Bionano for dummies

MB&C Course UCLL Workshop session-2
February 8th, 2024

Barbara Dewaele, PhD

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Clinical Laboratory Geneticist

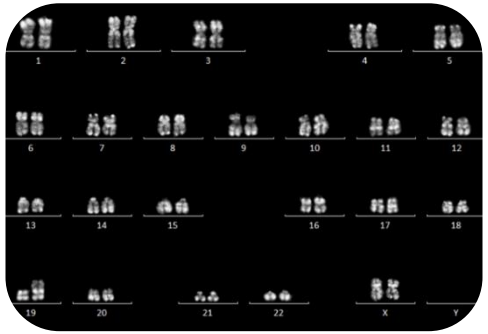
Supervisor of the Laboratory for Genetics of Hematological Malignancies

Center for Human Genetics, University Hospitals Leuven

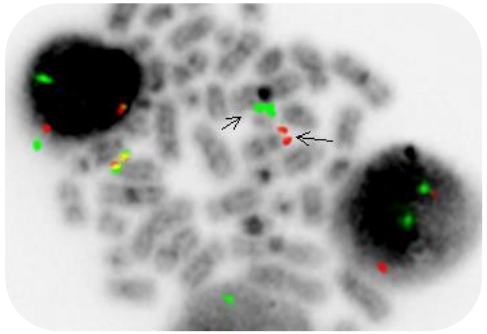
KU LEUVEN

CME

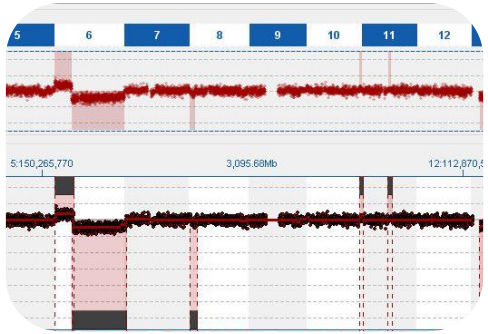
Current routine cytogenetic and molecular genetic testing procedures



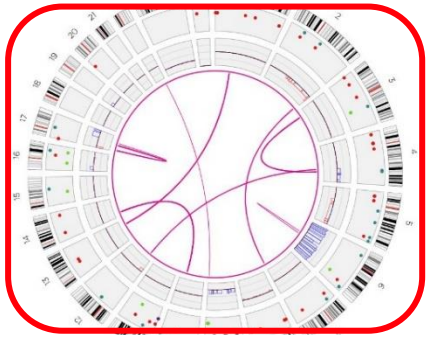
Karyotyping



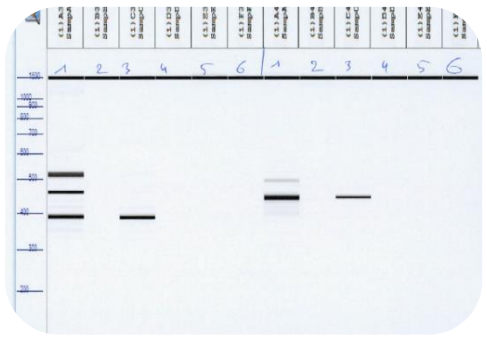
FISH



LPS or aCGH/SNPa

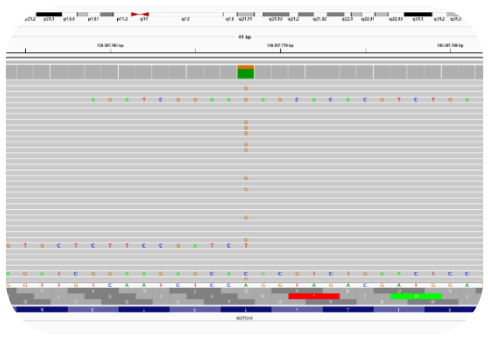


OGM



PCR

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



NGS targeted gene panel

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], Corrie 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

e of Medical Genetics, Center for Pathobiochemistry and Genetics, Medical University of Vienna.
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

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

IJC INTERNATIONAL JOURNAL of CANCER
itations: 5

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

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

enoist [4], /an Beek [5], huis [5], Wed Majdali [4], [5], Damien Sanlaville [7], ne Schluth-Bolard [7],

CANCER GENETICS AND EPIGENETICS

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

Wanda M. Gerding[1] | Marco Tembrink[1] | Verena Nilius-Eliliwi[1,2] |
Thomas Mika[2] | Fotic
Matthias Eckhardt[2] | M
Peter Reimer[3] | Roland

ORIGINAL ARTICLE
Molecular Genetics & Genomic Medicine
WILEY

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]

assessment of 52 hematological malignancy genomes by optical genome mapping

Kornelia Neveling [1], Tuomo Mantere [2], Susan Vermeulen [3], Michiel Oorsprong [3], Ronald van Beek [3], Ellen Kater-Baats [3], Marc Pauper [3], Guillaume van der Zande [3], Dominique Smeets [3], Daniel Olde Weghuis [3], Marian J.P.J. Stevens-Kroef [3], Alexander Hoischen [4]

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

TEST OF THE MONTH

AJH WILEY

Optical genome mapping for structural variation analysis in hematologic malignancies

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

Validation of Optical Genome Mapping for the Molecular Diagnosis of Facioscapulohumeral

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

now, Deqin

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 9], Lucienne Michaux [1], Barbara Dewael

Affiliations + expand

PMID: 35119131 DOI: 10.1002/ajh.26487

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
RESEARCH ARTICLE | NOVEMBER 23, 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 (Toronto General, Toronto Western, Princess Margaret, Toronto Rehab, Michener Institute)

Dr. Brynn Levy
COLUMBIA

Dr. Gordana Raca
Children's Hospital LOS ANGELES

Dr. Nikhil S. Sahajpal
Greenwood Genetic Center

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

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

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.

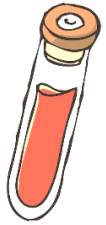
Review > Am J Hematol. 2024 Jan 2. doi: 10.1002/ajh.27175. Online ahead of print.

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

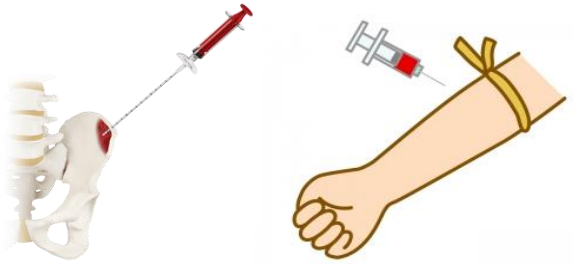
Brynn Levy [1], Rashmi Kanagal-Shamanna [2], Nikhil S Sahajpal [3], Kornelia Neveling [4] [5], Katrina Rack [6], Barbara Dewaele [6], Daniel Olde Weghuis [4], Marian Stevens-Kroef [4], Anna Puiggros [7] [8], Mar Mallo [9], Benjamin Clifford [10], Tuomo Mantere [11], Alexander Hoischen [4] [5] [12] [13],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

Optical Genome Mapping: wet laboratory workflow

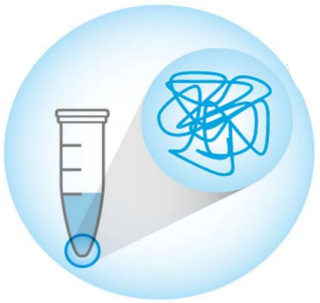
Count WBC and take 1,5 million cells

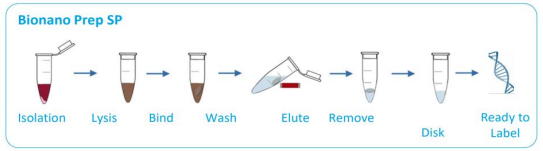


Bone marrow or blood (if invaded)

