Instructions For Use

Version: 1.2 *Ref: IFU-OmniSTR* *Revision date: 16 March 2023*

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

IDS-ASTR96

Research Use Only

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QUALITY MANAGEMENT SYSTEM

ISO 9001:2015 FM 711484
ISO 13485:2016 MD 711483

Symbols Used on Product Labels

Symbol	Description
	Manufacturer
	Use-by date
LOT	Lot number
REF	Reference number
RUO	Research Use Only
	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) ● (green)

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: IDX096-U01, IDX096-U02, IDX096-U03, IDX096-U04.	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 Eppendorf 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)	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 (600 cycles) or Verogen MiSeq FGx® Reagent Micro Kit (600 cycles)	Verogen / Illumina

General Precautions

Read the Material Safety Data Sheet (MSDS) and follow the handling instructions. Adhere to good laboratory practice and wear protective eyewear, gloves and lab coat when handling both the reagents supplied in this kit and other reagents required. Wash body parts with ample amount of water immediately if they come in contact with the reagents. Seek medical help if needed.

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

Protocol

1. Thermocycling Program

<table>
<tr><th>Temp</th><th>Duration</th><th>Ramp Rate
(from previous step)</th><th>Cycles</th></tr>
<tr><td colspan="4"></td></tr>
<tr><td>98 °C</td><td>2 minutes</td><td>N/A</td><td>1 x</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td>98 °C</td><td>10 seconds</td><td>Max</td><td rowspan="3">1 x</td></tr>
<tr><td>58 °C</td><td>10 minutes</td><td>0.1 °C/sec (or 2% of Max)</td></tr>
<tr><td>68 °C</td><td>1 minute</td><td>Max</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td>95 °C</td><td>10 seconds</td><td>Max</td><td rowspan="4">2 x</td></tr>
<tr><td>80 °C</td><td>1 second</td><td>Max</td></tr>
<tr><td>61 °C</td><td>90 minutes</td><td>0.1 °C/sec (or 2% of Max)</td></tr>
<tr><td>68 °C</td><td>1 minute</td><td>Max</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td>95 °C</td><td>10 seconds</td><td>Max</td><td rowspan="4">30 x</td></tr>
<tr><td>80 °C</td><td>1 second</td><td>Max</td></tr>
<tr><td>61 °C</td><td>2 minutes</td><td>0.5 °C/sec (or 10% of Max)</td></tr>
<tr><td>68 °C</td><td>30 seconds</td><td>Max</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td>68 °C</td><td>1 minute</td><td>Max</td><td>1 x</td></tr>
</table>

Heated lid at 105 °C.

Note: This protocol takes approximately 5-6 hours to complete, but may vary per thermal cycler used. When running an IDseek® kit 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)

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 sheets.

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 Eppendorf 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). This can be done by an electronic dispensing pipette.

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 and mix by short vortexing, followed by a quick spin. Verify that the color of the reaction mix is homogenously pink.

2.8. Start the RC-PCR program in the thermal cycler(s) and place the samples in the cycler as soon as it reaches a temperature of 60 °C. Then close the lid.

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

3. Purification

Depending on the sample types used, there are three library purification workflow strategies, to ensure the sample-to-sample read depth variation is limited to a minimum. Choose the workflow which is most applicable to your samples.

1. Reference samples with ~1 ng input (go to 3.1)
2. Samples with varying (but known) input quantities (go to 3.2)
3. Samples with unknown input, such as case samples (go to 3.3)

Note: The library purification involves one-sided size selection using magnetic beads, minimizing the number of reads lost to residual primers and primer-dimers.

Note: In cases where the PCR plate contains samples from more than one of the categories mentioned above, use pooling strategy 2 (go to 3.2).

3.1 Purification workflow for libraries created from HQ reference samples

This workflow is for high-quality samples with recommended input quantity (1 ng), e.g. reference samples.

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

3.1.2 Pool 5 µL RC-PCR product from each reaction into a 1.5 mL Eppendorf tube.

3.1.3 Mix well and transfer 40 µL of this pool into a new 1.5 mL Eppendorf tube.

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

3.1.5 Start the magnetic bead purification, using subsequent protocols Purification #1 and Purification #2 (see page 8).

3.1.6 Start the Elution protocol (see page 9).

Purification #1 (3.1)

a. Vortex the beads thoroughly to resuspend.
b. Add 100 µL beads solution to the 100 µL pool (from step 3.1.4) and mix well immediately by pipetting up and down 5 times.
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, or by flicking.
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 Eppendorf tube, ensuring not to transfer any of the beads.

