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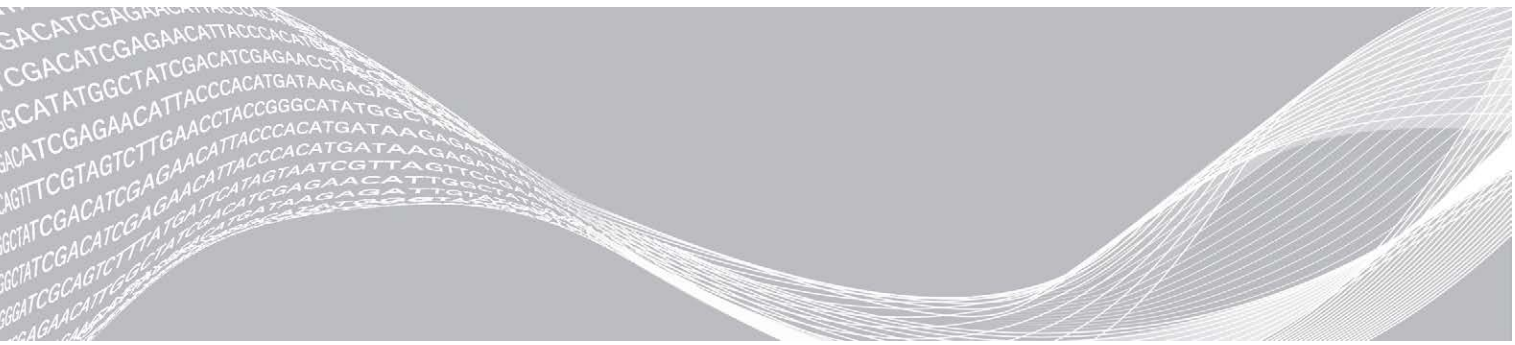
TruSeq Custom Amplicon Low Input Kit

Reference Guide



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June 2017

For Research Use Only– not for any clinical or therapeutic use in humans or animals. This product includes 2800M Control DNA manufactured by Promega Corporation for distribution by Illumina, Inc.

Revision History

Document	Date	Description of Change
Document # 1000000002191 v04	June 2017	Added thermal cycler programs for the hybridize oligo pool step. Added requirement for unique index combinations in each pool of a dual-pool protocol. Clarified the procedure for quantifying and diluting DNA. Updated the step for removing unbound oligos: • In the introduction, corrected the number of SW1 wash steps to three. • In the procedure, specified the HYB plate and keeping the plate on the magnetic stand when washing beads.
Document # 1000000002191 v03	March 2016	Added alternate dual-strand protocol. Updated PCR cycle number guidelines in Amplify Libraries. Modified the thermal cycler program in Hybridize Oligo Pool. Clarified ELE/ELB mixture instructions in Hybridize Oligo Pool. Corrected storage temperature for LNW1.
Document # 1000000002191 v02	February 2016	Corrected the volume of LNA1 to 44 µl in Normalize Libraries. Added temperatures to the thermal cycler program in Hybridize Oligo Pool.
Document # 1000000002191 v01	January 2016	Modified the thermal cycler program in Hybridize Oligo Pool. Removed reference to obsolete Experienced User Cards and added reference to the Custom Protocol Selector. Renamed and combined some procedures as needed to improve continuity.
Document # 1000000002191 v00	October 2015	Initial release

Table of Contents

Chapter 1 Overview

Introduction

This protocol explains how to prepare up to 96 uniquely indexed paired-end libraries of genomic DNA (gDNA) using the Illumina® TruSeq® Custom Amplicon Low Input Library Prep Kit. The kit supports sequencing targeted regions of the genome spanning upwards of 600 kb with up to 1536 amplicons in a single multiplex reaction. This highly targeted approach allows a wide range of applications for discovering, validating, and screening genetic variants rapidly and efficiently.

The TruSeq Custom Amplicon Low Input protocol offers:

- Streamlined 96-well based workflow amenable to automation.
- Multiplexing capability to amplify up to 1536 amplicons in one reaction and sequence up to 96 samples in one run.
- Fast and simple workflow to generate up to 1536 amplicons across 96 samples in one plate with less than 3 hours of hands-on time.

The TruSeq Custom Amplicon Low Input library prep supports:

- Qualified FFPE samples.
- Input DNA amount as low as 10 ng, depending on DNA quality.
- Customized design to create and manage projects using Illumina DesignStudio™ software online for a range of amplicon sizes and reference genomes.
- Automated data analysis to perform variant calling and analysis across all samples using simple on-instrument, automated analysis software.
- The convenience of a fully integrated DNA-to-data solution including online probe design and ordering, assay, sequencing, automated data analysis, and offline software for reviewing results.

Alternate Dual Strand Protocol

This guide includes an alternate dual strand protocol that adds a second, mirrored set of complementary amplicons to target both DNA strands at all loci. The alternate protocol allows preparation of up to 96 uniquely indexed paired-end libraries (48 libraries each in Pool A and Pool B) of gDNA. It is recommended for formalin-fixed, paraffin-embedded (FFPE) samples. You can configure the dual-strand protocol in DesignStudio during design configuration or after design completion.

DNA Input Recommendations

Quantify the input DNA and assess the DNA quality before beginning library prep. Greater input increases library yield and improves sequencing metrics, such as specificity (percent aligned reads).

Type of DNA	Supported Amplicon Size (bp)	DNA Quality	ΔCq	Input (ng)*
Genomic DNA	150, 175, 250	High	--	10
FFPE genomic DNA	150, 175	High	-1 to 1	10
		Medium	>1.0–2.5	20–50
		Low	2.5–4.0	50–100

* Input amount depends on ΔCq. For more information, see the *TruSeq FFPE DNA Library Prep QC Reference Guide (document # 1000000002136 v00)*.

Input DNA Quantification

Quantify the starting genomic material using a fluorescence-based quantification method, such as a Qubit dsDNA Assay Kit or PicoGreen. Do not use a UV-spectrometer-based method.

Fluorescence-based methods employ a dye specific to double-stranded DNA (dsDNA) and specifically and accurately quantify dsDNA, even when many common contaminants are present. In contrast, UV spectrometer methods based on 260 OD readings can overestimate DNA concentrations due to the presence of RNA and other contaminants common to gDNA preparations.

