

# Introducing the MiSeq FGx Reagent Micro Kit for Forensic Genomics

Sequence fewer samples, faster with the same optimized chemistry and high data quality as the MiSeq FGx Reagent Kit.

## Highlights

- Equivalent performance

  Delivers the same quality as the standard kit
- Enhanced flexibility for lower throughputs Sequence fewer libraries in each run.
- Backed by extensive testing
   Easily bring this technology into your forensic laboratory.

#### Introduction

The MiSeq FGx® Sequencing System is the leading solution for delivering next-generation sequencing (NGS) technology to forensic genomic scientists. The system was originally designed to be most cost-effective when sequencing ForenSeq® DNA Signature Prep libraries: 96 libraries prepared with DNA Primer Mix A (DPMA) or 32 libraries prepared with DNA Primer Mix B (DPMB) (Table 1). With the availability of the MiSeq FGx Reagent Micro Kit, laboratories have the economic option to sequence fewer libraries in each run.

The throughput needs of forensic laboratories, particularly those conducting casework, research, or missing persons identification, are often required to rapidly process a small number of samples. Partnered with the smaller library prep kit size of the 96-reaction ForenSeq DNA Signature Prep Kit, the MiSeq FGx Reagent Micro Kit represents an ideal solution for laboratories needing a lower throughput option.



**Figure 1:** Flow cell from the standard kit (left) and micro kit (right)

Table 1: Sample capacity

Reagent Kit	Samples per Run with DPMA	Samples per Run with DPMB
MiSeq FGx Reagent Kit	≤ 96	≤32
MiSeq FGx Reagent Micro Kit	≤ 36	≤ 12

Table 2: Sequencing metrics

Reagent Kit	Number of Paired Reads	Sequencing Time
MiSeq FGx Reagent Kit	12.5 million	~30 hours
MiSeq FGx Reagent Micro Kit	5 million	~22 hours



The MiSeq FGx Reagent Micro Kit is identical in manufacture to the MiSeq FGx Reagent Kit. With both kits, the best-in-class sequencing-by-synthesis (SBS) technology remains unaltered. The only difference is the surface area of the flow cell available for imaging (Figure 1). The MiSeq FGx System images the micro flow cell at approximately 35% of the standard flow cell area for a total output of 5 million reads. In contrast, the standard flow cell has an output of 12.5 million reads (Table 2).

### Equivalence between reagent kits

Verogen extensively tested the MiSeq FGx Reagent Micro Kit to ensure equivalence with the MiSeq FGx Reagent Kit. Multiple sequencing runs using a variety of samples assessed and confirmed reproducibility, repeatability, stochastic effects, and sensitivity. Mock mixtures confirmed that allele recovery and concordance was comparable between the two reagent kits. In all cases, the MiSeq FGx Reagent Micro Kit demonstrated performance equivalent to the MiSeq FGx Reagent Kit.

Additional studies established the recommended minimum and maximum sample guidelines when using the MiSeq FGx Reagent Micro Kit and ForenSeq DNA Signature Prep Kit (Table 1):

- For DPMA, 36 known samples with inputs 20–3000 pg were sequenced using the micro reagent kit and compared to 96 samples sequenced using the standard reagent kit and covering the same input range.
- For DPMB, 12 known samples with inputs 5–3000 pg were sequenced using the micro reagent kit and compared to 32 samples sequenced using the standard reagent kit in a 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 (Figures 2 and 3). The testing Verogen performed affirms the MiSeq FGx Reagent Kit and MiSeq FGx Reagent Micro Kit as equivalent choices.

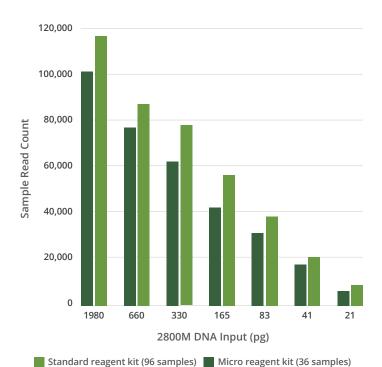
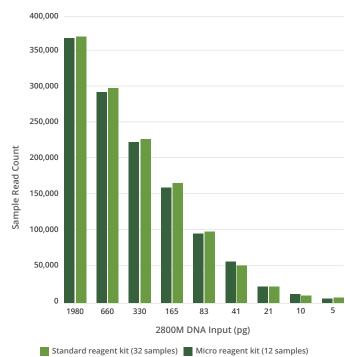


Figure 2: A comparison of the total number of reads per sample input amount using DPMA shows similar results between MiSeq FGx reagent kits. Verogen assessed the recommended maximum number of samples at different inputs. Reported inputs were adjusted from nominal based on quantification results.



**Figure 3:** A comparison of the total number of reads per sample input amount using DPMB shows similar results between MiSeq FGx reagent kits. Verogen assessed the recommended maximum number of samples at different inputs. Reported inputs were adjusted from nominal based on quantification results.



## Material modification guidance

For accredited forensic laboratories that have validated and adopted the Verogen MiSeq FGx Forensic Genomics Solution, onboarding the micro reagent 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 to the MiSeq FGx Reagent Kit and the tests demonstrated no substantive difference in performance. However, individual laboratories must assess their implementation requirements based on Scientific Working Group on DNA Analysis Methods (SWGDAM), European Network of Forensic Science Institutes (ENFSI), or other applicable guidelines.

Laboratories newly adopting the Verogen MiSeq FGx Forensic Genomics Solution have the option to choose the MiSeq FGx Reagent Micro Kit as their primary sequencing reagents. In these cases, Verogen recommends incorporating the micro reagent kit into the SWGDAM internal validation plan that is executed with any newly implemented system. Laboratories that previously validated the MiSeq FGx Forensic Genomics Solution with the standard MiSeq FGx Reagent Kit and want to switch to the MiSeq FGx Reagent Micro Kit can consider performing a material modification assessment such as the one described in the following section.

# **Example testing scheme**

The following experimental design serves as an example for laboratories that have adopted the ForenSeq DNA Signature Prep Kit with the MiSeq FGx Reagent Kit and want to conduct a material modification assessment to enable use of the MiSeq FGx Reagent Micro Kit. This design applies to DPMA or DPMB and confirms equivalent sample coverage between the standard reagent kit with 32 samples and the micro reagent kit with 12 samples.

#### Experimental design:

- Same libraries for both sequencing runs Reduce errors associated with library prep
- Input amounts prepared in triplicate of a standard 4000, 1000, 500, 250, 125, 63, 31, 16, 8 pg
- Mock sample or different standard at 1000 pg in triplicate
- MiSeq FGx Reagent Kit (32 samples)
   Positive control, negative control, and all samples prepared above
- MiSeq FGx Reagent Micro Kit (12 samples)
   Positive control, negative control, and a single replicate of each sample listed above

#### Conclusion

Providing a second option for forensic applications, the MiSeq FGx Reagent Micro Kit delivers the equivalent high-quality performance established with the MiSeq FGx Reagent Kit while enhancing flexibility for lower throughputs. Following our material modification guidance facilitates the process of bringing this sequencing technology into your laboratory.

Learn more at www.verogen.com/products/ miseq-fgx-sequencing-reagents.