Instructions For Use

Version: 1.1 *Ref: IFU-OmniSNP* *Revision date: 2024-01-17*

OmniSNP™

Identity Informative SNP Typing

For MPS Library Prep by Reverse Complement PCR

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

IDseek® OmniSNP™ Identity Informative SNP Typing Kit

REF IDS-SNP96

Research Use Only

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Symbols Used on Product Labels and in Instruction For Use

Symbol	Description
	Manufacturer
	Use-by date
	Lot number
	Reference number
	Temperature limit for storage
	Contains sufficient for <*n*> tests
	Matrix code containing the reference number, lot number and use-by date

Product Description

Forensic DNA profiling utilizes high-variable human identity SNPs, to discern small differences both within a population and among different populations.

IDseek® OmniSNP™ Identity Informative SNP Typing kit provides multiplex amplicon-based MPS library preparation for sequencing 85 short regions containing highly variable single nucleotide polymorphism (SNP) positions. This Reverse Complement Polymerase Chain Reaction (RC-PCR) based library prep kit contains all reagents to generate Illumina compatible libraries in a simple, sensitive, robust and safe method for cost-effective and high-quality SNP analysis. The short amplicon range makes the assay particularly useful for analysis with highly degraded DNA samples.

IDseek® OmniSNP™ Targets		
rs1005533	rs1886510	rs6955448
rs10092491	rs1979255	rs7041158
rs1015250	rs2040411	rs717302
rs1024116	rs2046361	rs719366
rs1028528	rs2056277	rs722098
rs1031825	rs2076848	rs722290
rs10488710	rs2107612	rs727811
rs1058083	rs2111980	rs729172
rs10773760	rs214955	rs733164
rs10776839	rs221956	rs735155
rs1109037	rs2269355	rs740598
rs13182883	rs2342747	rs740910
rs13218440	rs251934	rs763869
rs1335873	rs279844	rs8037429
rs1336071	rs2830795	rs8078417
rs1355366	rs2831700	rs826472
rs1357617	rs2920816	rs873196
rs1360288	rs321198	rs876724
rs1382387	rs354439	rs891700
rs1413212	rs3780962	rs901398
rs1454361	rs430046	rs907100
rs1463729	rs4364205	rs917118
rs1490413	rs445251	rs938283
rs1493232	rs4606077	rs964681
rs1498553	rs560681	rs987640
rs1523537	rs576261	rs9905977
rs1528460	rs6444724	rs993934
rs159606	rs6811238	rs9951171
rs1821380		

Reverse Complement PCR Kit Contents

NimaGen Part# IDS-SNP96 (store at -20 °C)	Contents
IDseek® OmniSNP™ Probe Panel (REF: PM-IDS-SNPID)	1x Tube (24 µL) ●
2x PCR Master Mix (Hot Start HiFi) (REF: MMHS096)	1x Tube (1150 µL) ○
IDS Probe Dilution Buffer (REF: PDB-IDS)	1x Tube (216 µL) ●

Required Materials, Not Included

Description	Vendor
Index Primer Plate, dehydrated. Choose one of the 4 available EasySeq™ Unique Dual Index plates for Illumina. Available REF: IDX96-U01, IDX96-U02, IDX96-U03, IDX96-U04. **Note:** The index sequences are available from the download section of the NimaGen website.	NimaGen
Adjustable Pipette Set (P10, P20, P100, P200, P1000)	Multiple Vendors
TapeStation, Bioanalyzer Instrument, incl. consumables.	Agilent
Ethanol Absolute, Molecular Biology Grade	Multiple Vendors
AmpliClean™ or AMPure XP Bead Solution	NimaGen / Beckman Coulter
General plasticware, DNAse free (1.5 mL tubes, pipette tips etc.)	Multiple Vendors
Mini Spinner for 1.5 mL tubes and 8-well PCR strips or PCR plates	Multiple Vendors
Magnetic stand for 1.5 mL tubes and/or 96-wells plates	Multiple Vendors / NimaGen
Water, PCR Grade	Multiple Vendors
Qubit Fluorometer incl. High Sensitivity consumables	Thermo Fisher Scientific
Thermocycler with heated lid, (0.2 mL standard PCR tubes), compatible with semi-skirted ABI style PCR plates and option for ramp rate programming. **Note:** Kit is validated for Applied Biosystems™ Veriti™, MiniAmp™ and SimpliAmp™ Thermal Cyclers.	Multiple Vendors
NaOH Solution (2 N), MPS grade	Multiple Vendors
Tris-HCl (200 mM), pH 7	Multiple Vendors
Low TE (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)	Multiple Vendors
Illumina MPS Sequencing Instrument (MiSeq®/MiSeq FGx®)	illumina®/ Verogen®
Illumina MiSeq® Reagent kit v2 (300-cycle) Or Illumina MiSeq® Reagent kit v3 (300-cycle) Or Verogen MiSeq FGx® Reagent Kit Or Verogen MiSeq FGx® Reagent Micro Kit	illumina®/ Verogen®