Assessing DNA Quality

Use the TruSeq FFPE DNA Library Prep QC Kit (Illumina catalog # FC-121-9999) to determine FFPE DNA quality and input amounts for the TruSeq Custom Amplicon Low Input libraries.

Input DNA Dilution

- Quantify and dilute DNA to the desired input amount based on the DNA type and quality. For more information, see *DNA Input Recommendations* on page 2.
- You can dilute and store more than the required amount of DNA for later use. Dilute DNA in RS1 and SS1 and store as described in *Quantify and Dilute DNA* on page 7.

Additional Resources

Visit the TruSeq Custom Amplicon Low Input Kit support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

The following documentation is available for download from the Illumina website.

Resource	Description
Custom Protocol Selector	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.
TruSeq Custom Amplicon Low Input Kit Checklist (document # 1000000002204)	Provides a checklist of steps for the experienced user.

Resource	Description
TruSeq Custom Amplicon Low Input Kit Consumables & Equipment List (document # 1000000002296)	Provides an interactive checklist of user-provided consumables and equipment.
TruSeq FFPE DNA Library Prep QC Reference Guide (document # 1000000002136)	Provides instructions on how to determine the fragmentation status and the amplification potential of FFPE-extracted gDNA samples using the TruSeq FFPE DNA QC Kit.

Chapter 2 Protocol

Introduction

This section describes the TruSeq Custom Amplicon Low Input Kit protocol.

- Prepare at least eight samples at a time for the 16-sample kit. For the 96-sample kit, prepare at least 16 samples at a time.
- Follow the protocol in the order described using the specified parameters.
- Before proceeding, confirm your kit contents and make sure that you have the required consumables and equipment. This protocol requires different magnetic stands for pre-PCR and post-PCR procedures.

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. For more information, see the TruSeq Custom Amplicon Low Input Kit support page.

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.

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.

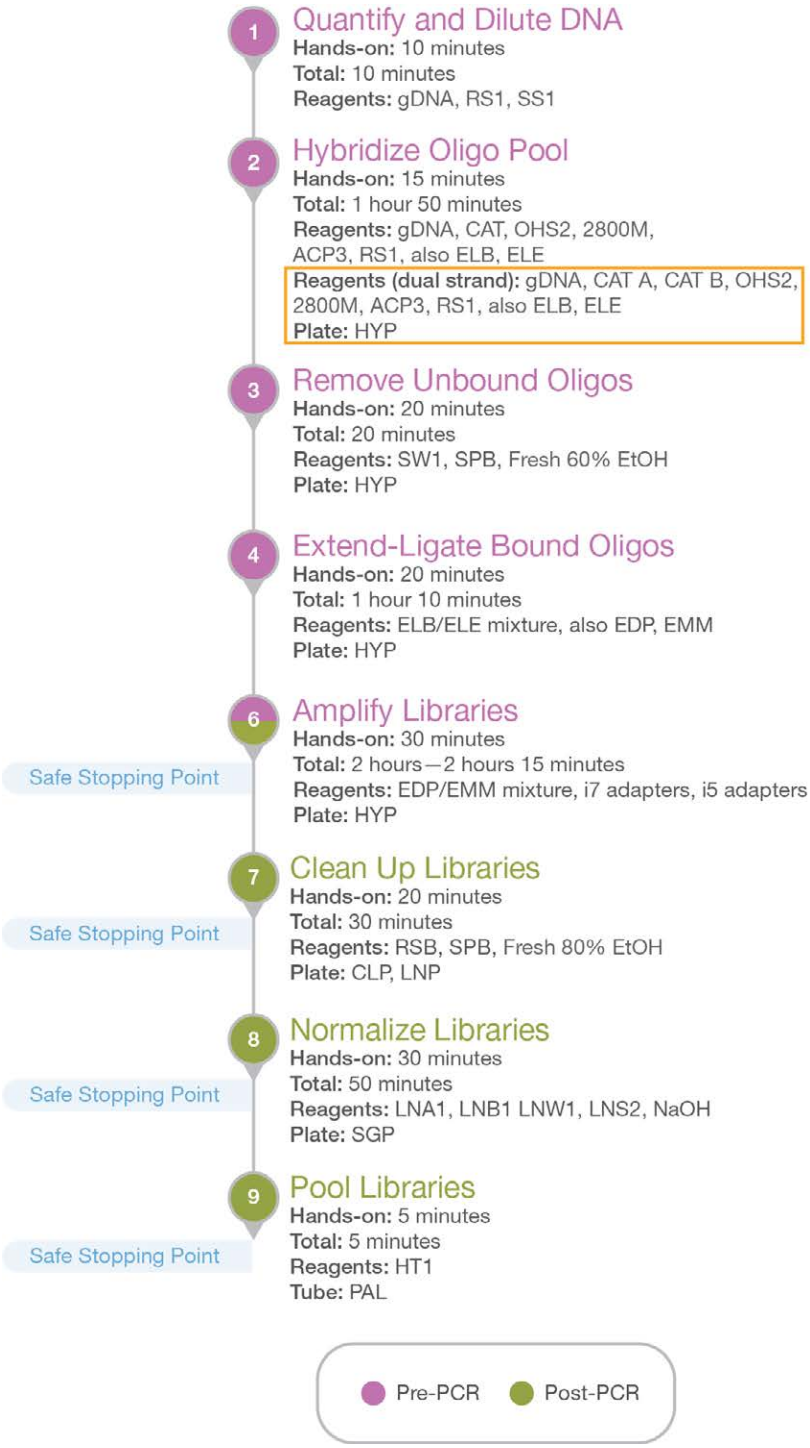
Handling Beads

- Pipette 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 magnetic stand for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Leave the plate on the magnetic stand until the instructions specify to remove it.
  - Do not agitate the plate while it is on the magnetic stand. Do not disturb the bead pellet.

Library Prep Workflow

The following figure illustrates the workflow for the TruSeq Custom Amplicon Low Input Kit.

Figure 1 TruSeq Custom Amplicon Low Input Kit Workflow



Quantify and Dilute DNA

This step quantifies and dilutes input DNA to the appropriate concentration in the required diluent for subsequent steps. For FFPE samples, prepare duplicate or triplicate DNA samples for increased sensitivity in variant calling.

