DEVELOPMENT OF **AN NGS ASSAY FOR *IGK*** THAT CAN BE **COMBINED WITH *IGH*** FOR IDENTIFYING CLONAL POPULATIONS IN LYMPHOID MALIGNANCIES

Niels C. Adams*[1], Gillian M. Pawlowsky[1], Ying Huang[2], Kasey Hutt[2], Michael Klass[2], Jeffrey Miller[1,2]

[1]LabPMM GmbH, Munich, Germany,
[2]Invivoscribe Technologies, Inc., San Diego, CA, United States

Introduction

Previously we developed next generation sequencing assays for the detection and characterization of clonality in B-cells, targeting the *IGH* locus with single step PCR approach followed by sequencing and analysis. These *IGH* assays were shown to be both sensitive and robust in a number of different laboratories and have demonstrated utility for tracking residual disease. *IGK* is also an important locus that can be used for the identification and tracking of clonality, as *IGK* rearrangements are stable markers that are retained even in lambda light chain expressing B cells, as Kde rearranges to Vk elements as well as to the recombination signal sequence (RSS) in the Jk5-Ck intron (INTR) prior to rearrangements within *IGL*. Accordingly, *IGK* is an ideal marker for tracking residual disease in B-lineage ALL.

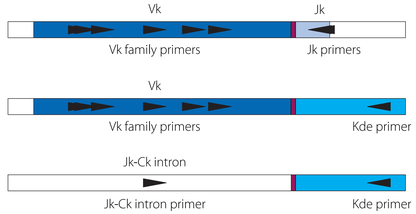
This poster describes the development of an assay for *IGK* that can be combined with *IGH* that will increase the chance of identifying clonal populations in lymphoid malignancies such as non-Hodgkin's Lymphoma and tracking a variety of B-cell tumours.

Methods

- Genomic DNA from peripheral blood, tonsil and bone marrow aspirates were tested for *IGK* gene rearrangements.
- Optimized V and J consensus primers targeted all *IGK* V and J region gene segments that are rearranged in lymphoid cells as well as the Kde rearrangements that precede lambda selection.
- Multiplex PCR master mixes simultaneously amplified *IGK* V-J gene rearrangements and incorporated sample identifying indices into amplicons, which facilitated combining and sequencing libraries generated testing up to 12 samples and controls in each run.
- Amplicon products were purified, quantified, the concentrations adjusted, pooled, and the harmonized libraries were loaded into the OneTouch 2. The enriched emulsion PCR libraries were sequenced using Ion 316 Chip Kit v2s and Ion PGM Sequencing 400 Kits.

FIGURE 1: ASSAY DESIGN

Single tube / single Step PCR with three arrangement types detected:



- Identical PCR programme to existing Lymphotrack assays *IGK*, *IGH* FR1 & *TRG* on the same plate.
- Assays can be combined with other assays using the same index.
- Analysis program separates *IGK* data from other assays.

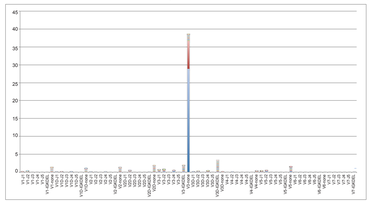
- Data were analysed using Invivoscribe's proprietary bioinformatics software (included with the test kits) on a standard personal computer.
- The bioinformatics package allows for *IGH* and *IGK* libraries with the same barcodes to be analysed on the same run.
- The PGM platform allows a sustainable, rapid turn around time, less than 36 hours from primary sample to result.

FIGURE 2: ASSAY WORKFLOW

From DNA to clonal sequence results in < 24 hours

FIGURE 3: CLINICAL DATA

Clinical samples that are not detected as clonal by *IGH* FR1 can be detected for clonality by *IGK*.



Total Reads 280190

	Rank	Sequence	Length	Raw count	V-gene	J-gene	% total reads	Cumulative %	V-Coverage (%)
1		AACAGGGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTTAGTCTCACCATCAGTGGCCTAGAGCCTGAAGATTCTGCAGTTTATTACTGTCAACAACGTAGCAACTGGCCTCTCACTTTCGGCGGA	144	81036	IGKV3-11_01	none	28.9218031	28.9218031	99.24
2		AACAGGGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTTACTCTCACCATCAGTAGCCTAGAGCCTGAAGATTCTGCAGTTTATTACTGTCAACAACGTAGCAACTGGCCTCTCACTTTCGGCGGA	144	21879	IGKV3-11_01	none	7.8086299	36.7304329	99.24

