# ForenSeq™ DNA Signature Prep

# Reference Guide

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# Revision History

| Document | Date | Description of Change |
|---|---|---|
| Material # 20000923<br>Document # 15049528 v01 | September 2015 | Updated introduction to indicate that DPMA contains primer pairs for 58 STRs and 94 identity-informative SNPs<br>Updated this document to current format for library prep documentation. Revised instructions to be more succinct<br>Changed reference from PCR Product to library following amplification<br>Removed reference to obsolete Experienced User Card and added reference to new protocol guide and checklist<br>Removed kit box and tube part numbers<br>Removed pipettes from *Consumables* as they are standard lab items<br>Removed thermal cycler from pre-PCR *Equipment*<br>Corrected 2800M Control Alleles in the *Loci* tables for the following.<br>• rs279844, DYS612, Y-GATA-H4, DXS10103<br>• rs1805007, rs1294331, rs1413212, rs993934<br>• rs1355366, rs2399332, rs1979255, rs2046361<br>• rs251934, rs338882, rs1336071, rs214955<br>• rs727811, rs763869, rs1463729, rs3780962<br>• rs735155, rs2111980, rs2920816, rs1335873<br>• rs1886510, rs354439, rs722290, rs1821380<br>• rs1382387, rs729172, rs1024116, rs1736442<br>• rs719366, rs1800407, rs2814778, rs3737576<br>• rs1876482, rs3827760, rs1229984, rs3811801<br>• rs870347, rs1871534, rs2196051, rs3814134<br>• rs1079597, rs1572018, rs1800414, rs2593595<br>• rs4411548, rs2042762, rs3916235, rs310644<br>Removed loci rs7520386 from Identity Informative SNPs *Loci* table<br>Corrected amplicon lengths for DXS8378 in the X Haplotype Markers *Loci* table<br>Changed *Loci* table headers from Target Start to Amplicon Start Position and Target End to Amplicon End Position and defined positions<br>Incorporated alternate procedures to prepare FTA Card into *Amplify and Tag Targets* procedures<br>Added 2800M as a positive template control to prepare FTA Card |
| Part # 15049528 Rev. D | February 2015 | • Updated introduction to indicate that DNA Primer Mix A supports 7 X haplotype markers<br>• Removed 1000 µl pipettes and tips from *Consumables and Equipment*<br>• In the *Loci* tables:<br>    • Moved SNPs rs16891982 and rs12913832 from phenotypic-informative SNP to ancestry-informative SNP and indicated that they are used for both predictions.<br>    • Corrected the vWA minimum and maximum amplicon length |

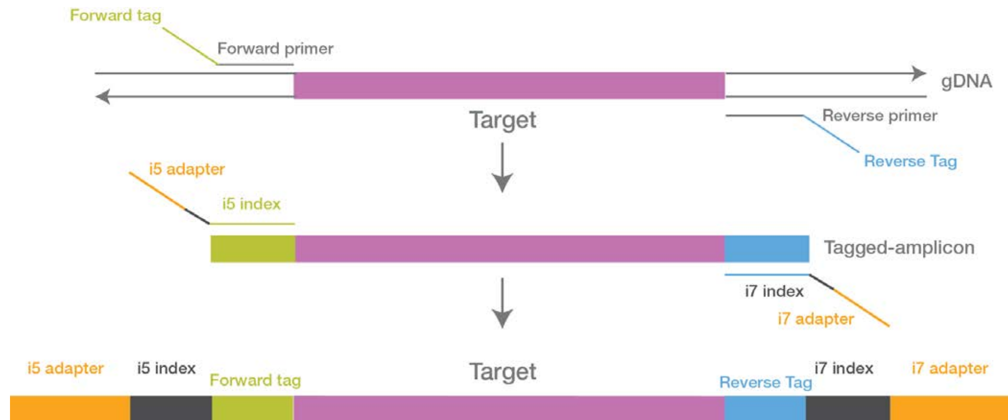
| Document | Date | Description of Change |
|---|---|---|
| Part # 15049528 Rev. C | January 2015 | • Updated *Loci* tables:<br>  • Revised autosomal STR, Y haplotype marker, and X haplotype marker amplicon lengths<br>  • Removed X haplotype marker DXS10148<br>  • Changed right column heading of identity, phenotypic, and ancestry-informative SNPs to 2800M Control Alleles<br>• Added number of reactions supported to *Kit Contents*<br>• Changed catalog numbers for kit, guide, and experienced user card<br>• Changed MiSeq FGx Reagent Kit name and catalog number |
| Part # 15049528 Rev. B | September 2014 | • Modified the reagent volumes in the *Amplify and Tag Targets* and *Prepare FTA Card* master mix tables to actual reagent volumes without overage<br>• Corrected locus D5S818 2800M Control alleles to 12,12<br>• Updated *Additional Resources* to remove updated support page url and remove web navigation instructions and written urls<br>• Separate seal and shake as separate substeps<br>• Updated SDS link to support.illumina.com/sds.html |
| Part # 15049528 Rev. A | August 2014 | Initial release. |

# Introduction

This protocol explains how to prepare DNA libraries using the reagents provided in the Illumina® ForenSeq™ DNA Signature Prep Kit to genotype database or casework reference samples in a single sequencing run.

A primer mix containing a pair of tagged oligos for each target sequence is mixed with the DNA sample. PCR cycles link the tags to copies of each target to form DNA templates consisting of the regions of interest flanked by universal primer sequences. The tags are used to attach indexed adapters, which are then amplified using PCR, purified, pooled into a single tube, and then sequenced. The index sequences allow the sequencing system to separate and isolate the data generated from each sample.

Figure 1 ForenSeq DNA Signature Prep Overview




Targeted primer mixes enable analysis of autosomal, Y- and X-chromosome Short Tandem Repeat (STR) targets, identity-informative SNPs, with the option to include ancestry-informative and phenotypic-informative SNPs depending on which primer mix is used. The ForenSeq DNA Signature Prep enables analysis of these markers on gDNA ranging from high-quality single source to difficult samples. This process is done within a single reaction with integrated indexing to support sequencing of up to 96 database or 32 casework samples per run. ForenSeq DNA Signature Prep applies the long paired-end read capability and high data quality of your Illumina sequencing system.

The ForenSeq DNA Signature Prep Kit offers:

- Multiplexing—Amplify STR and SNP amplicons in a single reaction, and sequence up to 96 samples in a single sequencing run.
- Two different primer mixes:
  - DNA Primer Mix A—Contains primer pairs for 58 STRs (including 27 autosomal STRs and 7 X and 24 Y haplotype markers) and 94 identity-informative SNPs.
  - DNA Primer Mix B—Contains all markers in DNA Primer Mix A, plus primer pairs for 56 ancestry-informative SNPs and 22 phenotypic-informative SNPs (2 ancestry-informative SNPs are also used for phenotype prediction).
- Library generation—Allows for simultaneous preparation of 96 samples to generate libraries of PCR products within a single plate. Each library is a collection of amplified DNA fragments from a single sample.