Consumables

- RS1 (Resuspension Solution 1)
- SS1 (Sample Stabilization Solution 1)
- Genomic DNA
- LoBind microcentrifuge tubes

About Reagents

- For later use, you can dilute and store more DNA than is required. Dilute DNA in RS1 and SS1 as described in the procedure.
- Prepare aliquots of diluted DNA in LoBind microcentrifuge tubes and store at -25°C to -15°C for up to 4 weeks.
- Store thawed diluted DNA at 2°C to 8°C for up to 2 weeks.
- Avoid repeatedly freezing and thawing diluted DNA.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
DNA	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Flick to mix, and then centrifuge briefly. Do not vortex.
RS1	15°C to 30°C	If stored at 2°C to 8°C, let stand for 30 minutes to bring to room temperature. Vortex to mix, and then centrifuge briefly.
SS1	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Flick to mix, and then centrifuge briefly.

Procedure

1 Quantify DNA using a fluorometric method, such as Qubit or PicoGreen.

2 Dilute DNA to 10–25 ng/µl in RS1, depending on the initial DNA concentration and the desired input DNA amount.
Diluting to 10–25 ng/µl is recommended for high-quality DNA. For medium- or low-quality DNA recommendations, see *DNA Input Recommendations* on page 2.

3 Requantify the diluted DNA using the same fluorometric quantification method.

4 Further dilute DNA in a LoBind tube as follows. For the dual strand protocol, prepare ~10% extra DNA to account for pipetting errors.

   a Dilute the desired input DNA amount in RS1 to result in a final volume of 4 µl.
   b Add 1 µl SS1 to the 4 µl diluted DNA.

Example: If you diluted high-quality DNA to 10 ng/µl in step 2, dilute the 10 ng/µl to 2.5 ng/µl in RS1 to result in 4 µl diluted DNA.

Hybridize Oligo Pool

This process 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

- CAT (Custom Amplicon Oligo Tube)
- [Dual strand protocol] CAT A (Custom Amplicon Oligo Tube A)
- [Dual strand protocol] CAT B (Custom Amplicon Oligo Tube B)
- OHS2 (Oligo Hybridization for Sequencing 2)
- 2800M (Control DNA 2800M)
- ACP3 (Control Oligo Pool)
- RS1 (Resuspension Solution 1)
- HYP (Hybridization Plate) barcode label
- Diluted DNA
- 96-well PCR plate
- Microseal 'A' adhesive film
- LoBind microcentrifuge tubes
- RNase/DNase-free eight-tube strips and caps
- Prepare for a later procedure:
  - ELB (Extension-Ligation Buffer)
  - ELE (Extension-Ligation Enzyme)
- Prepare for a later procedure:
  - SPB (Sample Purification Beads)
  - SW1 (Stringent Wash 1)

WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

About Reagents

- CAT (or CAT A and CAT B for dual strand)
  - You can dilute and store more than the required amount of CAT (or CAT A and B) to use later. Dilute CAT (or CAT A and B) in RS1 as described in the following procedure or *Procedure for 48 Samples (Dual Strand)* on page 10.
  - Use a multichannel pipette to dispense diluted CAT from a PCR eight-tube strip that contains 70 µl in each tube.

- Prepare aliquots of diluted CAT and store at -25°C to -15°C for up to 12 months.

- 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.
- 2800M
  - Include the 2800M control DNA in every batch of samples being prepared. Use of this control allows Illumina Technical Support to troubleshoot if assistance is needed. If this control is excluded from your assay, assistance cannot be provided.
  - ACP3 is specifically for use with the 2800M control DNA.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
DNA	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Flick to mix, and then centrifuge briefly. Do not vortex.
CAT or CAT A and CAT B (for dual strand)	-25°C to -15°C	Thaw at room temperature for 30 minutes. Vortex to mix, and then centrifuge briefly.
ACP3	-25°C to -15°C	Thaw at room temperature for 30 minutes. Vortex to mix, and then centrifuge briefly.
OHS2	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex thoroughly to mix, and then centrifuge briefly.
2800M	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Flick to mix, and then centrifuge briefly.
RS1	15°C to 30°C	If stored at 2°C to 8°C, let stand for 30 minutes to bring to room temperature. Vortex to mix, and then centrifuge briefly.
SPB	2°C to 8°C	Let stand to bring to room temperature in preparation for a later procedure. Do not exceed 25°C.
SW1	2°C to 8°C	Let stand to bring to room temperature in preparation for a later procedure.
ELB	-25°C to -15°C	Thaw at room temperature for 20 minutes, and then place on ice.
ELE	-25°C to -15°C	Place on ice.

2 Save the following HYB program for 25 µl reaction volume on a Bio-Rad thermal cycler:
- Choose the preheat lid option and set to 100°C.
- Step 1: 95°C for 3 minutes.
- Step 2: From 90°C, decrease by 0.5°C, hold for 30 seconds, ramp at 0.1°C per second.
- Step 3: Go to step 2 for 59x.
- Step 4: From 60°C, decrease by 0.5°C, hold for 1 minute, ramp at 0.1°C per second.
- Step 5: Go to step 4 for 19x.
- Step 6: From 50°C, decrease by 1°C, hold for 2 minutes, ramp at 0.1°C per second.
- Step 7: Go to step 6 for 9x.
- Step 8: From 40°C, hold for 10 minutes, ramp at 0.1°C per second.

This HYB program is a general program. For specific programming instructions, see *Thermal Cycler HYB Programs* on page 27.

3 Label a new 96-well PCR plate HYP.

Procedure for 96 Samples

1 In a LoBind microcentrifuge tube, dilute 2.5 µl CAT with 2.5 µl RS1 per sample well. Pulse vortex to mix, and then centrifuge briefly.

2 In a LoBind microcentrifuge tube, dilute 2.5 µl ACP3 with 2.5 µl RS1. Pulse vortex to mix, and then centrifuge briefly.

3 In a LoBind microcentrifuge tube, dilute 2 µl 2800M with 2 µl RS1 and 1 µl SS1. Pulse vortex to mix, and then centrifuge briefly.

