ForenSeq Kintelligence Kit

Reference Guide

VEROGEN PROPRIETARY
Document # VD2020053 Rev. B
March 2021

Revision History

Document #	Date	Description of Change
VD2020053 Rev. B	March 2021	Added a list of SNPs included in KPM, but not the analysis. Updated the legal notice to include Phusion.
VD2020053 Rev. A	February 2021	Initial release

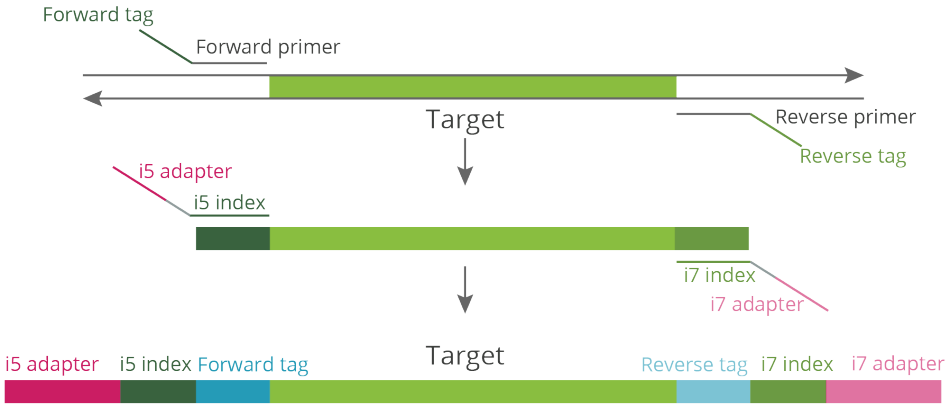
Table of Contents

Overview

Introduction

The Verogen ForenSeq® Kintelligence Kit prepares up to 12 paired-end, dual-indexed libraries for sequencing and long-range kinship analysis.

Figure 1 Assay overview



Kit Features

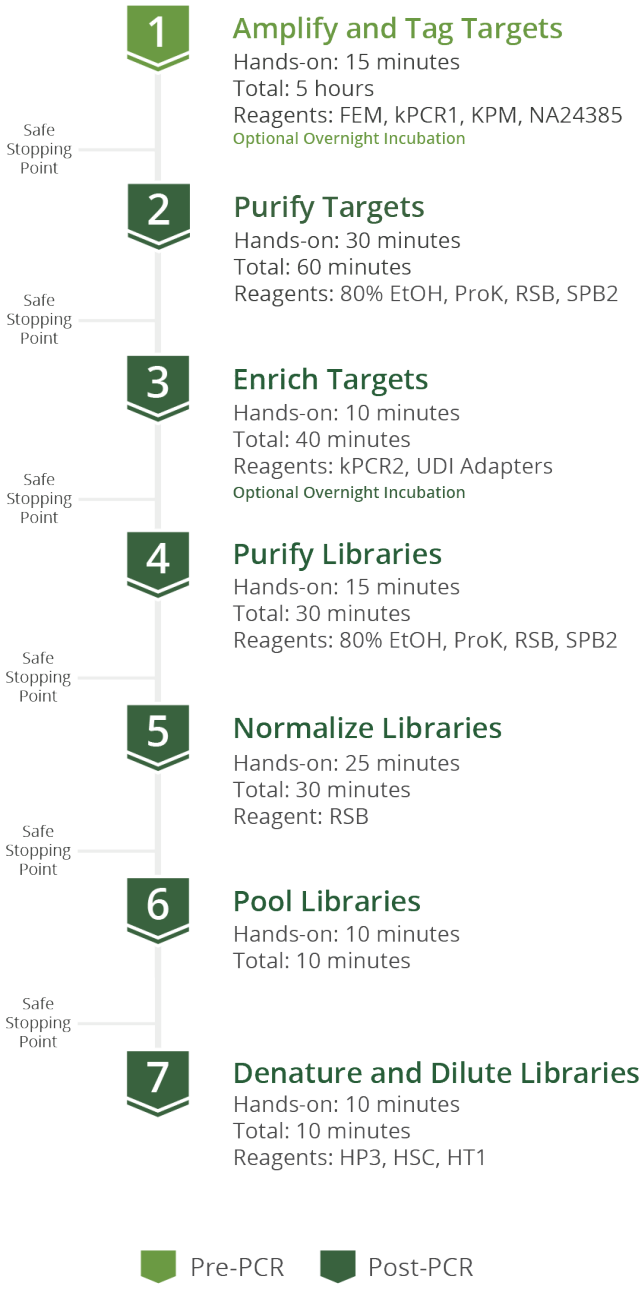
The ForenSeq Kintelligence Kit offers the following features:

- A forensically relevant set of single nucleotide polymorphisms (SNPs) that is directly compatible with GEDmatch PRO®.
- A 1 ng input requirement that allows preparation of libraries from diverse and low-quality DNA samples. A small amplicon size averaging < 150 bp improves outcomes.
- Unique Dual Index (UDI) adapters that have distinct, unrelated adapters for both index reads, preventing repeated sequences in a plate for optimum data recovery.
- An efficient protocol that simultaneously prepares all libraries in one plate. Each library is a collection of tagged, amplified DNA fragments from one sample.

