Optical Genome Mapping (OGM) from Bionano Genomics for dummies

MB&C Course UCLL Workshop session-4.3

February 6th, 2026

Barbara Dewaele, PhD

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Clinical Laboratory Geneticist

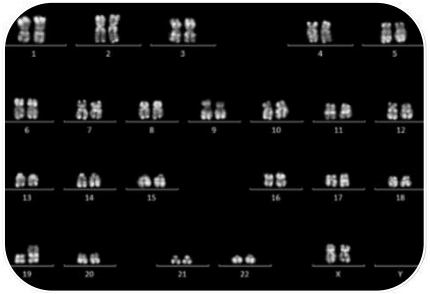
Supervisor of the Laboratory for Genetics of Hematological Malignancies

Center for Human Genetics, University Hospitals Leuven

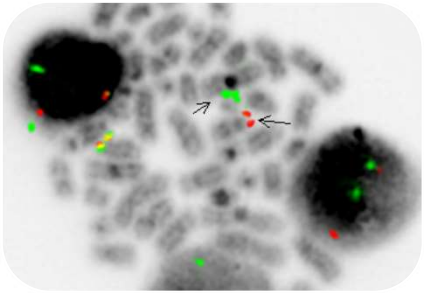
KU LEUVEN

CME

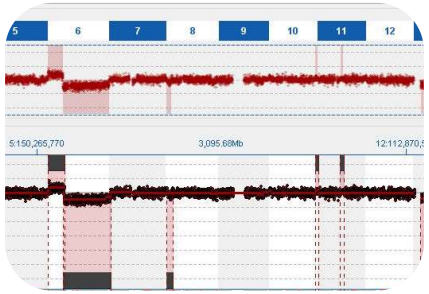
Current routine cytogenetic and molecular genetic testing procedures



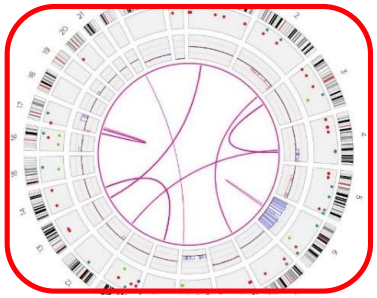
Karyotyping



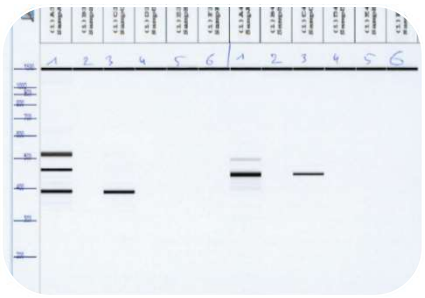
FISH



LPS or aCGH/SNPa

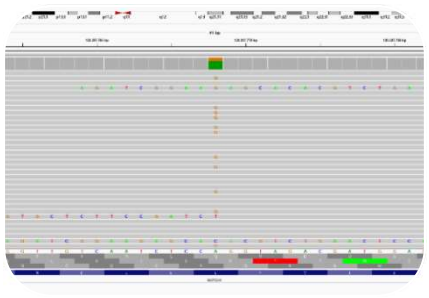


OGM



PCR

(Classical PCR, RT-PCR, Q-PCR, ddPCR, …)



NGS targeted gene panel

Optical Genome Mapping

PNAS

Single-molecule denaturation mapping of DNA in nanofluidic channels

Walter Reisner[a,b,c,2], Niels B. Larsen[b], Asli Silahtaroglu[d], Anders Kristensen[b], Niels Tommerup[d], Jonas O. Tegenfeldt[c,1], and Henrik Flyvbjerg[b,1]

[a]Department of Physics, McGill University, Montreal, QC, Canada; [b]Department of Micro- and Nanotechnology, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark; [c]Department of Physics, Division of Solid State Physics, Lund University, Box 118, S-221 00, Sweden; and [d]Department of Cellular and Molecular Medicine, Wilhem Johannsen Centre for Functional Genome Research, University of Copenhagen, Blegdamsvej 3B, Building 24.4, Copenhagen N, Denmark

Communicated by Robert H. Austin, Princeton University, Princeton, NJ, May 22, 2010 (received for review December 27, 2009)

Optical Genome Mapping

Journal of Pathology
J Pathol October 2021; **255**: 202–211
Published online 29 July 2021 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.5755

ORIGINAL PAPER

Optical genome mapping identifies a germline retrotransposon insertion in *SMARCB1* in two siblings with atypical teratoid rhabdoid tumors

Mariangela Sabatella[1†], Tuomo Mantere[2,3,4†], Esmé Waanders[5], Kornelia Neveling[2], Arjen R Mensenkamp[2,3], Freerk van Dijk[1], Jayne Y Hehir-Kwa[1], Ronnie Derks[2,3], Michael Kwint[2,3], Luke O'Gorman[2,3], Madalena Tropa Martins[1], Corne EM Gidding[1], Maarten H Lequin[6], Benno Küsters[7], Pieter Wesseling[1,8], Marcel Nelen[2,3], Jacklyn A Biegel[9,10], Alexander Hoischen[2,3,11‡], Marjolijn C Jongmans[1,5‡] and Roland P Kuiper[1,2*‡]

genes

MDPI

Article

Optical Genome Mapping in Routine Human Genetic Diagnostics—Its Advantages and Limitations

Paul Dremsek *, Thomas Schwarz, Beatrix Weil, Alina Malashka, Franco Laccone and Jürgen Neesen

GENES CHROMOSOMES & CANCER

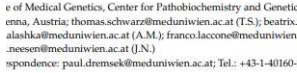
RESEARCH ARTICLE | Full Access

Optical genome mapping, a promising alternative to gold standard cytogenetic approaches in a series of acute lymphoblastic leukemias

Valentin Lestringant ✉, Nicolas Duployez, Dominique Penther, Isabelle Luquet, Coralie Derrieux, ... See all authors

Multicenter Study | Am J Hum Genet. 2021 Aug 5;108(8):1409-1422.
doi: 10.1016/j.ajhg.2021.05.012. Epub 2021 Jul 7.

Optical genome mapping enables constitutional chromosomal aberration detection



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enna, Austria; thomas.schwarz@meduniwien.ac.at (T.S.); beatrix.weil@medu
alashka@meduniwien.ac.at (A.M.); franco.laccone@meduniwien.ac.at (F.L.);
.neesen@meduniwien.ac.at (J.N.)
spondence: paul.dremsek@meduniwien.ac.at; Tel.: +43-1-40160-56554

CANCER GENETICS AND EPIGENETICS

IJC INTERNATIONAL JOURNAL of CANCER

itations: 5

Optical genome mapping reveals additional prognostic information compared to conventional cytogenetics in AML/MDS patients

ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine | WILEY

enoist [4], ran Beek [5], uis [5], Wed Majdali [4], [5], Damien Sanlaville [7], ne Schluth-Bolard [7],

Evaluation of optical genome mapping for detecting chromosomal translocation in clinical cytogenetics

Peng Dai[1] | Xiaofan Zhu[1] | Yanzheng Pei[2] | Peng Chen[3] | Jingjing Li[2] | Zhi Gao[1] | Yu Liang[2] | Xiangdong Kong[1]

The Journal of Molecular Diagnostics, Vol. 23, No. 11, November 2021

ELSEVIER

Validation of Optical Genome Mapping for the Molecular Diagnosis of Facioscapulohumeral

Thomas Mika[2] | Fotic
Matthias Eckhardt[2] | M
Peter Reimer[3] | Roland

cancers

MDPI

Article

Optical Genome Mapping: A Promising New Tool to Assess Genomic Complexity in Chronic Lymphocytic Leukemia (CLL)

Anna Puiggros [1,2,*], Silvia Ramos-Campoy [1,2], Joanna Kamaso [1,2], Mireia de la Rosa [1,2], Marta Salido [1,2], Carme Melero [1,2], María Rodríguez-Rivera [1,2], Sandrine Bougeon [3], Rosa Collado [4], Eva Gimeno [5,6], Rocío García-Serra [4,7], Sara Alonso [8], Marco Antonio Moro-García [9], María Dolores García-Malo [10], Xavier Calvo [1,2], Leonor Arenillas [1,2], Ana Ferrer [1,2], Tuomo Mantere [11,12], Alexander Hoischen [11,13], Jacqueline Schoumans [3] and Blanca Espinet [1,2,*]

blood advances

ISSUES | LATEST ARTICLES | GUIDELINES | COLLECTIONS | AU

Received: 17 February 2022 | Revised: 26 April 2022 | Accepted: 28 April 2022
DOI: 10.1002/ajh.26587

AJH WILEY

TEST OF THE MONTH

Optical genome mapping for structural variation analysis in hematologic malignancies

Adam C. Smith[1,2] | Kornelia Neveling[3] | Rashmi Kanagal-Shamanna[4]

Am J Hematol. 2022 Feb 4. doi: 10.1002/ajh.26487. Online ahead of print.

Optimizing the Diagnostic Workflow for Acute Lymphoblastic Leukemia by Optical Genome Mapping

Katrina Rack [1], Jolien De Bie [1, 2], Geneviève Ameye [1], Jan Cools [2, 3, 4], Kim De Keersmaecker [4, 5], Joris R Ver Heidi Segers [4, 5], Lucienne Michaux [1], Barbara Dewael

Affiliations + expand
PMID: 35119131 DOI: 10.1002/ajh.26487

RESEARCH ARTICLE | NOVEMBER 22, 2022

Optical Genome Mapping in Acute Myeloid Leukemia: A Multicenter Evaluation

Brynn Levy, Linda B. Baughn, Yassmine M. N. Akkari, Scott Chartrand, Brandon LaBarge, David F Claxton, Patrick Alan Lennon, Claudia Cujar, Ravindra Kolhe, Kate Kroeger, Beth Pitel, Nikhil Sahajpal, Malini Sathanoori, George Vlad, Lijun Zhang, Min Fang, Rashmi Kanagal-Shamanna, James R Broach ✉

International Consortium for Optical Genome Mapping in Hematologic Malignancies

Founding Members 2021– 2023

Dr. Adam C. Smith
UHN

Dr. Brynn Levy
COLUMBIA

Dr. Gordana Raca
Children's Hospital LOS ANGELES

Dr. Nikhil S. Sahajpal
Greenwood Genetic Center

Dr. Kornelia Neveling
Drs. Daniel Olde Weghuis
Dr. Marian Stevens-Kroef
Dr. Alexander Hoischen
Radboudumc university medical center

Dr. Tuomo Mantere
UNIVERSITY OF OULU

Dr. Katrina Rack
Dr. Barbara Dewaele
UZ LEUVEN
KU LEUVEN

Dr. Ravindra Kolhe
AUGUSTA UNIVERSITY

Dr. Rashmi Kanagal-Shamanna
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Dr. Anna Puiggros
Dr. Blanca Espinet
Hospital del Mar Barcelona

Dr. Mar Mallo
Dr. Francesc Solé
Josep Carreras LEUKAEMIA Research Institute

The International Consortium for Optical Genome Mapping in Hematologic Malignancies was established by a group of pioneering Optical Genome Mapping users. To combine their experiences and to develop a framework for the use of Optical Genome mapping in Clinical Laboratories — to harmonize the use of OGM and assist other laboratories in clinical adoption.

Review > Am J Hematol. 2024 Jan 2. doi: 10.1002/ajh.27175. Online ahead of print.

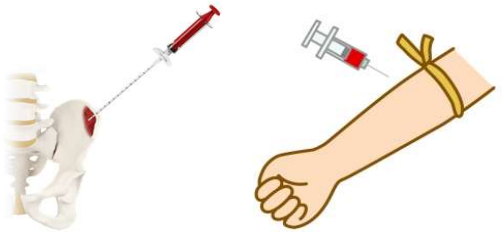
A framework for the clinical implementation of optical genome mapping in hematologic malignancies

Brynn Levy [1], Rashmi Kanagal-Shamanna [2], Nikhil S Sahajpal [3], Kornelia Neveling [4 5], Katrina Rack [6], Barbara Dewaele [6], Daniel Olde Weghuis [4], Marian Stevens-Kroef [4], Anna Puiggros [7 8], Mar Mallo [9], Benjamin Clifford [10], Tuomo Mantere [11], Alexander Hoischen [4 5 12 13], Blanca Espinet [7 8], Ravindra Kolhe [14], Francesc Solé [9], Gordana Raca [15], Adam C Smith [16 17]

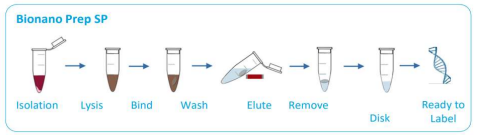
Optical Genome Mapping: wet laboratory workflow

Count WBC and take 1,5 million cells

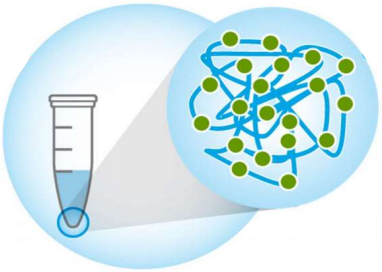
Bone marrow or blood (if invaded)

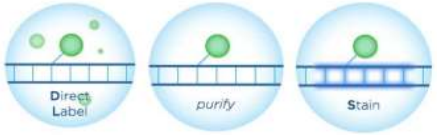


Extraction of UHMW DNA

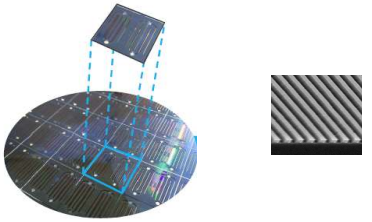


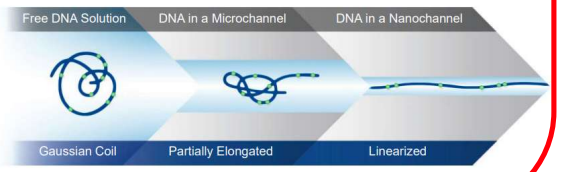
Label DNA at specific motifs



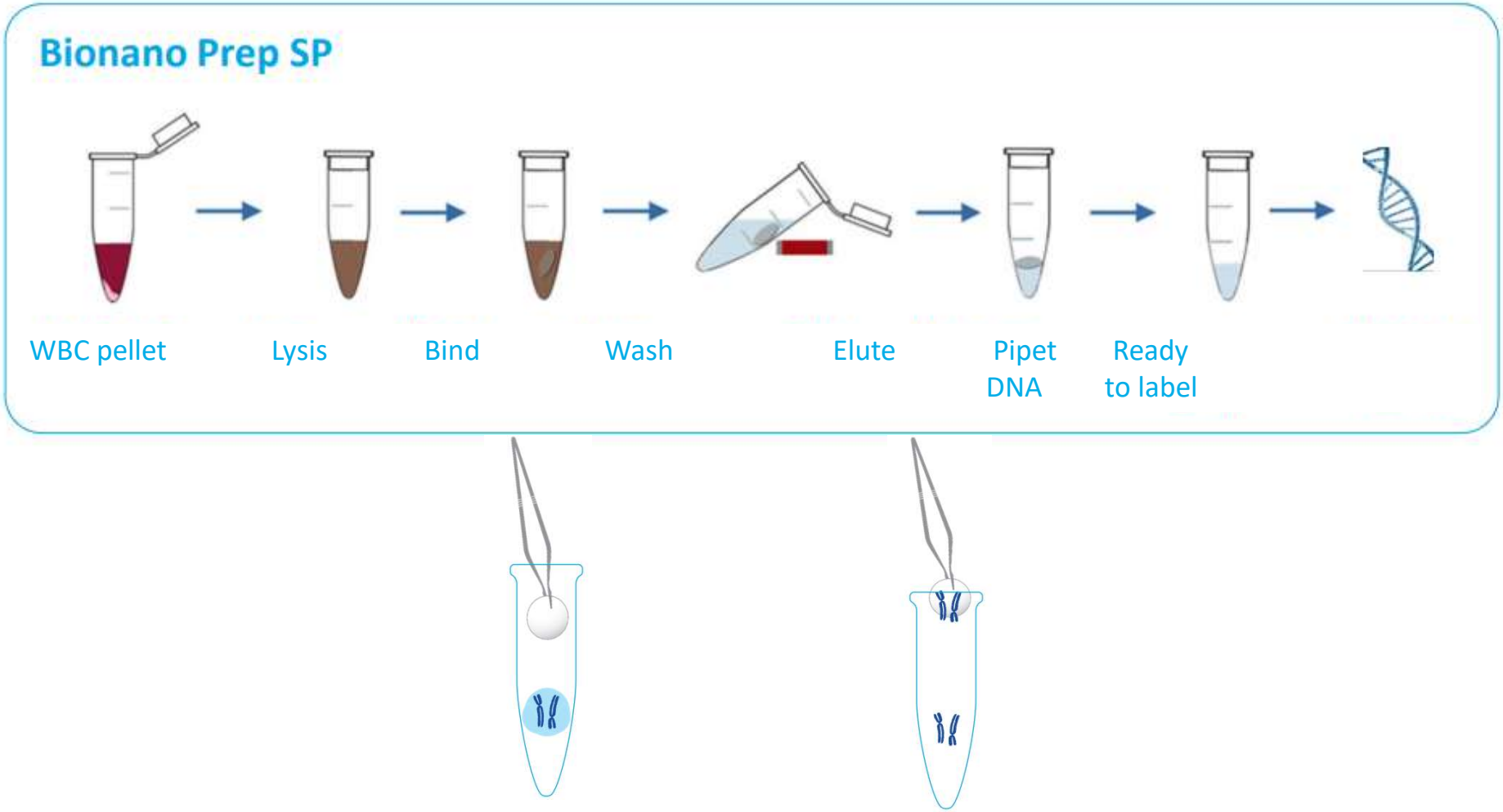


Transfer labeled DNA into Chip





Optical Genome Mapping: wet laboratory workflow



OGM requires extremely long molecules

Only dsDNA molecules that are longer than 150 kbp are assembled
Sample selection, proper storage and preservation are critical

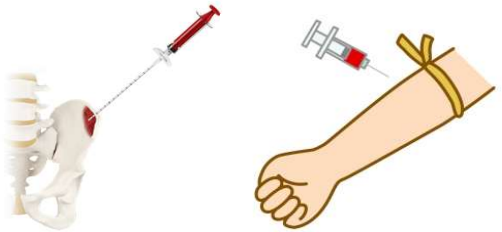
OGM: **not validated** for use on:

- cytogenetic fixed pellets
- formaline fixed specimens (FFPE)
- DNA from conventional DNA extraction methods

Optical Genome Mapping: wet laboratory workflow

Count WBC and take 1,5 million cells

Bone marrow or blood (if invaded)



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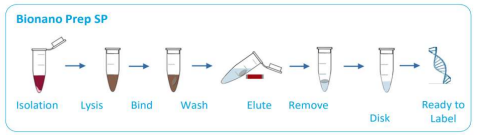

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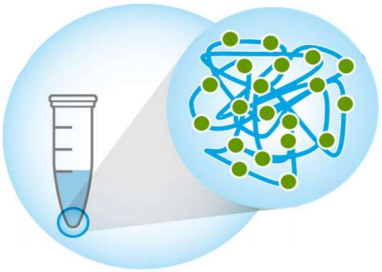

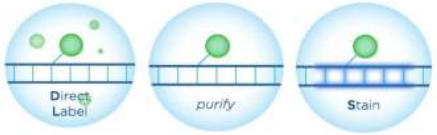

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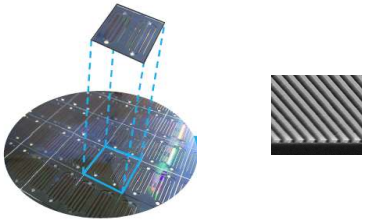
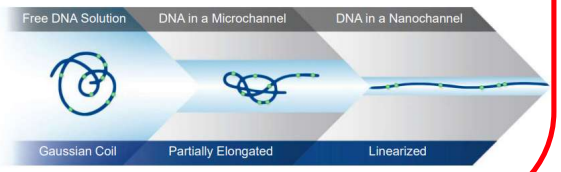
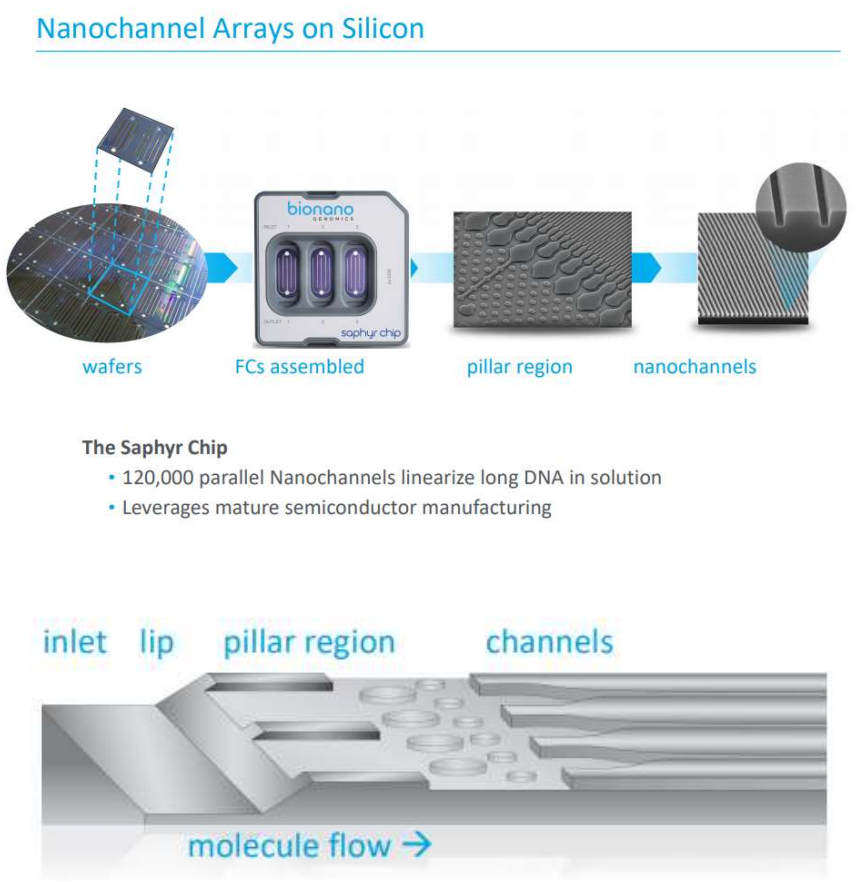
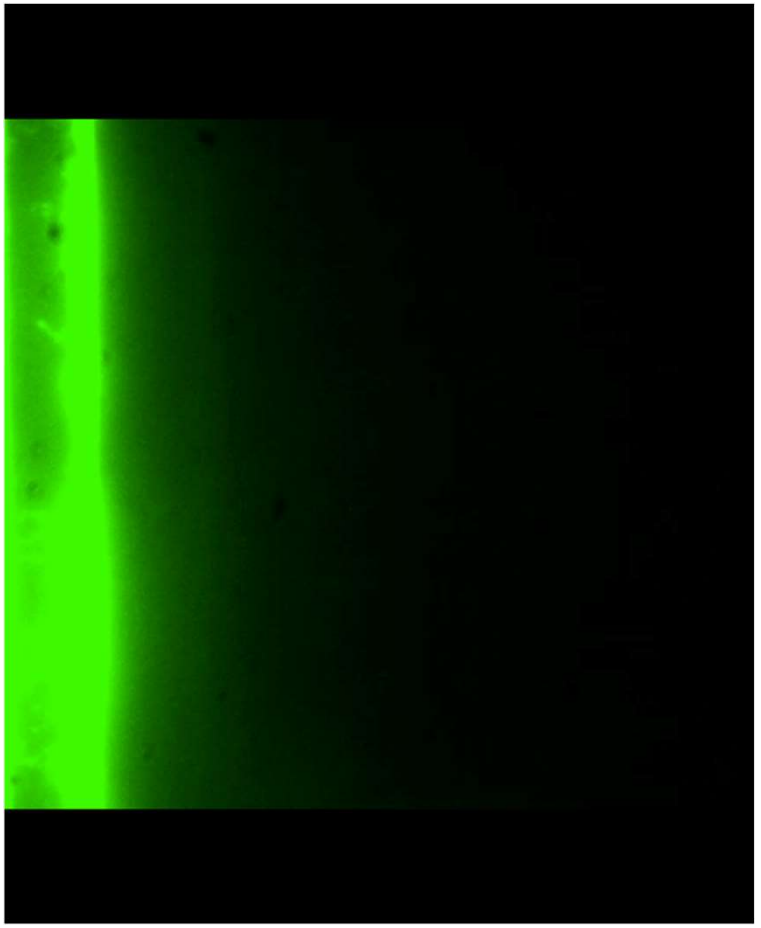
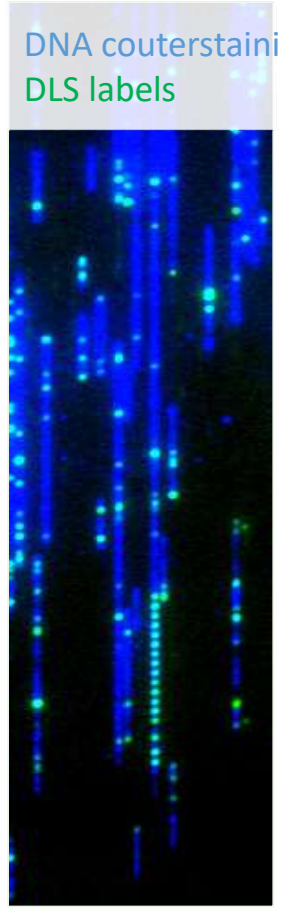




OGM is not sequencing based: visualisation of intact DNA molecules







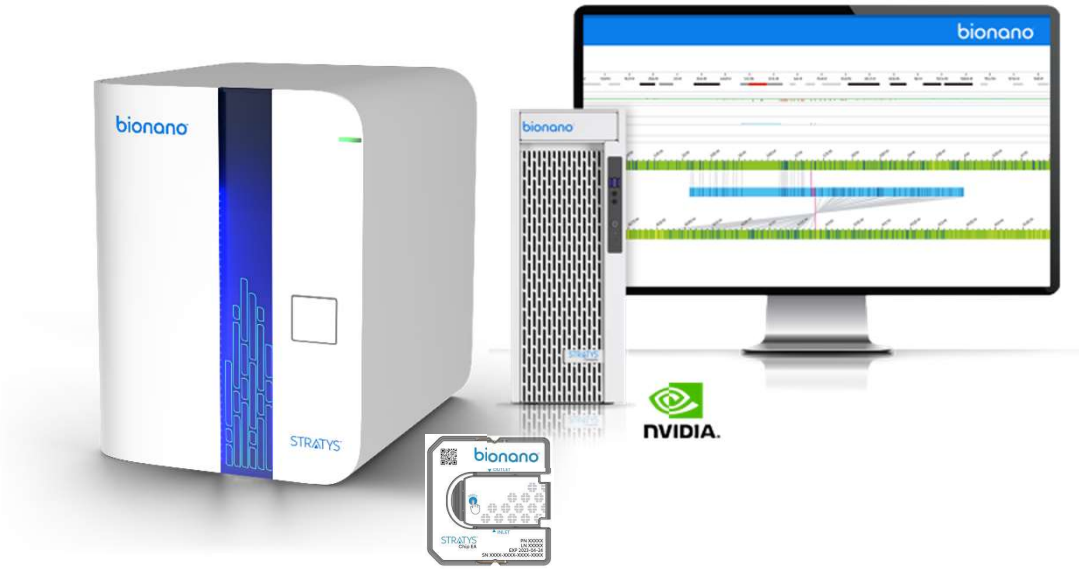
Length of DNA molecules:
150kb – 2.5Mb,
median size >350 kb

Saphyr *versus* Stratys

Saphyr: Batch-based system

- 6 samples/run on one Saphyr instrument
- 2 chips of 3 samples
- 48-72 hours for 12 samples at 400x
  (2 Saphyr devices)

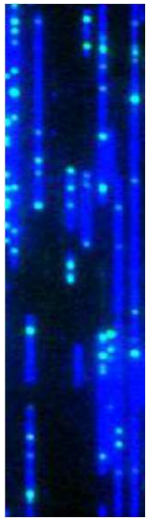
Stratys: more flexibility, increased speed?



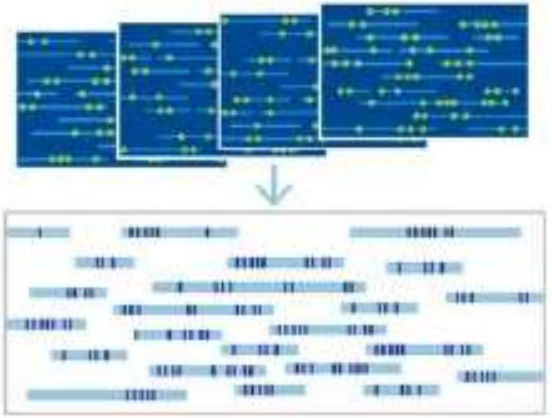
- Each sample on individual chip
- No batching required
- Possible to load 1 up to 12 samples
- “Jump the queue” for samples with high priority
- Continue loading
- 18-22 hours for 12 samples at 400x

Optical Genome Mapping: data analysis: De Novo Assembly Pipeline

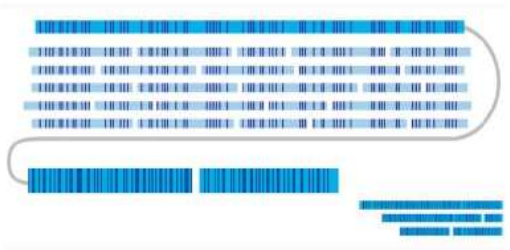
Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



Algorithms convert the raw images into .bnx files
=> population of molecules

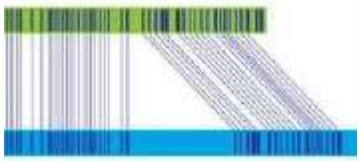


Algorithms align different molecules for constructing Consensus Genome Maps (.cmap files)



Cross mapping across a Reference

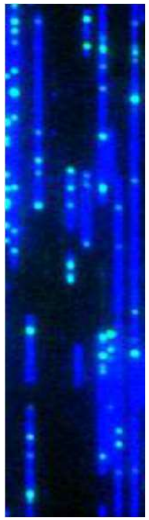
- Copy Number Aberration (CNA) profile
- Structural Variant (SV)



insertion

Optical Genome Mapping: data analysis: De Novo Assembly Pipeline

Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



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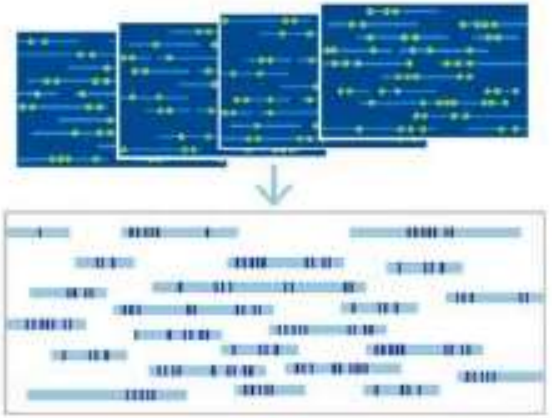

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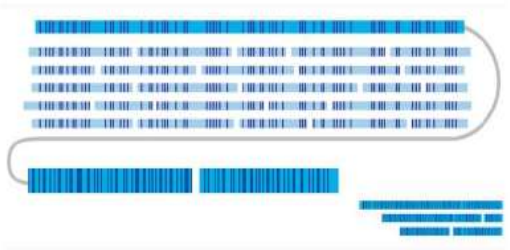

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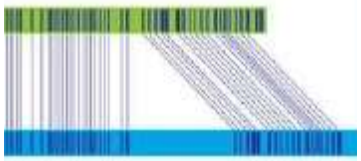
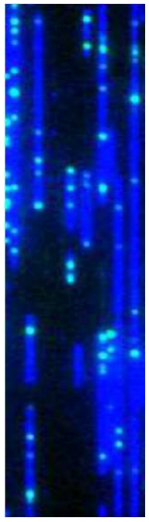


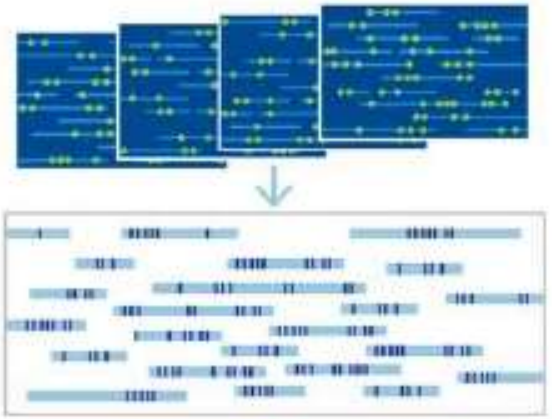
insertion

Optical Genome Mapping: data analysis: Rare Variant Analysis Pipeline

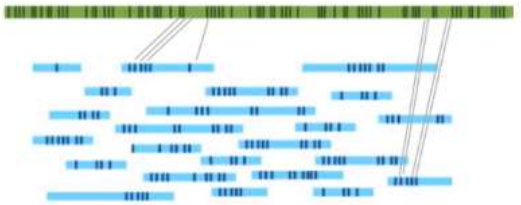
Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



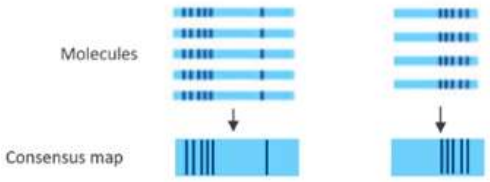
Algorithms convert the raw images into .bnx files
=> population of molecules



Single molecule alignment to the reference

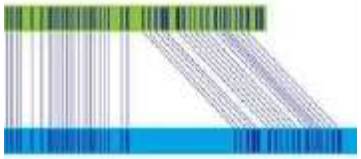


Molecules with SVs are clustered



Local alignment to reference to confirm SV

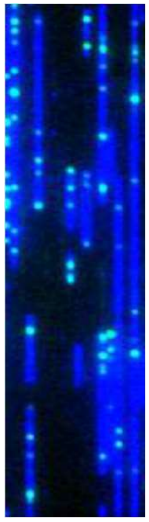
- CNA profile
- SV



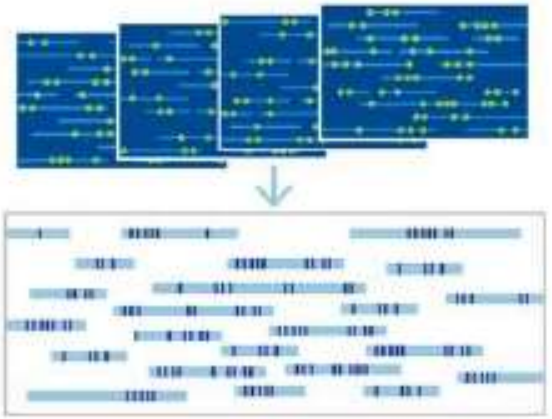
insertion

Optical Genome Mapping: data analysis: Rare Variant Analysis Pipeline

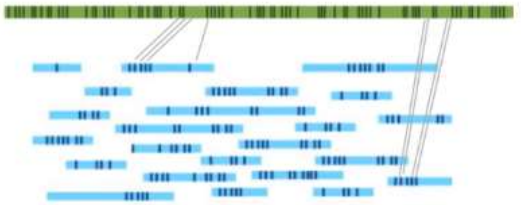
Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



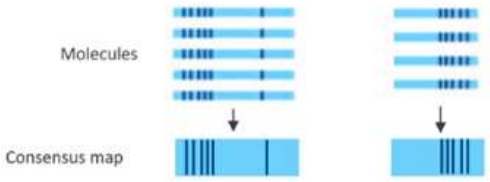
Algorithms convert the raw images into .bnx files
=> population of molecules



Single molecule alignment to the reference

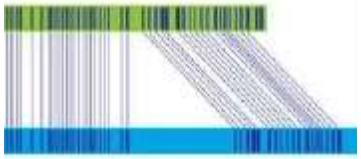


Molecules with SVs are clustered



Local alignment to reference to confirm SV

- CNA profile
- SV



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Optical Genome Mapping: data analysis: (reference) Guided Assembly Pipeline - LAF (low allele fraction variants)

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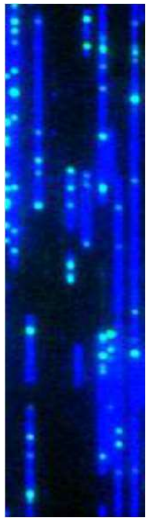

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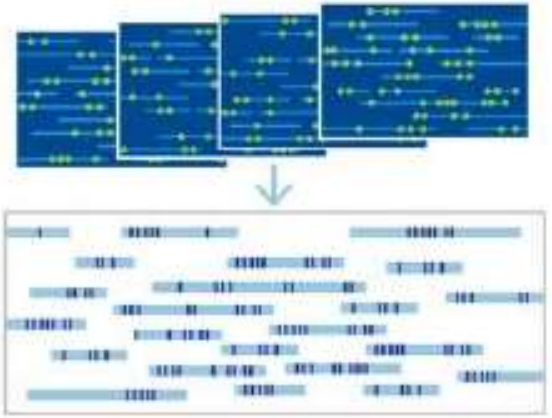


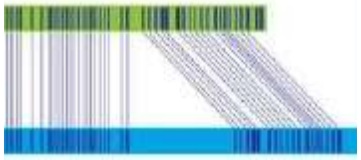
Algorithms align different molecules for constructing Consensus Genome Maps (.cmap files)

Reference genome is used as starting point for refinement and haplotype splitting (input CMAP files)

Consensus maps are refined through extend and merge stages

Align refined maps to the reference genome

- Copy Number Aberration (CNA) profile
- Structural Variant (SV)



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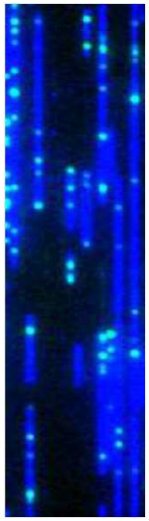

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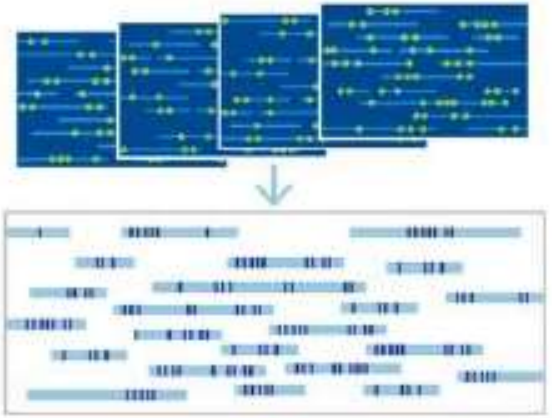

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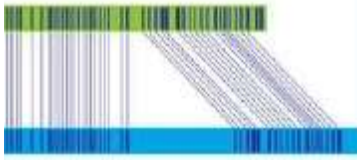

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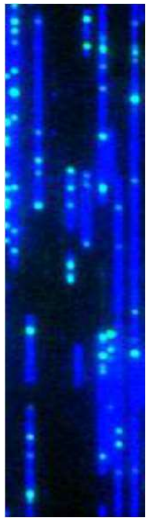

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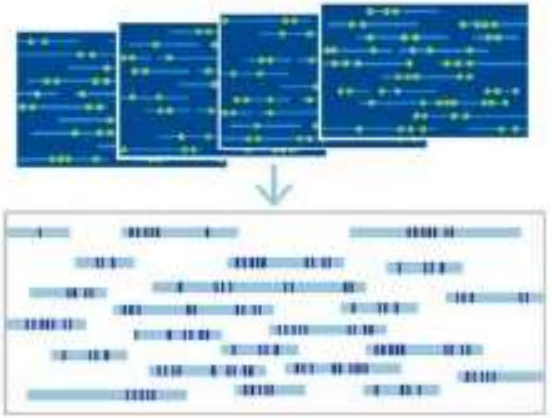

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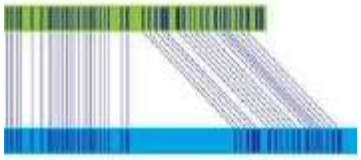


insertion

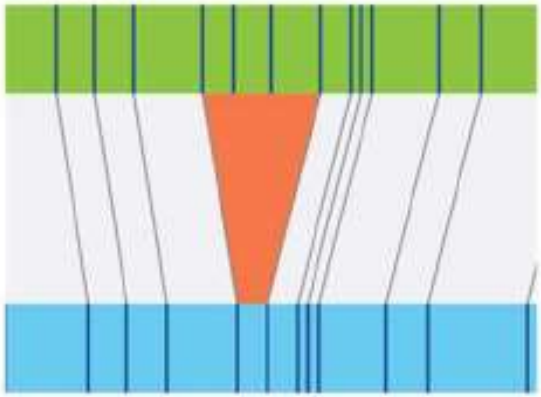
Comparison of the three pipelines

Feature	RVA	De Novo Assembly	Guided Assembly
Speed	Very Fast	Slow	Fast
Reference	Required	None	Used as a "Blueprint"
Detection	Large SVs, Low-allele fraction	New/Complex SVs	Low-Allele Fraction (5%)
Assembly	Targeted	Full	Full

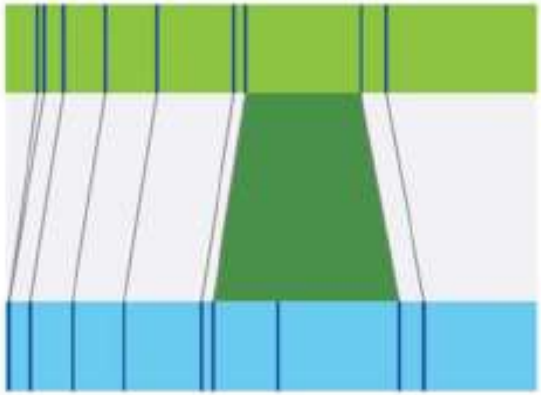
Optical Genome Mapping: calling structural aberrations

GAINS/LOSSES

Deletion

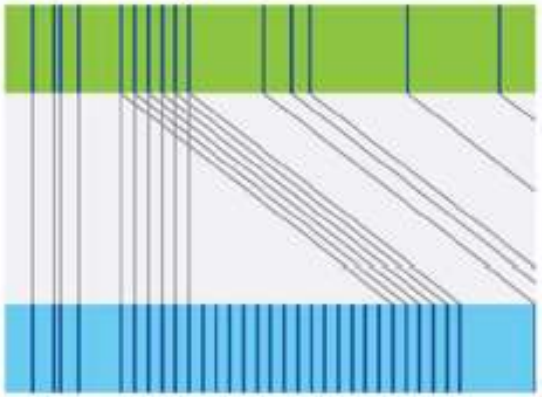


Insertion

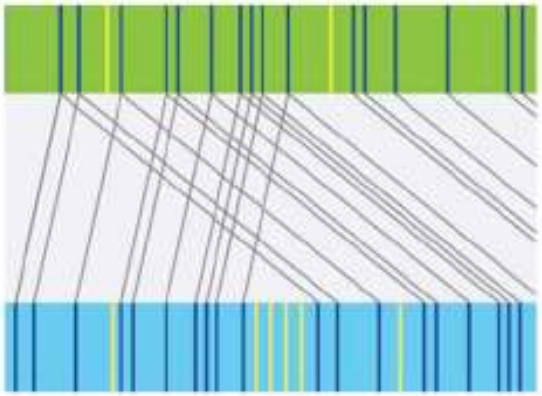


COPY NUMBER CHANGES

Repeat array expansion

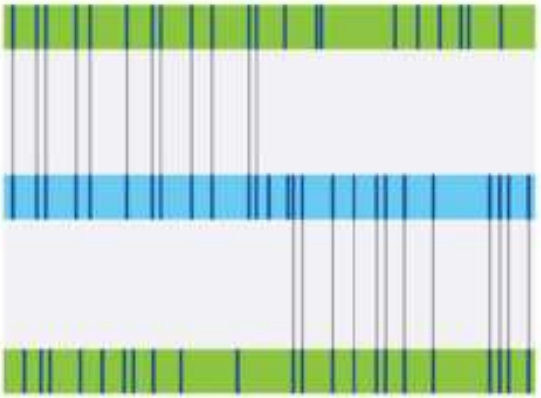


Tandem duplication

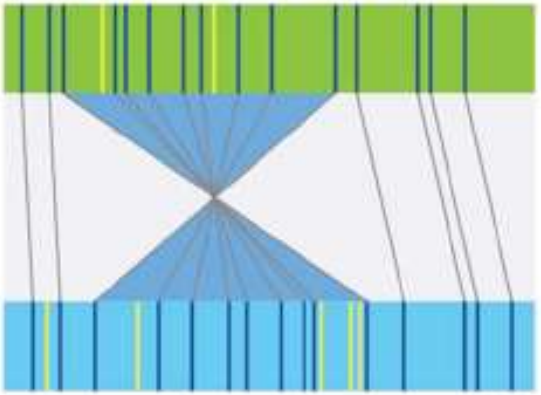


BALANCED

Translocation



Inversion



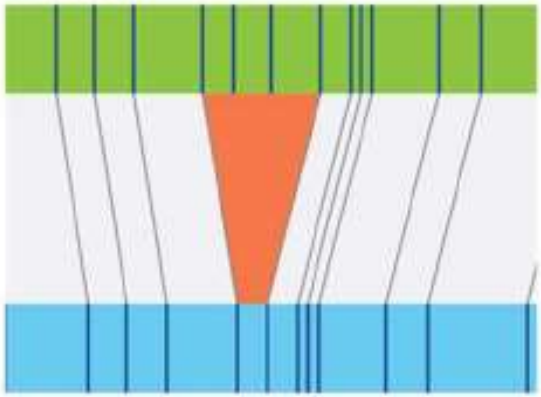
Reference genome (green)