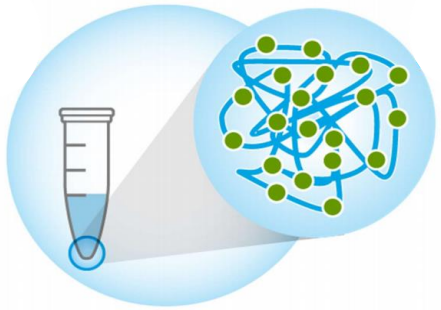


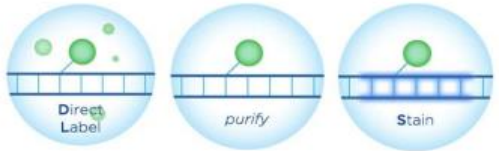
Extraction of UHMW DNA



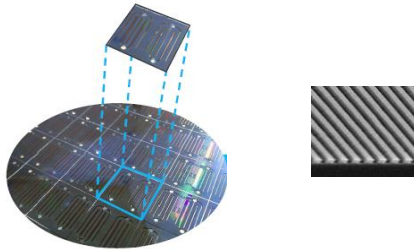


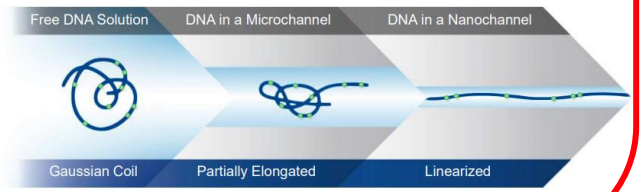
Label DNA at specific motifs



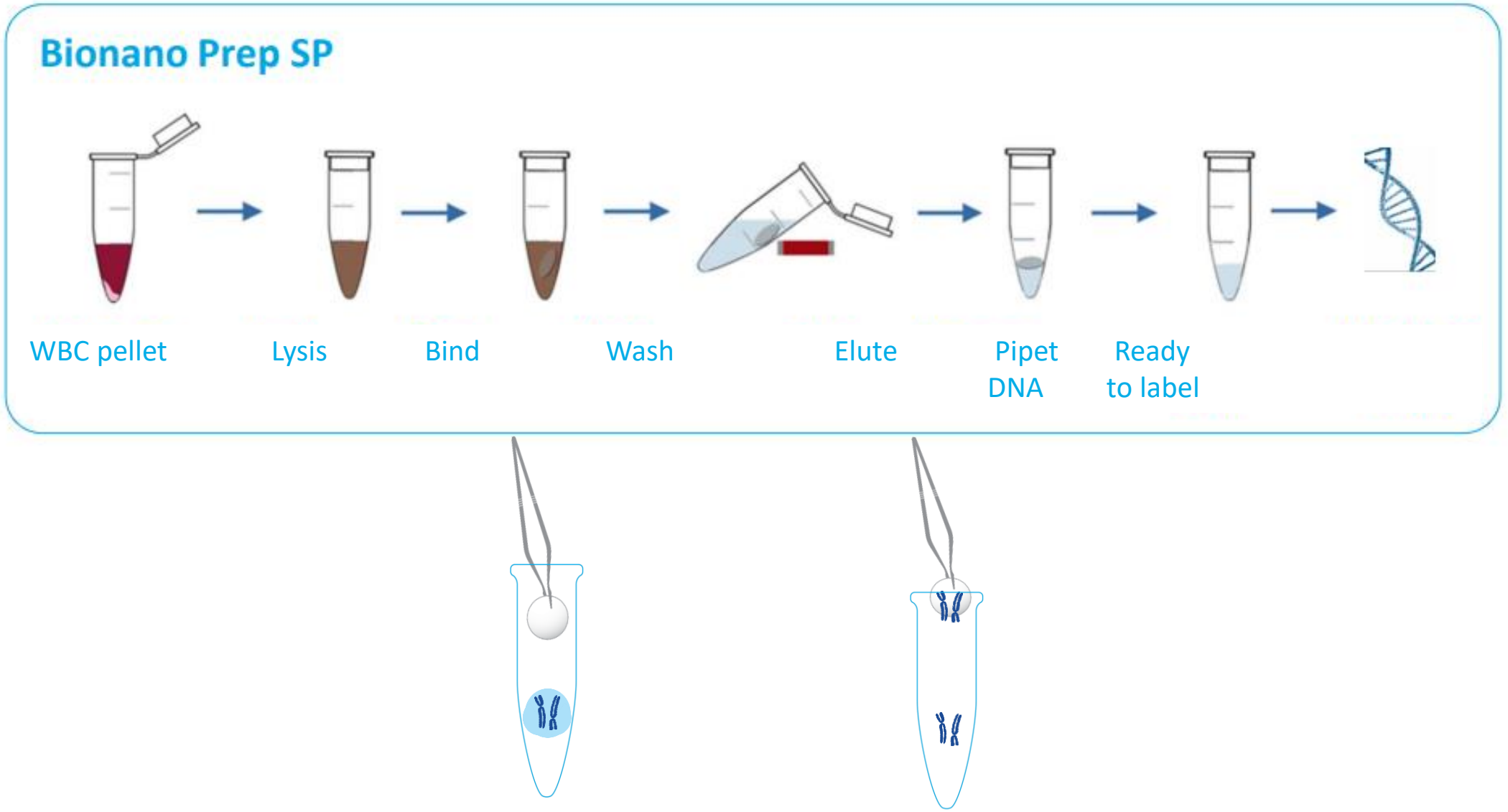


Transfer labeled DNA into Chip





Optical Genome Mapping: wet laboratory workflow



OGM requires extremely long molecules

Only dsDNA molecules that are longer than 150 kbp are assembled

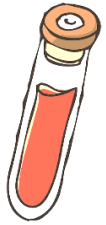
Sample selection, proper storage and preservation are critical

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



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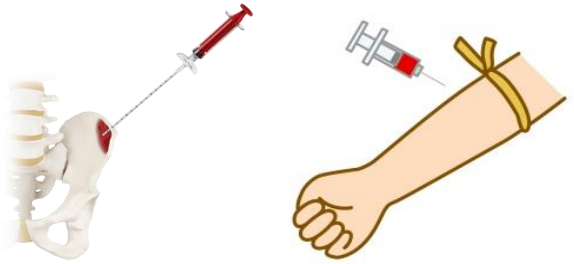

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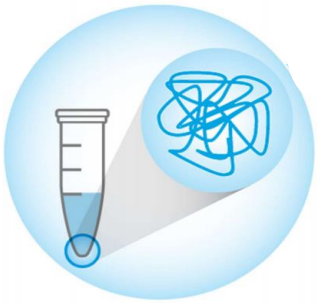

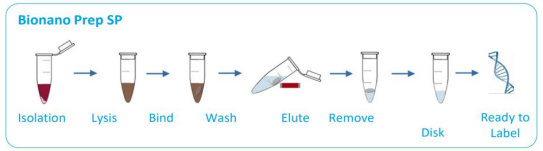

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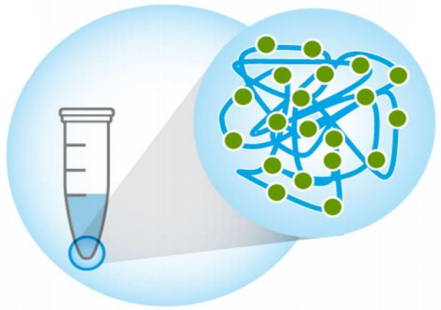
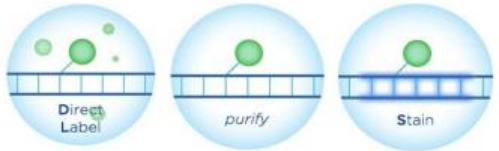
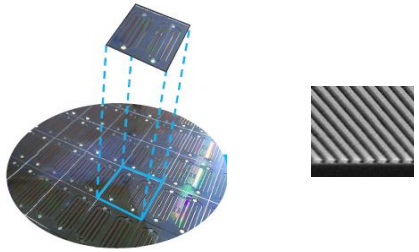
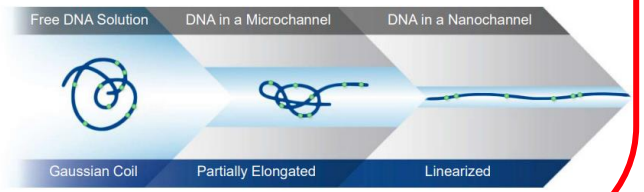




