A single-tube NGS assay for simultaneous detection of DNA and RNA biomarkers as a comprehensive solution for clinical management and guiding therapeutic intervention of Ph-like ALL patients

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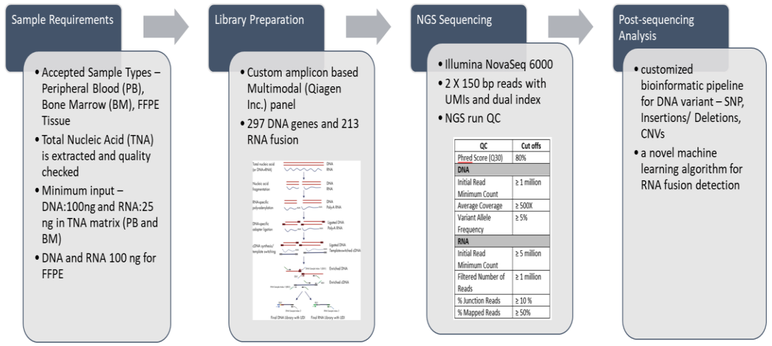
Background

Accurate molecular characterization of acute lymphoblastic leukemia (ALL) subtypes is crucial for clinical management and guiding therapeutic intervention. Over 20 B-ALL subtypes are defined by genetic variants and changes in gene expression. Upregulation of CRLF2 correlates with poor prognosis in 25-30% of Ph-like ALL. Upregulation of CRLF2 can be caused by gene fusion (e.g. IGH-CRLF2, P2RY8-CRLF2 or CSF2RA-CRLF2) or mutations in CRLF2, IKZF1. Most panel-based assays screen either DNA or RNA, requiring higher input material and multiple workflows leading to higher cost and processing time. We report a single tube NGS panel that uses total nucleic acid as input for simultaneous screening of DNA and RNA, providing a comprehensive genetic profile for diagnosis, and therapeutic guidance of Ph-like ALL patients.

Methods

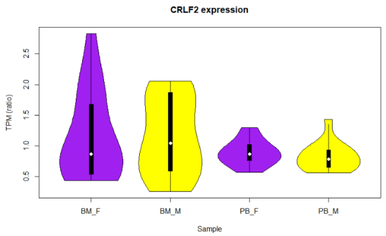


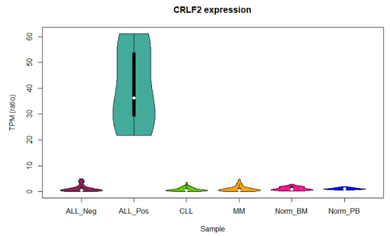
Conclusion

We demonstrate the use of a single tube multimodal NGS assay for comprehensive genomics profiling that simultaneously screens DNA and RNA for expression and variants. It is a powerful and cost-effective tool to help classify Ph-like B-ALL subgroup for clinical management and guiding therapeutic intervention.

Results

CRLF2 expression was tested across sample type, gender and clinical diagnosis to assure uniformity of assay performance in a pilot cohort.

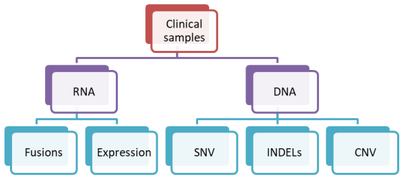




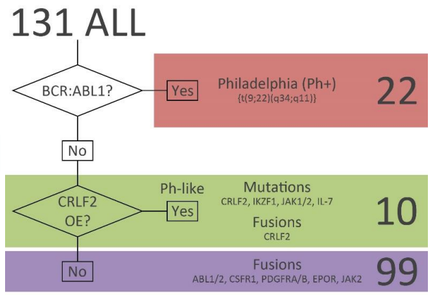
Next, we verified that % of lymphocyte has no impact on the our ability to reliably determine expression levels of CRLF2, and a general correlation between our NGS data with established qRT-PCR assay (n=20).

Sample	Type	Lymphocyte (%)	CRLF2 Exp NGS	Clinical Diagnosis
S01	BM	16.70%	NOE	Normal
S02	PB	3.80%	NOE	Normal
S03	BM	14.70%	NOE	Normal
S04	PB	32.3%	NOE	Normal
S05	BM	12.84%	OE	ALL

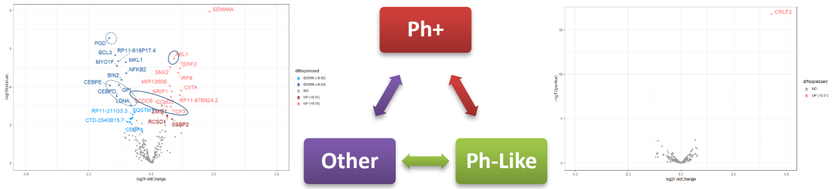
We then proceeded to investigate the robustness of our profiling assay with a clinical cohort.



Molecular events investigated in each sample.



Positive for BCR-ABL1 fusion and show elevated expression of ABL1, and miR-342-3p regulation.

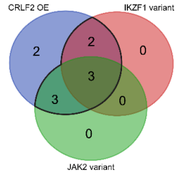


Enriched for genes linked with cancer associated metabolic reprograming (WikiPathways) and dysfunction of immune system (Human Phenotype Ontology).

Over expression of CRLF2, variants in IKZF1 and JAK2, enriched for genes under FLT3 regulation (Reactome).

Next we explored the molecular profile of each group to assess if we can generate molecular signatures.

Within the 10 CRLF2 OE samples, 8 had variants in either IKZF1 or JAK2, one had a pathogenic variant in CRLF2 and one had no pathogenic variants reported.



Finally, a pathway enrichment analysis highlighted the molecular mechanisms that are affected in our cohort.

Additional data will allow refinement of this profile and can lead to better clinical management.

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