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TruSeq Bovine Parentage Reference Guide

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Document # 1000000012390 v00
July 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 # 1000000012390 v00	July 2016	Initial release.

Table of Contents

Overview

Chapter 1

Introduction

This protocol explains how to prepare up to 96 uniquely indexed paired-end libraries of genomic DNA (gDNA) using the Illumina® TruSeq® Bovine Parentage Kit. The kit supports sequencing targeted regions of the bovine genome with up to 273 amplicons in a single multiplex reaction. This highly targeted approach enables a wide range of applications for discovering, validating, and screening genetic variants in a rapid and efficient manner.

The TruSeq Bovine Parentage library prep protocol offers:

- Multiplexing capability to amplify up to 273 amplicons in a single reaction and sequence up to 96 samples in a single sequencing run.
- Fast and simple workflow to generate up to 273 amplicons across 96 samples within a single plate with less than 3 hours hands on time.
- Streamlined 96-well based workflow amenable to automation.

The TruSeq Bovine Parentage library prep supports:

- Project creation and management using the Illumina online DesignStudio software for 273 amplicons and the UMD 3.1 bovine reference genome.
- Automated data analysis to perform variant calling and analysis across all samples using simple on-instrument, automated analysis software.
- Secondary analysis to convert variant calls to genotype calls using the Sequence Genotyper
- The convenience of a fully integrated DNA-to-data solution including online ordering, assay, sequencing, automated data analysis, and offline software for reviewing results.

DNA Input Recommendations

Type of DNA	Input	Concentration	A260/A280
High-quality genomic DNA	50 ng	10–25 ng/µl	1.8–2.0

Input DNA Quantification

Quantify the starting genomic material using a fluorescence-based quantification method, such as a Qubit dsDNA Assay Kit or PicoGreen, rather than a UV-spectrometer-based method. Fluorescence-based methods, which employ a double-stranded DNA (dsDNA) specific dye, specifically and accurately quantify dsDNA even in the presence of many common contaminants. In contrast, UV spectrometer methods based on 260 OD readings are prone to overestimating DNA concentrations due to the presence of RNA and other contaminants commonly found in genomic DNA (gDNA) preparations.

If necessary, dilute gDNA so that the concentration is between 10–25 ng/µl.

Assessing DNA Quality

The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity. This protocol is optimized for DNA with absorbance ratio values of 1.8–2.0.

Additional Resources

Visit the TruSeq Bovine Parentage 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 Bovine Parentage Protocol Guide (document # 1000000012391)	Provides instructions for the experienced user.
TruSeq Bovine Parentage Checklist (document # 1000000012394)	Provides a checklist of steps for the experienced user.
TruSeq Bovine Parentage Consumables & Equipment List (document # 1000000012395)	Provides an interactive checklist of user-provided consumables and equipment.
Sequence Genotyper Software Guide	Provides information about using the Sequence Genotyper software and available analysis options.
TruSeq Amplicon Quick Reference Card	Provides information about using the Illumina® Experiment Manager (IEM) to create and edit sample sheets compatible with your Illumina sequencing system and analysis software. For additional setup information, see the Questions & Answers section of the TruSeq Bovine Parentage support page.

Protocol

Chapter 2

Introduction

This chapter describes the TruSeq Bovine Parentage protocol.

- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- Review Best Practices from the TruSeq Bovine Parentage support page on the Illumina website.

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 TruSeq Bovine Parentage Kit 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.

