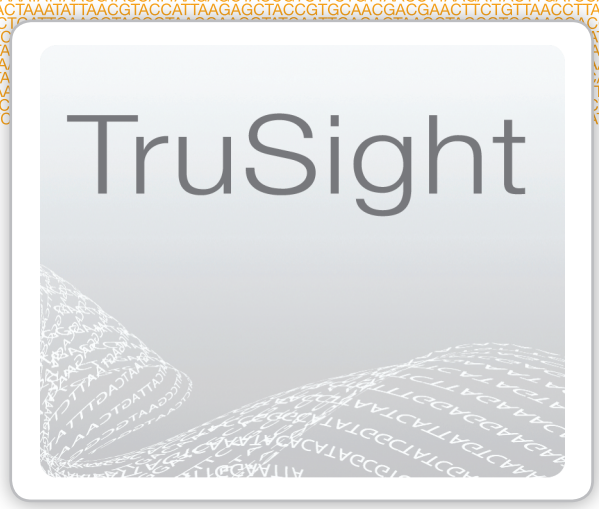
illumina®

TruSight® Myeloid Sequencing Panel Reference Guide



For Research Use Only. Not for use in diagnostic procedures.

ILLUMINA PROPRIETARY

Document # 15054779 v02
April 2016

Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html

Revision History

Document	Date	Description of Change

Document # 15054779 v02	April 2016	• Corrected the amplicon size in DNA Input Recommendations.
		• Added step in Hybridize Oligo Pool procedure to add ACP1 to ACD1.
		• Amplify Libraries procedure:
		  • Specified to add 25 µl 50 mM NaOH to each well of the filter plate.
		  • Specified 27 PCR cycles.
		• Corrected volumes of LNA1 and LNB1 in the Normalize Libraries procedure.
		• Changed wording in Kit Contents to say that TruSight Myeloid and the TruSeq Custom Amplicon Index Kit are required.
		• Specified heat block models in the Pre-PCR Equipment list.

Document # 15054779 v01	January 2016	• Changed the title of this document to TruSight Myeloid Sequencing Panel Reference Guide, and updated the kit name to TruSight Myeloid Sequencing Panel throughout. • Updated design of workflow diagram. • Added a safe stopping point before library normalization. • Renamed and combined some procedures as needed to improve continuity. • Simplified consumables information at the beginning of each section. • Revised step-by-step instructions to be more succinct. • Removed reference to obsolete Experienced User Cards and added references to Custom Protocol Selector and new protocol guide and checklist.
Part # 15054779 Rev. B	November 2014	• Clarified minimum batch size. The TruSight Myeloid Sequencing Panel does not provide enough reagents to process fewer than 8 samples at a time. • Clarified that unused volume is already included in calculation when preparing fewer than 96 samples and calculating volumes of TDP1 and PMM2.
Part # 15054779 Rev. A	May 2014	Initial release.

Table of Contents

Overview

Chapter 1

Introduction

This protocol explains how to prepare up to 96 indexed, pooled libraries using the Illumina® TruSight® Myeloid Sequencing Panel. The goal of this protocol is to hybridize a custom oligo pool to targeted regions of unfragmented gDNA. After extension and ligation, a polymerase chain reaction (PCR) amplifies the library template and adds indexes and sequence adapters to generate DNA libraries ready for clustering and sequencing.

The TruSight Myeloid protocol offers:

- A targeted approach for quickly and efficiently assessing various genetic variants.
- Multiplexing capability to generate up to 1536 amplicons in 1 reaction and sequence up to 96 libraries per sequencing run.
- Fast and easy workflow to prepare up to 96 pooled libraries in 1 plate with approximately 3 hours of hands-on time.
- Variant calling and analysis across all libraries using automated, on-instrument analysis software.
- Fully integrated DNA-to-data solution from assay, sequencing, and automated data analysis to offline software for reviewing results.

DNA Input Recommendations

Quantify the starting genomic DNA (gDNA) using a fluorescence-based quantification method, such as PicoGreen.

- Fluorescence-based methods employ dye specific to double-stranded DNA (dsDNA) and accurately quantify dsDNA, even in the presence of many common contaminants.
- Avoid UV spectrometer methods based on 260 OD readings, which can overestimate DNA concentrations due to the presence of RNA and other common contaminants.

The TruSight Myeloid protocol has been optimized for 50 ng of total gDNA.

DNA Type	Amplicon Size	Input (≥ 15 µl)	A260/A1280	FFPE DNA
High-quality gDNA	250 bp	50 ng	1.8–2.0	Not supported

Additional Resources

Visit the TruSight Myeloid Sequencing Panel support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

Resource	Description
Custom Protocol Selector	http://support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
TruSight Myeloid Sequencing Panel Protocol Guide (document # 1000000005004)	Provides only protocol instructions. The protocol guide is intended for experienced users. For new or less experienced users, see the TruSight Myeloid Sequencing Panel Reference Guide.
TruSight Myeloid Sequencing Panel Checklist (document # 1000000005005)	Provides a checklist of the protocol steps. The checklist is intended for experienced users. For new or less experienced users, see the TruSight Myeloid Sequencing Panel Reference Guide.