Purification #2 (3.1)

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. 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.

Elution (3.1)

- **a. On magnet:** Add 40 µL Low TE buffer to the tube and close.
- **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.** Carefully bring 30 µL of the clear solution into a new 1.5 ml Eppendorf tube, making sure not to transfer any of the beads.

The libraries are now ready for a quantitative and qualitative check, followed by MPS. Proceed to **4. Sequencing** (page 14).

3.2 Samples with varying (but known) input

The input quality and quantity of your samples will impact the PCR yield. To ensure low-input samples have appropriate read depth, it is required to normalise the read depth for each sample. If the input quantity of your samples is known, samples can be pooled based on the total input quantity of the PCR.

Note: The presence of inhibitors can impact PCR yield. If inhibitors are suspected, go to purification workflow 3.3.

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

3.2.2 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 Eppendorf tube. **Note: Perform all subsequent steps for each pool individually.**

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

3.2.3 Mix well and transfer 40 µL of the three pools into three corresponding 1.5 mL Eppendorf tubes.

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

3.2.5 Start the magnetic bead purification, using subsequent protocols Purification #1 and Purification #2 (see page 10 and page 11).

3.2.6 Start the Elution protocol (see page 11).

Purification #1 (3.2)

a. Vortex the beads thoroughly to resuspend.
b. Add 100 µL beads solution to the 100 µL pool (from step 3.2.4) and mix well immediately by pipetting up and down 5 times.
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, or by flicking.
m. Incubate for 2 minute.

On magnet:

n. 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 (3.2)

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. 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.

Elution (3.2)

a. On magnet: Add 40 µL Low TE buffer to the tube and close.
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. Carefully bring 30 µL of the clear solution into a new 1.5 ml Eppendorf tube, making sure not to transfer any of the beads.

The libraries are now ready for a quantitative and qualitative check, followed by MPS. Proceed to **4. Sequencing** (page 14).

3.3 Samples with unknown input

The input quality and quantity of your samples will impact the PCR yield. To ensure low-input samples have appropriate read depth, it is required to normalise the read depth for each sample. If the PCR input is unknown it is recommended to purify each sample individually – biological replicates may be pooled together and treated as a single sample.

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

3.3.2 Transfer 18 µL of each RC-PCR product to a new well of a clean plate.

3.3.3 Add 32 µL Low TE buffer or molecular grade H_2O to each well and mix well (total volume in each well is now 50 µL).

3.3.4 Start the magnetic bead purification, using subsequent protocols Purification #1 (see page 12) and Purification #2 (see page 13).

3.3.5 Start the Elution protocol (see page 13).

Purification #1 (3.3)

a. Vortex the beads thoroughly to resuspend.
b. To each well: add 50 µL beads solution to the 50 µL of sample (from step 3.3.3) and mix well immediately by pipetting up and down 5 times.
c. Incubate for 5 minutes.

On magnet:
d. Place the plate for 3 minutes on the magnetic 96-well block, or until the solution is fully cleared.

e. Remove and discard the liquid carefully without disturbing the beads.
f. Add 150 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 <u>without leaving traces of ethanol.</u>
j. Dry for 2-3 minutes at room temperature. **Do not over-dry.**
k. Add 55 µL Low TE buffer.

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

On magnet:
n. Wait for 3-5 minutes, or until the solution is fully cleared.
o. Carefully bring 50 µL of the clear solution to a fresh well of a plate, ensuring not to transfer any of the beads.

Purification #2 (3.3)

p. Add 50 µL resuspended beads solution to the 50 µL pool (from step o.) and mix well immediately by pipetting up and down 5 times.
q. Incubate for 5 minutes.

On magnet:
r. Place the plate for 3 minutes on the magnet or for the solutions to be fully cleared.

s. Remove and discard the liquid carefully without disturbing the beads.
t. Add 150 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.
x. Dry for 2-3 minutes at Room Temperature. **Do not over-dry**. Immediately continue with the Elution.