# DNA Input Recommendations

It is important to quantify the input DNA and assess the DNA quality before beginning the ForenSeq DNA Signature Prep protocol. Follow these DNA input recommendations:

- 1 ng of human genomic DNA (gDNA) input is recommended.
- Use a fluorometric based method for quantification, such as qPCR.
- The ForenSeq DNA Signature Prep Kit is compatible with lysates from buccal swabs and FTA card stains as DNA input.
    - If using crude lysates, 2 µl input material is required per sample. See *Equipment* on page 26 for recommended lysis buffers.
    - If using FTA paper, a 1.2 mm FTA card punch per sample is required.

# Additional Resources

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

| Resource | Description |
| --- | --- |
| *ForenSeq DNA Signature Prep Protocol Guide (document # 1000000001629)* | Provides only protocol instructions. The protocol guide is intended for experienced users. |
| *ForenSeq DNA Signature Prep Checklist (document # 1000000001630)* | Provides a checklist of the protocol steps. The checklist is intended for experienced users. |
| *MiSeq FGx Instrument Reference Guide (document # 15050524)* | Provides an overview of MiSeq FGx™ instrument components and software, instructions for performing sequencing runs, and procedures for proper instrument maintenance and troubleshooting. |
| *ForenSeq Universal Analysis Software User Guide (document # 15053876)* | Provides an overview of ForenSeq Universal Analysis Software (UAS), which performs secondary analysis such as demultiplexing, alignment, variant calling, and report generation. |

Visit the ForenSeq DNA Signature Prep Kit support page on the Illumina website for access to requirements and compatibility, additional documentation, frequently asked questions, and best practices.

- Review best practices before proceeding. See *Additional Resources* on page 7 for information about ForenSeq DNA Signature Prep Kit best practices on the Illumina website.
- Processing fewer than 8 samples at the same time, including positive and negative controls, can cause problems with pipetting accuracy, due to the small volumes used when preparing the master mix.
- Create a sample sheet to record the positions of each sample and index adapter. For more information, see the *ForenSeq Universal Analysis Software User Guide (document # 15053876)*.

NOTE
For indexing guidelines when pooling 16 or fewer libraries, see *Additional Resources* on page 7 for information about ForenSeq DNA Signature Prep Kit best practices on the Illumina website.

- Follow the protocol in the order shown using the specified volumes and incubation parameters.
- Confirm kit contents and make sure that you have the required equipment and consumables. For more information, see *Supporting Information* on page 24.

# 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.
- 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).

## Centrifugation

- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

# Library Prep Workflow

Figure 2 ForenSeq DNA Signature Prep Workflow

# Amplify and Tag Targets

This process amplifies and tags the gDNA using a ForenSeq oligonucleotide primer mix with regions specific to DNA sequences upstream and downstream of STRs and SNPs.

This protocol requires Control DNA 2800M and a negative PCR amplification control (nuclease-free water) in each experiment. If these controls are not included, troubleshooting support is limited.

NOTE
Processing fewer than 8 samples at the same time, including positive and negative controls, can affect pipetting accuracy due to the small volumes used when preparing the master mix.

## Consumables

- 2800M (Control DNA 2800M)
- One of the following:
  - DPMA (DNA Primer Mix A)
  - DPMB (DNA Primer Mix B)
- FEM (Enzyme Mix)
- PCR1 (PCR1 Reaction Mix)
- 1.5 ml microcentrifuge tubes (2)
- 96-well 0.3 ml PCR plate, skirted or semiskirted
- Human gDNA:
  - Purified DNA (1 ng per sample)
  - Crude lysate (2 µl per sample)
  - FTA card (1.2 mm punch per sample)
- [For FTA card] 1X TBE buffer (100 µl per FTA card punch)
- Microseal 'A' film
- Microseal 'B' adhesive seal
- Nuclease-free water
- [Optional] RNase/DNase-free 8-tube strip and caps

NOTE
Use Microseal 'A' when sealing the plate before placing on the thermal cycler. Use Microseal 'B' for other steps that require a sealed plate.

## About Reagents

- For information on the loci detected with DPMA and DPMB, see *Loci* on page 27.

# Preparation

1 Prepare the following consumables.

| Item | Storage | Instructions |
|---|---|---|
| DPMA or DPMB | -25°C to -15°C | Thaw at room temperature. |
| FEM | -25°C to -15°C | Thaw at room temperature. Return to storage after use. |
| PCR1 | -25°C to -15°C | Thaw at room temperature. |
| 1X TBE buffer | -25°C to -15°C | Thaw at room temperature. |
| 2800M | 2°C to 8°C | Let stand for 30 minutes to bring to room temperature. |

2 Create a sample sheet to record the positions of each sample and index adapter.

3 Save the following PCR1 program on the thermal cycler in the post-amplification area.

CAUTION
Failure to use the thermal ramping mode for your thermal cycler can have an adverse effect on results. See ramping modes for selected *Thermal Cyclers* on page 26.

- Choose the preheat lid option and set to 100°C
- 98°C for 3 minutes
- 8 cycles of:
  - 96°C for 45 seconds
  - 80°C for 30 seconds
  - 54°C for 2 minutes, with specified ramping mode
  - 68°C for 2 minutes, with specified ramping mode
- 10 cycles of:
  - 96°C for 30 seconds
  - 68°C for 3 minutes, with specified ramping mode
- 68°C for 10 minutes
- Hold at 10°C

NOTE
The PCR1 program takes approximately 3.5 hours and can be run overnight.

4 Label tube and plates with a marker as follows.
- [For Purified DNA or Crude lysate] Master Mix - 1.5 ml microcentrifuge tube
- [For FTA card] FTA Master Mix - 1.5 ml microcentrifuge tube
- FSP - PCR plate

## Procedure

1 Quantify gDNA using a fluorometric-based method.

2 **For purified DNA or crude lysate**—Perform the following steps:

a Dilute input material as follows.
  - [Purified DNA] Dilute 1 ng to 0.2 ng/µl with nuclease-free water.
  - [Crude lysate] Dilute 2 µl with 3 µl nuclease-free water.

b Create a master mix for 8 or more reactions in the Master Mix tube. Multiply each reagent volume by the number of reactions being prepared. Make 10% extra reagent for overage.
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMA or DPMB (5.0 µl)

c Pipette to mix and then centrifuge briefly.

d If processing more than 8 samples, evenly distribute the master mix into each well of an 8-tube strip, and then use a multichannel pipette to dispense.

e Add 10 µl master mix to each well of the FSP plate.

f Dilute 2 µl 2800M with 98 µl nuclease-free water in a new 1.5 ml microcentrifuge tube. Gently flick the tube and then centrifuge briefly.

g Add 5 µl diluted 2800M as a positive template control to the appropriate well according to the sample sheet.

h Add 5 µl nuclease-free water as a negative PCR amplification control to the appropriate well according to the sample sheet.

i Add samples to the wells according to the sample sheet. Pipette to mix.
  - [Purified DNA] Add 5 µl diluted DNA (0.2 ng/µl) sample to each well.
  - [Crude lysate] Add 5 µl diluted crude lysate sample to each well.

3 **For FTA card input material**—Perform the following steps:

a Place a 1.2 mm FTA card punch into each well of the FSP plate according to the sample sheet.

b Add 100 µl 1X TBE buffer.

c Place on a PCR tube storage rack.

d Shake at 1800 rpm for 2 minutes.

e Centrifuge at 1000 × g for 30 seconds.

f Remove and discard all supernatant.

g Add the following to the FTA Master Mix tube.
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMA or DMPB (5.0 µl)
  - Nuclease-free water (5.0 µl)

h Add 15 µl FTA master mix to an FTA punch.

i Add 15 µl FTA master mix to an empty well as a no template control.

j Dilute 2 µl 2800M with 98 µl nuclease-free water in a new 1.5 ml microcentrifuge tube. Gently flick the tube and then centrifuge briefly.

k Add 5 µl diluted 2800M as a positive template control to the appropriate well according to the sample sheet.