Protocol

Chapter 2

Introduction

This chapter describes the TruSight Myeloid Sequencing Panel protocol.

- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- Review Best Practices from the TruSight Myeloid Sequencing Panel support page on the Illumina website.
  - Include a common index in each column. A common index facilitates pipetting operations when dispensing index adapters and pooling indexed libraries later in the protocol.

NOTE

The TruSight Myeloid Sequencing Panel does not provide enough reagents to process fewer than 8 samples at a time. If you are processing less than 96 samples, only 6 freeze-thaw cycles are supported. When calculating smaller reagent amounts, the unused volume is already calculated in the totals listed in the protocol.

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. Different methods are available depending on the sequencing instrument you are using. See the TruSight Myeloid Sequencing Panel support page for more information.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Cross-Contamination

- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each row*** and ***each column***.
- Remove unused index adapter tubes from the working area.

Sealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps
- Apply the adhesive seal to cover the plate and seal with a rubber roller.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Microseal 'A' adhesive film is effective for thermal cycling and easy to cut when using fewer than 96 wells.

Plate Transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.

Centrifugation

- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.
  - To pellet beads, centrifuge at 280 × g for 1 minute.

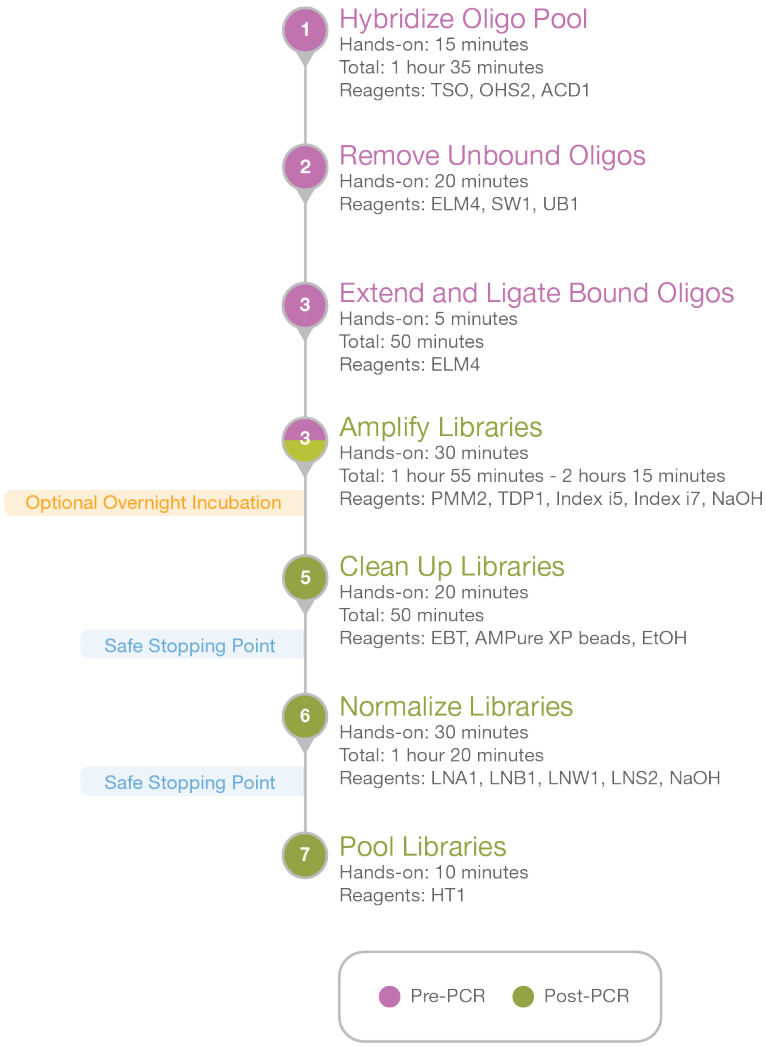
Handling Beads

- Pipette bead suspension slowly.
- When mixing, mix thoroughly.
- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
- When washing beads:
  - Use the appropriate magnet for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

The following diagram illustrates the workflow using the TruSight Myeloid Sequencing Panel. Safe stopping points are marked between steps.

Figure 1 TruSight Myeloid Sequencing Panel Workflow



Hybridize Oligo Pool

This step hybridizes a custom oligo pool that contains upstream and downstream oligos specific to your targeted regions of interest. Perform replicates to increase confidence in somatic variant calls.