4 Add 5 µl diluted 2800M to one well of the HYP plate.

5 Add 5 µl diluted ACP3 to the well that contains diluted 2800M as an assay control.

6 Add 5 µl RS1 to 1 well as a no template control.

7 Add 5 µl diluted DNA to the remaining wells.

8 Add 5 µl diluted CAT to all wells except the well containing 2800M.

9 Add 15 µl OHS2 to each well. Using a P20 pipette, pipette slowly to mix.

10 If bubbles form, centrifuge the plate at 100 × g for 20 seconds.

11 Place on the preprogrammed thermal cycler and run the HYB program.

12 Combine ELE and ELB as follows.
- [16-sample kit] Transfer 18 µl ELE to the ELB tube. Flick and invert to mix. Do not vortex.
- [96-sample kit] Transfer 137 µl ELE to the ELB tube. Flick and invert to mix. Do not vortex.

NOTE

Prepare the full amount of the ELE/ELB mixture for your kit, even if you are processing fewer than 16 or 96 samples. If necessary, store aliquots at -25°C to -15°C for up to 3 months. Do not allow more than six freeze-thaw cycles.

13 Place the ELB/ELE mixture on ice.
The mixture is used during the procedure for extending and ligating bound oligos.

Procedure for 48 Samples (Dual Strand)

1 In a LoBind microcentrifuge tube, dilute 2.5 µl CAT A with 2.5 µl RS1 per sample well. Pulse vortex to mix, and then centrifuge briefly.

2 In a LoBind microcentrifuge tube, dilute 2.5 µl CAT B with 2.5 µl RS1 per sample well. Pulse vortex to mix, and then centrifuge briefly.

3 In a LoBind microcentrifuge tube, dilute 2.5 µl ACP3 with 2.5 µl RS1. Pulse vortex to mix, and then centrifuge briefly.

4 In a LoBind microcentrifuge tube, diluteDilute 2 µl 2800M with 2 µl RS1 and 1 µl SS1. Pulse vortex to mix, and then centrifuge briefly.

5 Add 5 µl diluted 2800M to one well of the HYP plate.

6 Add 5 µl diluted ACP3 to the well that contains diluted 2800M as an assay control.

7 Add 5 µl RS1 to one well as a no template control.

8 Add 5 µl diluted DNA to wells on the left half of the plate, starting with column 1.

9 Add 5 µl diluted DNA to wells on the right half of the plate, starting with column 7. Do not add DNA to the well containing 2800M.

10 Add 5 µl diluted CAT A to each well containing DNA intended for CAT A.

11 Add 5 µl diluted CAT B to each well containing DNA intended for CAT B.

12 Add 15 µl OHS2 to each well. Pipette slowly to mix using a P20 pipette.

13 If bubbles form, centrifuge the plate at 100 × g for 20 seconds.

14 Place on the preprogrammed thermal cycler and run the HYB program.

15 Combine ELE and ELB as follows.
- [16 samples] Transfer 18 µl ELE to the ELB tube. Flick and invert to mix. Do not vortex.
- [96 samples] Transfer 137 µl ELE to the ELB tube. Flick and invert to mix. Do not vortex.

NOTE

Prepare the full amount of the ELE/ELB mixture for your kit, even if you are processing fewer than 16 or 96 samples. If necessary, store aliquots at -25°C to -15°C for up to 3 months. Do not allow more than six freeze-thaw cycles.

16 Place the ELB/ELE mixture on ice.
The mixture is used during the procedure for extending and ligating bound oligos.

Remove Unbound Oligos

This step uses SPB to remove unbound oligos from gDNA. Three wash steps with SW1 and one wash step with 60% ethanol ensure the complete removal of unbound oligos.

Consumables and Equipment

- SW1 (Stringent Wash 1)
- SPB (Sample Purification Beads)
- Freshly prepared 60% ethanol (EtOH)
- Magnetic Stand
  - DynaMag-96 Side Skirted Magnet (use with 96-well full-skirted PCR plates)
  - DynaMag-96 Side Magnet (use with Eppendorf 96-well twin.tec PCR plates)

WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

About Reagents

- Rinse SW1 over any beads on the side of the well.

- SPB
  - Make sure that beads are at room temperature.
  - Vortex SPB vigorously before each use.
  - When mixing, mix thoroughly.

Preparation

1 Transfer 3 ml SPB to a tube for pre-PCR use.

2 Transfer 6 ml SPB to a tube for post-PCR use.

3 Prepare 200 µl per well of fresh 60% ethanol from 100% ethanol.

Procedure

1 Add 25 µl SPB to each well of the HYB plate. Pipette slowly to mix.

2 Incubate at room temperature for 5 minutes.

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

4 Remove and discard all supernatant from each well.

5 Keeping the plate on the magnetic stand, wash beads three times as follows.

   a Add 80 µl SW1 to each well.
   b Incubate at room temperature for 30 seconds.
   c Remove and discard all supernatant from each well.

6 Use a 20 µl pipette to remove residual SW1 from each well.

7 Add 80 µl of 60% EtOH to each well.

8 Incubate at room temperature for 30 seconds.

9 Remove and discard all supernatant from each well.

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

11 Air-dry for up to 5 minutes. Proceed ***immediately*** to the next step.

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

- ELB/ELE mixture
- Microseal 'A' adhesive seal
- LoBind microcentrifuge tube
- Prepare for a later procedure:
  - EDP (Enhanced DNA Polymerase)
  - EMM (Enhanced Master Mix)

- Prepare for a later procedure:
  - Index i7 adapters (A7XX)
  - Index i5 adapters (A5XX)

About Reagents

- Do not allow more than six freeze-thaw cycles of EMM.
- ELB/ELE mixture
  - Invert and flick to mix, and then centrifuge briefly. Do not vortex.
  - Prepare full amount of the mixture even if processing fewer than 16 or 96 samples.
  - If needed, store aliquots at -25°C to -15°C for up to 3 months.
  - Do not allow more than six freeze-thaw cycles.

Preparation

1 Save the following EXT_LIG program for a 22 µl reaction volume on a thermal cycler:
  - Choose the preheat lid option and set to 100°C
  - 37°C for 45 minutes
  - 70°C for 20 minutes
  - Hold at 4°C

2 Prepare the following consumables.

Reagent	Storage	Instructions
ELB/ELE mixture	-25°C to -15°C	If frozen, thaw at room temperature and then place on ice. Invert and flick to mix, and then centrifuge briefly. Do not vortex.
EDP	-25°C to -15°C	Place on ice. Flick to mix, and then centrifuge briefly.
EMM	-25°C to -15°C	Thaw at room temperature for 20 minutes. Vortex to mix.
Index adapters (i7 and i5)	-25°C to -15°C	Thaw at room temperature for 20 minutes. Vortex each tube to mix. Centrifuge briefly using a 1.7 ml Eppendorf tube. Place on ice for a later procedure.