Protocol Steps

The following diagram lists the steps to prepare libraries with hands-on times, total times, and reagents. Safe stopping points are marked between steps.

Figure 2 Overview of the ForenSeq Kintelligence protocol



DNA Input Recommendations

Verogen recommends 1 ng human genomic DNA (gDNA) input. For challenging samples, such as partially degraded samples, you can use > 1 ng gDNA input to potentially increase the number of reads. Before starting the protocol, quantify the input gDNA using a fluorometric-based method or qPCR and assess quality.

The kit is compatible with gDNA extracted from samples such as bone, buccal swabs, hair, semen, and teeth. For all sample types, the input volume is 25 µl per sample.

Controls

Use nuclease-free water as a negative control and NA24385 Positive Amplification Control DNA (NA24385), which is included in the kit. If NA24385 is not included in each library prep and run, troubleshooting support is limited. For more information, see *Number of Samples* on page 9.

Additional Resources

This guide provides comprehensive information on the ForenSeq Kintelligence Kit with detailed protocol instructions. Visit the Documentation page on the Verogen website to download additional kit documentation and access the latest versions.

Resource	Description
ForenSeq Kintelligence Kit Checklist (document # VD2020051)	Provides concise protocol instructions for the experienced user.
ForenSeq Kintelligence Kit Materials List (document # VD2020052)	Lists the consumables and equipment needed to perform the protocol.

Protocol

Introduction

This chapter describes the ForenSeq Kintelligence protocol with step-by-step instructions to prepare libraries for sequencing. For an overview of the protocol with reagents and durations for each step, see *Protocol Steps* on page 6.

Before starting, confirm kit contents and make sure that you have the necessary reagents, consumables, and equipment. For a list of items, see *Materials* on page 23.

Number of Samples

Process at least three samples at a time, including positive and negative amplification controls. Preparing master mix for fewer than three samples can introduce pipetting inaccuracies due to small volumes.

Plan on sequencing up to three libraries on a MiSeq FGx® standard flow cell: one positive amplification control, one negative amplification control, and one sample. You can prepare the full 12 libraries and split them between four flow cells, if desired.

> The MiSeq FGx Reagent Kit provides the standard flow cell.

Pooling Recommendations

The kit includes six UDI adapters. When preparing 7-12 libraries, reuse the UDI adapters but ***do not*** combine libraries with the same adapter in a pool. For example, if you append UDI052 to Sample 1 and Sample 7 in the same preparation, keep the two samples in separate pools. For more information, see *Index Adapter Sequences* on page 26.

Tips and Techniques

Protocol Continuity

- Follow the steps in the order indicated using the specified volumes and incubation parameters.
- Unless a safe stopping point is specified, proceed immediately to the next step.

Plate Setup

- Create a sample sheet to record the position of each sample, control, and index adapter.
- Reference the sample sheet throughout the protocol to ensure proper plate setup.

The *Universal Analysis Software v2.0 Reference Guide (document # VD2019002)* provides detailed information on sample sheets and input of sample information.

Preventing Cross-Contamination

- Set up the *Amplify and Tag Targets* process in a pre-PCR environment. Perform all other processes in a post-PCR environment.
- Clean your workspace and equipment before starting work in the post-PCR area.
- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each well***.
- Remove unused index adapter tubes from the working area.

Sealing the Plate

- Apply a microseal to cover the plate and seal with a rubber roller. After each use, discard seals from plates.
- Use Microseal 'A' pressure film for thermal cycling.
- Use Microseal 'B' adhesive film for shaking, centrifuging, and long-term storage. These seals are effective at -40°C to 110°C.

Handling Beads

- For optimal performance and yield, confirm that beads are at room temperature before use.
- Aspirate and dispense beads slowly due to viscosity.
- Do not centrifuge plates and tubes containing beads, except when indicated.
- Vortex beads before use and frequently throughout the protocol to resuspend. Resuspended beads are evenly distributed and homogenous in color.
- If beads aspirate into pipette tips during supernatant removal, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).

