March 2024

ForenSeq® Kintelligence HT Kit Handbook

For sequencing and long-range kinship analysis using paired-end, dual-indexed libraries

Sample to Insight

Table of Contents

Kit Contents

ForenSeq Kintelligence HT Kit				
Catalog number				V16000190
No. of reactions				96
Reagent	**Description**	**Cap**	**Quantity**	**Storage temperature (°C)**
Box 1				
KPM	Kintelligence Primer Mix	Blue	9	2–8
NA24385	NA24385 Positive Amplification Control DNA	Black	1	2–8
PCR Additive	Additive for challenging samples	Pink	2	2–8
Box 2				
FEM	ForenSeq Enzyme Mix	Yellow	8	–25 to –15
kPCR1	Kintelligence PCR1 Reaction Mix	Green	9	–25 to –15
Box 3				
kPCR2	Kintelligence PCR2 Reaction Mix	Purple	9	–25 to –15
HP3	2 N NaOH	Orange	1	–25 to –15
HSC	Human Sequencing Control	Pink	1	–25 to –15
ProK	Proteinase K	Clear	1	–25 to –15
UDI Plate	Unique Dual Index Plate	–	1	–25 to –15
Box 4				
RSB	Resuspension Buffer	Purple	1	2–8
SPB2	Sample Purification Beads 2	Red	2	2–8

Shipping and Storage

The ForenSeq Kintelligence HT Kit is shipped in 4 boxes:

- Box 1 is shipped at 2–8°C. Upon receipt, all components in Box 1 should be stored immediately at 2–8°C in a constant-temperature refrigerator.
- Box 2 is shipped at –25°C to –15°C. Upon receipt, all components in Box 2 should be stored immediately at –25°C to –15°C in a constant-temperature freezer.
- Box 3 is shipped at –25°C to –15°C. Upon receipt, all components in Box 3 should be stored immediately at –25°C to –15°C in a constant-temperature freezer.
- Box 4 is shipped at 2–8°C. Upon receipt, all components in Box 4 should be stored immediately at 2–8°C in a constant-temperature refrigerator.

Under these conditions, the components are stable until the expiration date. The expiration date for the product is provided on the label and will vary based on the date of manufacture of the kit.

Intended Use

The ForenSeq Kintelligence HT Kit is intended for molecular biology applications in forensic, human identity, and paternity testing. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend that all users of QIAGEN® products adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

Each lot of ForenSeq Kintelligence HT Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The ForenSeq® Kintelligence HT Kit prepares up to 96 paired-end, dual-indexed libraries for sequencing and long-range kinship analysis.

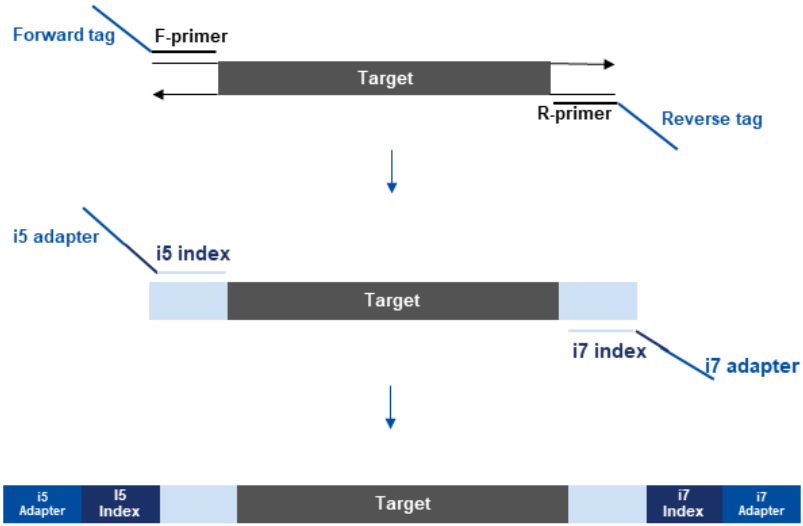

Figure 1. Assay overview.

The ForenSeq Kintelligence HT Kit offers the following features:

- A forensically relevant set of single nucleotide polymorphisms (SNPs) that is directly compatible with the ForenSeq Universal Analysis Software.
- A 1 ng input requirement for antemortem samples and a 500 pg input requirement for postmortem samples 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.

Description of protocols

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

Note: Times listed below assume a protocol consisting of 36 samples (the maximum number that can be prepared at one time). See "Important notes before starting" on page 22 for more information.