Consumables

- TSO (TruSight Oligos)
- OHS2 (Oligo Hybridization for Sequencing 2)
- ACD1 (Amplicon Control DNA 1)
- HYP (Hybridization Plate) barcode label
- gDNA (50 ng per sample)
- 96-well PCR plate, skirted
- Foil adhesive seals (2)

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For environmental, health, and safety information, see the SDS for this kit at support.illumina.com/sds.html.

About Reagents

- OHS2
  - Aspirate and dispense slowly due to the viscosity of the reagent.
  - Before each use, vortex thoroughly and then centrifuge briefly. Make sure that all precipitates have dissolved.
  - When mixing, mix thoroughly.
  - Do not mix with TSO for storage purposes. TSO is unstable when combined with other reagents.
- ACD1
  - Although the control oligo pool ACP1 is included with the kit, using it for the TruSight Myeloid Sequencing Panel protocol is not necessary. Use TSO with ACD1 as a positive control.
  - Include ACD1 and TSO in your assay to establish a baseline and monitor overall performance. Use of these controls enables Illumina Technical Support to troubleshoot if you need assistance.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
TSO	-25°C to -15°C	Thaw at room temperature.
OHS2	-25°C to -15°C	Thaw at room temperature.
ACD1	-25°C to -15°C	Thaw at room temperature.
gDNA	-25°C to -15°C	Thaw at room temperature.

2 Set a 96-well heat block to 95°C.

3 Preheat an incubator to 37°C.

4 Label a new 96-well PCR plate HYP.

Procedure

1 Add 5 µl ACD1 and 5 µl TE or water to 1 well of the HYP plate.

2 Add 10 µl gDNA to each remaining well.
For more diluted samples (eg, < 25 ng/µl), you can use up to 15 µl gDNA.

Table 1 Example Setup for High-Quality gDNA

Input (ng)	Volume (µl)	DNA Concentration (ng/µl)
50	10	5
50	up to 15	≥ 3.3

3 Add 5 µl TSO to each well containing gDNA.

4 Add 5 µl TSO to well containing ACD1.

5 Centrifuge at 1000 × g for 1 minute.

6 Add 35 µl OHS2 to each well. Pipette to mix.

7 Centrifuge at 1000 × g for 1 minute.

8 Place on the preheated heat block and incubate for 1 minute.

9 With the plate on the heat block, reset the temperature to 40°C and continue incubating for 80 minutes.

Remove Unbound Oligos

This step uses a filter to remove unbound oligos from gDNA. Two wash steps using SW1 ensure complete removal of unbound oligos. A third wash step using UB1 removes residual SW1 and prepares samples for extension and ligation.

Consumables

- ELM4 (Extension Ligation Mix 4)
- SW1 (Stringent Wash 1)
- UB1 (Universal Buffer 1)
- FPU (Filter Plate Unit) barcode label
- Filter plate with lid
- Adapter collar, reusable
- 96-well midi plate

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For environmental, health, and safety information, see the SDS for this kit at support.illumina.com/sds.html.

WARNING

This set of reagents contains ß-mercaptoethanol. Perform the following procedure in a hood or well-ventilated area.

About Reagents

- Incomplete drainage of SW1 compromises target enrichment specificity.

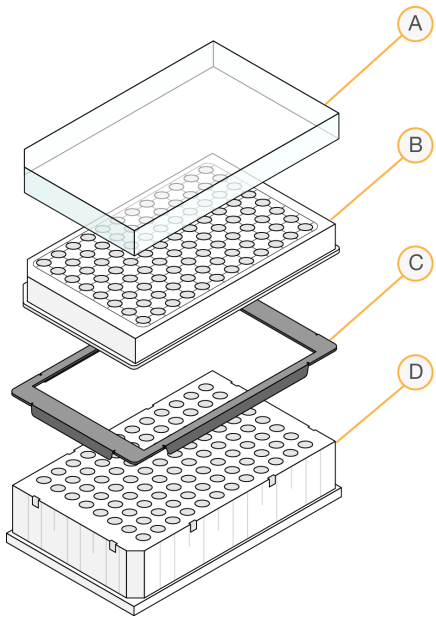
Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
ELM4	-25°C to -15°C	Let stand to bring to room temperature in preparation for a later procedure.
SW1	2°C to 8°C	Set aside at room temperature.
UB1	2°C to 8°C	Set aside at room temperature.

2 Assemble the filter plate unit (FPU) from top to bottom.

Figure 2 FPU Assembly



A Lid
B Filter plate
C Adapter collar
D Midi plate

3 Label the completed assembly FPU.

4 Wash the wells to be used in the assay as follows. Use new wells only.

a Add 45 µl SW1 to each well.
b Cover the FPU plate.
c Centrifuge at 2400 × g for 5 minutes.

5 If a significant amount (> 15 µl/well) of residual buffer remains in multiple wells (≥ 10 wells/plate), switch to a new filter plate.

