An Optimized NGS Workflow for Detection of FLT3 Internal Tandem Duplication (ITD) in AML Samples

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Introduction

FLT3 ITD, a prognostic and drug target, is present in ~15% of AML patients. FLT3 ITD is a known driver of the acute leukemia, and its presence has been associated with poor survival and shorter time to relapse. The standard assay for FLT3 ITD detection is the capillary electrophoresis (CE) method. However, the sensitivity of this method is limited to small sized ITDs. On the other hand, the Illumina TruSight Myeloid panel (TMP) is broadly used and nearly all NGS assays that can detect FLT3 ITD have been developed based on the CE method. Therefore, the comparison of the FLT3 ITD detection results between these two methods is of interest.

Materials and Methods

We tested 52 unique FLT3 ITDs ranging in size from 6bp to 300bp were detected in 79 clinical AML samples (a total of 230 clinical samples were tested) and 10 cell line samples using our enhanced TMP workflow. The Illumina TruSight Myeloid panel (TMP) is a broadly used off-the-shelf NGS assay that covers 54 genes involved in myeloid malignancies. Although the TMP assay does not detect small and single point mutations, large size ITD cannot be detected by the TMP assay. In addition, low level mutations were not detected by the TMP assay and further confirmed with an independent assay. A low-level hotspot mutations (<2%) can be accurately detected by the custom algorithm which is based on the custom designed PCR amplicon sequencing assay.

Results

Table 3. FLT3 ITD Detection in AML Samples (pilot study)

| Sample | Algorithm | Cigar | Caliper | ArcherDX | Standard CE
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|        |          |       |         |          | Low-level hotspot mutations (<2%) can be accurately detected by the enhanced TMP assay and confirmed using an independent Nextera XT assay, which could improve the custom designed PCR target regions. Drop off regions, such as the 52 bp ITD and 126bp ITD, were also recovered by the custom designed PCR target regions. Drop off regions, such as the GC enriched region within CEBPA, were also recovered by the custom designed PCR target regions. Drop off regions, such as the GC enriched region within CEBPA, were also recovered by the custom designed PCR target regions.

Table 4. Methods Comparison to Call FLT3 ITD

| Sample | Algorithm | Cigar | Caliper | ArcherDX | Standard CE
|--------|-----------|-------|---------|----------|------------------|
| AML-S2 |          |       |         |          | Low-level hotspot mutations (<2%) can be accurately detected by the enhanced TMP assay and confirmed using an independent Nextera XT assay, which could improve the custom designed PCR target regions. Drop off regions, such as the GC enriched region within CEBPA, were also recovered by the custom designed PCR target regions. Drop off regions, such as the GC enriched region within CEBPA, were also recovered by the custom designed PCR target regions.

Conclusions

- Molecular MD’s enhanced TMP assay for FLT3 detection allows for accurate and reliable detection of FLT3 ITD ranging in size from 6bp to 300bp.
- Limit of detection of the 126bp ITD is ~0.5%. FLT3 ITD mutation frequencies from all mixtures were confirmed by the Caliper assay which was in closest agreement with the Cigar analysis. All 3 runs detected the same ITD location with the same frequency.

References


For Further Information

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