Procedure

1 Remove plate from the magnetic stand.

2 Using a P100 or P200 pipette, add 22 µl ELB/ELE mixture to each well.

3 Using a pipette set to 20 µl or a P20 pipette, pipette to mix. Make sure that no beads remain in the pipette.

4 If bubbles form, centrifuge at 100 × g for 20 seconds.

5 Place on the thermal cycler and run the EXT_LIG program.

6 Combine EDP and EMM in a LoBind microcentrifuge tube as follows.

Number of Samples	EDP (µl)	EMM (µl)
1	1.1	21
16	17.6	334
48	53	1003
96	106	2006

Volumes include an additional 10%.

7 Pipette the EDP/EMM mixture to mix, and then centrifuge briefly. Place on ice for 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.

Each sample must have a unique index combination, including samples in separate pools for a dual pool design. Make sure that none of the samples in the CAT A pool have the same index combination as the samples in the CAT B pool.

Consumables

- EDP/EMM mixture
- Index i7 adapters (A7XX)
- Index i5 adapters (A5XX)
- Microseal 'A' adhesive film
- Microseal 'B' adhesive seal

NOTE

Use Microseal 'A' when sealing the plate before placing on the thermal cycler. Use Microseal 'B' for shaking, centrifuging, and long-term storage.

Preparation

1 Save the following PCR program on a thermal cycler. Use the following table to determine the number of cycles.

PCR cycles are based on 10 ng input DNA and the number of amplicons in the CAT.

- 95°C for 3 minutes
- X cycles of:
  - 98°C for 20 seconds
  - 67°C for 20 seconds
  - 72°C for 40 seconds
- 72°C for 1 minute
- Hold at 10°C

Plexity	Number of PCR Cycles (X) [1, 2]
< 96 amplicons	32
97–384 amplicons	29
385–700 amplicons	28

Plexity	Number of PCR Cycles (X) [1, 2]
701–999 amplicons	27
1000–1536 amplicons[3]	25

1 Add one cycle for FFPE samples and one cycle for < 10 ng DNA input.

2 To achieve desired library yield and specificity, optimize the PCR cycle number for your oligo pool.

3 Alignment specificity might be reduced for high-plexity panels designed with larger amplicon sizes.

NOTE

Process the 2800M/ACP3 control using the same conditions as the CAT.

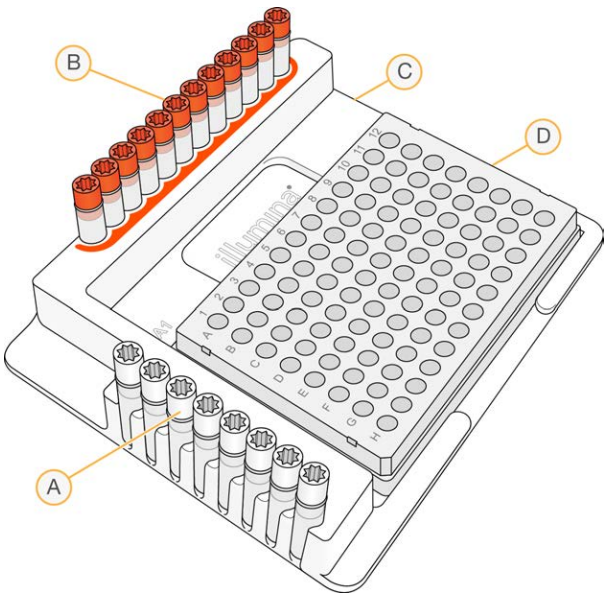
2 If using an iceless cooler, equilibrate the temperature to 2°C to 8°C.

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.

Figure 2 TruSeq Index Plate Fixture



A Rows A–H: Index 2 (i5) adapters (white caps)
B Columns 1–12: Index 1 (i7) adapters (orange caps)
C TruSeq Index Plate Fixture
D HYP plate

3 Place the HYP plate containing beads on a TruSeq Index Plate Fixture.

4 Add 4 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.

5 Add 4 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.

6 Place the plate on ice or iceless cooler.

7 Add 20 µl EDP/EMM mixture to each well. Pipette to mix.

8 Centrifuge at 280 × g for 1 minute.

9 Place the plate on ice or iceless cooler.

10 Immediately transfer to the post-PCR area.

11 Place on the preprogrammed thermal cycler and run the PCR program for the appropriate number of cycles.
The beads remain in the wells during PCR.

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

This step uses SPB (Sample Purification Beads) to purify the PCR products from other reaction components.

Consumables and Equipment

- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- Barcode labels
  - CLP (Cleanup Plate)
  - LNP (Library Normalization Plate)
- 96-well midi plates (2)
- Microseal 'B' adhesive seals
- Freshly prepared 80% ethanol (EtOH)
- Magnetic stand-96 (use with midi 96-well storage plates)

About Reagents

- Vortex SPB vigorously before use.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
SPB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Do not exceed 25°C.
RSB	15°C to 30°C	If frozen, thaw at room temperature for 20 minutes. Vortex to mix.

2 Prepare 400 µl per well fresh 80% ethanol from 100% ethanol.

3 Label a new midi plate CLP.

4 Label a new midi plate LNP.

Procedure

1 Centrifuge the HYP plate at 280 × g for 1 minute.

2 Transfer 45 µl of the supernatant from each well of the HYP plate to the corresponding well of the CLP plate. Transfer as few beads as possible.

3 Add 36 µl SPB to each well of the CLP plate.

4 Shake the plate at 1800 rpm for 2 minutes.

5 Incubate at room temperature for 5 minutes.

6 Centrifuge at 280 × g for 1 minute.

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 two times as follows.

   a Add 200 µl freshly prepared 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 Using a 20 µl pipette, remove residual EtOH from each well.