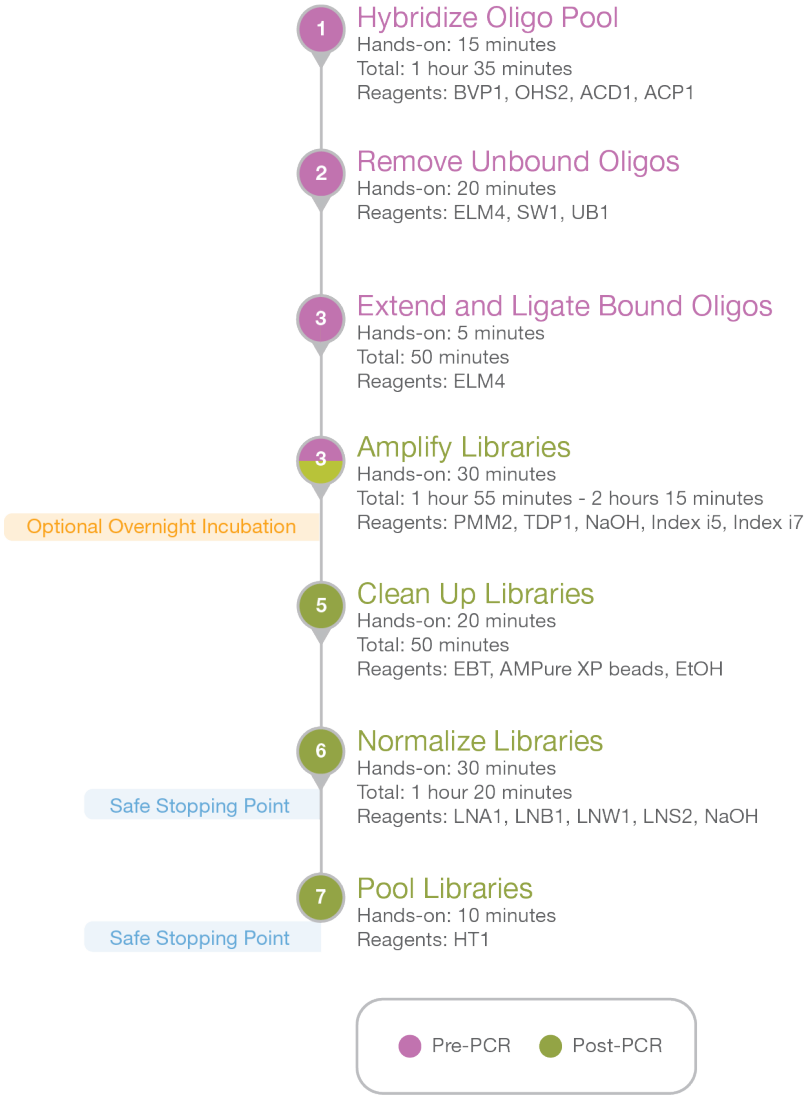
Handling Beads

- 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.

TruSeq Bovine Parentage Workflow

The following figure illustrates the TruSeq Bovine Parentage workflow. Safe stopping points are marked between steps.

Figure 1 TruSeq Bovine Parentage Workflow



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

- BVP1 (Bovine Parentage Oligo Tube)
- OHS2 (Oligo Hybridization for Sequencing 2)
- ACD1 (Amplicon Control DNA)
- ACP1 (Control Oligo Pool)
- HYP (Hybridization Plate) barcode label
- Diluted high-quality gDNA
- 96-well PCR plate
- Adhesive aluminum foil seal
- Microseal 'B' adhesive seals

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.
- ACD1 and ACP1
  - Include ACD1 and ACP1 in every batch of samples being prepared. Use of these controls enables Illumina Technical Support to troubleshoot if you need assistance. If these controls are excluded from your assay, assistance will not be provided.
  - Do not mix BVP1 and OHS2 for storage. If combined, BVP1 becomes unstable even when stored frozen.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
gDNA	-25°C to -15°C	Let stand for 30 minutes to bring to room temperature. Flick to mix, and then centrifuge briefly. Do not vortex.
BVP1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
ACD1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
ACP1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
OHS2	-25°C to -15°C	Thaw at room temperature. Vortex vigorously to mix. Inspect in front of a light. Make sure that all precipitates have dissolved.

2 Set a 96-well heat block to 95°C.

3 Preheat an incubator to 37°C to prepare for the extension-ligation step.

4 Apply the HYP barcode label to a new 96-well PCR plate.

Procedure

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

2 Add 50 ng (in < 10 µl volume) gDNA to each remaining well.
Add TE Buffer to bring the volume to 10 µl.

3 Add 5 µl ACP1 to the well containing ACD1.

4 Add 5 µl BVP1 to each well containing gDNA.

5 Apply Microseal 'B' and centrifuge at 1000 × g for 1 minute.

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

7 Apply the foil seal and 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 process removes unbound oligos from genomic DNA using a size-selection filter. Two wash steps using SW1 ensure complete removal of unbound oligos. A third wash step using UB1 removes residual SW1 and prepares samples for the next step.