OGM map sample (blue)

Optical Genome Mapping: calling structural aberrations

GAINS/LOSSES

Deletion



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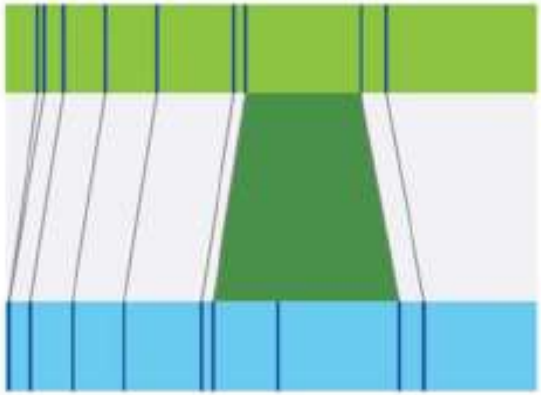

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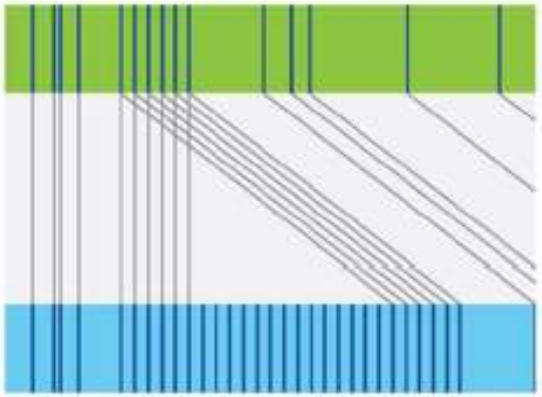

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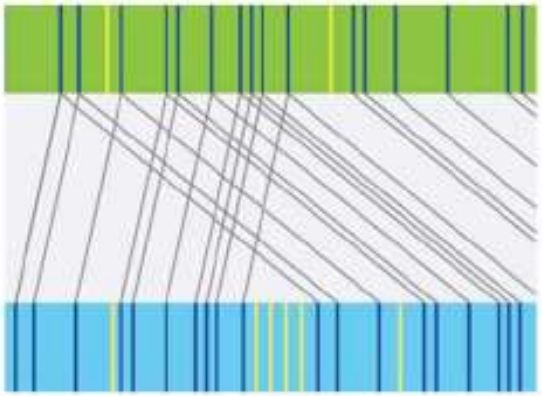

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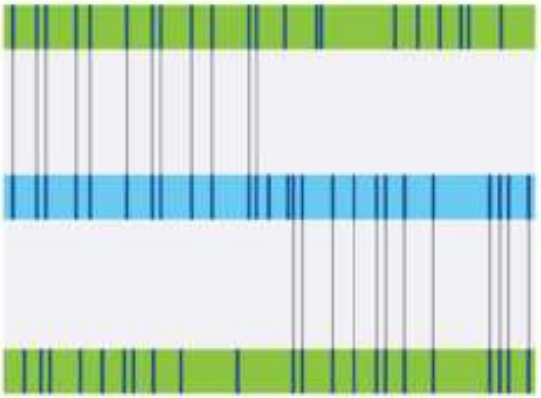

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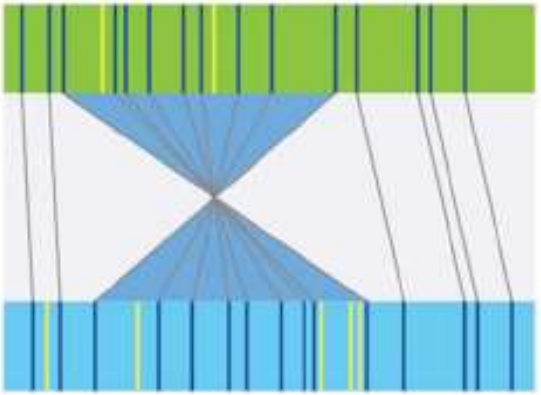
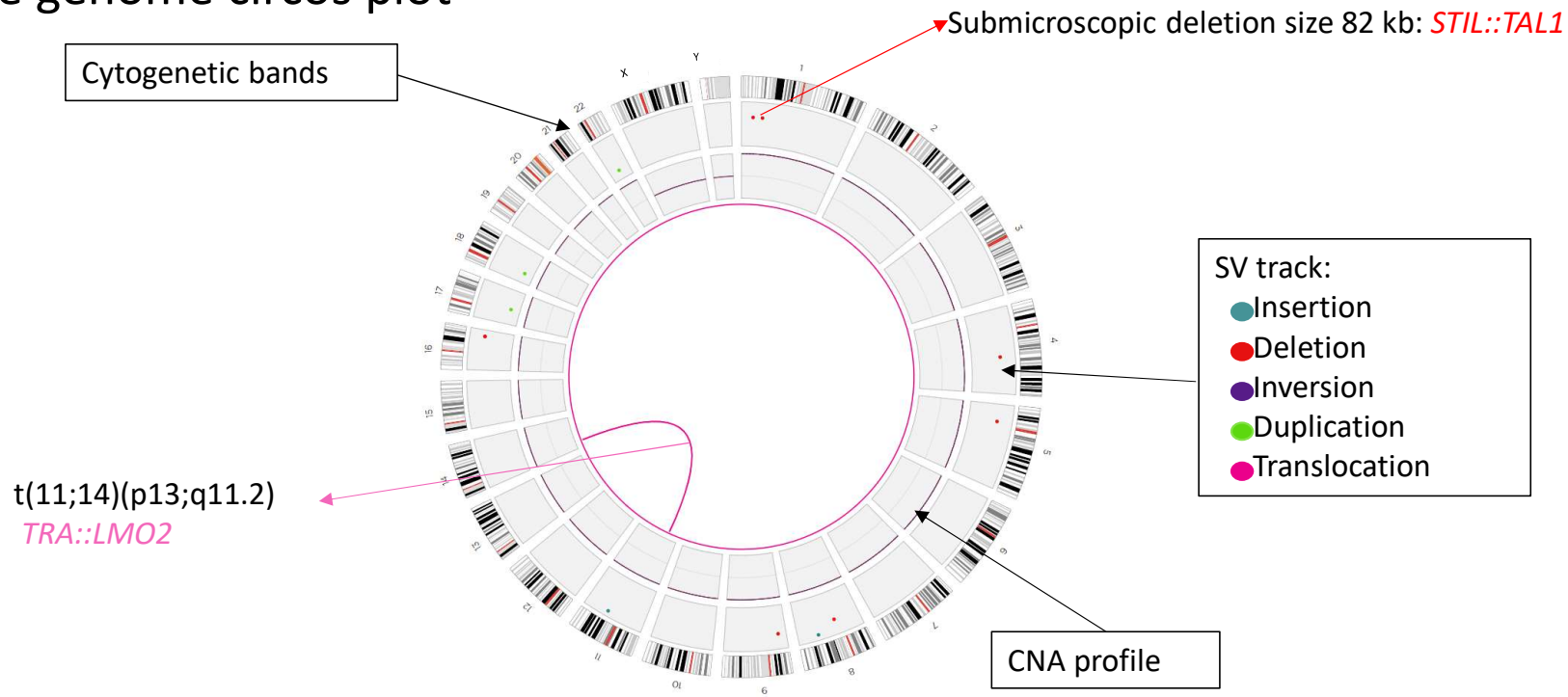


Reference genome (green)

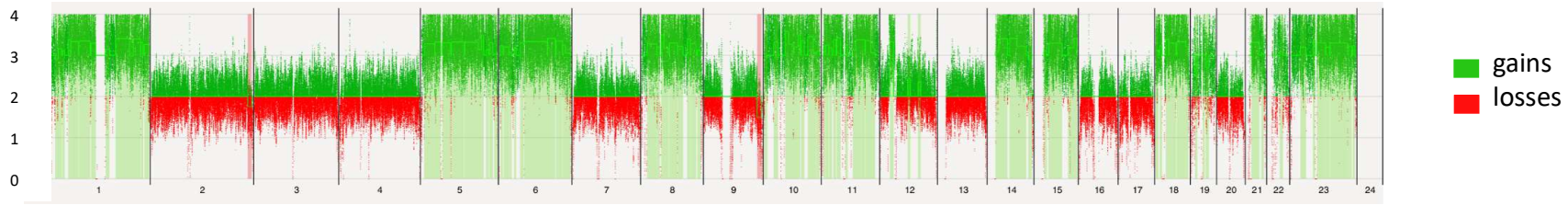
OGM map sample (blue)

OGM data visualisation: cancer: whole genome circos plot – whole genome CNA view

Whole genome circos plot



Whole genome CNA view



VALIDATION

Method validation:

(1) determining the type and number of samples to be tested;

(2) establishing test performance (e.g., analytic sensitivity, analytic specificity, accuracy and precision);

(3) demonstrating test reproducibility;

(4) determining the lower limit of detection (LLOD).

VALIDATION: cohort size and specimen types

- OGM =

=> novel

=> genome-wide

- A **sample size of 59** would produce sufficient data for complex genomic assays
- Test additional samples for **each specific clinical indication**
- **Normal** samples and samples with **different SV types**
- Test CNA's, aneuploidies, balanced and unbalanced translocations, insertions, inversions, insertions, …
- Test **different sample types** (blood, bone marrow, different tissue types, CD138+ enriched cell suspension, …)

Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing–based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American pathologists. *J Mol Diagn*. 2017;19:341-365.

Expected test performance

- Performance: you expect a sensitivity, specificity, precision and accuracy of >90% comparing OGM to SOC methods

TABLE 1 Performance calculations for methodological validation.

Parameter	How to calculate
Sensitivity/positive percentage agreement	TP/(TP + FN)
Specificity/negative percentage agreement	TN/(TN + FP)
Positive predictive value	TP/(TP + FP)
Negative predictive value	TN/(TN + FN)
Accuracy	(TP + TN)/(TP + TN + FP + FN)

Abbreviations: FN, false negative (type 2 error); FP, false positive (type 1 error); TN, true negative; TP, true positive.

Expected test performance

- Performance: you expect a sensitivity, specificity, precision and accuracy of >90% comparing OGM to SOC methods

Take into account the limitations of the technologies:
- OGM technology
- and all the other methods you compare with!! (e.g.: CBA detects CNA's starting from 5-10 Mb)

=> Often orthogonal confirmation using alternate methods will be required to confirm!
Make sure you have those technologies available: e.g. CBA, FISH, RNAseq, specific PCR's, …

OGM reproducibility

- Intra-run
- Inter-run
- Inter-instrument
- Inter-technologist
- Inter-analist

Measure both:

- technical performance: QA parameters
- analytical performance: reported variants

OGM limit of detection

- LLOD should be assessed for the different variant classes

- dilution series of cells
- dilution series of DNA
- *in silico* LLOD determination

Importantly: LLOD is dependent on:

- quality of the DNA
- the coverage

Clinical validation

- You may re-use the samples of the technical validation
- **Determine the diagnostic yield**

=> use clinically relevant abnormal results for each subtype of hematological malignancies (WHO, ICC, …) + normal cases

=> check concordance between OGM and SOC methods

- Include success rate, TAT, cost, … to assure the clinical benefits for the patient

- At the stage of implementation: do not forget to include a risk inventory!

Quality control parameters

- Samples
- Pre-analytical quality parameters
- Analytical quality parameters
- Post-analytical quality parameters

Quality control parameters

- Samples

- peripheral blood or bone marrow: collected in EDTA or in heparin (add DNA stabilizer asap)

- for longer storage: samples should be frozen at -80°C

- prepare multiple aliquots for storage

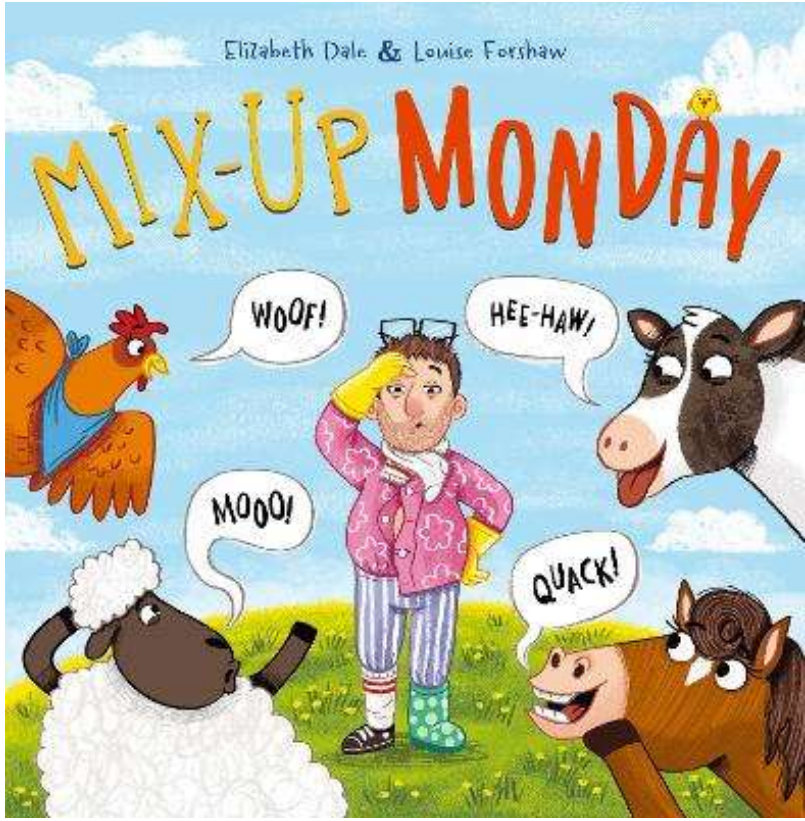
Quality control parameters

- Pre-analytic phase

- prevent DNA shearing during processing of the sample: never pipet the DNA harshly, never vortex it, …. It usually is viscous.

- make sure your DNA is homogeneous

- implement procedures to exclude sample mix-ups



Quality control parameters

- Pre-analytical phase

TABLE 2 Recommended targets for cell input, DNA concentration, and post-labeling DNA concentration.

parameter	Target	Common reasons for missed target
Input sample: cell count	1 500 000 viable cells/sample	• Improper sample handling, storage, stabilization • Low sample volume availability or paucicellular sample
DNA concentration	39–150 ng/µL	• Inaccurate cell input during DNA isolation • Excessive DNA mass loss during isolation related to inhibitory substances in lysate and/or fragmented DNA: ○ DNA mass fails to precipitate from lysate ○ DNA mass detaches from nanobind disk
DNA conc. coefficient of variation (CV) among three replicate measures $CV = \frac{\text{standard deviation}}{\text{mean}}$	≤0.30	• Isolated DNA needs more time and/or gentle mixing to homogenize • DNA is too concentrated
Labeled DNA concentration	4–16 ng/µL	• Inaccurate quantitation of input DNA • Low labeled DNA recovery from Direct Label and Stain (DLS) membrane

Quality control parameters

- Pre-analytic phase

- DNA isolated from frozen bone marrow aspirates: take longer to **homogenize**, may have lower N50 values

=> dead cells are present: generate degraded DNA and have protein contaminants

=> improve the quality by:

- including a centrifugation step
- by including apoptotic cell selection kits
- by sorting out the live cells (flow cytometry, microfluidics, …)

Quality control parameters

- Quality control parameters

⇒monitoring “in real time” during the run: Bionano Access Dashboard

⇒Check the molecule quality report

- DNA per scan (Gb) & Map Rate (%)

TABLE 3 Analytical quality metrics–the molecule quality report.

Parameter	Target	Common reasons for missed target
Effective coverage	≥340×	Effective coverage $= \frac{\text{total DNA} \times \lfloor \text{map rate} \rfloor}{\text{reference size}}$ So, • Inadequate total DNA in the data set • Low map rate (<70%)
N50 (≥150 kbp and minimum labels ≥9)	≥230 kb	• Deteriorated cell membrane integrity/DNA length from original sample • Excessive DNA shearing during sample prep or storage
N50 (≥20 kbp)	≥150 kb	
Map rate	≥70%	• Low label density/poor labeling efficiency • Short DNA molecules • DNA becoming stuck in the nanochannels

Quality control parameters

- Quality control parameters

⇒monitoring “in real time” during the run: Bionano Access Dashboard

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Quality control parameters

- Post-analytical quality parameters
- The analysis pipeline also generates a “informatics report”
- => check it to determine if the data meets the quality criteria established by your lab

TABLE 4 Post-analytic quality metrics and troubleshooting–Informatics report.

Parameter	Target	Common reasons for missed target
Sex	Consistent with indication	• Sex chromosome abnormalities could confound X/Y sex determination • Medical (e.g., transplantation) history may confound X/Y sex determination
Effective coverage of reference	≥300×	Effective coverage of reference (X) $= \frac{\text{total DNA aligned to the reference in pipeline}}{\text{reference size}}$ • Inadequate total DNA in the data set • Low map rate (<70%) • Poor analytical QC generally
CNV statistics: percent above expected (2 Mbp/6 Mbp window)	≤+20	• Poor analytical QC generally • Poor run performance • Poor label clean-up in DLS procedure • Expired or improperly stored Proteinase K used in DLS procedure
CNV statistics: correlation with label density	≤0.25	

Example 1: B-ALL: QA parameters

QA parameters

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

Molecule quality report (before analysis)

Molecule Quality Report

Reference	hg38_DLE1_0kb_0labels_masked_YPARs.cmap	Name of the reference genome this sample was aligned to

MQR Report Details

label	value	description
Version	1	Report version
Total DNA (≥ 150kbp)	1,320.24 Gbp	Total amount of DNA from molecules that are 150kbp or longer
Maximum molecule length	2.37 Mbp	The longest molecule detected during the chip run.
N50 (≥ 150kbp)	201 kbp	N50 of DNA molecules that are 150kbp or longer
N50 (≥ 20 kbp)	109.5 kbp	N50 of the molecules that are 20kbp or longer)
Total DNA (≥ 20kbp)	4,453.16 Gbp	Total amount of DNA from molecules that are 20 kbp or longer
Total DNA (≥ 150kbp and min sites ≥ 9)	1,271.98 Gbp	Same as other Total DNA fields, but molecules must have at least 9 labels
N50 (≥ 150kbp and min sites ≥ 9)	201.75 kbp	Same as other N50 fields, but molecules must have at least 9 labels
Label color	BNGFLGR001	Label color used for detection.
Site	CTTAAG	Recognition sequence of the enzyme used.
Average label density (≥ 150kbp)	14.32 /100kbp	Average number of labels per 100 kbp for the molecules that are 150kbp or longer
Enzyme	DLE-1	Name of the enzyme used in this sample.
Reference	hg38_DLE1_0kb_0labels_masked_YPARs.cmap	Name of the reference genome this sample was aligned to.
Reference Length	3,088,269,832 bp	Total length of reference sequence
Effective coverage	297.37	Total amount of aligned DNA divided by the size of the reference genome.
Map rate	72.2 %	Percentage of molecules that are 150kbp or longer mapped to the reference

For this case some QA parameters are subptimal

- DNA fragmenation (N50 values)
- Coverage a bit lower: not a problem usually for B-ALL with high % blasts

Example 1: B-ALL: QA parameters

Rare Variant Analysis Informatics Report(after analysis)

Molecules aligned to reference

label	value	description
Total number of molecules aligned	4,785,704	The number of molecules after filtering (≥ 150 kbp) that align to the in silico digested reference file (.cmap), e.g. GRCh37 or GRCh38
Total reference length	3,088.27	The summed length of the chromosomes (or other maps) in the specified reference.
Fraction of aligned molecules	0.87	The proportion of filtered molecules that align to the reference
Effective coverage of reference	279.42	The total length of molecules aligned to the reference divided by the length of the reference
Average confidence	25.96	The average alignment score for all the molecules that align to the reference.
Fraction aligned length	0.81	Length of fraction of aligned molecules

CNV Statistics

label	value	description
Sex	female	Sex determined based on coverage of sex chromosomes
Median Coverage	310.5	Median number of molecules covering each position genome-wide
Global coefficient of variation	0.073	Coefficient of variation in coverage genome-wide
Median local coefficient of variation (2Mbp)	0.063	Median coefficient of variation observed within 2Mbp intervals
Percent above expected (2 Mbp window)	8.3	Percent difference between the expected coefficient of variation in sample with low systematic biases (2 Mbp windows) and the observed coefficient of variation in the sample. If the observed percent different is greater than 20%, the sample should be considered to contain systematic bias.
Median local coefficient of variation (6Mbp)	0.068	Median coefficient of variation observed within 6Mbp intervals
Percent above expected (6 Mbp window)	15.51	Percent difference between the expected coefficient of variation in sample with low systematic biases (6 Mbp windows) and the observed coefficient of variation in the sample. If the observed percent different is greater than 20%, the sample should be considered to contain systematic bias.
Correlation with label density	0.11	Correlation between coverage of genomic regions and the label density in the given regions. Value greater than 0.25 indicates high systematic biases and sample may have more false positive CNV calls.
Wave template correlation	0.19	Correlation between the coverage of the query sample and the coverage profiles from known samples with large systematic bias. Value greater than 0.4 indicates high systematic biases

For this case some QA parameters are subptimal

- Coverage a bit lower: not a problem usually for B-ALL with high % blasts

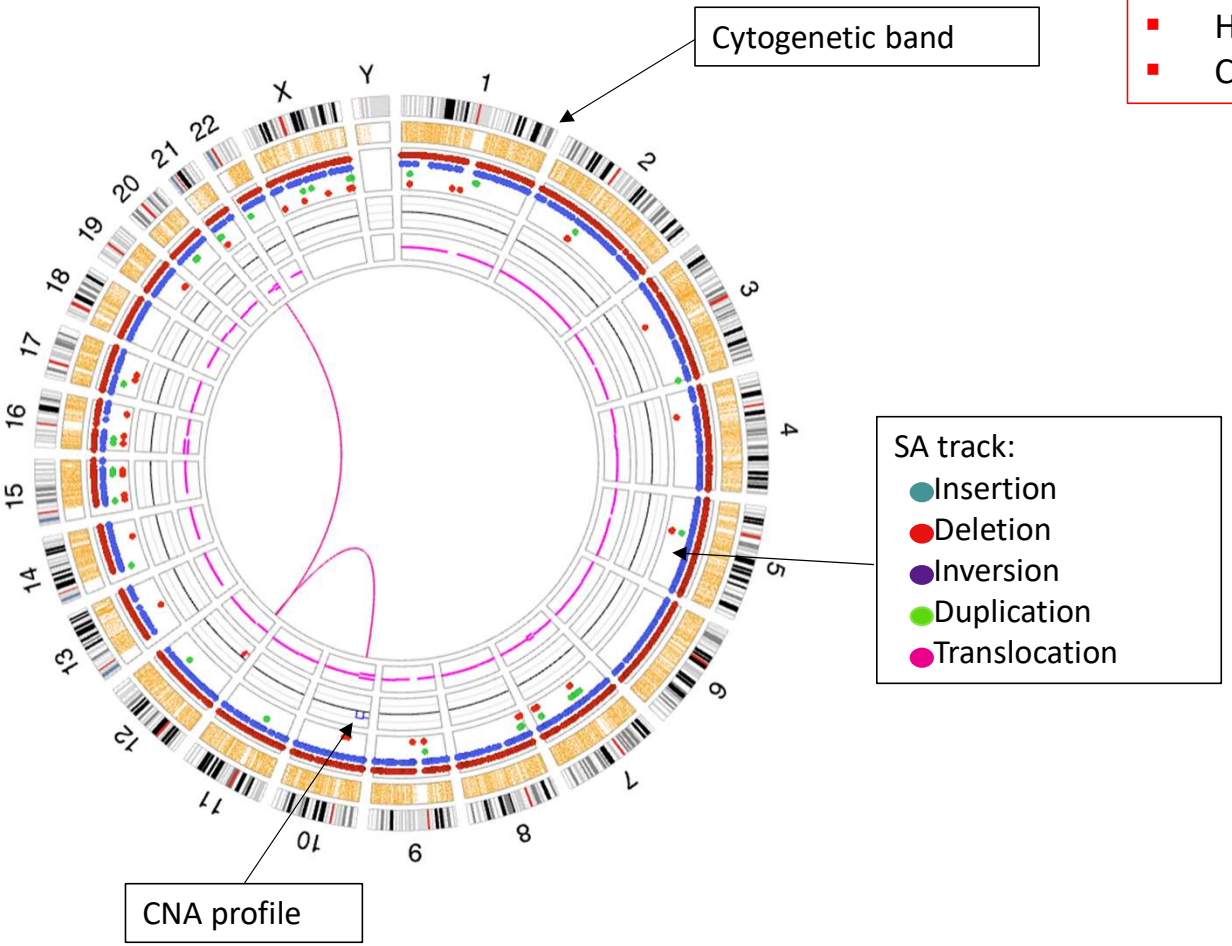
Example 1: B-ALL

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

Add karyotype: **Id:** 71668252
01/03/2018

Example 1: B-ALL

Whole genome circus plot, *De Novo Assembly*



- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

Filter settings UZ Leuven (confidential)

SV Filters

Feature SV Overlap Precision (Kbp):	12
SV Masking Filter:	all
VAF Filter:	0-1
Self Molecule Count:	1
% in Control Database:	2
% in Control Database for Enzyme:	2
SV Chimeric Score:	all
Found in Self Molecules:	yes
Overlap Genes:	all

CNV Filters

Feature CNV Overlap Precision (Kbp):	500
Copy Number Type:	all
Copy Number Confidence:	0
Copy Number Min Size (bp):	5.000.000
Copy Number Masking Filter:	all

Aneuploidy Filters

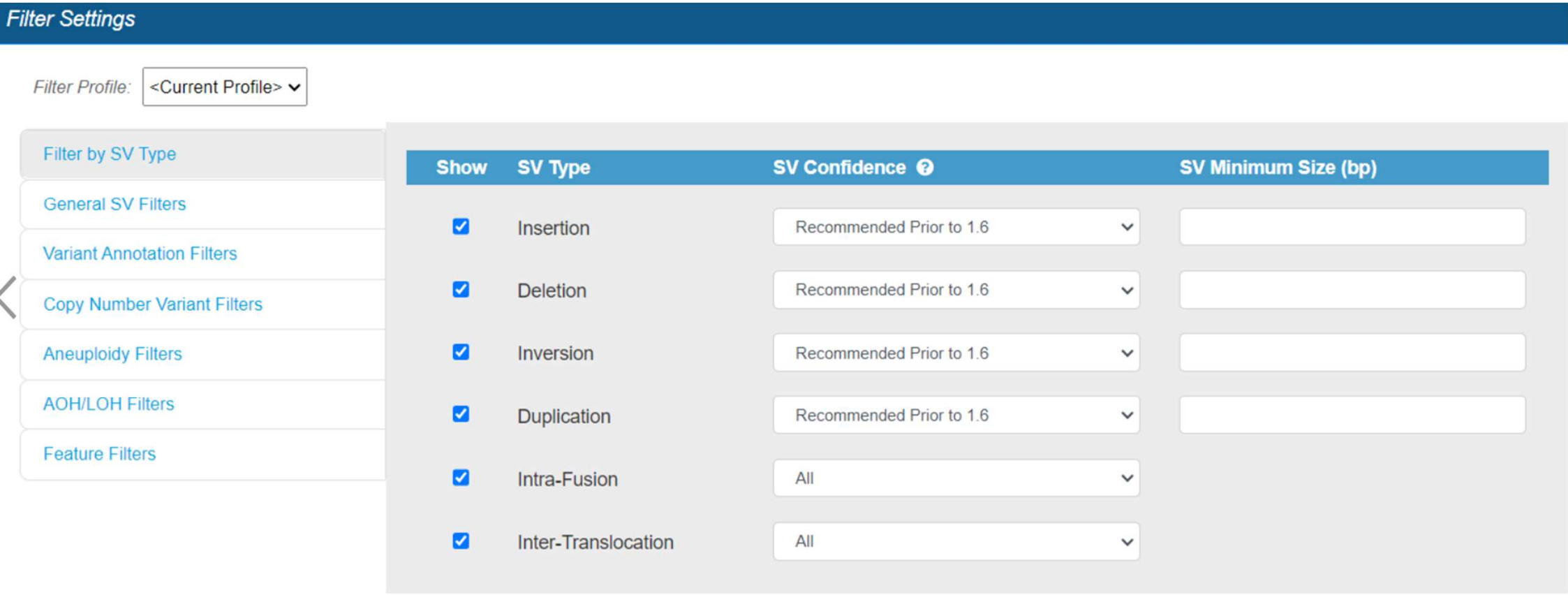
Aneuploidy Type:	all
Aneuploidy Confidence:	-1

AOH/LOH Filters

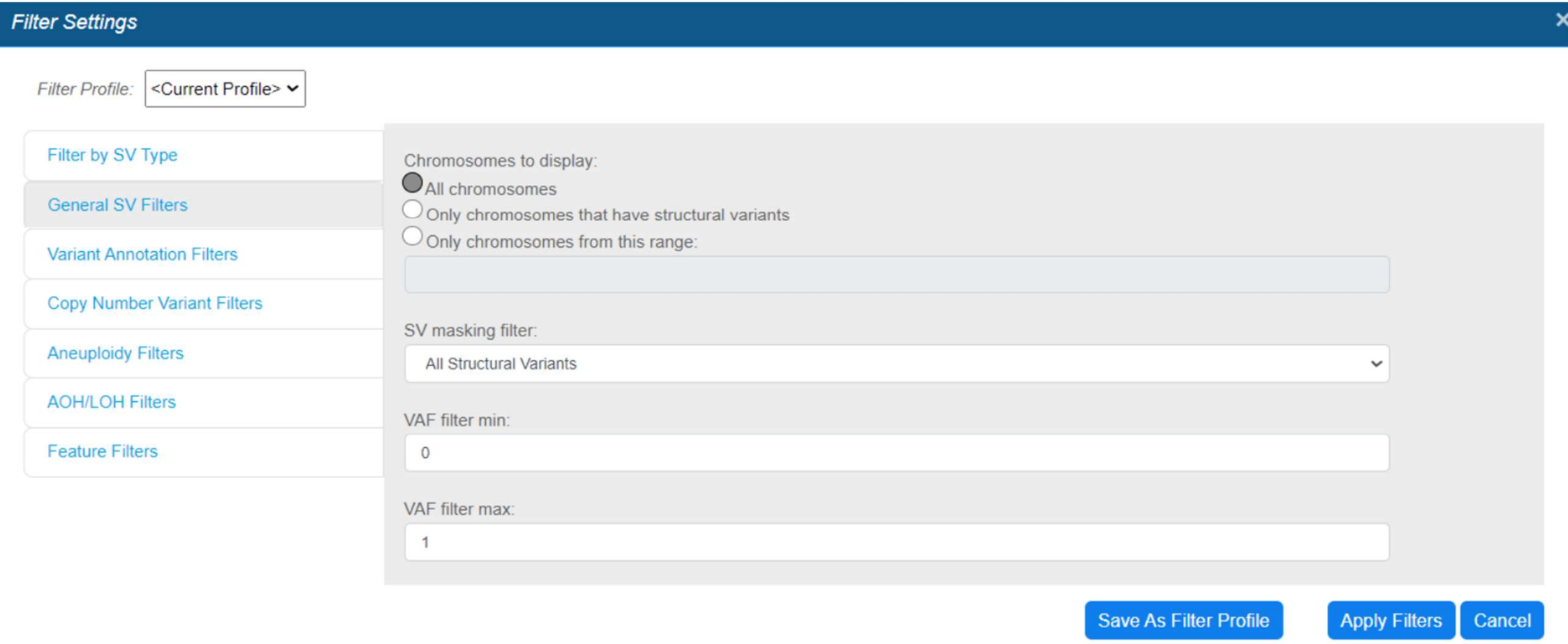
AOH/LOH Minimum Size (bp):	5.000.000

SV Filter	Confidence	Min Size (bp)
Insertion	0	
Deletion	0	
Inversion	0,01	
Duplication	-1	
Intra-Fusion	-1	
Inter-Translocation	-1	

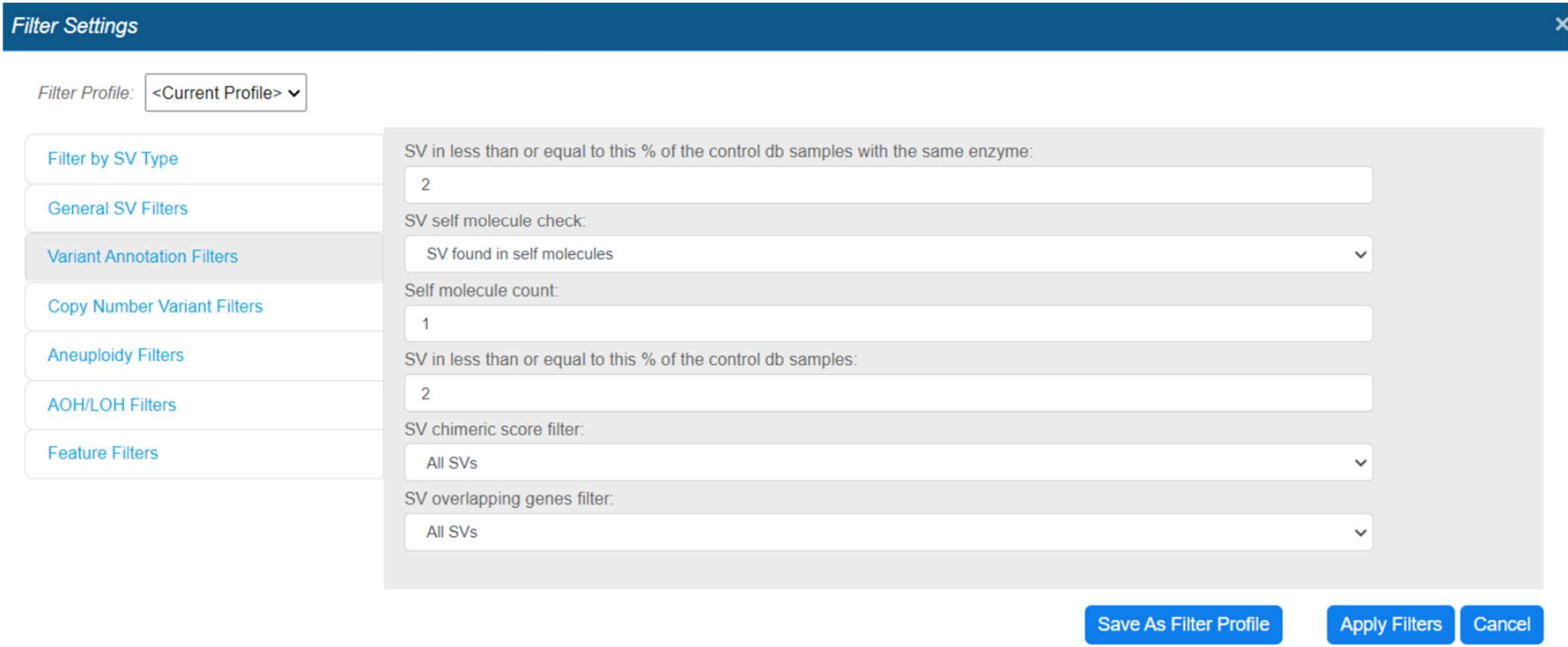
Filter settings UZ Leuven (confidential)



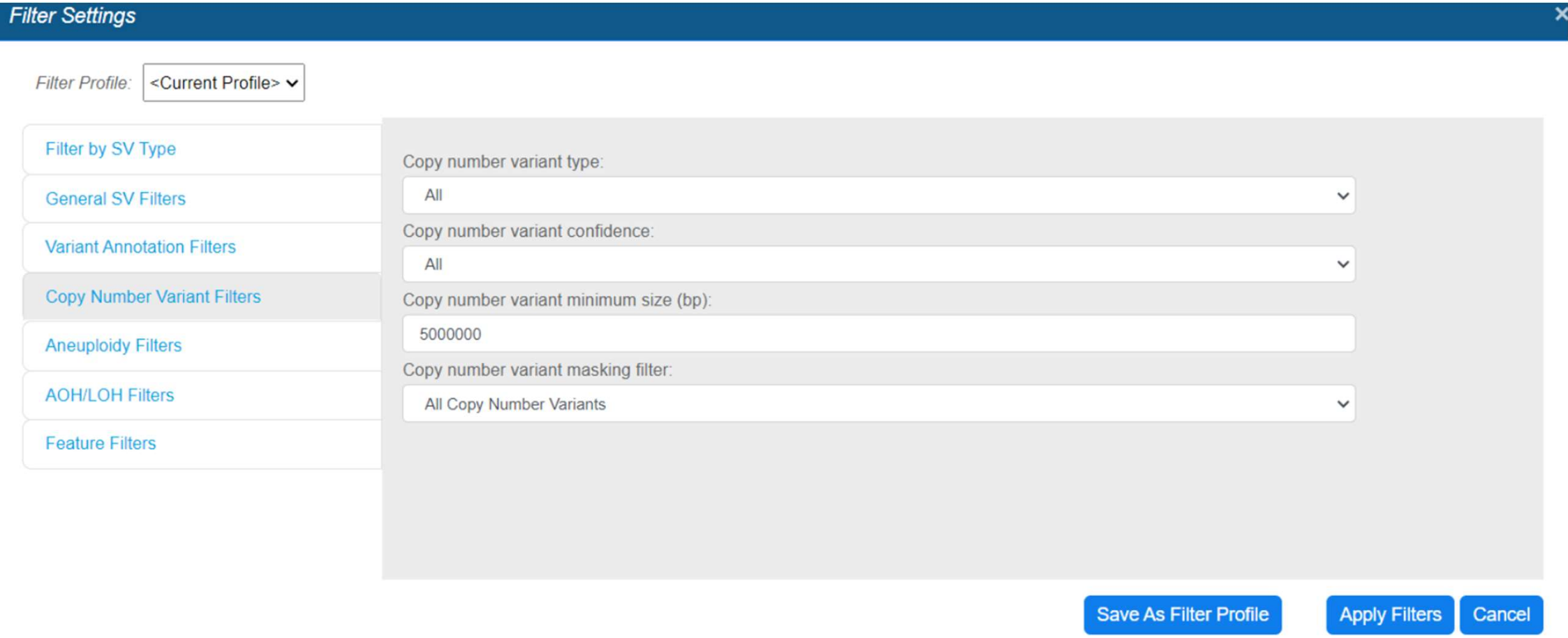
Filter settings UZ Leuven (confidential)



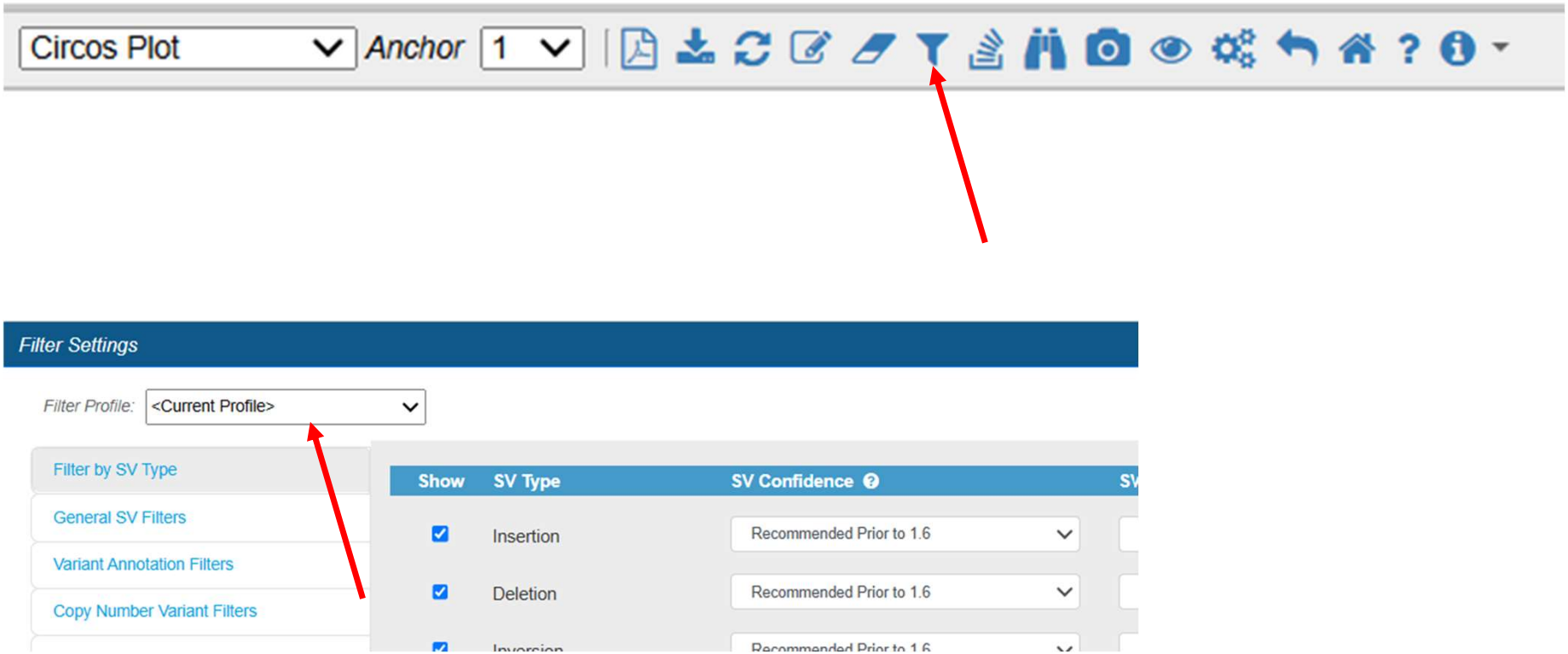
Filter settings UZ Leuven (confidential)



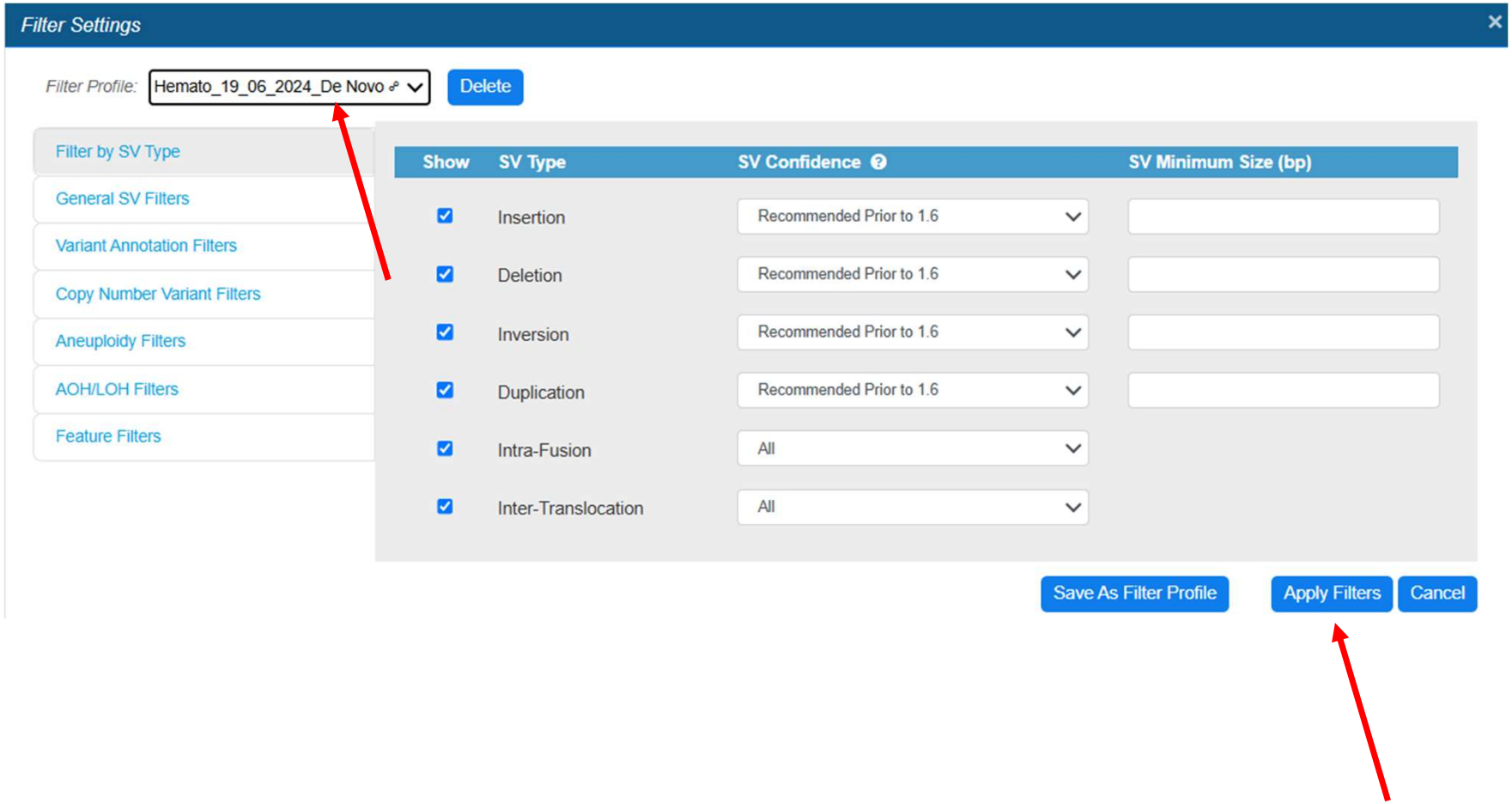
Filter settings UZ Leuven (confidential)