Procedure

1 Make sure that the heat block has cooled to 40˚C and the HYP plate seal is secure.

2 Remove from the heat block.

3 Centrifuge at 1000 × g for 1 minute.

4 Transfer each sample to the corresponding well of the FPU plate.

5 Cover and centrifuge at 2400 × g for 5 minutes.

6 Wash 2 times as follows.

a Add 45 µl SW1 to each sample well.
b Cover and centrifuge at 2400 × g for 5 minutes.
c If SW1 does not drain completely, centrifuge again for up to 10 minutes.

7 Discard flow-through.

8 Reassemble the FPU plate for continued use.

9 Add 45 µl UB1 to each sample well.

10 Cover and centrifuge at 2400 × g for 5 minutes.

11 If UB1 does not drain completely, centrifuge again for up to 10 minutes.

Extend and Ligate Bound Oligos

This step connects the hybridized upstream and downstream oligos. A DNA polymerase extends from the upstream oligo through the targeted region, followed by ligation to the 5' end of the downstream oligo using a DNA ligase. The result is the formation of products containing the targeted regions of interest flanked by sequences required for amplification.

Consumables

- ELM4 (Extension-Ligation Mix 4)
- Foil adhesive seal

Procedure

1. Add 45 µl ELM4 to each sample well of the FPU plate.
2. Incubate at 37°C for 45 minutes.
3. During incubation, proceed to the next step.

Amplify Libraries

This step amplifies the extension-ligation products and adds Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for cluster formation.

Consumables

- PMM2 (PCR Master Mix 2)
- Index i5 adapters (A5XX)
- Index i7 adapters (A7XX)
- TDP1 (TruSeq DNA Polymerase 1)
- IAP (Indexed Amplification Plate) barcode label
- Microseal 'A' adhesive film
- 50 mM NaOH (3.5 ml for 96 samples)
- 96-well PCR plate, skirted

About Reagents

- Always use a fresh dilution of NaOH.
- Do not store combined PMM2 and TDP1.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
PMM2	-25°C to -15°C	Thaw at room temperature.
Index adapters (i5 and i7)	-25°C to -15°C	Thaw at room temperature.

2 Prepare fresh 50 mM NaOH from 10 N NaOH.

3 Label a new PCR plate IAP.

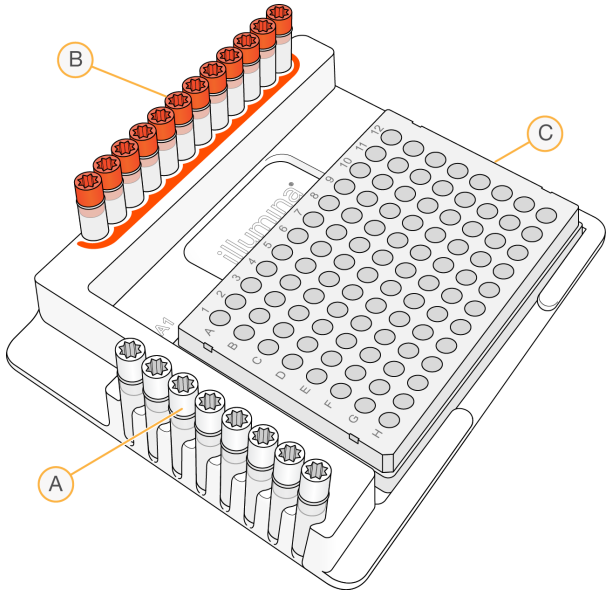
Procedure

1 Arrange the Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.

2 Arrange the Index 2 (i5) adapters in rows A–H of the TruSeq Index Plate Fixture.

3 Place the IAP plate on a TruSeq Index Plate Fixture.

Figure 3 TruSeq Index Plate Fixture



A Columns 1–12: Index 1 (i7) adapters (orange caps)
B Rows A–H: Index 2 (i5) adapters (white caps)
C IAP plate

4 Use a multichannel pipette to add 4 µl of each Index 1 (i7) adapter to each row. Replace the cap on each i7 adapter tube with a new orange cap.

5 Use a multichannel pipette to add 4 µl of each Index 2 (i5) adapter to each column. Replace the cap on each i5 adapter tube with a new white cap.

6 For 96 samples, add 56 µl TDP1 to 2.8 ml PMM2. For < 96 samples, calculate volumes of TDP1 and PMM2 needed.
Unused volume is already included in the calculation.