4 Centrifuge at 1000 × g for 30 seconds.

5 Transport to the post-PCR area.

6 Place on the thermal cycler and run the PCR1 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.

# Enrich Targets

This process amplifies the DNA and adds Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for cluster amplification.

The index adapters tag DNA with a unique combination of index sequences, which allow data from each tagged library to be separated during later analysis.

NOTE
This procedure is described using a 96-well PCR plate. However, when processing 8 libraries, it can be performed with an 8-tube strip.

## Consumables

- ForenSeq Index Plate Fixture Kit
- Index 1 (i7) adapters and orange tube caps
- Index 2 (i5) adapters and white tube caps
- PCR2 (PCR2 Reaction Mix)
- 1.7 ml microcentrifuge tubes (1 per index adapter tube)
- Microseal 'A' film
- Microseal 'B' adhesive seal

NOTE
Use Microseal 'A' when sealing the plate before placing on the thermal cycler. Use Microseal 'B' for other steps that require a sealed plate.

## About Reagents

- If processing more than 8 libraries at the same time, evenly distribute PCR2 to each well of an 8-tube strip, and then use a multichannel pipette to dispense.
- Add PCR2 slowly to each well to avoid creating air bubbles.

# Preparation

1 Prepare the following consumables.

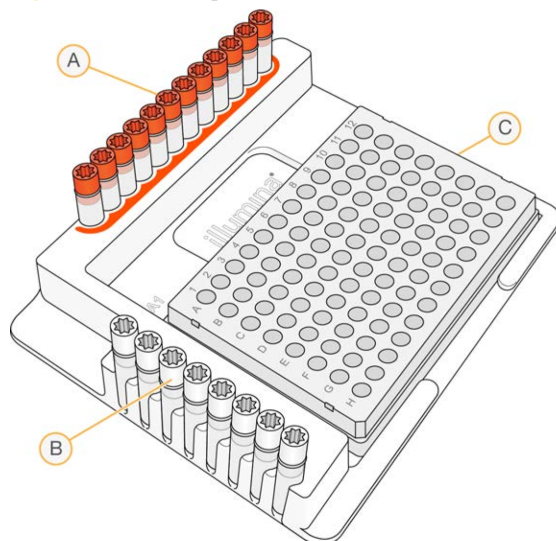
| Item | Storage | Instructions |
|---|---|---|
| Index adapters (i5 and i7) | -25°C to -15°C | Only remove adapters being used. Thaw at room temperature for 20 minutes.<br>Vortex each tube to mix. Centrifuge briefly using a 1.7 ml Eppendorf tube. |
| PCR2 | -25°C to -15°C | Thaw at room temperature. |

2 Save the following PCR2 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 98°C for 30 seconds
- 15 cycles of:
  - 98°C for 20 seconds
  - 66°C for 30 seconds
  - 68°C for 90 seconds
- 68°C for 10 minutes
- Hold at 10°C

## Procedure

1. Centrifuge at 1000 × g for 30 seconds.
2. Arrange Index 1 (i7) adapters in columns 1–12 of the ForenSeq Index Plate Fixture.
3. Arrange Index 2 (i5) adapters in rows A–H of the ForenSeq Index Plate Fixture.
4. Place the plate on the ForenSeq Index Plate Fixture.

Figure 3 ForenSeq Index Plate Fixture (96 libraries)




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

5. Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter to each column. Replace the cap on each i7 adapter tube with a new orange cap.
6. Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter to each row. Replace the cap on each i5 adapter tube with a new white cap.
7. Vortex PCR2 and then centrifuge briefly.
8. Add 27 µl PCR2 to each well.
9. Centrifuge at 1000 × g for 30 seconds.
10. Place on the preprogrammed thermal cycler and run the PCR2 program.

### SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

# Purify Libraries

This process uses SPB (Sample Purification Beads) to purify the amplified libraries from the other reaction components.

### Consumables

- RSB (Resuspension Buffer)
- SPB (Sample Purification Beads)
- 96-well 0.3 ml PCR plate, skirted or semiskirted
- 96-well midi plates (1 + 1 if processing 16–96 libraries)
- Freshly prepared 80% ethanol (EtOH)
- Microseal 'B' adhesive seals
- RNase/DNase-free reagent reservoirs (1 + 1 if processing more than 96 libraries)

### About Reagents

- Vortex SPB before each use.
- Vortex SPB frequently to make sure that beads are evenly distributed.
- Aspirate and dispense SPB slowly due to the viscosity of the solution.

## Preparation

1 Prepare the following consumables.

| Item | Storage | Instructions |
|---|---|---|
| RSB | 2°C to 8°C | Let stand for 30 minutes to bring to room temperature. |
| SPB | 2°C to 8°C | Let stand for 30 minutes to bring to room temperature. |

2 Label plates with a marker as follows.
- PBP - midi plate
- PLP - PCR plate

## Procedure

1 Do the following depending on the number of libraries your are preparing:

| Number of Libraries | Procedure |
|---|---|
| < 16 | Add 50 µl SPB × the number of libraries to a 1.7 ml microcentrifuge tube. |
| 16–96 | Add [50 µl SPB × (the number of libraries/8)] + 5 µl SPB to each well of a column of a new midi plate. |
| > 96 | Add (50 µl SPB × the number of libraries) + 200 µl SPB to a multichannel reagent reservoir. |

2 Add 45 µl SPB to each well of the PBP plate according to the sample sheet.

3 Centrifuge the FSP plate at 1000 × g for 30 seconds.

4 Transfer 45 µl to the corresponding well of the PBP plate, according to the sample sheet.

5 Shake at 1800 rpm for 2 minutes.

6 Incubate at room temperature for 5 minutes.

7 Place on the 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 freshly prepared 80% EtOH to each well.
   b Incubate on the magnetic stand for 30 seconds.
   c Remove and discard all supernatant from each well.
10 Centrifuge at 1000 × g for 30 seconds.
11 Place on the magnetic stand.
12 Use a 20 µl pipette to remove residual EtOH from each well.
13 Add 52.5 µl RSB to each well.
14 Shake at 1800 rpm for 2 minutes. If the beads are not resuspended, pipette to mix or repeat shake at 1800 rpm for 2 minutes.
15 Incubate at room temperature for 2 minutes.
16 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
17 Transfer 50 µl to the corresponding well of the PLP plate.
18 Centrifuge at 1000 × g for 30 seconds.

### SAFE STOPPING POINT

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

# Normalize Libraries

This process prepares DNA libraries for cluster generation to make sure that libraries of varying yields are equally represented within the sequencing run. This process assures that samples with varying input amounts or sample types achieve consistent cluster density to optimize the resolution of individual samples when pooled together. By normalizing the concentration of the libraries, while preserving the content of each library, post-PCR quantification and individual PCR product normalization are not necessary.