Filter settings UZ Leuven (confidential)

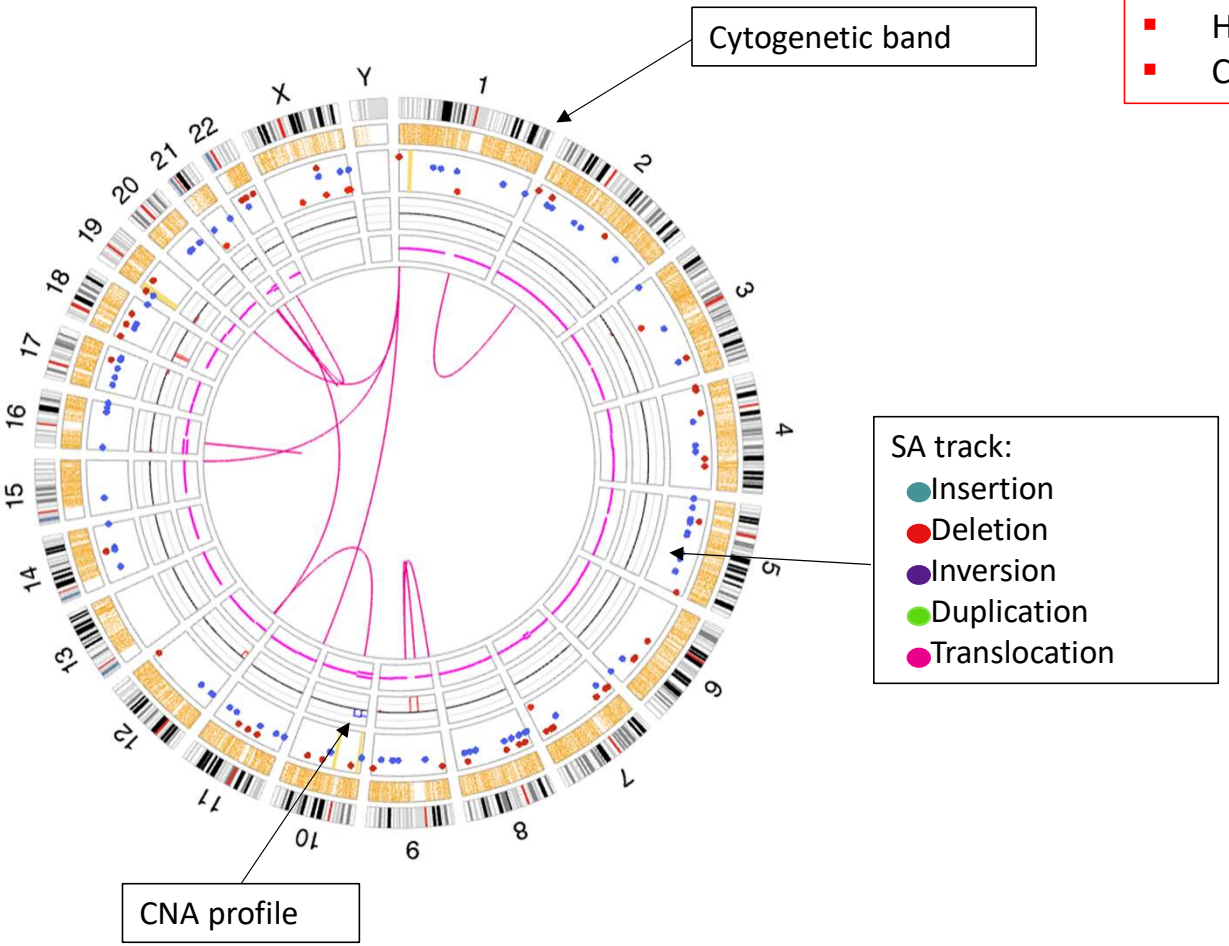


Filter settings UZ Leuven (confidential)



Example 1: B-ALL

Whole genome circus plot, *De Novo Assembly*



- Female, 2 years old
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- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

Example 1: B-ALL

Work in Excel: download data

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
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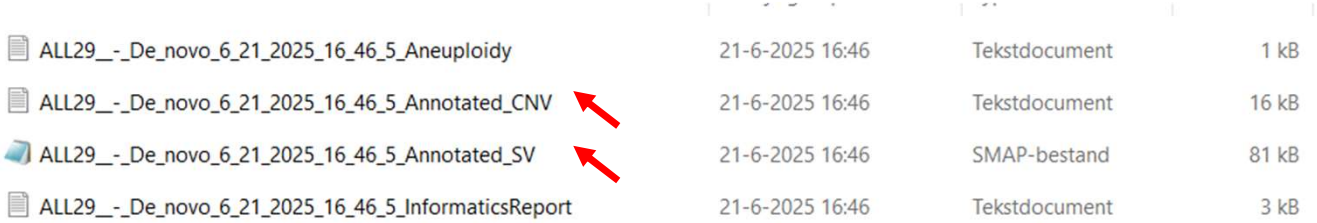
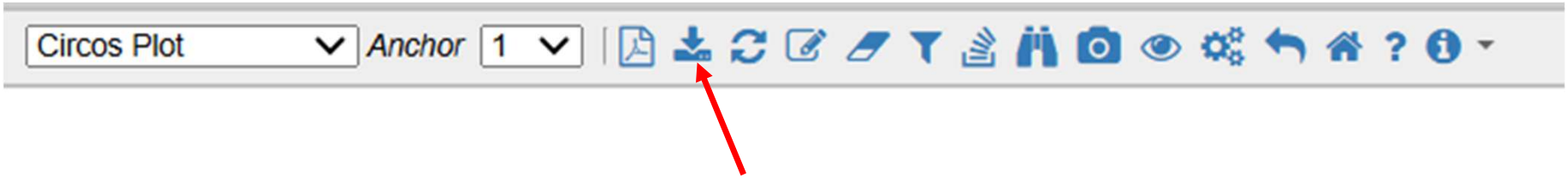


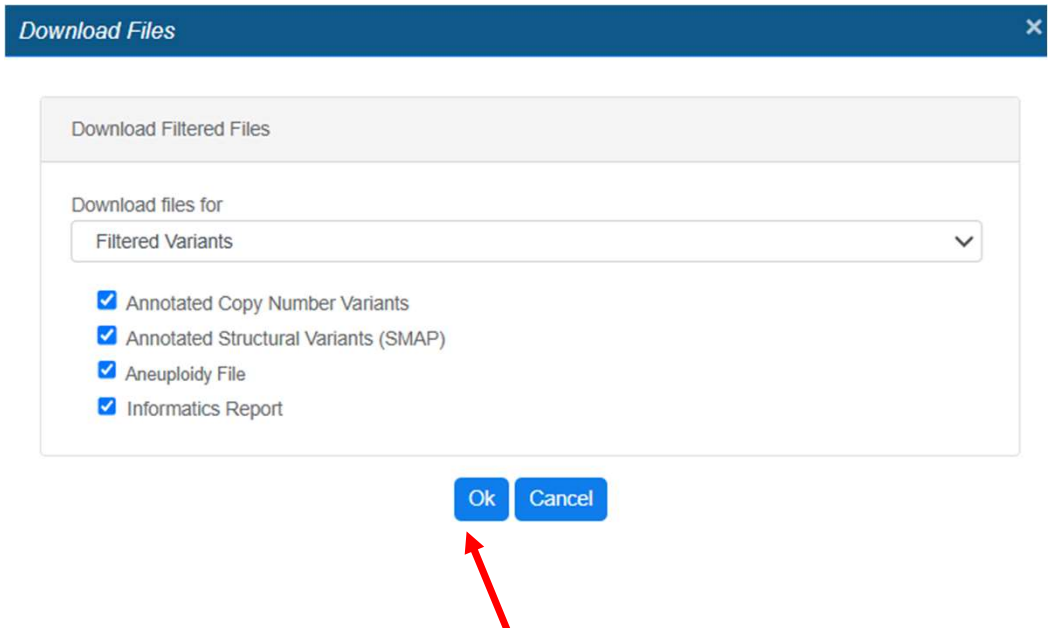
- Open files and copy to Excel
- There you can filter the data

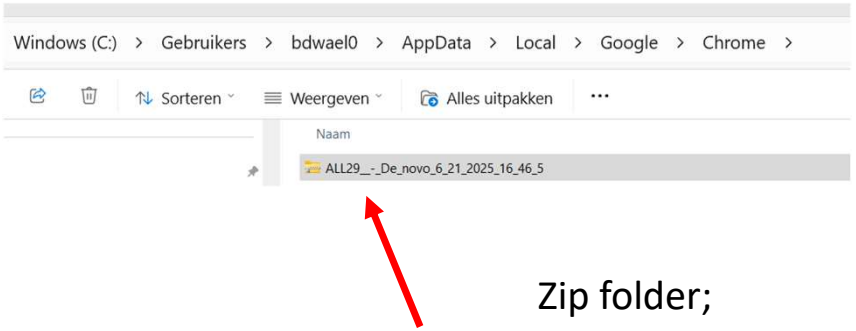
Example 1: B-ALL

Work in Excel: download data

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]







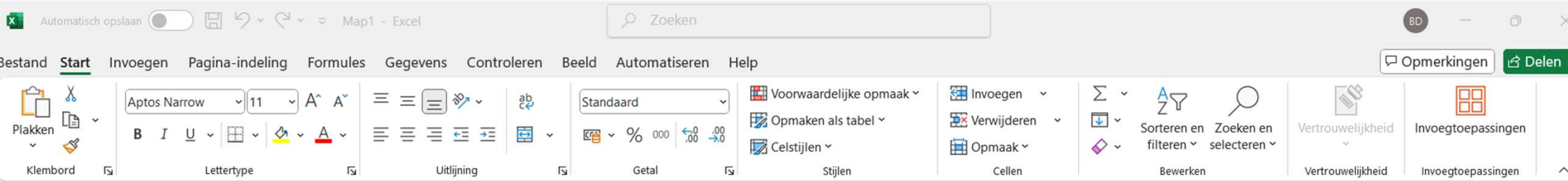
Zip folder; unpack

Example 1: B-ALL

Work in Excel: download data

I personally always start with the analysis of the CNV file

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



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2	# Version: 50ba3ddaea96215543add73d9c1d2564b97ba46a; installed on 2023-07-12 14:02:51																					
3	# annotation_source=Bionano Solve 3.8.1 CNV annotation pipeline																					
4	# command=run_cnv_annotation.py /home/bionano/access/local/jobs/996522/output/contigs/annotation/cnv_partition_0/inputs/vap_params.txt																					
5	# case_cnv_stats=/home/bionano/access/local/jobs/996522/output/contigs/alignmolvref/copynumber/cnv_chr_stats.txt case_cnv=/home/bionano/access/local/jobs/996522/output/contigs/annotation/cnv_partition_0/inputs/cnv_calls_exp.txt overlap_database																					
6	#Id	Chromoso	Start	End	Size	Type	fractionalCopyNumber	CopyNumb	Confidenc	CNFoldCh	Mask_over	Algorithm	OverlapGe	NearestNc	NearestNc	num_overl	UCSC_wel	ISCN				
7	17	3	48343148	54030407	5687260	loss	178.787.020.957.297	2	0.0	0.1060648	0.0	Region-ba	FBXW12;P	SPINK8	8488.0	6671.0	http://genc	ogm[GRCh38] 3p21.31p21.1(48343148_54030407)				
8	61	9	43289106	60867228	17578123	loss_mask	0.0493012481060519	0	0.0	0.9753493	1.0	Region-ba	-	SPATA31A	46179.0	6.0	http://genc	ogm[GRCh38] 9q11q12(43289106_60867228)x0~1				
9	74	9	127748196	133094807	5346612	loss	18.480.092.111.147.400	2	0.0	0.0759953	0.1191035	Region-ba	SH2D3C;M	RALGDS	1915.0	14796.0	http://genc	ogm[GRCh38] 9q34.11q34.13(127748196_133094				
10	78	10	18514	13874878	13856365	gain	273.069.296.811.665	3	1.0	0.3653464	0.0360845	Region-ba	TUBB8;ZM	LOC10272	1939.0	2875.0	http://genc	ogm[GRCh38] 10p15.3p13(18514_13874878)x2~3				
11	79	10	13883191	29311476	15428286	gain	28.755.808.895.305	3	1.0	0.4377904	0.0	Region-ba	FRMD4A;L	SVIL-AS1	97058.0	2490.0	http://genc	ogm[GRCh38] 10p13p11.23(13883191_29311476)x				
12	96	12	230737	9308760	9078024	loss	11.668.409.832.776.200	1	1.0	0.4165795	0.0312657	Region-ba	SLC6A13;L	SLC6A12	15581.0	28321.0	http://genc	ogm[GRCh38] 12p13.33p13.31(230737_9308760)x				
13	123	16	887824	6092059	5204236	loss	18.267.628.819.690.900	2	0.0	0.0866185	0.2421948	Region-ba	LMF1;LMF	GNG13	86091.0	5549.0	http://genc	ogm[GRCh38] 16p13.3(887824_6092059)x1~2				
14	134	17	74223205	82257988	8034784	loss	17.873.540.617.396.400	2	0.0	0.1063229	0.1941384	Region-ba	TTYH2;DN	MGC1627	8885.0	13483.0	http://genc	ogm[GRCh38] 17q25.1q25.3(74223205_82257988)				
15	136	18	15486705	20576802	5090098	loss_mask	0.0	0	0.0	1.0	1.0	Region-ba	-	LOC64466	155443.0	-	http://genc	ogm[GRCh38] 18p11.1q11.1(15486705_20576802)				
16	139	19	2068666	19097020	17028355	loss	178.713.525.084.046	2	0.0	0.1064323	0.1905494	Region-ba	MOB3A;IZ	MKNK2	16423.0	36976.0	http://genc	ogm[GRCh38] 19p13.3p13.11(2068666_19097020)				
17																						
18																						

Example 1: B-ALL

Work in Excel: download data

- Female, 2 years old
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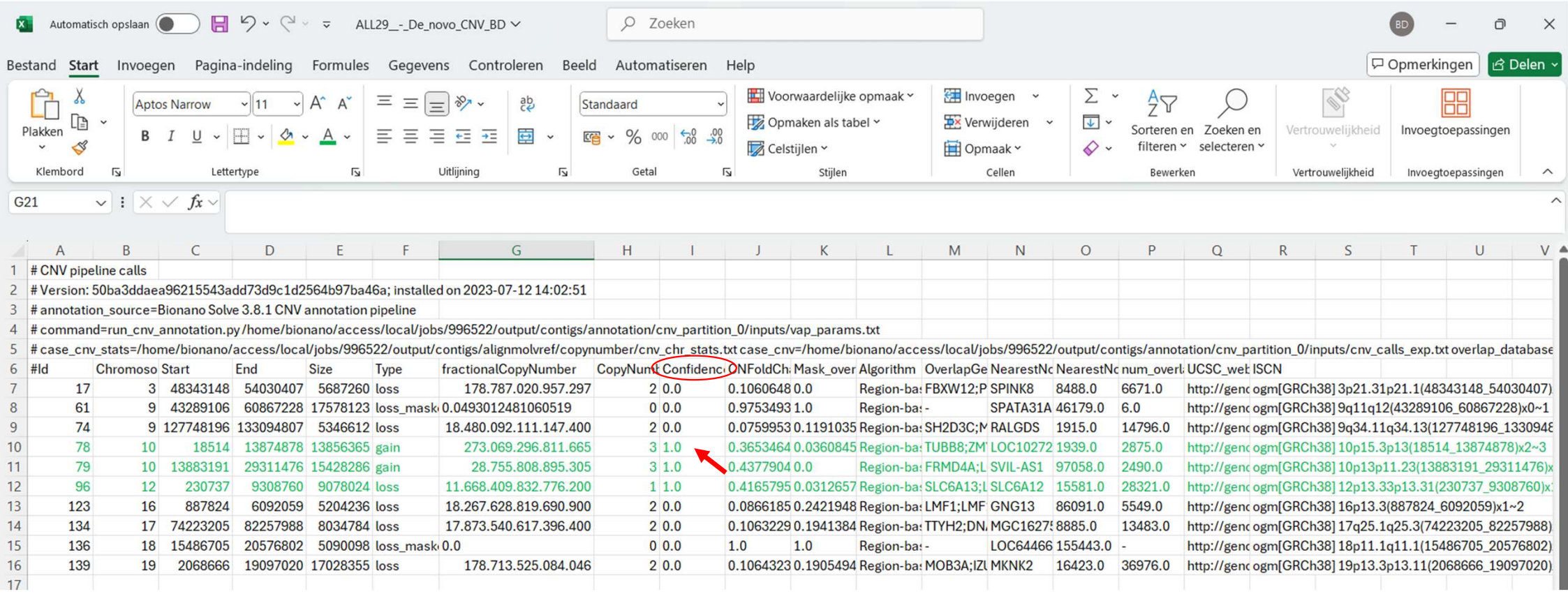
- Conventional karyotype: normal: 46,XX[20]



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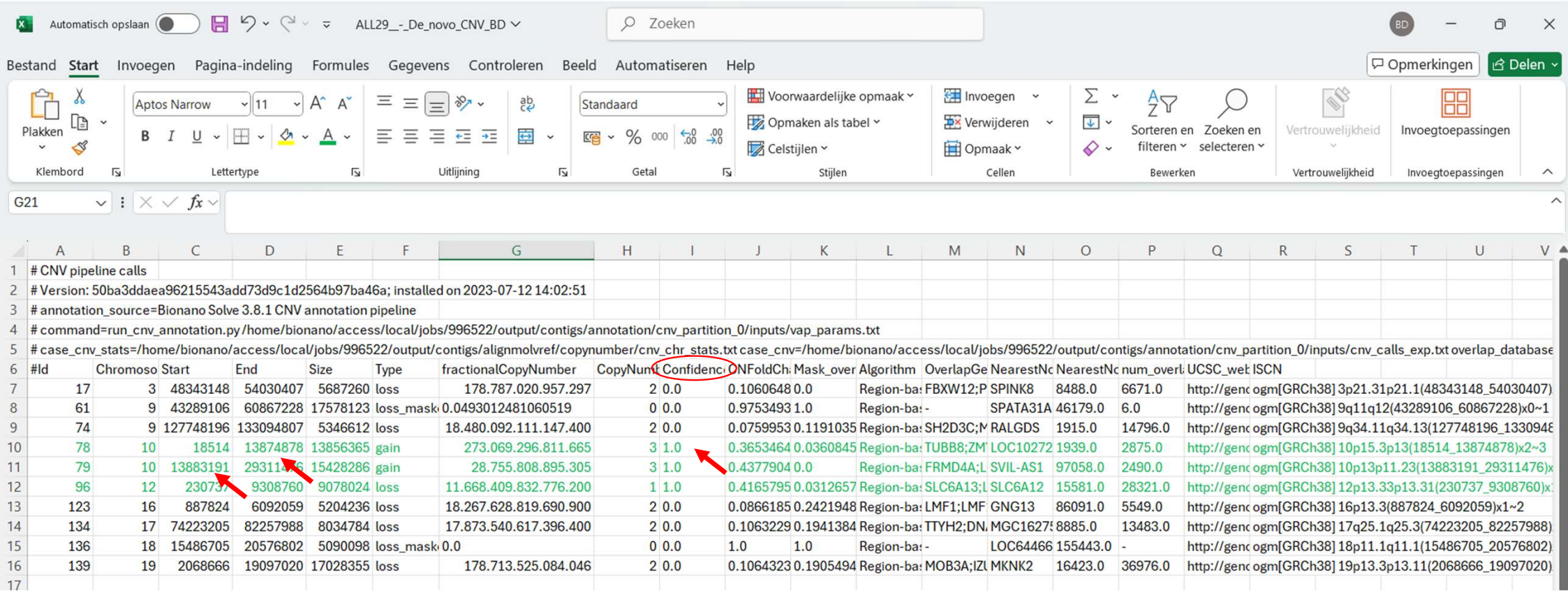
5	# case_cnv_stats=/home/bionano/access/local/jobs/996522/output/contigs/alignmolvref/copynumber/cnv_chr_stats.txt case_cnv=/home/bionano/access/local/jobs/996522/output/contigs/annotation/cnv_partition_0/inputs/cnv_calls_exp.txt overlap_database																					
6	#Id	Chromoso	Start	End	Size	Type	fractionalCopyNumber	CopyNumb	Confidenc	ONFoldCh	Mask_over	Algorithm	OverlapGe	NearestNc	NearestNc	num_overl	UCSC_wel	ISCN				
7	17	3	48343148	54030407	5687260	loss	178.787.020.957.297	2	0.0	0.1060648	0.0	Region-ba	FBXW12;P	SPINK8	8488.0	6671.0	http://genc	ogm[GRCh38] 3p21.31p21.1(48343148_54030407)				
8	61	9	43289106	60867228	17578123	loss_mask	0.0493012481060519	0	0.0	0.9753493	1.0	Region-ba	-	SPATA31A	46179.0	6.0	http://genc	ogm[GRCh38] 9q11q12(43289106_60867228)x0~1				
9	74	9	127748196	133094807	5346612	loss	18.480.092.111.147.400	2	0.0	0.0759953	0.1191035	Region-ba	SH2D3C;M	RALGDS	1915.0	14796.0	http://genc	ogm[GRCh38] 9q34.11q34.13(127748196_133094				
10	78	10	18514	13874878	13856365	gain	273.069.296.811.665	3	1.0	0.3653464	0.0360845	Region-ba	TUBB8;ZM	LOC10272	1939.0	2875.0	http://genc	ogm[GRCh38] 10p15.3p13(18514_13874878)x2~3				
11	79	10	13883191	29311476	15428286	gain	28.755.808.895.305	3	1.0	0.4377904	0.0	Region-ba	FRMD4A;L	SVIL-AS1	97058.0	2490.0	http://genc	ogm[GRCh38] 10p13p11.23(13883191_29311476)x				
12	96	12	230737	9308760	9078024	loss	11.668.409.832.776.200	1	1.0	0.4165795	0.0312657	Region-ba	SLC6A13;L	SLC6A12	15581.0	28321.0	http://genc	ogm[GRCh38] 12p13.33p13.31(230737_9308760)x				
13	123	16	887824	6092059	5204236	loss	18.267.628.819.690.900	2	0.0	0.0866185	0.2421948	Region-ba	LMF1;LMF	GNG13	86091.0	5549.0	http://genc	ogm[GRCh38] 16p13.3(887824_6092059)x1~2				
14	134	17	74223205	82257988	8034784	loss	17.873.540.617.396.400	2	0.0	0.1063229	0.1941384	Region-ba	TTYH2;DN	MGC1627	8885.0	13483.0	http://genc	ogm[GRCh38] 17q25.1q25.3(74223205_82257988)				
15	136	18	15486705	20576802	5090098	loss_mask	0.0	0	0.0	1.0	1.0	Region-ba	-	LOC64466	155443.0	-	http://genc	ogm[GRCh38] 18p11.1q11.1(15486705_20576802)				
16	139	19	2068666	19097020	17028355	loss	178.713.525.084.046	2	0.0	0.1064323	0.1905494	Region-ba	MOB3A;IZ	MKNK2	16423.0	36976.0	http://genc	ogm[GRCh38] 19p13.3p13.11(2068666_19097020)				
17																						

Example 1: B-ALL

Work in Excel: download data

- *Check confidence scores*
- *Sometimes merge CNVs*

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



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3	# annotation_source=Bionano Solve 3.8.1 CNV annotation pipeline																					
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6	#Id	Chromoso	Start	End	Size	Type	fractionalCopyNumber	CopyNum	Confidenc	ONFoldCh	Mask_over	Algorithm	OverlapGe	NearestNc	NearestNc	num_overl	UCSC_wel	ISCN				
7	17	3	48343148	54030407	5687260	loss	178.787.020.957.297	2	0.0	0.1060648	0.0	Region-ba	FBXW12;P	SPINK8	8488.0	6671.0	http://genc	ogm[GRCh38] 3p21.31p21.1(48343148_54030407)				
8	61	9	43289106	60867228	17578123	loss_mask	0.0493012481060519	0	0.0	0.9753493	1.0	Region-ba	-	SPATA31A	46179.0	6.0	http://genc	ogm[GRCh38] 9q11q12(43289106_60867228)x0~1				
9	74	9	127748196	133094807	5346612	loss	18.480.092.111.147.400	2	0.0	0.0759953	0.1191035	Region-ba	SH2D3C;M	RALGDS	1915.0	14796.0	http://genc	ogm[GRCh38] 9q34.11q34.13(127748196_133094				
10	78	10	18514	13874878	13856365	gain	273.069.296.811.665	3	1.0	0.3653464	0.0360845	Region-ba	TUBB8;ZM	LOC10272	1939.0	2875.0	http://genc	ogm[GRCh38] 10p15.3p13(18514_13874878)x2~3				
11	79	10	13883191	29311476	15428286	gain	28.755.808.895.305	3	1.0	0.4377904	0.0	Region-ba	FRMD4A;L	SVIL-AS1	97058.0	2490.0	http://genc	ogm[GRCh38] 10p13p11.23(13883191_29311476)x				
12	96	12	230737	9308760	9078024	loss	11.668.409.832.776.200	1	1.0	0.4165795	0.0312657	Region-ba	SLC6A13;L	SLC6A12	15581.0	28321.0	http://genc	ogm[GRCh38] 12p13.33p13.31(230737_9308760)x				
13	123	16	887824	6092059	5204236	loss	18.267.628.819.690.900	2	0.0	0.0866185	0.2421948	Region-ba	LMF1;LMF	GNG13	86091.0	5549.0	http://genc	ogm[GRCh38] 16p13.3(887824_6092059)x1~2				
14	134	17	74223205	82257988	8034784	loss	17.873.540.617.396.400	2	0.0	0.1063229	0.1941384	Region-ba	TTYH2;DN	MGC1627	8885.0	13483.0	http://genc	ogm[GRCh38] 17q25.1q25.3(74223205_82257988)				
15	136	18	15486705	20576802	5090098	loss_mask	0.0	0	0.0	1.0	1.0	Region-ba	-	LOC64466	155443.0	-	http://genc	ogm[GRCh38] 18p11.1q11.1(15486705_20576802)				
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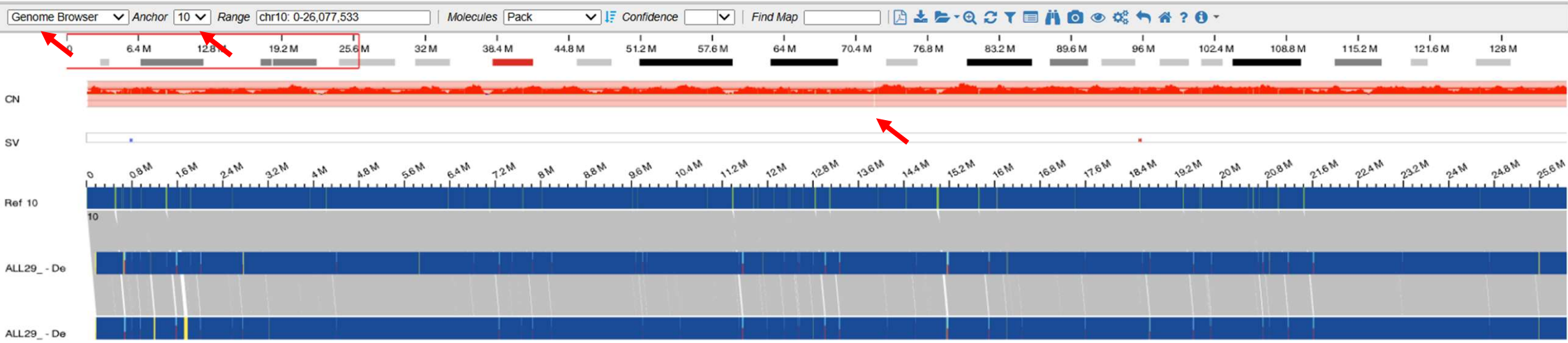
17																						

Example 1: B-ALL

Work in Excel: download data

Visual check in Bionano Access: Genome Browser
=> Chromosome 10

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



ogm[GRCh38]
10p15.3p13(18514_13874878)x3
10p13p11.23(13883191_29311476)x3

⇒ Merge to : 10p15.3p11.23(18514_29311476)x3
⇒ Gain on chromosome 10p

Example 1: B-ALL

Work in Excel: download data

- *Check confidence scores*
- *Sometimes merge CNVs*

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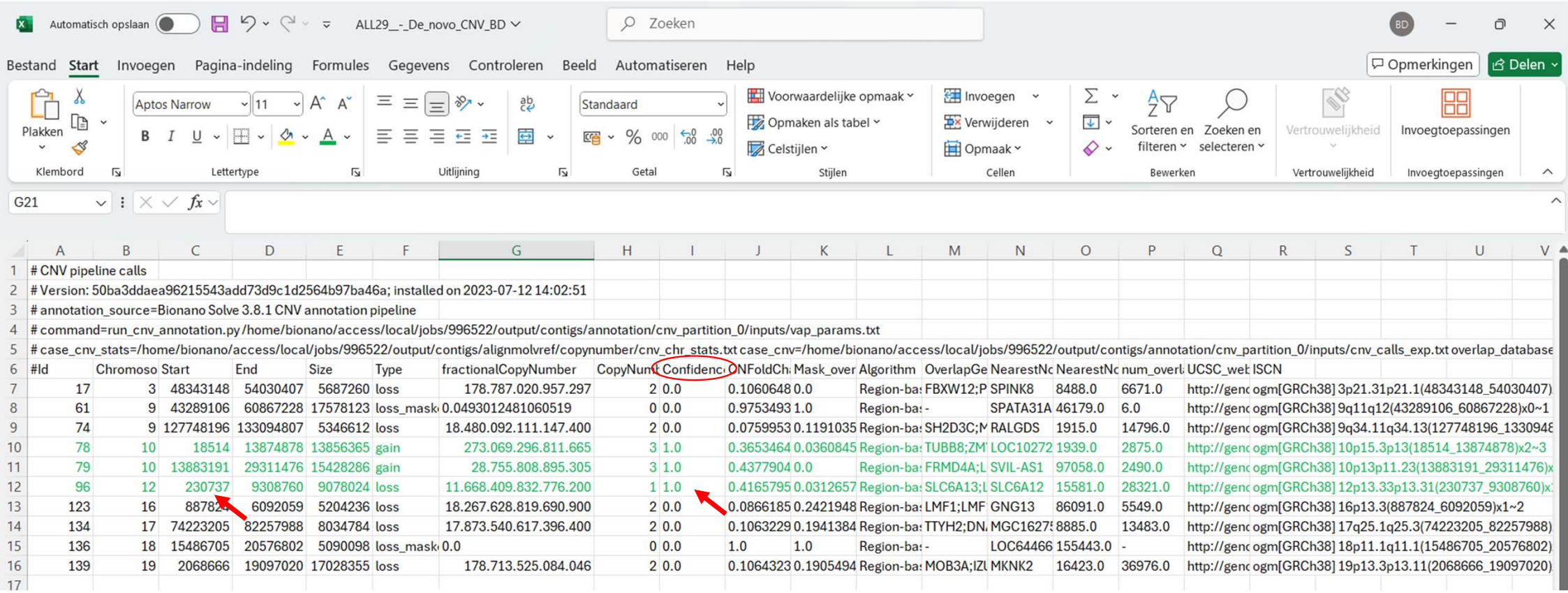

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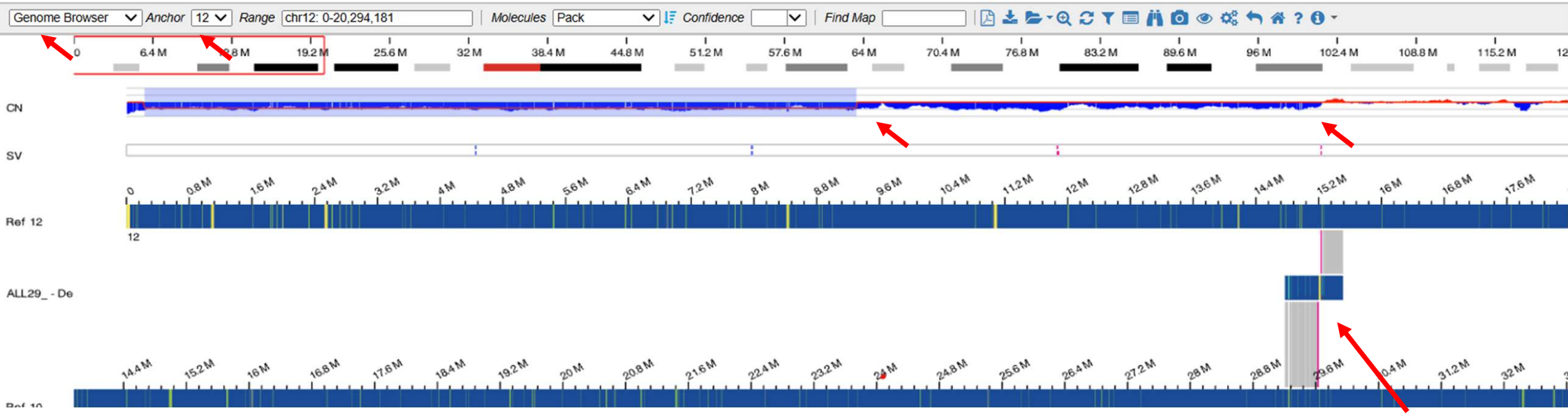
5	# case_cnv_stats=/home/bionano/access/local/jobs/996522/output/contigs/alignmolvref/copynumber/cnv_chr_stats.txt case_cnv=/home/bionano/access/local/jobs/996522/output/contigs/annotation/cnv_partition_0/inputs/cnv_calls_exp.txt overlap_database																					
6	#Id	Chromoso	Start	End	Size	Type	fractionalCopyNumber	CopyNum	Confidenc	ONFoldCh	Mask_over	Algorithm	OverlapGe	NearestNc	NearestNc	num_overl	UCSC_wel	ISCN				
7	17	3	48343148	54030407	5687260	loss	178.787.020.957.297	2	0.0	0.1060648	0.0	Region-ba	FBXW12;P	SPINK8	8488.0	6671.0	http://genc	ogm[GRCh38] 3p21.31p21.1(48343148_54030407)				
8	61	9	43289106	60867228	17578123	loss_mask	0.0493012481060519	0	0.0	0.9753493	1.0	Region-ba	-	SPATA31A	46179.0	6.0	http://genc	ogm[GRCh38] 9q11q12(43289106_60867228)x0~1				
9	74	9	127748196	133094807	5346612	loss	18.480.092.111.147.400	2	0.0	0.0759953	0.1191035	Region-ba	SH2D3C;M	RALGDS	1915.0	14796.0	http://genc	ogm[GRCh38] 9q34.11q34.13(127748196_133094				
10	78	10	18514	13874878	13856365	gain	273.069.296.811.665	3	1.0	0.3653464	0.0360845	Region-ba	TUBB8;ZM	LOC10272	1939.0	2875.0	http://genc	ogm[GRCh38] 10p15.3p13(18514_13874878)x2~3				
11	79	10	13883191	29311476	15428286	gain	28.755.808.895.305	3	1.0	0.4377904	0.0	Region-ba	FRMD4A;L	SVIL-AS1	97058.0	2490.0	http://genc	ogm[GRCh38] 10p13p11.23(13883191_29311476)x				
12	96	12	230737	9308760	9078024	loss	11.668.409.832.776.200	1	1.0	0.4165795	0.0312657	Region-ba	SLC6A13;L	SLC6A12	15581.0	28321.0	http://genc	ogm[GRCh38] 12p13.33p13.31(230737_9308760)x				
13	123	16	887824	6092059	5204236	loss	18.267.628.819.690.900	2	0.0	0.0866185	0.2421948	Region-ba	LMF1;LMF	GNG13	86091.0	5549.0	http://genc	ogm[GRCh38] 16p13.3(887824_6092059)x1~2				
14	134	17	74223205	82257988	8034784	loss	17.873.540.617.396.400	2	0.0	0.1063229	0.1941384	Region-ba	TTYH2;DN	MGC1627	8885.0	13483.0	http://genc	ogm[GRCh38] 17q25.1q25.3(74223205_82257988)				
15	136	18	15486705	20576802	5090098	loss_mask	0.0	0	0.0	1.0	1.0	Region-ba	-	LOC64466	155443.0	-	http://genc	ogm[GRCh38] 18p11.1q11.1(15486705_20576802)				
16	139	19	2068666	19097020	17028355	loss	178.713.525.084.046	2	0.0	0.1064323	0.1905494	Region-ba	MOB3A;IZ	MKNK2	16423.0	36976.0	http://genc	ogm[GRCh38] 19p13.3p13.11(2068666_19097020)				
17																						

Example 1: B-ALL

Work in Excel: download data

Visual check in Bionano Access: Genome Browser
=> Chromosome 12

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



ogm[GRCh38]
12p13.33p13.31(230737_9308760)x1

⇒ Elongate the deletion to : 12p13.33p12.3(230737_15233701)x1

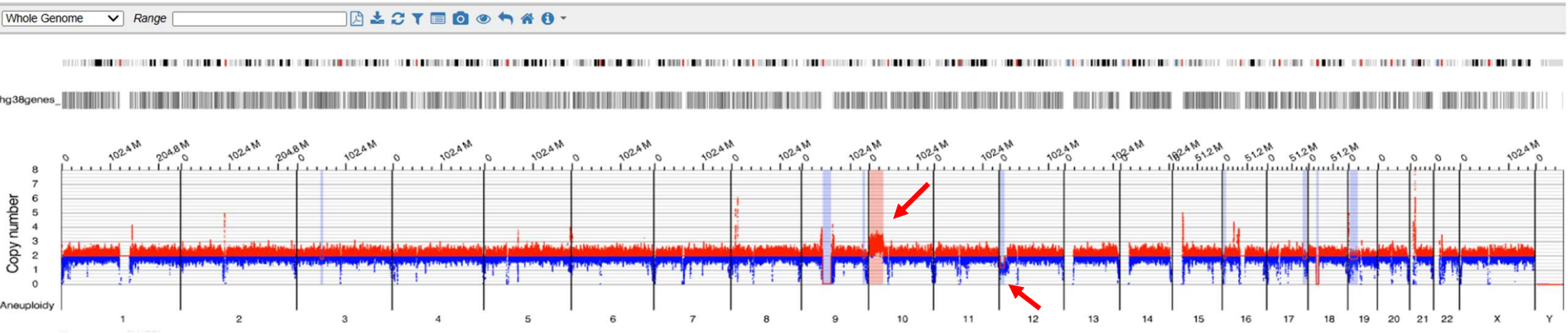
⇒ Loss on chromosome 12p; link to translocation with chr 10

Example 1: B-ALL

Work in Excel: download data

Always perform visual check of whole genome CNV profile

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

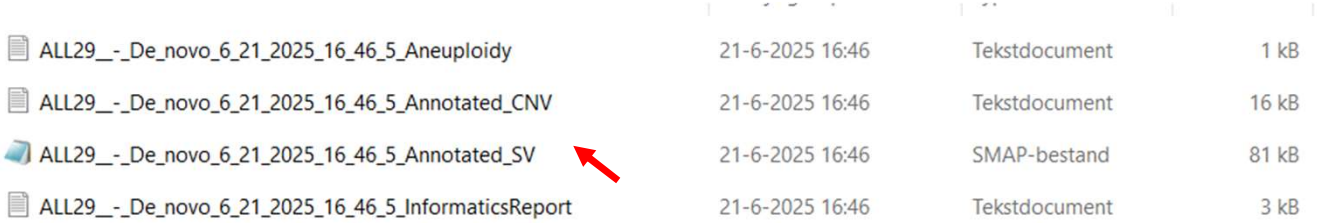


Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



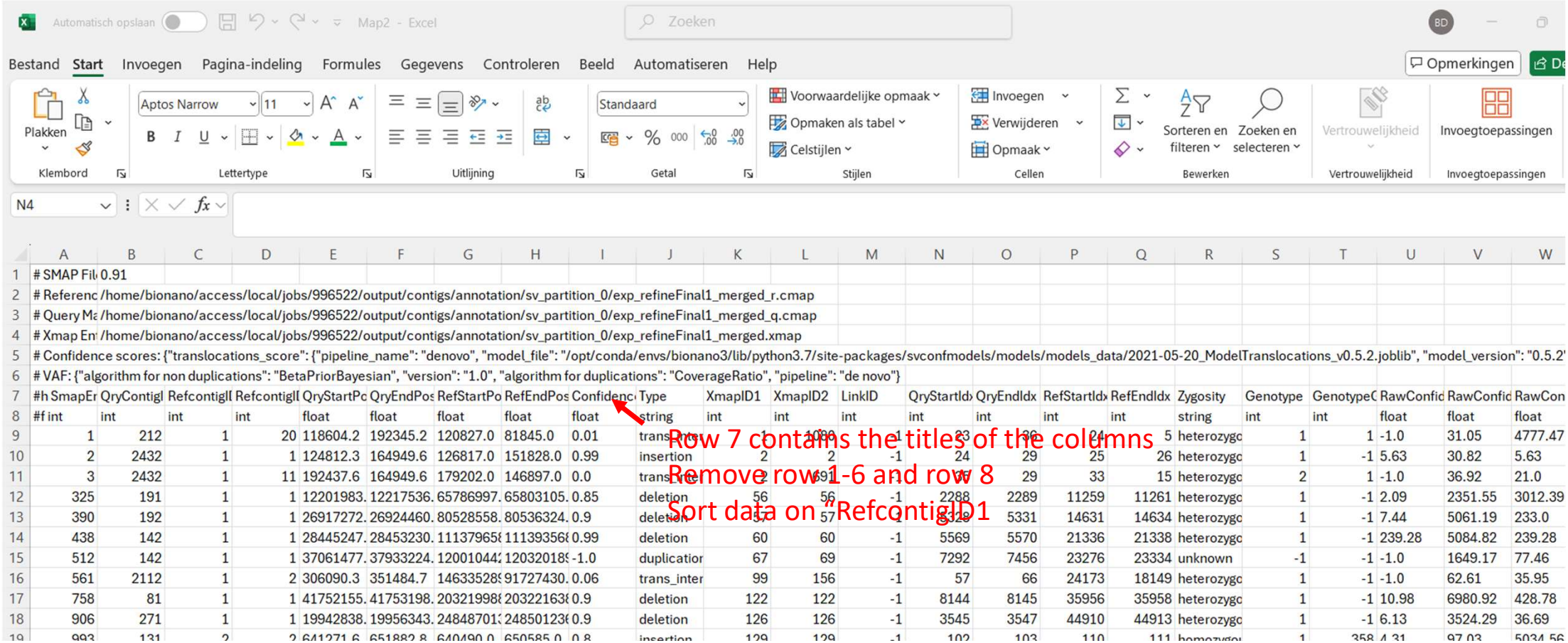
- Open files and copy to Excel
- There you can filter the data

Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



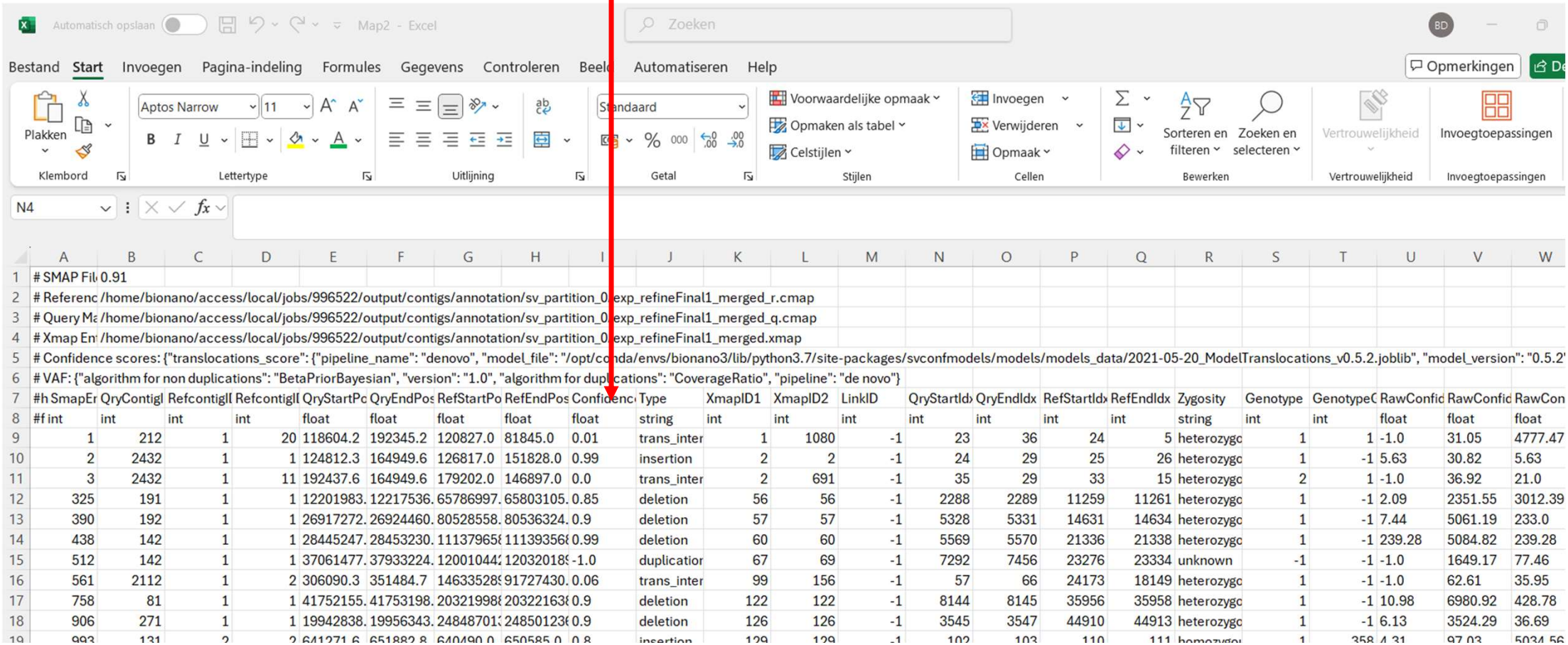
Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

Row 7 contains the titles of the columns
Remove row 1-6 and row 8
Sort data on “RefcontigID1

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



Automatisch opslaan — Map2 - Excel — Zoeken

Bestand Start Invoegen Pagina-indeling Formules Gegevens Controleren Beeld Automatiseren Help — Opmerkingen

Plakken | Klembord | Aptos Narrow | 11 | Lettertype | Uitlijning | Standaard | Getal | Voorwaardelijke opmaak | Opmaken als tabel | Celstijlen | Stijlen | Invoegen | Verwijderen | Opmaak | Cellen | Sorteren en filteren | Zoeken en selecteren | Bewerken | Vertrouwelijkheid | Invoegtoepassingen

N4

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	# SMAP Fil	0.91																					
2	# Referenc	/home/bionano/access/local/jobs/996522/output/contigs/annotation/sv_partition_0								exp_refineFinal1_merged_r.cmap													
3	# Query Ma	/home/bionano/access/local/jobs/996522/output/contigs/annotation/sv_partition_0								exp_refineFinal1_merged_q.cmap													
4	# Xmap En	/home/bionano/access/local/jobs/996522/output/contigs/annotation/sv_partition_0								exp_refineFinal1_merged.xmap													
5	# Confidence scores: {"translocations_score": {"pipeline_name": "denovo", "model_file": "/opt/conda/envs/bionano3/lib/python3.7/site-packages/svconfmodels/models/models_data/2021-05-20_ModelTranslocations_v0.5.2.joblib", "model_version": "0.5.2"																						
6	# VAF: {"algorithm for non duplications": "BetaPriorBayesian", "version": "1.0", "algorithm for duplications": "CoverageRatio", "pipeline": "de novo"}																						
7	#h SmapEr	QryContigI	RefcontigI	RefcontigI	QryStartPo	QryEndPos	RefStartPo	RefEndPos	Confidenc	Type	XmapID1	XmapID2	LinkID	QryStartIdx	QryEndIdx	RefStartIdx	RefEndIdx	Zygosity	Genotype	GenotypeC	RawConfid	RawConfid	RawCon
8	#f int	int	int	int	float	float	float	float	float	string	int	int	int	int	int	int	int	string	int	int	float	float	float
9	1	212	1	20	118604.2	192345.2	120827.0	81845.0	0.01	trans_inter	1	1080	-1	23	36	24	5	heterozygo	1	1	-1.0	31.05	4777.47
10	2	2432	1	1	124812.3	164949.6	126817.0	151828.0	0.99	insertion	2	2	-1	24	29	25	26	heterozygo	1	-1	5.63	30.82	5.63
11	3	2432	1	11	192437.6	164949.6	179202.0	146897.0	0.0	trans_inter	2	691	-1	35	29	33	15	heterozygo	2	1	-1.0	36.92	21.0
12	325	191	1	1	12201983.	12217536.	65786997.	65803105.	0.85	deletion	56	56	-1	2288	2289	11259	11261	heterozygo	1	-1	2.09	2351.55	3012.39
13	390	192	1	1	26917272.	26924460.	80528558.	80536324.	0.9	deletion	57	57	-1	5328	5331	14631	14634	heterozygo	1	-1	7.44	5061.19	233.0
14	438	142	1	1	28445247.	28453230.	11137965	11139356	0.99	deletion	60	60	-1	5569	5570	21336	21338	heterozygo	1	-1	239.28	5084.82	239.28
15	512	142	1	1	37061477.	37933224.	12001044	12032018	-1.0	duplicatior	67	69	-1	7292	7456	23276	23334	unknown	-1	-1	-1.0	1649.17	77.46
16	561	2112	1	2	306090.3	351484.7	14633528	91727430.	0.06	trans_inter	99	156	-1	57	66	24173	18149	heterozygo	1	-1	-1.0	62.61	35.95
17	758	81	1	1	41752155.	41753198.	20321998	20322163	0.9	deletion	122	122	-1	8144	8145	35956	35958	heterozygo	1	-1	10.98	6980.92	428.78
18	906	271	1	1	19942838.	19956343.	24848701	24850123	0.9	deletion	126	126	-1	3545	3547	44910	44913	heterozygo	1	-1	6.13	3524.29	36.69
19	993	131	2	2	641271.6	651882.8	640490.0	650585.0	0.8	insertion	129	129	-1	102	103	110	111	homozygo	1	358	4.31	97.03	5034.56

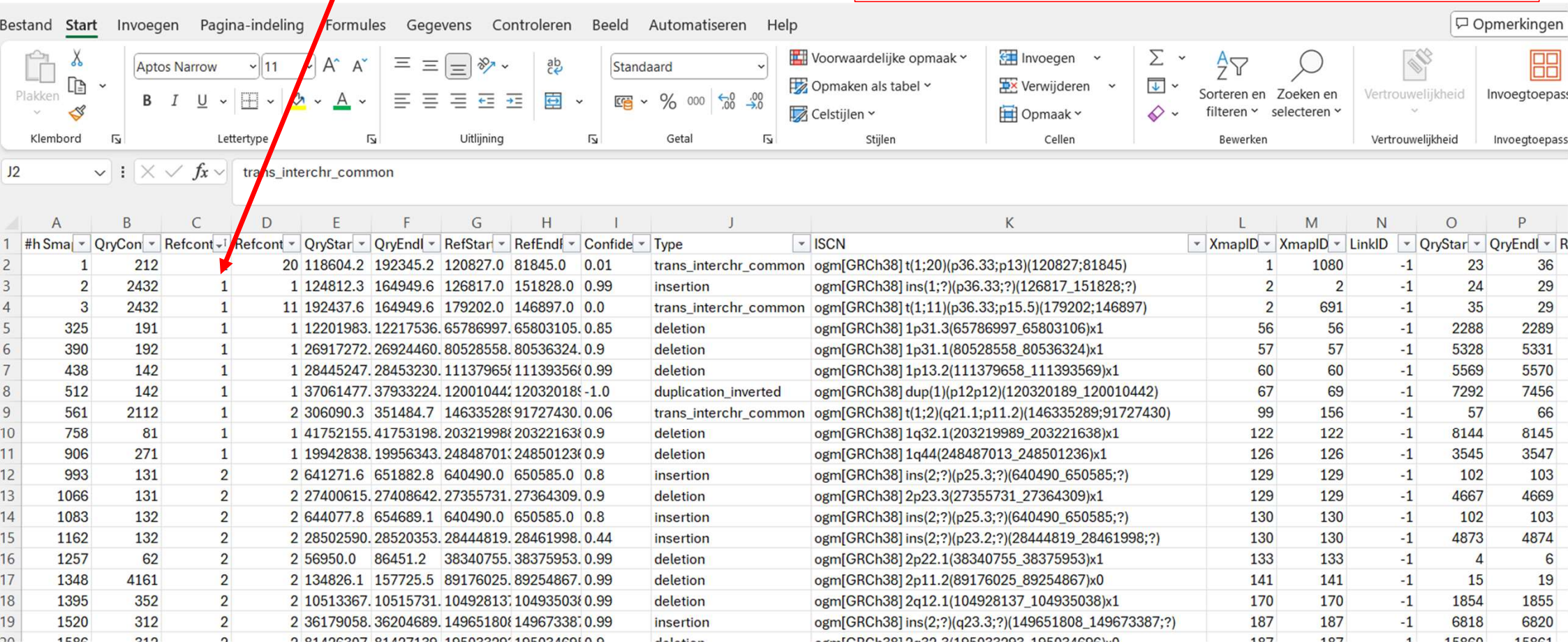
Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

Order of SVs per chromosome from 1-22; then X and Y

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	#h Sma	QryCon	Refcont	Refcont	QryStar	QryEndI	RefStar	RefEndI	Confide	Type	ISCN	XmapID	XmapID	LinkID	QryStar	QryEndI
2	1	212		20	118604.2	192345.2	120827.0	81845.0	0.01	trans_interchr_common	ogm[GRCh38] t(1;20)(p36.33;p13)(120827;81845)	1	1080	-1	23	36
3	2	2432	1	1	124812.3	164949.6	126817.0	151828.0	0.99	insertion	ogm[GRCh38] ins(1;?)(p36.33;?)(126817_151828;?)	2	2	-1	24	29
4	3	2432	1	11	192437.6	164949.6	179202.0	146897.0	0.0	trans_interchr_common	ogm[GRCh38] t(1;11)(p36.33;p15.5)(179202;146897)	2	691	-1	35	29
5	325	191	1	1	12201983.	12217536.	65786997.	65803105.	0.85	deletion	ogm[GRCh38] 1p31.3(65786997_65803106)x1	56	56	-1	2288	2289
6	390	192	1	1	26917272.	26924460.	80528558.	80536324.	0.9	deletion	ogm[GRCh38] 1p31.1(80528558_80536324)x1	57	57	-1	5328	5331
7	438	142	1	1	28445247.	28453230.	11137965	11139356	0.99	deletion	ogm[GRCh38] 1p13.2(111379658_111393569)x1	60	60	-1	5569	5570
8	512	142	1	1	37061477.	37933224.	12001044	12032018	-1.0	duplication_inverted	ogm[GRCh38] dup(1)(p12p12)(120320189_120010442)	67	69	-1	7292	7456
9	561	2112	1	2	306090.3	351484.7	14633528	91727430.	0.06	trans_interchr_common	ogm[GRCh38] t(1;2)(q21.1;p11.2)(146335289;91727430)	99	156	-1	57	66
10	758	81	1	1	41752155.	41753198.	20321998	20322163	0.9	deletion	ogm[GRCh38] 1q32.1(203219989_203221638)x1	122	122	-1	8144	8145
11	906	271	1	1	19942838.	19956343.	24848701	24850123	0.9	deletion	ogm[GRCh38] 1q44(248487013_248501236)x1	126	126	-1	3545	3547
12	993	131	2	2	641271.6	651882.8	640490.0	650585.0	0.8	insertion	ogm[GRCh38] ins(2;?)(p25.3;?)(640490_650585;?)	129	129	-1	102	103
13	1066	131	2	2	27400615.	27408642.	27355731.	27364309.	0.9	deletion	ogm[GRCh38] 2p23.3(27355731_27364309)x1	129	129	-1	4667	4669
14	1083	132	2	2	644077.8	654689.1	640490.0	650585.0	0.8	insertion	ogm[GRCh38] ins(2;?)(p25.3;?)(640490_650585;?)	130	130	-1	102	103
15	1162	132	2	2	28502590.	28520353.	28444819.	28461998.	0.44	insertion	ogm[GRCh38] ins(2;?)(p23.2;?)(28444819_28461998;?)	130	130	-1	4873	4874
16	1257	62	2	2	56950.0	86451.2	38340755.	38375953.	0.99	deletion	ogm[GRCh38] 2p22.1(38340755_38375953)x1	133	133	-1	4	6
17	1348	4161	2	2	134826.1	157725.5	89176025.	89254867.	0.99	deletion	ogm[GRCh38] 2p11.2(89176025_89254867)x0	141	141	-1	15	19
18	1395	352	2	2	10513367.	10515731.	10492813	10493503	0.99	deletion	ogm[GRCh38] 2q12.1(104928137_104935038)x1	170	170	-1	1854	1855
19	1520	312	2	2	36179058.	36204689.	14965180	14967338	0.99	insertion	ogm[GRCh38] ins(2;?)(q23.3;?)(149651808_149673387;?)	187	187	-1	6818	6820

Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

- Female, 2 years old
- Anemia, thrombopenia
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- Conventional karyotype: normal: 46,XX[20]

I first check the translocations

- Common/segdupe usually not retained (gen pop or known artefact)
- Others: check in Bionano access

112	7607	422	12	12	4074692.0	4084723.3	7970990.0	7981533.0	0.9	deletion	ogm[GRCh38] 12p13.31(7970990_7981533)x0	731	731	-1	753	754	1522
113	7627	421	12	12	7703757.2	7706378.8	4451423.0	4455774.0	0.99	deletion	ogm[GRCh38] 12p13.32(4451423_4455774)x0	732	732	-1	1390	1391	844
114	7637	421	12	12	4169462.6	4179494.0	7970990.0	7981533.0	0.9	deletion	ogm[GRCh38] 12p13.31(7970990_7981533)x0	732	732	-1	775	776	1522
115	7676	2002	12	10	278287.4	319187.7	15233701.	29628820.	0.47	translocation_interchr	ogm[GRCh38] t(12;10)(p12.3;p11.23)(15233701;29628820)	739	651	-1	60	68	2997
116	7677	2001	12	10	301842.6	342742.8	15233701.	29628820.	0.31	translocation_interchr	ogm[GRCh38] t(12;10)(p12.3;p11.23)(15233701;29628820)	740	649	-1	63	71	2997
117	7709	302	12	12	1085525.1	1100298.5	33656118.	33671650.	0.9	deletion	ogm[GRCh38] 12p11.1(33656118_33671651)x1	741	741	-1	156	158	7058
118	8062	491	12	12	971102.5	985949.1	13169389	13170818	0.5	insertion	ogm[GRCh38] ins(12;?)(q24.33;?)(131693898_131708189;?)	752	752	-1	95	96	26328
119	8063	491	12	12	805271.1	845156.2	13183397	13187291	0.42	insertion	ogm[GRCh38] ins(12;?)(q24.33;?)(131833971_131872917;?)	752	752	-1	75	76	26348
120	8503	22	14	14	3081288.2	3089385.1	21894406.	22523108.	0.99	deletion	ogm[GRCh38] 14q11.2(21894406_22523108)x1	776	778	-1	547	548	669
121	8510	21	14	14	3647790.5	3649734.5	22422983.	22451049.	0.99	deletion	ogm[GRCh38] 14q11.2(22422983_22451049)x1	777	777	-1	638	639	765
122	8563	21	14	14	41846898.	41851333.	60744080.	60749055.	0.9	deletion	ogm[GRCh38] 14q23.1(60744080_60749055)x1	777	777	-1	8122	8124	9076
123	8661	22	14	14	37103779.	37107441.	56610846.	56615013.	0.9	deletion	ogm[GRCh38] 14q22.3(56610846_56615013)x1	778	778	-1	7210	7212	8163
124	8662	22	14	14	37987512.	38002637.	57496444.	57510934.	0.44	insertion	ogm[GRCh38] ins(14;?)(q22.3;?)(57496445_57510934;?)	778	778	-1	7386	7387	8361
125	8777	3171	14	14	228016.3	238269.9	10653488	10658610	0.99	deletion	ogm[GRCh38] 14q32.33(106534885_106586104)x1	789	789	-1	28	29	17918
126	8878	792	15	15	2628278.6	2635529.7	30259276.	30267500.	0.9	deletion	ogm[GRCh38] 15q13.2(30259276_30267500)x1	835	835	-1	438	440	1886
127	9059	341	15	1	17100956.	17071668.	10189053	466235.5	0.0	trans_interchr_common	ogm[GRCh38] t(15;1)(q26.3;p36.33)(101890534;466236)	859	6	-1	3049	3044	16220
128	9089	342	15	1	17066086.	17036798.	10189053	466235.5	0.0	trans_interchr_common	ogm[GRCh38] t(15;1)(q26.3;p36.33)(101890534;466236)	860	7	-1	3039	3034	16220
129	9174	442	16	16	10284279.	10305902.	5111950.0	5134403.0	0.9	deletion	ogm[GRCh38] 16p13.3(5111950_5134403)x1	874	874	-1	1728	1729	577
130	9299	801	16	16	141457.3	141457.3	32706461.	32222510.	0.0	trans_intrachr_segdupe	ogm[GRCh38] fus(16;16)(p11.2;p11.2)(32706461;32222510)	939	937	-1	25	25	5476
131	9387	701	16	16	26423123.	26429644.	72714151.	72721251.	0.55	deletion	ogm[GRCh38] 16q22.2(72714152_72721251)x1	956	956	-1	4512	4513	10910

Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

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- Others: check in Bionano access

Nice labeling pattern

Example 1: B-ALL

Work in Excel: download data

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112	7607	422	12	12	4074692.0	4084723.3	7970990.0	7981533.0	0.9	deletion	ogm[GRCh38] 12p13.31(7970990_7981533)x0	731	731	-1	753	754	1522
113	7627	421	12	12	7703757.2	7706378.8	4451423.0	4455774.0	0.99	deletion	ogm[GRCh38] 12p13.32(4451423_4455774)x0	732	732	-1	1390	1391	844
114	7637	421	12	12	4169462.6	4179494.0	7970990.0	7981533.0	0.9	deletion	ogm[GRCh38] 12p13.31(7970990_7981533)x0	732	732	-1	775	776	1522
115	7676	2002	12	10	278287.4	319187.7	15233701.	29628820.	0.47	translocation_interchr	ogm[GRCh38] t(12;10)(p12.3;p11.23)(15233701;29628820)	739	651	-1	60	68	2997
116	7677	2001	12	10	301842.6	342742.8	15233701.	29628820.	0.31	translocation_interchr	ogm[GRCh38] t(12;10)(p12.3;p11.23)(15233701;29628820)	740	649	-1	63	71	2997
117	7709	302	12	12	1085525.1	1100298.5	33656118.	33671650.	0.9	deletion	ogm[GRCh38] 12p11.1(33656118_33671651)x1	741	741	-1	156	158	7058
118	8062	491	12	12	971102.5	985949.1	13169389	13170818	0.5	insertion	ogm[GRCh38] ins(12;?)(q24.33;?)(131693898_131708189;?)	752	752	-1	95	96	26328
119	8063	491	12	12	805271.1	845156.2	13183397	13187291	0.42	insertion	ogm[GRCh38] ins(12;?)(q24.33;?)(131833971_131872917;?)	752	752	-1	75	76	26348

Report this translocation: watch out for the ISCN: order of the chromosomes!

ogm[GRCh38] t(10;12)(p11.23;p12.3)(29628820;15233701)

Example 1: B-ALL

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10651	162	20	20	5509799.4	5523329.1	56819250.	56854774.	0.99	deletion	ogm[GRCh38] 20q13.31(56819250_56854774)x1	1113	1113	-1	744	745	10116
10680	161	20	20	25459666.	25466338.	36975084.	36985758.	0.99	deletion	ogm[GRCh38] 20q11.23(36975084_36985758)x1	1114	1114	-1	4078	4080	6446
10773	1121	21	21	990074.1	1024616.7	6215576.0	8100606.0	0.0	trans_intrachr_common	ogm[GRCh38] fus(21;21)(p12;p11.2)(6215576;8100606)	1129	1145	-1	162	165	116
10787	1122	21	21	549061.1	476935.5	7144161.0	5576643.0	0.0	trans_intrachr_common	ogm[GRCh38] fus(21;21)(p11.2;p12)(7144161;5576643)	1141	1126	-1	99	87	245
10794	1911	21	21	467974.3	477003.4	8853390.2	8867078.3	0.99	deletion	ogm[GRCh38] 21p11.2(8853390_8867078)x1	1153	1153	-1	44	45	404
10823	2621	21	21	11743.2	478484.3	9098731.0	9190519.0	-1.0	duplication_inverted	ogm[GRCh38] dup(21)(p11.2p11.2)(9190519_9098731)	1172	1171	-1	3	88	431
10875	1901	21	22	136070.6	136833.3	10373083.	11027065.	0.0	translocation_interchr	ogm[GRCh38] t(21;22)(p11.2;p11.2)(10373083;11027065)	1226	1266	-1	10	11	608
10981	421	21	12	305272.0	318789.5	35029693.	11870531.	0.18	translocation_interchr	ogm[GRCh38] t(21;12)(q22.12;p13.2)(35029693;11870531)	1249	732	-1	51	54	5300
10983	422	21	12	305272.0	318789.5	35029693.	11870531.	0.38	translocation_interchr	ogm[GRCh38] t(21;12)(q22.12;p13.2)(35029693;11870531)	1250	733	-1	51	54	5300
11063	432	21	21	11984630.	11993405.	46696723.	46709983.	0.99	deletion_nbase	ogm[GRCh38] 21q22.3(46696724_46709983)x0	1252	1252	-1	1829	1830	7220
11064	4570	21	12	80061.5	84578.8	35037114.	11881907.	0.07	translocation_interchr	ogm[GRCh38] t(21;12)(q22.12;p13.2)(35037114;11881907)	1253	735	-1	15	16	5302
11118	1011	22	22	566391.9	572698.5	16797434.	16803120.	0.82	insertion	ogm[GRCh38] ins(22;?)(q11.1;?)(16797434_16803120;?)	1317	1317	-1	88	89	584

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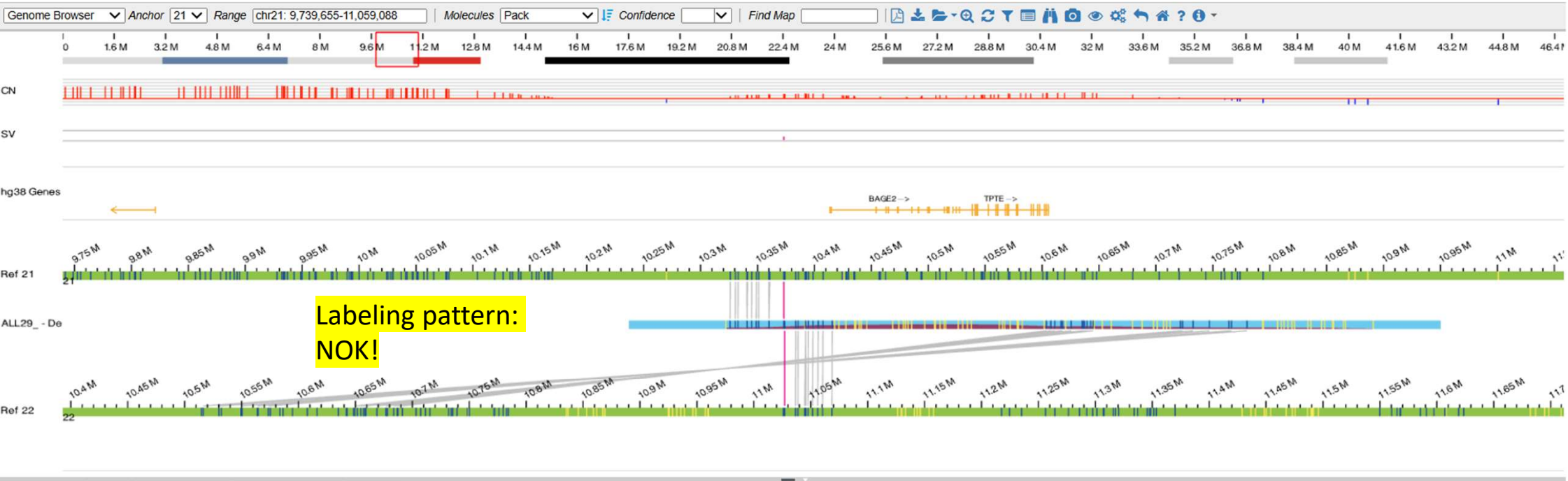

SV Annotation | Match | CNV Annotation | AOH/LOH Regions | Feature

Type	RefcontigID1	RefcontigID2	RefStartPos...	RefEndPos ...	Size (bp)	Confide...	SmapId	Present in % of control db samples	VAF	Present in % of control db samples with the same enzyme
translocation_interchr	21	22	10.373.083	11.027.065	-1	0,00	*10875*	0,0	0,230	0,0

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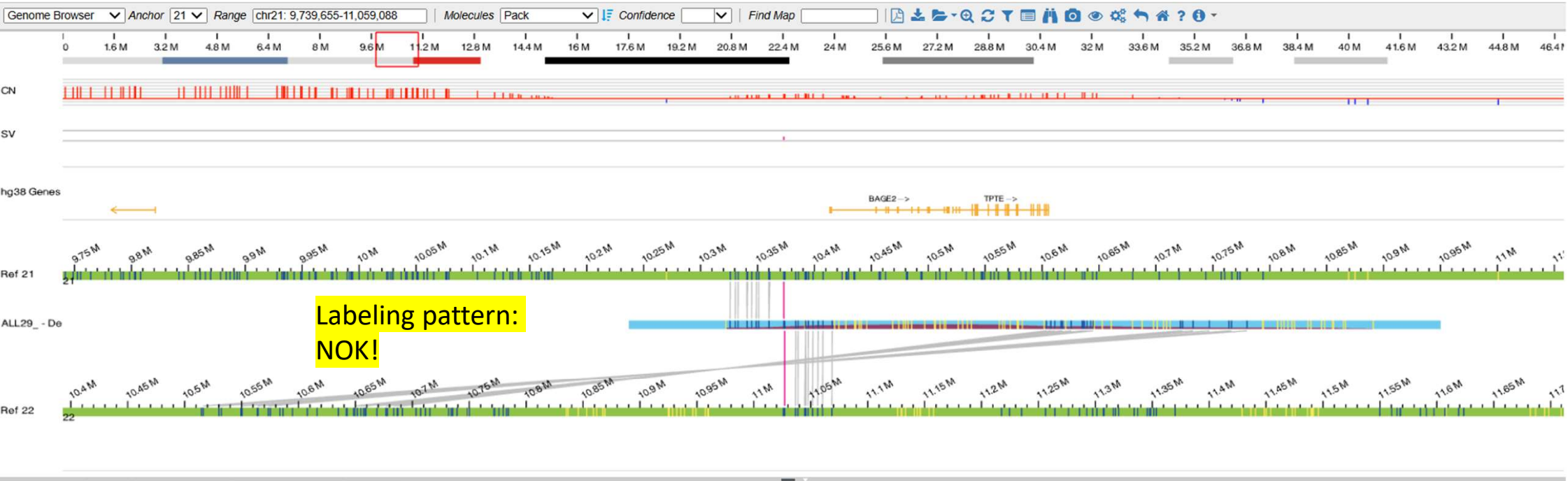

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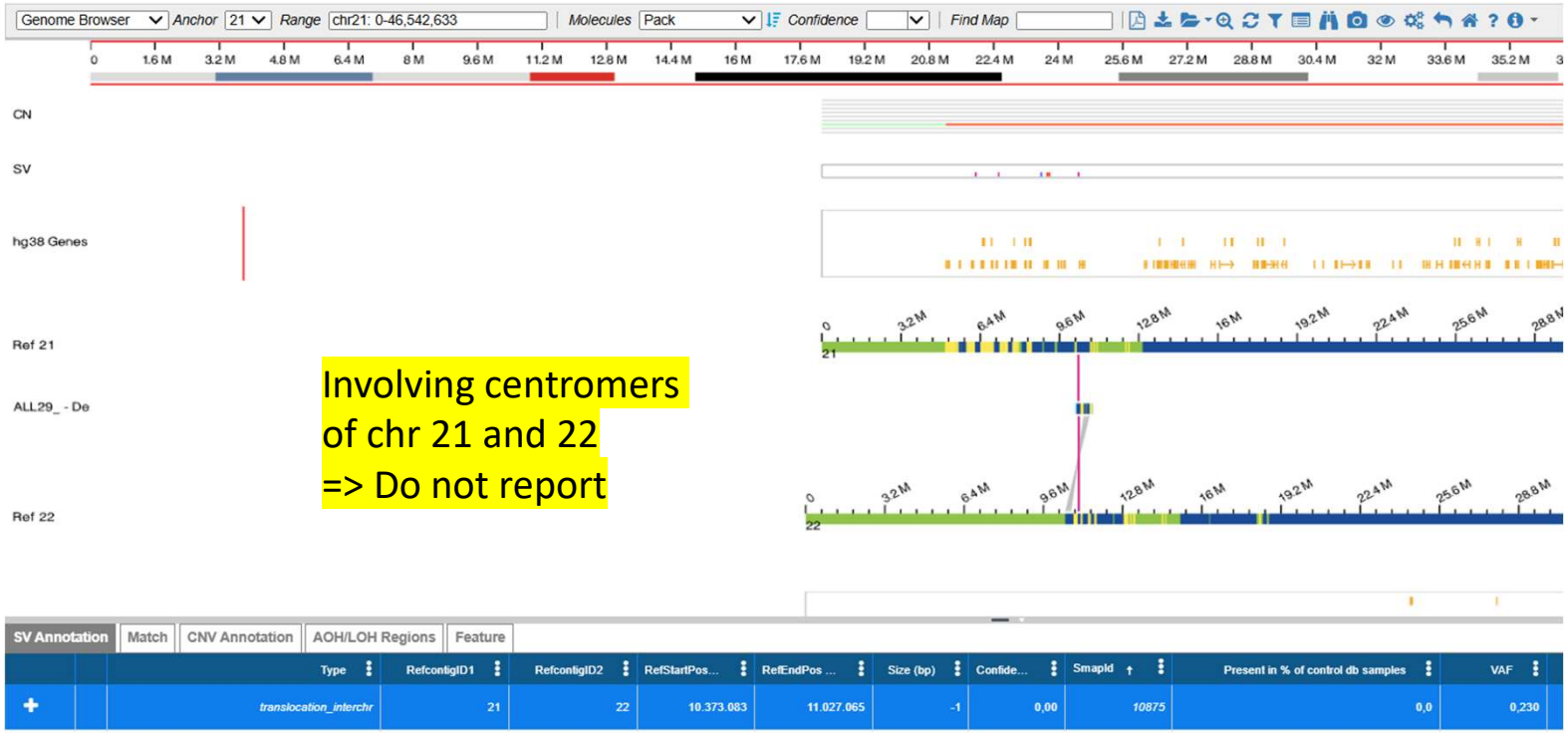

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10651	162	20	20	5509799.4	5523329.1	56819250.	56854774.	0.99	deletion	ogm[GRCh38] 20q13.31(56819250_56854774)x1	1113	1113	-1	744	745	10116	
10680	161	20	20	25459666.	25466338.	36975084.	36985758.	0.99	deletion	ogm[GRCh38] 20q11.23(36975084_36985758)x1	1114	1114	-1	4078	4080	6446	
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11063	432	21	21	11984630.	11993405.	46696723.	46709983.	0.99	deletion_nbase	ogm[GRCh38] 21q22.3(46696724_46709983)x0	1252	1252	-1	1829	1830	7220	
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Similar aberrations:
- Upper 2: same
- Last: reciprocal

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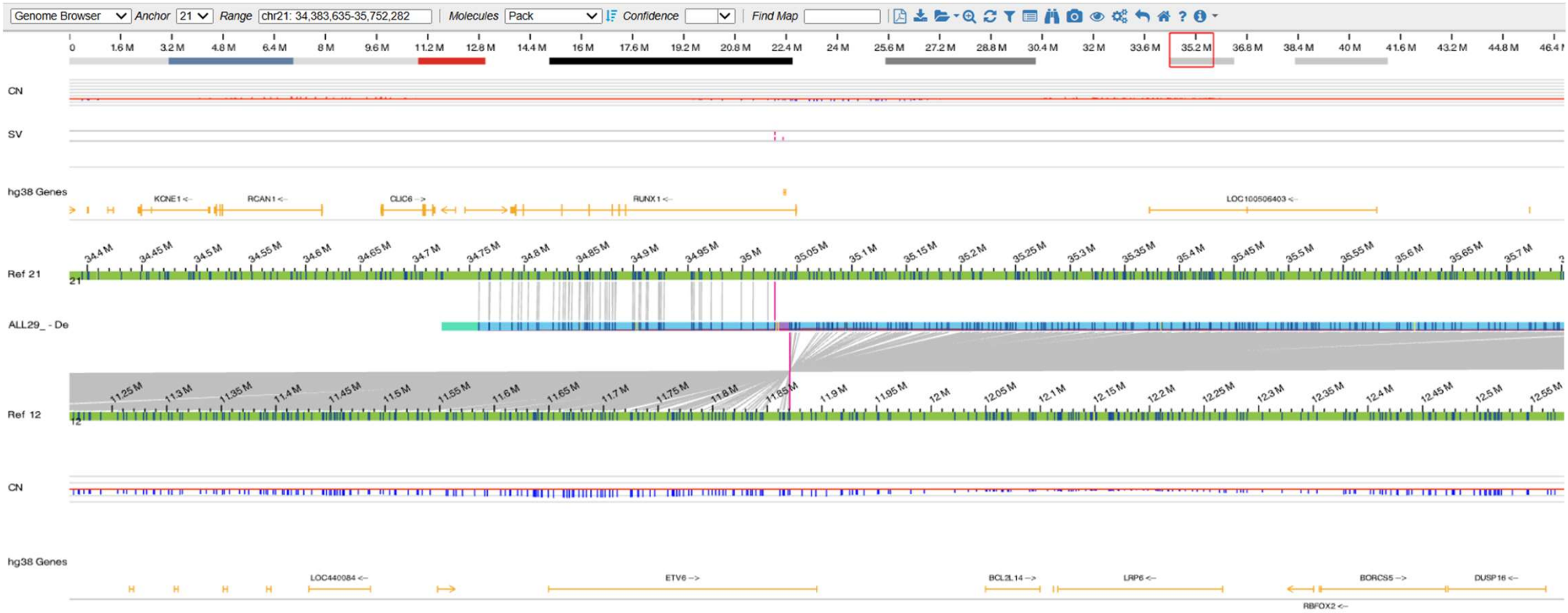

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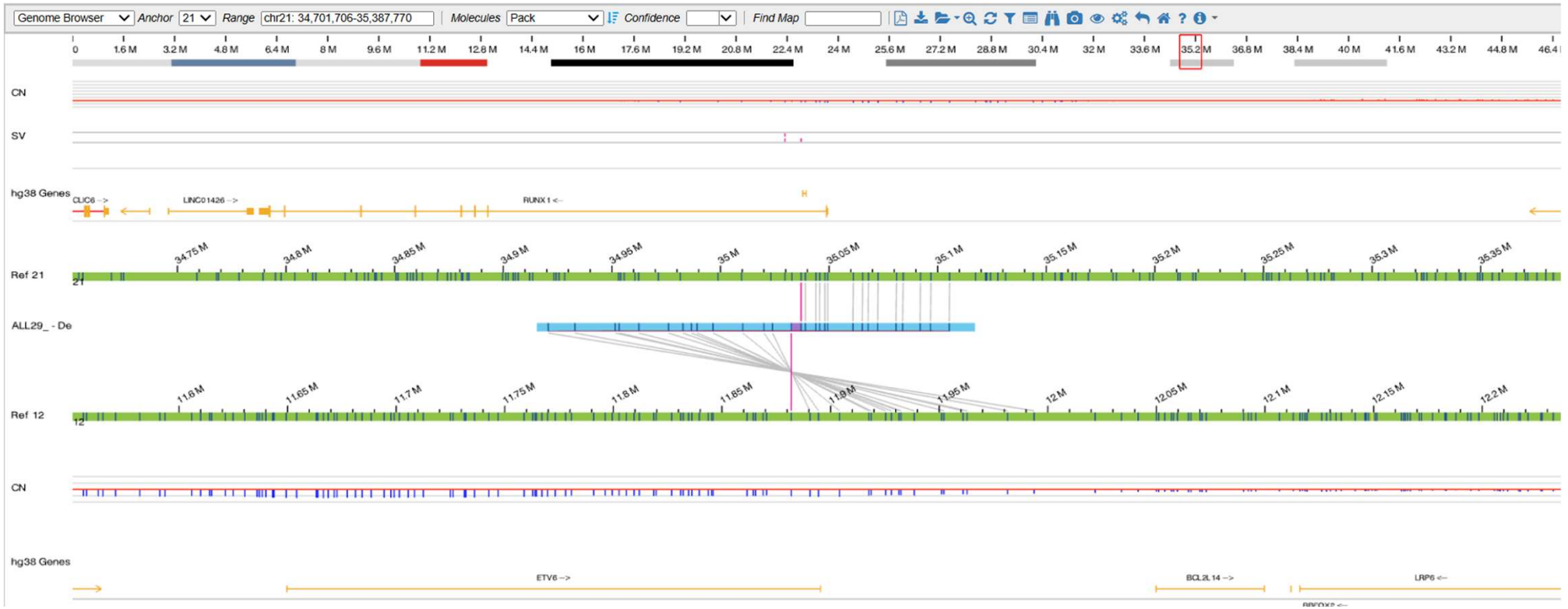

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ETV6 (TEL) and RUNX1 (AML1) breakpoints in the t(12;21) / 5 ETV6 - 3 RUNX1 fusion gene - Courtesy Hossein Mossafa.

