

# Introducing the MiSeq FGx™ Reagent Micro Kit for Forensic Applications

A kit optimized for running fewer samples, faster, with the same chemistry and data quality of the MiSeq FGx Reagent Kit.

## Introduction

The MiSeq FGx™ Forensic Genomics Solution is the industry-leading system for delivering massively parallel sequencing (MPS) to forensic genomic scientists. Until now, the system was designed to be most cost effective when sequencing 96 samples for DNA Primer Mix A (DPMA) or 32 samples for DNA Primer Mix B (DPMB) (Table 1). With the availability of the MiSeq FGx Reagent Micro Kit, laboratories now have the option of economically running fewer samples per sequencing run.

The throughput needs of forensic laboratories—particularly those conducting casework, research, or missing persons identification applications—frequently have the requirement to process a small number of samples rapidly. The MiSeq FGx Reagent Micro Kit, partnered with the smaller library preparation kit size of the 96-reaction ForenSeq™ DNA Signature Prep Kit, represents an ideal solution for those routinely needing a lower throughput option.

The MiSeq FGx Reagent Micro Kit is identical in its manufacture to the standard format reagent kit. With both formats, the best in class sequencing by synthesis technology is unaltered; the only difference being the surface area available for imaging. The MiSeq FGx Reagent Micro Kit images approximately 35% of the standard flow cell area, resulting in a total output of 5 million reads, as opposed to the 12.5 million reads of the standard flow cell (Table 2).

The ForenSeq Universal Analysis Software (UAS) v1.3 enables the use of the MiSeq FGx Reagent Micro Kit, in addition to a number of other features that empower laboratory operations. Previous versions of the software can be updated free of charge.



Figure 1: MiSeq FGx Standard Flow Cell (left) and Micro Flow Cell (right)

Table 1: Sample Capacity Comparison

Reagent Kit	Recommended Samples per Run with DPMA	Recommended Samples per Run with DPMB
MiSeq FGx Reagent Kit	Up to 96	Up to 32
MiSeq FGx Reagent Micro Kit	Up to 36	Up to 12

Table 2: Sequencing Metrics Comparison

Reagent Kit	Approximate Paired Reads	Approximate Sequencing Time
MiSeq FGx Reagent Kit	12.5 million	30 hours
MiSeq FGx Reagent Micro Kit	5.0 million	22 hours

## Equivalence Between Reagent Kits

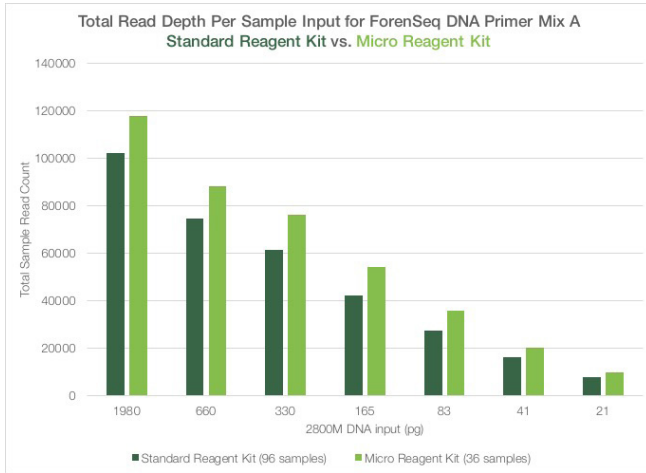
VeroGen scientists performed extensive testing of the MiSeq FGx Reagent Micro Kit to confirm equivalence with the MiSeq FGx Reagent Kit. Multiple sequencing runs were performed to assess and confirm reproducibility, repeatability, stochastic effects, and sensitivity using a variety of samples. Mock mixtures were created to confirm allele recovery and concordance was comparable between the reagent kits. In all cases, the MiSeq FGx Reagent Micro Kit performed equivalently to the standard MiSeq FGx Reagent Kit.

Additional studies were conducted to establish recommended sample minimum and maximum guidelines for the use of the MiSeq FGx Reagent Micro Kit (Table 1).

- For DPMA, 36 known samples with inputs ranging from 3000 pg to 20 pg were sequenced using the micro sequencing kit, and compared to 96 samples sequenced using the standard sequencing kit covering the same input range.
- For DPMB, 12 known samples with inputs ranging from 3000 pg to 5 pg were sequenced using the micro sequencing kit, and compared to 32 samples sequenced using the standard sequencing kit in the side-by-side comparison over several runs.