Amplify and Tag Targets

This process uses an oligonucleotide primer mix with regions specific to the DNA sequences upstream and downstream of SNP targets to tag and amplify the input gDNA.

Consumables

- FEM (ForenSeq Enzyme Mix)
- kPCR1 (Kintelligence PCR1 Reaction Mix)

- KPM (Kintelligence Primer Mix)
- NA24385 (NA24385 Positive Amplification Control DNA)
- Input gDNA
- Nuclease-free water
- 96-well PCR plate, semi-skirted
- 1.7 ml microcentrifuge tubes (3)
- Microseal 'A' film

About Reagents

- Do not vortex NA24385, FEM, or input gDNA.

Preparation

1. Prepare the following consumables:

Item	Storage	Instruction
NA24385	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Invert three times to mix, and then centrifuge briefly.
kPCR1	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
KPM	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex to mix, and then centrifuge.
FEM	-25°C to -15°C	Thaw at room temperature, and then centrifuge briefly. Return to storage immediately after use.

2. Save the following kPCR1 program on the thermal cycler in the post-amplification area. See Table 1 for lid temperatures and ramp modes.
   - Choose the preheat lid option and set to applicable temperature
   - 98°C for 3 minutes
   - 18 cycles of:
     - 96°C for 45 seconds
     - 80°C for 10 seconds
     - 54°C for 4 minutes with applicable ramp mode
     - 66°C for 90 seconds with applicable ramp mode
   - 68°C for 10 minutes
   - Hold at 4°C

Table 1 Lid temperatures and ramp modes

Thermal Cycler	Lid Temperature	Ramp Mode
ABI LTI Thermal Cycler 9700	105°C	8%

Thermal Cycler	Lid Temperature	Ramp Mode
Proflex 96-Well PCR System	100°C	0.2°C per second
Veriti 96-Well Thermal Cycler	100°C	4%

Total program time is ~4.5 hours and can continue overnight.

3. Label tubes and plates as follows.

Vessel	Label
1.7 ml tube	Master Mix
1.7 ml tube	Control DNA Dilution 1
1.7 ml tube	Control DNA Dilution 2
PCR plate	ForenSeq Sample Plate

Procedure

1. Using nuclease-free water, dilute 1 ng gDNA to 40 pg/µl. Gently pipette to mix.
2. In the Master Mix tube, combine the following volumes. Multiply each volume by the number of samples and add 10% for overage.
   - kPCR1 (18.5 µl)
   - KPM (5 µl)
   - FEM (1.5 µl)

   For example, for three samples prepare 82.5 µl Master Mix: 61 µl kPCR1, 16.5 µl KPM, and 5 µl FEM.
3. Pipette to mix, and then cap and centrifuge briefly.
4. Add 25 µl Master Mix to each sample well of the ForenSeq Sample Plate.
5. Dilute 10 ng/µl NA24385 stock:
   a. In the Control DNA Dilution 1 tube, combine the following volumes to prepare 50 µl 400 pg/µl NA24385:
      - 10 ng/µl NA24385 (2 µl)
      - Nuclease-free water (48 µl)

   b. Gently pipette to mix, and then cap and centrifuge briefly.
   c. In the Control DNA Dilution 2 tube, combine the following volumes to prepare 100 µl 40 pg/µl NA24385:
      - 400 pg/µl NA24385 (10 µl)
      - Nuclease-free water (90 µl)

   d. Gently pipette to mix, and then cap and centrifuge briefly.
6. Add 25 µl 40 pg/µl gDNA to each sample well of the ForenSeq Sample Plate. Pipette to mix.
7. Add 25 µl 40 pg/µl NA24385 to each positive amplification control well. Pipette to mix.
8. Add 25 µl nuclease-free water to each negative amplification control well. Pipette to mix.
9. Seal and centrifuge at 1000 × g for 30 seconds.

10. Place on the preprogrammed thermal cycler and run the kPCR1 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.

Purify Targets

This process combines purification beads with an enzyme to purify the amplified targets from other reaction components.

Consumables

- ProK (Proteinase K)
- RSB (Resuspension Buffer)
- SPB2 (Sample Purification Beads 2)
- Freshly prepared 80% EtOH
- 96-well midi plate
- 96-well PCR plate, semi-skirted
- PVC reagent reservoir
- Microseal 'B' film

About Reagents

- Aspirate and dispense ProK/SPB2 slowly due to viscosity.
- Prepare 80% EtOH fresh and discard after 1 day.

Preparation

1. Prepare the following consumables:

Item	Storage	Instructions
ProK	-25°C to -15°C	Let stand for 30 minutes to bring to room temperature. Invert to mix, and then centrifuge.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.
SPB2	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex for ***≥ 1 minute*** and invert to mix.