Results

- Data generated with the LymphoTrack® *IGK* assay and bioinformatics package reproducibly identified clonality and DNA sequences of *IGK* tVk-Jk, as well as Vk-Kde and INTR-Kde, gene rearrangements.
- Automated data outputs include frequency distributions and V-J gene usage.
- DNAs from cell lines serially diluted into polyclonal tonsil DNA, confirmed the linearity (R^2 >0.99) and low run-to-run variance of the assay.
- This assay may be used with genomic DNA from peripheral blood, bone marrow or FFPE.
- In addition to the assay development, the break point of the INTR element was also identified.
- Carrying out combined *IGH* and *IGK* testing increased the ability to define clonal populations by >11% of clinical samples tested compared to *IGH* alone in the clinical samples tested.

FIGURE 4: ASSAY CHARACTERISTICS

- Polyclonal templates have low individual frequences (<0.5%)
- Sequences present at 2.5% are easily identifiable
- Homopolymer regions can result in two sequences being reported
- Software solution for consolidation of sequences in beta testing
- Assays have good linearity (R^2 > 0.99)
- Little variation between runs and operators (R^2 > 0.99)

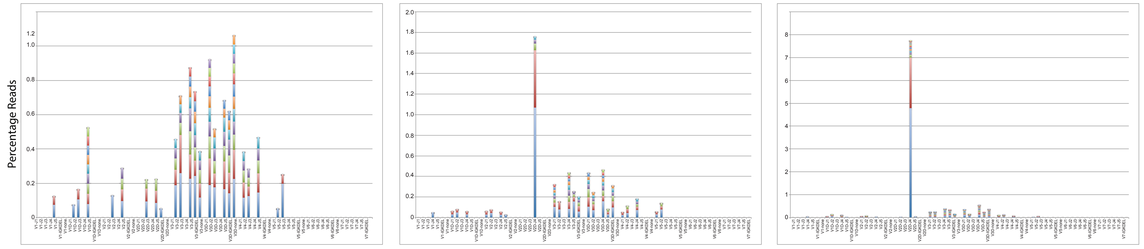
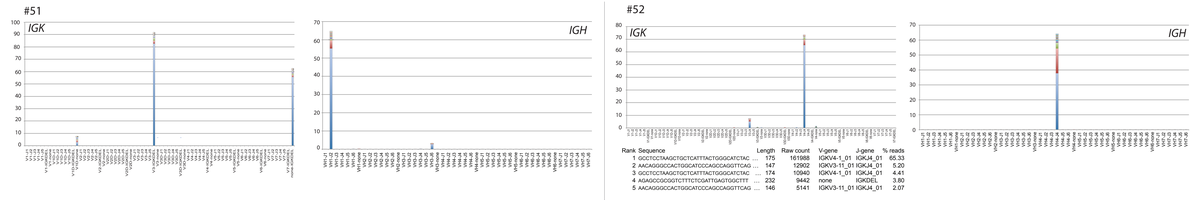


FIGURE 5: *IGK* AND *IGH* IN CLINICAL SAMPLES



Rank	Sequence	Length	Raw count	V-gene	J-gene	% reads
1	GCCTCCTAAGCTGCTCATTTACTGGGGCATCTAC ...	175	161988	IGKV4-1_01	IGK-J4_01	65.33
2	AACAGGGCCACTGGCATCCCAGCCAGGTTCAG ...	147	12902	IGKV3-11_01	IGK-J4_01	5.20
3	GCCTCCTAAGCTGCTCATTTACTGGGGCATCTAC ...	174	10940	IGKV4-1_01	IGK-J4_01	4.41
4	AGAGCCGCGGTCTTTCTCGATTGAGTGGCTTT ...	232	9442	none	IGKDEL	3.80
5	AACAGGGCCACTGGCATCCCAGCCAGGTTCAG ...	146	5141	IGKV3-11_01	IGK-J4_01	2.07

Conclusions

We have previously developed *IGH* and *TRG* assays for the Ion PGM.

We now add an NGS clonality assay for *IGK* that identifies clonal *IGK* Vk-Jk, as well as Vk-Kde and INTR-Kde, rearrangements and associated specific rearranged DNA sequences to the repertoire that can either be used in reflex testing to further characterise samples that are difficult to characterize by *IGH* alone.

Alternatively, a more time and cost efficient approach would be to run both assays at the same time on samples to be run simultaneously.

These assays are being developed to detect and monitor lymphoproliferative disease.

A further advantage is that the assays identify clonal lymphocyte populations by their unique DNA sequences that can subsequently be used to track clones in residual disease testing.