H049

KMT2A Fusion Detection for Measurable Residual Disease in Acute Myeloid Leukemia Using a Novel Multiplex Digital PCR Assay

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Introduction

KMT2A rearrangements (*KMT2A*r) are present in 5% to 10% of adult Acute Myeloid Leukemia (AML) and 15% to 20% of pediatric AML cases. *KMT2A*r AML is generally associated with poor prognosis and high rates of resistance and relapse following current standards of care.

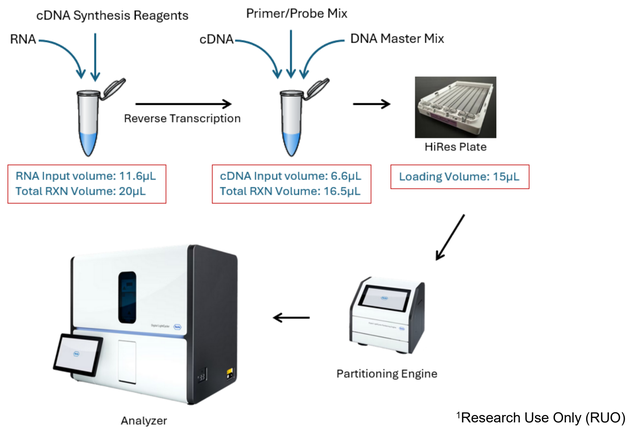
While *AF9* (*MLLT3*), *AF10* (*MLLT10*), *ELL*, and *AF6* (*AFDN*/*MLLT4*) are the four most prevalent *KMT2A*r in AML, over 100 different *KMT2A* fusion partners have been identified. Pre-hematopoietic cell transplantation (Pre-HCT) measurable residual disease (MRD) detection of *KMT2A* fusion transcripts is a strong prognostic indicator of *KMT2A*r AML, and targeting MRD positive *KMT2A*r patients pre-HCT using novel menin inhibitors is currently the focus of numerous clinical trials. Therefore, we have developed a multiplex digital PCR (dPCR) MRD assay for the detection of the top 4 *KMT2A*r in AML.

Materials & Methods

We designed a single multiplex dPCR primer/probe mix[1] capable of MRD detection and differentiation of *KMT2A*::*AF9*, *KMT2A*::*AF10*, *KMT2A*::*ELL*, and *KMT2A*::*AF6* fusion transcripts along with *ABL1* control gene transcripts for use on the Roche Digital LightCycler® platform. The Roche Digital LightCycler® has the capability to detect amplification in six unique channels, utilizing six different fluorophores. Hydrolysis probes specific for *KMT2A*, *AF9*, *AF10*, *ELL*, *AF6*, and *ABL1* were each designed using unique fluorophores for each gene. 2-Plex primer/probe mixes targeting a single *KMT2A* fusion along with *ABL1* were also produced for comparison to the multiplex

Channel	Reporter Dye	Target
1	Atto425	*KMT2A*
2	FAM	*AF9*
3	HEX	*AF10*
4	TexRed	*AF6*
5	Cy5	*ELL*
6	Cy5.5	*ABL1*

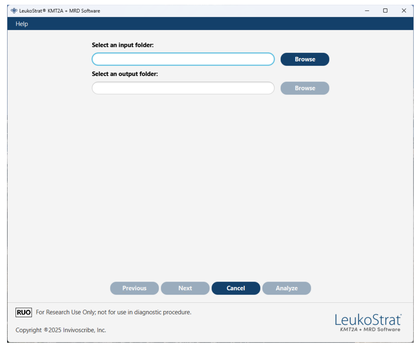
RNA extracted from human cell lines harboring *KMT2A* fusions (THP-1: *KMT2A*::*AF9*, OCI-AML2: *KMT2A*::*AF6*) and gBlock contrived *KMT2A*::*AF10* and *KMT2A*::*ELL* RNA were serially diluted into RNA extracted from a *KMT2A* wild type cell line (OCI-AML3) to dilution levels from 100% down to 0.001%. cDNA was synthesized from RNA dilutions using SuperScript™ IV Reverse Transcriptase (Thermo Fisher Scientific), combined with the primer/probe mix and Digital LightCycler® 5x DNA Master Mix, loaded onto a Digital LightCycler® High Resolution Nanowell Plate, and tested on the Digital LightCycler Analyzer.



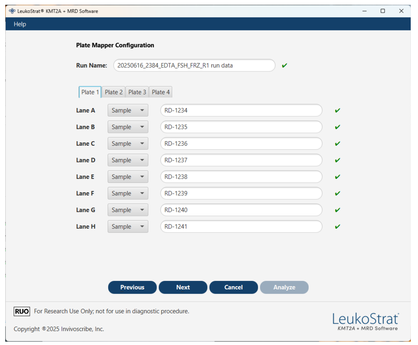
[1]Research Use Only (RUO)

Software Analysis

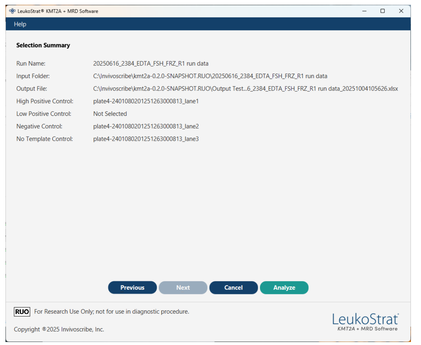
Raw fluorescence data was exported from the Roche Development Software® and analyzed using the LeukoStrat® *KMT2A* + MRD Software.[1] Results were reported as a ratio of: (*KMT2A*r Target Copies) / (*ABL1* Copies).



