CPP plate on bench (RT, 15 m):

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

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

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

  Incubate CPP plate on magnet (RT, 30 s) Let stand PCR plate on magnet (RT, 15 m):

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

 Shake CPP plate (1,800 rpm, 2 m) Incubate CPP plate on bench (RT, 2 m): Start \_\_\_\_\_ Stop \_\_\_\_\_ Incubate CPP 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: \_\_\_\_\_

 Shake DCT plate (1,000 rpm, 2 m) PDP Plate Barcode: \_\_\_\_\_ Centrifuge DCT plate (280 xg, 1 m)  Shake PDP plate (1,800 rpm, 2 m)