Targeted sequencing of *BRAF* by MinION in archival Formalin-Fixed Paraffin-Embedded specimens allows to discriminate between Hairy Cell Leukemia and Hair Cell Leukemia Variant

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Hairy cell leukemia (HCL) is a B-cell leukemia characterized by the pathological expansion of B-cell clones within the spleen, bone marrow, and peripheral blood. HCL is characterized by atypical cells that possess archetypical hair-like projections of the cytoplasm. An HCL-like lymphoproliferative disease has been recently recognized, which has been named HCL variant (HCL-V), and is characterized by similar cytological features to HCL and by a less indolent clinical behavior. On Immunohistochemistry (IHC), HCL cells are highly positive for **Annexin A1**, which is not expressed in HCL-V clones (Figure 1).

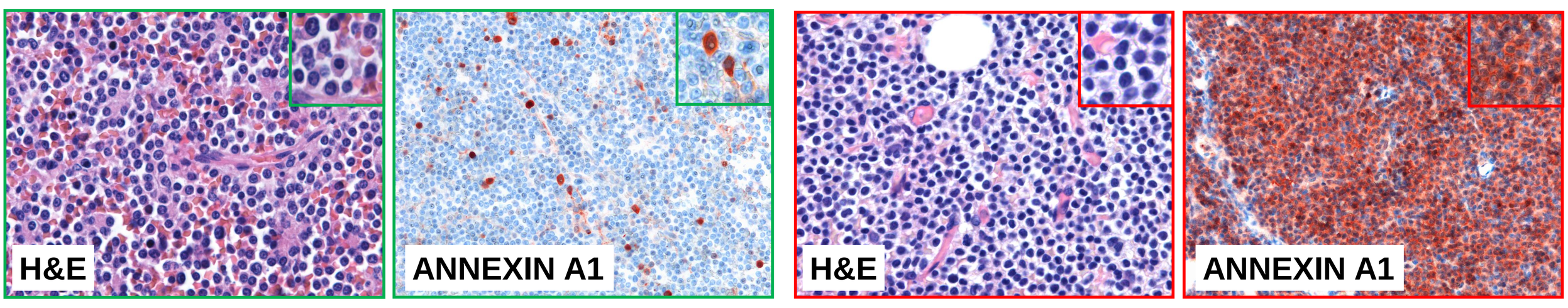


Fig.1: the two archival cases (diagnosed in 2013 et 2010) used for *BRAF* sequence analysis have been confirmed as HCL-V and HCL accordingly to their differential IHC expression of Annexin A1

The molecular hallmark of HCL is the ***BRAF*** mutation V600E (*c.1799T>A*), which is virtually constant in HCL clones representing a pathogenic driver of the disease. ***BRAF*** V600E is not found in HCL-V. (Verma S. et Al. Am J Clin Pathol. 2012; Tiacci E. et Al. N Engl J Med. 2011).

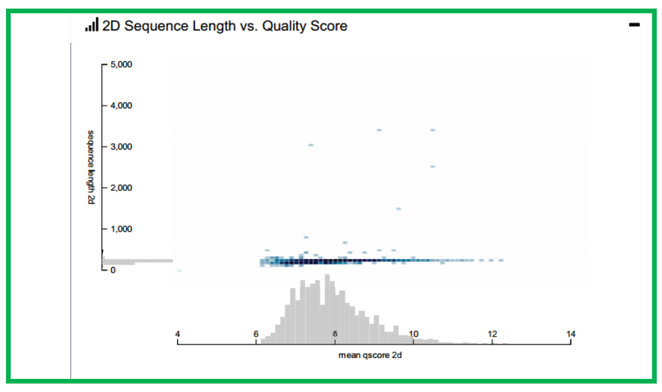
Formalin-Fixed Paraffin-Embedded (FFPE) tissues are the standard source of information for diagnosis in Pathology. Archival FFPE specimens represent an invaluable resource for biomedical research, because of their broad availability and of the possibility to be matched with clinical and follow-up data. FFPE specimens are usually employed for histochemical and immunophenotypical analyses. The use of nucleic acids derived from FFPE samples would be highly desirable, but methods based on their extraction are flawed by the high degree of DNA and RNA fragmentation and by chemical-induced mutations (Hedegaard J. et Al. PLoS One. 2014).