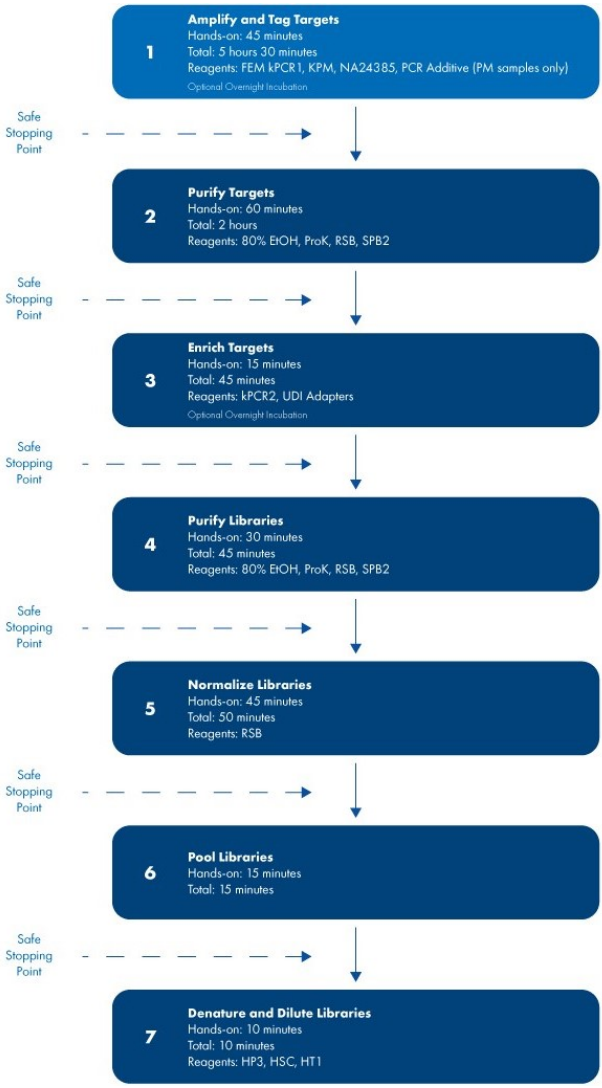

Figure 2. Overview of the ForenSeq Kintelligence HT protocol.

Acronyms

Abbreviations	Definition
EtOH	Ethanol
FEM	ForenSeq Enzyme Mix
gDNA	Genomic DNA
HP3	2 N NaOH
HSC	Human Sequencing Control
HT1	Hybridization Buffer
kPCR1	Kintelligence PCR1 Reaction Mix
kPCR2	Kintelligence PCR2 Reaction Mix
KPM	Kintelligence Primer Mix
NA24385	NA24385 Positive Amplification Control DNA
NaOH	Sodium hydroxide
ProK	Proteinase K
RSB	Resuspension Buffer
SPB2	Sample Purification Beads 2
UDI	Unique Dual Index

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment

- 20 µL pipettes
- 200 µL pipettes
- 1000 µL pipettes
- Benchtop microcentrifuge
- Magnetic stand-96 (Life Technologies, part no. AM10027)
- Microplate centrifuge
- Quantus™ Fluorometer (Promega, cat. no. E6150)
- Rubber roller
- Thermal cycler, 96-well with heated lid (see "Thermal cyclers" on page 12)
- Thermoshaker, one of the following:
  - BioShake iQ (QInstruments, item no. 1808-0506)
  - BioShake XP (QInstruments, item no. 1808-0505)
- Vortexer
- Optional: 20 µL multichannel pipettes
- Optional: 200 µL multichannel pipettes

Consumables

- 1.5 mL LoBind microcentrifuge tubes (VWR, cat. no. 80077-230)
- 15 mL conical tubes
- 20 µL barrier pipette tips
- 200 µL barrier pipette tips
- 1000 µL barrier pipette tips
- 96-well deep-well storage plates (midi plates) (Thermo Fisher Scientific, part no. AB-0765, individually sealed)
- 96-well twin.tec PCR plates, semiskirted (Eppendorf, cat. no 951020303; or VWR, cat. no. 89136-706)
- Ethyl alcohol, pure (Sigma-Aldrich, cat. no. E7023)
- Microseal 'A' sealing film (Bio-Rad, cat. no. MSA5001)
- Microseal 'B' sealing film, adhesive, optical (Bio-Rad, cat. no. MSB1001)
- MiSeq FGx® Reagent Kit (QIAGEN, cat. no. 15066817)
- Multichannel reagent reservoirs, PVC, disposable (VWR, cat. no. 89094-688)
- Nuclease-free water
- QuantiFluor® ONE dsDNA System (Promega, cat. no. E4870)

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
ABI LTI thermal cycler 9700*	9600 emulation	Heated
QIAamplifier 96-well thermal cycler	Standard	Heated, constant at 100°C
Proflex 96-well PCR System	Not applicable	Heated, constant at 100°C
Veriti 96-well thermal cycler	Standard	Heated, constant at 100°C

* Only gold heat blocks are supported.