Consumables and Equipment

- ELM4 (Extension-ligation Mix 4)
- SW1 (Stringent Wash 1)
- UB1 (Universal Buffer 1)
- Filter plate with lid
- Adapter roller
- Midi plate

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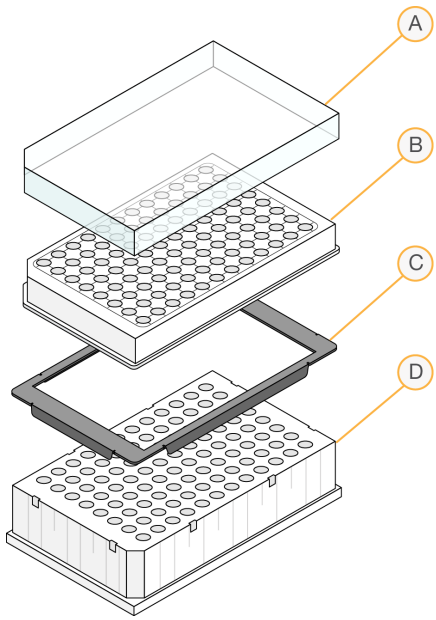
Preparation

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 10 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 2 minutes.

6 Wash 2 times as follows.

a Add 45 µl SW1 to each sample well.
b Cover and centrifuge at 2400 × g for 2 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 2 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. Apply the seal and 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)
- 50 mM NaOH (less than one week old; prepared from 10 N NaOH)
- 96-well skirted PCR plate
- IAP (Indexed Amplification Plate) barcode label
- Microseal 'A' adhesive film
- Microseal 'B' adhesive seal (for 2°C to 8°C storage)

About Reagents

- PMM2/TDP1
  - Combine PMM2 and TDP1 immediately before use. Do not combine and store the combined PMM2/TDP1 mixture.
  - When mixing, mix thoroughly.

Preparation

1 Prepare the following consumables.

Reagent	Storage	Instructions
PMM2	-25° to -15° C	Thaw at room temperature for 20 minutes. Vortex to mix, and then centrifuge briefly.
Index i5 adapters (A5XX)	-25° to -15° C	Let stand for 20 minutes to bring to room temperature. Vortex to mix, and then centrifuge briefly using a 1.7 ml Eppendorf tube.
Index i7 adapters (A7XX)	-25° to -15° C	Let stand for 20 minutes to bring to room temperature. Vortex to mix, and then centrifuge briefly using a 1.7 ml Eppendorf tube.

2 Prepare fresh 50 mM NaOH.

3 Save the following PCR program on a thermal cycler.
- 95°C for 3 minutes
- 25 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

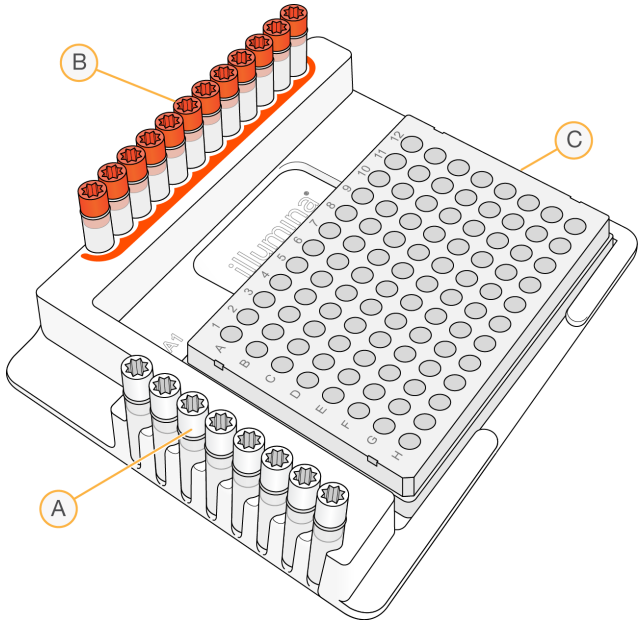
4 Apply the IAP label to a new 96-well PCR plate.

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 3 TruSeq Index Plate Fixture



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

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. Replace the cap on each i7 adapter tube with a new orange cap.

5 Using a multichannel pipette, 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 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 to each well. 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 each well of the IAP plate.

