Poster # H038

High-Throughput Multiplex MRD Assay for Prognostic Markers in AML Utilizing DNBSEQ-G99 Sequencer

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Background

Acute Myeloid Leukemia (AML) is a complex and dynamic hematological malignancy marked by significant genetic diversity, making monitoring Measurable Residual Disease (MRD) a critical aspect of patient management. We have developed a novel **AML Multiplex MRD Assay** (**AMM Assay**) capable of detecting MRD for the *FLT3* Internal Tandem Duplication (*FLT3*-ITD) mutation and Nucleophosmin 1 (*NPM1*) mutation. In addition, the assay is also capable of detecting *FLT3* tyrosine kinase domain (*FLT3*-TKD) mutations.

The AMM Assay was developed for the DNBSEQ-G99 NGS sequencer (Complete Genomics, San Jose) and SM+ PE300 sequencing kit. It allows multiplexing of up to 48 samples per flowcell with a fast turnaround time of 3 days from gDNA to data analysis while requiring relatively low DNA input. Results were processed using in-house developed bioinformatics pipelines. Results for the 3 targeted regions are generated for each of the samples tested (i.e. 1 reaction = 3 results).

The AMM Assay is a Research Use Only (RUO) tool capable of detecting three key mutations associated with AML pathogenesis. Its performance is demonstrated by testing cell line DNA dilutions and clinical samples.

Methods

Assay Workflow

- **Sample Type and Input volume**
  - 0.25 mL for Bone Marrow Aspirates
  - 0.5 mL for Peripheral Blood
- **Sample DNA Input: 350 ng – 700 ng**
- **Two Step PCR:**
  - PCR 1 primers: Gene Specific Primer (GSP) with Adapter sequence
  - PCR 2 primers*: Up to 48 from 96 available indices with Adapter sequence
- **Multiplex capability:** Up to 48 per flow cell
- **gDNA Sample to Result:** ~3 days
- **Sequencer:** DNBSEQ-G99RS
- **Sequencing Kit:** DNBSEQ-G99RS High -throughput Sequencing Reagent Set (PE300 SM+), early released lot
- **Invivoscribe Bioinformatics Software:**
  - For *FLT3*-ITD (MRD): ITD length (9-252bp), Sequence and VRF
  - For *FLT3*-TKD: Mutation Type (D835/I836) and VRF
  - For *NPM1* (MRD): Mutation Type (A, B, D or "Other"), Sequence and VRF

*Available in pre-aliquoted 96 well plate

Results: G99 Run Metric Summary

Run #	Spike-in Control* (%)	Total reads (M)	Q30 (%)	Chip Productivity (%)	Multiplexed (n sample)
1	30.0	121.1	93.1	84.1	32
2	12.5	104.9	94.2	73.2	49

*Standard Library Reagent-TP (V5)

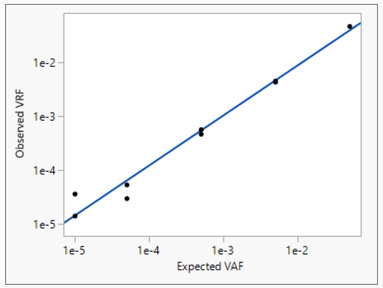
Results: LoD with Cell Line DNA

- A total of five positive panel members, containing three different cell line DNA at various VAF levels, were tested in two-replicates each.
- Testing was performed with 700 ng gDNA/rxn.
- Negative control and NTC-1 (for PCR 1) and NTC-2 (for PCR 2) were tested in 1 replicate each

Panel Member	Expected VAF *FLT3*-ITD	Expected VAF *NPM1*	Expected VAF *FLT3*-TKD
1	5.00E-05	5.00E-02	1.00E-02
2	1.00E-05	5.00E-03	5.00E-03
3	5.00E-02	5.00E-04	1.00E-03
4	5.00E-03	5.00E-05	5.00E-04
5	5.00E-04	1.00E-05	5.00E-02

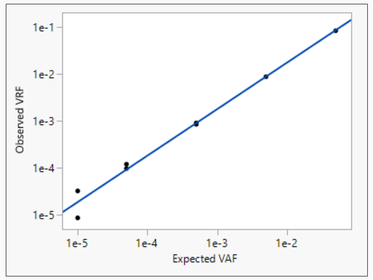
FLT3-ITD

- Dilution panel was prepared with cell line carrying 30 bp mutation
- R^2 = 0.99
- Estimated LoD = 1E-05



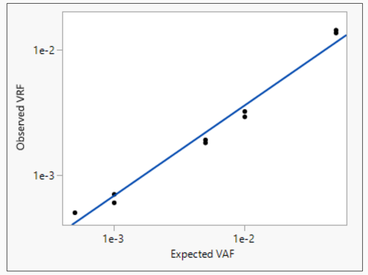
NPM1

- Dilution panel was prepared with cell line carrying Type A mutation
- R^2 = 0.99
- Estimated LoD = 1E-05



FLT3-TKD

- Dilution panel was prepared with cell line carrying D835Y mutation
- R^2 = 0.98
- Estimated LoD = 1E-03



- Negative controls and NTCs showed no reads for any of the three targets.

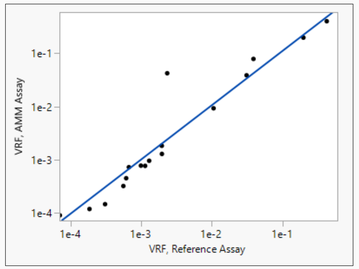
Results: Clinical Sample Performance

- A total of 41 gDNA from suspected AML samples were tested by the AMM Assay. For samples that had a result from a comparator assay (used as the "reference") for either *FLT3*-ITD and/or *NPM1*, a concordance evaluation and comparison of VRF values were performed.
- For *FLT3* TKD, no such comparison was made due to the unavailability of a comparator assay.
- Testing was performed with 350 ng gDNA/rxn.

FLT3-ITD

		Reference Assay +	Reference Assay -	Total
AMM Assay	+	19	0	22
	-	0	3	

- Overall Agreement = 100.0% (22/22)
- ITD length detected = 100.0% match
- R^2 = 0.92

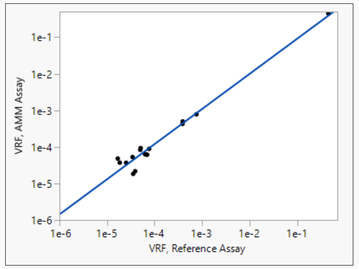


NPM1

		Reference Assay +	Reference Assay -	Total
AMM Assay	+	16	1	21
	-	2	2	

- Overall Agreement = 85.7% (18/21)*
- Mutation Type detected = 100.0% match
- R^2 = 0.97

*Three (3) discordant samples were with VAR near the Reference Assay LoD.



FLT3-TKD

- Out of 41 clinical AML suspected samples tested, two samples tested Positive for D835V mutation.

Conclusion

- The AML Multiplex MRD Assay (AMM Assay) offers a comprehensive 3-in-1 test allowing simultaneous detection of the three critical AML-associated mutations: *FLT3*-ITD, *NPM1*, and *FLT3*-TKD.
- Estimated LoD for the AMM assay for three targets are 1E-05, 1E-05 and 1E-03 for *FLT3*-ITD, *NPM1*, and *FLT3*-TKD, respectively.
- The AMM Assay demonstrated good concordance with the comparator assays, yielding 100.0% and 85.7% agreement for *FLT3*-ITD and *NPM1*, respectively.

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