Important Notes

DNA input recommendations

QIAGEN recommends 1 ng human genomic DNA (gDNA) input when preparing antemortem or reference samples and 500 pg gDNA input depending on sample purity when preparing postmortem or unknown samples. For challenging samples, such as partially degraded samples, you can use >1 ng gDNA input to potentially increase the number of reads, although with some bone samples, higher detection can be achieved by using as little as 50 pg of gDNA. 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, rooted hair, semen, and teeth. For antemortem samples, the input volume is 25 µL per sample; for postmortem samples, the input volume is 20 µL.

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 "Important notes before starting" on page 22.

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.

Note: The *Universal Analysis Software User Guide for Kintelligence HT Kit Module* 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.
- When adding or transferring samples, change tips between each sample.
- When adding adapters or primers, change tips between each well.
- When processing smaller sample batches of less than 96 libraries at a time, prevent aerosolization by resealing the utilized, pierced UDI wells with Microseal "B" adhesive seal. Trim excess seal with scissors.
- Do not apply Microseal "B" to unused UDI wells as this will impact the integrity of the single-use pierceable foil.

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.
- Vortex beads before use for at least 1 min, and frequently throughout the protocol to resuspend. Resuspended beads are evenly distributed and homogeneous in color.
- Aspirate and dispense beads slowly due to viscosity.
- Do not centrifuge plates and tubes containing beads, except when indicated.
- Do not let beads sit in reservoirs, and do not reuse reservoirs for beads.
- 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 (around 2 min).

Index adapter sequences

Table 1 lists the 8 bp sequences for the Unique Dual Index (UDI) adapters included in the ForenSeq Kintelligence HT Kit. Each adapter combines an Index 1 (i7) and Index 2 (i5) sequence.

- Index 1 adapter:

  `CAAGCAGAAGACGGCATACGAGAT[i7]GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA`

- Index 2 adapter:

  `/5Biosg/AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT`

Table 1. Sequences for the UDI adapters

Index name	Index 1 bases	Index 2 bases	Plate well location
UDI0001	ATCACGAT	AGCGCTAG	A1
UDI0002	CGATGTAT	GATATCGA	A2

Table 1. Sequences for the UDI adapters (continued)

Index name	Index 1 bases	Index 2 bases	Plate well location
UDI0003	TTAGGCAT	CGCAGACG	A3
UDI0004	TGACCAAT	TATGAGTA	A4
UDI0005	ACAGTGAT	AGGTGCGT	A5
UDI0006	GCCAATAT	GAACATAC	A6
UDI0007	CAGATCAT	ACATAGCG	A7
UDI0008	ACTTGAAT	GTGCGATA	A8
UDI0009	GATCAGAT	CCAACAGA	A9
UDI0010	TAGCTTAT	TTGGTGAG	A10
UDI0011	GGCTACAT	CGCGGTTC	A11
UDI0012	CTTGTAAT	TATAACCT	A12
UDI0013	AGTCAAAT	AAGGATGA	B1
UDI0014	AGTTCCAT	GGAAGCAG	B2
UDI0015	ATGTCAAT	TCGTGACC	B3
UDI0016	CCGTCCAT	CTACAGTT	B4
UDI0017	GTAGAGAT	ATATTCAC	B5
UDI0018	GTCCGCAT	GCGCCTGT	B6
UDI0019	GTGAAAAT	ACTCTATG	B7
UDI0020	GTGGCCAT	GTCTCGCA	B8
UDI0021	GTTTCGAT	AAGACGTC	B9
UDI0022	CGTACGAT	GGAGTACT	B10
UDI0023	GAGTGGAT	ACCGGCCA	B11
UDI0024	GGTAGCAT	GTTAATTG	B12

Table 1. Sequences for the UDI adapters (continued)

Index name	Index 1 bases	Index 2 bases	Plate well location
UDI0025	ACTGATAT	AACCGCGG	C1
UDI0026	ATGAGCAT	GGTTATAA	C2
UDI0027	ATTCCTAT	CCAAGTCC	C3
UDI0028	CAAAAGAT	TTGGACTT	C4
UDI0029	CAACTAAT	CAGTGGAT	C5
UDI0030	CACCGGAT	TGACAAGC	C6
UDI0031	CACGATAT	CTAGCTTG	C7
UDI0032	CACTCAAT	TCGATCCA	C8
UDI0033	CAGGCGAT	CCTGAACT	C9
UDI0034	CATGGCAT	TTCAGGTC	C10
UDI0035	CATTTTAT	AGTAGAGA	C11
UDI0036	CCAACAAT	GACGAGAG	C12
UDI0037	CGGAATAT	AGACTTGG	D1
UDI0038	CTAGCTAT	GAGTCCAA	D2
UDI0039	CTATACAT	CTTAAGCC	D3
UDI0040	CTCAGAAT	TCCGGATT	D4
UDI0041	GACGACAT	CTGTATTA	D5
UDI0042	TAATCGAT	TCACGCCG	D6
UDI0043	TACAGCAT	ACTTACAT	D7
UDI0044	TATAATAT	GTCCGTGC	D8
UDI0045	TCATTCAT	AAGGTACC	D9
UDI0046	TCCCGAAT	GGAACGTT	D10