## Consumables

- HP3 (2N-NaOH)
- LNA1 (Library Normalization Additives 1)
- LNB1 (Library Normalization Beads 1)
- LNS2 (Library Normalization Storage Buffer 2)
- LNW1 (Library Normalization Wash 1)
- 1.5 ml microcentrifuge tube
- One of the following:
  - 1.5 ml microcentrifuge tube
  - 15 ml conical tube
- 96-well 0.3 ml PCR plate, skirted or semiskirted
- 96-well midi plate
- Microseal 'B' adhesive seals
- Nuclease-free water
- RNase/DNase-free Reagent Reservoir

WARNING

This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin.

LNA1 and LNW1 contain ß-mercaptoethanol and prolonged exposure can be toxic to the nervous system and cause organ damage.

Perform this procedure in a hood or well-ventilated area if desired. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see how to access safety data sheets (SDSs) in *Technical Assistance* on page 35.

Supernatant, excess LNA1/LNB1 Master Mix, and tips used to pipette LNA1 and LNB1 are hazardous waste. Discard in accordance with the governmental safety standards for your region.

## About Reagents

- After vortexing, hold LNA1 in front of a light and make sure that no crystals are present and all precipitate has dissolved.
- After vortexing, make sure that LNB1 beads are well-resuspended and no pellet remains at the bottom of the tube.
- It is critical to resuspend the LNB1 bead pellet at the bottom of the tube. Use a 1000 µl pipette to make sure that the beads are homogeneously resuspended and that there is no bead mass at the bottom of the tube. Resuspension is essential for achieving consistent cluster density to optimize the resolution of individual libraries when pooled together.
- The library that remains in the PLP plate can be stored. Seal the PLP plate and store at -25°C to -15°C for up to 1 year.

## Preparation

1. Prepare the following consumables.

| Item | Storage | Instructions |
|---|---|---|
| HP3 | -25°C to -15°C | Thaw at room temperature. |
| LNA1 | -25°C to -15°C | Thaw at room temperature. Vortex with intermittent inversion. |
| LNB1 | 2°C to 8°C | Let stand for 30 minutes to bring to room temperature. Vortex for at least 1 minute, inverting 5 times every 15 seconds. Pipette to mix until the bead pellet at the bottom is resuspended. |
| LNW1 | 2°C to 8°C | Let stand for 30 minutes to bring to room temperature. |
| LNS2 | 15°C to 30°C | Remove from storage. |

2. Label tubes and plates with a marker as follows.
   - LNA1/LNB1 Master Mix - 1.5 ml microcentrifuge tube or 15 ml conical tube
   - NWP - midi plate
   - NLP - PCR plate
3. Dedicate separate hazardous waste disposal containers for liquids and solids.

## Procedure

1. Create a master mix in the LNA1/LNB1 Master Mix tube.
   - LNA1 (46.8 µl) (Example: 8 reactions 374 µl)
   - LNB1 (8.5 µl) (Example: 8 reactions 68 µl)
2. Vortex and then invert the tube several times to mix.
3. Pour into a reagent reservoir.
4. Transfer 45 µl to each well of the NWP plate that will contain a library according to the sample sheet.
5. Place the PLP plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
6. Transfer 20 µl from each well of the PLP plate to the corresponding well of the NWP plate.
7. Shake at 1800 rpm for 30 minutes.
8. Prepare 0.1 N HP3 in a new 1.5 ml microcentrifuge tube.
   - Nuclease-free water (33.3 µl) (Example: 8 reactions 266 µl)
   - HP3 (1.8 µl) (Example: 8 reactions 14 µl)
9. Invert the tube several times to mix.
10. Add 30 µl LNS2 to each well of the NLP plate that will contain a library according to the sample sheet
11. Place the NWP plate on the magnetic stand and wait until the liquid is clear (~2 minutes).
12. Remove and discard all supernatant from each well.
13. Remove from the magnetic stand.

14 Add 45 µl LNW1 to each well.
15 Shake at 1800 rpm for 5 minutes.
16 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
17 Remove and discard all supernatant from each well.
18 Repeat steps 14–17 for a total of 2 washes.
19 Remove from the magnetic stand.
20 Centrifuge at 1000 × g for 30 seconds.
21 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
22 Use a 20 µl pipette to remove residual supernatant from each well.
23 Remove from the magnetic stand.
24 Add 32 µl freshly prepared 0.1 N HP3 to each well.
25 Shake at 1800 rpm for 5 minutes. If the beads are not resuspended, pipette to mix or repeat shake at 1800 rpm for 5 minutes.
26 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
27 Transfer 30 µl to the corresponding well of the NLP plate. Pipette to mix.
28 Centrifuge at 1000 × g for 30 seconds.

### 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 process combines equal volumes of normalized library to create a pool of libraries that are sequenced together on the same flow cell.

### Consumables

- 1.5 ml microcentrifuge tube
- Microseal 'B' adhesive seal
- RNase/DNase-free 8-tube strip and caps

## Preparation

1. Determine which libraries to pool for sequencing.
   - For casework samples, do not pool more than 32 libraries, including the positive and negative controls.
   - For database samples, do not pool more that 96 libraries.
2. Label the tube PNL with a marker.

## Procedure

1. Transfer 5 µl of each library to a new 8-tube strip.
2. Store the plate in the post-PCR area at -25°C to -15°C for up to 30 days.
3. Transfer the contents of each well of the 8-tube strip to the PNL tube.
4. Vortex and then centrifuge briefly.

### SAFE STOPPING POINT

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

# Denature and Dilute Libraries

This process dilutes the libraries in HT1 (Hybridization Buffer), adds HSC (Human Sequencing Control), and heat denatures the libraries in preparation for sequencing.

NOTE
Perform this process immediately before loading the library onto the reagent cartridge to ensure efficient template loading on the flow cell.

### Consumables

- HP3 (2N-NaOH)
- HSC (Human Sequencing Control)
- 1.5 ml microcentrifuge tubes (2)
- MiSeq FGx Reagent Kit contents:
  - HT1 (Hybridization Buffer)
  - Reagent cartridge
- Nuclease-free water

### About Reagents

- Follow *Prepare the Reagent Cartridge* instructions in the *MiSeq FGx Instrument Reference Guide (document # 15050524).*

## Preparation

1. Prepare the following consumables.

| Item | Storage | Instructions |
|---|---|---|
| HP3 | -25°C to -15°C | Thaw at room temperature. |
| HSC | -25°C to -15°C | Thaw at room temperature. |
| HT1 | -25°C to -15°C | Thaw at room temperature. |
| Reagent cartridge | -25°C to -15°C | Thaw at room temperature. |

2. Preheat the microheating system to 96°C.
3. Prepare either of the following:
   - Remove a tube benchtop cooler from -25°C to -15°C storage or ice bucket.
   - Prepare an ice-water bath by combining 3 parts ice and 1 part nuclease-free water.
4. Label tubes with a marker as follows.
   - HSC mixture
   - DNL

## Procedure

1. Create an HSC denaturation reaction in the HSC mixture tube.
   - HSC (2 µl)
   - HP3 (2 µl)
   - Nuclease-free water (36 µl)
2. Vortex and then centrifuge briefly.
3. Incubate at room temperature for 5 minutes.
4. Add 591 µl HT1 to the DNL tube.