2. Prepare the appropriate volume of fresh 80% EtOH from absolute ethanol:
   - If you plan to complete *Purify Libraries* within the day, prepare 1.5 ml per sample.
   - If you plan to stop before starting *Purify Libraries*, prepare 1 ml per sample.
3. Label plates as follows.

Plate Type	Label
Midi	Purification Bead Plate 1
PCR	Purified Targets Plate

4. Prepare the SPB2 tube for a first or subsequent use:
   - For first-time use, add 7.5 µl ProK to the SPB2 tube. Select the checkbox on the SPB2 label to indicate the addition.
   - For a subsequent use, make sure the checkbox on the SPB2 label is selected.
5. Vortex the ProK/SPB2 tube for ≥ 1 minute and invert several times to mix.

Procedure

Clean Up Targets

1. Add 75 µl ProK/SPB2 to each well of the Purification Bead Plate 1.
2. Transfer 45 µl reaction from each well of the ForenSeq Sample Plate to the corresponding well of the Purification Bead Plate 1.
3. Discard the ForenSeq Sample Plate.
4. Seal the Purification Bead Plate 1 and shake at 1800 rpm for 2 minutes.
5. Incubate at room temperature for 10 minutes.
6. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
7. Centrifuge at 1000 × g for 30 seconds.
8. Place on the magnetic stand and wait until the liquid is clear (~1 minute).
9. Remove and discard all supernatant.
10. Keep on the magnetic stand and wash as follows.
    a. Add 200 µl fresh 80% EtOH to each well.
    b. Incubate for 30 seconds.
    c. Remove and discard all supernatant.
11. Wash a **second** time.
12. Seal and centrifuge at 1000 × g for 30 seconds.
13. Place on the magnetic stand and wait until the liquid is clear (~1 minute).
14. With a 20 µl pipette, remove residual EtOH from each well.
15. Remove from the magnetic stand.
16. Add 30 µl RSB to each sample well.
17. Seal and shake at 1800 rpm for 2 minutes.
18. If the beads are not fully resuspended, pipette to mix or reshake at 1800 rpm for 2 minutes.
19. Incubate at room temperature for 2 minutes.
20. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
21. Transfer 28 µl supernatant from each well of the Purification Bead Plate 1 to a fresh well in the ***same plate***.
    Some bead carryover into the second cleanup is normal.

Perform Second Cleanup

1. Add 45 µl ProK/SPB2 to each sample well.
2. Seal and shake at 1800 rpm for 2 minutes.
3. Incubate at room temperature for 5 minutes.
4. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
5. Centrifuge at 1000 × g for 30 seconds.
6. Place on the magnetic stand and wait until the liquid is clear (~1 minute).
7. Remove and discard all supernatant.
8. Keep on the magnetic stand and wash as follows.
    a. Add 200 µl fresh 80% EtOH to each well.
    b. Incubate for 30 seconds.
    c. Remove and discard all supernatant.
9. Wash a **second** time.
10. Seal and centrifuge at 1000 × g for 30 seconds.
11. Place on the magnetic stand and wait until the liquid is clear (~1 minute).
12. With a 20 µl pipette, remove residual EtOH from each well.
13. Remove from the magnetic stand.
14. Add 27 µl RSB to each sample well.
15. Seal and shake at 1800 rpm for 2 minutes.
16. If the beads are not fully resuspended, pipette to mix or reshake at 1800 rpm for 2 minutes.
17. Incubate at room temperature for 2 minutes.
18. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
19. Transfer 25 µl supernatant from each well of the Purification Bead Plate 1 to the corresponding well of the Purified Targets Plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C overnight.

Enrich Targets

This process amplifies the DNA and adds the UDI adapters and sequences required for cluster generation. The UDI adapters tag DNA with a unique combination of sequences that identify each sample for analysis.

Consumables

- kPCR2 (Kintelligence PCR2 Reaction Mix)
- UDI adapters
- Microseal 'A' film

Preparation

1. Prepare the following consumables:

Item	Storage	Instructions
kPCR2	-25°C to -15°C	Let stand for 30 minutes to bring to room temperature. Vortex to mix, and then centrifuge.
UDI adapters	-25°C to -15°C	Remove only the adapters being used. Let stand for 30 minutes to bring to room temperature. Vortex to mix, and then centrifuge.