Table 1. Sequences for the UDI adapters (continued)

Index name	Index 1 bases	Index 2 bases	Plate well location
UDI0047	GTTCCAAT	AATTCTGC	D11
UDI0048	ACCTTGGC	GGCCTCAT	D12
UDI0049	ATATCTCG	ATCTTAGT	E1
UDI0050	GCGCTCTA	GCTCCGAC	E2
UDI0051	AACAGGTT	ATACCAAG	E3
UDI0052	GGTGAACC	GCGTTGGA	E4
UDI0053	CAACAATG	CTTCACGG	E5
UDI0054	TGGTGGCA	TCCTGTAA	E6
UDI0055	AGGCAGAG	AGAATGCC	E7
UDI0056	GAATGAGA	GAGGCATT	E8
UDI0057	TGCGGCGT	CCTCGGTA	E9
UDI0058	CATAATAC	TTCTAACG	E10
UDI0059	GATCTATC	ATGAGGCT	E11
UDI0060	AGCTCGCT	GCAGAATC	E12
UDI0061	CGGAACTG	CACTACGA	F1
UDI0062	TAAGGTCA	TGTCGTAG	F2
UDI0063	TTGCCTAG	ACCACTTA	F3
UDI0064	CCATTCGA	GTTGTCCG	F4
UDI0065	ACACTAAG	ATCCATAT	F5
UDI0066	GTGTCGGA	GCTTGCGC	F6
UDI0067	TTCCTGTT	AGTATCTT	F7
UDI0068	CCTTCACC	GACGCTCC	F8

Table 1. Sequences for the UDI adapters (continued)

Index name	Index 1 bases	Index 2 bases	Plate well location
UDI0069	GCCACAGG	CATGCCAT	F9
UDI0070	ATTGTGAA	TGCATTGC	F10
UDI0071	ACTCGTGT	ATTGGAAC	F11
UDI0072	GTCTACAC	GCCAAGGT	F12
UDI0073	CAATTAAC	CGAGATAT	G1
UDI0074	TGGCCGGT	TAGAGCGC	G2
UDI0075	AGTACTCC	AACCTGTT	G3
UDI0076	GACGTCTT	GGTTCACC	G4
UDI0077	TGCGAGAC	CATTGTTG	G5
UDI0078	CATAGAGT	TGCCACCA	G6
UDI0079	ACAGGCGC	CTCTGCCT	G7
UDI0080	GTGAATAT	TCTCATTC	G8
UDI0081	AACTGTAG	ACGCCGCA	G9
UDI0082	GGTCACGA	GTATTATG	G10
UDI0083	CTGCTTCC	GATAGATC	G11
UDI0084	TCATCCTT	AGCGAGCT	G12
UDI0085	AGGTTATA	CAGTTCCG	H1
UDI0086	GAACCGCG	TGACCTTA	H2
UDI0087	CTCACCAA	CTAGGCAA	H3
UDI0088	TCTGTTGG	TCGAATGG	H4
UDI0089	TATCGCAC	CTTAGTGT	H5
UDI0090	CGCTATGT	TCCGACAC	H6

Table 1. Sequences for the UDI adapters (continued)

Index name	Index 1 bases	Index 2 bases	Plate well location
UDI0091	GTATGTTC	AACAGGAA	H7
UDI0092	ACGCACCT	GGTGAAGG	H8
UDI0093	TACTCATA	CCTGTGGC	H9
UDI0094	CGTCTGCG	TTCACAAT	H10
UDI0095	TCGATATC	ACACGAGT	H11
UDI0096	CTAGCGCT	GTGTAGAC	H12

Unanalyzed SNPs

The following SNPs are included in KPM, but are not analyzed in Universal Analysis Software (UAS). QIAGEN 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	

Protocol

This chapter describes the ForenSeq Kintelligence HT 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 "Description of protocols" on page 7.

Before starting, confirm kit contents and make sure that you have the necessary reagents, consumables, and equipment. For a list of items, see "Equipment and Reagents to Be Supplied by the User" on page 10.

Important: Do not reuse UDI adapters to minimize the likelihood of pooling samples with the same UDI or cross- contaminating UDIs in the UDI plate.