Transfer labeled DNA into Chip



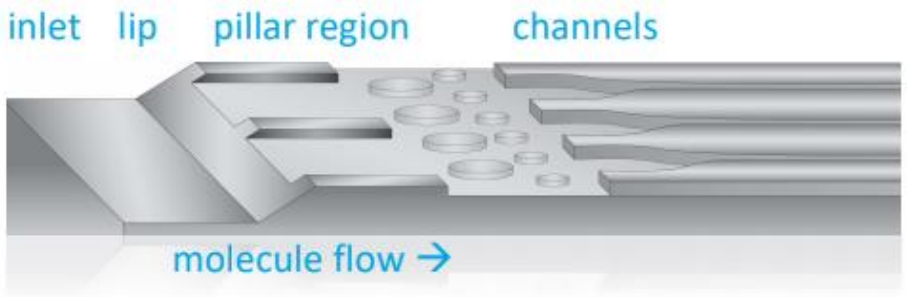


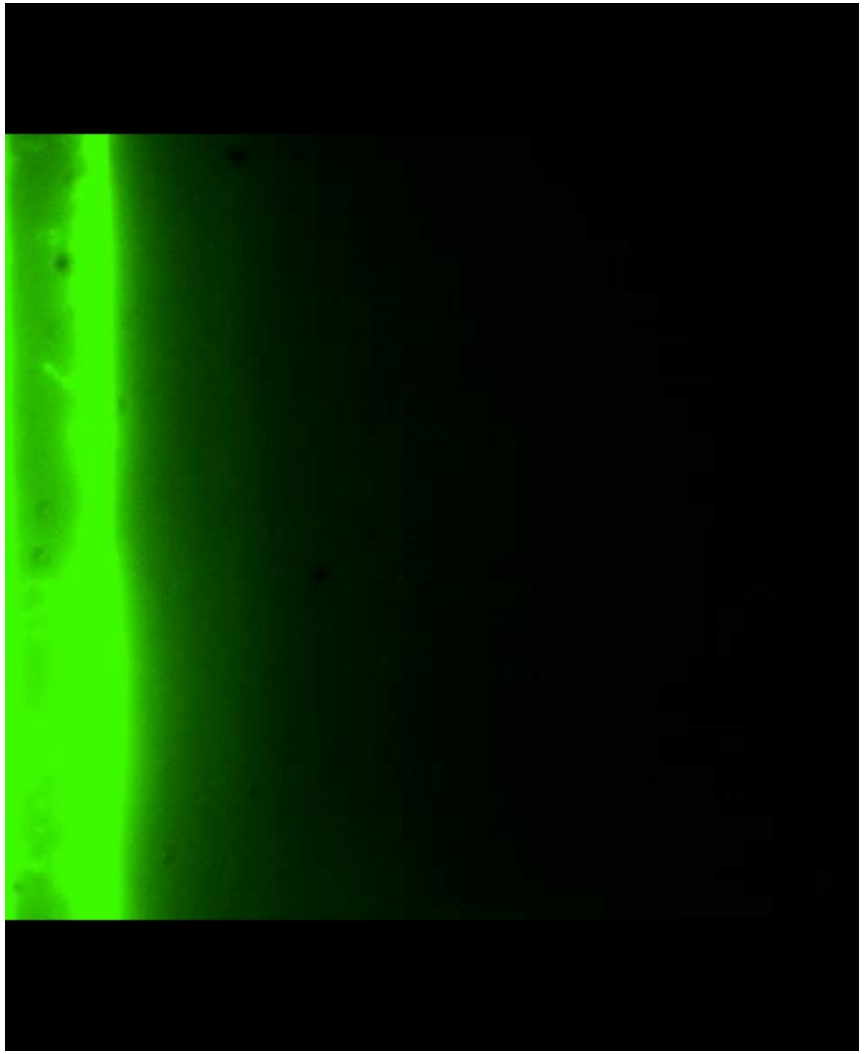
OGM is not sequencing based: visualisation of intact DNA molecules

Nanochannel Arrays on Silicon

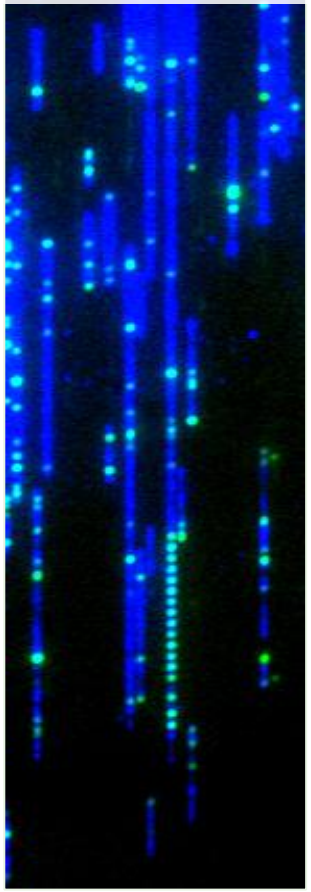
The Saphyr Chip

- 120,000 parallel Nanochannels linearize long DNA in solution
- Leverages mature semiconductor manufacturing





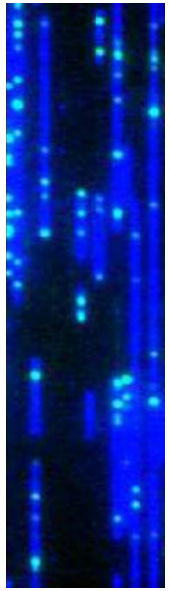
DNA couterstaining
DLS labels



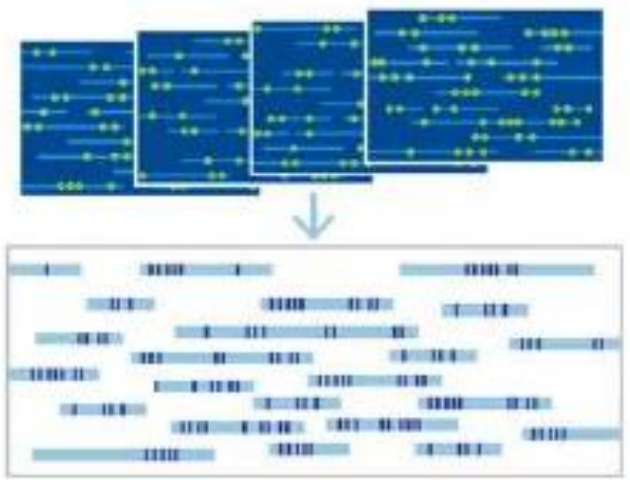
Length of DNA molecules:
150kb – 2.5Mb,
median size >350 kb

Optical Genome Mapping: data analysis: De Novo Assembly Pipeline

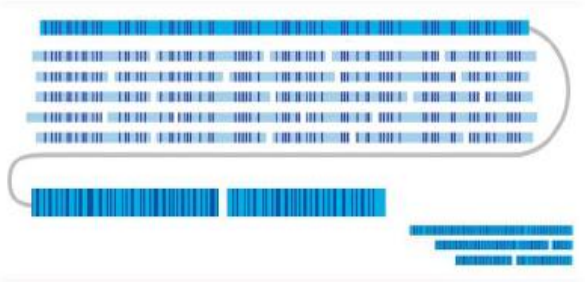
Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



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

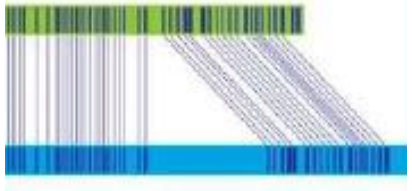


Algorithms align different molecules for constructing Consensus Genome Maps (.cmap files)



Cross mapping across a Reference

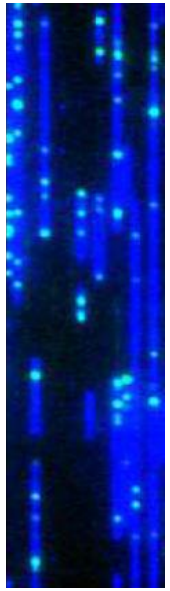
- Copy Number Aberration (CNA) profile
- Structural Variant (SV)