2. Save the following kPCR2 program on the thermal cycler. See Table 2 for lid temperatures.
   - Choose the preheat lid option and set to applicable temperature
   - 98°C for 30 seconds
   - 15 cycles of:
     - 98°C for 20 seconds
     - 66°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 1 minute
   - Hold at 4°C

Table 2 Lid temperatures

Thermal Cycler	Lid Temperature
ABI LTI Thermal Cycler 9700	105°C
Proflex 96-Well PCR System	100°C
Veriti 96-Well Thermal Cycler	100°C

Total program time is ~30 minutes.

Procedure

1. Seal and centrifuge the Purified Targets Plate at 1000 × g for 30 seconds.
2. Add 5 µl UDI adapter to each sample well.
   The total volume per well is 30 µl.
3. Briefly centrifuge kPCR2, and then pipette to mix.
4. Add 20 µl kPCR2 to each well.
5. Pipette to mix.
6. Seal and centrifuge at 1000 × g for 30 seconds.
7. Place on the preprogrammed thermal cycler and run the kPCR2 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 purification beads to purify the amplified libraries from other reaction components.

Consumables

- ProK (Proteinase K)
- RSB (Resuspension Buffer)
- SPB2 (Sample Purification Beads 2)
- Freshly prepared 80% EtOH
- 96-well midi plate
- 96-well PCR plate, skirted or semi-skirted
- PVC reservoir
- Microseal 'B' film

Preparation

1. Prepare the following consumables:

Item	Storage	Instructions
ProK	-25°C to -15°C	Let stand for 30 minutes to bring to room temperature. Invert to mix, and then centrifuge.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.
SPB2	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex for ***≥ 1 minute*** and invert to mix.

2. If 80% EtOH was not prepared within the day, discard and prepare 0.5 ml fresh 80% EtOH per sample.
3. Label plates as follows.

Plate Type	Label
Midi	Purification Bead Plate 2
PCR	Purified Library Plate

4. Prepare the SPB2 tube for a first or subsequent use:
   - For first-time use, add 7.5 µl ProK to the SPB2 tube. Select the checkbox on the SPB2 label to indicate the addition.
   - For a subsequent use, make sure the checkbox on the SPB2 label is selected.
5. Vortex the ProK/SPB2 tube for ≥ 1 minute and invert several times to mix.

Procedure

1. Add 45 µl ProK/SPB2 to each well of the Purification Bead Plate 2.
2. Transfer 45 µl reaction from each well of the Purified Targets Plate to the corresponding well of the Purification Bead Plate 2.
3. Seal and shake at 1800 rpm for 2 minutes.
4. Incubate at room temperature for 5 minutes.
5. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
6. Remove and discard all supernatant.
7. Keep on the magnetic stand and wash as follows.
    a. Add 200 µl fresh 80% EtOH to each well.
    b. Incubate for 30 seconds.
    c. Remove and discard all supernatant.
8. Wash a **second** time.
9. Seal and centrifuge at 1000 × g for 30 seconds.
10. Place on the magnetic stand and wait until the liquid is clear (~1 minute).
11. With a 20 µl pipette, remove residual EtOH from each well.
12. Remove from the magnetic stand.
13. Add 52.5 µl RSB to each sample well.
14. Seal and shake at 1800 rpm for 2 minutes.
15. If the beads are not fully resuspended, pipette to mix or reshake at 1800 rpm for 2 minutes.
16. Incubate at room temperature for 2 minutes.
17. Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
18. Transfer 50 µl supernatant from each well of the Purification Bead Plate 2 to the corresponding well of the Purified Library Plate.
19. Seal and 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 1 year.

Normalize Libraries

This process quantifies libraries and checks the quality. Accurate quantification helps ensure optimum cluster density on the flow cell and a similar number of read counts.

Consumables

- RSB (Resuspension Buffer)
- One of the following vessels:

- 1.7 ml microcentrifuge tube
- 96-well PCR plate, skirted or semi-skirted

Preparation

1. Prepare the following consumable:

Item	Storage	Instructions
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

2. Label the applicable vessel:

Vessel	Label
1.7 ml tube	Normalized Library
PCR plate	Normalized Library Plate

Procedure

1. Place the Purified Library Plate on the magnetic stand.
2. Quantify libraries using a fluorometric method, such as QuantiFluor ONE (recommended), AccuCleer, PicoGreen, or Qubit.
3. If concentration is > 0.75 ng/µl, prepare RSB to dilute each library to 0.75 ng/µl as follows. For additional guidance, see *Example Calculations* on page 20.
   a. Use the formula $C_1V_1/C_2=V_2$ to calculate the value for V_2, where:
      - C_1 is the library quantification result
      - V_1 is 8 µl undiluted library
      - C_2 is 0.75 ng/µl
      - V_2 is the final volume of diluted library
   b. Calculate the requisite volume of RSB (V_2 - 8 µl).
   c. Add the calculated volume of RSB to the corresponding well of the Normalized Library Plate or tube. Use the tube when the library is ≥ 15 ng/µl.

