

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)
 Incubate IMP plate (30°C, 30 m):
 Start _____ Stop _____
 Vortex AMPure XP Beads
 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 _____
 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)
 Incubate ALP plate (37°C, 30 m):
 Start _____ Stop _____

ATL Reagent: _____
 CTA Reagent (optional): _____
 RSB Reagent: _____

Project: _____
 Batch: _____
 Date: _____

4 Ligate Adapters

Date/Time: _____ CTL Reagent (optional): _____

Operator: _____ DNA Adapter Plate _____ or
 DNA Adapter Indices:

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

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

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

Vortex AMPure XP Beads

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 _____

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

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

Vortex AMPure XP Beads

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

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 _____

Let stand CAP plate on bench (RT, 2 m):
 Start _____ Stop _____

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

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) TSP1 Plate Barcode: _____
 Thermal cycle (PCR Program): Start _____ Stop: _____
 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 magnet (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: _____