In both cases, the total number of reads per sample for each input level were very similar between the flow cells with concordance confirmed (Figure 2 and Figure 3).

The testing performed by VeroGen affirms the MiSeq FGx Reagent Kit and MiSeq FGx Reagent Micro Kit as equivalent choices for sequencing on a MiSeq FGx sequencing platform.



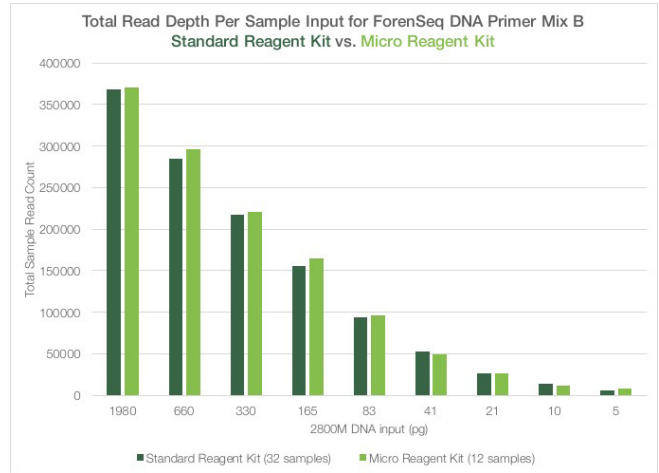
**Figure 2: Comparison of the total amount of reads per sample input using ForenSeq DNA Primer Mix A**—The maximum recommended multiplex for the MiSeq FGx Reagent Kit and the MiSeq FGx Reagent Micro Kit were assessed at different sample inputs. Reported inputs were adjusted from nominal based on quantification results.

## Material Modification Guidance

For accredited forensic genomics laboratories that have validated and adopted the Verogen MiSeq FGx Forensic Genomics Solution, the adoption of the micro sequencing kit represents no change to the technology or function of the system. Verogen has conducted internal testing to compare the performance of the MiSeq FGx Reagent Micro Kit with the standard MiSeq FGx Reagent Kit, and these tests have demonstrated no substantive difference in performance. However, individual laboratories should assess their own requirements for implementation based on SWGDAM, ENFSI, or other applicable guidelines.

Laboratories that are newly adopting the Verogen MiSeq FGx Forensic Genomics Solution can choose to use the MiSeq FGx Reagent Micro Kit as their primary sequencing format. In that case, Verogen recommends that these laboratories incorporate the micro sequencing kit into the SWGDAM internal validation plan that would be executed with any newly implemented system.

For laboratories that have previously validated the MiSeq FGx Forensic Genomics Solution with the standard MiSeq FGx Reagent Kit and want to use the MiSeq FGx Reagent Micro Kit going forward, consider performing a material modification assessment, such as the one described in the following section.



**Figure 3: Comparison of the total amount of reads per sample input using ForenSeq DNA Primer Mix B**—The maximum recommended multiplex for the MiSeq FGx Reagent Kit and the MiSeq FGx Reagent Micro Kit were assessed at different sample inputs. Reported inputs were adjusted from nominal based on quantification results.

## Example Testing Scheme

The following experimental design is provided as an example for laboratories that have already adopted the ForenSeq DNA Signature Prep Kit using the standard MiSeq FGx Reagent Kit and would like to conduct a material modification assessment to enable the MiSeq FGx Reagent Micro Kit. This design is applicable to either DPMA or DPMB and confirms equivalent sample coverage between the standard sequencing kit (with 32 samples) and the micro sequencing kit (with 12 samples).

### Experimental Design

- **Use the same libraries for both sequencing runs**  
Reduce error associated with sample preparation
- **Input amounts (pg) prepared in triplicate of a standard**  
4000, 1000, 500, 250, 125, 63, 31, 16, 8
- **Mock sample or different standard at 1000 pg in triplicate**
- **MiSeq FGx Reagent Kit (32 samples)**  
Pos Control, Neg Control, All samples prepared above
- **MiSeq FGx Reagent Micro Kit (12 samples)**  
Pos Control, Neg Control, Single replicate of each sample above

## Ordering Information

Product	Verogen Catalog No.
MiSeq FGx Reagent Kit	TG-143-1001
MiSeq FGx Reagent Micro Kit	TG-143-1002
ForenSeq DNA Signature Prep Kit (384 reactions)	TG-450-1001
ForenSeq DNA Signature Prep Kit (96 reactions)	TG-450-1002

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