11 Remove from the magnetic stand and air-dry for 5 minutes.

12 Add 25 µl RSB to each well.

13 Shake the plate 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 Centrifuge at 280 × g for 1 minute.

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

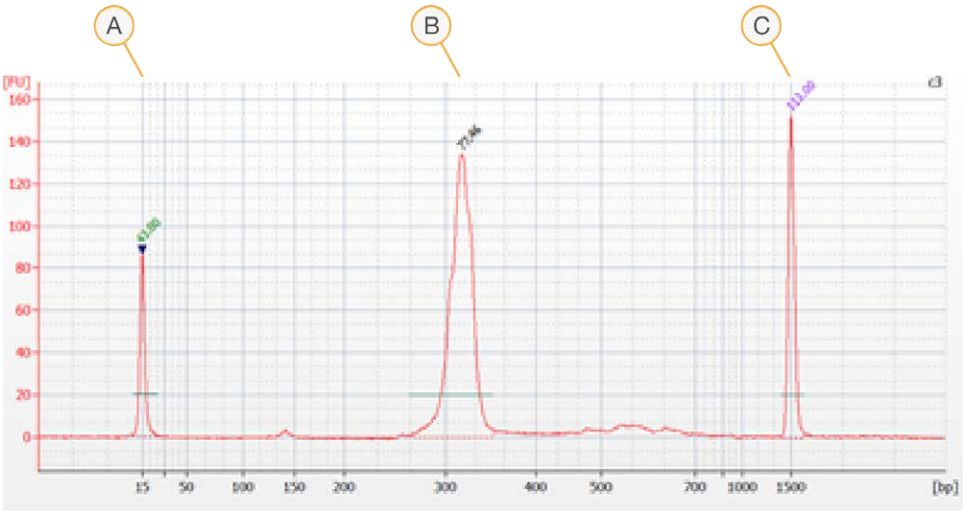
18 Transfer 20 µl purified library from each well of the CLP plate to the corresponding well of the LNP plate.

19 From the remaining liquid in the CLP plate, run an aliquot of the samples and control on any of the following methods:
   - 5 µl on a 4% agarose gel.
   - For up to six samples, 1 µl on an Agilent Bioanalyzer using a DNA 1000 chip.
   - For more than 16–96 samples, 2 µl on an Advanced Analytical Fragment Analyzer using the Standard Sensitivity NGS Fragment Analysis Kit.
   - For 2–96 samples, 1 µl on an Agilent 2200 TapeStation using the D1000 ScreenTape assay.

Table 1 Expected PCR Product Sizes

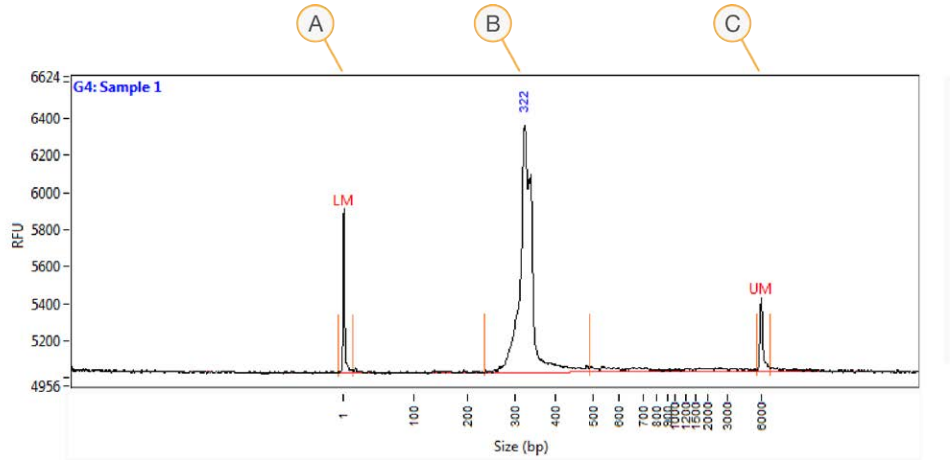
Amplicon Size	PCR Product Size
150 bp	~280 bp
175 bp	~310 bp
250 bp	~350 bp
2800M	~310 bp

Figure 3 Bioanalyzer Trace Example



A Marker
B Expected PCR Product for 200 bp amplicons (~350 bp)
C Marker

Figure 4 Fragment Analyzer Example (Showing Expected 2800M/ACP3 PCR Product)



A Marker
B Expected PCR Product for 200 bp amplicons (~350 bp)
C Marker

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 6 months.

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 potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS 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

- When mixing, mix thoroughly.
- Mix only the amounts of LNA1 and LNB1 required for the current experiment.
- Use a P1000 pipette to transfer LNB1 to LNA1.
- 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.

Reagent	Storage	Instructions
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 Add 44 µl LNA1 per library to a new 15 ml conical tube.

2 Use a P1000 pipette to resuspend LNB1.

3 Transfer 8 µl LNB1 per library to the 15 ml conical tube of LNA1. Invert to mix.

4 Add 45 µl LNA1/LNB1 to each well of the LNP plate.
Each well contains 20 µl library.

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

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 two 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.

10 Use a 20 µl pipette to remove residual LNW1 from each well.

11 Remove from the magnetic stand.

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

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 each well of the SGP plate.

16 Transfer 30 µl supernatant from each well of the LNP plate to the corresponding well of 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

This step pools libraries by combining equal volumes of normalized libraries in one tube.

NOTE

With the dual strand protocol, two libraries represent each sample and are generated from CAT A and CAT B. To analyze the results of each sample, the CAT A and CAT B libraries for each sample must be run together on the same flow cell.

Consumables

- PAL (Pooled Amplicon Library) barcode label
- Microcentrifuge tube
- RNase/DNase-free eight-tube strips and caps

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 Label a new Eppendorf tube PAL.

Procedure

1 Centrifuge at 1000 × g for 1 minute.

2 Transfer 5 µl of each library to an eight-tube strip, column by column.

3 Seal the plate and store at -25°C to -15°C.

4 Transfer the contents of the eight-tube strip to the PAL tube. Pipette to mix.

5 Denature and dilute the library pool to the appropriate loading concentration for the sequencing run. For instructions, see the denature and dilute libraries guide for your instrument.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C for up to 7 days.