5. Transfer 7 µl from the PNL tube to the DNL tube. Pipette to mix.
6. Cap the PNL tube and store at -25°C to -15°C for up to 30 days. Exceeding 30 days in storage results in a significant reduction of cluster density.
7. Transfer 2 µl HSC mixture to the DNL tube. Pipette to mix. Do not store HSC mixture long term, which results in a significant reduction of cluster density.
8. Vortex and then centrifuge briefly.
9. Place on the 96°C microheating system for 2 minutes.
10. Invert the tube several times to mix.
11. Immediately place in the ice-water bath or on the -25°C to -15°C benchtop cooler for 5 minutes.
12. Immediately load the entire contents onto the reagent cartridge. For more information, see the *Load Sample Libraries Onto Cartridge* instructions in the *MiSeq FGx Instrument Reference Guide (document # 15050524)*.

# Supporting Information

The protocols provided in this guide assume that you are familiar with the contents of this section and that you have the required equipment and consumables.

## Kit Contents

Make sure that you have all the ForenSeq DNA Signature Prep Kit (catalog # TG-450-1001) reagents identified in this section before starting the protocol. Each kit supports 384 reactions and contains 3 boxes.

### Pre-PCR Box 1, Store as specified

| Quantity | Reagent | Description | Storage Temperature |
|---|---|---|---|
| 2 | 2800M | Control DNA 2800M | 2°C to 8°C |
| 8 | PCR1 | PCR1 Reaction Mix | -25°C to -15°C |
| 8 | FEM | Enzyme Mix | -25°C to -15°C |
| 8 | DPMA | DNA Primer Mix A | -25°C to -15°C |
| 8 | DPMB | DNA Primer Mix B | -25°C to -15°C |

### Post-PCR Box 2, Store at -25°C to -15°C

| Quantity | Reagent | Description |
|---|---|---|
| 4 | LNA1 | Library Normalization Additives 1 |
| 4 | LNS2 | Library Normalization Storage Buffer 2 |
| 8 | LNW1 | Library Normalization Wash 1 |
| 3 | HP3 | HP3 2N-NaOH |
| 8 | PCR2 | PCR2 Reaction Mix |
| 1 | HSC | Human Seq Control |
| 1 | A501 | A501 Index Adapter |
| 1 | A502 | A502 Index Adapter |
| 1 | A503 | A503 Index Adapter |
| 1 | A504 | A504 Index Adapter |
| 1 | A505 | A505 Index Adapter |
| 1 | A506 | A506 Index Adapter |
| 1 | A507 | A507 Index Adapter |
| 1 | A508 | A508 Index Adapter |
| 1 | R701 | R701 Index Adapter |
| 1 | R702 | R702 Index Adapter |
| 1 | R703 | R703 Index Adapter |
| 2 | R704 | R704 Index Adapter |
| 1 | R705 | R705 Index Adapter |
| 1 | R706 | R706 Index Adapter |
| 1 | R707 | R707 Index Adapter |
| 1 | R708 | R708 Index Adapter |
| 1 | R709 | R709 Index Adapter |
| 1 | R710 | R710 Index Adapter |
| 1 | R711 | R711 Index Adapter |
| 1 | R712 | R712 Index Adapter |
| 1 | — | i7 Index Tube Caps, Orange |
| 1 | — | i5 Index Tube Caps, White |

## Post-PCR Box 3, Store at 2°C to 8°C

| Quantity | Reagent | Description |
|---|---|---|
| 4 | LNB1 | Library Normalization Beads 1 |
| 1 | RSB | Resuspension Buffer |
| 2 | SPB | Sample Purification Beads |

# 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.

## Consumables

| Consumable | Supplier |
|---|---|
| 1.5 ml microcentrifuge tubes | General lab supplier |
| 1.7 ml microcentrifuge tubes | General lab supplier |
| 15 ml conical tube | General lab supplier |
| 20 µl barrier pipette tips | General lab supplier |
| 200 µl barrier pipette tips | General lab supplier |
| 96-well 0.3 ml semiskirted PCR plates | Eppendorf Twin-Tec, part # 951020303 or VWR, part # 89136-706 |
| 96-well storage plates, round well, 0.8 ml ('midi' plate) | Fisher Scientific, part # AB-0859 |
| Ethanol 200 proof (absolute) for molecular biology (500 ml) | Sigma-Aldrich, part # E7023 |
| Microseal 'A' film | Bio-Rad, part # MSA-5001 |
| Microseal 'B' adhesive seals | Bio-Rad, part # MSB-1001 |
| MiSeq FGx Reagent Kit | Illumina, catalog # TG-143-1001 |
| Nuclease-free water | General lab supplier |
| PCR tube storage rack (If using an FTA card as input material) | VWR, part # 80086-074 |
| One of the following, if using crude lysates as input material:<br>• QuickExtract DNA Extraction Solution<br>• SwabSolution Kit | <br><br>• Epicentre, catalog # QE09050<br>• Promega, catalog # DC8271 |
| RNase/DNase-free 8-tube strips and caps | General lab supplier |
| RNase/DNase-free multichannel reagent reservoirs, disposable | Labcor, part # 730-001 |

## Equipment

| Equipment | Supplier/Description | Pre-PCR | Post-PCR |
|---|---|---|---|
| 1.5 ml tube benchtop cooler | VWR, catalog # 414004-286 | | X |
| 96-well thermal cycler (with heated lid) | See *Thermal Cyclers*. | | X |
| Benchtop microcentrifuge | General lab supplier | X | X |
| ForenSeq Index Plate Fixture | Illumina, catalog # FC-451-1001 | | X |
| Magnetic stand-96 | Life Technologies, part # AM10027 or general lab supplier | | X |
| Microplate centrifuge | General lab supplier | X | X |
| 1.5 ml 96-well heating system | General lab supplier | | X |
| One of the following high-speed thermal mixers:<br>• BioShake iQ<br>• BioShake XP | Q instruments, catalog #:<br>• 1808-0506<br>• 1808-0505 | | X |
| Vortexer | General lab supplier | X | X |

### Thermal Cyclers

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

| Thermal Cycler | Temp Mode | Lid Temp | Vessel Type | Ramp Mode |
|---|---|---|---|---|
| ABI LTI thermal cycler 9700 | 9600 emulation | Heated | Polypropylene plates and tubes | 8% |
| Bio-Rad | Calculated | Heated, Constant at 100°C | Polypropylene plates and tubes | 0.2°C per second |
| Eppendorf Mastercycler Pro S | Gradient S, Simulated Tube | Heated | Plate | 2% |

## Index Sequences

The kit contains the following the index adapter sequences.

| Index 1 (i7) | Sequence | Index 1 (i7) | Sequence |
|---|---|---|---|
| R701 | ATCACGAT | R707 | CAGATCAT |
| R702 | CGATGTAT | R708 | ACTTGAAT |
| R703 | TTAGGCAT | R709 | GATCAGAT |
| R704 | TGACCAAT | R710 | TAGCTTAT |
| R705 | ACAGTGAT | R711 | GGCTACAT |
| R706 | GCCAATAT | R712 | CTTGTAAT |

| Index 2 (i5) | Sequence |
|---|---|
| A501 | TGAACCTT |
| A502 | TGCTAAGT |
| A503 | TGTTCTCT |
| A504 | TAAGACAC |
| A505 | CTAATCGA |
| A506 | CTAGAACA |
| A507 | TAAGTTCC |
| A508 | TAGACCTA |

## Loci

NOTE

- The amplicon length does not include 120 bp for Illumina adapter sequences. The amplicon start and end positions are the 1-based endpoints of the entire amplicon including the sequence matching primers on the hg19 human reference genome.
- All the loci in DNA Primer Mix A are also included in DNA Primer Mix B.

