Instructions For Use

Version: 1.0 *Revision date: 30 Aug, 2022*

OmniSTR™ Global

Autosomal STR Profiling Kit

MPS library prep by Reverse Complement PCR

NimaGen.

Innovators in DNA Sequencing Technologies

Product and Company Information

Product name:	IDseek® OmniSTR™ Global Autosomal STR Profiling Kit
SKU:	IDS-ASTR96
Product use:	Research Use Only
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Product Use

Multiplex amplicon-based MPS library preparation for sequencing 28 autosomal Short Tandem Repeat (STR) targets, one Y chromosomal STR and Amelogenin. This Reverse Complement Polymerase Chain Reaction (RC-PCR) based library prep kit contains all reagents to generate Illumina® compatible libraries in a simple, sensitive and robust method for fast and cost-effective STR analysis.

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**

Kit Content

Note: One complete kit consist of two part numbers, to be ordered separately:

1. NimaGen Part# IDS-ASTR96 (store at -20 °C)	Content
RC-PCR probe panel (OmniSTR™ Global Autosomal STR Profiling Kit)	1x Tube (24 µL)
2x PCR Mastermix (HiFi, Hotstart)	1x Tube (1150 µL)
IDseek® Probe Dilution Buffer	1x Tube (216 µL)

2. NimaGen Part# IDX96-U0x*	Content
IDX Index Primer Plate, dehydrated. *Choose one of the 4 available Index Plates for Illumina®.* *Available SKU: IDX96-U01, IDX96-U02, IDX96-U03, IDX96-U04. Semi-skirted, "ABI style" PCR Plates, containing 96 different dehydrated, colored, Unique Dual Index primer pairs, ready to use.	Sealed, breakable 96-well plate 12 strips of 8 caps

Note: When ordering multiple IDseek® kits, any combination of UDI indexes from the 4 available Index plates can be used in a single Illumina run. This enables to combine different applications in one run

Needed, but not included:

Description	Vendor
Adjustable Pipette Set (P10, P20, P200, P1000)	Multiple Vendors
TapeStation, Bioanalyzer Instrument, incl. consumables.	Agilent®
Optionally: Agarose Gel system	Multiple Vendors
Ethanol absolute, mol. biol. grade	Multiple Vendors
AmpliClean™ or AMPureXP® 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 Eppendorf tubes	Multiple Vendors
PCR Grade Water	Multiple Vendors
Qubit® Fluorometer including High Sensitivity consumables	Thermofisher®
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™, SimpliAmp and MiniAmp Thermal Cycler	Multiple Vendors
NaOH solution (2 N)	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®)	Illumina®
Illumina® MiSeq® Reagent kit v3 (600-cycle)	Illumina®

General precautions

Use a Pre-PCR environment for setting up the RC-PCR reaction. Pooling, cleaning and library preparation should be performed in a Post-PCR environment

1. Thermocycling program

Temp:	Duration:	Ramping rate: (from prev. 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

Heated lid at 105 °C

Depending on the instrument, this protocol takes 5-6 hours to complete

Note: When running a IDseek® kit 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 Index 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.

2.1 Thaw on ice:

- 1x RC-PCR OmniSTR™ probe panel (Black Cap)
- 1x Probe Dilution buffer (Green cap)
- 2x HiFi Mastermix (White cap)

Note: The 2x HiFi Mastermix contains isostabilizers 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 2x HiFi Mastermix is fully thawed and thoroughly mixed before use.

2.2. Take the IDX PCR plate 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 Illumina® sample sheets.

2.3. Prepare in a fresh 1.5 mL Eppendorf tube the Probe-Polymerase premix 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 2x HiFi Mastermix per reaction (White cap)

Example: 24 samples + 10% extra volume*

- **Probe-Polymerase premix:**
  - **5.28 µL RC-PCR probe panel**
  - **47.52 µL Probe Dilution buffer**
  - **264 µL 2x HiFi Mastermix**

*** It is recommended to allow for a 10% extra when preparing the mastermix to correct for pipetting loss. The kit contains extra reagent for this.**

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

2.5. Dispense 12 µL of Probe-Polymerase premix (from step 2.3) to each well of the plate/strip(s).

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

2.7. Close the tube strips carefully with caps (included in the kit) and mix by short vortexing, followed by a quick spin.

2.8. Verify that the color of the reaction mix is homogenously pink.

2.9. Start the RC-PCR program in the thermal cycler(s) and place the samples in the cycler when the block is between 60 °C and 98 °C. Then close the lid.

The samples have now been amplified and tagged with sample specific indexes and sequencing adapters. From this point, PCR products can be pooled together in a single tube and purified by a bead purification to remove primers and salt.

3. Pool, Purify and Sequence

3.1. Bring the beads solution (AmpliClean™ or AMpureXP®) to room temperature.

3.2. Combine 5 µL RC-PCR products from all the reactions in a 1.5 mL Eppendorf tube.

Note: In order to decrease read depth variation between samples with low and high quantity/quality, optionally a pooling strategy can be followed, based on the quantity of the final specific individual PCR products. Please contact NimaGen for recommendations.