The kit includes 96 UDI adapters. After preparing libraries, cover the used UDIs with Microseal 'B' before re-storing the plate at the appropriate temperature. For more information, see "Index adapter sequences" on page 15.

Important points before starting

- You can process a minimum of 12 and a maximum of 36 samples at a time including positive and negative amplification controls. This decreases the likelihood of inaccuracies due to incubation times.
- QIAGEN recommends preparing postmortem and antemortem samples in separate batches. This decreases the likelihood of inaccuracies due to differences in the workflow such as incubation times.
- Plan on sequencing up to 12 libraries of postmortem samples or 36 libraries of antemortem samples on a MiSeq FGx® standard flow cell: one positive amplification control, one negative amplification control, and 10 postmortem or 34 antemortem samples. You can

prepare 36 libraries and split them between 3 flow cells for postmortem samples or one flow cell for antemortem samples, if desired.

Protocol step 1: 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)
- PCR Additive (for postmortem samples only)
- NA24385 (NA24385 Positive Amplification Control DNA)
- Input gDNA
- Nuclease-free water
- 96-well PCR plate, semi-skirted
- 1.5 mL LoBind microcentrifuge tubes (3)
- Microseal 'A' film

About reagents

- Do not vortex NA24385, FEM, PCR Additive, or input gDNA.
- Prepare fresh dilutions of NA24385 every time.

Preparation

1. Prepare the following consumables:

Item	Storage temperature (°C)	Instructions
NA24385	2–8	Let stand for 30 min to bring to room temperature. Invert 3 times to mix, and then centrifuge briefly.
kPCR1	–25 to –15	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
KPM	2–8	Let stand for 30 min (or longer if necessary) to bring to room temperature. Vortex to mix, and then centrifuge.
FEM	–25 to –15	Thaw at room temperature, and then centrifuge briefly. Return to storage immediately after use.
PCR Additive*	2–8	Let stand for 30 min to bring to room temperature. Invert 3 times to mix, and then centrifuge briefly.

* Applicable only for postmortem samples.

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

- 68°C for 10 min
- Hold at 4°C

Table 2. Lid temperature and ramp modes

Thermal cycler	Temperature mode	Lid temperature (°C)	Ramp mode
ABI LTI Thermal Cycler 9700	9600 emulation	105	8%
Proflex 96-Well PCR System	Not applicable	100	0.2°C/s
QIAamplifier 96-Well Thermal Cycler	Standard	100	0.1°C/s
Veriti 96-Well Thermal Cycler	Standard	100	4%

Note: Total program time is approximately 4.5 h and can continue overnight.

3. Label tubes and plates as follows:

Vessel	Label
1.5 mL tube	Master Mix
1.5 mL tube	Control DNA Dilution 1
1.5 mL tube	Control DNA Dilution 2
PCR plate	ForenSeq Sample Plate

Procedure

Antemortem samples

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)

   As an example, for 12 samples, prepare 330 µL Master Mix with 244.2 µL kPCR1, 66 µL KPM, and 19.8 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. To prepare the positive amplification control, 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 for 30 s at 100 × *g*.
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 2–8°C for up to 2 days. Alternatively, leave the thermal cycler on overnight.

Postmortem samples

1. Using nuclease-free water, dilute 500 pg gDNA to 25 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)
   - PCR Additive (5 µL)
   - FEM (1.5 µL)

   As an example, for 12 samples, prepare 396 µL Master Mix using 244.2 µL kPCR1, 66 µL KPM, 66 µL PCR Additive solution, and 19.8 µL FEM.
3. Pipette to mix, and then cap and centrifuge briefly.

4. Add 30 µL Master Mix to each sample well of the ForenSeq Sample Plate.
5. To prepare the positive amplification control, 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 80 µL 25 pg/µL NA24385:
        - 400 pg/µL NA24385 (5 µL)
        - Nuclease-free water (75 µL)
    d. Gently pipette to mix, and then cap and centrifuge briefly.
6. Add 20 µL 25 pg/µL gDNA to each sample well of the ForenSeq Sample Plate. Pipette to mix.
7. Add 20 µL 25 pg/µL NA24385 to each positive amplification control well. Pipette to mix.
8. Add 20 µL nuclease-free water to each negative amplification control well. Pipette to mix.
9. Seal and centrifuge for 30 s at 100 × *g*.
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 2–8°C for up to 2 days. Alternatively, leave the thermal cycler on overnight.

Protocol step 2: 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 temperature (°C)	Instructions
ProK	–25 to –15	Let stand for 30 min to bring to room temperature. Invert to mix, and then centrifuge.
RSB	2–8	Let stand for 30 min (or longer if necessary) to bring to room temperature. Vortex and invert to mix.
SPB2	2–8	Let stand for 30 min (or longer if necessary) to bring to room temperature. Immediately prior to dispensing into midi plate, vortex for ≥1 min, and invert to mix.