The following loci are detected using DNA Primer Mix A or DNA Primer Mix B.

Amelogenin—A genetic marker that confirms the gender of the donor of the biological sample. Its size range is 106–112 bp and the control DNA is male.

Table 1 Identity Informative SNPs

| Locus | Amplicon Length (bp) | Chromosome | Amplicon Start Position | Amplicon End Position | 2800M Control Alleles |
|---|---|---|---|---|---|
| rs10495407 | 109 | 1 | 238439234 | 238439342 | G |
| rs1294331 | 85 | 1 | 233448359 | 233448443 | GA |
| rs1413212 | 64 | 1 | 242806767 | 242806830 | G |
| rs1490413 | 98 | 1 | 4367256 | 4367353 | A |
| rs560681 | 90 | 1 | 160786641 | 160786730 | AG |
| rs891700 | 115 | 1 | 239881850 | 239881964 | AG |
| rs1109037 | 118 | 2 | 10085691 | 10085808 | G |
| rs12997453 | 100 | 2 | 182413195 | 182413294 | A |
| rs876724 | 119 | 2 | 114945 | 115063 | C |
| rs907100 | 115 | 2 | 239563542 | 239563656 | CG |
| rs993934 | 120 | 2 | 124109120 | 124109239 | C |
| rs1355366 | 119 | 3 | 190806041 | 190806159 | AG |
| rs1357617 | 120 | 3 | 961696 | 961815 | AT |
| rs2399332 | 157 | 3 | 110300999 | 110301155 | AC |

| Locus | Amplicon Length (bp) | Chromosome | Amplicon Start Position | Amplicon End Position | 2800M Control Alleles |
|---|---|---|---|---|---|
| rs4364205 | 98 | 3 | 32417576 | 32417673 | G |
| rs6444724 | 120 | 3 | 193207306 | 193207425 | T |
| rs1979255 | 102 | 4 | 190318007 | 190318108 | G |
| rs2046361 | 120 | 4 | 10968994 | 10969113 | A |
| rs279844 | 167 | 4 | 46329584 | 46329750 | AT |
| rs6811238 | 120 | 4 | 169663541 | 169663660 | G |
| rs13182883 | 169 | 5 | 136633252 | 136633420 | AG |
| rs159606 | 104 | 5 | 17374845 | 17374948 | A |
| rs251934 | 97 | 5 | 174778619 | 174778715 | T |
| rs338882 | 157 | 5 | 178690599 | 178690755 | C |
| rs717302 | 110 | 5 | 2879333 | 2879442 | G |
| rs13218440 | 170 | 6 | 12059928 | 12060097 | AG |
| rs1336071 | 120 | 6 | 94537182 | 94537301 | G |
| rs214955 | 120 | 6 | 152697629 | 152697748 | G |
| rs727811 | 115 | 6 | 165045254 | 165045368 | A |
| rs321198 | 165 | 7 | 137029715 | 137029879 | T |
| rs6955448 | 120 | 7 | 4310285 | 4310404 | CT |
| rs737681 | 120 | 7 | 155990742 | 155990861 | T |
| rs917118 | 109 | 7 | 4456953 | 4457061 | C |
| rs10092491 | 116 | 8 | 28411037 | 28411152 | CT |
| rs2056277 | 104 | 8 | 139399038 | 139399141 | C |
| rs4606077 | 151 | 8 | 144656710 | 144656860 | CT |
| rs763869 | 85 | 8 | 1375576 | 1375660 | CT |
| rs1015250 | 117 | 9 | 1823702 | 1823818 | G |
| rs10776839 | 103 | 9 | 137417271 | 137417373 | G |
| rs1360288 | 119 | 9 | 128967994 | 128968112 | C |
| rs1463729 | 99 | 9 | 126881396 | 126881494 | GA |
| rs7041158 | 115 | 9 | 27985907 | 27986021 | C |
| rs3780962 | 94 | 10 | 17193284 | 17193377 | T |
| rs735155 | 170 | 10 | 3374133 | 3374302 | A |
| rs740598 | 120 | 10 | 118506839 | 118506958 | AG |
| rs826472 | 153 | 10 | 2406511 | 2406663 | T |
| rs964681 | 105 | 10 | 132698394 | 132698498 | CT |
| rs10488710 | 118 | 11 | 115207134 | 115207251 | CG |
| rs1498553 | 111 | 11 | 5708981 | 5709091 | CT |
| rs2076848 | 118 | 11 | 134667502 | 134667619 | AT |
| rs901398 | 90 | 11 | 11096173 | 11096262 | T |
| rs10773760 | 99 | 12 | 130761623 | 130761721 | AG |
| rs2107612 | 103 | 12 | 888262 | 888364 | AG |
| rs2111980 | 94 | 12 | 106328186 | 106328279 | G |
| rs2269355 | 65 | 12 | 6945881 | 6945945 | C |
| rs2920816 | 157 | 12 | 40862976 | 40863132 | T |
| rs1058083 | 76 | 13 | 100038193 | 100038268 | AG |
| rs1335873 | 109 | 13 | 20901665 | 20901773 | T |
| rs1886510 | 116 | 13 | 22374646 | 22374761 | CT |
| rs354439 | 170 | 13 | 106938320 | 106938489 | T |
| rs1454361 | 118 | 14 | 25850765 | 25850882 | AT |

| Locus | Amplicon Length (bp) | Chromosome | Amplicon Start Position | Amplicon End Position | 2800M Control Alleles |
|---|---|---|---|---|---|
| rs4530059 | 170 | 14 | 104769099 | 104769268 | G |
| rs722290 | 101 | 14 | 53216686 | 53216786 | G |
| rs873196 | 114 | 14 | 98845506 | 98845619 | CT |
| rs1528460 | 115 | 15 | 55210664 | 55210778 | T |
| rs1821380 | 118 | 15 | 39313343 | 39313460 | G |
| rs8037429 | 63 | 15 | 53616876 | 53616938 | T |
| rs1382387 | 89 | 16 | 80106318 | 80106406 | GT |
| rs2342747 | 104 | 16 | 5868645 | 5868748 | AG |
| rs430046 | 119 | 16 | 78016980 | 78017098 | C |
| rs729172 | 104 | 16 | 5606153 | 5606256 | C |
| rs740910 | 113 | 17 | 5706552 | 5706664 | A |
| rs8078417 | 143 | 17 | 80461847 | 80461989 | CT |
| rs938283 | 98 | 17 | 77468433 | 77468530 | T |
| rs9905977 | 170 | 17 | 2919324 | 2919493 | G |
| rs1024116 | 98 | 18 | 75432317 | 75432414 | A |
| rs1493232 | 75 | 18 | 1127945 | 1128019 | A |
| rs1736442 | 153 | 18 | 55225698 | 55225850 | G |
| rs9951171 | 119 | 18 | 9749789 | 9749907 | G |
| rs576261 | 76 | 19 | 39559780 | 39559855 | AC |
| rs719366 | 170 | 19 | 28463281 | 28463450 | T |
| rs1005533 | 158 | 20 | 39487066 | 39487223 | A |
| rs1031825 | 126 | 20 | 4447416 | 4447541 | C |
| rs1523537 | 117 | 20 | 51296076 | 51296192 | C |
| rs445251 | 119 | 20 | 15124865 | 15124983 | CG |
| rs221956 | 97 | 21 | 43606933 | 43607029 | C |
| rs2830795 | 114 | 21 | 28608089 | 28608202 | A |
| rs2831700 | 79 | 21 | 29679639 | 29679717 | A |
| rs722098 | 101 | 21 | 16685561 | 16685661 | AG |
| rs914165 | 156 | 21 | 42415865 | 42416020 | AG |
| rs1028528 | 78 | 22 | 48362256 | 48362333 | AG |
| rs2040411 | 68 | 22 | 47836378 | 47836445 | A |
| rs733164 | 120 | 22 | 27816711 | 27816830 | AG |
| rs987640 | 120 | 22 | 33559450 | 33559569 | AT |