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

a Using fine tips, pipette to mix the NaOH in the first column of the FPU plate.
b Transfer 20 µl NaOH to the corresponding column of the IAP plate. Pipette to mix.
c Transfer remaining columns from the FPU to the IAP plate.
d Discard the waste collection midi plate.

NOTE
Set aside the metal adapter collar for future use. If you partially used an FPU plate, mark the used wells and store the FPU plate and lid in a sealed plastic bag.

10 Apply Microseal 'A' and 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

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 (Cleanup Plate)
  - LNP (Library Normalization Plate)
- 96-well midi plates (2)
- Microseal 'B' adhesive seals
- Freshly prepared 80% ethanol (EtOH)
- Magnetic stand-96

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 40 ml (for 96 samples) fresh 80% ethanol from 100% ethanol.

3 Apply the CLP barcode label to a new midi plate.

4 Apply the LNP barcode label to a new midi plate.

Procedure

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

2 [Optional] Run an aliquot of the library and control using any of the following methods:

- 5 µl on 4% agarose gel
- 1 µl on an Agilent Bioanalyzer using a DNA 1000 chip
- 2 µl on an Advanced Analytical Fragment Analyzer using the Standard Sensitivity NGS Fragment Analysis Kit

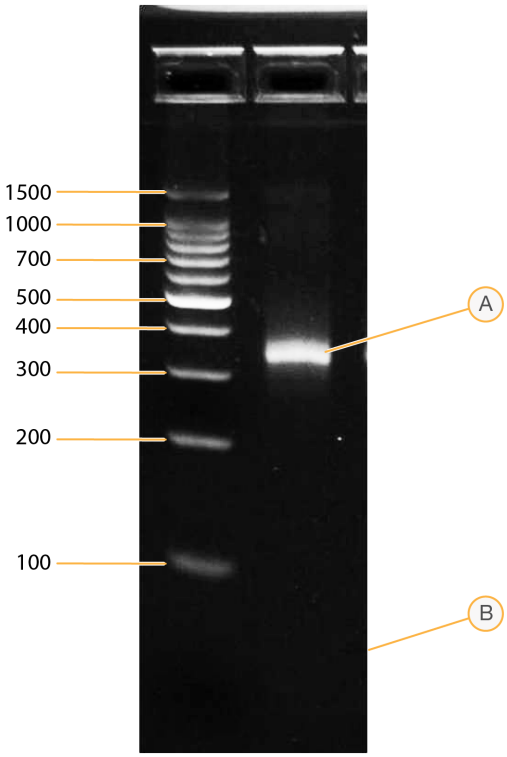
Expect the PCR product size to be 200–300 bp.

NOTE

Assess library quality by gel electrophoresis, Fragment Analyzer, or Bioanalyzer for oligo pools being used for the first time. You do not need to assess the quality of every sample in the experiment.

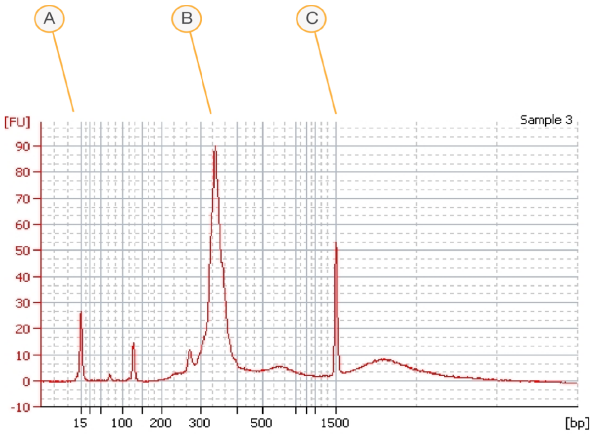
To enable Illumina Technical Support to troubleshoot if you need assistance, assess the quality of the control reaction generated with the ACD1 and ACP1. Process the control reaction using the same conditions as BVP1.