Elution (3.3)

a. On magnet: Add 30 µL Low TE buffer to the wells.
b. Off magnet: Resuspend the beads.
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 25 µL of the clear solution into a new 1.5 ml Eppendorf tube making sure not to transfer any of the beads.

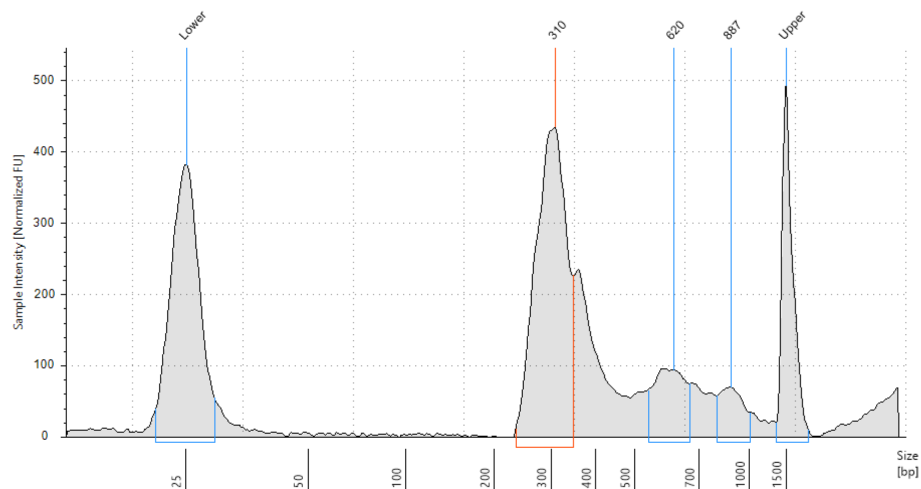
The libraries are now ready for a quantitative and qualitative check, followed by MPS. Proceed to **4. Sequencing** (page 14).

4. Sequencing

4.1. Determine the final concentration of the library or libraries by a double Qubit (HS) measurement according to manufacturer's manual.

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

Note: The number of samples that can be sequenced on a flow cell depends on the type of flow cell and sample. For reference samples we advise a minimum of 30.000 reads per sample. For traces, mixed samples or samples with low RC-PCR input (<250 pg), we advise a minimum of 300.000 reads per sample. Use the NimaGen pooling calculator to determine how many samples can be run on a flow cell and in what ratio to pool them.

Note: When sequencing STR, it is recommended to only use a maximum 75% of the flow cell for STR targets. The remaining 25% should be filled with non-STR targets in order to increase base diversity, this may be achieved by loading PhiX.

Customer Support

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

Appendix 1: Illumina MiSeq® Sequencer Protocol

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

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

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

2. Prepare the sample sheet. Select workflow 'Generate FASTQ'. Use the following adapter sequences for trimming in the sample sheet:

   Adapter: GCGAATTTCGACGATCGTTGCATTAACTCGCGAA
   AdapterRead2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

3. Dilute the DNA to 4 nM using 10 mM Tris-HCl, pH 8.5/0.1% Tween20 (Illumina EBT Buffer) or Low TE.
4. Prepare fresh 0.2 N NaOH.
5. **Optional:** pool different libraries based on the NimaGen pooling calculator.
6. Mix 5 µL of the 4 nM library with 5 µL of 0.2 N NaOH, vortex, spin down.
7. Incubate for 5 minutes at room temperature.
8. Add 10 µL of 200 mM Tris-HCl pH 7.0 to hydrolyse the NaOH.
9. Add 980 µL ice cold HT1 to the DNA/NaOH mix to dilute the DNA to 20 pM.
10. Dilute the 20 pM library with ice cold HT1 to 8 pM (for v3 kits) in a new tube by combining:
    a. 240 µL of the library (20 pM)
    b. 280 µL of HT1
    c. 80 µL of phiX control (20 pM)
    d. Invert to mix and then pulse centrifuge.
11. 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.
12. Load the MiSeq® cartridge:
    a. Load 600 µL of **the library** to the **Load Samples** well.

Set up and start the MiSeq® run: Clean the flow cell according to the instructions, follow on-screen instructions to load and start the instrument.

For further information on using MiSeq®, please see Illumina Systems Reference Guides:

- MiSeq® System Guide
- MiSeq® Denature and Dilute Libraries Guide
- Illumina Experiment Manager (IEM) User Guide

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