7 Invert to mix.

8 When incubation is complete, remove the FPU plate from the incubator and remove the seal.

9 Cover and centrifuge at 2400 × g for 5 minutes.

10 Use a multichannel pipette to add 25 µl 50 mM NaOH to each well of the filter plate. Pipette to mix.

11 Incubate at room temperature for 5 minutes.

12 During incubation, transfer 22 µl PMM2/TDP1 master mix to each well of the IAP plate.

13 Transfer samples eluted from the FPU plate to the IAP plate as follows.

a Pipette to mix the NaOH in the first column.
b Transfer 20 µl from the FPU plate to the corresponding column of the IAP plate. Pipette to mix.
c Discard the waste collection midi plate.

14 Centrifuge the IAP plate at 1000 × g for 1 minute.

15 Transfer to the post-amplification area.

16 Perform PCR on a thermal cycler using the following program:
- 95°C for 3 minutes
- 27 cycles of:
  - 95°C for 30 seconds
  - 66°C for 30 seconds
  - 72°C for 60 seconds
- 72°C for 5 minutes
- Hold at 10°C

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 2°C to 8°C overnight.

Clean Up Libraries

This step uses AMPure XP beads to purify the PCR products from other reaction components.

Consumables and Equipment

- EBT (Elution Buffer with Tris)
- AMPure XP beads
- Barcode labels
  - CLP (Clean-up Plate)
  - LNP (Library Normalization Plate)
- Freshly prepared 80% ethanol (EtOH) (40 ml per 96 samples)
- 96-well midi plates (2)
- Microseal 'B' adhesive film

About Reagents

- Always prepare fresh 80% EtOH for wash steps.

Preparation

1. Prepare the following consumables.

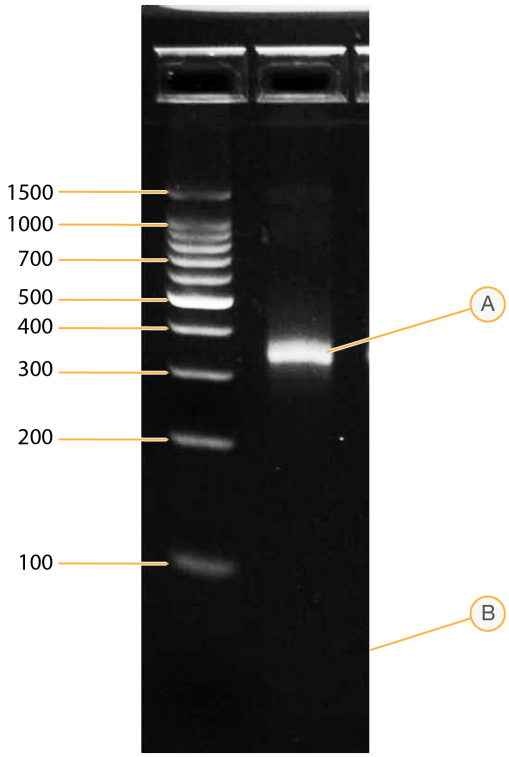
Reagent	Storage	Instructions
AMPure XP beads	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2. Prepare fresh 80% EtOH from absolute ethanol.
3. Label 2 new midi plates CLP and LNP.

Procedure

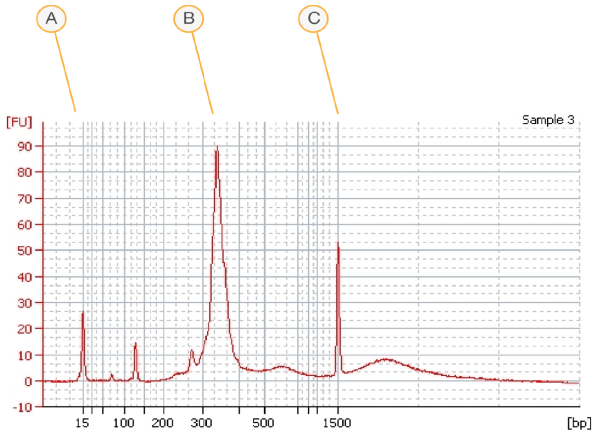
1. Centrifuge the IAP plate at 1000 × g for 1 minute.
2. Run an aliquot of the libraries on 4% agarose gel (5 µl) or Bioanalyzer (1 µl). Expected PCR product size for 250 bp amplicons is ~350 bp.

Figure 4 Agarose Gel Example



A Expected PCR product for 250 bp amplicons (~350 bp)
B Primers

Figure 5 Bioanalyzer Example



A Marker
B Expected PCR product for 250 bp amplicons (~350 bp)
C Marker

3 Add 45 µl AMPure XP beads to each well of the CLP plate.

4 Transfer all the supernatant from each well of the IAP plate to the corresponding well of the CLP plate.

5 Shake at 1800 rpm for 2 minutes.

6 Incubate at room temperature for 10 minutes.

7 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

8 Remove and discard all supernatant from each well.

9 Wash 2 times as follows.

   a Add 200 µl of 80% EtOH to each sample well.
   b Incubate on the magnetic stand for 30 seconds.
   c Remove and discard all supernatant from each well.