Supporting Information

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
2800M	Control DNA 2800M
ACP3	Amplicon Control Oligo Pool 3
CAT	Custom Amplicon Oligo Tube
CAT A CAT B	Custom Amplicon Oligo Tube A Custom Amplicon Oligo Tube B
CLP	Clean-up Plate
EDP	Enhanced DNA Polymerase
ELB	Extension-Ligation Buffer
ELE	Extension-Ligation Enzyme
EMM	Enhanced Master Mix
HT1	Hybridization Buffer
HYP	Hybridization 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
RS1	Resuspension Solution 1
RSB	Resuspension Buffer
SPB	Sample Purification Beads
SGP	Storage Plate
SS1	Sample Stabilization Solution 1
SW1	Stringent Wash 1

Kit Contents

Make sure that you have all the reagents identified in this section before proceeding to the library preparation procedures. A TruSeq Custom Amplicon Low Input Kit and a TruSeq Custom Amplicon Index Kit are required.

Kit Name	Catalog #
TruSeq Custom Amplicon Low Input Kit (16 samples)	FC-134-2002
TruSeq Custom Amplicon Low Input Kit (96 samples)	FC-134-2001
TruSeq Custom Amplicon Index Kit (96 indexes, 384 samples)	FC-130-1003

TruSeq Custom Amplicon Low Input Kit Contents (16 samples, FC-134-2002) (96 samples, FC-134-2001)

Box 1, Store in the Pre-PCR Area

Quantity	Reagent	Description	Storage Temperature
1	ACP3	Amplicon Control Oligo Pool 3	-25°C to -15°C
1	ELE	Extension-Ligation Enzyme	-25°C to -15°C
1	ELB	Extension-Ligation Buffer	-25°C to -15°C
1	EDP	Enhanced DNA Polymerase	-25°C to -15°C
1	EMM	Enhanced Master Mix	-25°C to -15°C
1	2800M	2800M Control DNA	2°C to 8°C

This box also contains the HYP barcode label.

Box 2

Quantity	Reagent	Description	Storage Area	Storage Temperature
1	SS1	Sample Stabilization Solution 1	Pre-PCR	2°C to 8°C
1	SPB	Sample Purification Beads	Pre-PCR	2°C to 8°C
1	OHS2	Oligo Hybridization for Sequencing Reagent 2	Pre-PCR	2°C to 8°C
1	SW1	Stringent Wash 1	Pre-PCR	2°C to 8°C
1	RS1	Resuspension Solution 1	Pre-PCR	15°C to 30°C
1	LNB1	Library Normalization Beads 1	Post-PCR	2°C to 8°C

Box 3, Store in the Post-PCR Area

Quantity	Reagent	Description	Storage Temperature
1	HT1	Hybridization Buffer	-25°C to -15°C
1	LNA1	Library Normalization Additives 1	-25°C to -15°C
1	LNW1	Library Normalization Wash 1	2°C to 8°C
1	LNS2	Library Normalization Storage Buffer 2	15°C to 30°C
1	RSB	Resuspension Buffer	15°C to 30°C

This box also contains plate barcode labels.

TruSeq Custom Amplicon Index Kit (96 indexes, 384 samples) (FC-130-1003)

Box 1, Store at -25°C to -15°C in the Pre-PCR Area

Quantity	Reagent	Description
1	A501	i5 Index Adapter
1	A502	i5 Index Adapter
1	A503	i5 Index Adapter
1	A504	i5 Index Adapter
1	A505	i5 Index Adapter
1	A506	i5 Index Adapter
1	A507	i5 Index Adapter
1	A508	i5 Index Adapter
1	A701	i7 Index Adapter
1	A702	i7 Index Adapter
1	A703	i7 Index Adapter
1	A704	i7 Index Adapter
1	A705	i7 Index Adapter
1	A706	i7 Index Adapter
1	A707	i7 Index Adapter
1	A708	i7 Index Adapter
1	A709	i7 Index Adapter
1	A710	i7 Index Adapter
1	A711	i7 Index Adapter
1	A712	i7 Index Adapter

Index Adapter Replacement Caps, Store at 15°C to 30°C in the Pre-PCR Area

Quantity	Description
1	i5 Index Tube Caps, White
1	i7 Index Tube Caps, Orange

Consumables and Equipment

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

The protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Use a dedicated set of consumables and equipment for pre-PCR and post-PCR procedures. Different magnetic stands are needed for pre-PCR and post-PCR procedures.

Consumables

Consumable	Supplier
10 N NaOH, molecular biology grade[1]	General lab supplier
20 µl barrier pipette tips	General lab supplier
20 µl multichannel pipettes	General lab supplier
20 µl single channel pipettes	General lab supplier
200 µl barrier pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
1000 µl barrier pipette tips	General lab supplier
1000 µl multichannel pipettes	General lab supplier
1000 µl single channel pipettes	General lab supplier
One of the following plate types: • Hard-Shell 96-Well Skirted PCR Plates, low-profile, skirted • Eppendorf 96-Well twin.tec PCR Plates, semiskirted	One of the following suppliers, depending on plate type: • Bio-Rad, catalog # HSP-9601 • Fisher Scientific, catalog # E9-510-20303
96-well storage plates, 0.8 ml (midi plate)	Fisher Scientific, catalog # AB-0859 or AB-0765
Adhesive seal roller	General lab supplier
Conical tubes, 15 ml	General lab supplier
DNA molecular weight markers	General Lab Supplier
Ethanol, 100% for molecular biology	General lab supplier
Ice bucket	General Lab Supplier
LoBind microcentrifuge tubes, 1.5 ml	Eppendorf, part # 022431021
Microseal 'A' film	Bio-Rad, catalog # MSA-5001
Microseal 'B' adhesive seals	Bio-Rad, catalog # MSB-1001
PCR grade water	General lab supplier
RNase/DNase-free eight-tubes strips and caps	General lab supplier
TruSeq FFPE DNA Library Prep QC Kit	Illumina catalog # FC-121-9999
One of the following library quality assessment methods: • 4% Agarose gel • Standard Sensitivity NGS Fragment Analysis Kit (1–6000 bp) • DNA 1000 Kit	One of the following suppliers, depending on method: • General lab supplier • Advanced Analytical Technologies, part # DNF-473 • Agilent Technologies, catalog # 5067–1504
[Optional] TruSeq Index Plate Fixture Kit[2]	Illumina, catalog # FC-130-1005

[1] Prepare from tablets or use a standard solution.