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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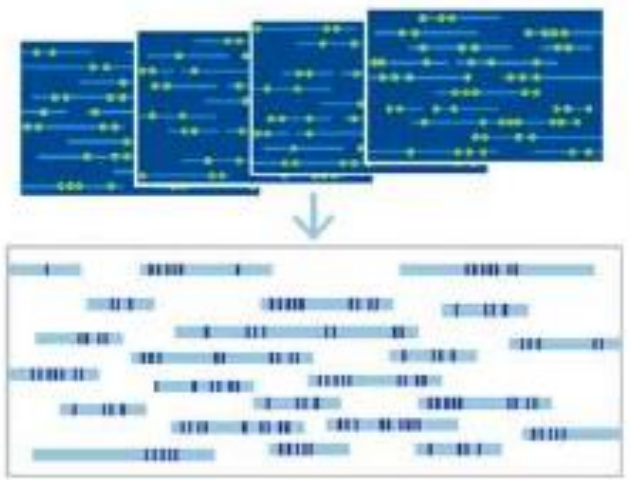

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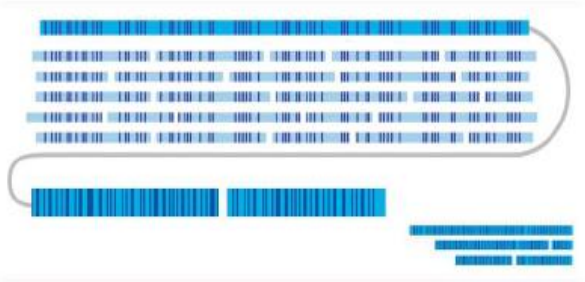

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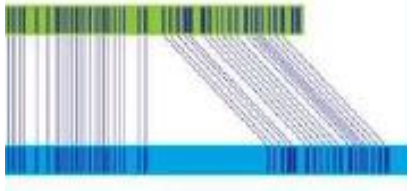
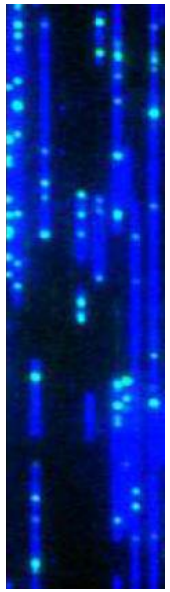
- Structural Variant (SV)



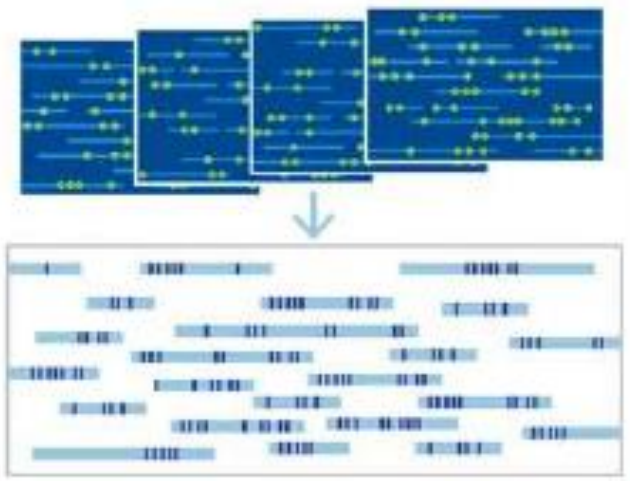
insertion

Optical Genome Mapping: data analysis: Rare Variant Analysis Pipeline

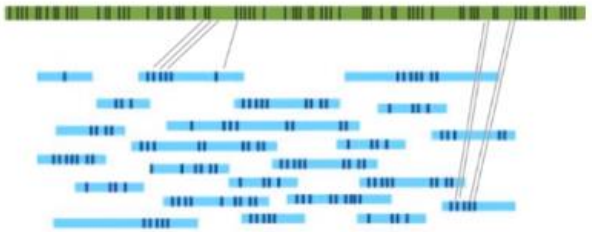
Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



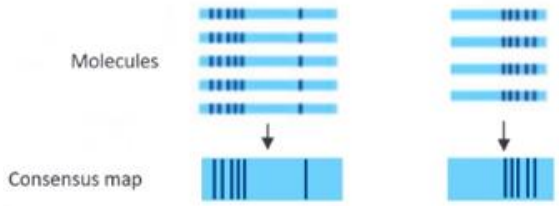
Algorithms convert the raw images into .bnx files
=> population of molecules



Single molecule alignment to the reference



Molecules with SVs are clustered



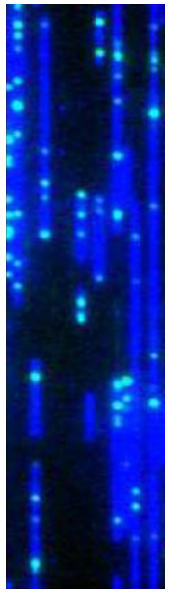
Local alignment to reference to confirm SV

- CNA profile
- SV

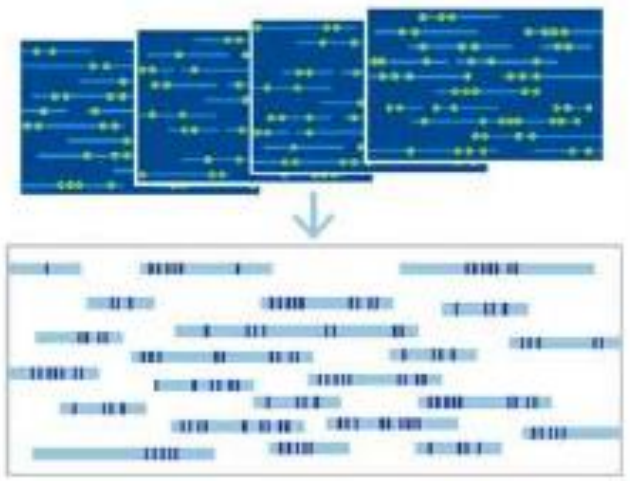
insertion

Optical Genome Mapping: data analysis: Rare Variant Analysis Pipeline

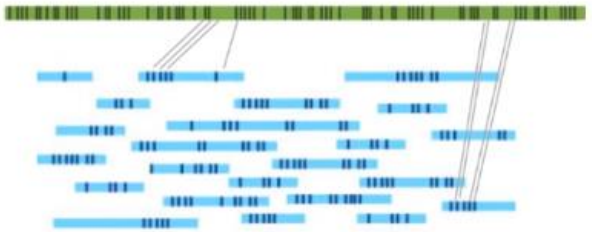
Raw Image Data: Direct observation of DLS labels on long DNA molecules
(150 kb up to 3 Mb)



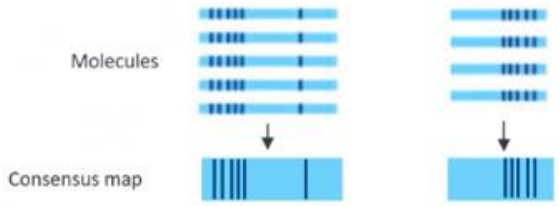
Algorithms convert the raw images into .bnx files
=> population of molecules



