Validation and clinical implementation of next generation sequencing for routine IGH and TCRG clonality assessment

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Introduction

IGH and TCR gamma clonality testing is an important component in the diagnosis of lymphoproliferative disorders. Capillary electrophoresis (CE) of multiplexed PCR products is a common method of analysis. Although robust, simple and highly reproducible, it does not provide a full characterization of clonal sequences and lacks sensitivity and specificity to track clones in subsequent samples. We describe our clinical validation and implementation of an NGS based assay for initial clonal characterization and minimal residual disease assessment through patient specific clone tracking.

Methods

Blood, bone marrow and FFPE tissue samples submitted for routine clonality assessment were selected. DNA was extracted and tested using both standard CE and LymphoTrack® *IGH* +*TRG* – MiSeq assays (InVivoScribe). Positive, negative and no template controls were run with all assays. Sensitivity and LOD were assessed based on dilution studies for detection of initial clonal population and subsequently for tracking of a pre-characterized clone at minimal residual disease levels.

Results

A total of 160 samples were analyzed including 126 clonal (defined by initial CE testing, 92 IGH, 34 TCRG) and 34 non-clonal samples with 94% concordance between the 2 methods. Discordant results (clonal by CE, non clonal by NGS) were attributable to pseudoclonality in the post treatment setting. Based on a minimum input of 50ng of high quality DNA, analytical sensitivity was 5% for diagnostic samples (un-characterized clone) with good Inter and intra-assay reproducibility. Further dilution studies to establish LOD for tracking a previously characterized clone showed accurate detection at 1x10-5 with 1-2ug DNA input. The mean number of reads per sample was approximately 500,000.

Conclusion

Assessment of clonality by NGS methods provides significant improvement over existing clonality assays using fragment analysis by CE. Sensitivity for detection of a diagnostic clone is similar to the CE assays but provides full characterization of the clone to enable tracking in subsequent samples at the MRD level. NGS testing readily resolved pseudoclonality calls in post-treatment samples by differentiating clonal products of same size but different sequences interpreted as clonal by the CE method. However, the LymphoTrack® assays remain expensive and with higher TAT compared to CE.

Acknowledgements

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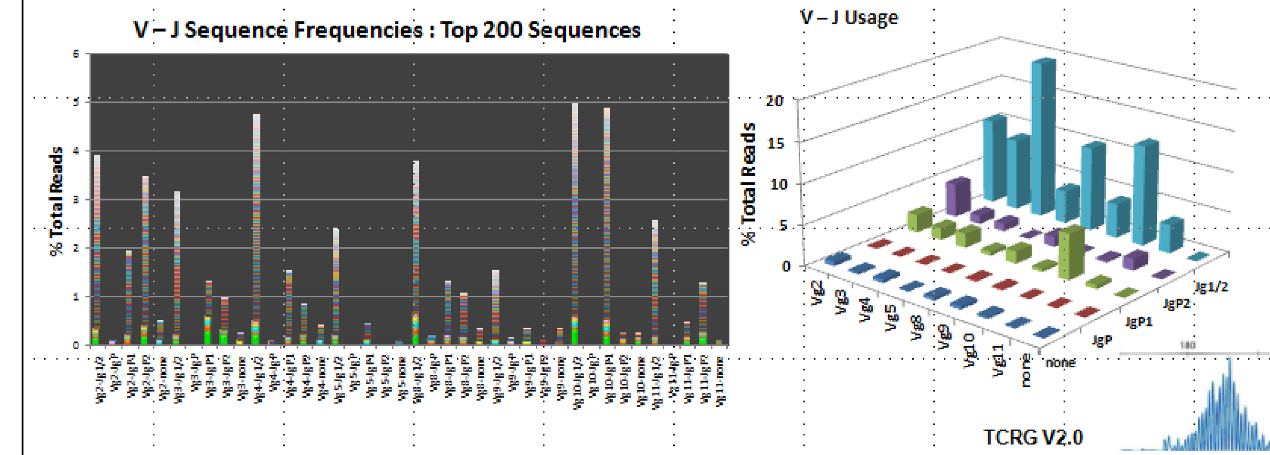
Results