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Report this translocation: watch out for the ISCN: order of the chromosomes!

ogm[GRCh38]
t(12;21)(p13.2;q22.12)(11870531~11881907;35029693~35037114)(ETV6::RUNX1)[VAF0.57]

Example 1: B-ALL

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Now perform analysis of SV file

- Check in Bionano Access: circos plot of chromosomes of interest

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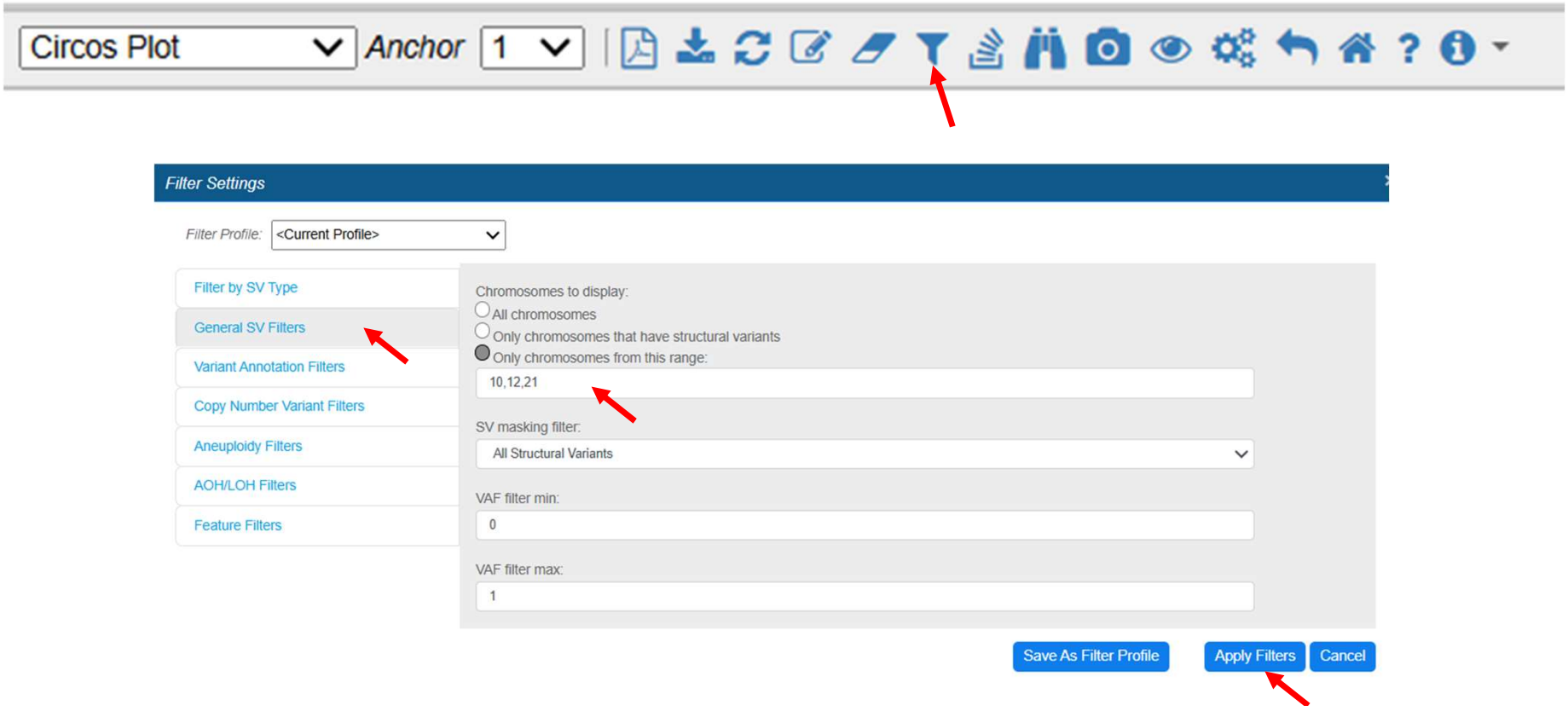
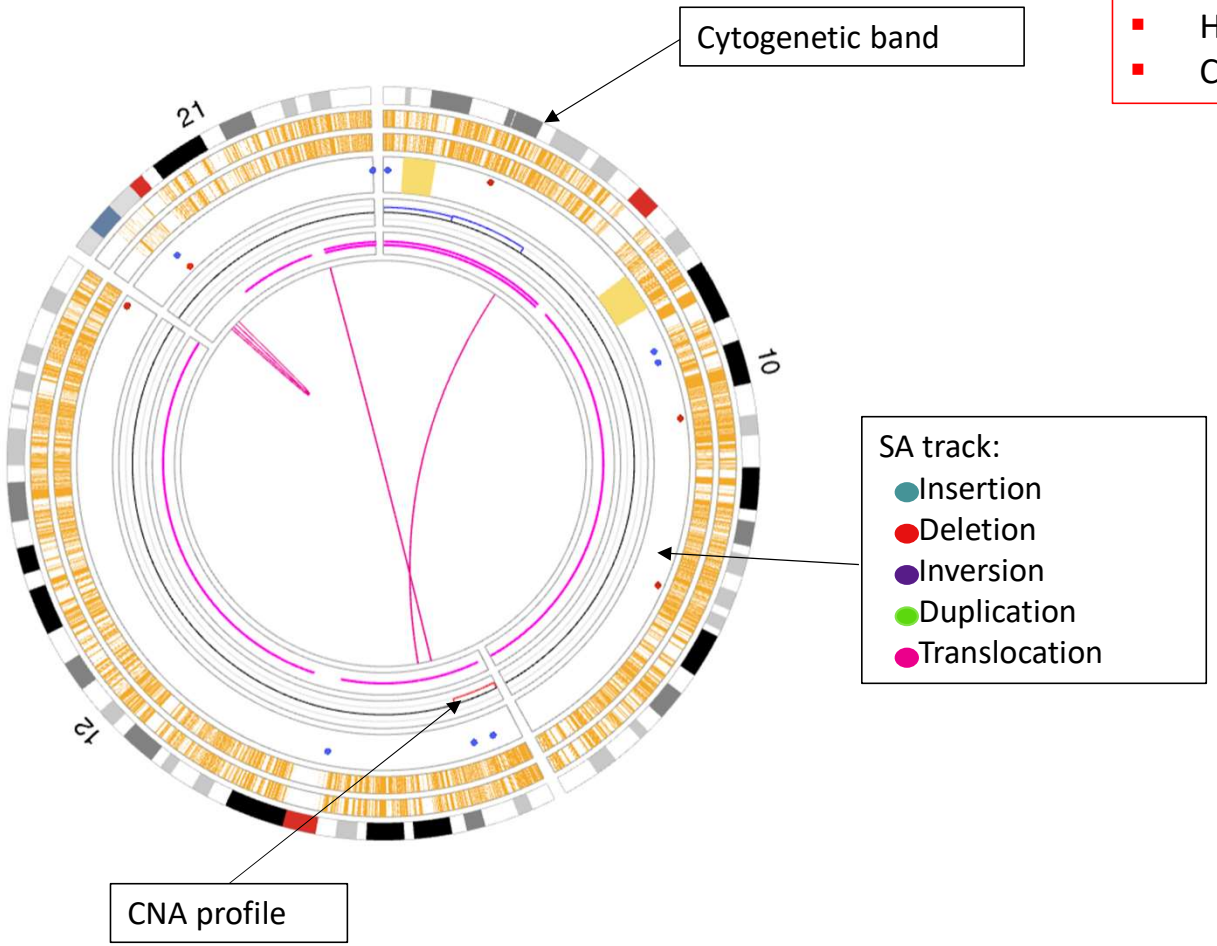


Example 1: B-ALL

Whole genome circus plot, *De Novo Assembly*



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- Conventional karyotype: normal: 46,XX[20]

From this you can appreciate:

- The t(12;21) is balanced and leads to ETV6::RUNX1
- Which often goes together with del(12)(p12) on the other chromosome
  => in this case by:
  t(10;12)(p11.23;p12.3)

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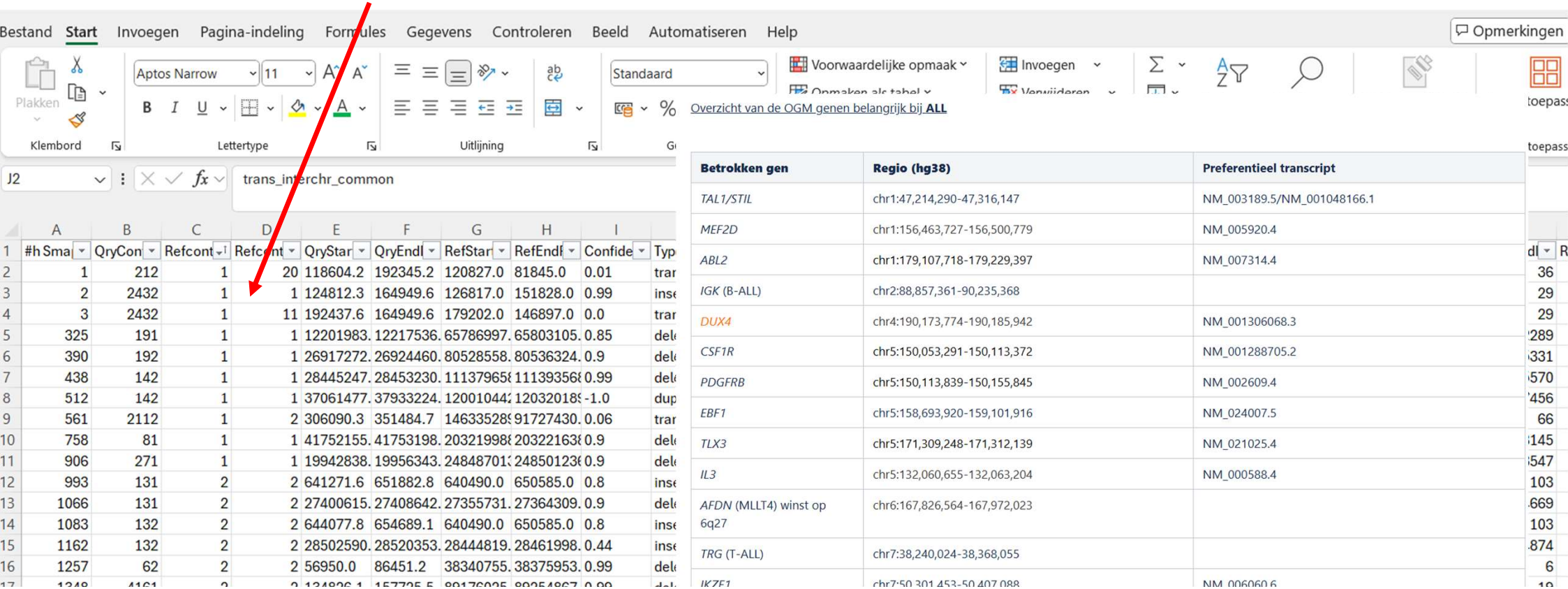
Order of SVs per chromosome from 1-22; then X and Y

So far: only checked translocations

Now: look at the deletions!!!! In Leuven: only specific gene regions are checked (gene list per pathology)



#h Sma	QryCon	Refcont	Refc	QryStar	QryEndI	RefStar	RefEndI	Confide	Typ
1	212	1	20	118604.2	192345.2	120827.0	81845.0	0.01	trar
2	2432	1	1	124812.3	164949.6	126817.0	151828.0	0.99	inse
3	2432	1	11	192437.6	164949.6	179202.0	146897.0	0.0	trar
325	191	1	1	12201983.	12217536.	65786997.	65803105.	0.85	del
390	192	1	1	26917272.	26924460.	80528558.	80536324.	0.9	del
438	142	1	1	28445247.	28453230.	11137965	11139356	0.99	del
512	142	1	1	37061477.	37933224.	12001044	12032018	-1.0	dup
561	2112	1	2	306090.3	351484.7	14633528	91727430.	0.06	trar
758	81	1	1	41752155.	41753198.	20321998	20322163	0.9	del
906	271	1	1	19942838.	19956343.	24848701	24850123	0.9	del
993	131	2	2	641271.6	651882.8	640490.0	650585.0	0.8	inse
1066	131	2	2	27400615.	27408642.	27355731.	27364309.	0.9	del
1083	132	2	2	644077.8	654689.1	640490.0	650585.0	0.8	inse
1162	132	2	2	28502590.	28520353.	28444819.	28461998.	0.44	inse
1257	62	2	2	56950.0	86451.2	38340755.	38375953.	0.99	del

Betrokken gen	Regio (hg38)	Preferentieel transcript
TAL1/STIL	chr1:47,214,290-47,316,147	NM_003189.5/NM_001048166.1
MEF2D	chr1:156,463,727-156,500,779	NM_005920.4
ABL2	chr1:179,107,718-179,229,397	NM_007314.4
IGK (B-ALL)	chr2:88,857,361-90,235,368	
DUX4	chr4:190,173,774-190,185,942	NM_001306068.3
CSF1R	chr5:150,053,291-150,113,372	NM_001288705.2
PDGFRB	chr5:150,113,839-150,155,845	NM_002609.4
EBF1	chr5:158,693,920-159,101,916	NM_024007.5
TLX3	chr5:171,309,248-171,312,139	NM_021025.4
IL3	chr5:132,060,655-132,063,204	NM_000588.4
AFDN (MLLT4) winst op 6q27	chr6:167,826,564-167,972,023	
TRG (T-ALL)	chr7:38,240,024-38,368,055	
IKZF1	chr7:50,301,453-50,407,088	NM_006060.6

Example 1: B-ALL

Work in Excel: download data

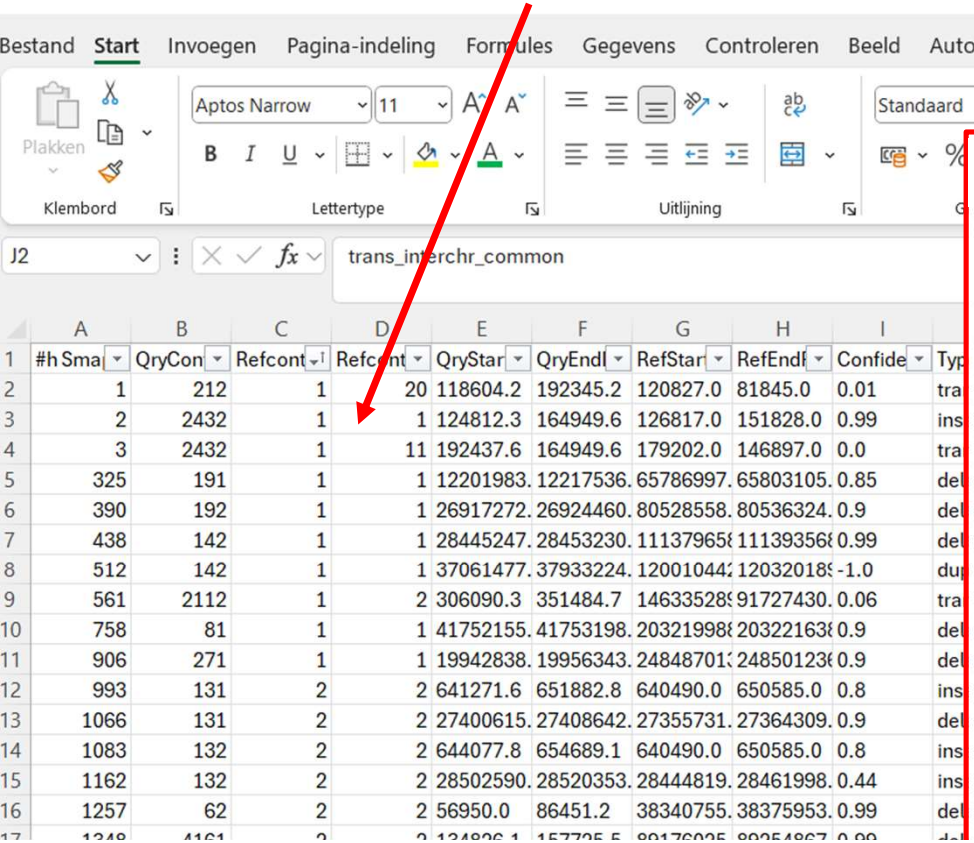
Now perform analysis of SV file

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

Order of SVs per chromosome from 1-22; then X and Y

So far: only checked translocations

Now: look at the deletions!!!! In Leuven: only specific gene regions are checked (gene list per pathology)



	A	B	C	D	E	F	G	H	I	
1	#h Sma	QryCon	Refcont	Refcont	QryStar	QryEndI	RefStar	RefEndI	Confide	Typ
2	1	212	1	20	118604.2	192345.2	120827.0	81845.0	0.01	tra
3	2	2432	1	1	124812.3	164949.6	126817.0	151828.0	0.99	ins
4	3	2432	1	11	192437.6	164949.6	179202.0	146897.0	0.0	tra
5	325	191	1	1	12201983.	12217536.	65786997.	65803105.	0.85	del
6	390	192	1	1	26917272.	26924460.	80528558.	80536324.	0.9	del
7	438	142	1	1	28445247.	28453230.	11137965	11139356	0.99	del
8	512	142	1	1	37061477.	37933224.	12001044	12032018	-1.0	dup
9	561	2112	1	2	306090.3	351484.7	14633528	91727430.	0.06	tra
10	758	81	1	1	41752155.	41753198.	20321998	20322163	0.9	del
11	906	271	1	1	19942838.	19956343.	24848701	24850123	0.9	del
12	993	131	2	2	641271.6	651882.8	640490.0	650585.0	0.8	ins
13	1066	131	2	2	27400615.	27408642.	27355731.	27364309.	0.9	del
14	1083	132	2	2	644077.8	654689.1	640490.0	650585.0	0.8	ins
15	1162	132	2	2	28502590.	28520353.	28444819.	28461998.	0.44	ins
16	1257	62	2	2	56950.0	86451.2	38340755.	38375953.	0.99	del

UZ Leuven: OGM list with important genes/regions for ALL cases

Betrokken gen	Regio (hg38)	Preferentieel transcript
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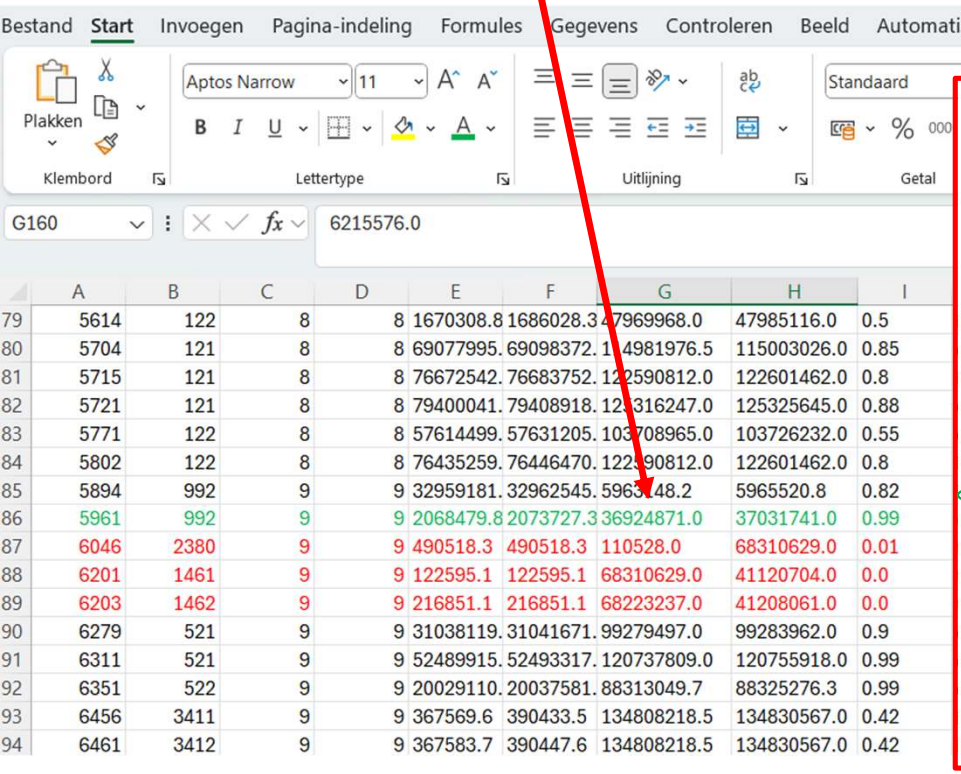
Now: look at the deletions!!!! In Leuven: only specific gene regions are checked (gene list per pathology)



	A	B	C	D	E	F	G	H	I
79	5614	122	8	8	1670308.8	1686028.3	47969968.0	47985116.0	0.5
80	5704	121	8	8	69077995.	69098372.	114981976.5	115003026.0	0.85
81	5715	121	8	8	76672542.	76683752.	122590812.0	122601462.0	0.8
82	5721	121	8	8	79400041.	79408918.	125316247.0	125325645.0	0.88
83	5771	122	8	8	57614499.	57631205.	103708965.0	103726232.0	0.55
84	5802	122	8	8	76435259.	76446470.	122590812.0	122601462.0	0.8
85	5894	992	9	9	32959181.	32962545.	5963148.2	5965520.8	0.82
86	5961	992	9	9	2068479.8	2073727.3	36924871.0	37031741.0	0.99
87	6046	2380	9	9	490518.3	490518.3	110528.0	68310629.0	0.01
88	6201	1461	9	9	122595.1	122595.1	68310629.0	41120704.0	0.0
89	6203	1462	9	9	216851.1	216851.1	68223237.0	41208061.0	0.0
90	6279	521	9	9	31038119.	31041671.	99279497.0	99283962.0	0.9
91	6311	521	9	9	52489915.	52493317.	120737809.0	120755918.0	0.99
92	6351	522	9	9	20029110.	20037581.	88313049.7	88325276.3	0.99
93	6456	3411	9	9	367569.6	390433.5	134808218.5	134830567.0	0.42
94	6461	3412	9	9	367583.7	390447.6	134808218.5	134830567.0	0.42

UZ Leuven: OGM list with important genes/regions for ALL cases

TRG (T-ALL)	chr7:38,240,024-38,368,055	
IKZF1	chr7:50,301,453-50,407,088	NM_006060.6
TRB (T-ALL)	chr7:142,299,011-142,813,287	
MYC	chr8:127,736,231-127,742,951	NM_002467.6
CDKN2A	chr9:21,965,752-21,997,324	NM_000077.5
CDKN2B	chr9:22,000,903-22,011,313	NM_004936.4
PAX5	chr9:36,831,269-37,036,268	NM_016734.3
ABL1	chr9:130,713,016-130,887,670	NM_005157.6/NM_007313.2
NUP214	chr9:131,125,586-131,234,663	NM_005085.4
PICALM	chr11:85,957,175-86,069,084	NM_007166.4
KMT2A	chr11:118,434,492-118,528,832	NM_001197104.2
ETV6	chr12:11,647,601-11,897,386	NM_001987.5

Example 1: B-ALL

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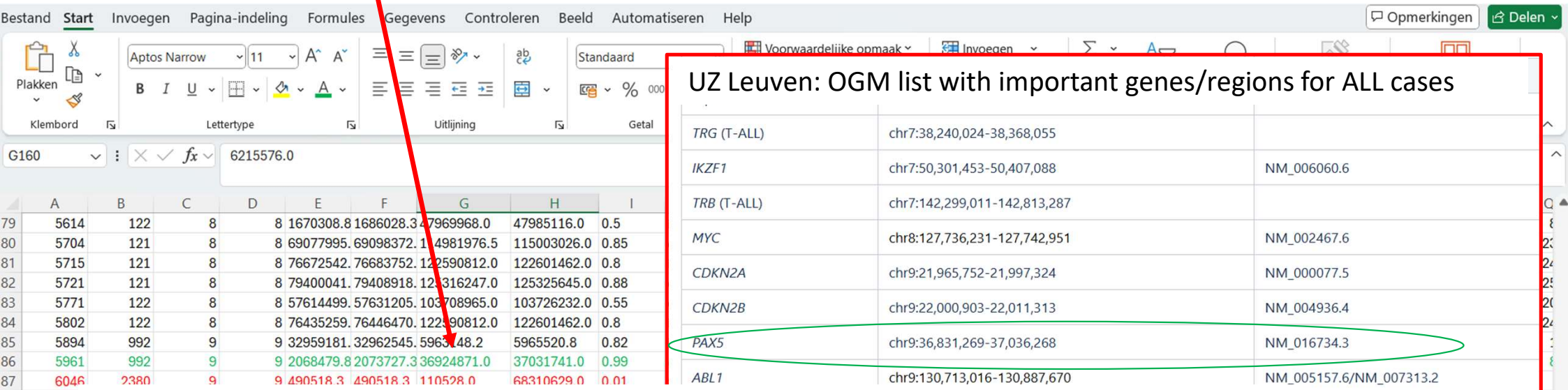
Now: look at the deletions!!!! In Leuven: only specific gene regions are checked (gene list per pathology)



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TRG (T-ALL)	chr7:38,240,024-38,368,055	
IKZF1	chr7:50,301,453-50,407,088	NM_006060.6
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PAX5	chr9:36,831,269-37,036,268	NM_016734.3
ABL1	chr9:130,713,016-130,887,670	NM_005157.6/NM_007313.2
		05085.4
		07166.4
		01197104.2
		01987.5

Report this deletion: check in IGV: use Mane transcript for *PAX5*: NM_016734.3

ogm[GRCh38]

9p13.2(36924871_37031741)x1[0.42], => loss of exon 2-6 of *PAX5*

Example 1: B-ALL

Work in Excel: download data

Now perform analysis of SV file

Order of SVs per chromosome from 1-22; then X and Y

So far: only checked translocations

Now: lo

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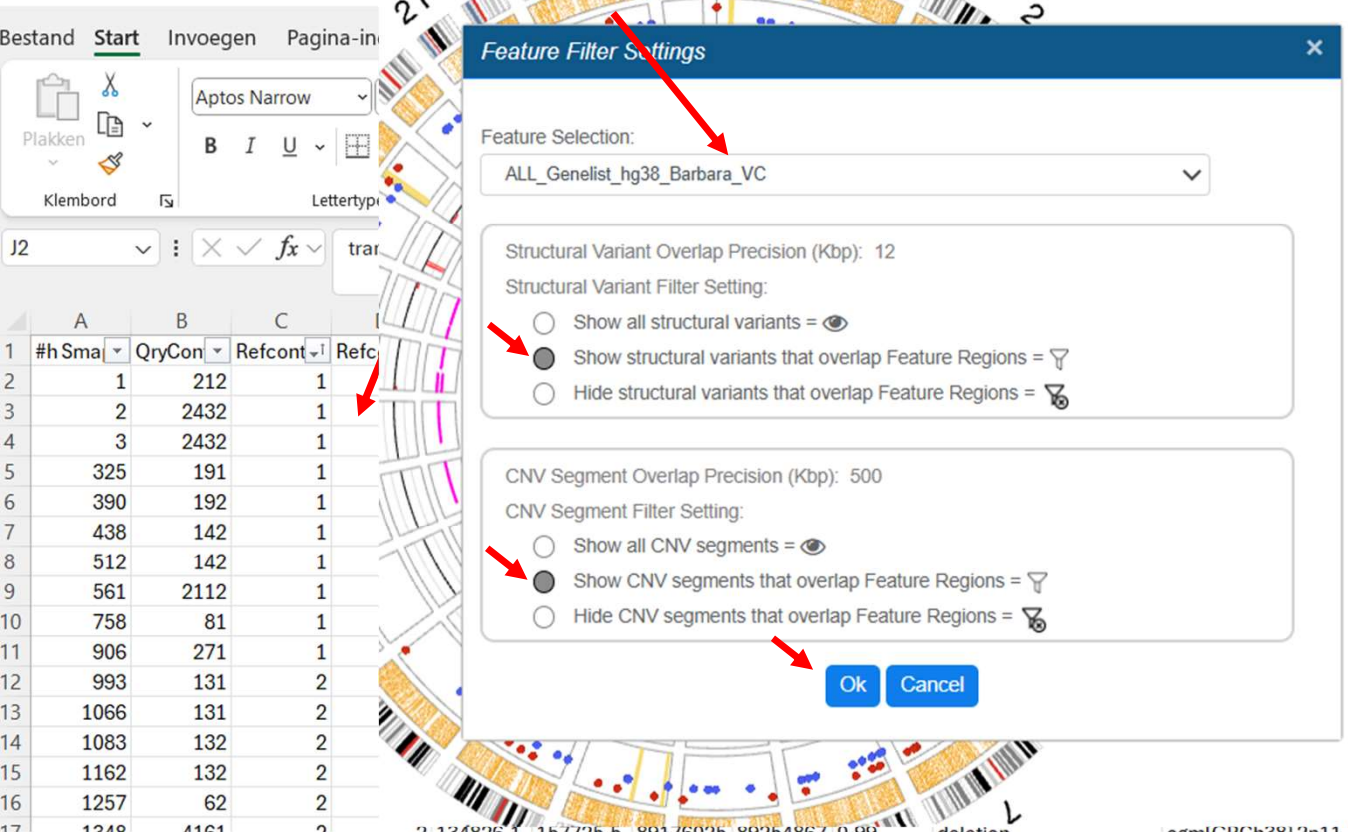

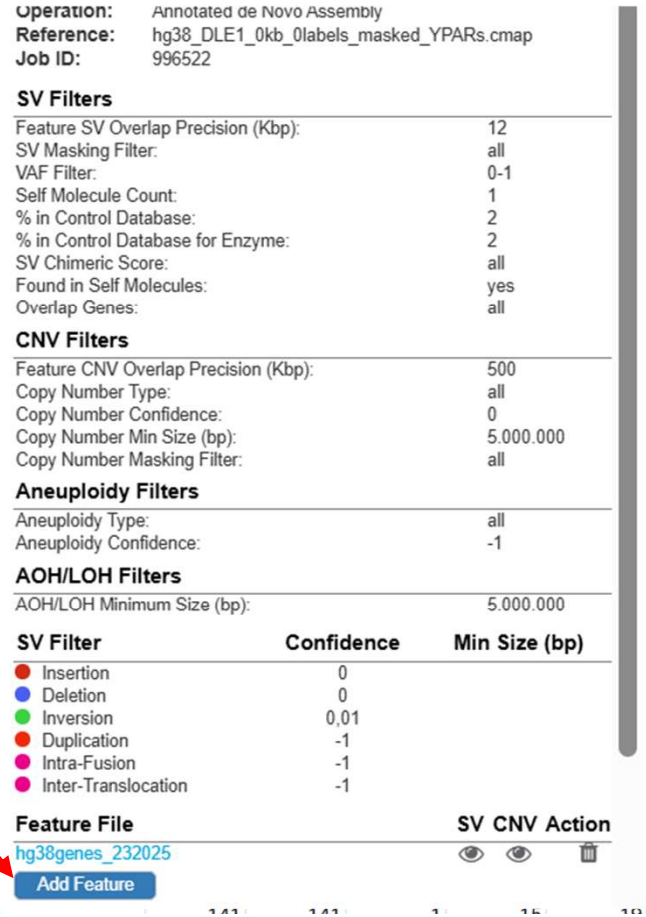

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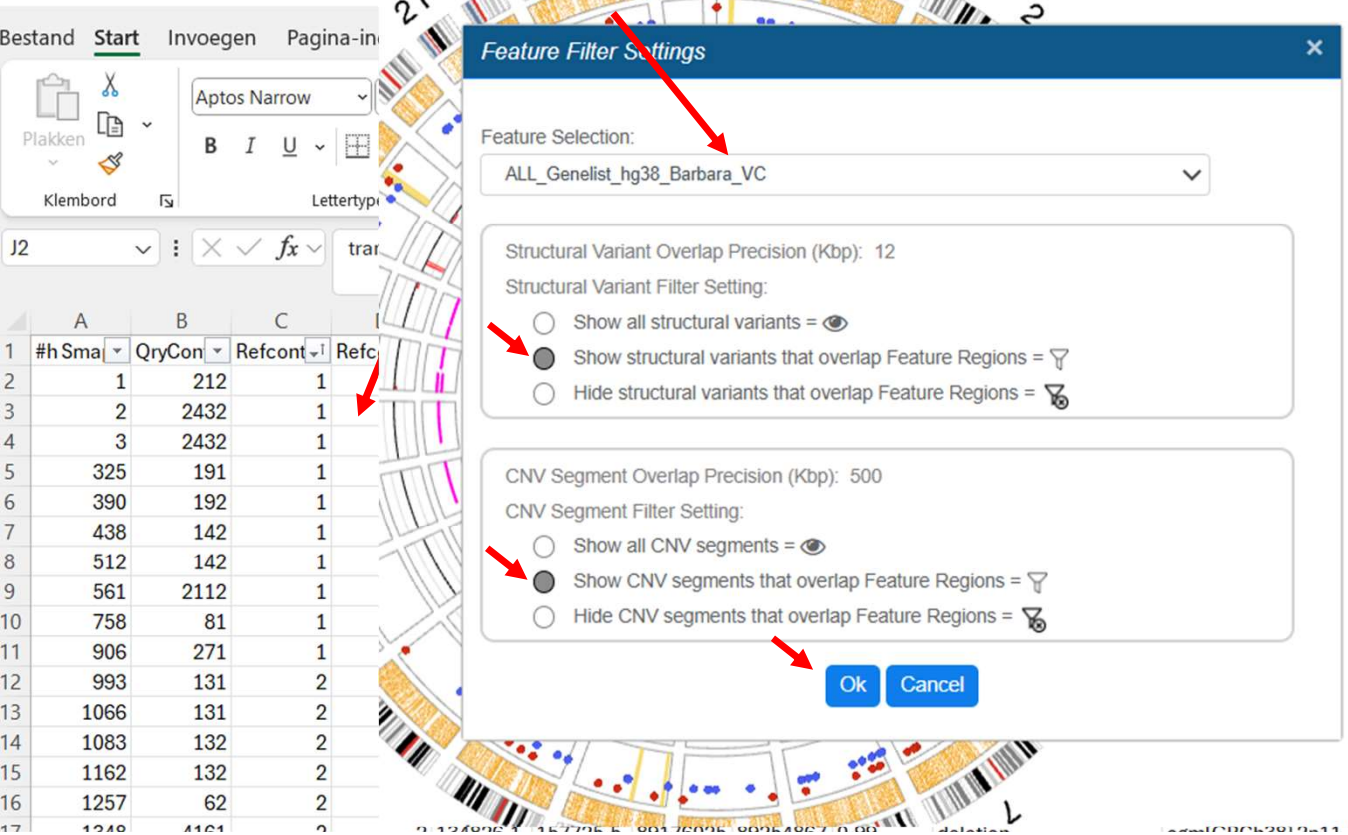
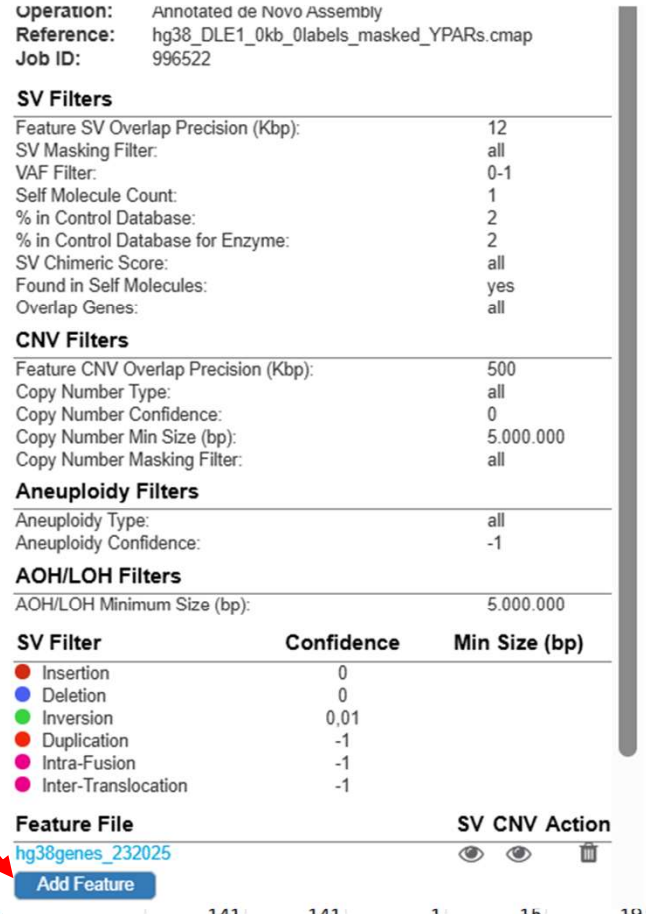
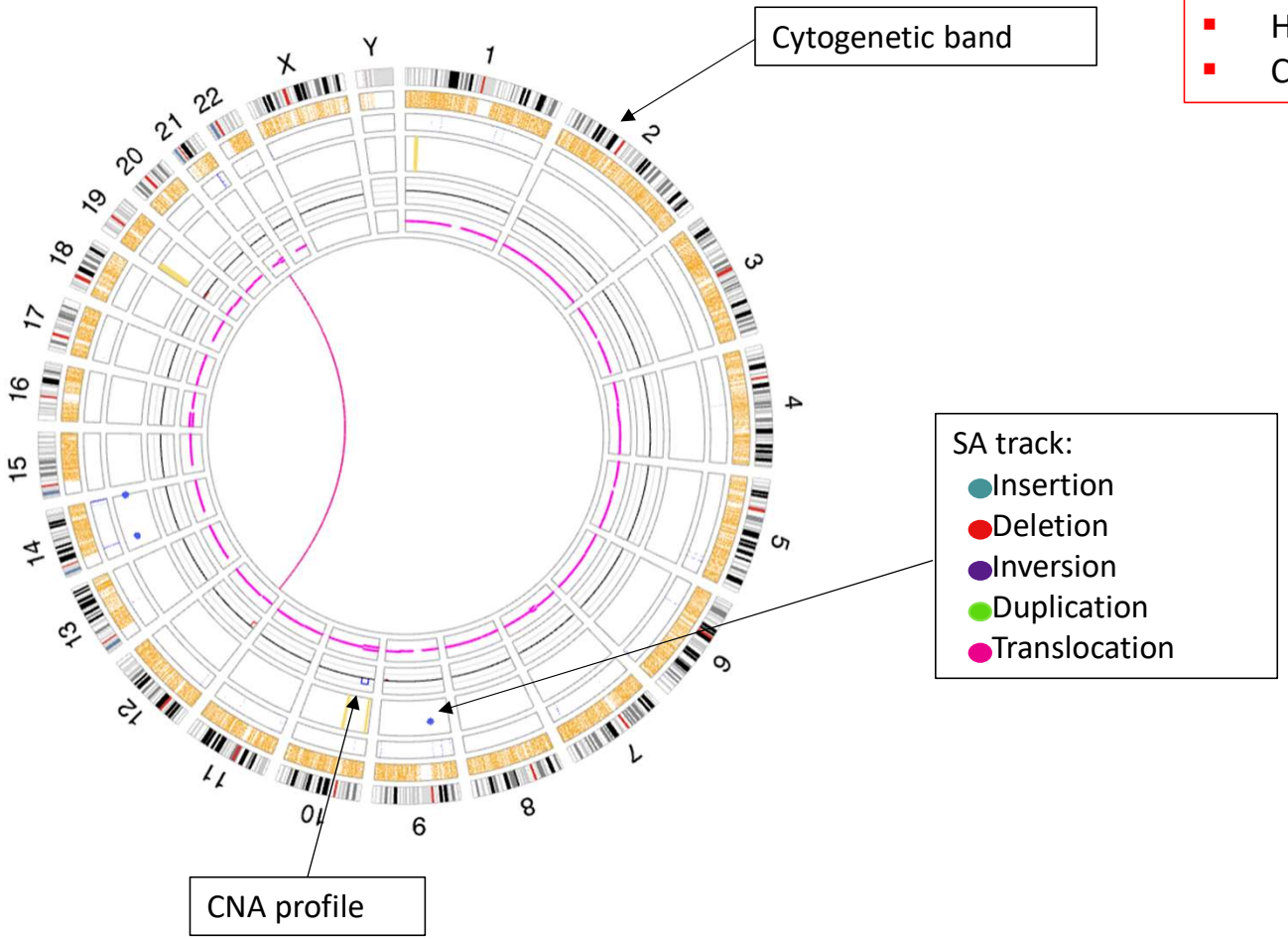




Example 1: B-ALL

Whole genome circus plot, *De Novo Assembly*



- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

After filtering using the bed file:

Reduced number of aberrations visible

(be careful: also the t(10;12) has been filtered out)

Example 1: B-ALL

Whole genome circus plot, *De Novo Assembly*

- Female, 2 years old
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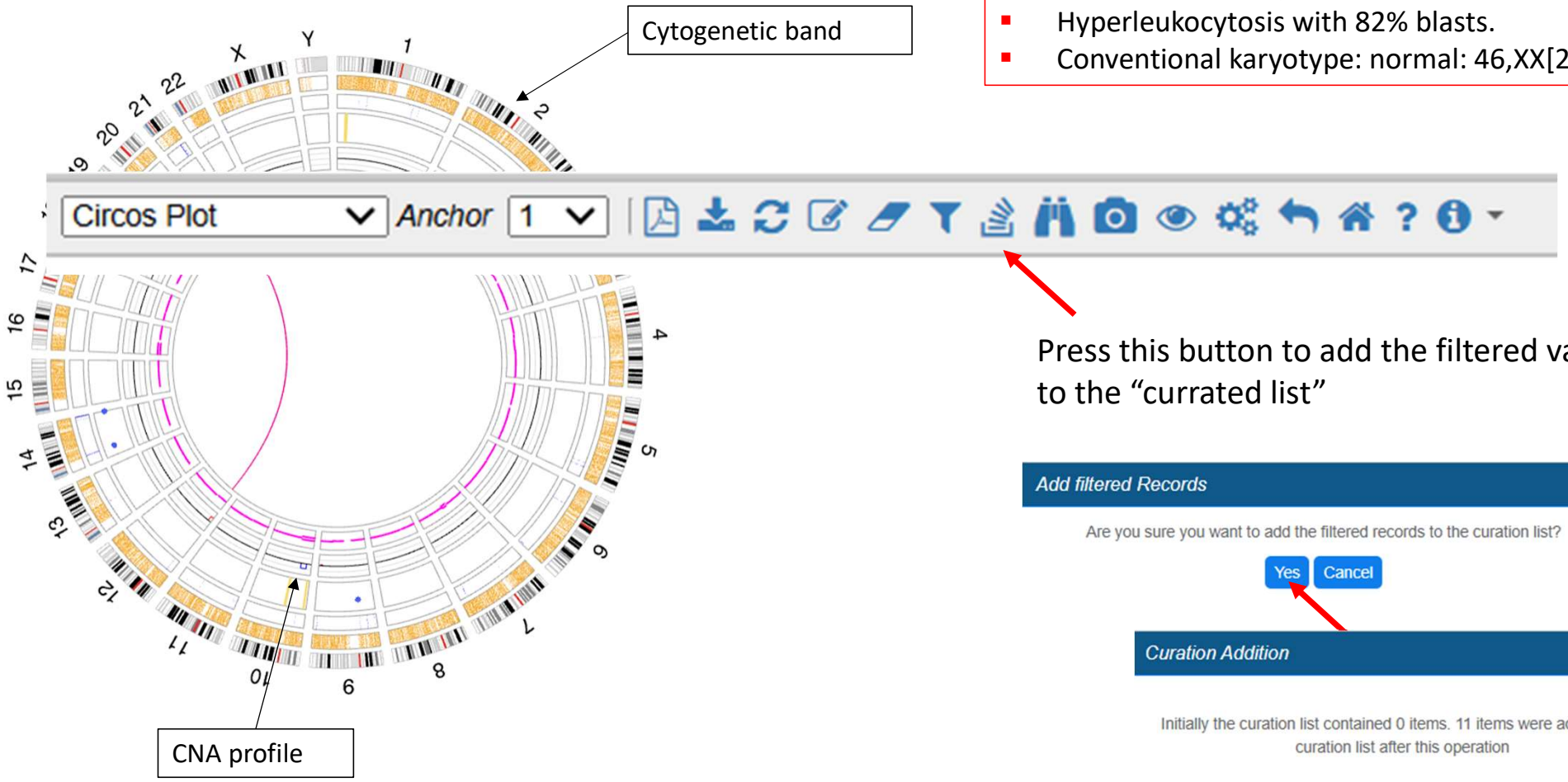
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]



Press this button to add the filtered variants to the “currated list”

Example 1: B-ALL

De Novo Assembly

Work in Bionano Access: Variant classifier

Now perform analysis of SVs

- Female, 2 years old
- Anemia, thrombopenia
- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

Curated Variant List

Reconcile records | Review complete | Remove records | Remove all records

classification	Notes	Type	SVID	RefStartPos (...	RefEndPos (bp)	Size (bp) (bp)	Present in % of ...	overlap Genes	Putative Gene Fusion	ISCN	Chr	Det...
Unclassified		loss	74	127.748.196	133.094.807	5.346.612	0	SH2D3C;MIR3960;MIR2861;CDK9;FPGS;...		ogm[GRCh38] 9q3...	9	
Unclassified		gain	79	13.883.191	29.311.476	15.428.286	0	FRMD4A;LOC101928453;MIR4293;MIR12...		ogm[GRCh38] 10p...	10	
Unclassified		loss	96	230.737	9.308.760	9.078.024	0	SLC6A13;LOC102723544;KDM5A;CCDC7...		ogm[GRCh38] 12p...	12	
Unclassified		loss	139	2.068.666	19.097.020	17.028.355	0	MOB3A;IZUMO4;AP3D1;DOT1L;PLEKHJ1;...		ogm[GRCh38] 19p...	19	
Unclassified		deletion	5961	36.924.871	37.031.741	101.622	0	PAX5	-	ogm[GRCh38] 9p1...	9	
Unclassified		deletion	8503	21.894.406	22.523.108	620.605	1	TRD-AS1	-	ogm[GRCh38] 14q...	14	
Unclassified		deletion	8510	22.422.983	22.451.049	26.122	0	TRD-AS1	-	ogm[GRCh38] 14q...	14	
Unclassified		deletion	8777	106.534.885	106.586.104	40.965	0	-	-	ogm[GRCh38] 14q...	14	
Unclassified		translocation_interchr	10981	11.870.531	35.029.693	-1	0	RUNX1;ETV6	RUNX1-ETV6	ogm[GRCh38] t(21...	12;21	
Unclassified		translocation_interchr	10983	11.870.531	35.029.693	-1	0	RUNX1;ETV6	RUNX1-ETV6	ogm[GRCh38] t(21...	12;21	
Unclassified		translocation_interchr	11064	11.881.907	35.037.114	-1	0	RUNX1;ETV6	RUNX1-ETV6	ogm[GRCh38] t(21...	12;21	

Example 1: B-ALL

De Novo Assembly

Work in Bionano Access: Variant classifier

Now perform analysis of SVs

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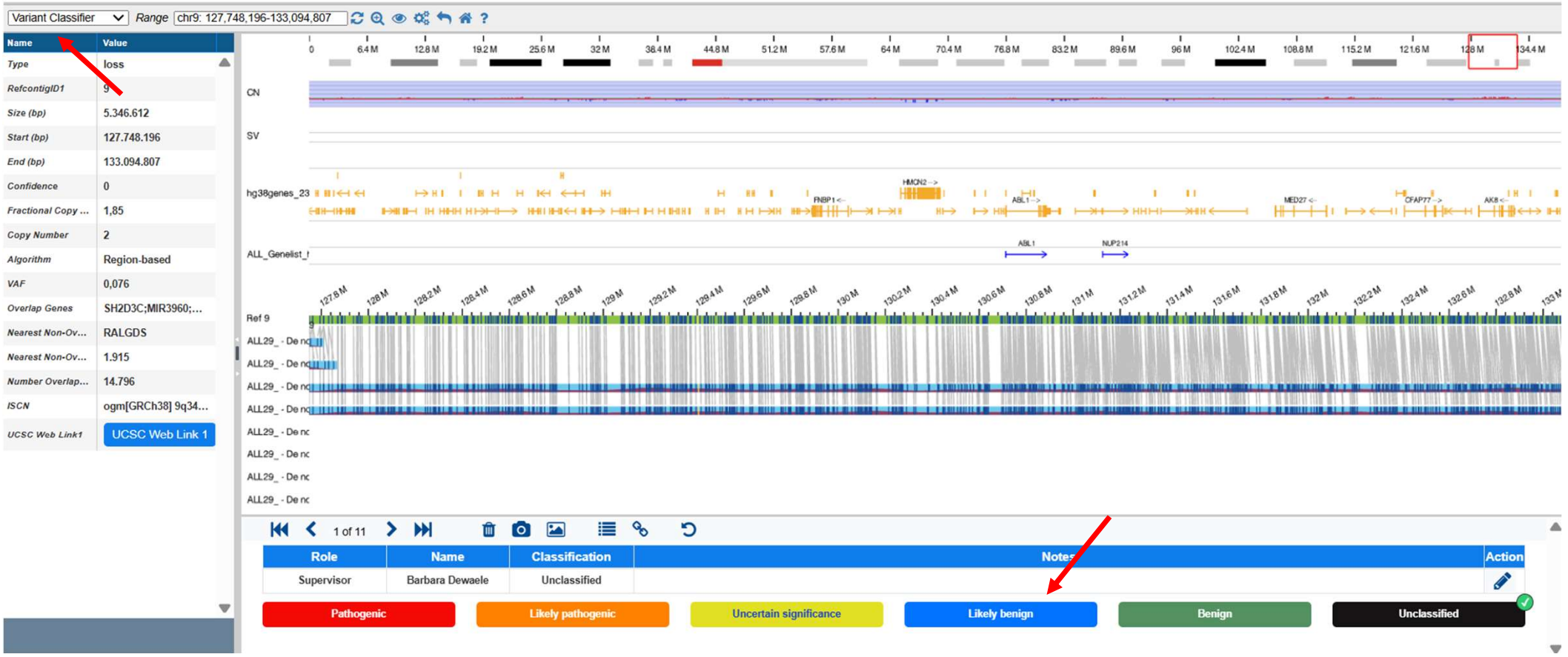

Example 1: B-ALL

De Novo Assembly

Work in Bionano Access: Curated variant list: after variant classifier:

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- Conventional karyotype: normal: 46,XX[20]

Curated Variant List

Reconcile records | Review complete | Remove records | Remove all records

classification	Notes	Type	Chr	RefStartPos (...	RefEndPos (bp)	Size (bp) (bp)	ISCN	SVID	overlap Genes	Putative Gene Fusion	Present in % of ...	Det...
Likely benign		loss	9	127.748.196	133.094.807	5.346.612	ogm[GRCh38] 9q3...	74	SH2D3C;MIR3960;MIR2861;CDK9;FPGS;...		0	
Pathogenic		gain	10	13.883.191	29.311.476	15.428.286	ogm[GRCh38] 10p...	79	FRMD4A;LOC101928453;MIR4293;MIR12...		0	
Pathogenic		loss	12	2[illegible]737	9.308.760	9.078.024	ogm[GRCh38] 12p...	96	SLC6A13;LOC102723544;KDM5A;CCDC7...		0	
Likely benign		loss	19	2[illegible]068.666	19.097.020	17.028.355	ogm[GRCh38] 19p...	139	MOB3A;IZUMO4;AP3D1;DOT1L;PLEKHJ1;...		0	
Pathogenic		deletion	9	36.924.871	37.031.741	101.622	ogm[GRCh38] 9p1...	5961	PAX5	-	0	
Likely benign		deletion	14	21.894.406	22.523.108	620.605	ogm[GRCh38] 14q...	8503	TRD-AS1	-	1	
Likely benign		deletion	14	22.422.983	22.451.049	26.122	ogm[GRCh38] 14q...	8510	TRD-AS1	-	0	
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Pathogenic		translocation_interchr	12;21	11.870.531	35.029.693	-1	ogm[GRCh38] t(21...	10981	RUNX1;ETV6	RUNX1-ETV6	0	
Pathogenic		translocation_interchr	12;21	11.870.531	35.029.693	-1	ogm[GRCh38] t(21...	10983	RUNX1;ETV6	RUNX1-ETV6	0	
Pathogenic		translocation_interchr	12;21	11.881.907	35.037.114	-1	ogm[GRCh38] t(21...	11064	RUNX1;ETV6	RUNX1-ETV6	0	

ALWAYS perform a visual check of Xp22.33 and Yp11.32 to exclude the *P2RY8::CRLF2* fusion

Only makes sense in De Novo pipeline:

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- Hyperleukocytosis with 82% blasts.
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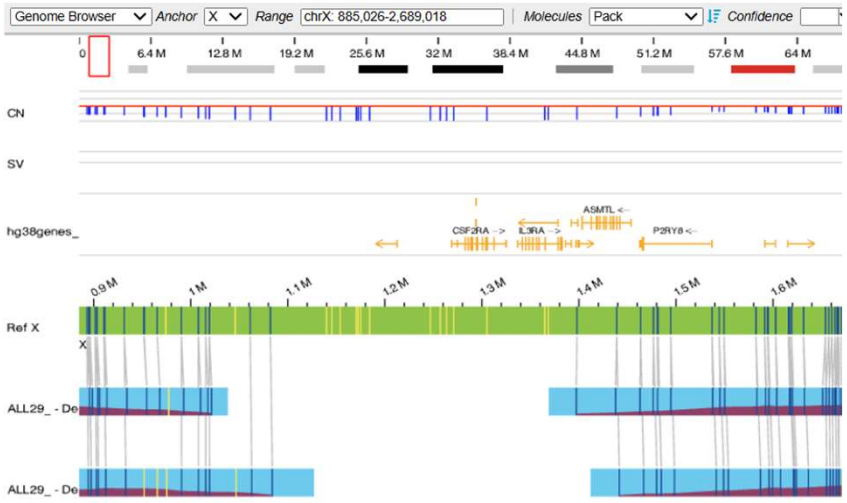
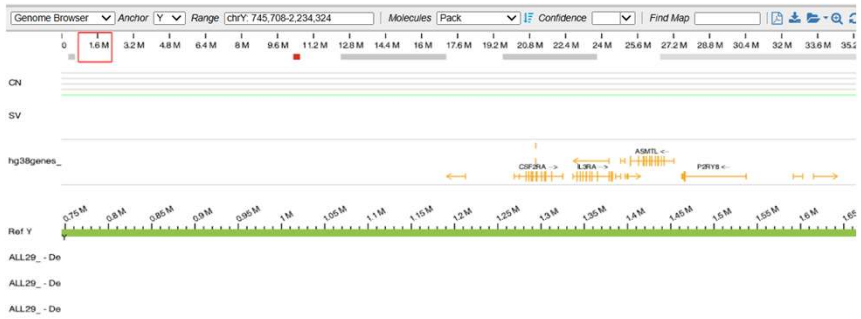


Xp22.33: normal pattern

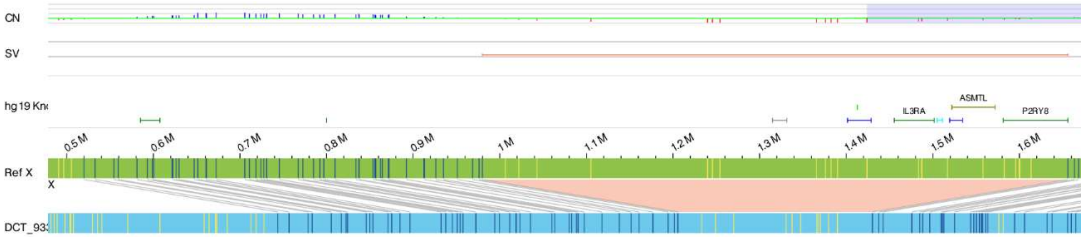


Yp11.32: no molecules (female)

ALWAYS perform a visual check of Xp22.33 and Yp11.32 to exclude the *P2RY8::CRLF2* fusion

Example of a positive case:
(not always detected by pipelines, Guided Assembly very promising!!!)

