DETECTING CHROMOSOMAL ABERRATIONS IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS BY EXOME SEQUENCING: A COPY NUMBER APPROACH

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In addition to the identification of somatic point mutations and small insertions and deletions in the protein coding regions, exome sequencing allows to detect chromosomal aberrations of tumor DNA. Although challenging due to the experimental variability of the exon target enrichment, we were able to confirm various chromosomal aberrations in acute myeloid leukemia (AML) patients including a trisomy 13, an *MLL/AF9* rearrangement with a partial loss of the *MLL* and downstream genes, and a complex karyotype with unbalanced translocations and monosomy 7.

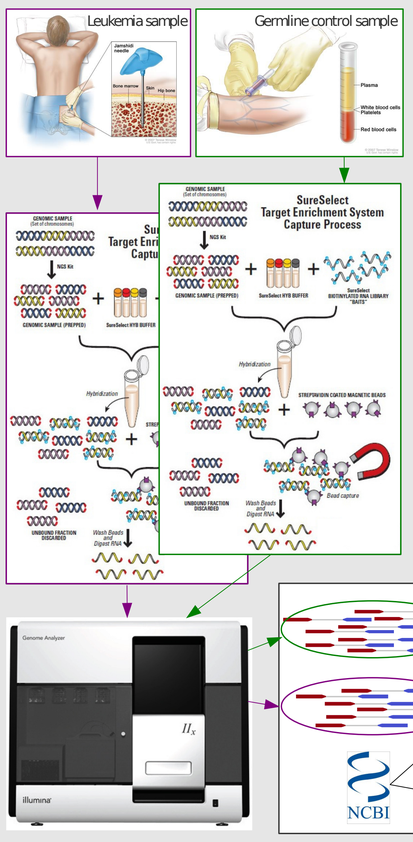

Fig. 1: Overall workflow from patient's samples to Copy Number Alterations.

Sample preparation:
- Extraction of genomic DNA from tumor and germline control samples
- Fragmentation to an average size of 150 bp using a Bioruptor sonicator (Diagenode)
- Enrichment of protein coding regions using the Agilent human all exons 50Mb kit

Sequencing:
- 84 bp paired-end sequencing on a Genome Analyzer IIx platform (Illumina)
- Mapping to the *Human hg18* reference genome using *BWA* (Li and Durbin, Bioinformatics 2009)
- Removing duplicated read pairs (PCR artifacts) using *Samtools* (Li et al., Bioinformatics 2009)

CNA calling:
- Defining exon read depths using *GATK: DepthOfCoverage* (McKenna et al., Genome Res. 2010)
- A linear regression model builds the tumor sample coverage as a linear function of the control sample coverage (Rigaill et al., Bioinformatics 2012)
- Maximum-likelihood segmentation using a Bayesian Information Criterion adapted for segmentation problems (Zhang and Siegmund, Biometrics 2007)

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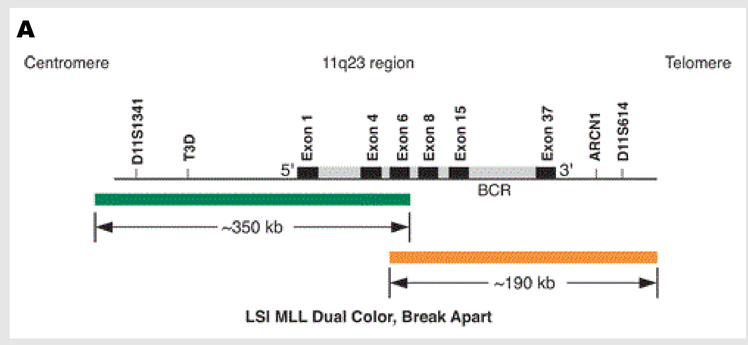

