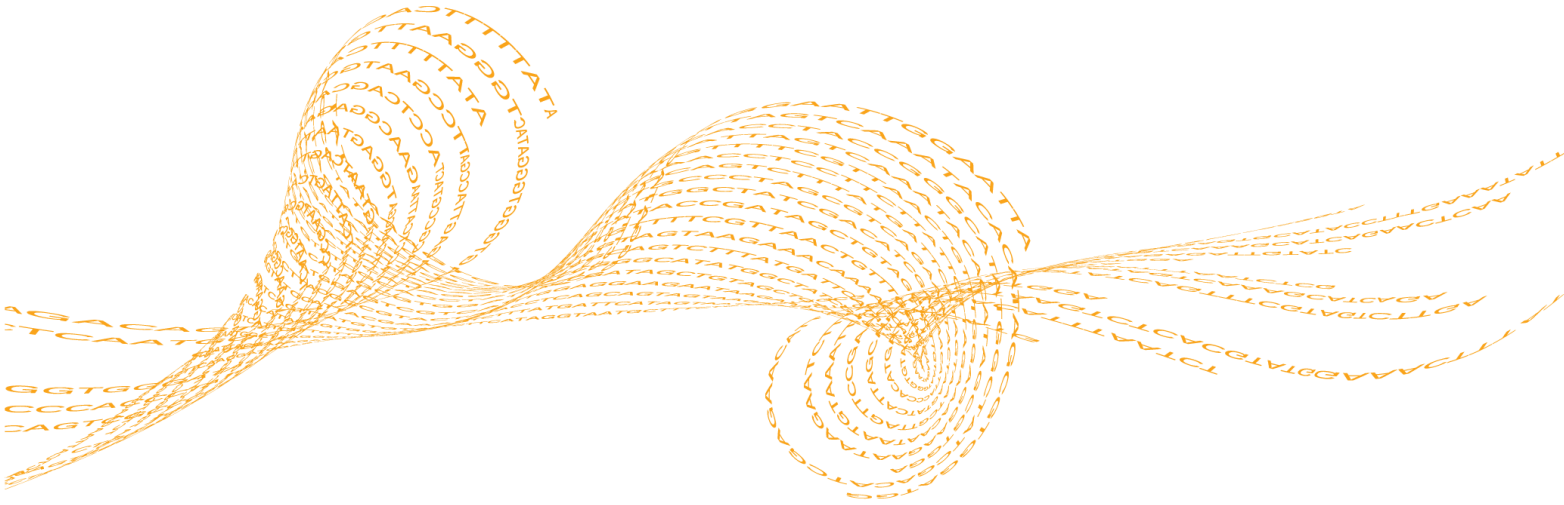
TruSeq Custom Amplicon v1.5

Protocol Guide

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



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Hybridize Oligo Pool

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.

Preparation

1. Set a 96-well heat block to 95°C.
2. Preheat an incubator to 37°C to prepare for the extension-ligation step.

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 ACP1 to the well containing ACD1.
4. Add 5 µl CAT to each well containing gDNA.
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

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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 2 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 2 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 Save the following PCR program on a thermal cycler using the appropriate number of PCR cycles.
- 95°C for 3 minutes
- X 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

Table 1 50–99 ng

Plexity	Number of PCR Cycles (X)		
	150/175 bp	250 bp	425 bp
< 96 amplicons	32	33	33
97–384 amplicons	28	28	29
385–768 amplicons	26	27	28
769–1536 amplicons	25	26	27

Table 2 100–250 ng

Amplicon Size	Number of PCR Cycles (X)		
	150/175 bp	250 bp	425 bp
< 96 amplicons	29	30	30
97–384 amplicons	25	25	26
385–768 amplicons	23	24	25
769–1536 amplicons	22	23	24

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 plate on a TruSeq Index Plate Fixture.

4 Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter down each column.

5 Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter across each row.

6 Remove the FPU plate from the incubator and do the following.

a Replace the aluminum foil seal with the filter plate lid.
b Centrifuge at 2400 × g for 2 minutes.
c Add 25 µl 50 mM NaOH. Pipette to mix.
d Incubate at room temperature for 5 minutes.

7 Add 56 µl TDP1 to a full tube (2.8 ml) of PMM2. Invert to mix.

8 Transfer 22 µl PMM2/TDP1 mixture to the IAP plate.

9 Transfer eluted samples from the FPU plate to the IAP plate.

10 Centrifuge at 1000 × g for 1 minute.

11 Transfer the IAP plate to the post-amplification area.

12 Place on the preprogrammed thermal cycler and run the PCR program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

Procedure

1. Centrifuge the IAP plate at 1000 × g for 1 minute.
2. Run an aliquot of library and control on 4% agarose gel (5 µl) or Bioanalyzer (1 µl).
3. Add the appropriate volume of AMPure XP beads to each well of 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.

Normalize Libraries

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WARNING

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

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. Transfer 800 µl LNB1 to the tube of LNA1.
4. Add 45 µl LNA1/LNB1 to the LNP plate.
5. Shake at 1800 rpm for 30 minutes.
6. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
7. Remove and discard all supernatant.
8. Remove from the magnetic stand.
9. Wash 2 times with 45 µl LNW1.
10. Use a 20 µl pipette to remove residual LNW1.
11. Remove from the magnetic stand.
12. Add 30 µl fresh 0.1 N NaOH.
13. Shake at 1800 rpm for 5 minutes.
14. Place the LNP plate on a magnetic stand and wait until the liquid is clear (~2 minutes).
15. Add 30 µl LNS2 to the SGP plate.
16. Transfer 30 µl supernatant from the LNP plate to the SGP plate.
17. 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

Procedure

1. Centrifuge at 1000 × g for 1 minute.
2. Transfer 5 µl of each library to an 8-tube strip, column by column.
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 sequencing 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
CAT	Custom Amplicon Oligo Tube
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	Indexed 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
PMM2	PCR Master Mix 2
SGP	Storage Plate
SW1	Stringent Wash 1
TDP1	TruSeq DNA Polymerase 1
UB1	Universal Buffer 1

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 3 Illumina General Contact Information

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

Table 4 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**.

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