



Fast Ramping Thermal Cyclers Enable an 8-Hour MassARRAY® Workflow

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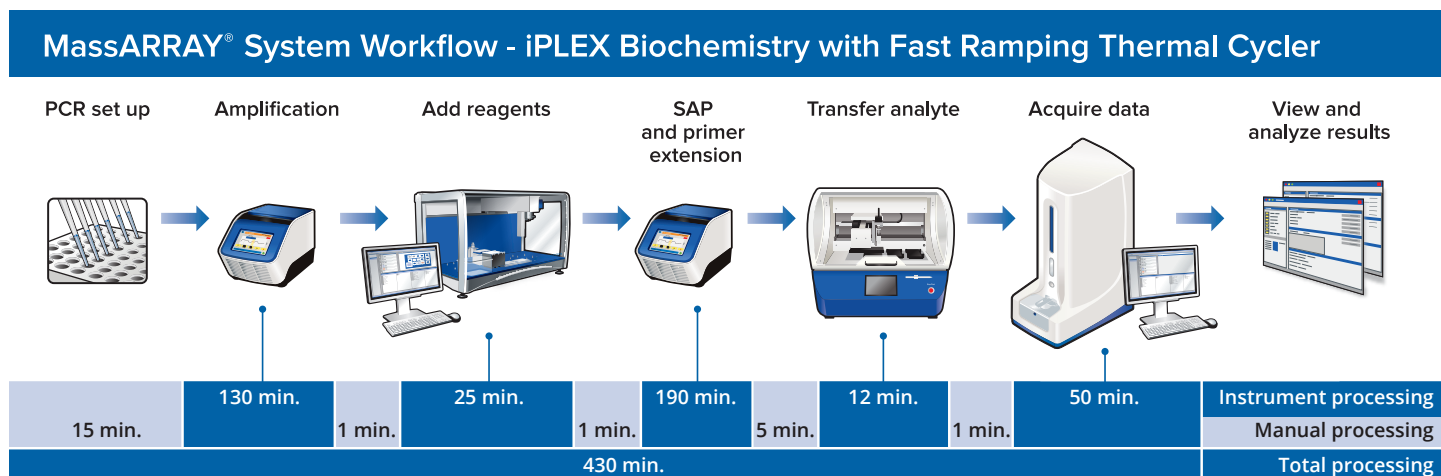
INTRODUCTION

Agena Bioscience's MassARRAY® System, based on MALDI-TOF mass spectrometry, is a scalable platform with a suite of research applications for quantitative and qualitative nucleic acid analysis. It provides flexible assay design, fast time-to-results, and the ability to run from tens to thousands of samples daily. These features make it an ideal genetic analysis system for validation and fine mapping studies in basic and translational research settings.

Starting with purified DNA or RNA, genetic analysis with iPLEX® biochemistry on the MassARRAY System can be

accomplished in a single day. The workflow requires minimal hands-on time (< 30 minutes), but with standard thermal cyclers the overall processing time can exceed 9.5 hours. This application note details an accelerated workflow with fast ramping thermal cyclers, which reduces the cycling time by almost 2 hours, enabling the entire workflow to be completed in a single eight-hour shift (Figure 1). The adapted protocol was tested against two Agena Bioscience panels to demonstrate robustness and assess equivalency with the Applied Biosystems® Veriti® thermal cycler (Veriti).

Figure 1. Accelerated iPLEX Workflow on the MassARRAY System



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METHODS

The iPLEX process relies on exponential amplification of the target sequence, followed by linear amplification in which a single base extension (SBE) reaction is performed. A significant portion of the workflow time involves PCR thermal cycling. With standard thermal cyclers, such as the Applied Biosystems GeneAmp® PCR System 9700 (GeneAmp 9700), the amplification step requires 2 hours, 40 minutes and the primer extension step takes 3 hrs, 40 minutes, due to the ramping speed (2°C/second). If the ramping time were minimized, the turnaround time for the iPLEX workflow would be reduced (**Figure 1**). New thermal cyclers with higher ramping rates (up to 6°C/second) are now available. Higher ramping rates allow faster cycling times and thus a reduction in overall workflow time.

Agena Bioscience selected the Veriti 384-well format. The maximum ramping rate is 4°C/second, which is twice as fast as the GeneAmp 9700. Moreover, we demonstrate here that the increased ramp speed translates to an overall reduction in thermal cycler time of nearly 2 hours.