10 Use a 20 µl pipette to remove residual EtOH from each well.

11 Remove from the magnetic stand and air-dry for 10 minutes.

12 Add 30 µl EBT to each well.

13 Shake at 1800 rpm for 2 minutes.

14 Make sure that all beads are resuspended. If necessary, pipette to mix and repeat the shaking step.

15 Incubate at room temperature for 2 minutes.

16 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

17 Transfer 20 µl supernatant from each well of the CLP plate to the corresponding well of the LNP plate.

18 Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 3 days. Alternatively, store at -25°C to -15°C for up to 7 days.

Normalize Libraries

This step normalizes the quantity of each library for balanced representation in pooled libraries. Only samples containing DNA require processing through the subsequent steps.

Consumables and Equipment

- LNA1 (Library Normalization Additives 1)
- LNB1 (Library Normalization Beads 1)
- LNW1 (Library Normalization Wash 1)
- LNS2 (Library Normalization Storage buffer 2)
- SGP (Storage Plate) barcode label
- 0.1 N NaOH (freshly prepared)
- 96-well PCR plate, skirted
- 15 ml conical tube
- Microseal 'B' adhesive seals
- Magnetic stand-96 (use with midi 96-well storage plates)

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For environmental, health, and safety information, see the SDS for this kit at support.illumina.com/sds.html.

WARNING

This set of reagents contains ß-mercaptoethanol. Perform the following procedure in a hood or well-ventilated area.

About Reagents

- Use a P1000 pipette to transfer LNB1 to LNA1.
- When mixing, mix thoroughly.
- Mix only the amounts of LNA1 and LNB1 required for the current experiment.
- Store remaining LNA1 and LNB1 separately at their respective temperatures.
- Make sure that LNB1 is resuspended before use. Homogeneous resuspension is essential for consistent cluster density on the flow cell.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
LNA1	-25°C to -15°C	Thaw at room temperature. Let stand for 30 minutes to bring to room temperature. Vortex to mix. Inspect in front of a light. Make sure that all precipitate has dissolved.
LNB1	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex for at least 1 minute. Invert intermittently to resuspend. Make sure that the bottom of the tube is free of pellets.
LNW1	2°C to 8°C	Thaw at room temperature. Let stand for 30 minutes to bring to room temperature.
LNS2	15°C to 30°C	If frozen, thaw at room temperature for 20 minutes. Vortex to mix.

2 Prepare fresh 0.1 N NaOH.

3 Label a new 96-well plate SGP.

Procedure

1 For 96 samples, add 4.4 ml LNA1 to a new 15 ml conical tube.

2 Use a P1000 pipette to resuspend LNB1.

3 For 96 samples, transfer 800 µl LNB1 to the 15 ml conical tube of LNA1. Invert to mix. The LNB1/NA1 mix is sufficient for 96 libraries.

4 Add the LNA1/LNB1 mix to a trough.

5 Add 45 µl LNA1/LNB1 to each well of the LNP plate.

6 Shake at 1800 rpm for 30 minutes.
Durations other than 30 minutes can affect library representation and cluster density.

7 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).

8 Remove and discard all supernatant.

9 Remove from the magnetic stand.

10 Wash 2 times as follows.

   a Add 45 µl LNW1 to each library well.
   b Shake at 1800 rpm for 5 minutes.
   c Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
   d Remove and discard all supernatant.

11 Remove residual LNW1 from each well.

12 Remove from the magnetic stand.

13 Add 30 µl fresh 0.1 N NaOH to each well.

14 Shake at 1800 rpm for 5 minutes.

15 If the libraries are not resuspended, pipette to mix, and then shake at 1800 rpm for 5 minutes.

16 Place the LNP plate on a magnetic stand and wait until the liquid is clear (~2 minutes).

17 Add 30 µl LNS2 to each well of the SGP plate.

18 Transfer 30 µl supernatant from each well of the LNP plate to the corresponding well of the SGP plate.

19 Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 30 days.

Pool Libraries

Pooling libraries combines equal volumes of normalized libraries in a single tube. After pooling, dilute and heat-denature the library pool before loading libraries for the sequencing run.

Consumables

- HT1 (Hybridization Buffer)
- PAL (Pooled Amplicon Library) barcode label
- Eppendorf tubes, screw top (2)
- PCR 8-tube strip

About Reagents

- Store the PAL tube at -25°C to -15°C for later use.

Preparation

1. If the SGP plate was stored frozen, prepare as follows.
   a. Thaw at room temperature.
   b. Centrifuge at 1000 × g for 1 minute.
   c. Pipette to mix.
2. To prepare for the sequencing run, begin thawing reagents according to the instructions for your instrument.
3. Label a new Eppendorf tube PAL.