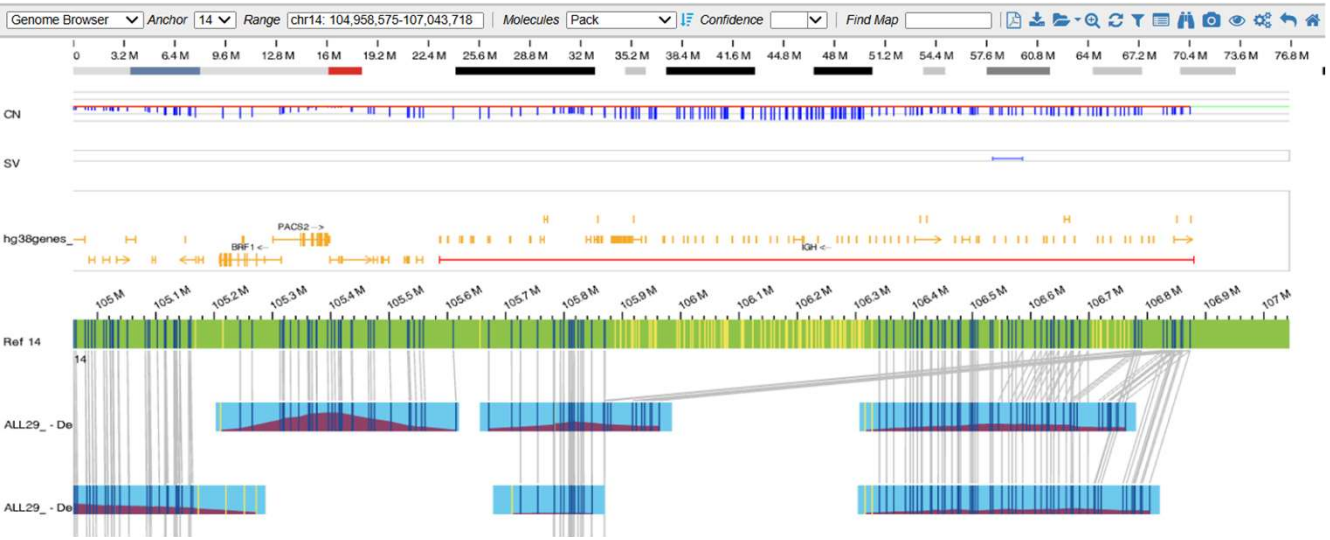
Xp22.33: *P2RY8::CRLF2*



Yp11.32: *P2RY8::CRLF2*

ALWAYS perform a visual check of 14q32 to exclude fusion involving IGH

Only makes sense in De Novo pipeline:



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- Hyperleukocytosis with 82% blasts.
- Conventional karyotype: normal: 46,XX[20]

14q32.33: normal pattern

Just fysiological intrachr variants

Example 1: B-ALL

Work in Excel: download data

- Female, 2 years old
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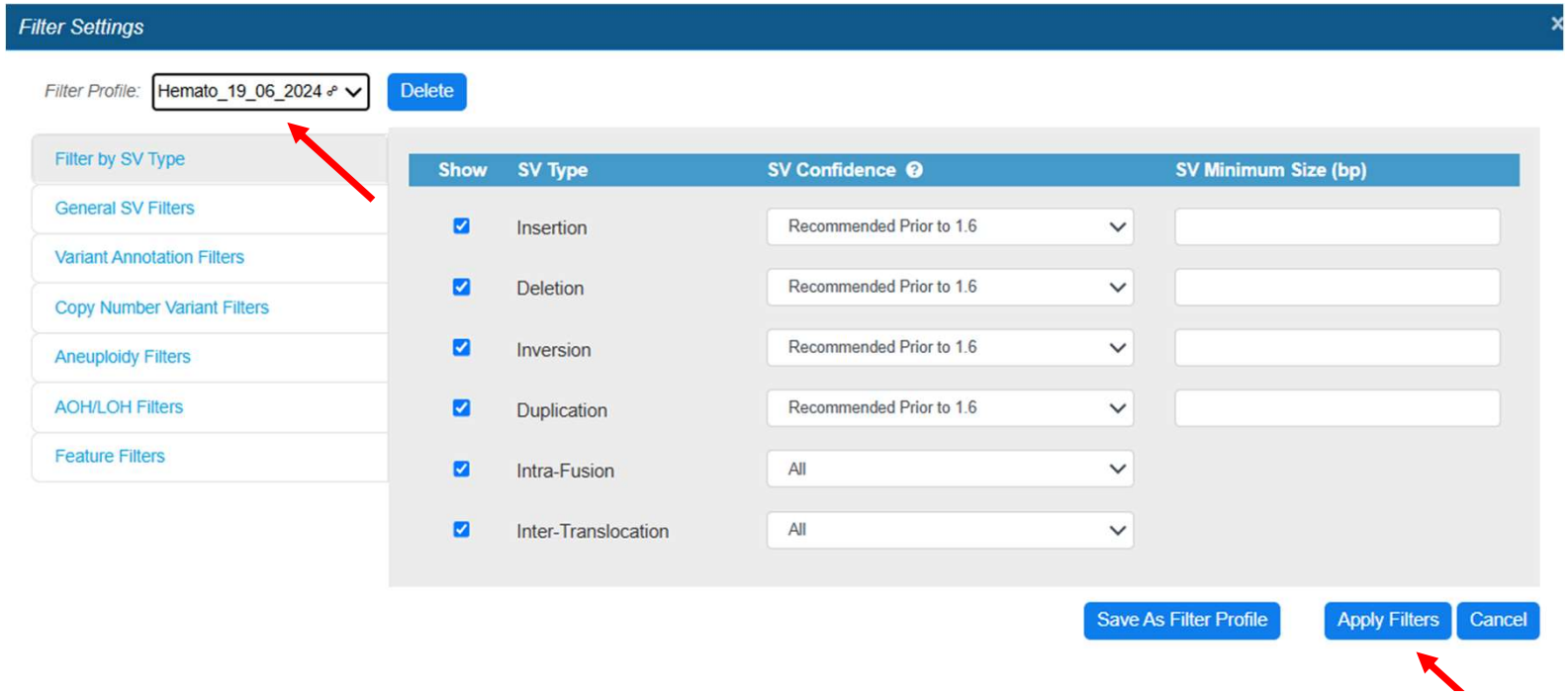
- Conventional karyotype: normal: 46,XX[20]

Repeat the entire process for the RVA analysis (except for check for *P2RY8::CRLF2)*

(check CNV and SV files)



Example 1: B-ALL

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- Conventional karyotype: normal: 46,XX[20]

Final result:

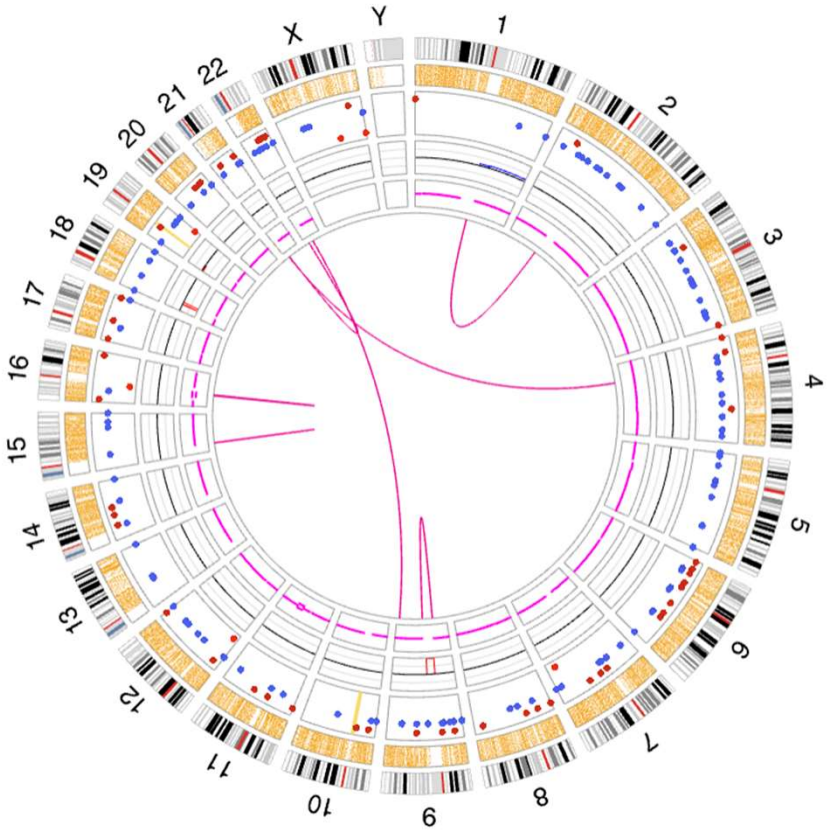
ogm[GRCh38]
9p13.2(36924871_37031741)x1[0.42], => loss of exon 2-6 of *PAX5*
10p15.3p11.23 (18514_29311476)x2~3[0.44],
t(10;12)(p11.23;p12.3)(29628820;15233701)[VAF0.35],
12p13.33p12.3(230737_15233701)(ETV6)x1[0.42], => *loss of ETV6*
t(12;21)(p13.2;q22.12)(11870531~11881907;35029693~35037114)(ETV6::RUNX1)[VAF0.57]

WHO:
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1* fusion
Associated with a very favourable prognosis

Example 2: precursor B-ALL

QA parameters: optimal

De Novo Assembly: circos plot
Work in Excel: download data



- Female, 11 years old
- Anemia, neutropenia/monocytopenia, thrombocytopenia
- Hyperleukocytosis with 93% lymphoblasts.
- Conventional karyotype:
  46,XX,t(9;22)(q34;q11)[8]/46,sl,add(16)(p12)[2]

Example 2: precursor B-ALL

QA parameters: optimal

Work in Excel: download data

- Female, 11 years old
- Anemia, neutropenia/monocytopenia, thrombocytopenia
- Hyperleukocytosis with 93% lymphoblasts.
- Conventional karyotype: 46,XX,t(9;22)(q34;q11)[8]/46,sl,add(16)(p12)[2]

Final result:

ogm[GRCh38]
?1q21.1q44(143278152_248943333)x2~3[VAF0.14],
7p12.2(50324505_50399656)x1[VAF0.47], => loss of exon 4-7 of *IKZF1*
9p21.3(21925732~21960270_22165462)x1[VAF0.53], => loss of CDKN2A/2B
9p13.2(36759965_37121784)x1[VAF0.41], => loss of *PAX5*
t(9;22)(q34.12;q11.23)(130709559;23244051)(BCR::ABL1)[VAF0.39],
12q21.33(91882647_92157466)x1[VAF0.42], => loss of exon 2 of *BGT1*
12p13.33p12.3(230737_15233701)(ETV6)x1[0.42], => *loss of ETV6*
13q14.2(48407665_48609400)x1[VAF0.24], => loss of exon 18-27 of *RB1*
13q14.2(48408876_48510295)x1[VAF0.46] , => loss of exon 18-27 of *RB1*

WHO:

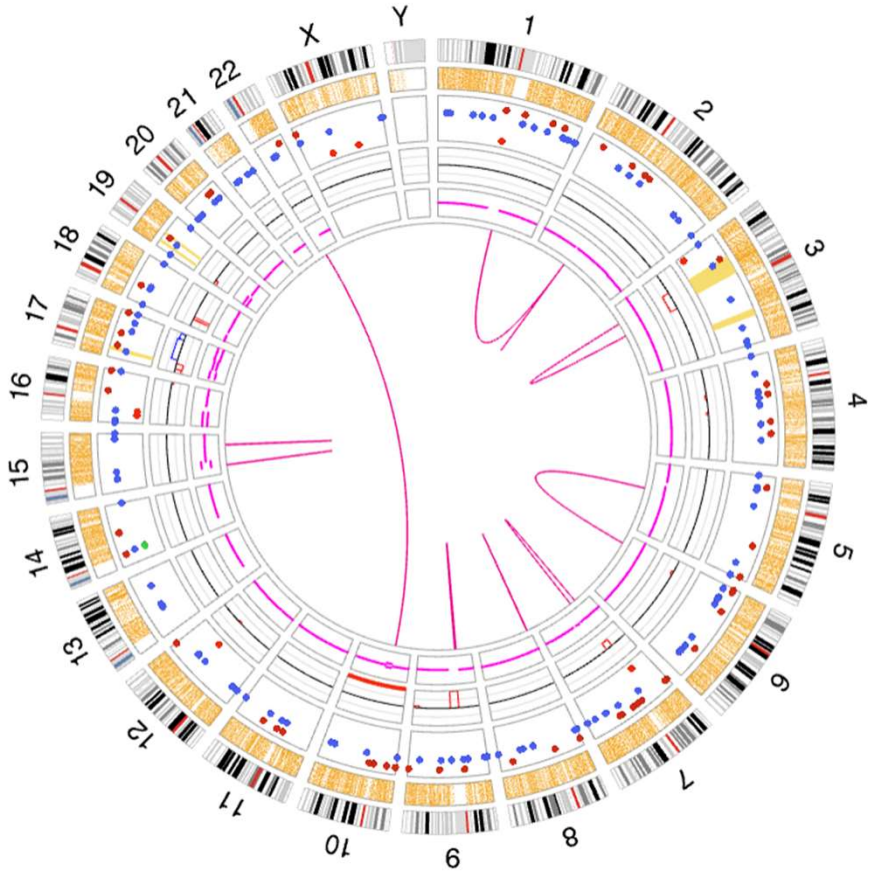
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1* fusion

Incorporation of TKIs in the treatment; improved the long-term survival rates for this leukaemia
IKZF1 alterations are associated with a poor response to TKIs and worse overall survival

Example 3: B-ALL

QA parameters: optimal

De Novo Assembly: circos plot



- Female, 4 years old
- Anemia
- Hyperleukocytosis with 92% blasts.
- Conventional karyotype: failure: 46,XX[2]

Example 3: B-ALL

QA parameters: optimal

De Novo Assembly: circos plot
Work in Excel: download data

- Female, 4 years old
- Anemia
- Hyperleukocytosis with 92% blasts.
- Conventional karyotype: failure: 46,XX[2]

Final result:

ogm[GRCh38]
t(X;14)(p22.33;q32.33)(1184290;105829284)(IGH::CRLF2),
3p21.31p12.2(44931202_81718429)x1[VAF0.43],
7p14.2p12.1(36400310_50676122)x1[VAF0.44], => loss of *IKZF1*
12p13.2(11635931_11819702)x1[VAF0.42], => loss of *ETV6* exon 1-2
12q21.33(91882647_92157466)x1[VAF0.48], => loss of *BTG1* exon 2
17p13.3p11.2(66653_19154549)x1[VAF0.38], => loss of *TP53*
17q11.1q25.3(26692353_81131977)x3[VAF0.38],

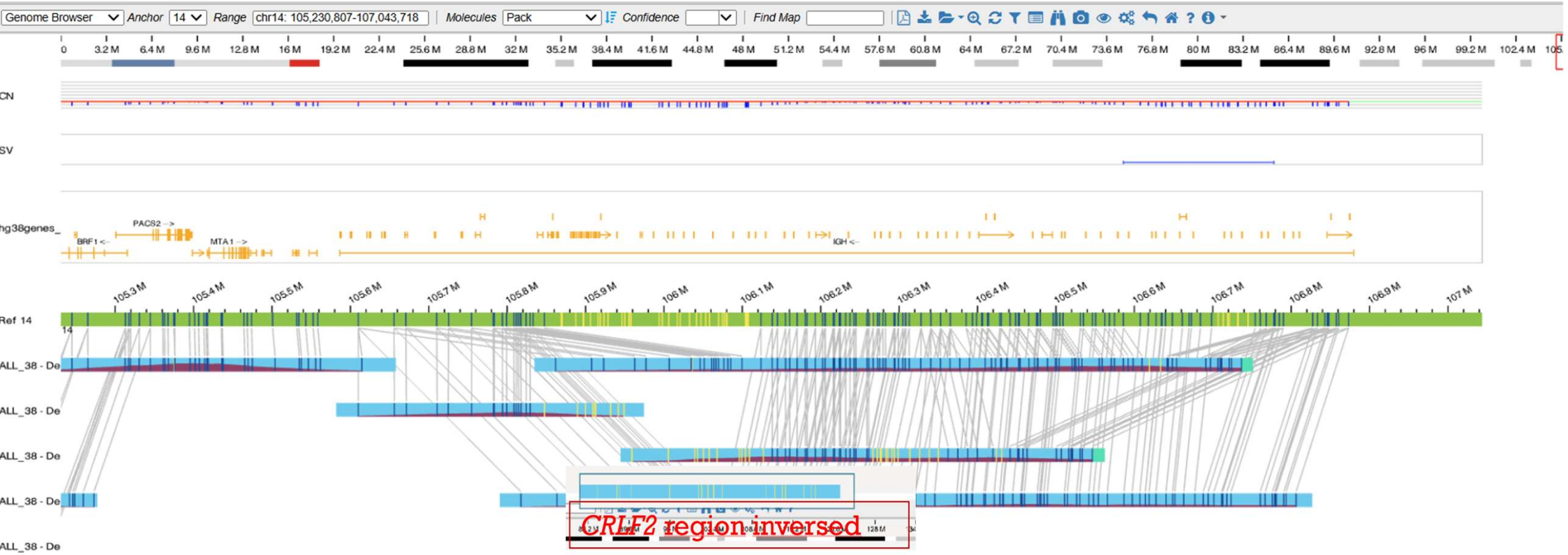
WHO:
B-lymphoblastic leukaemia/lymphoma with BCR::ABL1-like features
associated with high-risk clinical features
Recent studies have shown promising outcomes for B-ALL/LBL with *BCR*::*ABL1*-like features with the addition of tyrosine kinase inhibitors directed against specific genomic lesions

Example 3: B-ALL

QA parameters: optimal

De Novo Assembly: circos plot

- Female, 4 years old
- Anemia
- Hyperleukocytosis with 92% blasts.
- Conventional karyotype: failure: 46,XX[2]

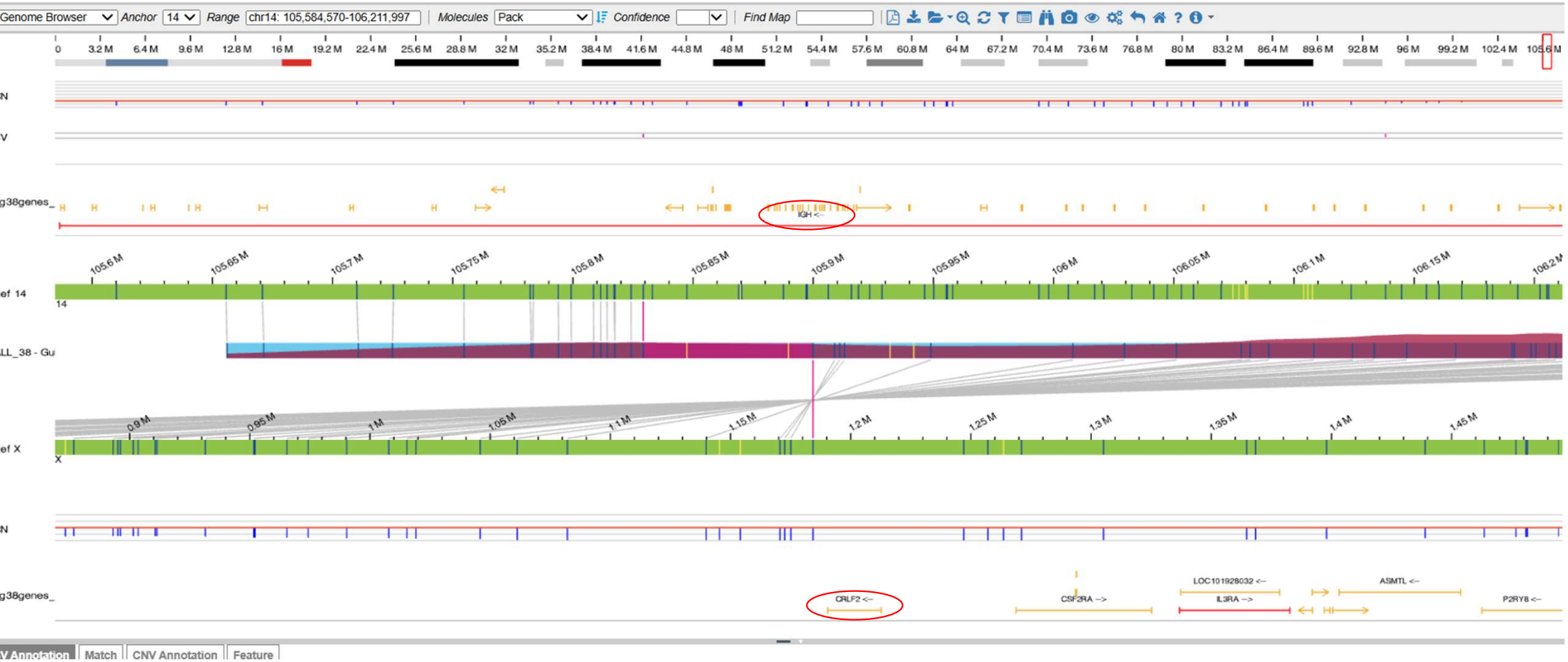


Exemple 3: B-ALL: OGM: version 1.8.1: the fusion *IGH::CRLF2* is still not detected by pipeline

- with version 1.8.1 , fusion *IGH::CRLF2* is not detected nor with the RVA nor with the De Novo.
- So we checked whether the « Guided Assembly» could detect it

Example 3: B-ALL: OGM: Guided Assembly: the fusion *IGH::CRLF2* is detected

Breakpoint on chromosome 14 : in *IGH*



Example 3: B-ALL: OGM: Guided Assembly: the fusion *IGH::CRLF2* is detected

Breakpoint on chromosome X : in *CRLF2*

Quality control parameters

- Bioinformatic pipelines

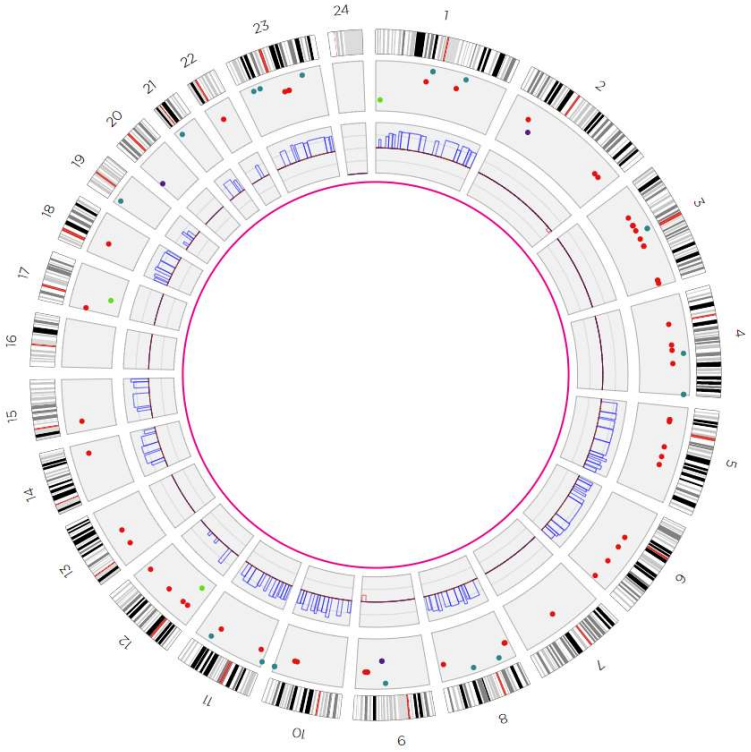
	Lower size limit	LOH	LLOD
De Novo Assembly	500 bp	yes	20-25% VAF
RVA	5 kb • Insertions: 5-50 kb • Deletions: > 7 kb • Translocations: ≥ 70 kb • Inversions: ≥ 100 kb • Duplications: ≥ 150 kb	no	5% VAF at 300x coverage

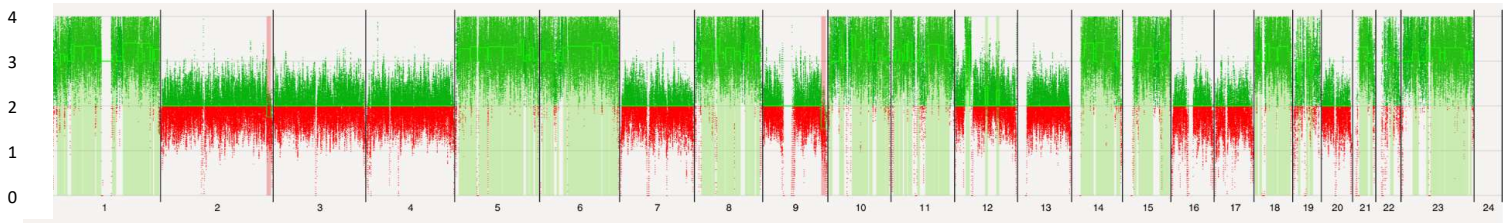
=> the **"De Novo Assembly"** pipeline is required for the analysis of **Acute Lymphoblastic Leukemia** cases!!!
=> the best is to also run an RVA to be able to pick up the aberrations present at low VAF

=> for other hematological malignancies: usually the RVA alone is sufficient

Example 4: OGM identified hyperdiploidy in a B-ALL case with “normal” karyotype

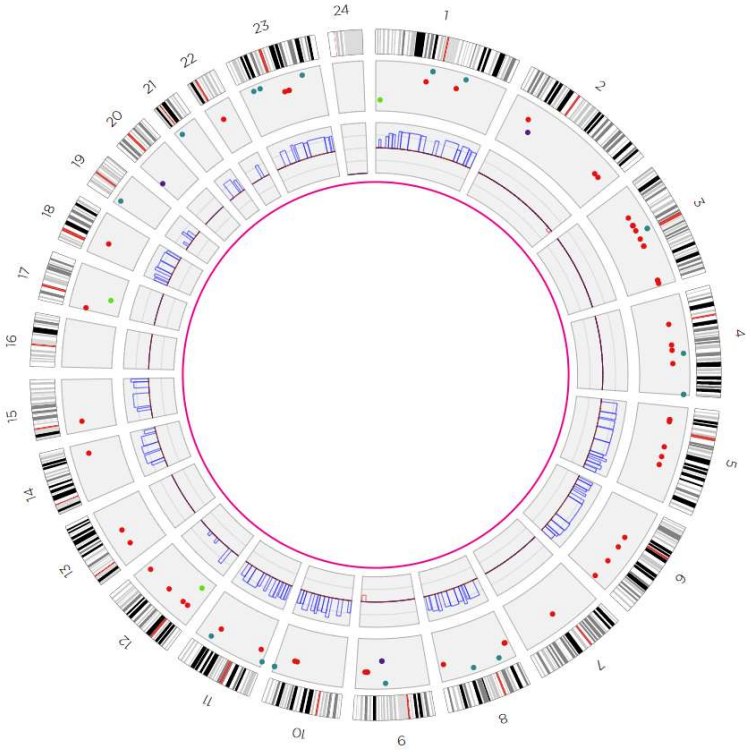
- Female, 18 years old
- 69% blasts in blood
- Karyotype: normal: late sample receipt



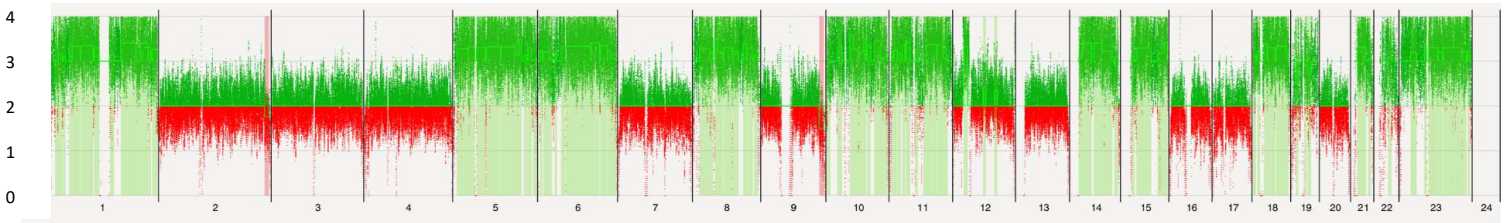


gains
losses

Example 4: OGM identified hyperdiploidy in a B-ALL case with “normal” karyotype



- Female, 18 years old
- 69% blasts in blood
- Karyotype: normal: late sample receipt
- FISH: monoallelic loss of 9q34 and 12p13 and monosomy 7
- CGH array: 36,XX,-2,-3,-4,-7,-9,-12,-13,-16,-17,-20
  => low hypodiploidy
  => high risk



Treatment protocols in ALL require extensive genetic testing:

Aberrations with clinical significance in terms of risk:

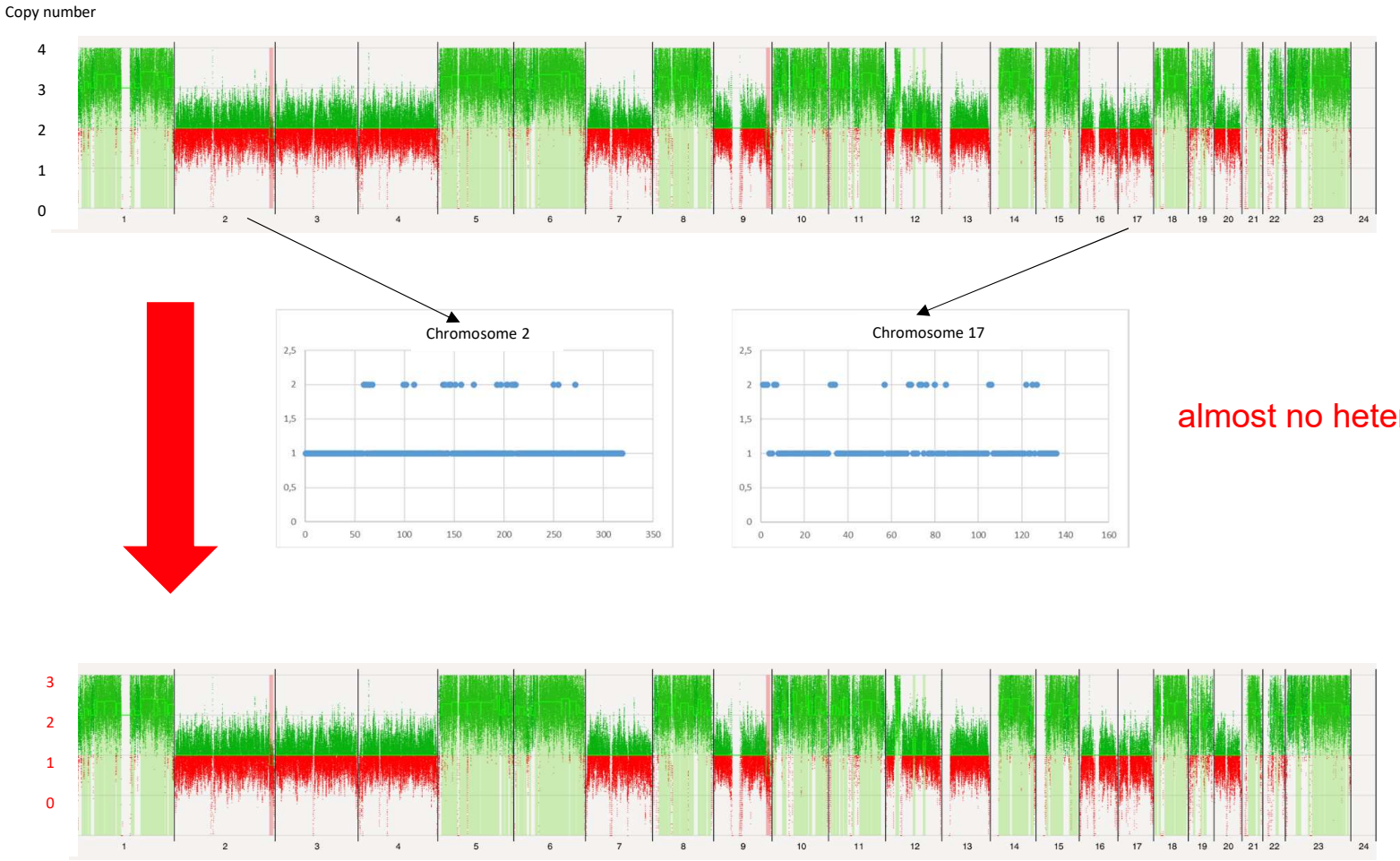
Good risk abnormalities	*Standard risk abnormalities*	*Intermediate risk abnormalities*	*High risk abnormalities*
High hyperdiploidy (>50chr)	t(1;19)(q23;p13) *TCF3::PBX1*	t(X;14)(p22;q32)/t(Y;14)(p11;q32) *IGH::CRLF2*	Near haploidy (25-29 chr)
TAL1 abnormalities]	15q13-15 rearrangements	del(X)(p22.33)/del(Y)(p11.32) *P2RY8::CRLF2*	Low hypodiploidy (30-39 chr)
t(2;8)(p11;q24) *IGK::MYC*			High hypodiploidy (<44, poor)
t(7;10)(q34;p24) *TRB::TLX1**			Trisomy 5
t(8;14)(q24;q32) *IGH::MYC*			del(5)(q32q33.3) *EBF1, PDGFRB*
t(8;14)(q24;q11) *IGL::MYC*			t(5;9)(q22;q34) *SNX2::ABL1*
dic(9;12)(p13;p13) *PAX5::ETV6*			t(5;14)(q35;q32) *BCL11B::TLX3*
t(10;14)(q24;q11) *TRA/TRD::TLX1**			del(7p12.2) *IKZF1*
t(12;21)(p13;q22) *ETV6::RUNX1*			t(7;19)(q34;p13) *TRB::LYL1*
del(21)(q22.2) *ERG*			dic(9;20)(p13;q11) *PAX5*
			del(9)(p23.3) *CDKN2A*°
			t(9;22)(q34;q11) *BCR::ABL1*^
			10p12 aberrations *MLLT10*
			11q23 aberrations *KMT2A*
			t(14;18)(q32;q21) *IGH::BCL2*
			t(17;19)(q22;q13) *TCF3:HLF*^

*better than other T-ALL
°prognosis variable
^extremely poor
chromosomal aberrations are shown on the left-hand side and involved genes on the right-hand side

- recurrent structural rearrangements
- whole chromosome CNA
- submicroscopic deletions

Table 1
Adeapted from: Cancer cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells, Foutrh Edition. Page 202-204.