   Libraries ≤ 0.75 ng/µl are used at the existing concentration and do not require diluting.

4. Transfer 8 µl of each purified library from the Purified Library Plate to the corresponding well of the Normalized Library Plate or tube.

   The result is a Normalized Library Plate or tube containing 0.75 ng/µl libraries.

Example Calculations

DNA Input	Library Concentration (C_1)	Library Volume (V_1)	Normalized Library Concentration (C_2)	RSB Volume (V_2 - V_1)
1 ng gDNA	1.5 ng/µl	8 µl	0.75 ng/µl	8 µl
Negative control	0.25 ng/µl	8 µl	0.25 ng/µl	0 µl

SAFE STOPPING POINT

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

Pool Libraries

This process combines equal volumes of normalized libraries to create a pool of libraries that are sequenced together on the same flow cell. For more information, see *Pooling Recommendations* on page 9.

Consumables

- 1.7 ml microcentrifuge tube
- Microseal 'B' film

Preparation

1. Select ≤ 3 libraries to pool for sequencing.
   Three is the maximum number of ForenSeq Kintelligence libraries that a standard flow cell supports.
2. Label a new 1.7 ml tube Pooled Libraries.

Procedure

1. Transfer 5 µl of each library to the Pooled Libraries tube.
2. Store remaining normalized libraries as follows.
   a. Seal the Normalized Library Plate or cap the Normalized Library tube.
   b. Store in the post-PCR area at -25°C to -15°C for ≤ 30 days.
3. Cap and vortex the Pooled Libraries tube to mix, 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 6 months.

Denature and Dilute Libraries

This process adds a sequencing control and uses a sodium hydroxide (NaOH)-based method to denature and dilute libraries. Denaturing and diluting ensures the concentration of NaOH in the final library does not exceed 1 mM. Higher concentrations can inhibit hybridization to the flow cell and decrease cluster density.

Start this process when you are ready to prepare sequencing reagents and set up the run. Delays can impact template loading.

Consumables

- HP3 (2 N NaOH)
- HSC (Human Sequencing Control)
- MiSeq FGx Reagent Kit contents:
  - HT1 (Hybridization Buffer)
  - Reagent cartridge
- Nuclease-free water
- Pooled libraries
- 1.7 ml microcentrifuge tubes (4)

Preparation

1. Prepare the reagent cartridge per instructions in the *MiSeq FGx Sequencing System Reference Guide (document # VD2018006)*.
2. Prepare the following consumables:

Item	Storage	Instructions
HP3	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
HSC	-25°C to -15°C	Let stand for 30 minutes to bring to room temperature. Invert to mix, and then centrifuge.
HT1	-25°C to -15°C	Thaw at room temperature, and then vortex to mix.

3. Label four new 1.7 ml tubes as follows.
   - 12 pM Denatured Library
   - 20 pM Denatured Library
   - Denatured HSC
   - 0.2 N NaOH

Procedure

1. In the 0.2 N NaOH tube, combine the following volumes to prepare 0.1 ml 0.2 N NaOH:
   - Nuclease-free water (90 µl)
   - HP3 (10 µl)

2. Invert the tube several times to mix. Use within ***12 hours***.
   Freshly diluted NaOH is essential to the denaturation process.
3. In the Denatured HSC tube, combine the following volumes to prepare denatured HSC:
   - HSC (2 µl)
   - 0.2 N NaOH (2 µl)
4. Cap and vortex to mix, and then centrifuge briefly.
5. Incubate at room temperature for 5 minutes.
6. Add 36 µl HT1 to the Denatured HSC tube.
   You can store denatured HSC at room temperature for ≤ 1 day.
7. In the 20 pM Denatured Library tube, combine the following volumes:
   - 0.75 ng/µl library pool (5 µl)
   - 0.2 N NaOH (5 µl)
8. Cap and vortex briefly, and then centrifuge briefly.
9. Incubate at room temperature for 5 minutes.
10. Add 990 µl HT1 to the 20 pM Denatured Library tube to prepare 1 ml 20 pM denatured library.
    You can store the 20 pM denatured library at -15°C to -25°C for ≤ 3 weeks.
11. In the 12 pM Denatured Library tube, combine the following volumes to dilute the 20 pM library to 12 pM:
    - 20 pM library pool (360 µl)
    - HT1 (238 µl)
    - Denatured HSC (2 µl)
12. Cap and vortex to mix, and then centrifuge briefly.
13. Immediately transfer the entire volume to the reagent cartridge per instructions in the *MiSeq FGx Sequencing System Reference Guide (document # VD2018006)*.