Single molecule alignment to the reference



Molecules with SVs are clustered



Local alignment to reference to confirm SV

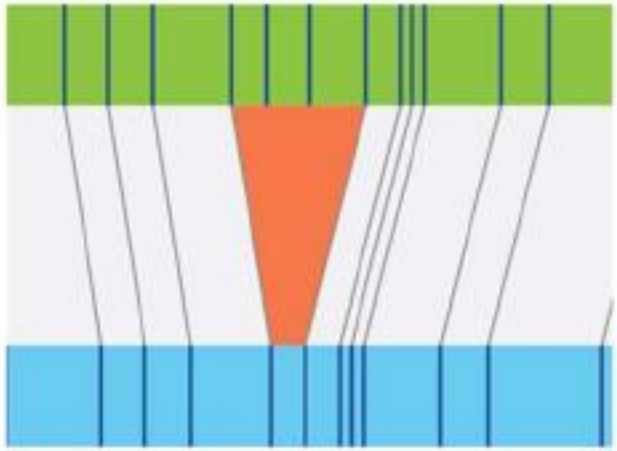
- CNA profile
- SV

insertion

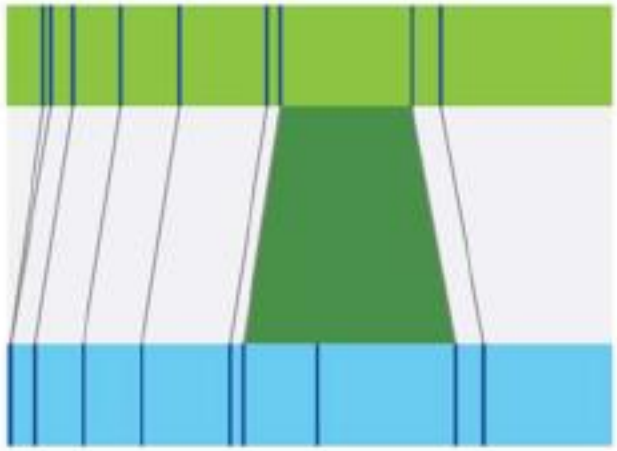
Optical Genome Mapping: calling structural aberrations

GAINS/LOSSES

Deletion

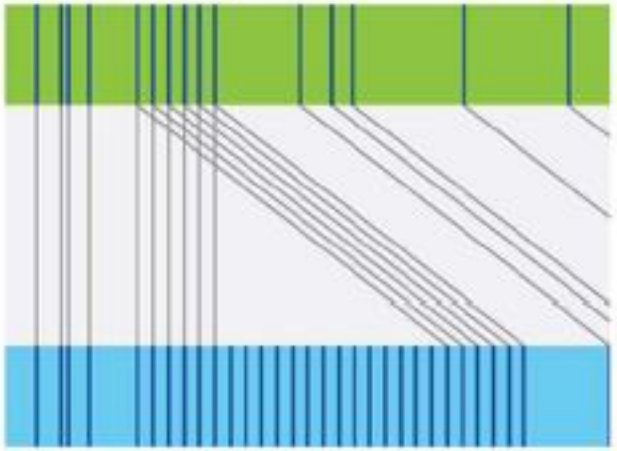


Insertion

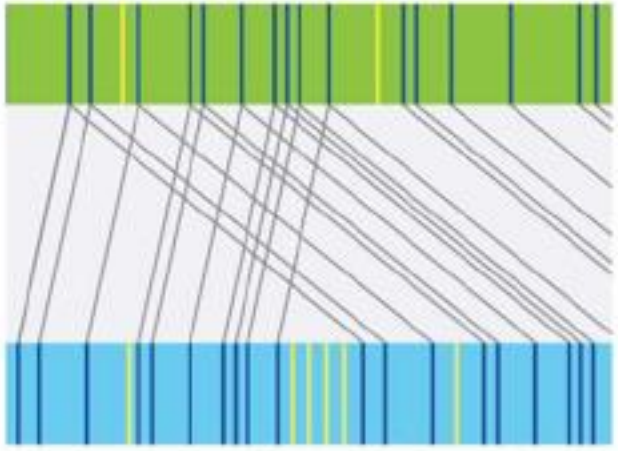


COPY NUMBER CHANGES

Repeat array expansion

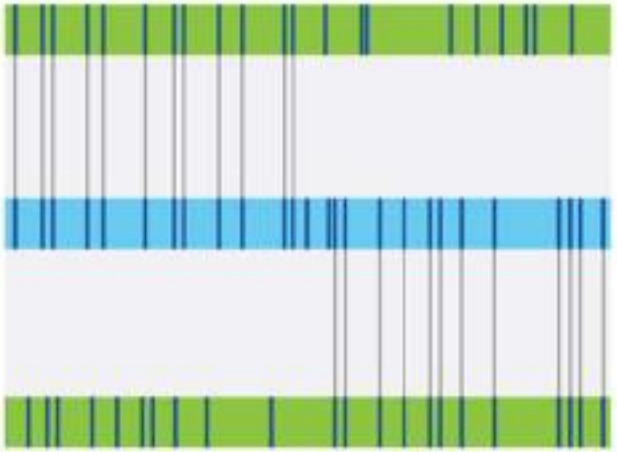


Tandem duplication

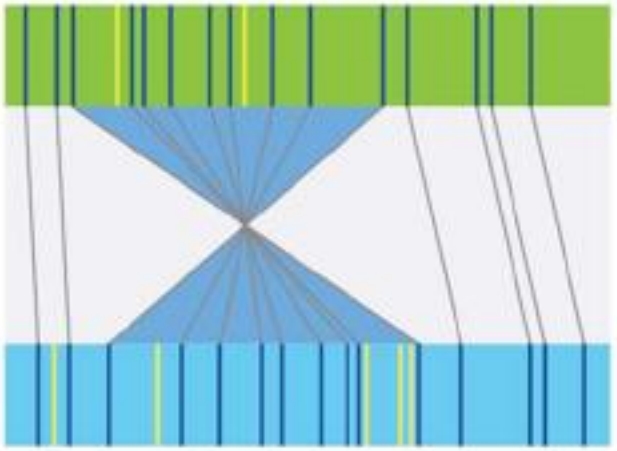


BALANCED

Translocation



Inversion



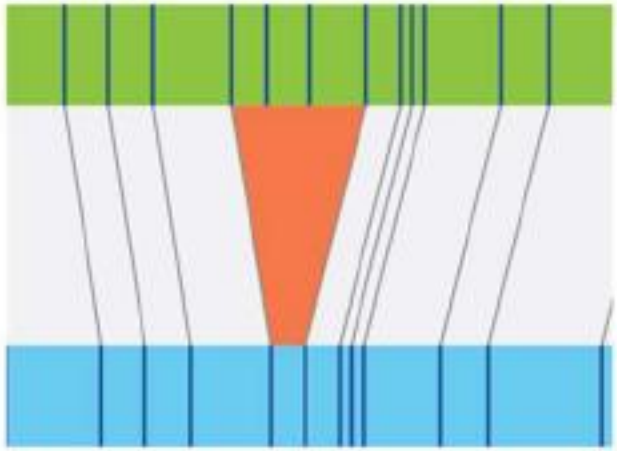
Reference genome (green)

OGM map sample (blue)

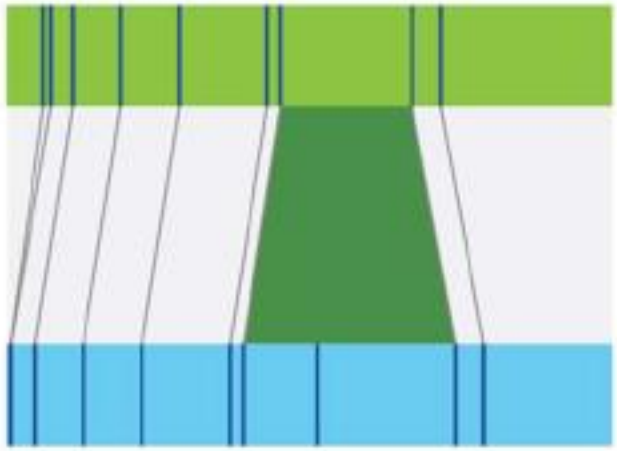
Optical Genome Mapping: calling structural aberrations