Our laboratory tested the Veriti to determine whether this instrument would improve the standard workflow time without negatively impacting data quality. The process was evaluated using two of Agena Bioscience's panels: OncoFOCUS™ Panel and iPLEX PGx Pro Panel. Standard cycling parameters for each panel were used (see panel user guides). The Veriti reduced the cycling time by nearly 2 hours, as predicted (**Table 1**).

Table 1. The observed time for each step of the standard iPLEX workflow using the Veriti and GeneAmp 9700 thermal cyclers.

Step	GeneAmp 9700 (hr)	Veriti (hr)
PCR	2:40	2:10
SAP	0:50	0:50
SBE	3:40	2:20

RESULTS

Equivalency Testing and Performance with the OncoFOCUS Panel

The OncoFOCUS Panel was run against a universal test cohort utilized during beta evaluation; it provides historical data with a pre-defined outcome. Workflow steps were initiated when the slowest thermal cycler had finished the previous step in order to accurately compare cycling times. Allelotyping results were compared to one another as well as to data generated during the OncoFOCUS Panel beta studies (which used the GeneAmp 9700 only).

The Veriti and GeneAmp 9700 thermal cyclers resulted in the expected calls and mutation frequencies (**Table 2**). In fact, the results of the current study and the OncoFOCUS Panel beta studies are concordant, presenting correlation values of >0.99 (**Table 3**). Moreover, results from the current study are concordant with the beta evaluation, with the correlations for the Veriti results slightly higher than the correlations for the GeneAmp 9700 results.

Table 2. Equivalency testing results for the GeneAmp 9700 and Veriti thermal cyclers for samples with a mutation identified.

Sample*	Mutation	Expected Frequency	Frequency (GeneAmp 9700)	Frequency (Veriti)
1	NRAS Q61L	0.49	0.47	0.46
2	BRAF V600E	0.18	0.17	0.16
3	KRAS G13D/N	0.72	0.68	0.67
4	EGFR T751I, KRAS G12A	0.32, 0.67	0.32, 0.56	0.32, 0.63
5	BRAF V600E, EGFR P753S	0.91, 0.99	0.84, 1.0	0.90, 1.0

*Unexpected mutations were detected in neither control NTC nor wild type DNA templates.

Table 3. Mutant ID correlation across both the Veriti/GeneAmp 9700 equivalency test and the OncoFOCUS Panel beta study (correlation, r).

	Equivalency Test		Beta Study (GeneAmp 9700 Only)			
	GeneAmp 9700	Veriti	Agena Bioscience	Site 1	Site 2	Site 3
GeneAmp 9700	1.000	0.994	0.992	0.992	0.994	0.995
Veriti	0.994	1.000	0.993	0.993	1.000	0.991
Agena	0.992	0.993	1.000	1.000	0.994	0.989
Site 1	0.992	0.993	1.000	1.000	0.994	0.989
Site 2	0.994	1.000	0.994	0.994	1.000	0.991
Site 3	0.995	0.991	0.989	0.989	0.991	1.000

Equivalency Testing and Performance with the iPLEX PGx Pro Panel

Forty-eight HapMap samples were evaluated using the Veriti and the data was compared to data generated during iPLEX PGx Pro Panel beta evaluation.

The results from the equivalency test and beta evaluation were concordant with one another (**Table 4**).

Table 4. Comparison of data generated during GeneAmp 9700/Veriti equivalency testing and data generated during iPLEX PGx Pro Panel beta studies.

	Equivalency Test	Beta Study (GeneAmp 9700 Only)		
	Veriti	Site 1	Site 2	Site 3
Average Call Rate	.975	.982	.975	.980
Extension Rate	.917	.931	.936	.920
Genotype Concordance	.997	.998	.994	.996

SUMMARY

Two of Agena Bioscience's panels were tested to evaluate the performance of the Applied Biosystems Veriti thermal cycler. In order to ascertain equivalency, the results were compared with historical data for these panels that was derived using the Applied Biosystems GeneAmp PCR System 9700.

- Allelotyping and genotyping data produced by the Veriti was highly concordant with the GeneAmp 9700.

- The Veriti thermal cycler reduces the workflow time by nearly 2 hours.
- The MassARRAY workflow can be accomplished in 8 hours using the Veriti thermal cycler.

In summary, the Veriti thermal cycler is highly recommended as a replacement for the GeneAmp 9700 thermal cycler for those who wish to reduce the overall cycling time and process.

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