2. Prepare the appropriate volume of fresh 80% EtOH from absolute ethanol:
   - If you plan to complete "Protocol step 4: Purify libraries" within the day, prepare 1.5 mL per sample.
   - If you plan to stop before starting "Protocol step 4: 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 15 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 min and invert several times to mix. Immediately dispense into the midi plate.

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 min.
5. Incubate at room temperature for 10 min.
6. Place on the magnetic stand and wait until the liquid is clear (approximately 5 min).
7. Centrifuge at 100 × *g* for 30 s.
8. Place on the magnetic stand and wait until the liquid is clear (approximately 1 min).
9. **Important**: 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. Avoid disturbing the bead pellet.
    b. Incubate for 30 s.
    c. Remove and discard all supernatant.
11. Wash a second time.
12. Seal and centrifuge for 30 s at 100 × *g*.
13. Place on the magnetic stand and wait until the liquid is clear (approximately 1 min).

14. **Important**: With a 20 µL pipette, remove all residual EtOH from each well.
15. Remove from the magnetic stand.
16. Add 30 µL RSB to the bottom of each sample well.
17. Seal and shake for 2 min at 1800 rpm.
18. **Important**: If the beads are not fully resuspended, pipette to mix or reshake for 2 min at 1800 rpm.
19. Incubate at room temperature for 2 min.
20. Centrifuge for 30 s at 100 × *g*.
21. Place on the magnetic stand and wait until the liquid is clear (approximately 5 min).
22. Transfer 28 µL supernatant from each well of the Purification Bead Plate 1 to a fresh well in the same plate.

    Note: Some bead carryover into the second cleanup is normal.

Perform second cleanup

1. Remove from the magnetic stand and add 45 µL ProK/SPB2 to each sample well.
2. Seal and shake at 1800 rpm for 2 min.
3. Incubate at room temperature for 5 min.
4. Place on the magnetic stand and wait until the liquid is clear (approximately 2 min).
5. Centrifuge for 30 s at 100 × *g*.
6. Place on the magnetic stand and wait until the liquid is clear (approximately 1 min).
7. **Important**: 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. Avoid disturbing the bead pellet.
    b. Incubate for 30 s.
    c. Remove and discard all supernatant.
9. Wash a second time.
10. Seal and centrifuge for 30 s at 100 × *g*.
11. Place on the magnetic stand and wait until the liquid is clear (around 1 min).
12. **Important**: With a 20 µL pipette, remove all residual EtOH from each well.
13. Remove from the magnetic stand.
14. Add 27 µL RSB to the bottom of each sample well.
15. Seal and shake for 2 min at 1800 rpm.
16. **Important**: If the beads are not fully resuspended, pipette to mix or reshake for 2 min at 1800 rpm.
17. Incubate at room temperature for 2 min.
18. Centrifuge for 30 s at 100 × *g*.
19. Place on the magnetic stand and wait until the liquid is clear (approximately 5 min).
20. 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.

Protocol step 3: 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 temperature (°C)	Instructions
kPCR2	–25 to –15	Let stand for 30 min (or longer if necessary) to bring to room temperature. Vortex to mix, and then centrifuge.
UDI adapters	–25 to –15	Remove the adapter plate. Let stand for 30 min (or longer if necessary) to bring to room temperature. Shake at 1800 rpm for 2 min, and then centrifuge.

2. Save the following kPCR2 program on the thermal cycler. See Table 3 on the facing page for lid temperatures.
    - Choose the preheat lid option and set to applicable temperature
    - 98°C for 30 s

- 15 cycles of:
  - 98°C for 20 s
  - 66°C for 30 s
  - 72°C for 30 s
- 72°C for 1 min
- Hold at 4°C

Table 3. Lid temperature settings for supported thermal cyclers

Thermal cycler	Lid temperature (°C)
ABI LTI Thermal Cycler 9700	105
Proflex 96-Well PCR System	100
QIAamplifier 96-Well Thermal Cycler	100
Veriti 96-Well Thermal Cycler	100

Note: Total program time is approximately 30 min.

Procedure

1. Seal and centrifuge the Purified Targets Plate for 30 s at 100 × *g*.
2. Add 5 µL UDI adapter to each sample well. See Table 4 (next page) for the plate layout.

   Note: 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, avoiding excess carry-over of viscous kPCR2 to the sample wells. Pipette to mix.
5. Seal and centrifuge for 30 s at 100 × *g*.
6. Place on the preprogrammed thermal cycler and run the kPCR2 program.