GAINS/LOSSES

Deletion



Insertion



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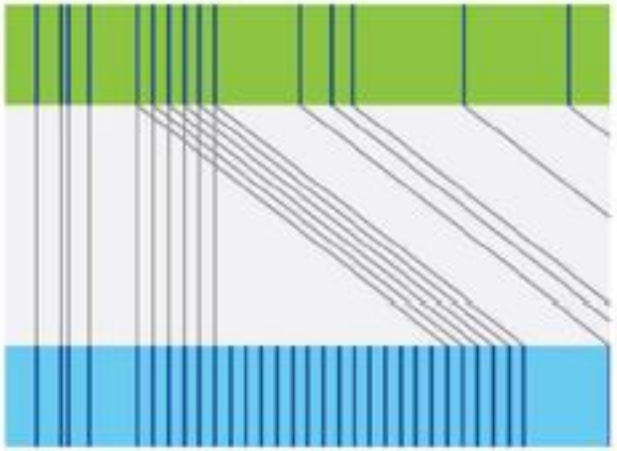

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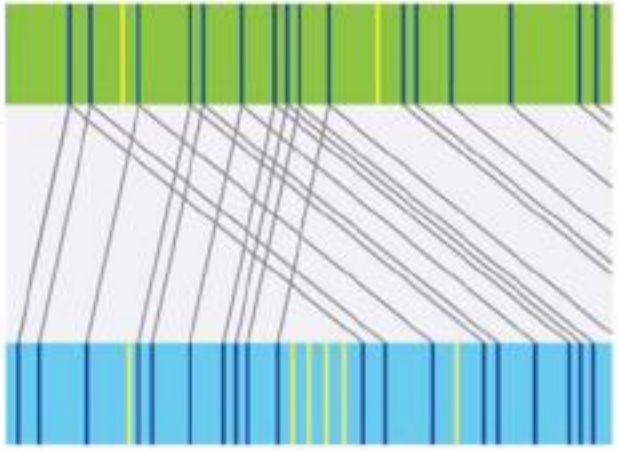

BALANCED

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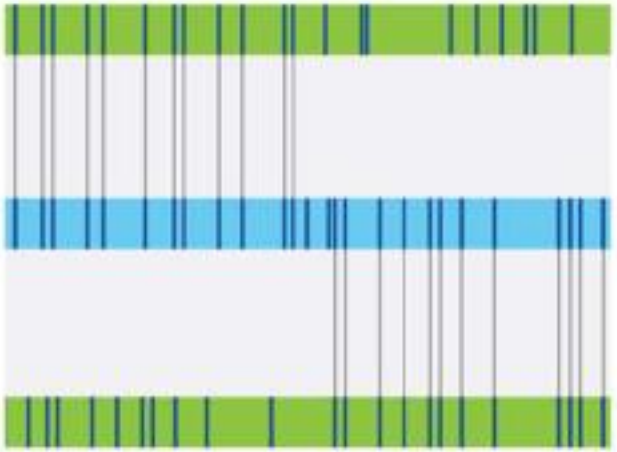
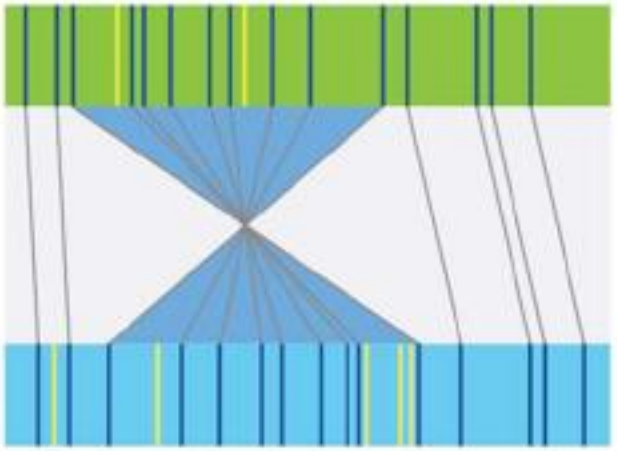


Inversion

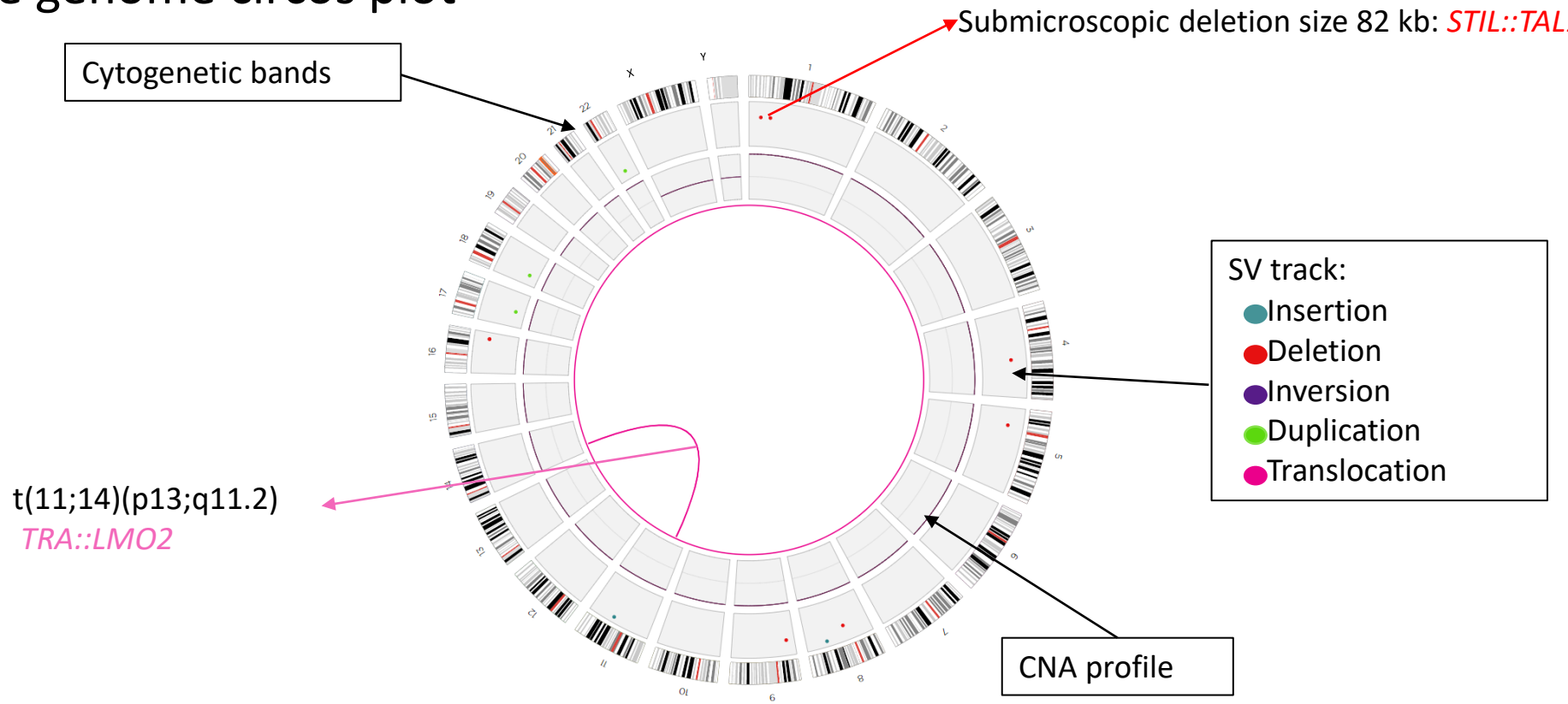


Reference genome (green)

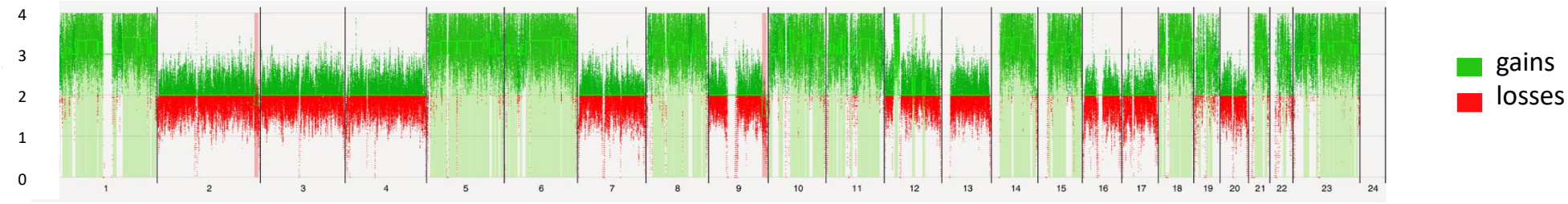
OGM map sample (blue)