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

Purification #1

a. Vortex beads thoroughly to resuspend.
b. Add 100 µL beads solution to the 100 µL pool (from step 3.4) and mix well immediately by pipetting up and down 5 times.
c. **Off magnet:** Incubate for 5 minutes.
d. **On magnet:** Place tube for 3 minutes or until the solution is fully cleared.

e. Remove and discard 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. (Optionally a quick spin can be performed, then place tube back on magnet and remove last traces of ethanol).
j. Dry with open cap for 2-3 minutes at Room Temperature. **Do not over-dry.**
k. **On magnet**: Add 110 µL Low TE buffer.
l. **Off magnet:** Resuspend the beads by pipetting up and down or flicking.
m. **Off magnet:** Incubate for 2 minutes.
n. **On magnet**: Wait for 3-5 minutes or until the solution is fully cleared.
o. Carefully bring 100 µL of the clear solution to a new 1.5 ml Eppendorf tube, ensuring not to transfer any of the beads.

Purification #2

p. Add 100 µL resuspended beads solution to the 100 µL pool (from step o) and mix well immediately by pipetting up and down 5 times
q. **Off magnet**: Incubate for 5 minutes.
r. **On magnet**: Place tube for 3 minutes or for the solutions to be fully cleared.

s. Remove and discard 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. (Optionally a quick spin can be performed, then place tube **back on magnet** and remove last traces of ethanol).
x. Dry with open cap for 2-3 minutes at Room Temperature. **Do not over-dry**. Immediately continue with Step 3.5.

3.5. Elution

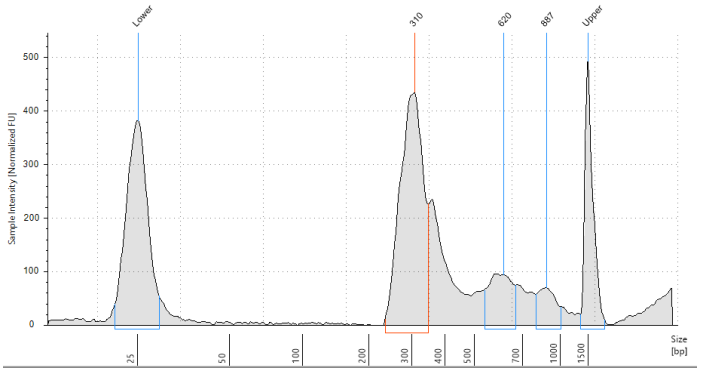
a. **On magnet:** Add 40 µL Low TE buffer to the tube and close.
b. **Off magnet:** Resuspend the beads by flicking or short vortexing.
c. **Off magnet**: Incubate for 2 minutes.
d. **On magnet**: Wait for 3-5 minutes or until the solution is fully cleared.
e. Carefully bring 30 µL of the clear solution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

Libraries are now ready for quantitation and qualification, followed by MPS.

3.6. Determine the final concentration of the library by a duplo Qubit® (HS) measurement according to manufacturer's manual.

3.7. Verify the library on TapeStation or Bioanalyzer, according to the manufacturer's protocol. If needed, dilute the pool. Example: For TapeStation High Sensitivity kit, dilute to ~2 ng/µL.

Example of a clean library on TapeStation:



3.8. Perform Sequencing on an Illumina® MiSeq® platform, according to the manufacturer's manual. Use 300-10-10-300 sequencing scheme. Appendix 1 outlines the detailed Illumina® MPS protocol.

Customer Support:

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

APPENDIX 1: ILLUMINA® SEQUENCER PROTOCOLS

A: Illumina® MiSeq® protocol

Use Illumina® MiSeq® v3 kit for 600 cycles (2x300 bp run).

Ref: MiSeq® System Denature and Dilute Libraries Guide #15039740 v03.

1. Thaw DNA sample/library, buffer HT1, and MiSeq® cartridge.

NOTE: MiSeq® cartridge should be thawed by submerging it in (but not covering it completely with) water at room temperature. Thawing takes ~ 1.5hr, do not use warm water as it degrades the enzymes. Store other components of MiSeq® kit in 4°C refrigerator until ready to start the MiSeq® run.

2. Prepare sample sheet. Workflow: Generate FASTQ. Use the following adapter sequences for trimming in the sample sheet:
   Adapter: GCGAATTTCGACGATCGTTGCATTAACTCGCGAA
   AdapterRead2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

3. Dilute DNA to 2 nM using 10 mM Tris-HCl, pH 8.5/0.1% Tween20 (Illumina® EBT Buffer).
4. Prepare fresh 0.2 N NaOH.
5. Mix 10 µL of the 2 nM library with 10 µL of 0.2N NaOH, vortex, spin down.
6. Incubate for 5 minutes at room temperature.
7. Add 10 µL of 200 mM Tris-HCl pH 7.0 to hydrolyse the NaOH.
8. Add 970 µL ice cold HT1 to DNA/NaOH mix to dilute DNA to 20 pM.
9. Dilute the 20 pM library with ice cold HT1 to 8 pM (for v3 kits) in a new tube:
   a. Dilute 400 of library (20 pM) with 600 µL of HT1
      (Total volume is now 1000 µL of 8 pM loading concentration)
   b. Invert to mix and then pulse centrifuge.
10. Mix the MiSeq® cartridge by inverting 10x, make sure the reagents do not contain ice. After mixing, tap the cartridge on the bench 2-3 times to dislodge any air trapped in the bottom of the tubes.
11. Load MiSeq® cartridge:
    a. Optional but recommended, use PhiX control: Add 3% of PhiX control to the library
    b. Load 600 µL of **the library** to the **Load Samples** well.
12. Set up and start MiSeq® run: Clean flow cell according to instructions, follow on-screen instructions to load and start instrument.

Illumina® Systems Reference Guides

- **MiSeq® System Guide**
- **MiSeq® Denature and Dilute Libraries Guide**
- **Illumina® experiment manager**

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