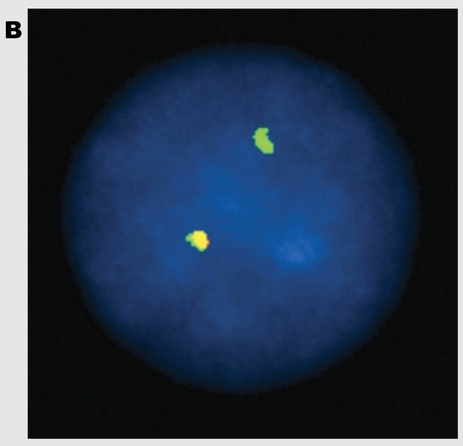

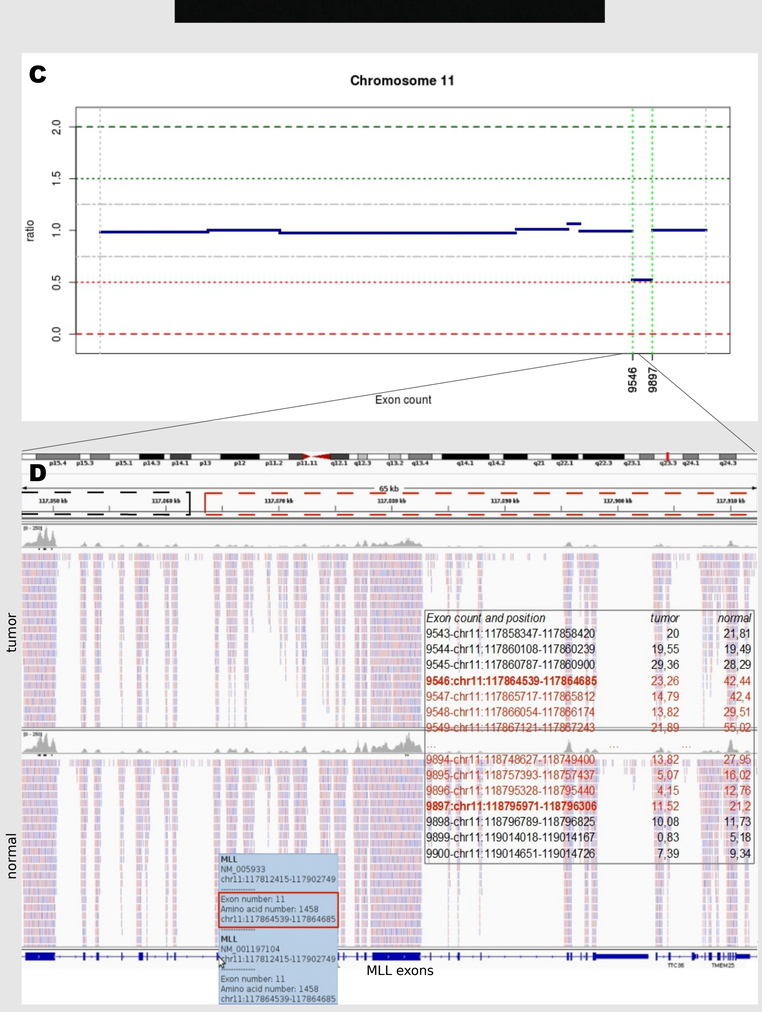

Fig. 2: Detecting Copy Number Alterations in an AML patient carrying an *MLL/AF9* gene fusion with break apart of 3' *MLL* and downstream genes.
A) Probe map of an *MLL* break apart rearrangement probe for fluorescence in situ hybridization (FISH)
B) Example result of FISH indicating the 3' *MLL* break apart
C) Segmentation of chromosome 11 with reduced coverage ranging from exon #9,546 to exon #9,897 (ratio ~0.5), indicating somatic gene copy loss of 3' *MLL* and downstream genes
D) Visual validation of the breakpoint in *MLL* using the IGV browser. Segments are coloured with black for equivalent coverage and red for reduced coverage

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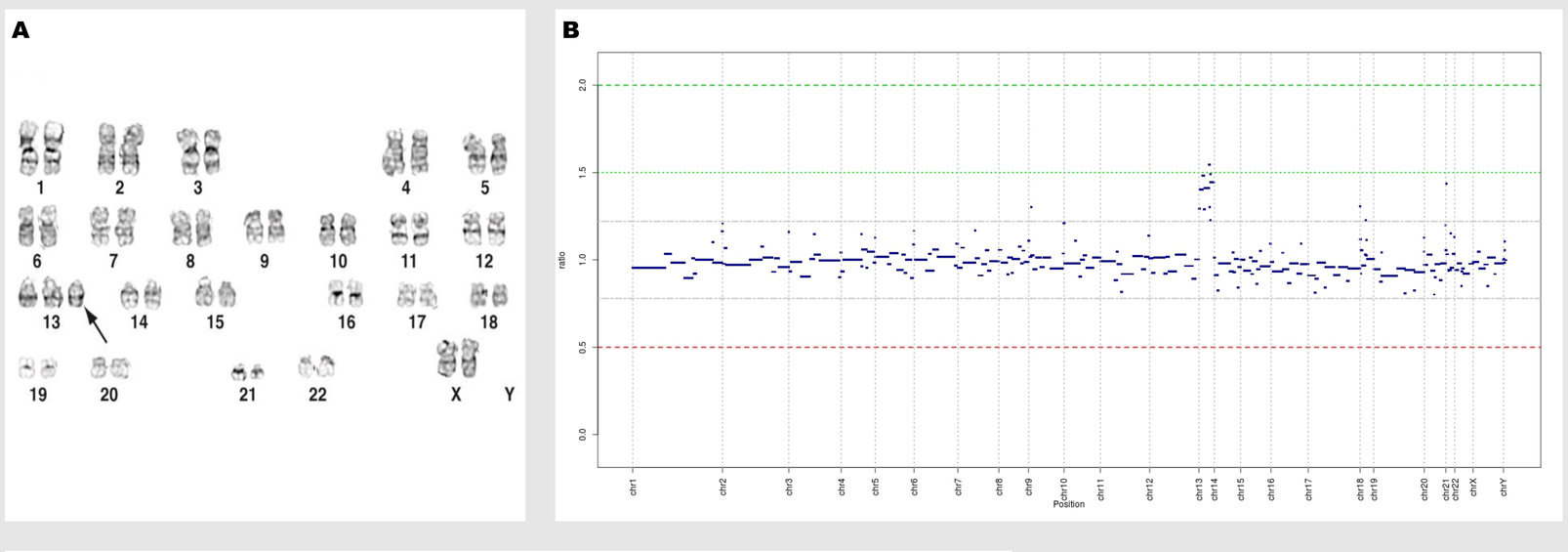

Fig. 3: Results of the approach to detect CNAs in AML patients carrying a trisomy 13 and with complex karyotype.
A) Example karyogram of an AML patient carrying a trisomy 13
B) CNA analysis of an AML patient with a trisomy 13. The coverage of almost all exons on chromosome 13 are elevated in tumor vs. remission sample (ratio ~1.5)
C) CNA analysis of an AML patient with complex karyotype including unbalanced translocations t(1;2)(p12;q37), t(5;17)(q11;q11), t(8;11)(q24;q12) and a monosomy 7 resulting in corresponding copy number changes on chromosomes 1, 11 (ratio ~1.5) and 5, 7, 17 (ratio ~0.5)

Vardimann, Am J Clin Pathol, 2009