AMPLIFICATION OF *BRAF* region of interest after DNA extraction

>Amp_1 Seq2 + Seq1 ==> gi|224589819|ref|NC_000007.13| Homo sapiens chromosome 7, GRCh37.p2 primary reference assembly
TAGTAACTCAGCAGCATCTCAGGgccaaaaatttaatcagtggaaaaatagcctcaattcttaccatccacaaaatggatccagacaactgttcaaactgatgggacccactccatcgagatttc[**A**/**T**]ctgtagctagaccaaaatcacctatttttactgtgaggtctt catgaagaaatatatctgaggtgtagtaagtaaaggaaaacagtagatctcattttt**CCTATCAGAGCAAGCATTATGAAGAG**

MinION analysis

HCL-V



2D Basecalling Summary

This report shows the outcome of the workflow for each read. Select a row to filter reads with any Exit Status or Run ID from the rest of the report

Exit Status - Read Counts

Exit Status	Total	Filtered
Workflow successful	139	139
No template data found	1	1
No complement data found	1137	1137
Calibration strand detected	2	2
2D basecall failed quality filters	804	804
2D basecall could not be performed	41	41
1D basecall could not be performed	15	15

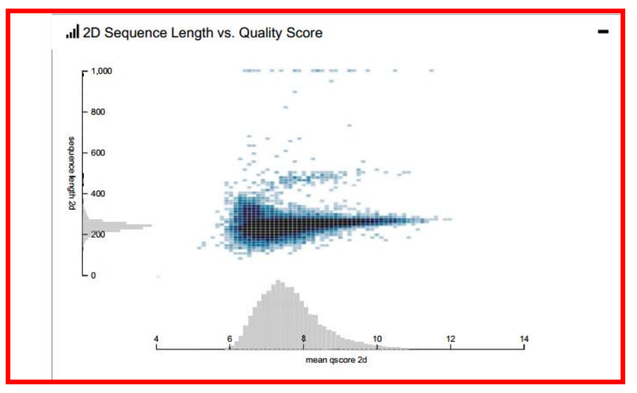
HCL

2D Basecalling Summary

This report shows the outcome of the workflow for each read. Select a row to filter reads with any Exit Status or Run ID from the rest of the report

Exit Status - Read Counts

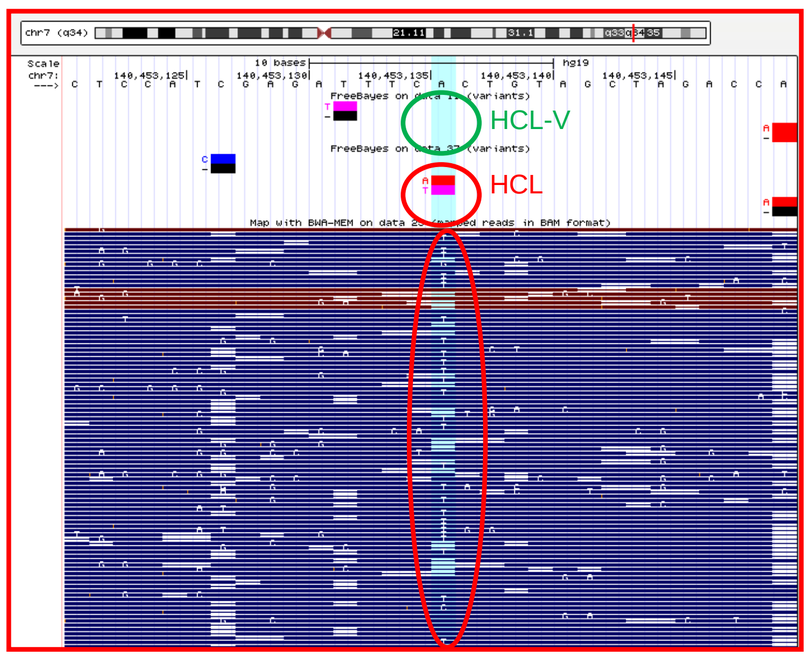
Exit Status	Total	Filtered
Workflow successful	1327	1327
No template data found	68	68
No complement data found	25228	25228
Calibration strand detected	3	3
2D basecall failed quality filters	16291	16291
2D basecall could not be performed	804	804
1D basecall could not be performed	241	241