[2] A reusable part for setting up index adapters.

Equipment

Pre-PCR Equipment

Equipment	Supplier
Iceless cooler for 96-well plates	General lab supplier
96-well thermal cycler (with heated lid) See *Thermal Cyclers*.	General lab supplier
One of the following magnets, depending on the type of PCR plate: • DynaMag-96 Side Skirted Magnet (use with 96-well full-skirted PCR plates) • DynaMag-96 Side Magnet (use with Eppendorf 96-well twin.tec PCR plates)	Life Technologies: • Catalog # 12027 • Catalog # 12331D
Microplate centrifuge	General lab supplier

Post-PCR Equipment

Equipment	Supplier
Magnetic stand-96 (use with midi 96-well storage plates)	Life Technologies, catalog # AM10027
One of the following: • BioShake iQ high-speed thermal mixer • BioShake XP high-speed lab shaker	Q.Instruments: • Order # 1808-0506 • Order # 1808-0505
Microplate centrifuge	General lab supplier
One of the following library quality assessment methods: • Fragment Analyzer Automated CE System • 2100 Bioanalyzer Desktop System • Gel electrophoresis supplies and apparatus • Agilent 2200 TapeStation	One of the following suppliers, depending on method: • Advanced Analytical Technologies, part # FSv2-CE2 or FSv2-CE10 • Agilent Technologies, catalog # G2940CA • General lab supplier • Agilent Technologies, catalog # G2964AA
Heat block for 1.5 ml centrifuge tubes	General lab supplier

Thermal Cyclers

Use the following recommended settings for selected thermal cycler models. Before performing library prep, validate any thermal cyclers not listed.

NOTE

The Bio-Rad thermal cyclers might provide superior specificity.

Thermal Cycler	Block Type	Ramp Rate	Lid Temp	Block Rate	Vessel Type
Bio-Rad DNA Engine Tetrad 2	Standard	0.1°C	Heated, Constant at 100°C	--	Bio-Rad Hard-Shell 96-Well Skirted PCR Plates, low-profile, skirted
Bio-Rad S1000	Standard	0.1°C	Heated, Constant at 100°C	--	Bio-Rad Hard-Shell 96-Well Skirted PCR Plates, low-profile, skirted

Thermal Cycler	Block Type	Ramp Rate	Lid Temp	Block Rate	Vessel Type
Bio-Rad C1000	Standard	0.1°C	Heated, Constant at 100°C	--	Bio-Rad Hard-Shell 96-Well Skirted PCR Plates, low-profile, skirted
Bio-Rad T100	Standard	0.1°C	Heated, Constant at 100°C	--	Eppendorf twin.tec PCR Plate 96, semiskirted
Applied Biosystems GeneAmp PCR System 9700	Gold	1%	Heated, Constant at 100°C	9600 for HYB and EXT_LIG programs or MAX for PCR program	Eppendorf twin.tec PCR Plate 96, semiskirted
Applied Biosystems Veriti 96-Well	Alloy	2% to 2.1%	Heated, Constant at 100°C	--	Eppendorf twin.tec PCR Plate 96, semiskirted

Thermal Cycler HYB Programs

The thermal cycler program for the hybridize oligo pool step varies by thermal cycler model. The following tables provide the programs for selected models. Use these tables as a reference when saving the HYB program.

Bio-Rad DNA Engine Tetrad 2, S1000, C1000, and T100

Step	Ramp Speed	Increment (°C)	Temperature (°C)	Hold
1	--	--	95	3 minutes
2	0.1°C/s	-0.5	90	30 seconds
3			Go to step 2 for 59x	
4	0.1°C/s	-0.5	60	1 minute
5			Go to step 4 for 19x	
6	0.1°C/s	-1	50	2 minutes
7			Go to step 6 for 9x	
8	0.1°C/s	--	40	10 minutes

Applied Biosystems GeneAmp PCR System 9700

- Initial hold at 95°C for 3 minutes.
- Select 41-temperature, 2-cycle protocol. Because the minimum cycle number is two, make sure that you include the pause step to prevent the thermal cycler from returning to 95°C.
- Set the temperature and time as listed in the following table.
- Modify the ramp rate to 1% per temperature.
- Add a pause at the end of the cycle.
- Remove the HYB plate during the pause step.
- When performing the protocol, set the time to 90 minutes so that the HYB plate does not remain at 40°C for more than 10 minutes.

Step	Ramp Speed (%)	Temperature (°C)	Hold
1	None	95	3 minutes
2	1	95	45 seconds
3	1	90	45 seconds
4	1	85	45 seconds
5	1	80	45 seconds
6	1	75	1 minute
7	1	74	1 minute
8	1	73	1 minute
9	1	72	1 minute
10	1	71	1 minute
11	1	70	1 minute
12	1	69	2 minutes
13	1	68	2 minutes
14	1	67	2 minutes
15	1	66	2 minutes
16	1	65	2 minutes
17	1	64	2 minutes
18	1	63	2 minutes
19	1	62	2 minutes
20	1	61	2 minutes
21	1	60	2 minutes
22	1	59	2 minutes
23	1	58	2 minutes
24	1	57	2 minutes
25	1	56	2 minutes
26	1	55	2 minutes
27	1	54	2 minutes
28	1	53	2 minutes
29	1	52	2 minutes
30	1	51	2 minutes
31	1	50	2 minutes
32	1	49	3 minutes
33	1	48	3 minutes
34	1	47	3 minutes
35	1	46	3 minutes
36	1	45	3 minutes
37	1	44	3 minutes
38	1	43	3 minutes
39	1	42	3 minutes

Step	Ramp Speed (%)	Temperature (°C)	Hold
40	1	41	3 minutes
41	1	40	10 minutes
42	--	Pause	99:59

Applied Biosystems Veriti 96-Well Thermal Cycler

Step	Ramp Speed	Increment (°C)	Temperature (°C)	Hold
1	--	--	95	3 minutes
2	2.1%	-0.5	90	30 seconds
3		Go to step 2 for 59x		
4	2%	-0.5	60	1 minute
5		Go to step 4 for 19x		
6	2.1%	-1	50	2 minutes
7		Go to step 6 for 9x		
8	2%	--	40	10 minutes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.635.9898	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

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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