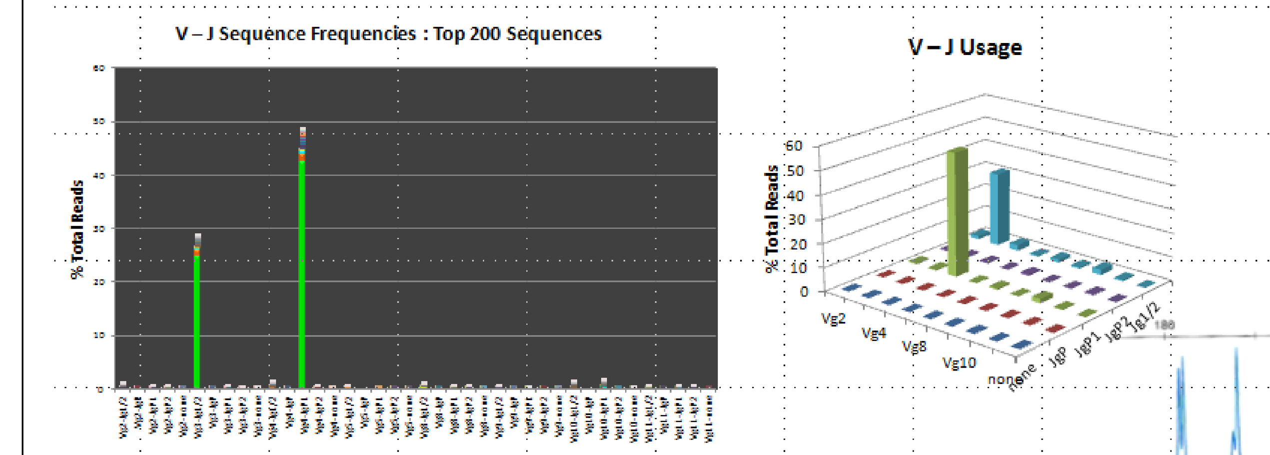
Lymphotrack read summary for polyclonal sample