Materials

Kit Contents and Storage

This section identifies the reagents provided in the following kit. When you receive your kit, promptly store reagents at the indicated temperatures.

In addition to the kit, completing the protocol requires third-party consumables and equipment. For a list of items, see *Consumables and Equipment* on page 24.

Kit Name	Verogen Part #
ForenSeq Kintelligence Kit (12 Reactions)	V16000120

ForenSeq Kintelligence Kit (12 Reactions) (V16000120)

Box 1

Quantity	Reagent	Description	Cap	Storage*
1	KPM	Kintelligence Primer Mix	Blue	2°C to 8°C
1	NA24385	NA24385 Positive Amplification Control DNA	Black	2°C to 8°C

* Shipped at -25°C to -15°C.

Box 2

Quantity	Reagent	Description	Cap	Storage
2	FEM	ForenSeq Enzyme Mix	Yellow	-25°C to -15°C
1	kPCR1	Kintelligence PCR1 Reaction Mix	Green	-25°C to -15°C

Box 3

Quantity	Reagent	Description	Cap	Storage
1	kPCR2	Kintelligence PCR2 Reaction Mix	Purple	-25°C to -15°C
1	HP3	2 N NaOH	Orange	-25°C to -15°C
1	HSC	Human Sequencing Control	Pink	-25°C to -15°C

Quantity	Reagent	Description	Cap	Storage
1	ProK	Proteinase K	Clear	-25°C to -15°C
1	UDI052	UDI Adapter Set 52	Yellow	-25°C to -15°C
1	UDI060	UDI Adapter Set 60	Yellow	-25°C to -15°C
1	UDI077	UDI Adapter Set 77	Yellow	-25°C to -15°C
1	UDI079	UDI Adapter Set 79	Yellow	-25°C to -15°C
1	UDI080	UDI Adapter Set 80	Yellow	-25°C to -15°C
1	UDI096	UDI Adapter Set 96	Yellow	-25°C to -15°C

Box 4

Quantity	Reagent	Description	Cap	Storage
1	RSB	Resuspension Buffer	Purple	2°C to 8°C
1	SPB2	Sample Purification Beads 2	Red	2°C to 8°C

Consumables and Equipment

Consumables

Consumable	Supplier
1.7 ml microcentrifuge tubes	General lab supplier
15 ml conical tubes	General lab supplier
20 µl barrier pipette tips	General lab supplier
200 µl barrier pipette tips	General lab supplier
1000 µl barrier pipette tips	General lab supplier
96-well deep well storage plates (midi plates)	Fisher Scientific, part # AB-0765
96-well twin.tec PCR plates, semi-skirted	One of the following suppliers: • Eppendorf, catalog # 951020303 • VWR, catalog # 89136-706
Ethyl alcohol, pure	Sigma-Aldrich, catalog # E7023
Microseal 'A' sealing film	Bio-Rad, catalog # MSA5001
Microseal 'B' sealing film, adhesive, optical	Bio-Rad, catalog # MSB1001
MiSeq FGx Reagent Kit	Verogen part # 15066817
Nuclease-free water	General lab supplier

Consumable	Supplier
QuantiFluor ONE dsDNA System	Promega, catalog # E4870
[Optional] Multichannel reagent reservoirs, PVC, disposable	Labcor, part # 730-001

Equipment

Equipment	Supplier	Pre-PCR	Post-PCR
20 µl pipettes	General lab supplier	X	X
200 µl pipettes	General lab supplier	X	X
1000 µl pipettes	General lab supplier		X
Benchtop microcentrifuge	General lab supplier	X	X
Magnetic stand-96	Life Technologies, part # AM10027		X
Microplate centrifuge	General lab supplier	X	X
One of the following thermoshakers: • BioShake iQ • BioShake XP	QInstruments, item #: • 1808-0506 • 1808-0505		X
Quantus Fluorometer	Promega, catalog # E6150		X
Rubber roller	General lab supplier	X	X
Thermal cycler, 96-well with heated lid	See *Thermal Cyclers*		X
Vortexer	General lab supplier	X	X
[Optional] 20 µl multichannel pipettes	General lab supplier	X	X
[Optional] 200 µl multichannel pipettes	General lab supplier	X	X

Thermal Cyclers

The following table lists supported thermal cyclers with recommended settings. If your laboratory has an unlisted thermal cycler, evaluate the thermal cycler before performing the protocol.