Table 4. UDI plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI0001	UDI0002	UDI0003	UDI0004	UDI0005	UDI0006	UDI0007	UDI0008	UDI0009	UDI0010	UDI0011	UDI0012
B	UDI0013	UDI0014	UDI0015	UDI0016	UDI0017	UDI0018	UDI0019	UDI0020	UDI0021	UDI0022	UDI0023	UDI0024
C	UDI0025	UDI0026	UDI0027	UDI0028	UDI0029	UDI0030	UDI0031	UDI0032	UDI0033	UDI0034	UDI0035	UDI0036
D	UDI0037	UDI0038	UDI0039	UDI0040	UDI0041	UDI0042	UDI0043	UDI0044	UDI0045	UDI0046	UDI0047	UDI0048
E	UDI0049	UDI0050	UDI0051	UDI0052	UDI0053	UDI0054	UDI0055	UDI0056	UDI0057	UDI0058	UDI0059	UDI0060
F	UDI0061	UDI0062	UDI0063	UDI0064	UDI0065	UDI0066	UDI0067	UDI0068	UDI0069	UDI0070	UDI0071	UDI0072
G	UDI0073	UDI0074	UDI0075	UDI0076	UDI0077	UDI0078	UDI0079	UDI0080	UDI0081	UDI0082	UDI0083	UDI0084
H	UDI0085	UDI0086	UDI0087	UDI0088	UDI0089	UDI0090	UDI0091	UDI0092	UDI0093	UDI0094	UDI0095	UDI0096

Safe stopping point

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

Important: When processing fewer than 96 libraries, reseal only the used or pierced UDI plate wells with Microseal "B". Do not seal unpierced or unused UDI wells as this will impact the integrity of the single-use pierceable foil. The remaining UDIs can be utilized with subsequent library preparations.

Protocol step 4: 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 temperature (°C)	Instructions
ProK	–25 to –15	Let stand for 30 min to bring to room temperature. Invert to mix, and then centrifuge.
RSB	2–8	Let stand for 30 min (or longer if necessary) to bring to room temperature. Vortex and invert to mix.
SPB2	2–8	Let stand for 30 min (or longer if necessary) to bring to room temperature. Immediately prior to dispensing into midi plate, vortex for ≥1 min, 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

* This procedure assumes you are reusing the same midi plate from "Protocol step 2: Purify targets".

4. Prepare the SPB2 tube for a first or subsequent use:
   - For first-time use, add 15 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 min and invert several times to mix. Immediately dispense into the midi plate.

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 for 2 min at 1800 rpm.
4. Incubate at room temperature for 5 min.
5. Place on the magnetic stand and wait until the liquid is clear (approximately 5 min).
6. **Important**: 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. Avoid disturbing the bead pellet.
    b. Incubate for 30 s.
    c. Remove and discard all supernatant.
8. Wash a second time.
9. Seal and centrifuge for 30 s at 100 × *g*.
10. Place on the magnetic stand and wait until the liquid is clear (approximately 1 min).
11. **Important**: With a 20 µL pipette, remove all residual EtOH from each well.
12. Remove from the magnetic stand.
13. Add 52.5 µL RSB to the bottom of each sample well.
14. Seal and shake for 2 min at 1800 rpm.
15. **Important**: If the beads are not fully resuspended, pipette to mix or reshake for 2 min at 1800 rpm.
16. Incubate at room temperature for 2 min.

17. Centrifuge for 30 s at 100 × *g*.
18. Place on the magnetic stand and wait until the liquid is clear (around 5 min).
19. Transfer 50 µL supernatant from each well of the Purification Bead Plate 2 to the corresponding well of the Purified Library Plate.
20. Seal and centrifuge for 30 s at 100 × *g*.

Safe stopping point

If you are stopping, seal the plate and store at –25°C to –15°C for up to 1 year.

Protocol step 5: 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:
  - 1.5 mL LoBind microcentrifuge tube
  - 96-well PCR plate, skirted or semi-skirted

Preparation

1. Prepare the following consumable:

Item	Storage temperature (°C)	Instructions
RSB	2–8	Let stand for 30 min (or longer if necessary) to bring to room temperature. Vortex and invert to mix.

2. Label the applicable vessel:

Vessel	Label
1.5 mL tube	Normalized Library
PCR plate	Normalized Library Plate

Procedure

Antemortem samples

1. Place the Purified Library Plate on the magnetic stand.
2. Quantify libraries using a fluorometric method, such as QuantiFluor ONE (recommended), AccuClear®, 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 the facing page.

   a. Use the formula

   $$V_2 = \frac{C_1 V_1}{C_2}$$

   where V_2 is the final volume of diluted library, C_1 is the library quantification result, V_1 is the undiluted library at 8 µL, and C_2 is 0.75 ng/µL.