Table 2 Autosomal STRs

| Locus | Minimum Amplicon Length (bp) | Maximum Amplicon Length (bp) | Chromosome | 2800M Control Alleles |
|---|---|---|---|---|
| D1S1656 | 141 | 189 | 1 | 12,13 |
| TPOX | 85 | 145 | 2 | 11,11 |
| D2S441 | 144 | 180 | 2 | 10,14 |
| D2S1338 | 114 | 182 | 3 | 22,25 |
| D3S1358 | 138 | 186 | 3 | 17,18 |
| D4S2408 | 93 | 117 | 4 | 9,9 |
| FGA | 150 | 306 | 22 | 20,23 |
| D5S818 | 102 | 150 | 5 | 12,12 |
| CSF1PO | 85 | 129 | 5 | 12,12 |

| Locus | Minimum Amplicon Length (bp) | Maximum Amplicon Length (bp) | Chromosome | 2800M Control Alleles |
|---|---|---|---|---|
| D6S1043 | 163 | 227 | 6 | 12,20 |
| D7S820 | 135 | 179 | 7 | 8,11 |
| D8S1179 | 86 | 138 | 8 | 14,15 |
| D9S1122 | 108 | 140 | 9 | 12,12 |
| D10S1248 | 128 | 172 | 10 | 13,15 |
| TH01 | 100 | 148 | 11 | 6,9.3 |
| vWA | 132 | 192 | 12 | 16,19 |
| D12S391 | 237 | 281 | 12 | 18,23 |
| D13S317 | 138 | 186 | 13 | 9,11 |
| PentaE | 362 | 467 | 15 | 7,14 |
| D16S539 | 132 | 180 | 16 | 9,13 |
| D17S1301 | 114 | 142 | 17 | 11,12 |
| D18S51 | 140 | 227 | 18 | 16,18 |
| D19S433 | 154 | 212 | 19 | 13,14 |
| D20S482 | 125 | 165 | 20 | 14,15 |
| D21S11 | 158 | 276 | 20 | 29,31.2 |
| PentaD | 209 | 293 | 21 | 12,13 |
| D22S1045* | 193 | 229 | 21 | 16,16 |

* Interpret the locus D22S1045 with caution. More imbalance in read counts may be seen between alleles of a heterozygote than observed at other loci. Consider multilocus genotype when determining the presence of a DNA mixture.

Table 3 Y Haplotype Markers

| Locus | Minimum Amplicon Length (bp) | Maximum Amplicon Length (bp) | Chromosome | 2800M Control Alleles |
|---|---|---|---|---|
| DYF387S1 | 123 | 155 | Y | 37,38 |
| DYS19 | 261 | 345 | Y | 14 |
| DYS385a-b | 316 | 354 | Y | 13,16 |
| DYS389I | 231 | 275 | Y | 14 |
| DYS389II | 255 | 299 | Y | 31 |
| DYS390 | 242 | 286 | Y | 24 |
| DYS391 | 123 | 167 | Y | 10 |
| DYS392 | 346 | 358 | Y | 13 |
| DYS437 | 178 | 210 | Y | 14 |
| DYS438 | 144 | 169 | Y | 9 |
| DYS439 | 199 | 239 | Y | 12 |
| DYS448 | 288 | 324 | Y | 19 |
| DYS460 | 356 | 380 | Y | 11 |
| DYS481 | 102 | 129 | Y | 22 |
| DYS505 | 154 | 194 | Y | 11 |
| DYS522 | 294 | 334 | Y | 12 |
| DYS533 | 198 | 258 | Y | 12 |
| DYS549 | 214 | 262 | Y | 13 |
| DYS570 | 162 | 214 | Y | 17 |
| DYS576 | 183 | 235 | Y | 18 |
| DYS612 | 215 | 248 | Y | 29 |

| Locus | Minimum Amplicon Length (bp) | Maximum Amplicon Length (bp) | Chromosome | 2800M Control Alleles |
|---|---|---|---|---|
| DYS635 | 214 | 306 | Y | 21 |
| DYS643 | 115 | 115 | Y | 10 |
| Y-GATA-H4 | 151 | 203 | Y | 11 |

Table 4 X Haplotype Markers

| Locus | Minimum Amplicon Length (bp) | Maximum Amplicon Length (bp) | Chromosome | 2800M Control Alleles |
|---|---|---|---|---|
| DXS10074 | 211 | 309 | X | 21 |
| DXS10103 | 161 | 185 | X | 18 |
| DXS10135 | 228 | 334 | X | 28 |
| DXS7132 | 176 | 208 | X | 13 |
| DXS7423 | 147 | 215 | X | 15 |
| DXS8378 | 430 | 462 | X | 12 |
| HPRTB | 193 | 237 | X | 12 |

In addition to the SNPs and haplotype markers above, the following loci are also detected when using DNA Primer Mix B. These are not present in DNA Primer Mix A.

Table 5 Phenotypic Informative SNPs

| Locus | Amplicon Length (bp) | Chromosome | Amplicon Start Position | Amplicon End Position | 2800M Control Alleles |
|---|---|---|---|---|---|
| rs28777 | 92 | 5 | 33958916 | 33959007 | A |
| rs12203592 | 110 | 6 | 396273 | 396382 | C |
| rs4959270 | 161 | 6 | 457655 | 457815 | AC |
| rs683 | 120 | 9 | 12709246 | 12709365 | AC |
| rs1042602 | 113 | 11 | 88911659 | 88911771 | AC |
| rs1393350 | 99 | 11 | 89010977 | 89011075 | G |
| rs12821256 | 119 | 12 | 89328278 | 89328396 | CT |
| rs12896399 | 73 | 14 | 92773627 | 92773699 | G |
| rs2402130 | 120 | 14 | 92801169 | 92801288 | A |
| rs1800407 | 119 | 15 | 28230246 | 28230364 | G |
| N29insA | 112 | 16 | 89985688 | 89985799 | C |
| rs1110400 | 213 | 16 | 89985774 | 89985986 | T |
| rs11547464 | 213 | 16 | 89985774 | 89985986 | G |
| rs1805005 | 173 | 16 | 89986044 | 89986216 | G |
| rs1805006 | 173 | 16 | 89986044 | 89986216 | C |
| rs1805007 | 213 | 16 | 89985774 | 89985986 | C |
| rs1805008 | 213 | 16 | 89985774 | 89985986 | C |
| rs1805009 | 227 | 16 | 89986484 | 89986710 | G |
| rs201326893_ Y152OCH | 213 | 16 | 89985774 | 89985986 | C |
| rs2228479 | 173 | 16 | 89986044 | 89986216 | G |
| rs885479 | 213 | 16 | 89985774 | 89985986 | G |
| rs2378249 | 118 | 20 | 33218028 | 33218145 | A |

