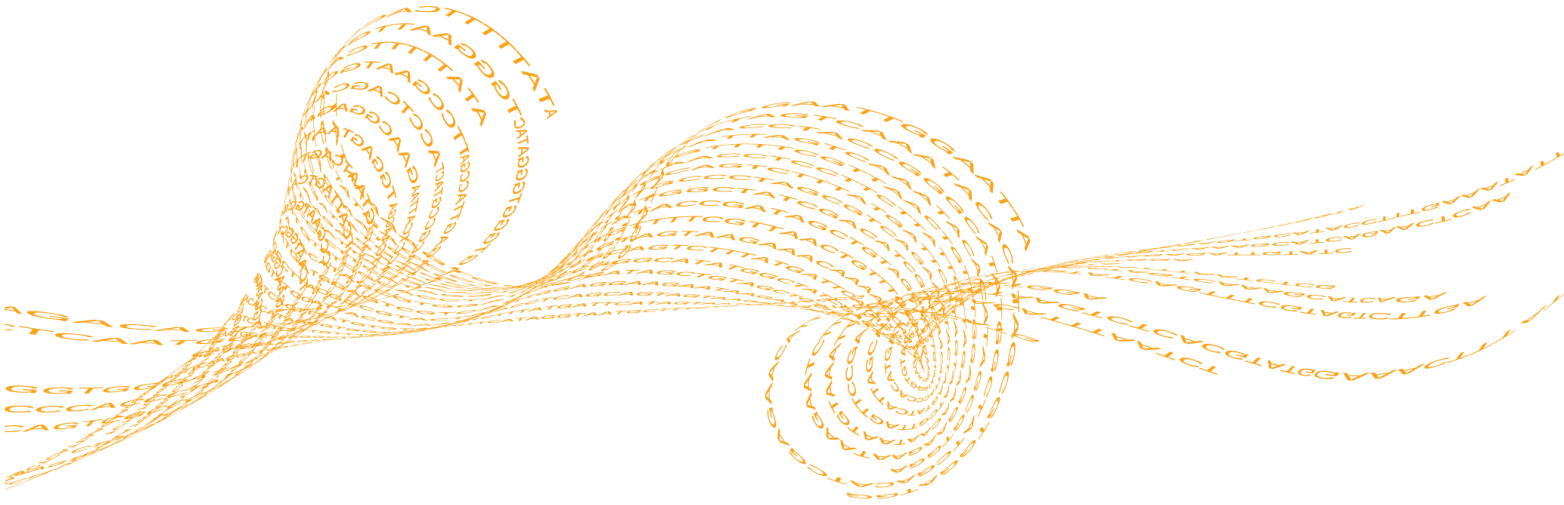
TruSight Myeloid Sequencing Panel

Protocol Guide

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



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Document # 1000000005004 v01
April 2016

Hybridize Oligo Pool

Preparation

1. Set a 96-well heat block to 95°C.
2. Preheat an incubator to 37°C.
3. 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.
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. 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

Preparation

1. Assemble the filter plate unit (FPU) from top to bottom as follows: lid, filter plate, adapter collar, and midi plate.
2. Wash the wells to be used in the assay with 45 µl SW1.

Procedure

1. Make sure that the heat block has cooled to 40˚C.
2. Remove from the heat block.
3. Centrifuge at 1000 × g for 1 minute.
4. Transfer each sample to the FPU plate.
5. Cover and centrifuge at 2400 × g for 5 minutes.
6. Wash 2 times with 45 µl SW1.
7. Discard flow-through.
8. Reassemble the FPU plate.
9. Add 45 µl UB1.
10. Cover and centrifuge at 2400 × g for 5 minutes.

Extend and Ligate Bound Oligos

Procedure

1. Add 45 µl ELM4 to the FPU plate.
2. Incubate at 37°C for 45 minutes.

Amplify Libraries

Preparation

1. Prepare fresh 50 mM NaOH from 10 N NaOH.
2. 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.
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.
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 the filter plate.
11. Incubate at room temperature for 5 minutes.
12. Transfer 22 µl PMM2/TDP1 master mix to 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 IAP plate. Pipette to mix.
    c. Discard the waste collection midi plate.
14. Centrifuge 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

Preparation

1 Prepare fresh 80% EtOH from absolute ethanol.

2 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 libraries on 4% agarose gel (5 µl) or Bioanalyzer (1 µl).

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

4 Transfer all the supernatant from the IAP plate to 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 with 200 µl 80% EtOH.

10 Use a 20 µl pipette to remove residual EtOH.

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

12 Add 30 µl EBT.

13 Shake at 1800 rpm for 2 minutes.

14 Incubate at room temperature for 2 minutes.

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

16 Transfer 20 µl supernatant from the CLP plate to the LNP plate.

17 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

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.

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 tube of LNA1.

4 Add the LNA1/LNB1 mix to a trough.

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

6 Shake at 1800 rpm for 30 minutes.

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 with 45 µl LNW1.

11 Remove residual LNW1.

12 Remove from the magnetic stand.

13 Add 30 µl fresh 0.1 N NaOH.

14 Shake at 1800 rpm for 5 minutes.

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

16 Add 30 µl LNS2 to the SGP plate.

17 Transfer 30 µl supernatant from the LNP plate to the SGP plate.

18 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

Preparation

1. If the SGP plate was stored frozen, thaw at room temperature and then centrifuge at 1000 × g for 1 minute. Pipette to mix.

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.
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.

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

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

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

Table 2 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.

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