Thermal Cycler	Temperature Mode	Lid Temperature	Vessel Type
ABI LTI thermal cycler 9700*	9600 emulation	Heated	Polypropylene plates
Proflex 96-well PCR System	Not applicable	Heated, constant at 100°C	Polypropylene plates
Veriti 96-well thermal cycler	Standard	Heated, constant at 100°C	Polypropylene plates

* Only gold heat blocks are supported.

Index Adapter Sequences

The following table lists the 8 bp sequences for the UDI adapters included in the ForenSeq Kintelligence Kit. Each adapter combines an Index 1 (i7) and Index 2 (i5) sequence.

Index Name	Index 1 Bases	Index 2 Bases
UDI052	GGTGAACC	GCGTTGGA
UDI060	AGCTCGCT	GCAGAATC
UDI077	TGCGAGAC	CATTGTTG
UDI079	ACAGGCGC	CTCTGCCT
UDI080	GTGAATAT	TCTCATTC
UDI096	CTAGCGCT	GTGTAGAC

Unanalyzed SNPs

The following SNPs are included in KPM, but are not analyzed in Universal Analysis Software (UAS). Verogen deselected these SNPs due to relatively poor performance or inclusion in pseudoautosomal regions of human sex chromosomes.

rs1012268	rs175860	rs3093457	rs4601986	rs666649	rs7966062
rs1017557	rs1845732	rs3093480	rs4673011	rs6765044	rs7986143
rs10191614	rs1919763	rs3093493	rs4704560	rs6795829	rs7986958
rs10192706	rs1973880	rs3093534	rs4779695	rs6826691	rs7992347
rs10243282	rs2037999	rs3093535	rs4787646	rs6826948	rs802480
rs10767570	rs2099102	rs322942	rs4792592	rs6883351	rs8042524
rs10802523	rs2102339	rs32720	rs4798537	rs698060	rs8064753
rs10846980	rs2159318	rs35699777	rs4803455	rs6993473	rs8089809
rs10932860	rs2163374	rs365515	rs4825454	rs7080901	rs8104762
rs11200087	rs2163534	rs3741007	rs4870448	rs7136439	rs902957
rs11243900	rs2205598	rs3810694	rs4925498	rs7251963	rs9288091
rs11590081	rs2217438	rs3883043	rs4929992	rs7266062	rs9305450
rs11617701	rs2292061	rs38930	rs4959539	rs7296849	rs9315143
rs11672485	rs2294069	rs3894377	rs518569	rs7309	rs9450273
rs11763147	rs2369423	rs3907818	rs5766384	rs731477	rs9519679
rs1177935	rs2522051	rs3912966	rs5771862	rs7353574	rs952092
rs1191684	rs252308	rs3912967	rs5940618	rs7388463	rs954657
rs12051139	rs2608382	rs4063769	rs5983800	rs7429010	rs9564411
rs12187185	rs265052	rs4092077	rs5983831	rs7479949	rs9644545
rs12540927	rs2834332	rs4107159	rs6031237	rs7501530	rs970263
rs12703731	rs2856229	rs4121676	rs6038639	rs7694098	rs977385
rs13164902	rs2876248	rs4235203	rs6133869	rs773325	rs9786160
rs13304286	rs2908049	rs4279783	rs625052	rs7796255	rs9786224
rs1356761	rs2941484	rs4312742	rs627461	rs7798323	rs9786240

rs1454746	rs2953818	rs4333836	rs6465305	rs779921	rs9786855
rs1512371	rs2969087	rs4427223	rs6468549	rs7812820	rs9812368
rs1514644	rs306875	rs4495225	rs6535065	rs7822979	rs982757
rs1585552	rs306883	rs4518813	rs656111	rs785143	rs993183
rs1593872	rs308837	rs4593087	rs657452	rs7874668	

Technical Support

For technical assistance, contact Verogen Technical Support.

	Contact Information
Address	11111 Flintkote Avenue San Diego CA 92121 USA
Website	www.verogen.com
Email	techsupport@verogen.com
Telephone	+1.833.837.6436 toll-free (North America) +1.858.285.4101 tel +44 (0) 208 054 8706 (United Kingdom)

Safety data sheets (SDS)–Available for download from verogen.com/documentation.

Product documentation–Available for download from verogen.com/documentation.

VEROGEN

Meet any challenge

Verogen is a dedicated developer of human identification products for sequencing and analysis of forensic genomic samples. Working closely with the forensics community, Verogen places exceptional value on flexible, scalable solutions that deliver results when you need them most.

Verogen

+1.833.837.6436 toll-free
(North America)

+1.858.285.4101
(outside North America)

techsupport@verogen.com

www.verogen.com