   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.

   Note: 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.

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.

Postmortem Samples

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 > 0.45 ng/µL, prepare RSB to dilute each library to 0.45 ng/µL as follows. For additional guidance, see "Example calculations" below.

   a. Use the formula

$$V_2 = \frac{C_1 V_1}{C_2}$$

   where V_2 is the final volume of diluted library, C_1 is the library quantification result, V_1 is the undiluted library at 8 µL, and C_2 is 0.45 ng/µL.

   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.

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 0.45 ng/µL libraries.

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.

Example Calculations

DNA input	Library concentration C_1 (ng/µL)	Library volume V_1 (µL)	Normalized library concentration C_2 (ng/µL)	RSB Volume $V_2 - V_1$ (µL)
1 ng gDNA	1.5	8	0.75	8
Negative control	0.25	8	0.25	0

Protocol step 6: 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.

Consumables

- 1.5 mL LoBind microcentrifuge tube
- Microseal 'B' film

Preparation

1. Select ≤12 postmortem or ≤36 antemortem libraries to pool for sequencing.

   Note: The maximum number of ForenSeq Kintelligence HT libraries that a standard flow cell supports is 12 postmortem or 36 antemortem.

2. Label a new 1.5 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.

Protocol step 7: 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.

Important: 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
- 1.5 mL LoBind microcentrifuge tubes (4)

Preparation

1. Prepare the reagent cartridge per instructions in the *MiSeq FGx Sequencing System Reference Guide*.

2. Prepare the following consumables:

Item	Storage temperature (°C)	Instructions
ProK	–25 to –15	Thaw at room temperature. Vortex to mix, and then centrifuge briefly.
RSB	–25 to –15	Let stand for 30 min to bring to room temperature. Invert to mix, and then centrifuge.
SPB2	–25 to –15	Thaw at room temperature, and then vortex to mix.

3. Label 4 new 1.5 mL tubes as follows.
   - 12 pM Denatured Library
   - 20 pM Denatured Library
   - Denatured HSC
   - 0.2 N NaOH

Procedure

Antemortem samples

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.

   Note: 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. Pipette to mix, cap, and then centrifuge briefly.

   Important: Do not vortex HSC with 0.2 N NaOH.

5. Incubate at room temperature for 5 min.
6. Add 36 µL HT1 to the Denatured HSC tube.

   Note: You can store denatured HSC at room temperature for ≤1 day.

7. Pipette to mix.
8. 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)
9. Pipette to mix, cap, and then centrifuge briefly.

   Important: Do not vortex Pooled Libraries with 0.2 N NaOH.

10. Incubate at room temperature for 5 min.
11. Add 990 µL HT1 to the 20 pM Denatured Library tube to prepare 1 mL 20 pM denatured library.

    Note: You can store the 20 pM denatured library at –15°C to –25°C for ≤3 weeks.

12. 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)

13. Pipette to mix, cap, and then centrifuge briefly.
14. Immediately transfer the entire volume to the reagent cartridge per instructions in the *MiSeq FGx Sequencing System Reference Guide*.

Postmortem samples

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 h.

    Note: 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. Pipette to mix, cap, and then centrifuge briefly.

    Important: Do not vortex HSC with 0.2 N NaOH.
5. Incubate at room temperature for 5 min.
6. Add 36 µL HT1 to the Denatured HSC tube.

    Note: You can store denatured HSC at room temperature for ≤1 day.
7. Pipette to mix.
8. In the 20 pM Denatured Library tube, combine the following volumes:
    - 0.45 ng/µL library pool (5 µL)
    - 0.2 N NaOH (5 µL)

9. Pipette to mix, cap, and then centrifuge briefly.

   Important: Do not vortex Pooled Libraries with 0.2 N NaOH.

10. Incubate at room temperature for 5 min.

11. Add 490 µL HT1 to the 20 pM Denatured Library tube to prepare 0.5 mL 20 pM denatured library. Pipette to mix.

    Note: You can store the 20 pM denatured library at –15°C to –25°C for ≤3 weeks.

12. 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 (236 µL)
    - Denatured HSC (4 µL)

13. Pipette to mix, cap, and then centrifuge briefly.

14. Immediately transfer the entire volume to the reagent cartridge per instructions in the *MiSeq FGx Sequencing System Reference Guide*.

Technical Support

For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
ForenSeq Kintelligence HT Kit (96)	Prepares up to 96 paired-end, dual-indexed libraries for sequencing and long-range kinship analysis.	V16000190
	Related product	
MiSeq FGx Reagent Kit	Supports up to 12.5 million paired-end reads for deep sequencing or high-throughput sample processing	15066817

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Document Revision History

Date	Changes
03/2024	Initial release

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