General Precautions

Read the Material Safety Data Sheet (MSDS) and follow the handling instructions. Adhere to good laboratory practice when handling both the reagents supplied in this kit and other reagents required.

Use a Pre-PCR environment for setting up the RC-PCR. Sample pooling, purification and library quantification should be performed in a Post-PCR environment.

Protocol

1. Thermocycling Program

Temp	Duration	Ramp Rate (from previous step)	Cycles
98 °C	2 minutes	N/A	1 x
98 °C	10 seconds	Max	1 x
58 °C	10 minutes	0.1 °C/sec (or 2% of Max)	
68 °C	1 minute	Max	
95 °C	10 seconds	Max	2 x
80 °C	1 second	Max	
58 °C	90 minutes	0.1 °C/sec (or 2% of Max)	
68 °C	1 minute	Max	
95 °C	10 seconds	Max	38 x
80 °C	1 second	Max	
58 °C	2 minutes	0.5 °C/sec (or 10% of Max)	
68 °C	30 seconds	Max	
68 °C	1 minute	Max	1 x
16 °C	∞	Max	1 x

Heated lid at 105 °C.

Note: This protocol takes approximately 6-7 hours to complete, but may vary per thermal cycler used. When running this protocol for the first time, start the cycling program as a dummy run, to check the predicted duration of 6-7 hours.

2. Reverse Complement PCR

In a single, closed tube reaction, the target specific RC-probes are working as a template to extend the UDI primers to synthesize functional, tailed and indexed PCR primers. This will be followed by two long hybridization/extension steps of 90 minutes and subsequently a further DNA amplification of the target regions, meanwhile synthesizing more primers.

2.1 Thaw on ice:

- RC-PCR Probe Panel (Black cap)
- Probe Dilution Buffer (Green cap)
- HiFi Master Mix (White cap)

Note: The HiFi Master Mix contains iso-stabilizers and may not freeze completely, even when stored at -15 °C to -25 °C. It may contain precipitates when thawed at +2 °C to +8 °C. Always ensure that the Master Mix is fully thawed and thoroughly mixed before use.

2.2. Take the IDX PCR plate of choice and break off the number of strips needed.

Note: Register the indexes used (IDX set/strip-column number and well position for each sample). Download the index details for setting up the Illumina sample sheet.

Note: Before breaking off 8-well strips, cut the seal at the breaking line with a sharp knife.

2.3. Prepare in a fresh 1.5 mL tube the RC-PCR mix by combining and mixing:

- 0.2 µL RC-PCR Probe Panel per reaction (Black cap)
- 1.8 µL Probe Dilution Buffer per reaction (Green cap)
- 10 µL HiFi Master Mix per reaction (White cap)

Example: 24 samples + 10% extra volume*

- 5.28 µL RC-PCR Probe Panel
- 47.52 µL Probe Dilution Buffer
- 264 µL HiFi Master Mix

*It is recommended to allow for a 10% excess when preparing the RC-PCR mix to correct for any pipetting loss. The kit contains extra reagent to facilitate this.

2.4. Remove the seal from the PCR plate or strip(s).

2.5. Dispense 12 µL of the RC-PCR mix (from step 2.3) to each well of the plate/strip(s).

2.6. Add to each well: 8 µL of DNA solution (optimal: 1 ng total DNA input).

2.7. Close the tube strips **carefully** with the caps provided, there should be an audible click. Mix by short vortexing, followed by a quick spin. Verify that the colour of the reaction mix is homogenously pink.