Procedure

1. Transfer 5 µl of each library from the SGP plate to an 8-tube strip, column by column.
2. Seal the plate and store at -25°C to -15°C.
3. Transfer the contents of the 8-tube strip to the PAL tube. Pipette to mix.
4. Denature and dilute pooled libraries to the loading concentration for the instrument you are using. See the denature and dilute libraries guide for your instrument.

Supporting Information

Appendix A

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

Acronyms

Acronym	Definition
ACD1	Amplicon Control DNA 1
ACP1	Amplicon Control Oligo Pool 1
TSO	TruSight Oligos
CLP	Clean-up Plate
EBT	Elution Buffer with Tris
ELM4	Extension Ligation Mix 4
FPU	Filter Plate Unit
HT1	Hybridization Buffer
HYP	Hybridization Plate
IAP	Index Amplification Plate
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNP	Library Normalization Plate
LNS2	Library Normalization Storage Buffer 2
LNW1	Library Normalization Wash 1
OHS2	Oligo Hybridization for Sequencing Reagent 2
PAL	Pooled Amplicon Library

Kit Contents

Make sure that you have all reagents identified in this section before proceeding to the library preparation procedures. The following kits are required.

Kit Name	Catalog #
TruSight Myeloid	FC-130-1010
TruSeq Custom Amplicon Index Kit	FC-130-1003

TruSight Myeloid Sequencing Panel Kit Contents (96 Samples) (FC-130-1010)

Box 1

Quantity	Reagent	Description	Storage Temperature	Area
1	ACD1	Amplicon Control DNA 1	-25°C to -15°C	Pre-amp
1	ACP1	Amplicon Control Oligo Pool 1	-25°C to -15°C	Pre-amp
1	OHS2	Oligo Hybridization for Sequencing Reagent 2	-25°C to -15°C	Pre-amp
1	ELM4	Extension Ligation Mix 4	-25°C to -15°C	Pre-amp
1	PMM2	PCR Master Mix 2	-25°C to -15°C	Pre-amp
1	TDP1	TruSeq DNA Polymerase 1	-25°C to -15°C	Pre-amp
1	SW1	Stringent Wash 1	2°C to 8°C	Pre-amp
1	UB1	Universal Buffer 1	2°C to 8°C	Pre-amp
5 each	--	Barcode labels for FPU, HYP, and IAP	Room temperature	Pre-amp

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For environmental, health, and safety information, see the SDS for this kit at support.illumina.com/sds.html.

Box 2

Quantity	Reagent	Description	Storage Temperature	Area
1	--	Filter plate with lid	Room temperature	Pre-amp
1	LNB1	Library Normalization Beads 1	2°C to 8°C	Post-amp

Box 3

Quantity	Reagent	Description	Storage Temperature	Area
1	HT1	Hybridization Buffer	-25°C to -15°C	Post-amp
1	LNA1	Library Normalization Additives 1	-25°C to -15°C	Post-amp
1	LNW1	Library Normalization Wash 1	2°C to 8°C	Post-amp

Quantity	Reagent	Description	Storage Temperature	Area
1	LNS2	Library Normalization Storage Buffer 2	Room temperature	Post-amp
1	EBT	Elution Buffer with Tris	Room temperature	Post-amp
5 each	--	Barcode labels for CLP, DAL, LNP, PAL, and SGP	Room temperature	Post-amp

TruSight Myeloid Custom Amplicon Oligos Box, Store at -25°C to -15°C

Quantity	Reagent	Description	Area
1	TSO	TruSight Oligo Tube	Pre-amp

TruSeq Custom Amplicon Index Kit Contents (96 Indexes, 384 Samples)(FC-130-1003)

TruSeq Custom Amplicon Index Kit, Store at -25°C to -15°C

Quantity	Description	Area
8	i5 Index Primers, A501 to A508	Pre-amp
12	i7 Index Primers, A701 to A712	Pre-amp

Index Adapter Replacement Caps, Store at Room Temperature

Quantity	Reagent Name	Area
48	i5 Index Tube Caps, White	Pre-amp
32	i7 Index Tube Caps, Orange	Pre-amp

Additional Components

Illumina Consumable	Catalog #	Storage Temperature	Area
TruSeq Custom Amplicon Filter Plate[1]	FC-130-1006	Room temperature	Pre-amp
TruSeq Index Plate Fixture and Collar Kit[2]	FC-130-1007	Room temperature	Pre-amp

