Technical Application note

Edition 1, 2025-11

Compatibility of NGSgo products with the MiSeq™ i100 System

MiSeq i100 System is a benchtop sequencer with multiple benefits over the previous MiSeq System. GenDx verified that its key products, **NGSgo-MX11-3**, **NGSgo-ProntoAmp, NGSgo-MX6-1 and NGSgo-AmpX v2 and v3** in combination with the **NGSgo Library Full Kit (LFK)**, provide high-quality, concordant, and reproducible results on this platform.

The outcome confirms that laboratories using the above mentioned GenDx products can confidently transition to the MiSeq i100 System without altering their established NGSgo workflows or data quality expectations.

Illumina MiSeq i100 System

The MiSeq i100 System is designed to offer faster turnaround times compared to the legacy MiSeq System with all sequencing runs completing in approximately 24 hours or less. It has simplified logistics including room temperature stored cartridges offering improved usability and workflow flexibility. The system offers high-quality data supporting a smooth transition for existing legacy MiSeq System users

Advantages

- **Shorter run time:** ~4-24 hours
- **Room temperature cartridge storage**
- **Higher sequencing output**, enabling larger batch sizes
- **Equivalent data quality and typing concordance** to MiSeq System
- **Simplified setup and maintenance** with integrated flow cells and no washes

Objective of this technical note

This note presents the study results that confirm that NGSgo-MX11-3, NGSgo-ProntoAmp, NGSgo-MX6-1 and NGSgo-AmpX libraries, prepared using the NGSgo Library Full Kit (LFK), deliver high-quality and concordant HLA typing results on the MiSeq i100 System, comparable to those obtained with the legacy MiSeq System.

Methods

A comparison between MiSeq and MiSeq i100 Systems was performed by amplifying 58 cell-line DNA samples from the Genetic Testing Reference Materials Coordination Program sample panel (GeT-RM HLA58 high diversity panel) using NGSgo-MX11-3 and NGSgo-ProntoAmp. Amplicons were processed using the NGSgo Library Full Kit (LFK) and sequenced on both MiSeq platforms. Additionally, MiSeq i100 System compatibility was investigated using NGSgo-MX6-1 (58 samples) and NGSgo-AmpX v2 and v3 (both 7 samples) amplicons, also processed with the NGSgo Library Full Kit. Data was analyzed using NGSengine and results were evaluated based on predefined acceptance criteria.

Results

Data generated using the MiSeq i100 System, using either NGSgo-MX11-3 or NGSgo-ProntoAmp libraries as input, yielded high-quality data that was comparable to data from the MiSeq System (**Table 1**). All metrics investigated (indicated in green) passed internal quality standards.

NGSgo-MX6-1 and NGSgo-AmpX (v2 and v3) were evaluated on the MiSeq i100 System to confirm performance consistent with the quality standards. Data met all predefined acceptance criteria, and showed 100% typing concordance (**Table 2**).

A modest reduction in mappability was observed for the MiSeq i100 System compared to the MiSeq System. This is likely due to XLEAP-SBS chemistry's enhanced sensitivity, which preferentially clusters short DNA fragments. The modest reduction in mappability is not expected to have an impact on the typing result, given that the increased data output per run compensates for the minor decrease in mappability. Moreover, it allows for sequencing more samples in parallel (e.g., up to 96 for NGSgo-ProntoAmp, NGSgo-MX11-3, NGSgo-MX6-1, and NGSgo-AmpX).

Table 1. Data generated using NGSgo-MX11-3 and NGSgo-ProntoAmp libraries as input on a 2x 150bp paired end run.

		NGSgo-MX11-3 (n=58)		NGSgo-ProntoAmp (n=58)	
		MiSeq	MiSeq i100	MiSeq	MiSeq i100
Typing	Typing Concordance	98,3% (536/545)	98,3% (536/545)	100% (545/545)	100% (545/545)
	Exon Mismatches	0	0	0	0
Illumina Run Metrics	Cluster Passing Filter	91.05%	84.23%	91.28%	82.32%
	PF Reads	31M	6.7M (5M flow cell)	26M	33M (25M flow cell)
	QV30 base calls	91.3%	98.0%	92.9%	96.6%
	Runtime	~24 hours	~7 hours	~24 hours	~7 hours
NGSengine Quality Metrics	Mean Sample Mappability (±95% CI)	78,8% (±0.7%)	74,1% (±0.7%)	79,6% (±0.8%)	74,3% (±1.0%)
	Mean Exon Lowest Read Depth (±95% CI)	464 (±26)	458 (±22)	444 (±16)	500 (±17)
	Mean Noise (±95% CI)	2,7% (±0.2%)	3% (±0.2%)	2.1% (±0.1%)	2.0% (±0.1%)
	Mean Delta Signal-To-Noise (±95% CI)	37,3% (±0.9%)	37,6% (±0.8%)	39,5% (±0.6%)	40.2% (±0.5%)
	Mean Estimated Second Allele (±95% CI)	44,1% (±0.7%)	44,8% (±0.6%)	46,1% (±0.4%)	46.5% (±0.3%)

Table 2. Data generated using NGSgo-MX6-1 and NGSgo-AmpX libraries as input on a 2x 150bp paired end run.

		NGSgo-MX6-1 (n=58)	NGSgo-AmpX (n=7)	
		LongMix	v2	v3
Typing	**Typing Concordance**	**100 % (348/348)**	**100% (73/73)**	**100% (66/66)**
	Exon Mismatches	**0**	**0**	**0**
Illumina Run Metrics	**Cluster Passing Filter**	**83,07%**	**85.49%**	**85.49%**
	PF Reads	**33M (25M flow cell)**	**34M (25M flow cell)**	**34M (25M flow cell)**
	QV30 base calls	**95.3%**	**90.5%**	**90.5%**
	Runtime	**~7 hours**	**~7 hours**	**~7 hours**
NGSengine Quality Metrics	**Mean Sample Mappability (±95% CI)**	**97.2% (±2.4%)**	**96.1% (±1.5%)**	**94,9% (±3.2%)**
	Mean Exon Lowest Read Depth (±95% CI)	**511 (±83)**	**245 (±26)**	**211 (±30)**
	Mean Noise (±95% CI)	**1.6% (±0.2%)**	**2.3% (±0.2%)**	**2.8% (±0.3%)**
	Mean Delta Signal-To-Noise (±95% CI)	**38.1% (±2.5%)**	**38.1% (±1.7%)**	**37.9% (±1.6%)**
	Mean Estimated Second Allele (±95% CI)	**44.1% (±2.1%)**	**45.0% (±1.4%)**	**45.2% (±1.2%)**

Conclusion

The data presented in this tech note demonstrate that the MiSeq i100 System generates high-quality data for NGSgo-MX11-3, NGSgo-ProntoAmp, NGSgo-MX6-1, and NGSgo-AmpX amplicons processed with the NGSgo Library Full Kit.

In all instances, the MiSeq i100 System produced reliable typing results, with an overall typing concordance between 98.3 and 100%, thereby meeting the GenDx data quality acceptance criteria.

These outcomes confirm that laboratories using any of these NGSgo products in combination with the NGSgo Library Full Kit can confidently transition to the MiSeq i100 System without altering their established NGSgo workflows or data quality expectations.

Overall, the MiSeq i100 System delivers equivalent or improved sequencing performance, shorter runtime, and higher output across all evaluated GenDx NGSgo products, confirming robust compatibility and reproducibility.

Contact

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