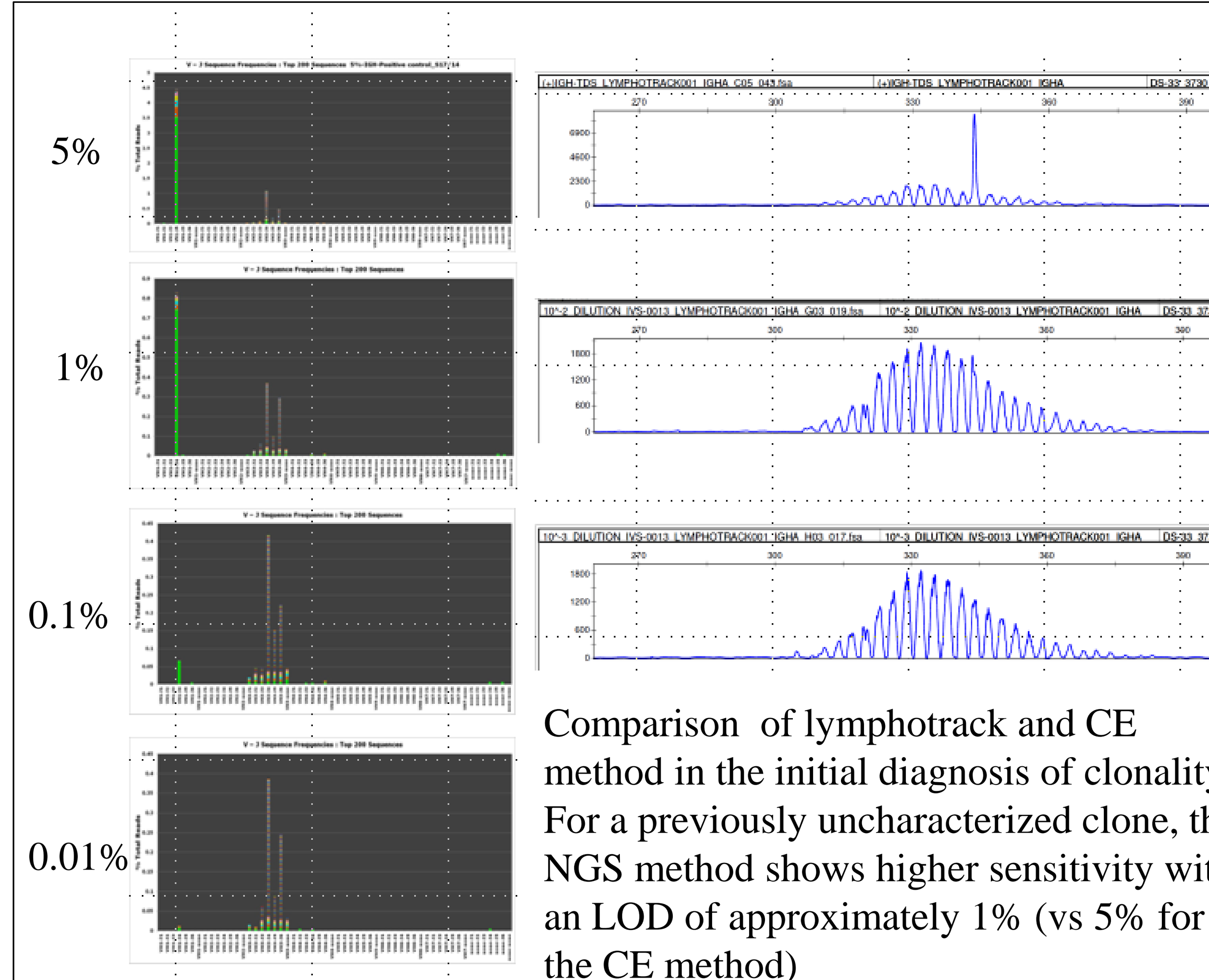
Total count	453,969						
Rank	**Sequence**	**Length**	**Raw count**	**V-gene**	**J-gene**	**% total reads**	**Cumulative %**
1	TGGGTAAGA	129	1257	Vg10	Jg1/2	0.2768912	0.2768912
2	GGACTCAGT	106	1110	Vg3	JgP1	0.2445101	0.5214012
3	GGACTCAGT	121	940	Vg3	JgP2	0.2070626	0.7284638
4	GGAATCAGC	146	898	Vg4	Jg1/2	0.1978109	0.9262747
5	GGAATCAGT	140	835	Vg8	Jg1/2	0.1839333	1.1102080
6	TGGGTAAGA	121	799	Vg10	JgP1	0.1760032	1.2862112
7	GGAGTCAGT	116	733	Vg2	JgP2	0.1614648	1.4476759
8	GGACTCAGT	111	677	Vg3	JgP1	0.1491291	1.5968051
9	GGAATCAGT	133	629	Vg8	Jg1/2	0.1385557	1.7353608
10	GAAGACTAA	128	629	Vg11	JgP2	0.1385557	1.8739165



Lymphotrack read summary for clonal sample (biclonal/biallelic)