Example 4: correction baseline based on zygosity of structural variants



After correction (baseline reset): 36,XX,-2,-3,-4,-7,-9,-12,-13,-16,-17,-20

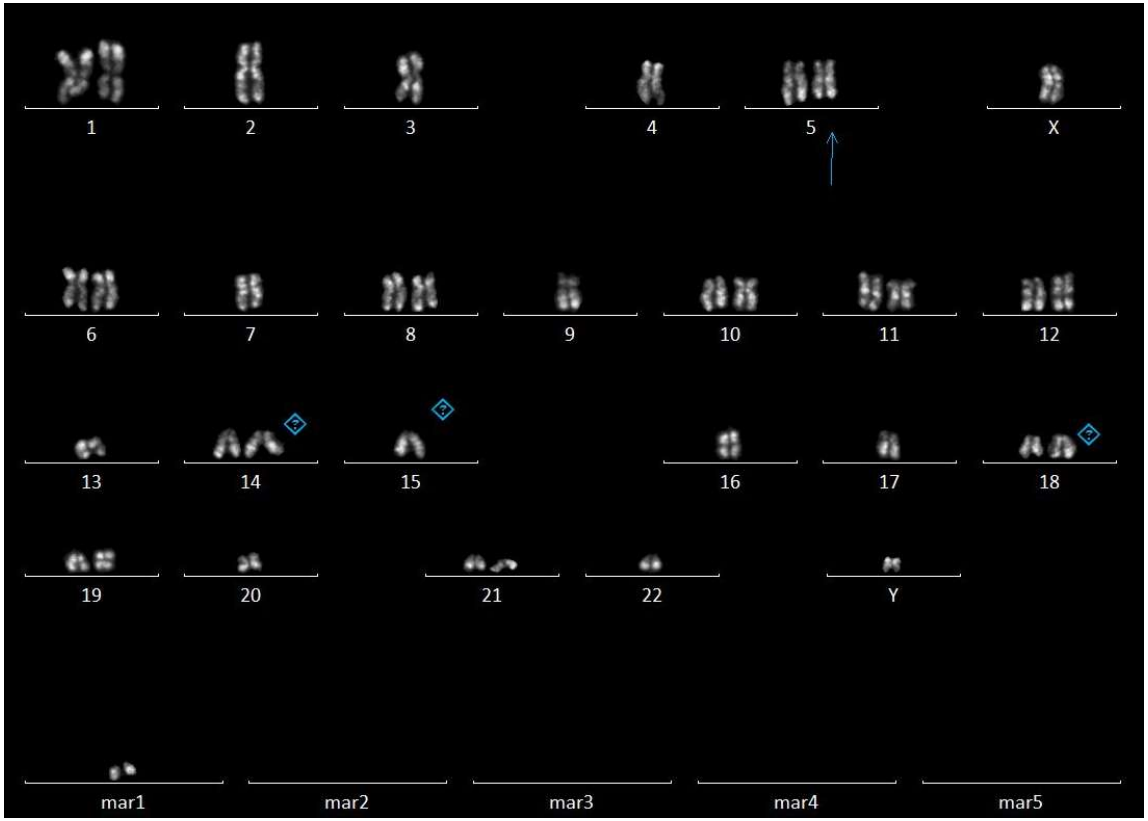
=> low hypodiploid karyotype

Example 5: correction baseline based on zygosity of structural variants

- Male, 63 years old
- 90% blasts in bone marrow
- Flow: pre-B-ALL
- Karyotype:

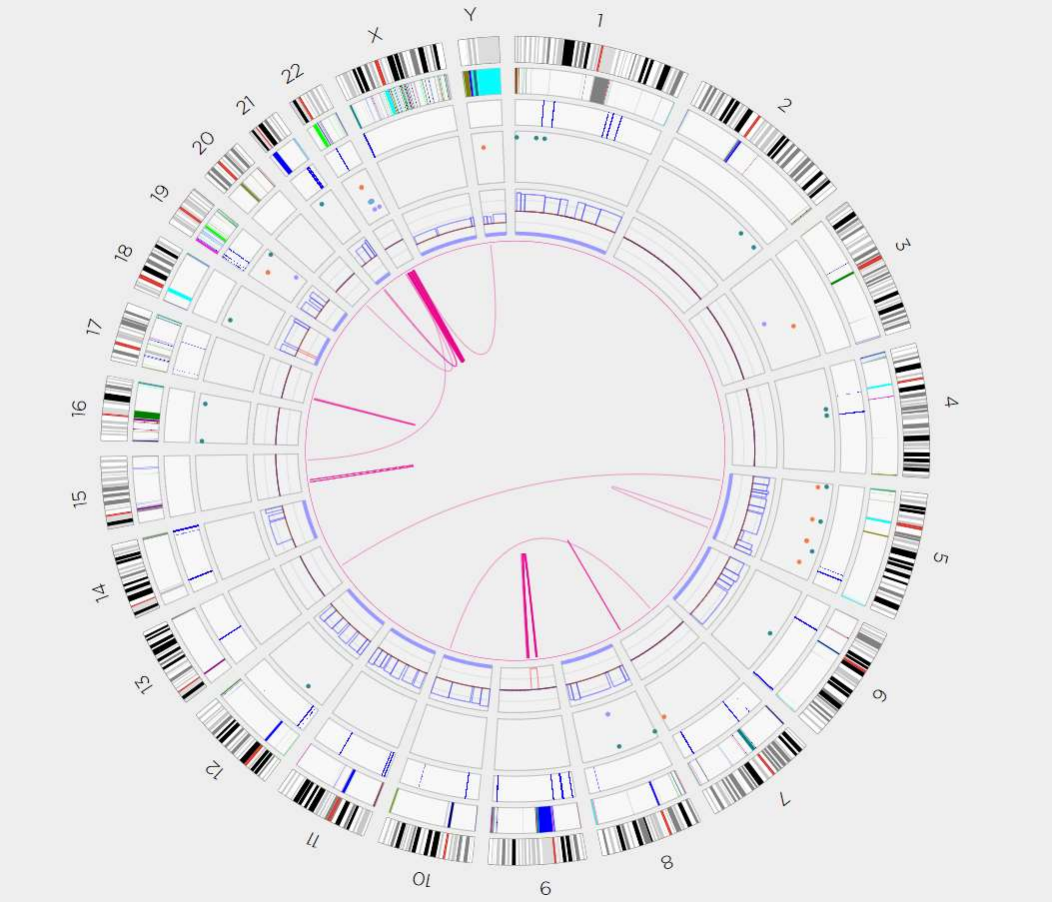
36,XY,-2,-3,-4,del(5)(q31q33),-7,-9,-13,-15,-16,-17,-20,-22,+mar,inc[6]/46,XY[7]

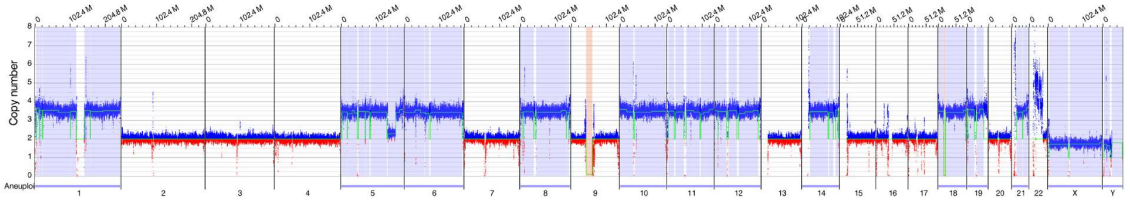
- Low hypodiploid clone. Prognosis: adverse. Add NGS to exclude TP53 mutation



Example 5: correction baseline based on zygosity of structural variants

- Male, 63 years old
- 90% blasts in bone marrow
- Flow: pre-B-ALL

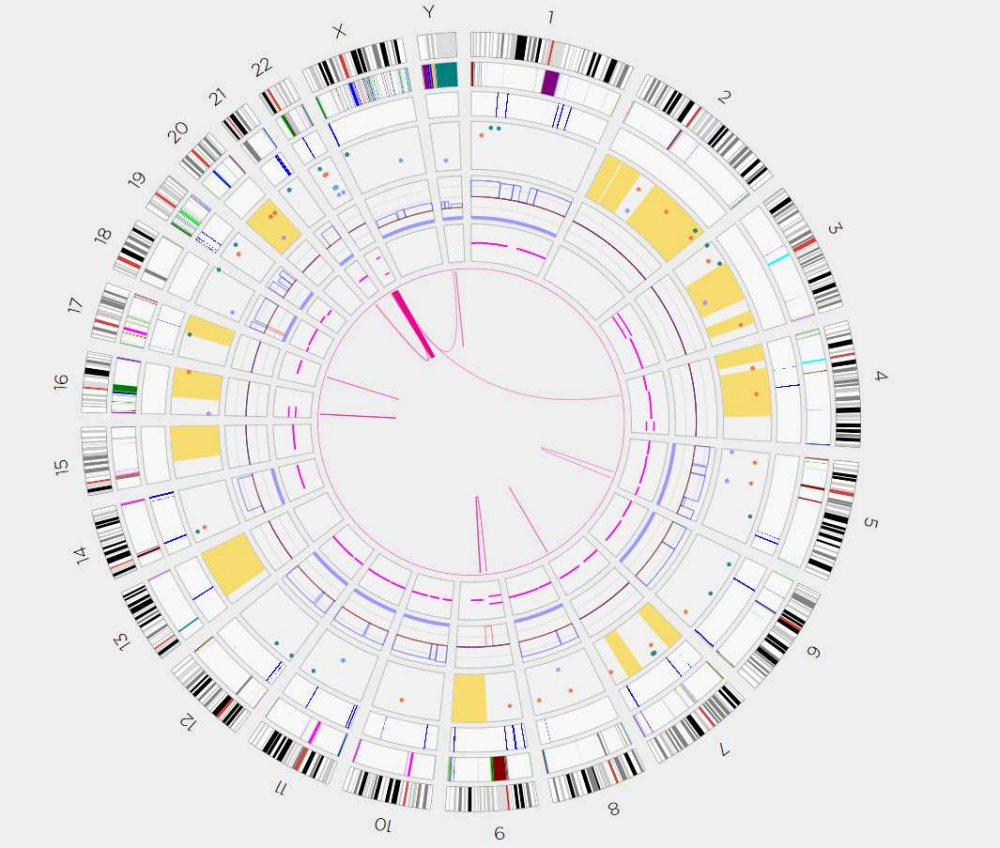


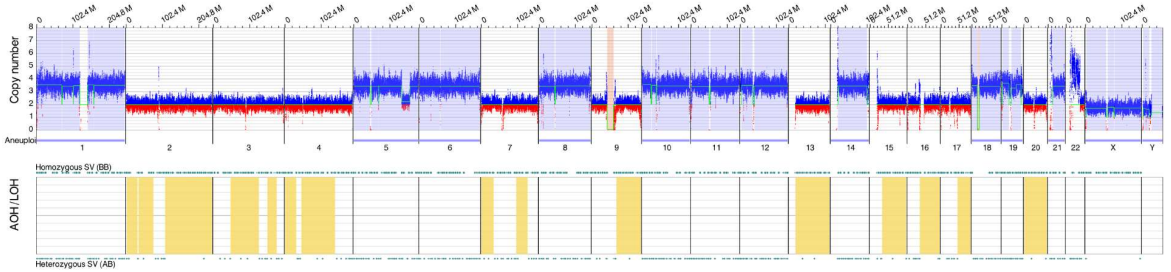


If you only run the RVA:
Seems like hyperdiploidy: gain of multiple chromosomes: gain of #1, gain of #8, etc

Example 5: correction baseline based on zygosity of structural variants

- Male, 63 years old
- 90% blasts in bone marrow
- Flow: pre-B-ALL



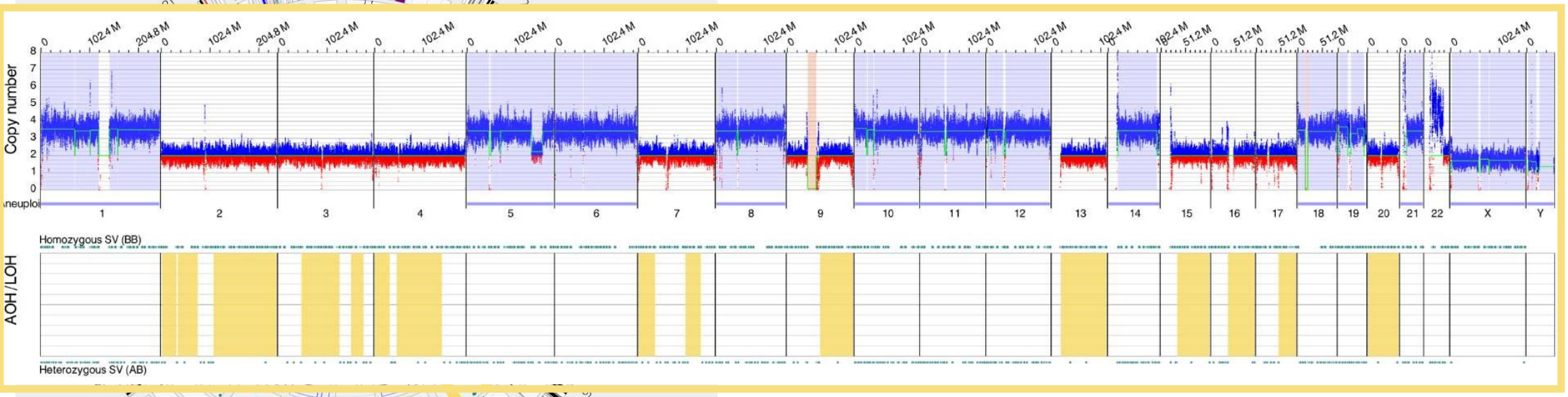


If you run the De Novo:

Indicates that there is LOH of chromosomes 2, 3, 4 etc …
Indication for hypodiploidy cfr conventional karyotype!

Example 5: correction baseline based on zygosity of structural variants

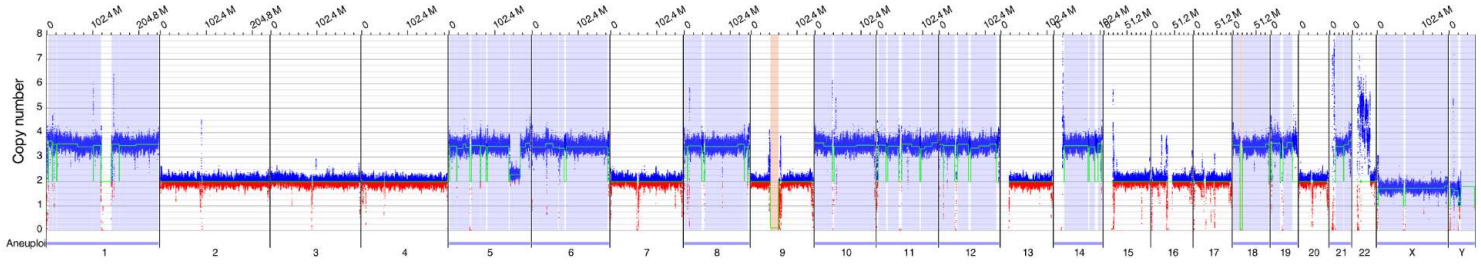
- Male, 63 years old
- 90% blasts in bone marrow
- Flow: pre-B-ALL



If you run the De Novo:

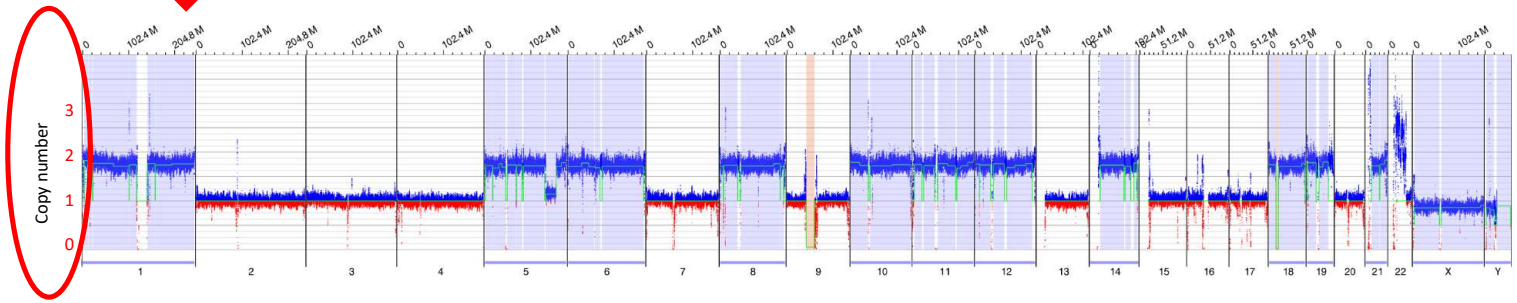
Indicates that there is LOH of chromosomes 2, 3, 4 etc …
Indication for hypodiploidy cfr conventional karyotype!

Example 5: correction baseline based on zygosity of structural variants



gains
losses

almost no heterozygous variants for
chromosomes 2, 3, 4, 7, 9, 13, 15, 16, 17 and 20



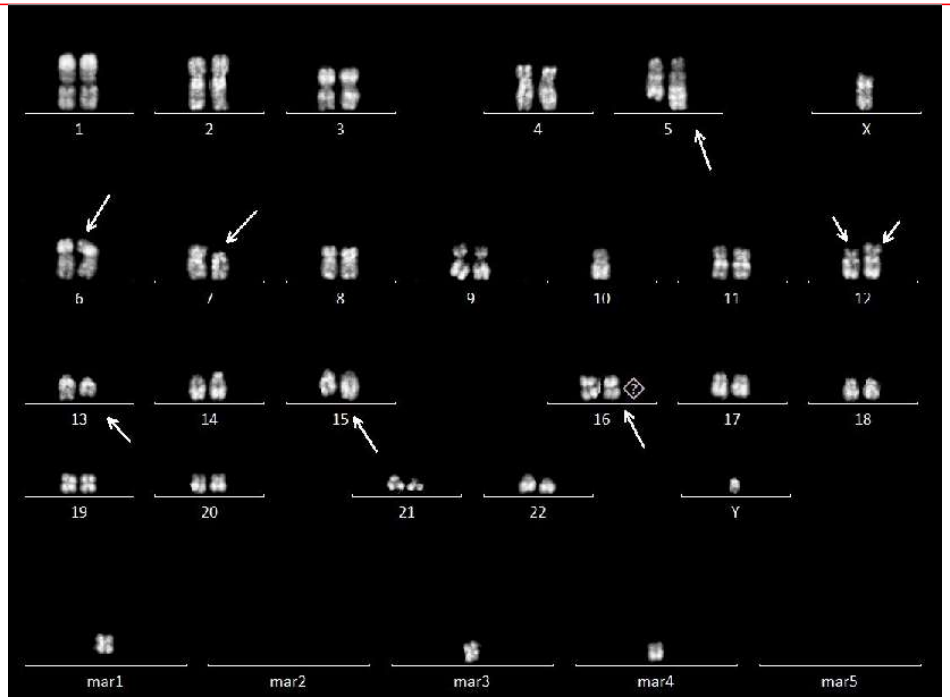
After correction (baseline reset):
Karyotype according to OGM: 36,XY,-2,-3,-4,del(5)(q31.1q33.3),-7,-9,-13,-15,-16,-17,-20,(22p11.2q13.1)cth,del(22)(q13.1q13.33)
WHO: "B-lymphoblastic leukaemia/lymphoma with low-hypodiploidy".

Example 6: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

- Male, 13 years old
- 90% blasts in bone marrow
- Flow: B-ALL relapse
- Karyotype:

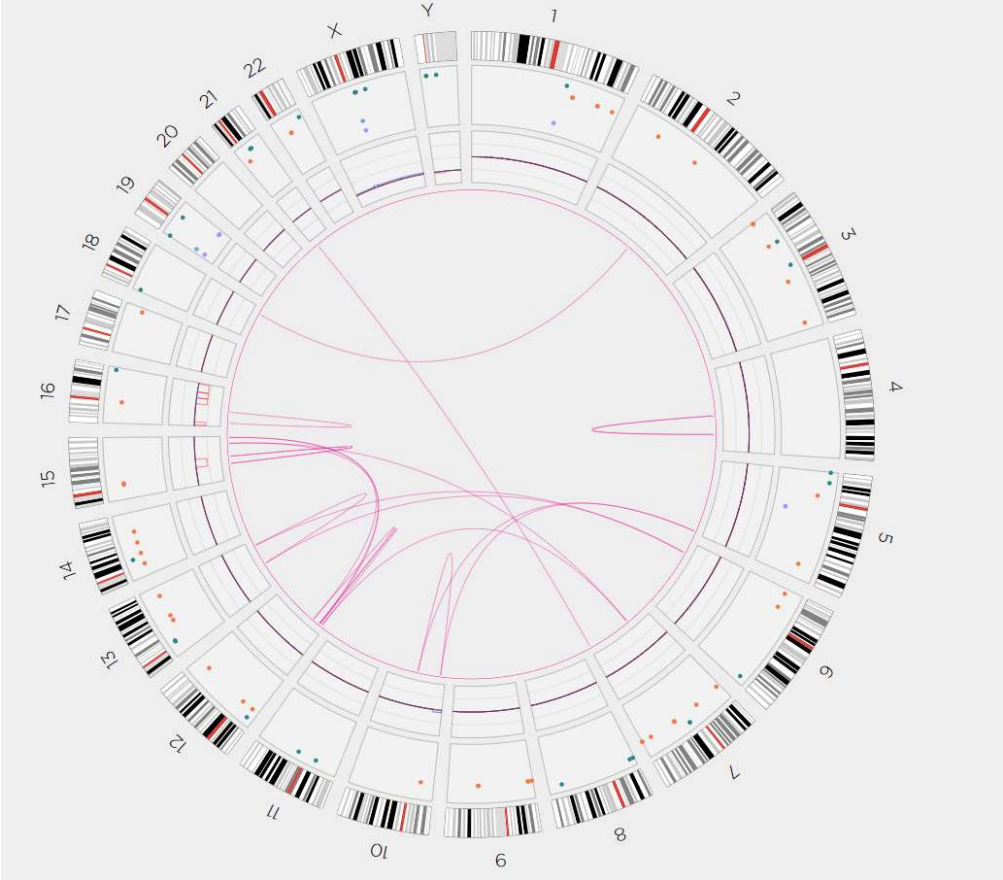
39-48,XY,der(5)t(5;?10)(q3?;q?),?t(6;13)(p21;q14),del(7)(p11) or der(7)t(7;15)(p11;q26),add(12)(p13),del(12)(p12),add(15)(q26) or der(15)t(7;15)(p11;q26),-16[3],?add(16)(p13)[7],+mar1,+mar2[3],inc[cp10]//46,XX[4]
In total: 4/80 mitoses with donor hematopoiesis

- Conclusión: persisting aberrations with clonal evolution



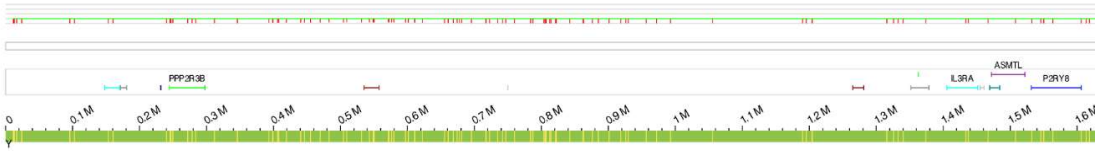
Example 6: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

- Male, 13 years old
- 90% blasts in bone marrow
- Flow: B-ALL relapse
- OGM/Bionano: **Rare variant pipeline**: very complex pseudodiploid karyotype comparable to the conventional karyotype



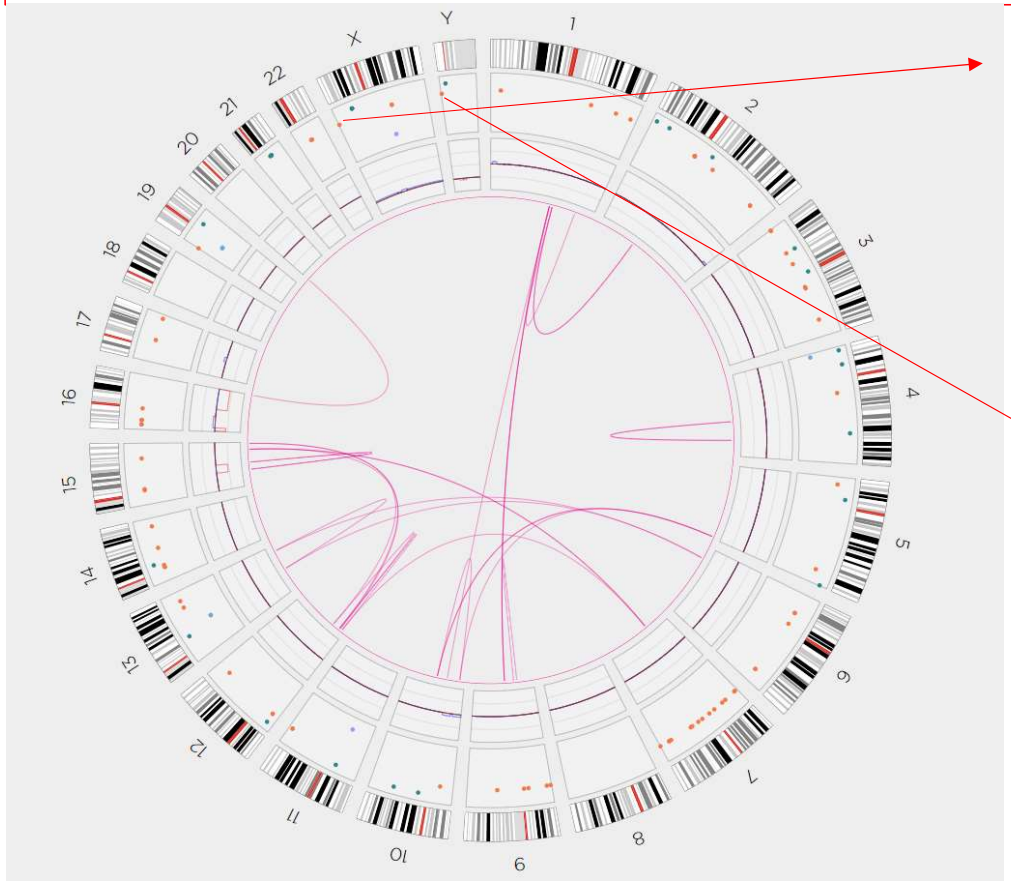
Xp22.33: completely normal

Yp11.32: completely normal

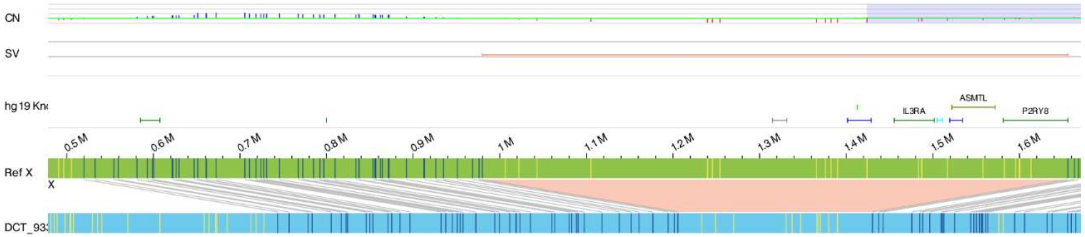


Example 6: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

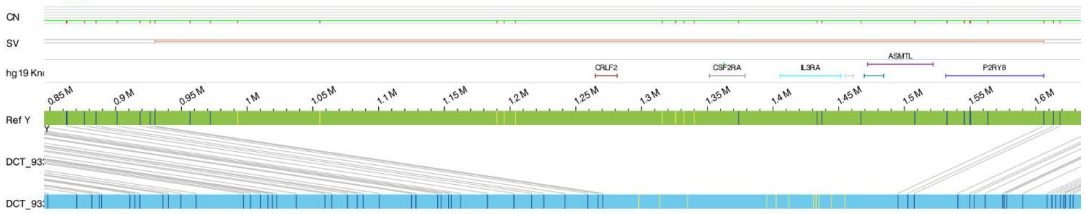
- Male, 13 years old
- 90% blasts in bone marrow
- Flow: B-ALL relapse
- OGM/Bionano: **De Novo Assembly pipeline**: very complex pseudodiploid karyotype comparable to the conventional karyotype
- De Novo Assembly detects a deletion on Xp22.33 and Yp11.32: resulting in **the *CRLF2::P2RY8* fusion gene**!



Xp22.33: *CRLF2::P2RY8*

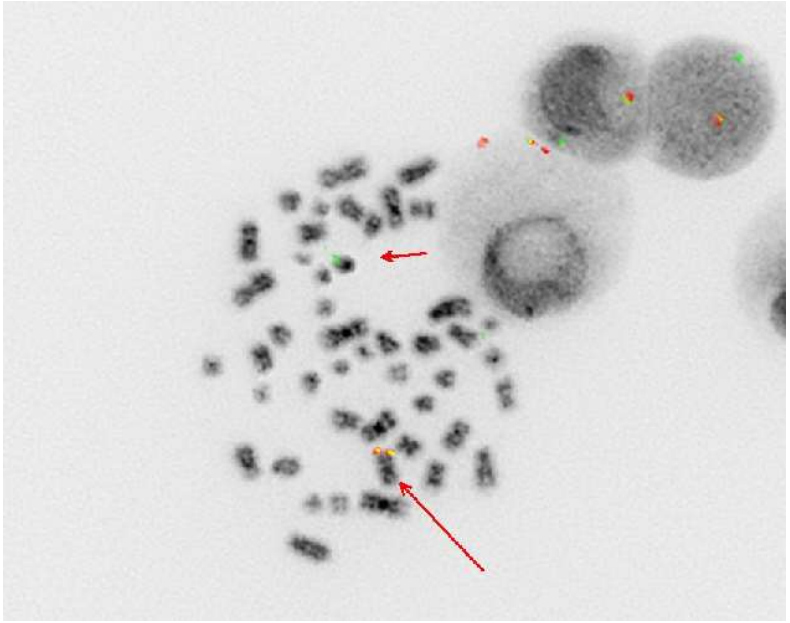


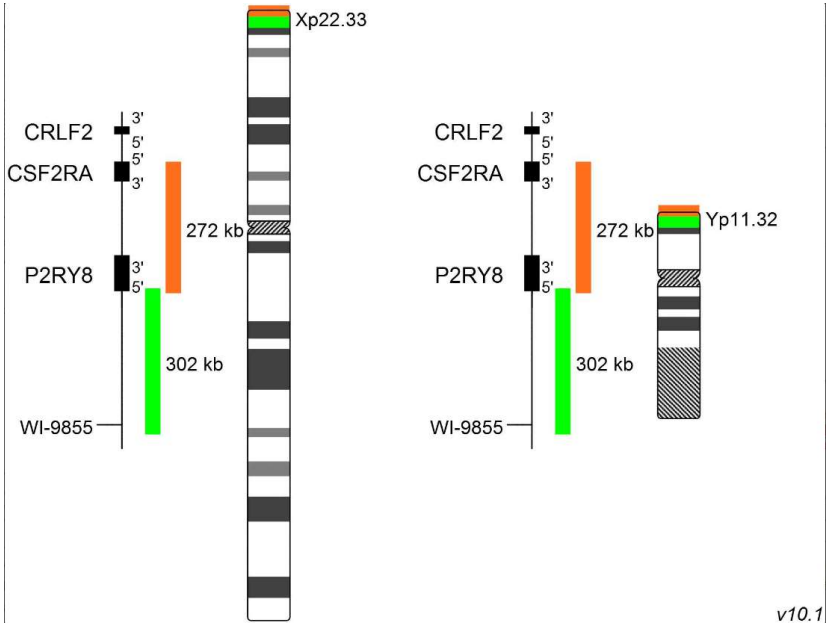
Yp11.32: *CRLF2::P2RY8*



Example 6: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

- Male, 13 years old
- 90% blasts in bone marrow
- Flow: B-ALL relapse
- **the *CRLF2::P2RY8* fusion was confirmed with FISH**





FISH using the probe: XL CRLF2 DC BA [Xp22-Yp11, Metasystems] on 200 interphase nuclei and 10 metaphases:
- an unbalanced rearrangement of Yp11/CRLF2, with loss of the 5'cen CRLF2 signal in ~90% of nuclei and 7/10 metaphases
- 3/10 metphases with female karyotype (donor cells)

➔ **FISH confirmed the cytogenetic cryptic deletion on Yp11**, seen with OGM and leading to ***CRLF2::P2RY8***

Example 6: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

- Male, 13 years old
- 90% blasts in bone marrow
- Flow: B-ALL relapse
- **the *CRLF2::P2RY8* fusion was confirmed with FISH**

- **WHO entity: "B-lymphoblastic leukaemia/lymphoma, BCR-ABL1-like", prognosis: adverse.**

- **Important remark: regions** Xp22.33 and Yp11.32 need a visual inspection for all ALL cases: sometimes the software does not call the CRLF2::P2RY8 fusion although you can see it upon visual inspection

Analysis

- Carefully validate and determine the filter settings you want to use
- Check your filter settings before every analysis 
- Recommendation:

- use the setting “ALL STRUCTURAL VARIANTS” and “ALL COPY NUMBER VARIANTS” with a 1-2% control base threshold
(I do not recommend checking only the “NON-MASKED VARIANTS”)
- discard non-relevant SV’s or CNA’s then manually

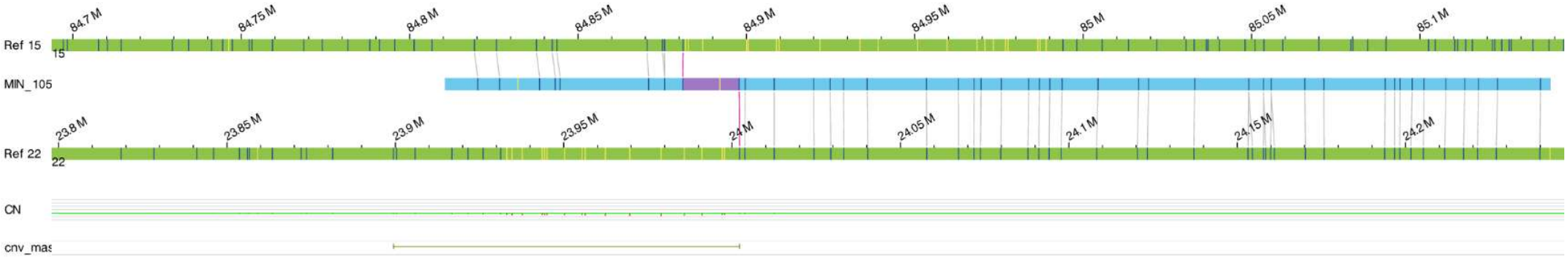
Analysis: variant review

Criteria for manual review:

- Check all the SV's that were retained by the filters manually in the software
- Confirm real CV's
- Eliminate artefacts and false positives

=> Reasons for false positives/artefacts: poor alignment due to:
- N-base gaps in reference genome
- segmental duplications
- repetitive sequences (e.g. transposons)
- centromeres and telomeres: regions with highly repetitive nature

Analysis: variant review: example of a probably false translocation:



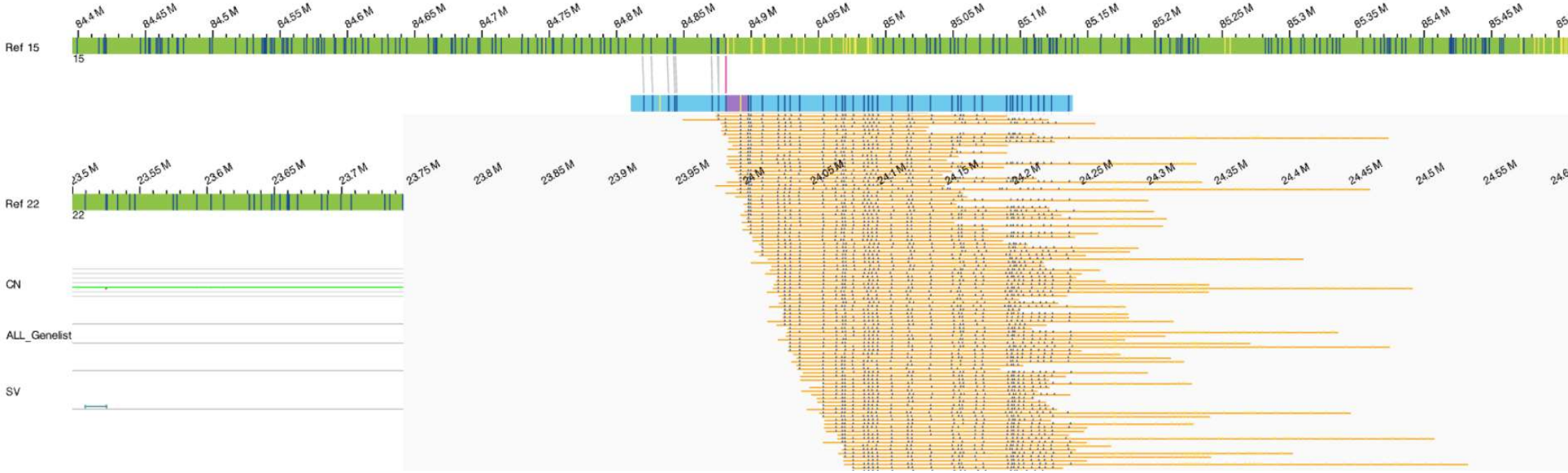
translocation_interchr: t(15;22)(q25.3;q11.23): example of translocation I would discard

=> not enough labels at left breakpoint, not exact match, + overlap with CNV masked region

=> “fail” for parameter “Fail_assembly_chimeric_score”

=> not seen with conventional karyotyping

Analysis: variant review: example of a probably false translocation:



Check the raw data: right mouse click: show molecules

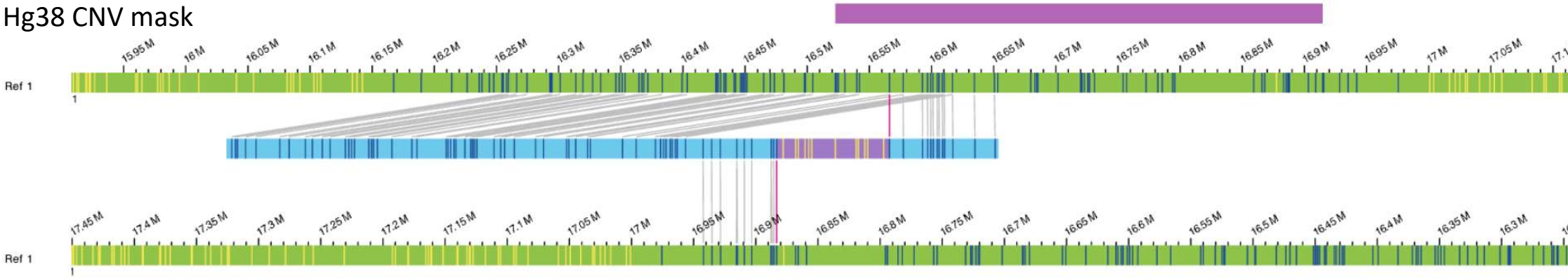
Analysis: variant review: fail assembly chimeric score

A flag used to denote whether there might be a potential chimeric join at the variant locus. This denotes whether a minimal chimeric quality score of 35 and coverage of 10X have been achieved around each SV breakpoint. A value of 'pass' means that the two criteria have been met; a 'fail' denotes the criteria not met; and a 'not_applicable' value denotes that the check has not been performed. Notice that this check is performed only for inversion and translocation calls.

Note: a chimeric quality score of a label on a genome map is the percent of molecules that align to both sides of the label out of all molecules that align on either side near this label.

Self_molecule_count: The number of molecules supporting the SV. Currently at "recommended" value of "5", but in Leuven we do not filter on this initially. We take it into account in the decision together with other parameters

Analysis: variant review: example of a false translocation:

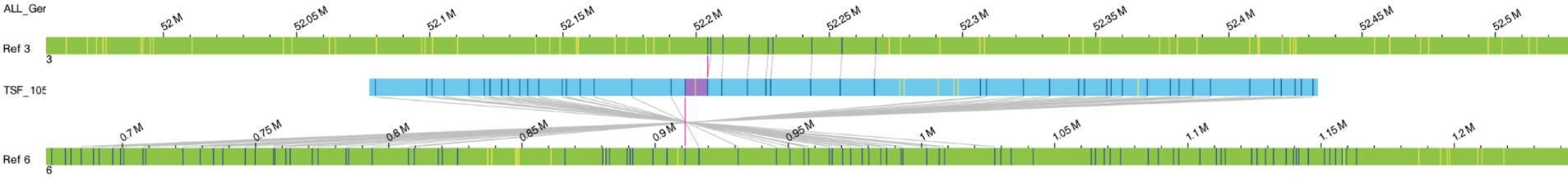


translocation_intrachr: ogm[GRCh38] t(1;1)(p36.13;p36.13): example of translocation I would discard

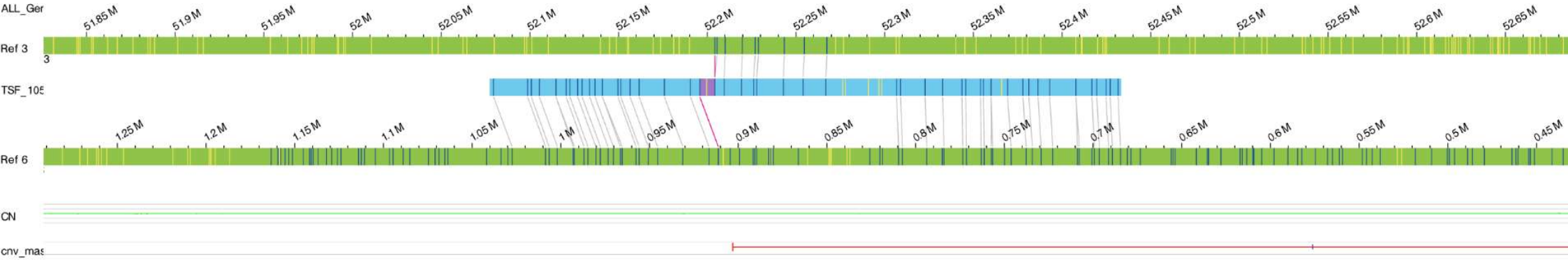
=> in region of CNV mask (purple)

=> seen in many samples

Analysis: variant review: example of a false translocation:



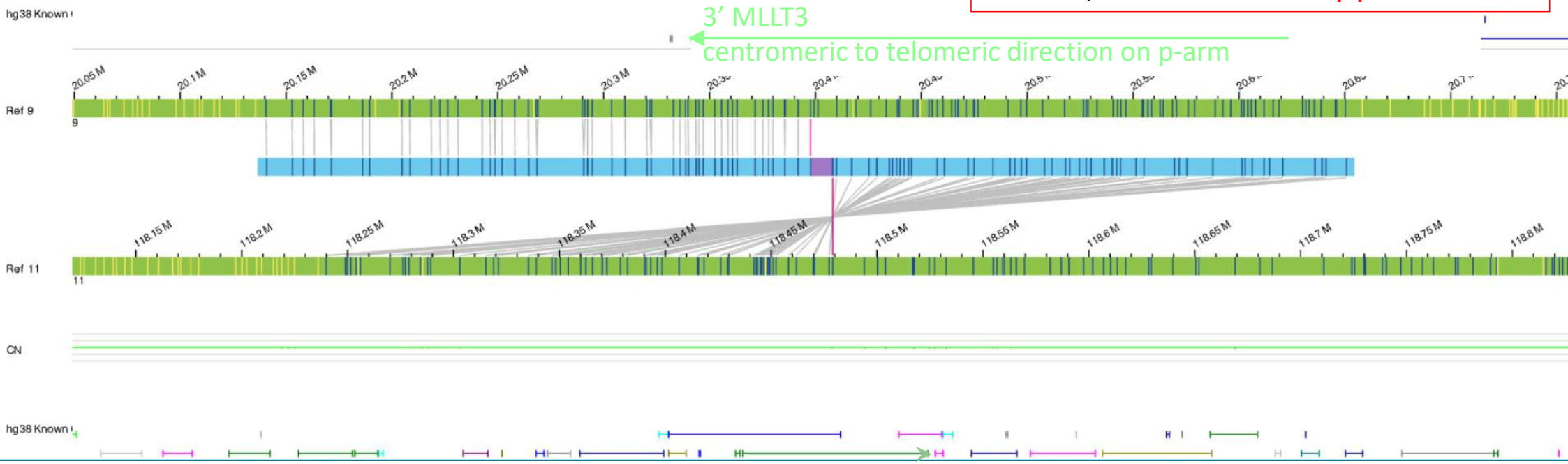
Same as above, but inverted, so that you can better perform a visual inspection:



translocation_interchr: t(3;6)(p21.2;p25.3): example of a “translocation” I would discard
=> not exact match, maybe small insertion but???, could just be miss alignment + overlap with CNV masked region
=> “fail” for parameter “Fail_assembly_chimeric_score”

Analysis: variant review: example of a balanced translocation

- Male, 75 years old
- 80% blasts in bone marrow; pancytopenia
- Flow: AML
- OGM/Bionano: **Rare variant pipeline**



ogm[GRCh38]
t(9;11)(p21.3;q23.3)(20397688;118479068) [5’KMT2A::3’MLLT3]
"AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*" (ICC 2022); Prognosis intermediate (ELN 2022 Döhner et al.)