[1] Highly recommended

[2] Required and reusable

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

Consumables

Consumable	Supplier
10 N NaOH (prepare from tablets or use a standard solution)	General lab supplier
96-well skirted PCR plates, 0.2 ml, polypropylene	Bio-Rad, Part # MSP-9601
96-well storage plates, 0.8 ml (midi plates)	Fisher Scientific, Part # AB-0859 Fisher Scientific, Part # AB-0765
Agencourt AMPure XP, 60 ml kit	Beckman Coulter, Part # A63881/A63880
Foil seals	Beckman Coulter, Part # 538619
Conical tubes, 15 ml	General lab supplier
Eppendorf microcentrifuge tubes, screw top	General lab supplier
Ethanol, 200 proof for molecular biology	General lab supplier
Microseal 'A' adhesive seals	Bio-Rad, Part # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, Part # MSB-1001
PCR 8-tube strips	General lab supplier
Solution basin, PVC, non-sterile (trough)	Labcor, Part# 730-001
Agarose gel (2% for 250 bp and 425 bp amplicons, or 4% for 150 bp, 175 bp, and 250 bp amplicons)	General Lab Supplier
DNA 1000 Kit for Bioanalyzer	Agilent 5067–1504 (for 300 samples)
DNA molecular weight markers	General Lab Supplier
Ice bucket	General Lab Supplier

Pre-PCR Equipment

Equipment	Supplier
37° incubator	Forced Air Oven, VWR International or comparable

Equipment	Supplier

Heat block, 96-well:
• SciGene TruTemp Heating System or
 	
• Hybex Microsample Incubator
	• Illumina, catalog #
	  • SC-60-503 (110 V) or
	  • SC-60-504 (220 V)
	• SciGene, catalog #
	  • 1057-30-0 (115 V) or
	  • 1057-30-2 (230 V)

Tabletop centrifuge	General lab supplier

Post-PCR Equipment

Equipment	Supplier
Magnetic stand-96	Invitrogen DynaMag™-96 Side Skirted
Post-PCR plate shaker	Q Instruments BioShake iQ high-speed thermoshaker, part # 1808-0506, or Q Instruments BioShake XP high-speed lab shake, part # 1808-0505
Tabletop centrifuge	General lab supplier
Gel electrophoresis supplies and apparatus	General lab supplier
Heat block for 1.5 ml centrifuge tubes	General lab supplier
Bioanalyzer System	Agilent Technologies

Thermal Cyclers

The following table lists the recommended settings for the recommended thermal cycler, and other comparable models. If your lab has a thermal cycler that is not listed, validate the thermal cycler before performing the protocol.

Thermal Cycler	Temp Mode	Lid Temp	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Calculated	Heated, Constant at 100°C	Polypropylene plates and tubes
MJ Research DNA Engine Tetrad	Calculated	Heated	Plate
Eppendorf Mastercycler Pro S	Gradient S, Simulated Tube	Heated	Plate

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 2 Illumina General Contact Information

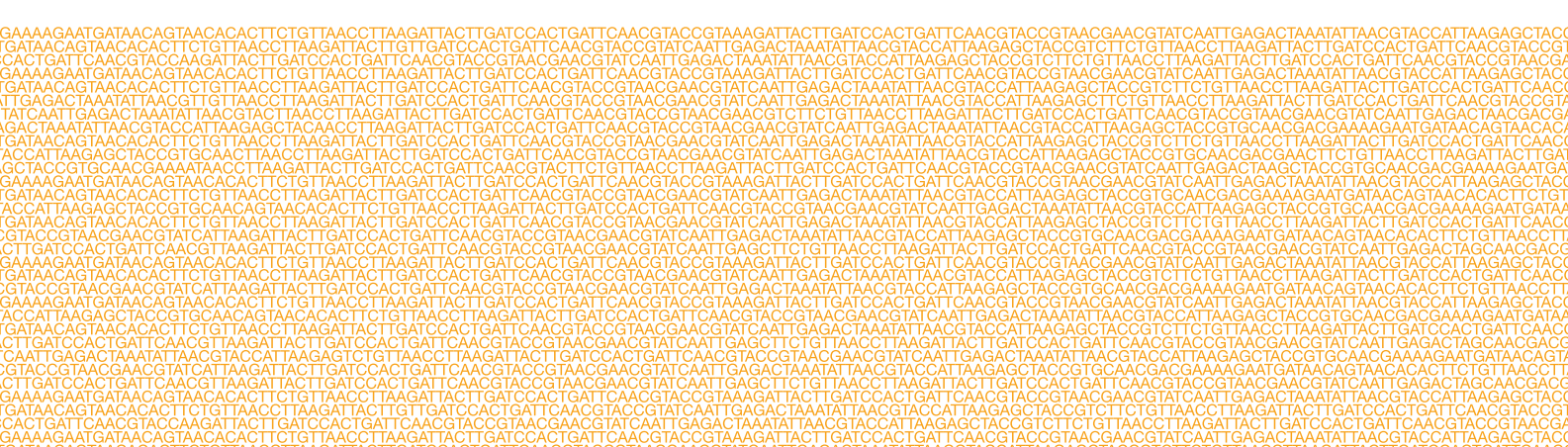
Website	www.illumina.com
Email	techsupport@illumina.com

Table 3 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com