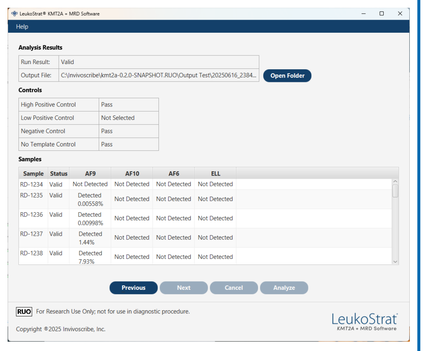
Input/Output folders are selected



A run name is assigned, controls are selected, and samples names are assigned



A selection summary is presented

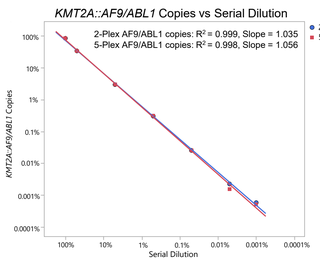


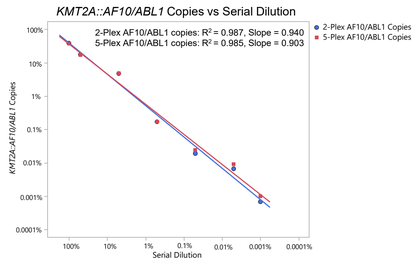
Results for (*KMT2A*r Target Copies) / (*ABL1* Copies) are displayed In the user interface. Additional data is provided in a .csv export

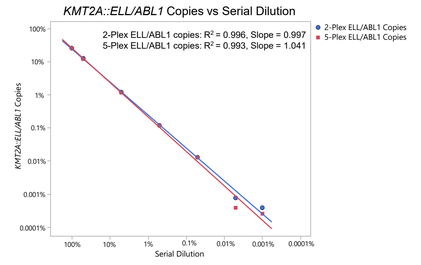
Results

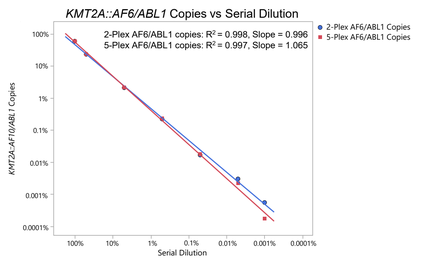
KMT2A::*AF9, KMT2A*::*AF10, KMT2A*::*ELL, and KMT2A*::*AF6* fusions were successfully detected in a single dPCR reaction down to the 0.001% dilution using the Digital LightCycler® High Resolution Nanowell Plate, which contains ~100,000 partitions. *KMT2A*::*AF9* dilution series results are shown below. The measured ratio of *KMT2A*r to *ABL1* matched the expected RNA dilutions over 5 orders of magnitude. For each of the 4 targets, excellent performance and linearity was observed and successful detection of *KMT2A* fusion transcripts in the 0.001% RNA dilution was demonstrated in a single dPCR reaction.

Consistency in *KMT2A*r/*ABL1* ratio was observed in results between 2-plex and 5-plex primer/probe mixes for all four fusion targets.









Coefficient of determination and detection ratio of the lowest dilution using the 5-plex primer/probe mix were: *KMT2A*::*AF9* (R^2=0.998, Detection Ratio = 0.000506%); *KMT2A*::*AF10* (R^2=0.985, Detection Ratio = 0.000908%); *KMT2A*::*ELL* (R^2=0.993, Detection Ratio = 0.000254%); *KMT2A*::*AF6* (R^2=0.997, Detection Ratio = 0.000176%).

Serial Dilution	*KMT2A*:*AF9* / *ABL1* Copies	*KMT2A*:*AF10* / *ABL1* Copies	*KMT2A*:*ELL* / *ABL1* Copies	*KMT2A*:*AF6* / *ABL1* Copies
100%	83.1%	37.5%	25.4%	59.2%
50%	33.5%	17.1%	12.1%	23.2%
5%	2.96%	4.77%	1.16%	2.13%
0.5%	0.311%	0.169%	0.119%	0.230%
0.05%	0.0254%	0.0239%	0.0126%	0.0177%
0.005%	0.00154%	0.00908%	0.000383%	0.00223%
0.001%	0.000506%	0.000980%	0.000254%	0.000176%

Conclusion

Invivoscribe's LeukoStrat® *KMT2A* + MRD Assay[1] is highly sensitive and capable of detecting and differentiating the top four *KMT2A* fusion partners in AML for both screening and MRD. This assay unlocks new MRD detection sensitivity potential in a single reaction by leveraging dPCR technology, making it an accurate and cost-effective choice for monitoring MRD in study subjects with *KMT2A*r AML.