Total count	572,268						
Rank	**Sequence**	**Length**	**Raw count**	**V-gene**	**J-gene**	**% total reads**	**Cumulative %**
1	GGAATCAGC	122	241712	Vg4	JgP1	42.2375530	42.2375530
2	GGACTCAGT	138	141811	Vg3	Jg1/2	24.7805224	67.0180754
3	GGAATCAGC	122	8247	Vg4	JgP1	1.4411080	68.4591835
4	GGACTCAGT	138	5451	Vg3	Jg1/2	0.9525257	69.4117092
5	GGAATCAGC	121	3590	Vg4	JgP1	0.6273285	70.0390377
6	GGACTCAGT	137	2399	Vg3	Jg1/2	0.4192092	70.4582468
7	GAATCAGCC	121	1669	Vg4	JgP1	0.2916466	70.7498934
8	GGAATCAGC	123	1308	Vg4	JgP1	0.2285642	70.9784576
9	GGAATCAGC	143	1104	Vg4	Jg1/2	0.1929166	71.1713743
10	GGAATCAGT	141	997	Vg8	Jg1/2	0.1742191	71.3455933

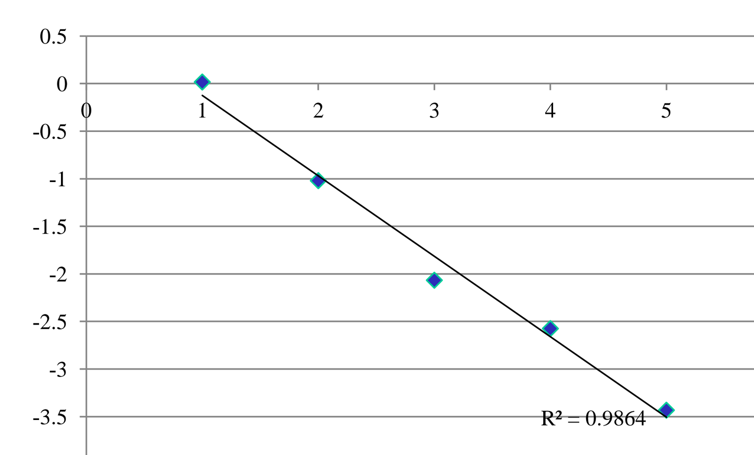




Comparison of lymphotrack and CE method in the initial diagnosis of clonality. For a previously uncharacterized clone, the NGS method shows higher sensitivity with an LOD of approximately 1% (vs 5% for the CE method)

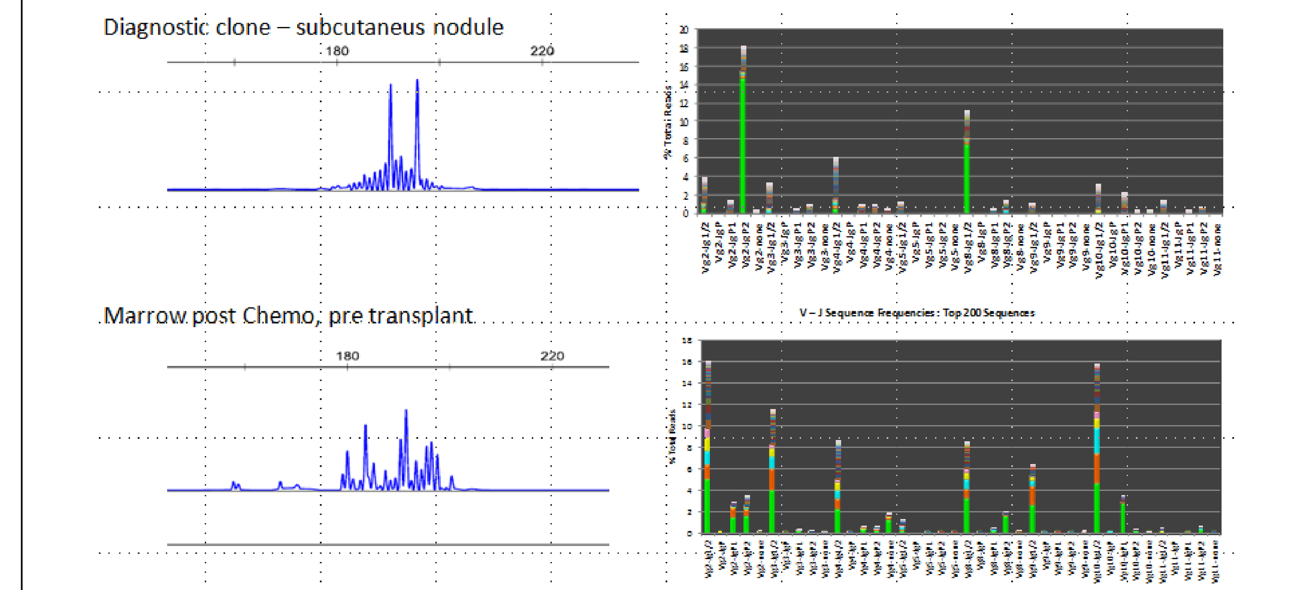
IGH dilution study (cell line - 1ug input)