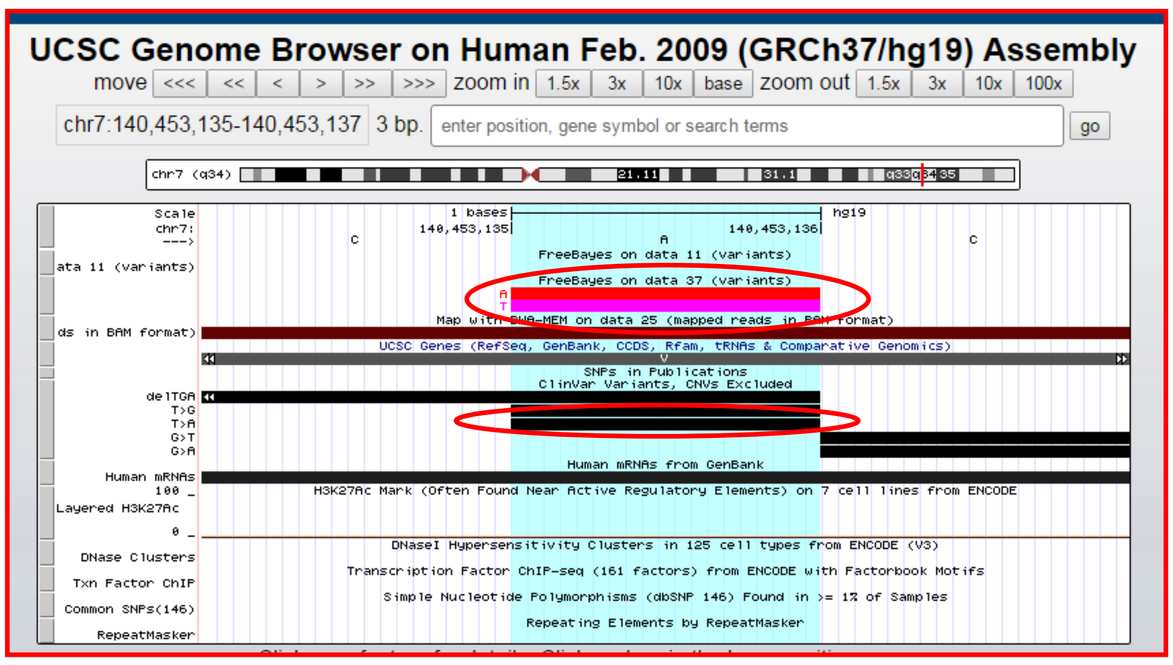


Data analysis://usegalaxy.org/

VCF for HCL-V and HCL

Aligned BAM In HCL





It is the same Clinical Variant

In order to analyze if MinION could be used to sequence DNA from suboptimal or critical samples, such as archival FFPE specimens, we investigated the feasibility of determining the mutational status of *BRAF* to discriminate between HCL and HCL-V.

We successfully applied MinION-based targeted NGS to routine diagnostic FFPE samples allowing the specific detection of *BRAF* V600E (*c.1799T>A*) mutation in HCL. This paves the way for MinION adoption in molecular pathology for research and/or diagnostic uses.