Analysis: variant review: example of a balanced translocation

- Male, 75 years old
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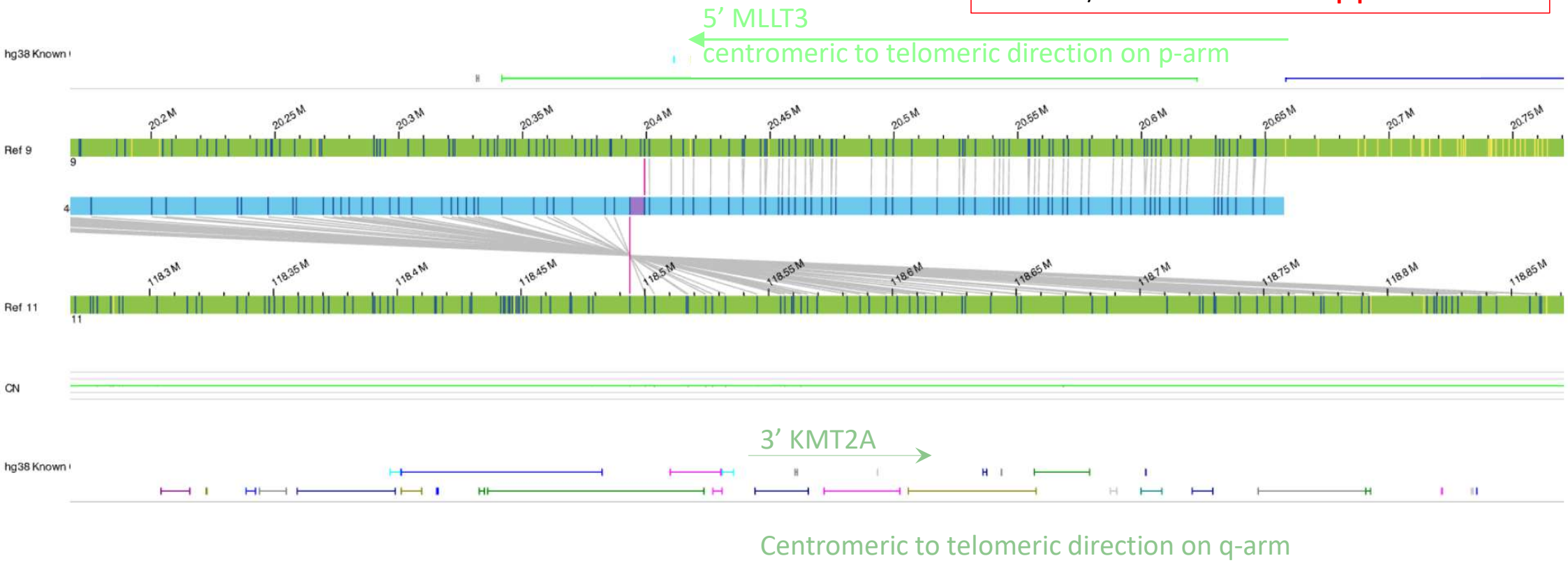
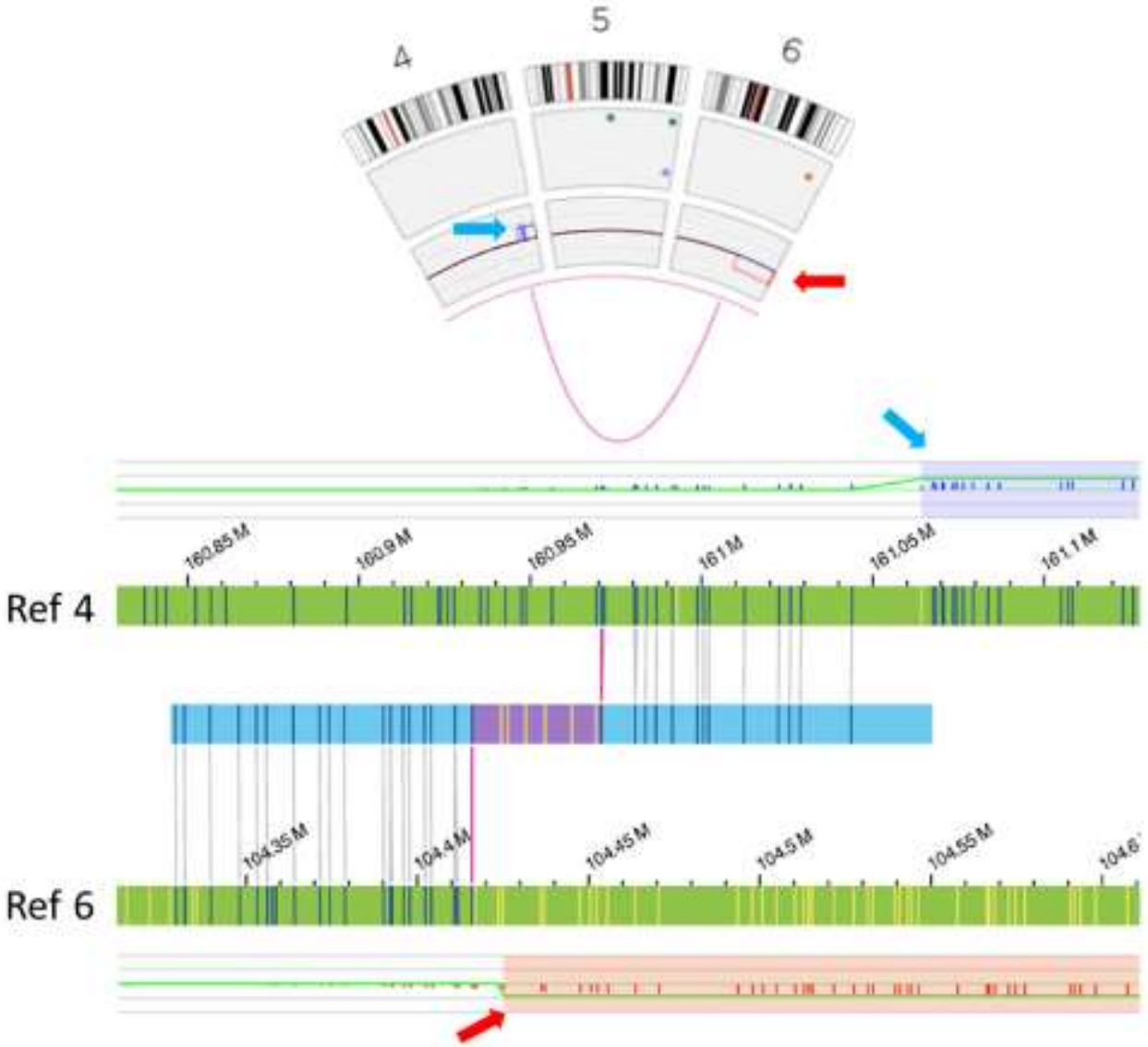
- Flow: AML
- OGM/Bionano: **Rare variant pipeline**

You can also see the reciprocal translocation in Access:



Analysis: variant review: example of an unbalanced translocation



Analysis: variant review: example of a deletion

- Male, 66 years old
- 90% blasts in bone marrow
- Flow: AML
- Karyotype: 46,XY,del(20)(q11q13)[10]
  Conclusion: pseudiploid clone with deletion 20q. Recurrent in myeloid malignancies. ELN 2022: intermediate risk
- OGM/Bionano: **Rare variant pipeline**

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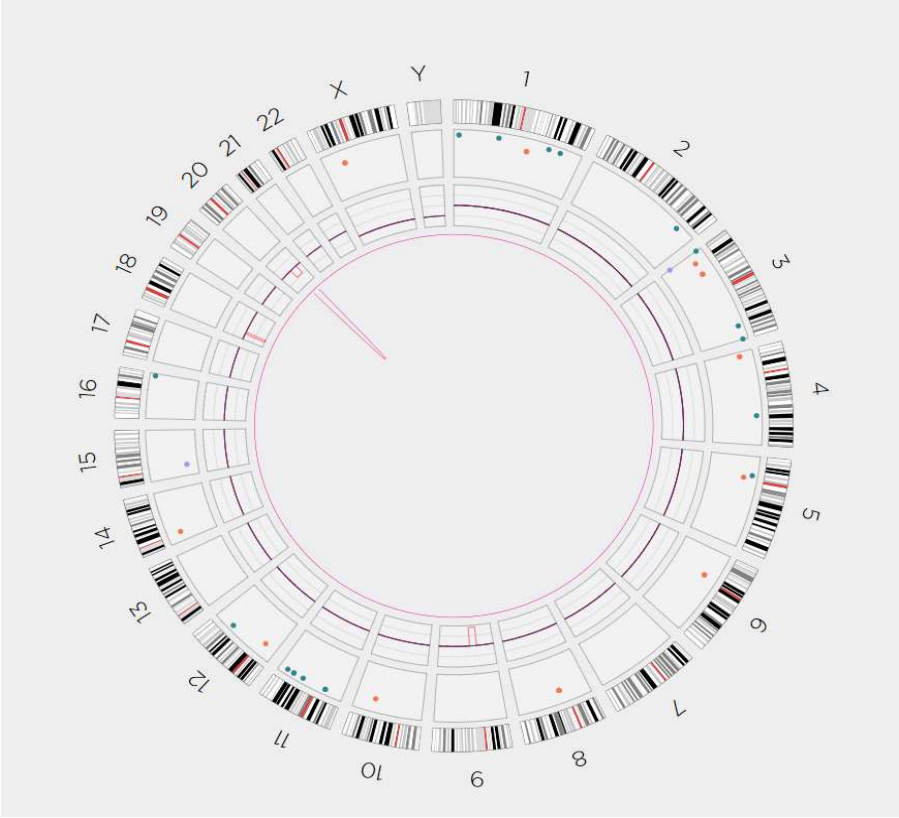
OGM

Rare Variant Pipeline analysis

1. Analysis CNVs
2. Analysis SVs



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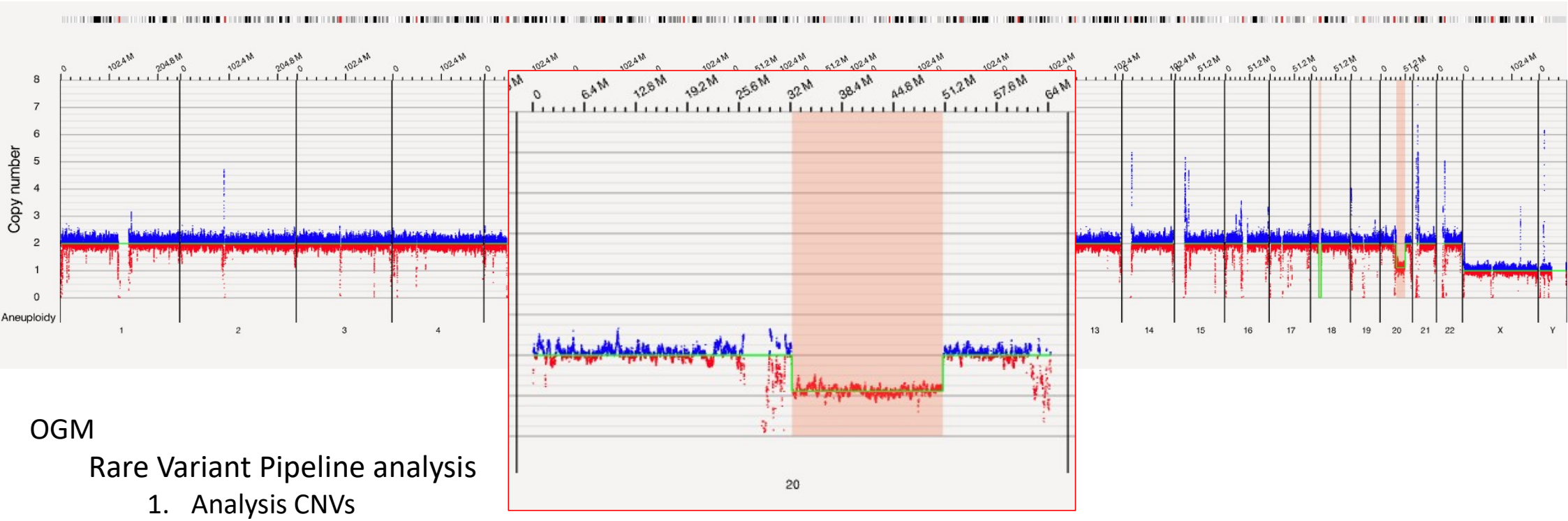

OGM

Rare Variant Pipeline analysis

1. Analysis CNVs

Analysis: variant review: example of a deletion

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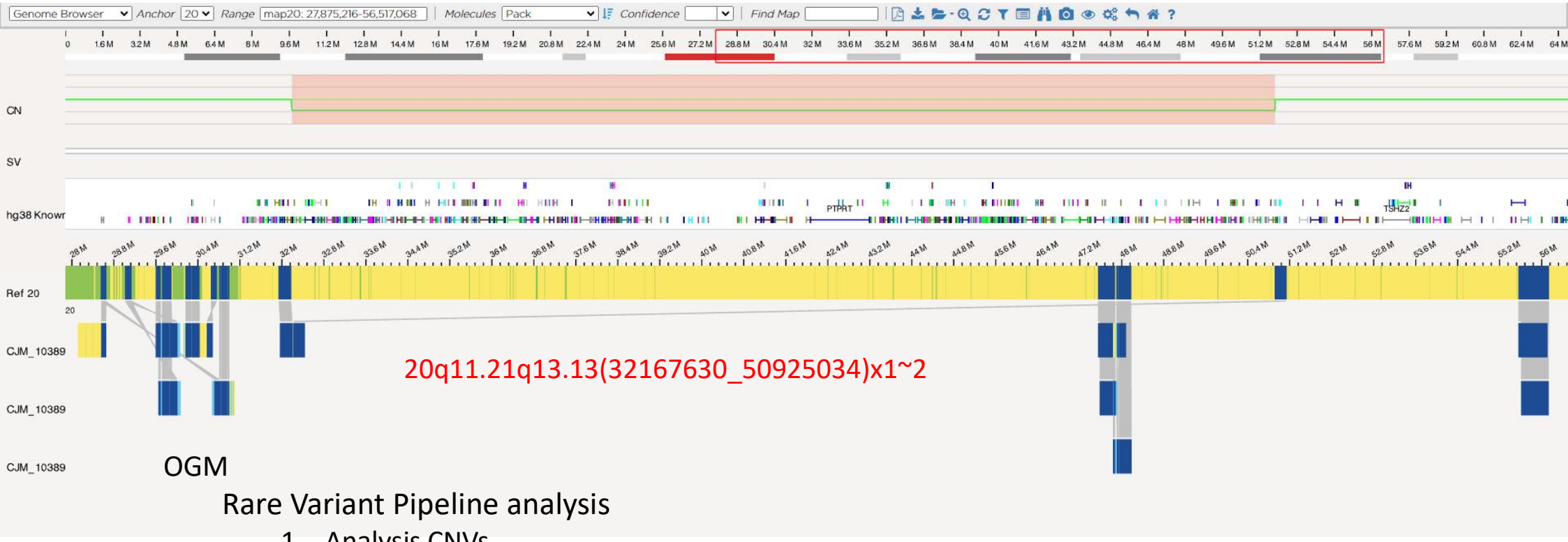
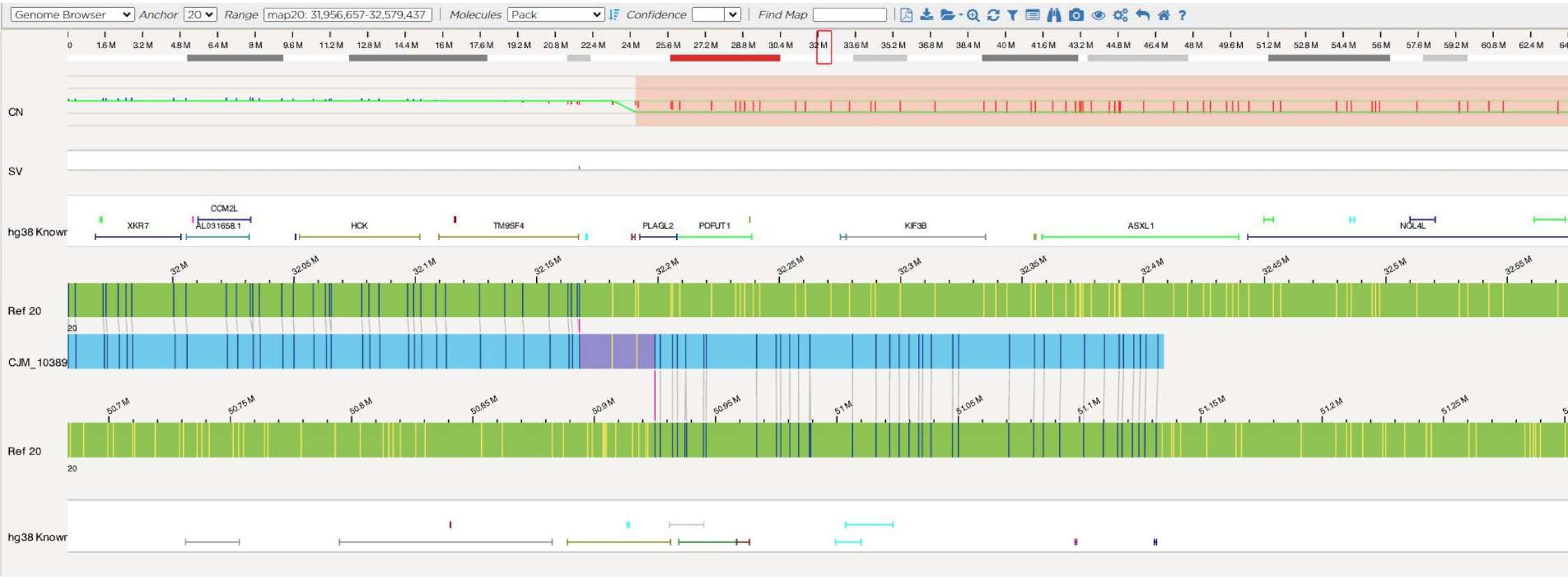


OGM

Rare Variant Pipeline analysis

1. Analysis CNVs

Analysis: variant review: example of a deletion

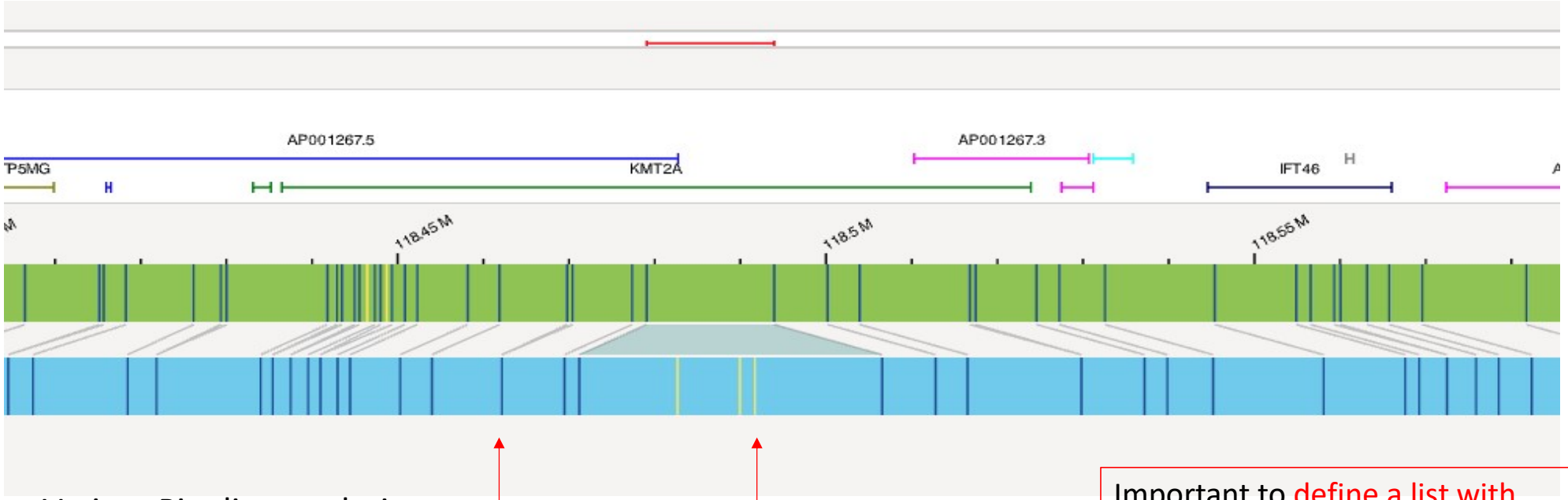


3685	11132	16	16	248524.8	275128.2	85.615.102,00	85.622.085,00	0.99	insertion	19620.4	0.42	CJM_1038	19621	3	GSE1	AC092127.1	19383.0	-	yes	65	-	ogm[GRCh38] ins(16;?)(q24.1;?)
4073	17852	20	20	223519.4	254658.4	32.167.630,00	50.925.034,00	0.99	translocation_intrachr	-1.0	0.44	CJM_1038	-1	-1	ADNP	TM9SF4	375.0	-	yes	71	http://ger	ogm[GRCh38] fus(20;20)(q11.21;q13.13)
4103	15482	21	21	241028.0	259736.8	5.562.690,00	7.071.865,00	0.0	trans_intrachr_segdupe	-1.0	0.11	CJM_1038	-1	-1	CU633967.1;C	Y_RNA	20751.0	CU633967.	yes	110	http://ger	ogm[GRCh38] fus(21;21)(p12;p11.2)

20q11.21q13.13(32167630_50925034)x1~2

Analysis: variant review: example of a duplication

- Male, 66 years old
- 90% blasts in bone marrow
- Flow: AML
- Karyotype: 46,XY,del(20)(q11q13)[10]
  Conclusion: pseudiploid clone with deletion 20q. Recurrent in myeloid malignancies. ELN 2022: intermediate risk
- OGM/Bionano: **Rare variant pipeline**



OGM

Rare Variant Pipeline analysis

2. Analysis of SVs

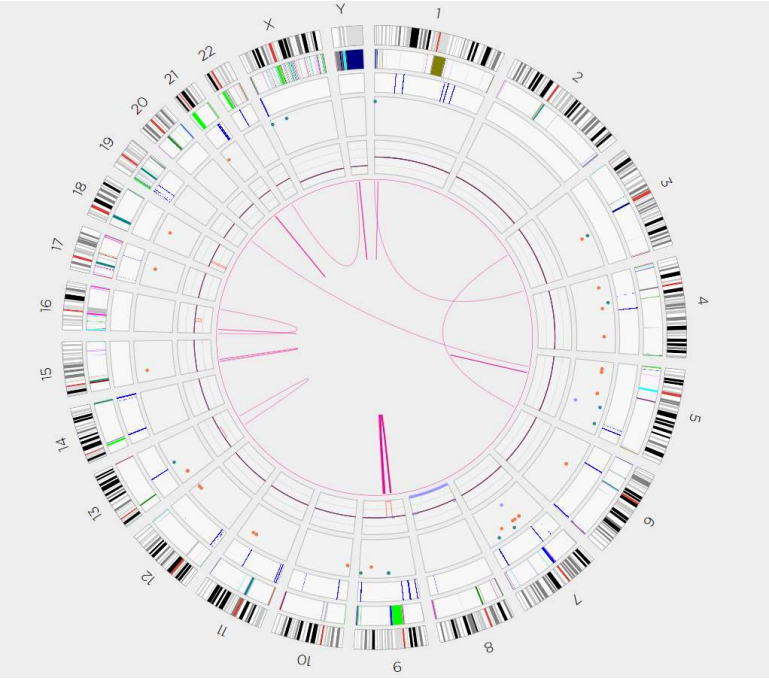
11q23.3q23.3(118470405_118479068)x3 [KMT2A-PTD]

Important to define a list with important genes per pathology: check this list in detail for each case!!

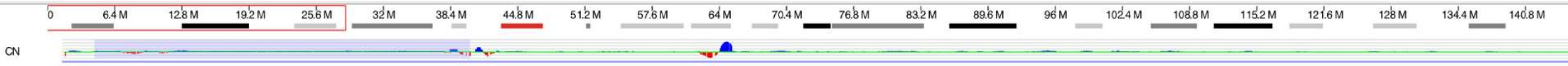
Analysis: allways check the “Whole Genome” view

Example of case with AML. Bone marrow contained clot, so needed to work with blood sample
Bone marrow: 40% blasts, blood: 22% blasts.

Trisomy 8 and deletion of 13q is much clearer in “whole genome” view than in circos plot



Genome browser: unclear if there is a trisomy 8:



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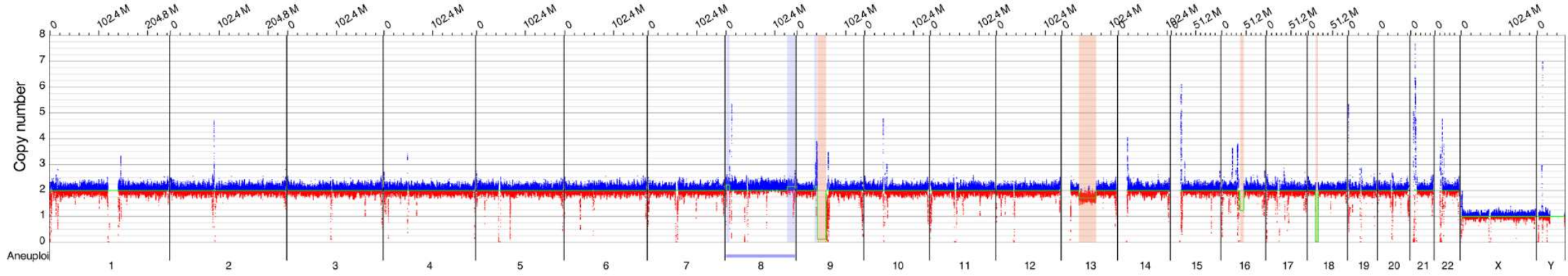
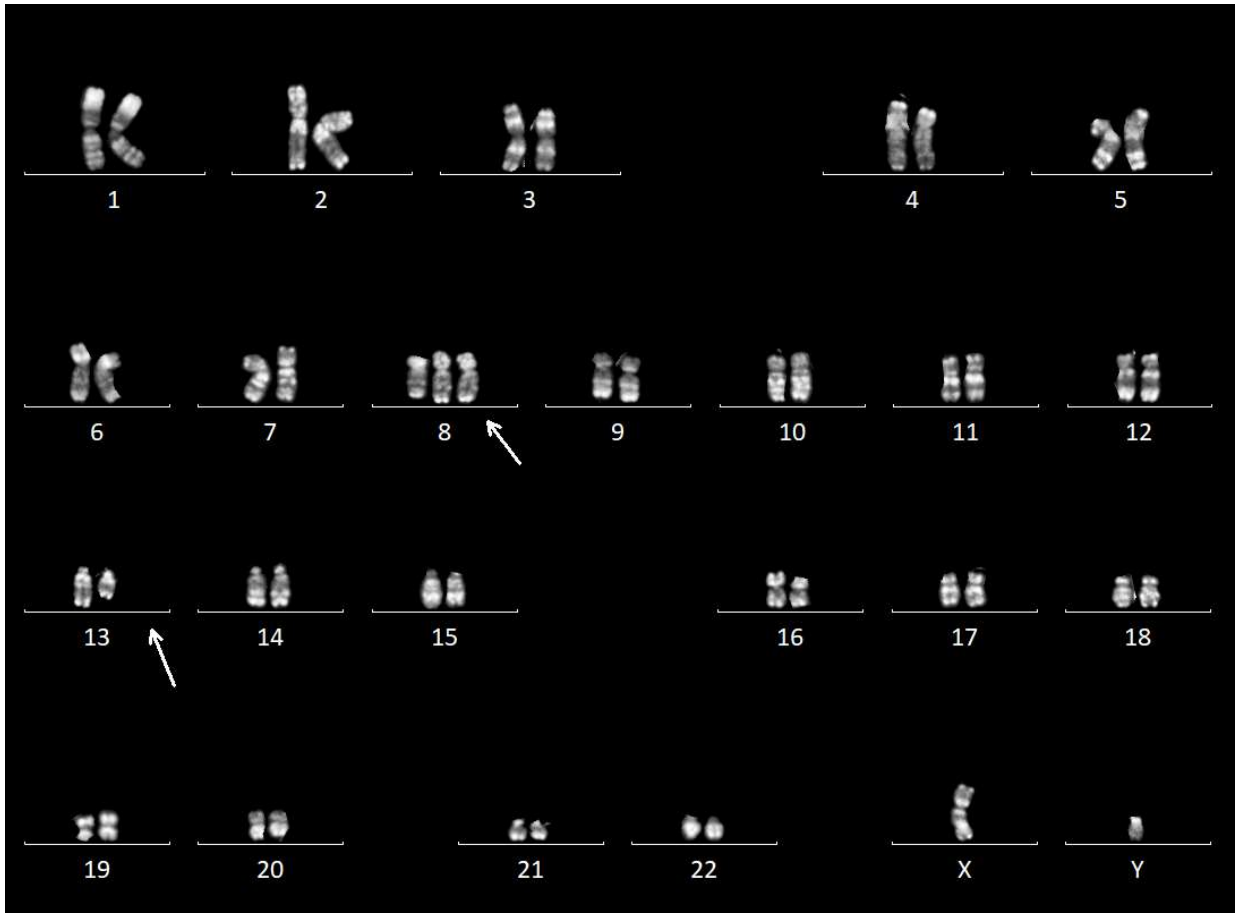
Example of case with AML
Trisomy 8 and deletion of 13q is much clearer in “whole genome” view than in circos plot



Analysis: allways check the “Whole Genome” view

Example of case with AML
Trisomy 8 and deletion of 13q: confirmed with conventional karyotype:
46,XY,del(13)(q13q22)[6]/47,sl,+8[2]/46,XY[2]



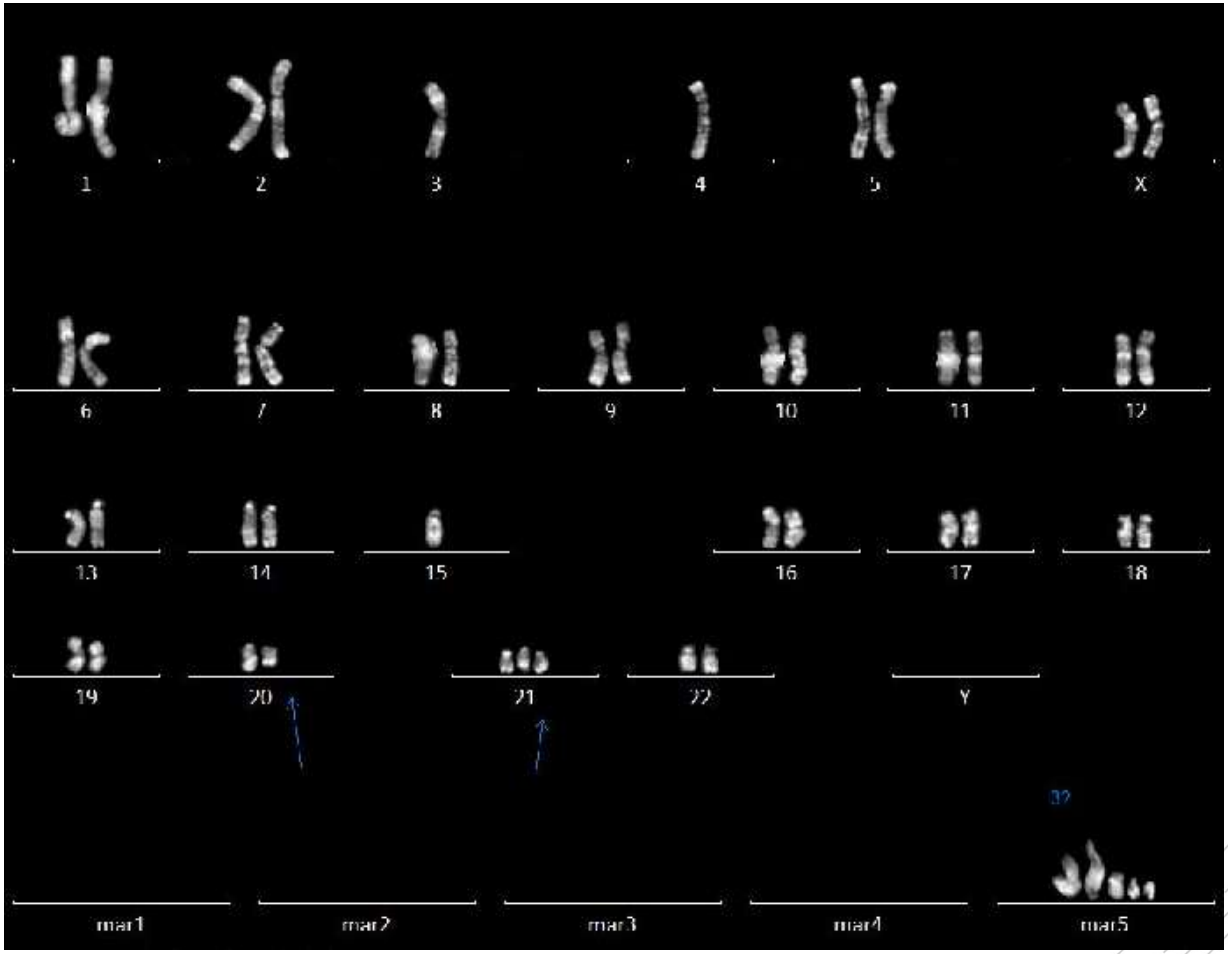
Del(13)(q13q22) in 8 out of 10 metaphases.

Subclone with trisomy 8 in 2 out of 10 metaphases.

CASE REPORT

Woman, 48 y
MDS-IB2 (13% bl)

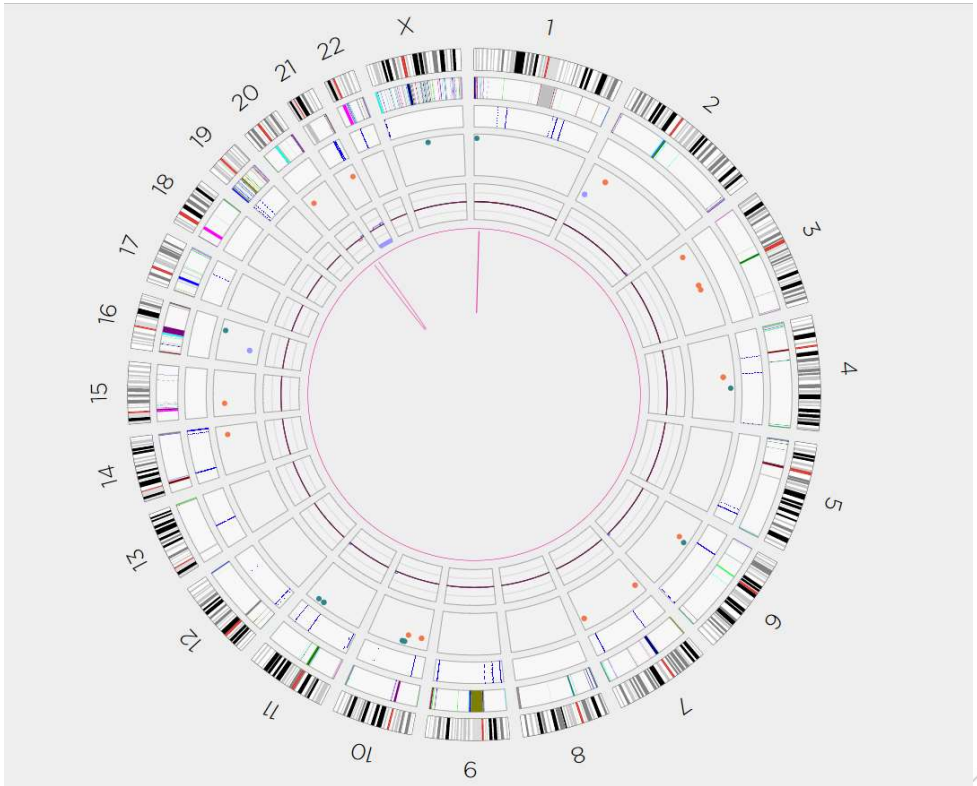
Conventional karyotype:

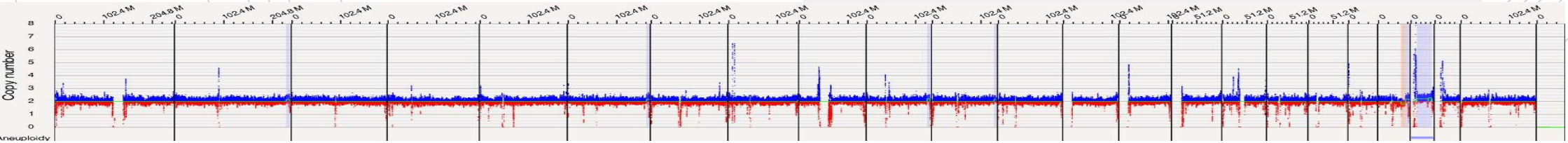


CASE REPORT

Woman, 48 y
MDS-IB2 (13% bl)

- **Conventional karyotype:** 47-49,XX,inc[2]
- OGM:



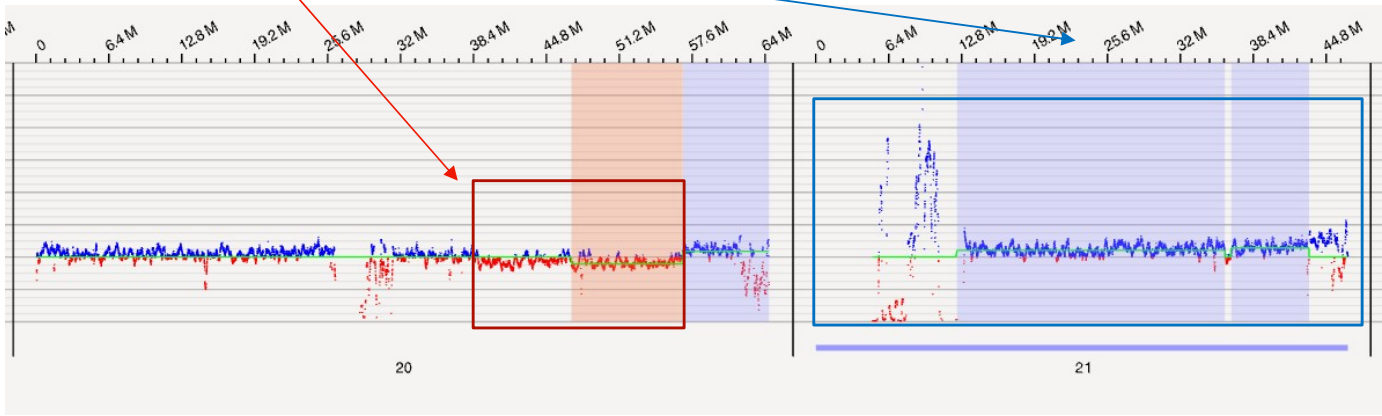


CASE REPORT

Woman, 48 y MDS-IB2 (13% bl)

- **Conventional karyotype:** 47-49,XX,inc[2]
- OGM:

ogm[GRCh38]
20q11.23q13.31(38709036_56550158)x1~2,
(21)x2~3,



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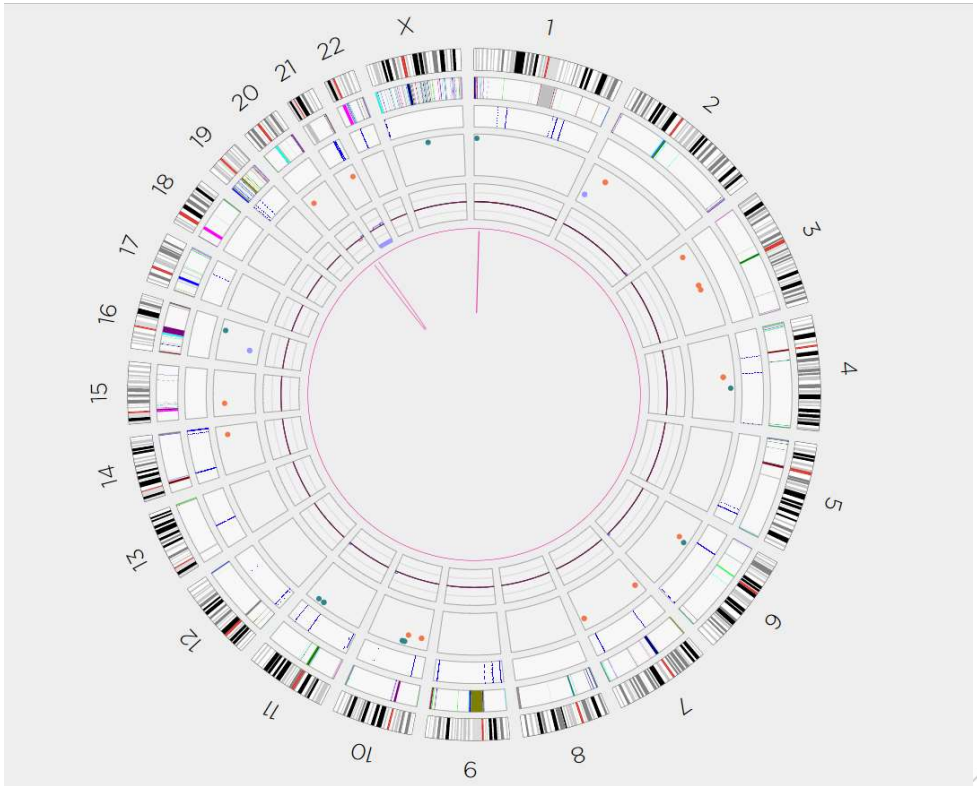

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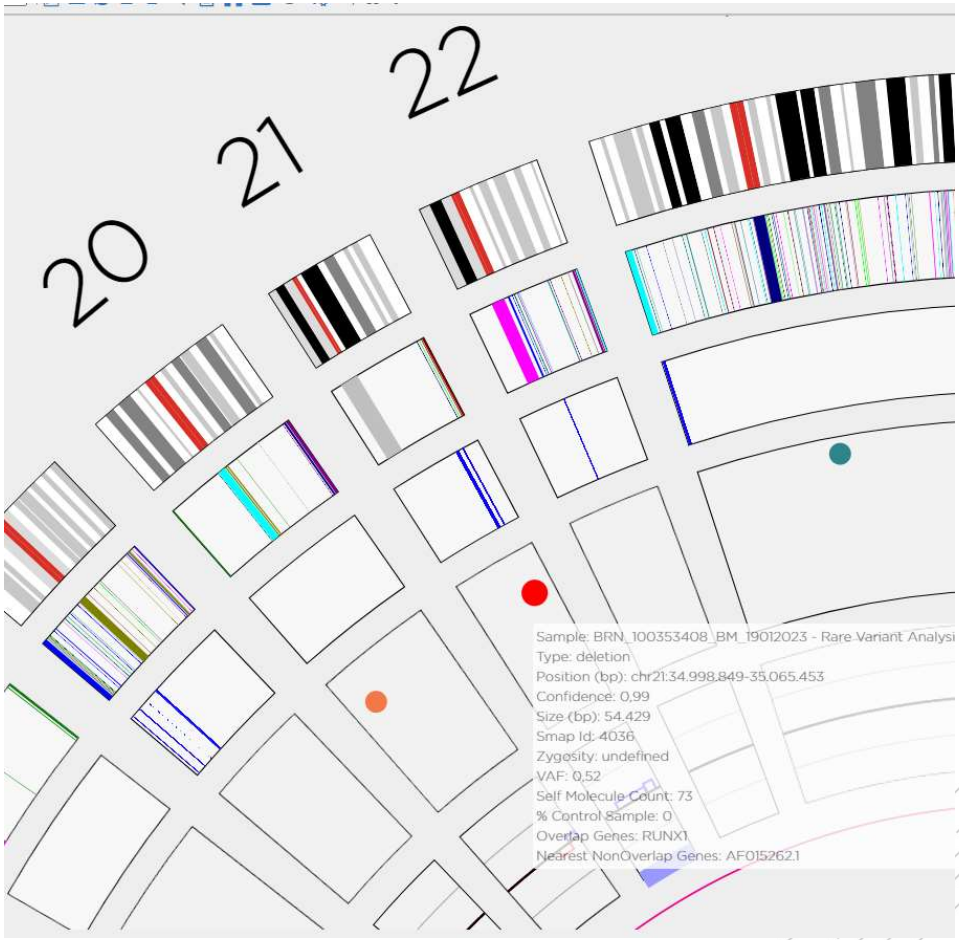

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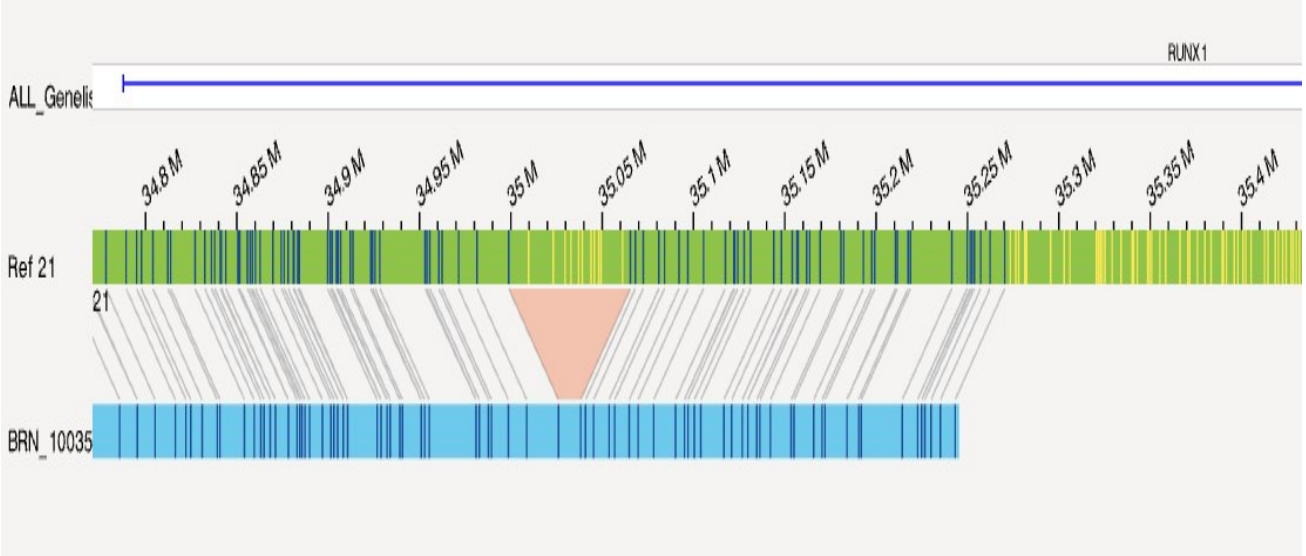

CASE REPORT

Woman, 48 y
MDS-IB2 (13% bl)

- **Conventional karyotype:** 47-49,XX,inc[2]
- OGM:

ogm[GRCh38]
20q11.23q13.31(38709036_56550158)x1~2,
(21)x2~3,
21q22.12(34998849_35065453)x1~2, [*RUNX1* exon 1-2; NM_001754.4]

⇒ **Loss of exon 1-2 of the RUNX1 gene**
⇒ **Loss of function type; tumor suppressor gene RUNX1**
⇒ **Included in IPSS-M, major impact prognosis**

CASE REPORT

Woman, 48 y MDS-IB2 (13% bl)

⇒ Such deletions also occur (constitutionally) in families with platelet disorders and/or predisposition to myeloid hematologic malignancies

- A constitutional abnormality cannot be excluded in this case.
- To be integrated with
  - the family history
  - personal history (previous thrombocytopenia, cfr "ITP" since 2015).
  - the constitutional character could be investigated by MLPA on hair if induction not initiated (a dozen with bulb, case discussed with Dr Sc H Brems).

(Ref: Song et al. 1999, Nature Genetics, 23: 166-175; Brown et al. 2020, Blood Adv, 4:1131-1144; Almazni et al. 2021, Platelets, Feb22:1-4).

CASE REPORT

Woman, 48 y MDS-IB2 (13% bl)

⇒ DNA (fibroblasts)

- Analysis with MLPA (SALSA MLPA P437-B1)

- Deletion in *RUNX1* DETECTED with MLPA in DNA from cultured fibroblasts

(Ref: Song et al. 1999, Nature Genetics, 23: 166-175; Brown et al. 2020, Blood Adv, 4:1131-1144; Almazni et al. 2021, Platelets, Feb22:1-4).

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CME

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International Consortium on OGM
Adam Smith *et al.*