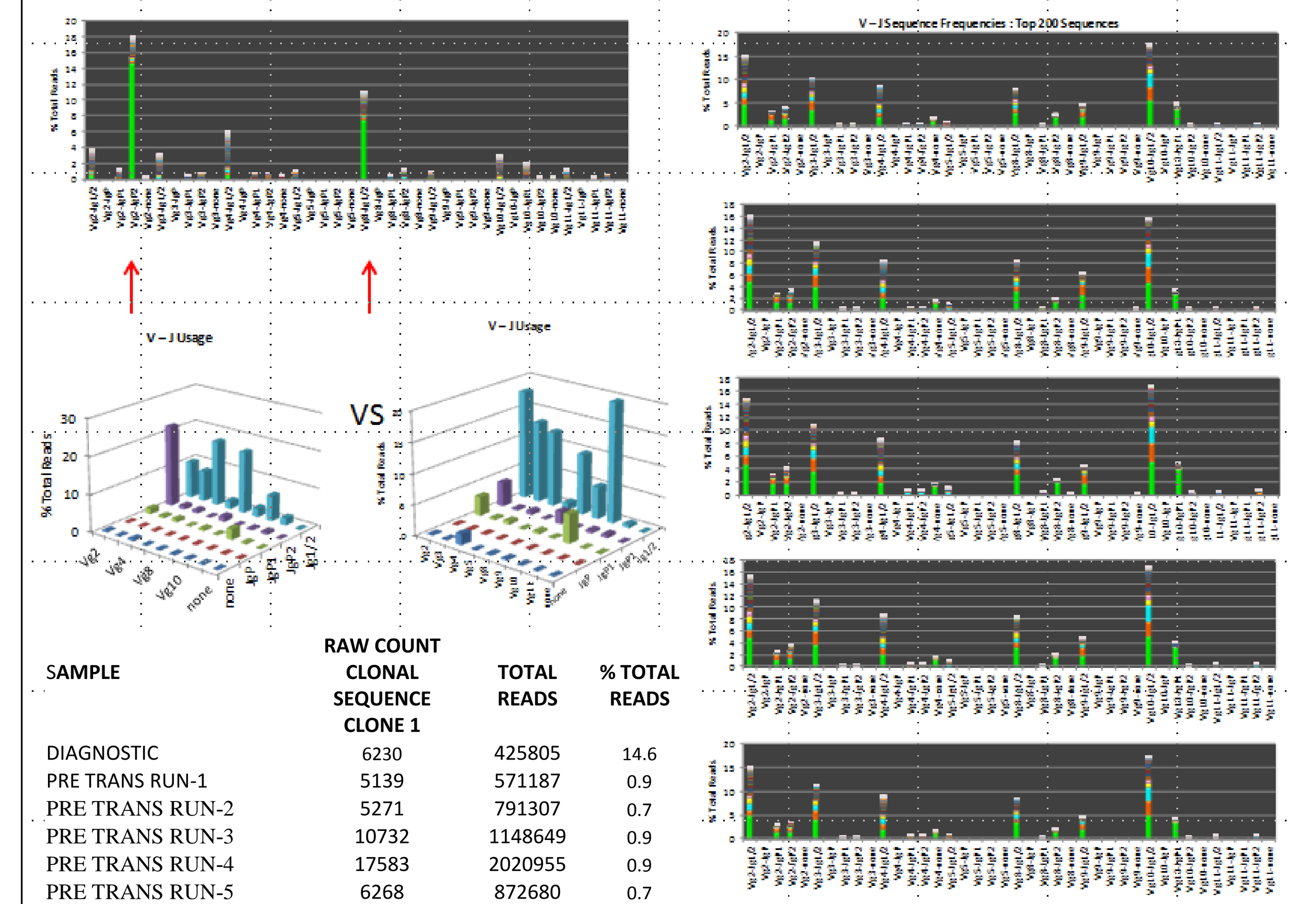
Dilution (IVS-0013 into IVS 0000)	target sequence run 1	Target Sequence run 2	Total reads run 1	Total reads run 2	Total target sequence	Total reads (run 1+2)	Calculated % target reads
1% (1/100)	5152	5898	498,330	565,180	11050	1,063,510	1.039012
0.1% (1/1000)	381	447	396,417	470,979	828	867,396	0.095458
0.01% (1/10,000)	64	27	637,782	422,769	91	1,060,551	0.008580
0.001% (1/100,000)	17	4	431,755	356,569	21	788,324	0.002664
0.0001% (1/1,000,000)	0	3	425,060	387,118	3	812,178	0.000369