Figure 4 Agarose Gel Example



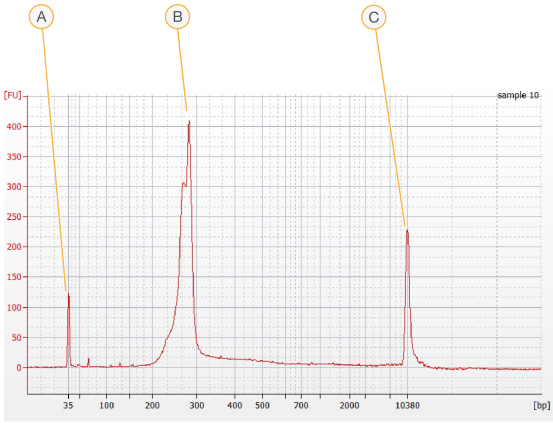
A Expected ACP1/ACD1 PCR product (~350 bp)
B Primers

Figure 5 Bioanalyzer Example (ACP1)



A Marker
B Expected ACP1/ACD1 PCR product for 250bp amplicons (~350bp)
C Marker

Figure 6 Bioanalyzer Example (BVP1)



A Marker
B Expected BVP1/gDNA PCR product (~250 bp)
C Marker

3 Add 60 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 Apply the seal and 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 Apply the seal and 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 Apply the seal and centrifuge at 1000 × g for 1 minute.

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. 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 Transfer 800 µl LNB1 to the 15 ml conical tube of LNA1. Invert to mix.

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

5 Apply the seal and 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 2 times as follows.

   a Add 45 µl LNW1 to each library well.
   b Apply the seal and 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 Apply the seal and shake at 1800 rpm for 5 minutes.

14 If the libraries are not resuspended, pipette to mix, and then 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 each well of the SGP plate.

17 Transfer 30 µl supernatant from each well of the LNP plate to the corresponding well of the SGP plate. Pipette to mix.

18 Apply the seal and 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 denature the library pool before loading libraries for the sequencing run.

Consumables

- PAL (Pooled Amplicon Library) barcode label
- LoBind microcentrifuge tube
- RNase/DNase-free 8-tube strips and caps

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. Centrifuge at 1000 × g for 1 minute.
2. Transfer 5 µl of each library to an 8-tube strip, column by column. 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
BVP1	Bovine Parentage 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

Kit Contents

Make sure that you have all the reagents identified in this section before proceeding to the library preparation procedures. The TruSeq Custom Amplicon Index Kit is included in the TruSeq Bovine Parentage Kit.

TruSeq Bovine Parentage Kit Contents (20004795)

Box 1—Store in the Pre-PCR Area

This box also contains the HYP, FPU, and IAP barcode labels.

Quantity	Reagent	Description	Storage Temperature
1	ACD1	Amplicon Control DNA 1	-25°C to -15°C
1	ACP1	Amplicon Control Oligo Pool 1	-25°C to -15°C
1	OHS2	Oligo Hybridization for Sequencing Reagent 2	-25°C to -15°C
1	ELM4	Extension Ligation Mix 4	-25°C to -15°C
1	PMM2	PCR Master Mix 2	-25°C to -15°C
1	TDP1	TruSeq DNA Polymerase 1	-25°C to -15°C
1	SW1	Stringent Wash 1	2°C to 8°C
1	UB1	Universal Buffer 1	2°C to 8°C

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—Store in the Pre-PCR Area

This box is shipped at room temperature. As soon as you receive your kit, remove LNB1 from box 2 and store at 2°C to 8°C in the post-amplification area. ***Keep the filter plate in the pre-PCR area at room temperature.***

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

Box 3—Store in the Post-PCR Area

This box also contains the CLP, LNP, SGP, PAL, DAL barcode labels.

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	Room temperature
1	EBT	Elution Buffer with Tris	Room temperature

Box 4—Store in the Pre-PCR Area

Quantity	Reagent	Description	Storage Temperature
1	BVP1	Bovine Parentage Oligos	-25°C to -15°C

TruSeq Custom Amplicon Index Kit Contents

Box 1—Store in Pre-PCR Area

Quantity	Reagent Name	Storage Temperature
8 tubes	i5 Index Primers, A501 to A508	-25°C to -15°C
12 tubes	i7 Index Primers, A701 to A712	-25°C to -15°C

Box 2—Store in Pre-PCR Area

Quantity	Reagent Name	Storage Temperature
1 box	i5 Index Tube Caps, White	Room temperature
1 box	i7 Index Tube Caps, Orange	Room temperature

Additional Components

Consumable	Catalog #	Storage Temperature	Area
TruSeq Index Plate Fixture Kit (Recommended)	FC-130-1005	Room temperature	Pre-PCR
TruSeq Custom Amplicon Filter Plate (Required)	FC-130-1006	Room temperature	Pre-PCR
TruSeq Index Plate Fixture and Collar Kit (Recommended)	FC-130-1007	Room temperature	Pre-PCR

Consumables and Equipment

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

NOTE

- Use a dedicated set of consumables and equipment for pre-PCR and post-PCR procedures.
- The TruSeq Bovine Parentage library prep protocol requires different magnetic stands for pre-PCR and post-PCR procedures.