2.8. Place the samples in the thermal cycler(s) and start the RC-PCR program.

After the RC-PCR, samples have been amplified and tagged with sample-specific indexes and sequencing adapters. From this point, RC-PCR product purification is performed using a magnetic bead based purification to remove primers, dimers and salts.

3. Purification

The purification involves one-sided size selection using magnetic beads, minimizing the number of reads lost to residual primers and primer-dimers. The input quality and quantity of your samples will impact the PCR yield. Samples can be pooled based on the total input quantity of the PCR to ensure low-input samples have appropriate read depth.

3.1. Bring the magnetic bead solution (AmpliClean™ or AMpure XP) to room temperature.

3.2. Create three separate pools based on the input quantity of each reaction. Pool 10 µL RC-PCR product from each reaction in the same input range into a 1.5 mL tube.

- Pool 1: ± 1000 – 250 pg
- Pool 2: ± 250 – 64 pg
- Pool 3: < 64 pg and NTC

Note: Perform all subsequent steps for each pool individually.

Note: Before pooling, optionally check the unpurified PCR products on agarose.

3.3. Mix well and transfer 40 µL of this pool to a new 1.5 mL tube.

3.4. Add 60 µL Low TE buffer or molecular grade H_2O to the tube and mix well (total volume is now 100 µL).

3.5. Bead purification:

Purification

a. Vortex the beads thoroughly to resuspend.
b. Add 100 µL bead solution to the 100 µL pool (from step 3.4) and mix well immediately by pipetting up and down at least 5 times or by short vortexing.
c. Incubate for 5 minutes.

On magnet:
d. Place the tube for 3 minutes on the magnet, or until the solution is fully cleared.

e. Remove and discard the liquid carefully, without disturbing the beads.
f. Add 300 µL (freshly prepared) 75% ethanol, without disturbing the beads.
g. Wait for 1 minute.

h. Repeat steps **e.**, **f.** and **g.** for a second ethanol wash step.
i. Carefully remove all liquid without leaving traces of ethanol. (Optional: quick spin, then place the tube **back on the magnet** and remove the last traces of ethanol).
j. Dry with open cap for 2-3 minutes at room temperature. **Do not over-dry.** Immediately continue with the Elution.

3.6. Elution:

a. **On magnet:** Add 40 µL Low TE buffer to the tube and close the tube.
b. **Off magnet:** Resuspend the beads by flicking, or by short vortexing.
c. **Off magnet**: Incubate for 2 minutes.
d. **On magnet**: Wait for 3-5 minutes, or until the solution is fully cleared.
e. **On magnet:** Carefully bring 30 µL of the clear solution into a new 1.5 mL tube, making sure not to transfer any of the beads.

The libraries are now ready for a quantitative and qualitative check, followed by NGS.