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

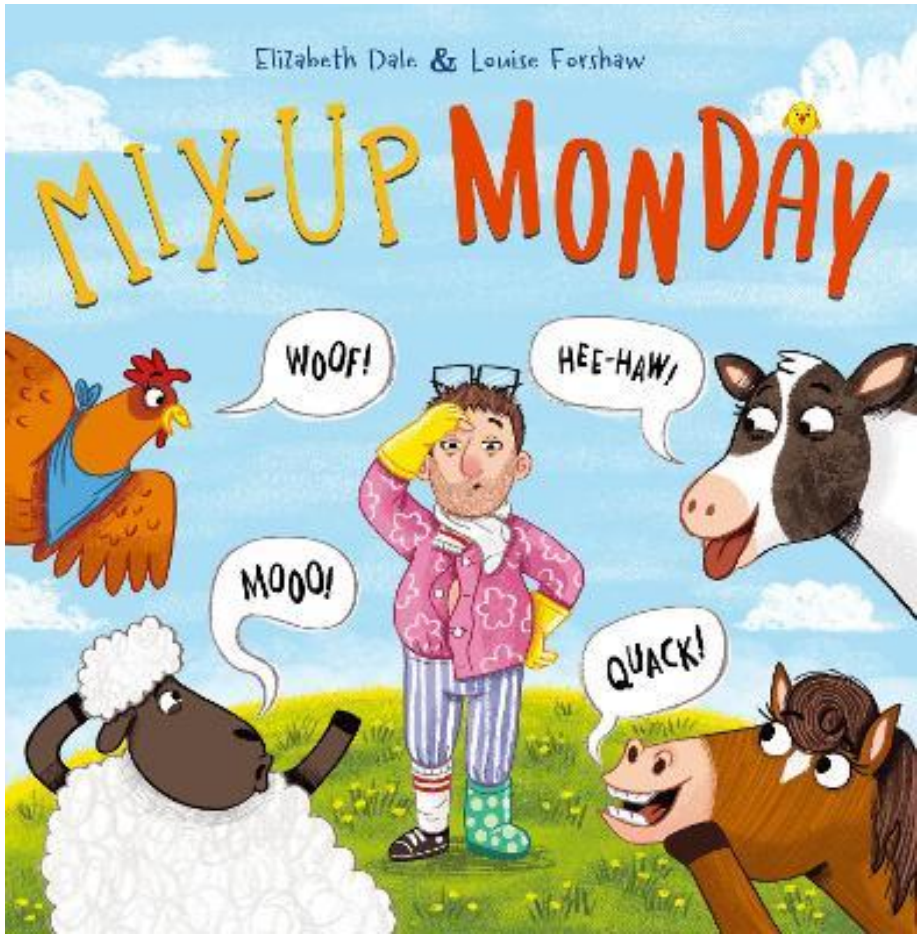
Quality control parameters

- 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 during the analytical phase

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

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 $= \frac{\text{total DNA} \times [\text{map rate}]}{\text{reference size}}$ 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 during the analytical phase

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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	

Quality control parameters

- Bioinformatic pipelines

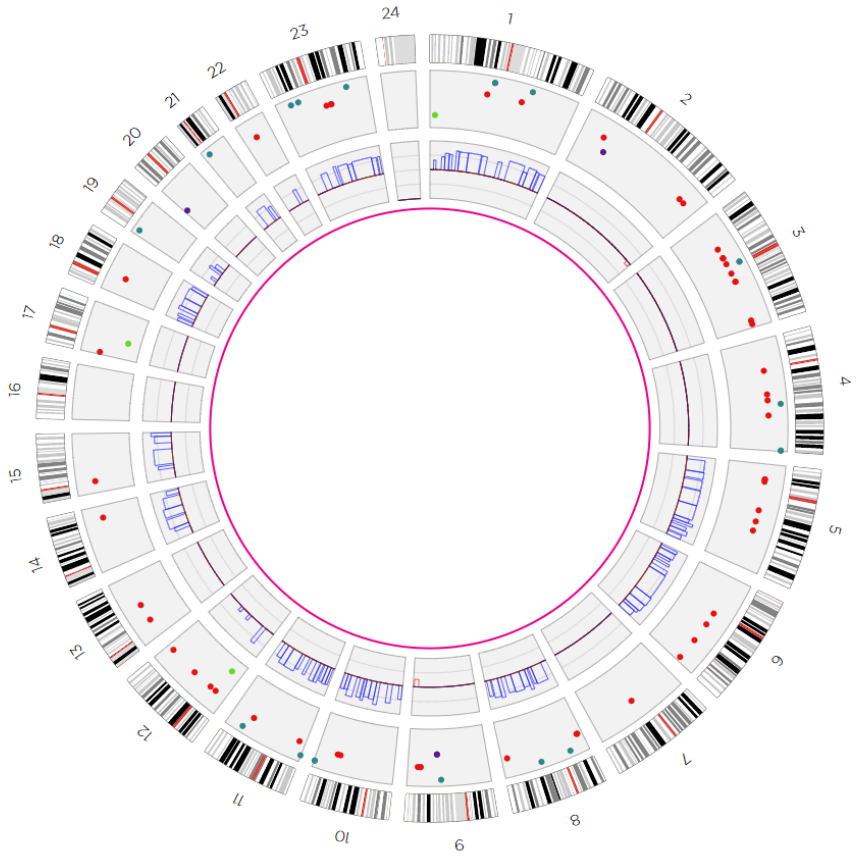
	Lower size limit	LOH	LLOD
De Novo Assembly	500 bp	yes	20-25% VAF
RVA	5 kb • Insertions: 5-50 kb • Deletions: > 7 kb • Translocations: ≥ 70 kb • Inversions: ≥ 100 kb • Duplications: ≥ 150 kb	no	5% VAF at 300x coverage

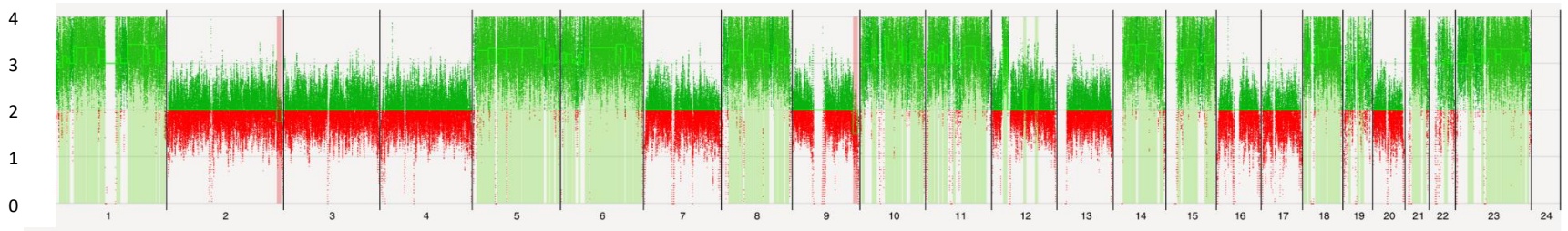
=> the **"De Novo Assembly"** pipeline is required for the analysis of **Acute Lymphoblastic Leukemia** cases!!!
=> the best is to also run an RVA to be able to pick up the aberrations present at low VAF