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, nonsterile (trough)	Labcor, Part# 730-001
One of the following (for library quality assessment): • Standard Sensitivity NGS Fragment Analysis Kit (1–6000 bp) • DNA 1000 Kit • 4% Agarose gel	 • Advanced Analytical Technologies, part # DNF-473 • Agilent Technologies, catalog # 5067–1504 • General lab supplier
DNA molecular weight markers	General lab supplier
Ice bucket	General lab supplier

Pre-PCR Equipment

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

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
Heat block for 1.5 ml centrifuge tubes	General lab supplier
One of the following (for library quality assessment): • Gel electrophoresis supplies and apparatus • Bioanalyzer System • Fragment Analyzer System	 • General lab supplier • Agilent Technologie • Advanced Analytical Technologies

Thermal Cyclers

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

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

Index Sequences

The TruSeq Custom Amplicon Index Kit contains the following indexed adapter sequences.

i7 Index Adapter	Sequence
A701	ATCACGAC
A702	ACAGTGGT
A703	CAGATCCA
A704	ACAAACGG
A705	ACCCAGCA
A706	AACCCCTC
A707	CCCAACCT
A708	CACCACAC
A709	GAAACCCA
A710	TGTGACCA
A711	AGGGTCAA
A712	AGGAGTGG

i5 Index Adapter	Sequence
A501	TGAACCTT
A502	TGCTAAGT
A503	TGTTCTCT
A504	TAAGACAC
A505	CTAATCGA
A506	CTAGAACA
A507	TAAGTTCC
A508	TAGACCTA

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

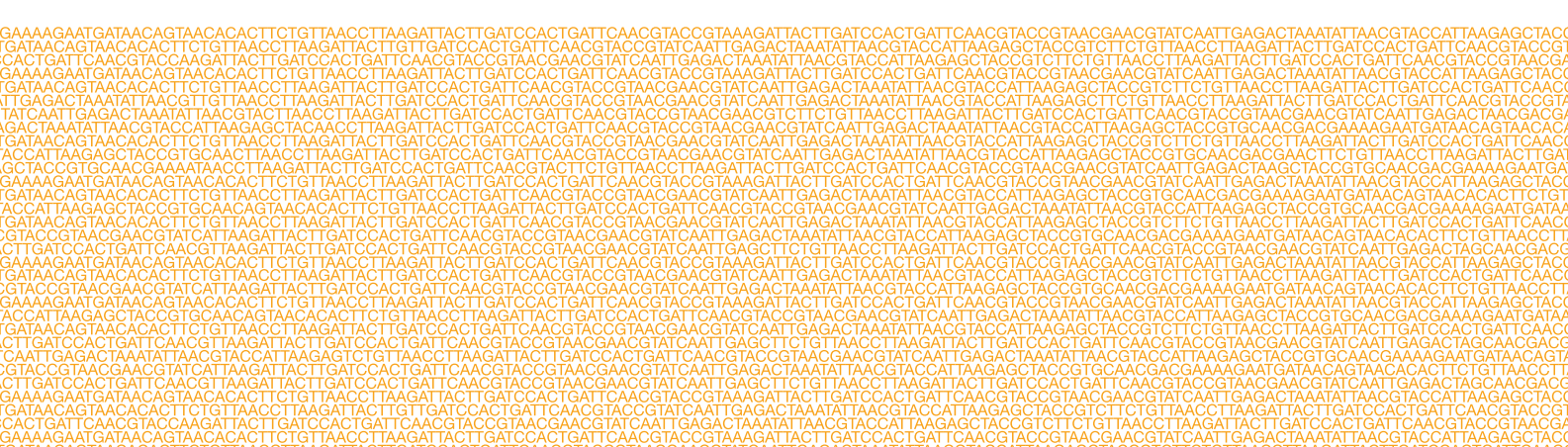
Website	www.illumina.com
Email	techsupport@illumina.com

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.

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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