MRD case study #2

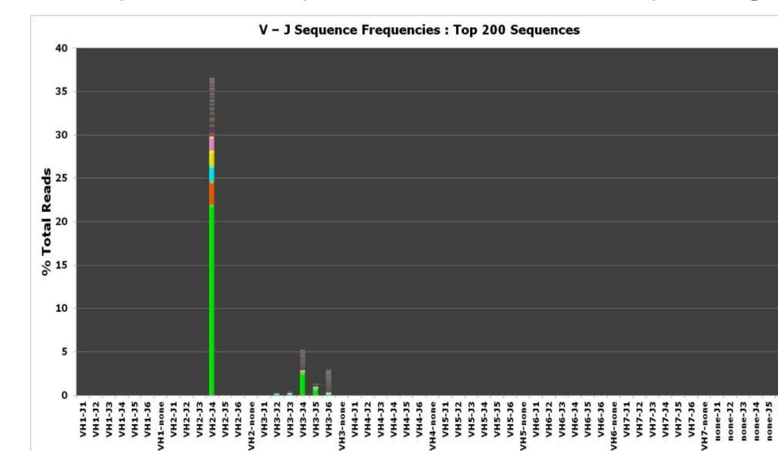
57 yo male with growing subcutaneous nodule in the arm diagnosed as typical T cell proliferation most consistent with angioimmunoblastic T cell lymphoma. Bone marrow submitted for evaluation of disease, pretransplant





SAMPLE	RAW COUNT CLONAL SEQUENCE CLONE 1	TOTAL READS	% TOTAL READS
DIAGNOSTIC	6230	425805	14.6
PRE TRANS RUN-1	5139	571187	0.9
PRE TRANS RUN-2	5271	791307	0.7
PRE TRANS RUN-3	10732	1148649	0.9
PRE TRANS RUN-4	17583	2020955	0.9
PRE TRANS RUN-5	6268	872680	0.7

MRD case study #1 - 51 yo male with newly diagnosed plasma cell neoplasm



Diagnostic sample
Bone marrow: Plasma cell myeloma, involving 80-90% marrow cellularitySmall monotypic B-cell population detected by flow cytometry

Post treatment sample
Bone marrow: Morphology: no evidence . Flow cytometry: Minute abnormal plasma cell population detected by flow cytometry (0.035%)
Lymphotrack: 0.12% (744/632030, 99% identity)

References

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