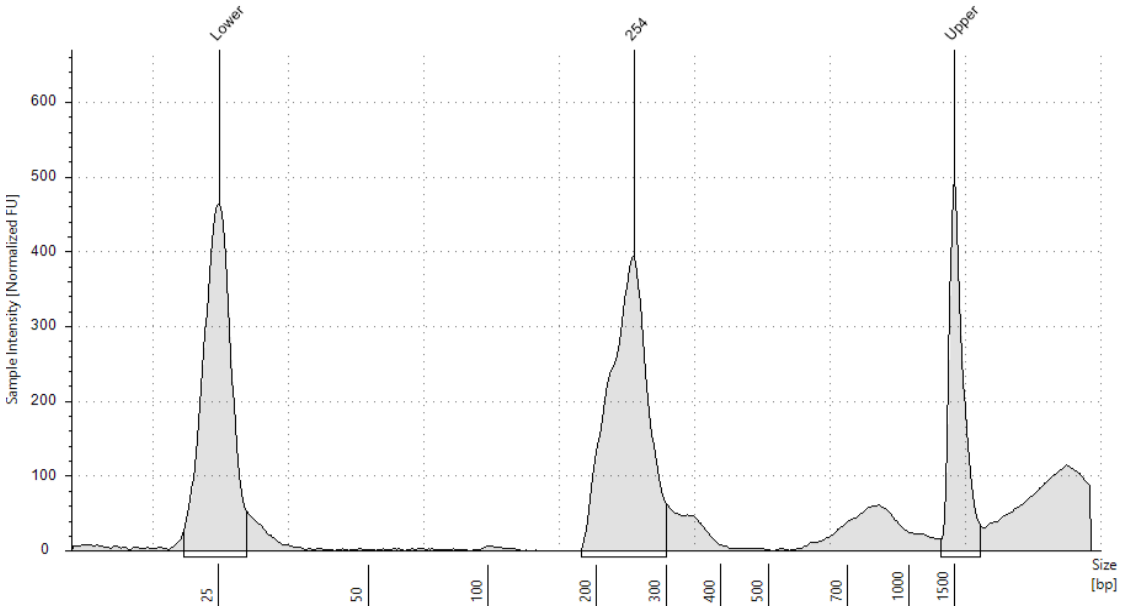
4. Sequencing

4.1. Determine the final concentration of the library or libraries by a double Qubit (HS) measurement:

- **a.** Bring the Qubit reagents to room temperature.
- **b.** Label the Qubit tubes on the lid according to the number of samples to be used plus 2 standards.
- **c.** Dilute the Qubit dsDNA HS Reagent 1:200 in Qubit dsDNA HS Buffer for each sample/ standard. It is recommended to allow for >10% excess when preparing the working solution to correct for any pipetting loss.
- **d.** For the standards: mix 190 µL of the working solution with 10 µL of the standard.
- **e.** For the samples: mix 180-199 µL of the working solution with 1-20 µL sample (total 200 µL).
- **f.** Vortex the tubes thoroughly and incubate the tubes for 2 minutes.
- **g.** Measure the standards and the samples using the 'dsDNA High Sensitivity' settings making sure to select the correct sample volume used in step **e.**.

4.2. **Optional but recommended:** Perform a qualitative verification of the library on TapeStation or Bioanalyzer, according to the manufacturer's protocol. If needed, dilute the pool. E.g. for TapeStation High Sensitivity kit, dilute to ~2 ng/µL.

Example of a clean library on TapeStation:



4.3. Perform sequencing on an Illumina® platform or MiSeq™ FGx in research mode, according to the manufacturer's manual.

- **a.** We recommend a length of 220 bp for calculating library molarity.
- **b.** We advise to maintain a minimal read depth of 80.000 reads per sample for a minimal of 100x coverage.
- **c.** A spike-in of 5% PhiX is recommended for QC purposes.
- **d.** We advise to start with a lower loading concentration for the initial sequence run and adjust in subsequent runs if needed. This avoids overclustering and potentially failure of the run. For the MiSeq a concentration of 8 pM is recommended. See table 1 for sequencing guidelines.

Table 1 Illumina sequencer and sample multiplexing guidelines

Sequencer	Reagent kit	Run setup	Number of samples*	Paired-end reads	Library concentration**
MiSeq	V2 Nano 300 cycles	151-10-10-151	12	1 million	8 pM
MiSeq	V2 Micro 300 cycles	151-10-10-151	48	4 million	8 pM
MiSeq	V2 300 cycles	151-10-10-151	176	15 million	8 pM
MiSeq	V3 300 cycles	151-10-10-151	301	25 million	8 pM
MiSeq	MiSeq FGx® Reagent Kit	151-10-10-151	301	25 million	8 pM
MiSeq	MiSeq FGx® Reagent Micro Kit	151-10-10-301	96	8 million	8 pM

* Pristine samples of recommended input.
** See point **4.3.d.**

Data Analysis

The IDseek® OmniSNP™ kit does not come with predetermined data analysis software, instead the user is given full freedom to implement the analysis tools which best suits their needs. The open-source software STRait Razor online and STRait Razor v3 (The University of North Texas Health Science Centre) include the OmniSNP™ kit as a preset library.

Customer Support

For technical questions, assistance, or to suggest enhancements, please contact us at techsupport@nimagen.com.

Revision History

Section	Summary of changes	Version	Date
All	New document.	1.0	2023-12-22
p11-12	Corrected an error in 'Run setup' in table 1. Adjustment to sequencing guidelines.	1.1	2024-01-17

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