Table 6 Ancestry Informative SNPs

| Locus | Amplicon Length (bp) | Chromosome | Amplicon Start Position | Amplicon End Position | 2800M Control Alleles |
|---|---|---|---|---|---|
| rs2814778 | 120 | 1 | 159174650 | 159174769 | A |
| rs3737576 | 98 | 1 | 101709521 | 101709618 | A |
| rs7554936 | 106 | 1 | 151122413 | 151122518 | CT |
| rs10497191 | 101 | 2 | 158667153 | 158667253 | C |
| rs1834619 | 84 | 2 | 17901444 | 17901527 | G |
| rs1876482 | 120 | 2 | 17362526 | 17362645 | C |
| rs260690 | 115 | 2 | 109579681 | 109579795 | A |
| rs3827760 | 108 | 2 | 109513546 | 109513653 | T |
| rs6754311 | 98 | 2 | 136707920 | 136708017 | CT |
| rs798443 | 84 | 2 | 7968221 | 7968304 | A |
| rs12498138 | 119 | 3 | 121459545 | 121459663 | G |
| rs1919550 | 117 | 3 | 121364112 | 121364228 | A |
| rs1229984 | 120 | 4 | 100239288 | 100239407 | G |
| rs3811801 | 114 | 4 | 100244261 | 100244374 | C |
| rs4833103 | 95 | 4 | 38815462 | 38815556 | AC |
| rs7657799 | 116 | 4 | 105375396 | 105375511 | T |
| rs7722456 | 114 | 5 | 170202901 | 170203014 | T |
| rs870347 | 119 | 5 | 6844995 | 6845113 | T |
| rs16891982* | 108 | 5 | 33951621 | 33951728 | G |
| rs192655 | 70 | 6 | 90518235 | 90518304 | AG |
| rs3823159 | 119 | 6 | 136482701 | 136482819 | A |
| rs917115 | 71 | 7 | 28172543 | 28172613 | T |
| rs1462906 | 84 | 8 | 31896545 | 31896628 | C |
| rs1871534 | 71 | 8 | 145639652 | 145639722 | C |
| rs2196051 | 120 | 8 | 122124216 | 122124335 | T |
| rs6990312 | 111 | 8 | 110602270 | 110602380 | G |
| rs3814134 | 104 | 9 | 127267664 | 127267767 | T |
| rs4918664 | 168 | 10 | 94920962 | 94921129 | A |
| rs1079597 | 167 | 11 | 113296227 | 113296393 | G |
| rs174570 | 120 | 11 | 61597179 | 61597298 | C |
| rs2238151 | 113 | 12 | 112211753 | 112211865 | CT |
| rs671 | 136 | 12 | 112241658 | 112241793 | G |
| rs1572018 | 116 | 13 | 41715225 | 41715340 | AG |
| rs2166624 | 71 | 13 | 42579949 | 42580019 | AG |
| rs7326934 | 96 | 13 | 49070482 | 49070577 | G |
| rs7997709 | 85 | 13 | 34847693 | 34847777 | T |
| rs9522149 | 119 | 13 | 111827125 | 111827243 | C |
| rs200354 | 165 | 14 | 99375246 | 99375410 | G |
| rs12439433 | 100 | 15 | 36219979 | 36220078 | G |
| rs1426654 | 92 | 15 | 48426457 | 48426548 | A |
| rs1800414 | 116 | 15 | 28196969 | 28197084 | A |
| rs735480 | 108 | 15 | 45152321 | 45152428 | T |
| rs12913832* | 119 | 15 | 28365523 | 28365641 | AG |
| rs459920 | 78 | 16 | 89730800 | 89730877 | T |
| rs11652805 | 119 | 17 | 62987113 | 62987231 | T |
| rs17642714 | 118 | 17 | 48726060 | 48726177 | AT |

| Locus | Amplicon Length (bp) | Chromosome | Amplicon Start Position | Amplicon End Position | 2800M Control Alleles |
|---|---|---|---|---|---|
| rs2593595 | 102 | 17 | 41056210 | 41056311 | TC |
| rs4411548 | 158 | 17 | 40658440 | 40658597 | G |
| rs4471745 | 67 | 17 | 53568849 | 53568915 | G |
| rs2042762 | 83 | 18 | 35277568 | 35277650 | A |
| rs3916235 | 120 | 18 | 67578894 | 67579013 | AG |
| rs4891825 | 106 | 18 | 67867615 | 67867720 | AG |
| rs7226659 | 149 | 18 | 40488180 | 40488328 | G |
| rs7251928 | 200 | 19 | 4077044 | 4077243 | A |
| rs310644 | 89 | 20 | 62159472 | 62159560 | A |
| rs2024566 | 88 | 22 | 41697312 | 41697399 | A |

* Also used for phenotype prediction.

## Acronyms

| Acronym | Definition |
|---|---|
| 2800M | Control DNA 2800M |
| A7XX | i7 Index Adapter |
| A50X | i5 Index Adapter |
| DNL | Diluted Normalized Libraries |
| DPMA | DNA Primer Mix A |
| DPMB | DNA Primer Mix B |
| FEM | Enzyme Mix |
| FSP | ForenSeq Sample Plate |
| HP3 | 2N NaOH |
| HSC | Human Sequencing Control |
| HT1 | Hybridization Buffer |
| LNA1 | Library Normalization Additives 1 |
| LNB1 | Library Normalization Beads 1 |
| LNS2 | Library Normalization Storage Buffer 2 |
| LNW1 | Library Normalization Wash 1 |
| NLP | Normalized Library Plate |
| NWP | Normalization Working Plate |
| PBP | Purification Bead Plate |

| Acronym | Definition |
|---|---|
| PCR1 | PCR1 Reaction Mix |
| PCR2 | PCR2 Reaction Mix |
| PLP | Purified Library Plate |
| PNL | Pooled Normalized Libraries |
| RSB | Resuspension Buffer |
| SPB | Sample Purification Beads |

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 7 Illumina General Contact Information

| | |
|---|---|
| **Website** | www.illumina.com |
| **Email** | techsupport@illumina.com |

Table 8 Illumina Customer Support Telephone Numbers

| Region | Contact Number | Region | Contact Number |
|---|---|---|---|
| North America | 1.800.809.4566 | Italy | 800.874909 |
| Australia | 1.800.775.688 | Netherlands | 0800.0223859 |
| Austria | 0800.296575 | New Zealand | 0800.451.650 |
| Belgium | 0800.81102 | Norway | 800.16836 |
| Denmark | 80882346 | Spain | 900.812168 |
| Finland | 0800.918363 | Sweden | 020790181 |
| France | 0800.911850 | Switzerland | 0800.563118 |
| Germany | 0800.180.8994 | United Kingdom | 0800.917.0041 |
| Ireland | 1.800.812949 | 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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