Instructions For Use

Version: 2.0 *Ref: IFU-OmniSTR* *Revision date: 2023-12-22*

OmniSTR™ Global

Autosomal STR Profiling Kit

For MPS Library Prep by Reverse Complement PCR

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

IDseek® OmniSTR™ Global Autosomal STR Profiling Kit

REF IDS-ASTR96

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 autosomal short tandem repeat (STR) markers to establish identity of missing persons, confirm kinship, and link persons of interest to crime scenes. Gender identification can be performed in conjunction with STR typing, using PCR products generated from the Amelogenin gene on both the X- and Y-chromosome.

IDseek® OmniSTR™ Global Autosomal STR Profiling Kit provides multiplex amplicon-based MPS library preparation for sequencing 28 autosomal STR targets, one Y-chromosomal STR and the Amelogenin gene. 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 STR analysis and sex determination.

IDseek® OmniSTR™ Targets		
Amelogenin	D20S482	D8S1179
CSF1PO	D21S11	D9S1122
D10S1248	D22S1045	DYS391
D12S391	D2S1338	FGA
D13S317	D2S441	Penta D
D16S539	D3S1358	Penta E
D17S1301	D4S2408	SE33
D18S51	D5S818	TH01
D19S433	D6S1043	TPOX
D1S1656	D7S820	VWA

Reverse Complement PCR Kit Contents

NimaGen Part# IDS-ASTR96 (store at -20 °C)	Contents
IDseek® OmniSTR™ Probe Panel (REF: PM-IDS-STR)	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)
Positive Control (REF: PCTRL-2800M)	1x Tube (100 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 v3 (600-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	
61 °C	90 minutes	0.1 °C/sec (or 2% of Max)	
68 °C	1 minute	Max	
95 °C	10 seconds	Max	30 x
80 °C	1 second	Max	
61 °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 5-6 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 5-6 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)
- Positive control (Orange 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 either 8 µL of DNA solution (optimal: 1 ng total DNA input) or 8 µL of positive control (Orange cap).

Note: the positive control contains enough volume to set up 12 reactions.

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 purifications:

Purification #1

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.**
k. Add 110 µL Low TE buffer.

Off magnet:
l. Resuspend the beads by pipetting up and down, by flicking or by short vortexing.
m. Incubate for 2 minutes.

On magnet:
n. Wait for 3-5 minutes, or until the solution is fully cleared.
o. Carefully bring 100 µL of the clear solution into a new 1.5 mL tube, ensuring not to transfer any of the beads.

Purification #2

Off magnet:
p. Add 100 µL resuspended bead solution to the 100 µL pool (from step **o.**) and mix well immediately by pipetting up and down at least 5 times or by short vortexing.
q. Incubate for 5 minutes.

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

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

v. Repeat steps **s.**, **t.** and **u.** for a second ethanol wash step.
w. 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).
x. 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.

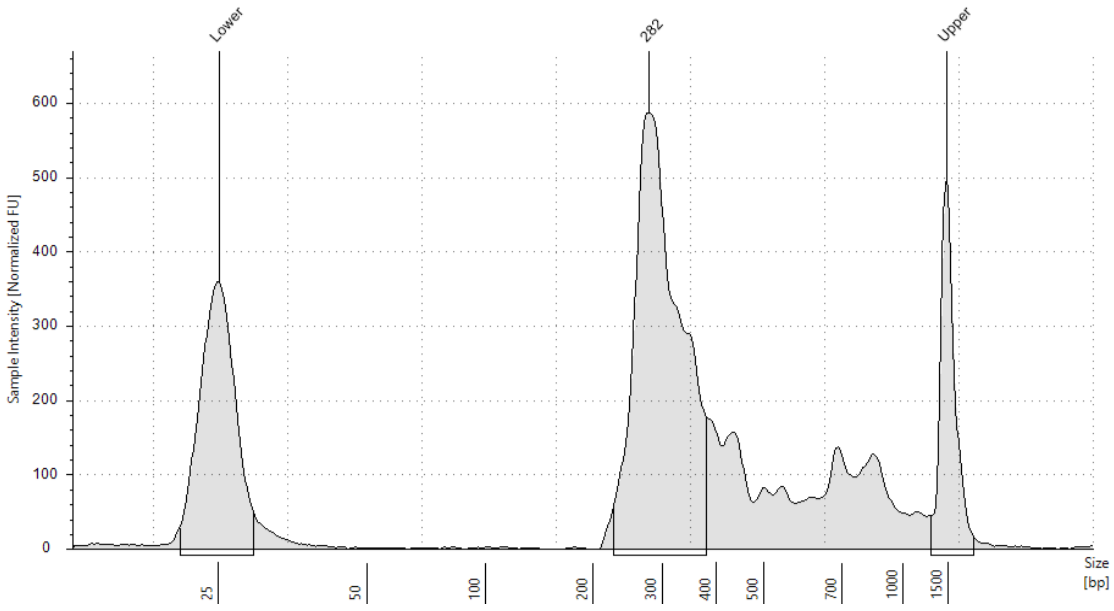
4. Sequencing

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.

Recommended: pool different libraries based on the NimaGen IDseek® OmniSTR™ Global Library Calculator

- **a.** We recommend a length of 320 bp for calculating library molarity.
- **b.** We advise to maintain a minimal read depth of 30.000 reads per sample for reference samples or a minimum of 300.000 reads per sample for traces, mixed samples or samples with low RC-PCR input (<250 pg).
- **c.** A spike-in of 25% PhiX is required for QC purposes and to act as a safeguard against colour-space issues.

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**
			Reference	challenging		
MiSeq	V3 600 cycles	301-10-10-301	625*	62	25 million	8 pM
MiSeq	MiSeq FGx® Reagent Kit	301-10-10-301	625*	62	25 million	8 pM
MiSeq	MiSeq FGx® Reagent Micro Kit	301-10-10-301	200	20	8 million	8 pM

* Maximum number of indices available (n=384).
** Based on Illumina recommendations and assuming onboard denature and dilution.

Data Analysis

The IDseek® OmniSTR™ 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. Several options already include the OmniSTR™ kit as a preset library including commercial software solutions such as MixtureAce™ (NicheVision®), or open-source software such as FDSTools (Netherlands Forensic Institute), STRait Razor online and STRait Razor v3 (The University of North Texas Health Science Centre).

Due to its size, larger alleles of SE33 cannot be sequenced fully from one side and could require merging of the two individual reads into a single long fragment for downstream analysis. The read 1 and 2 fastq files can be merged using a tool such as 'Fast Length Adjustment of Short reads' (FLASh) (Centre for Computational Biology, John Hopkins University).

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	First Version.	1.0	2022-08-30
All	New layout. Included positive control. Included suggestions for downstream analysis. Simplified chapter 3: purification.	2.0	2023-12-22

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