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

Example 1: 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

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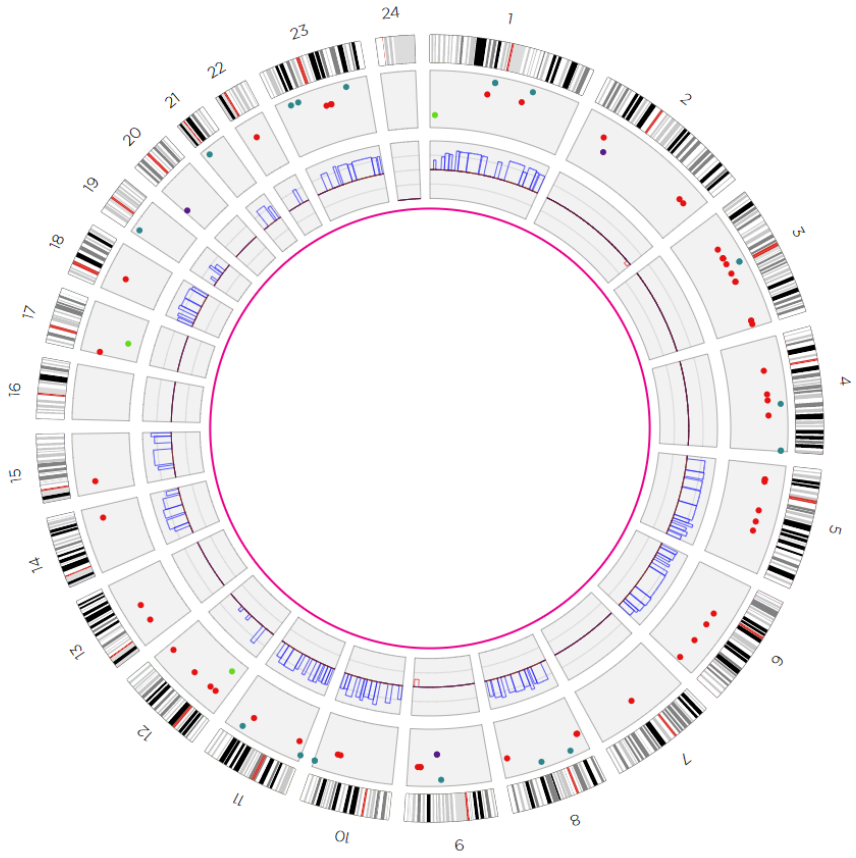
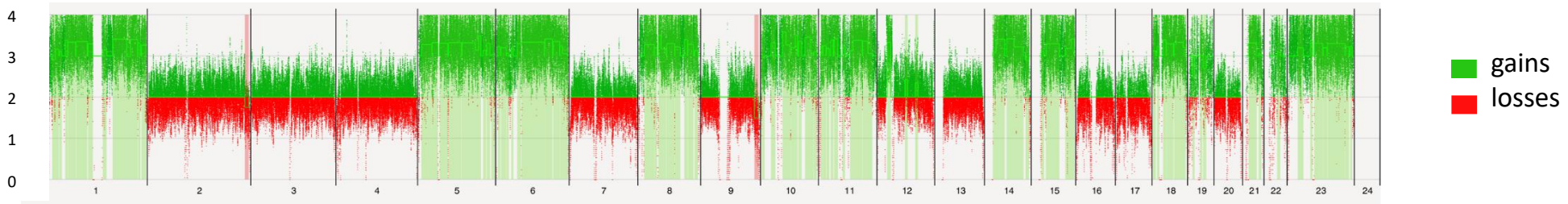


- Female, 18 years old
- 69% blasts in blood
- Karyotype: normal: late sample receipt
- FISH: monoallelic loss of 9q34 and 12p13 and monosomy 7
- CGH array: 36,XX,-2,-3,-4,-7,-9,-12,-13,-16,-17,-20
  => low hypodiploidy
  => high risk



Treatment protocols in ALL require extensive genetic testing:

Aberrations with clinical significance in terms of risk:

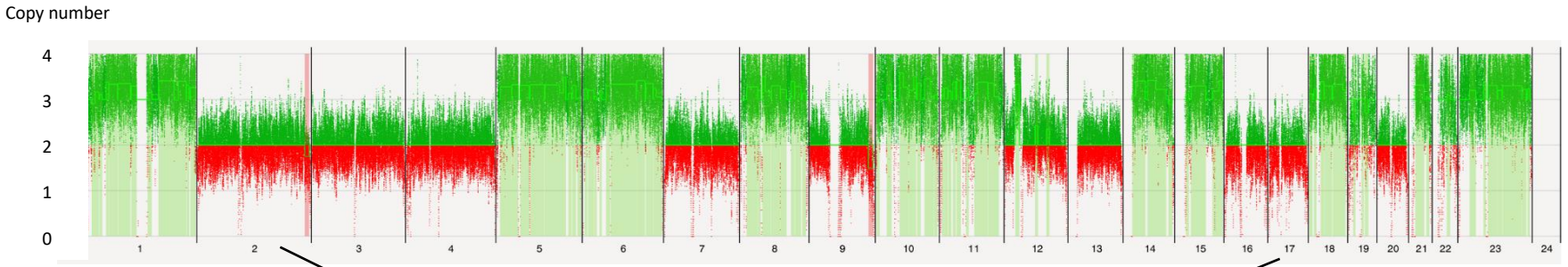
Good risk abnormalities	*Standard risk abnormalities*	*Intermediate risk abnormalities*	*High risk abnormalities*
High hyperdiploidy (>50chr)	t(1;19)(q23;p13) *TCF3::PBX1*	t(X;14)(p22;q32)/t(Y;14)(p11;q32) *IGH::CRLF2*	Near haploidy (25-29 chr)
TAL1 abnormalities]	15q13-15 rearrangements	del(X)(p22.33)/del(Y)(p11.32) *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

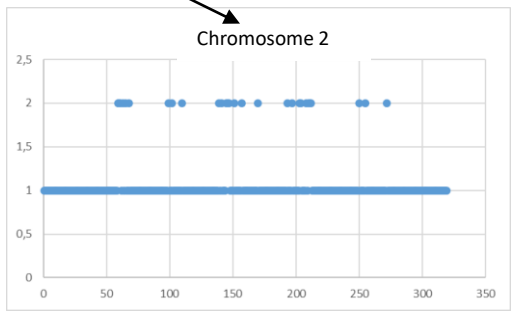
- recurrent structural rearrangements
- whole chromosome CNA
- submicroscopic deletions

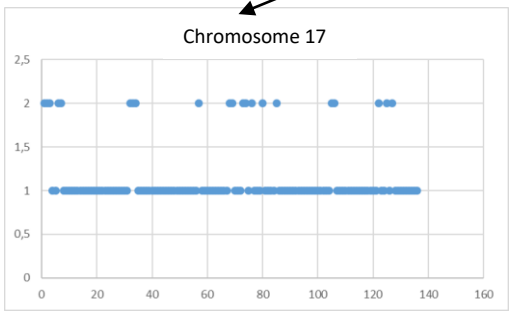
Table 1
Adeapted from: Cancer cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells, Foutrh Edition. Page 202-204.

Example 1: correction baseline based on zygosity of structural variants

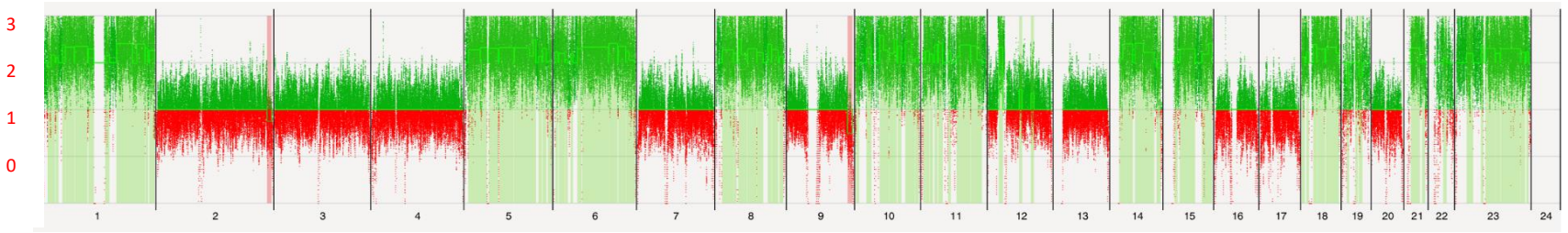


gains
losses





almost no heterozygous variants



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

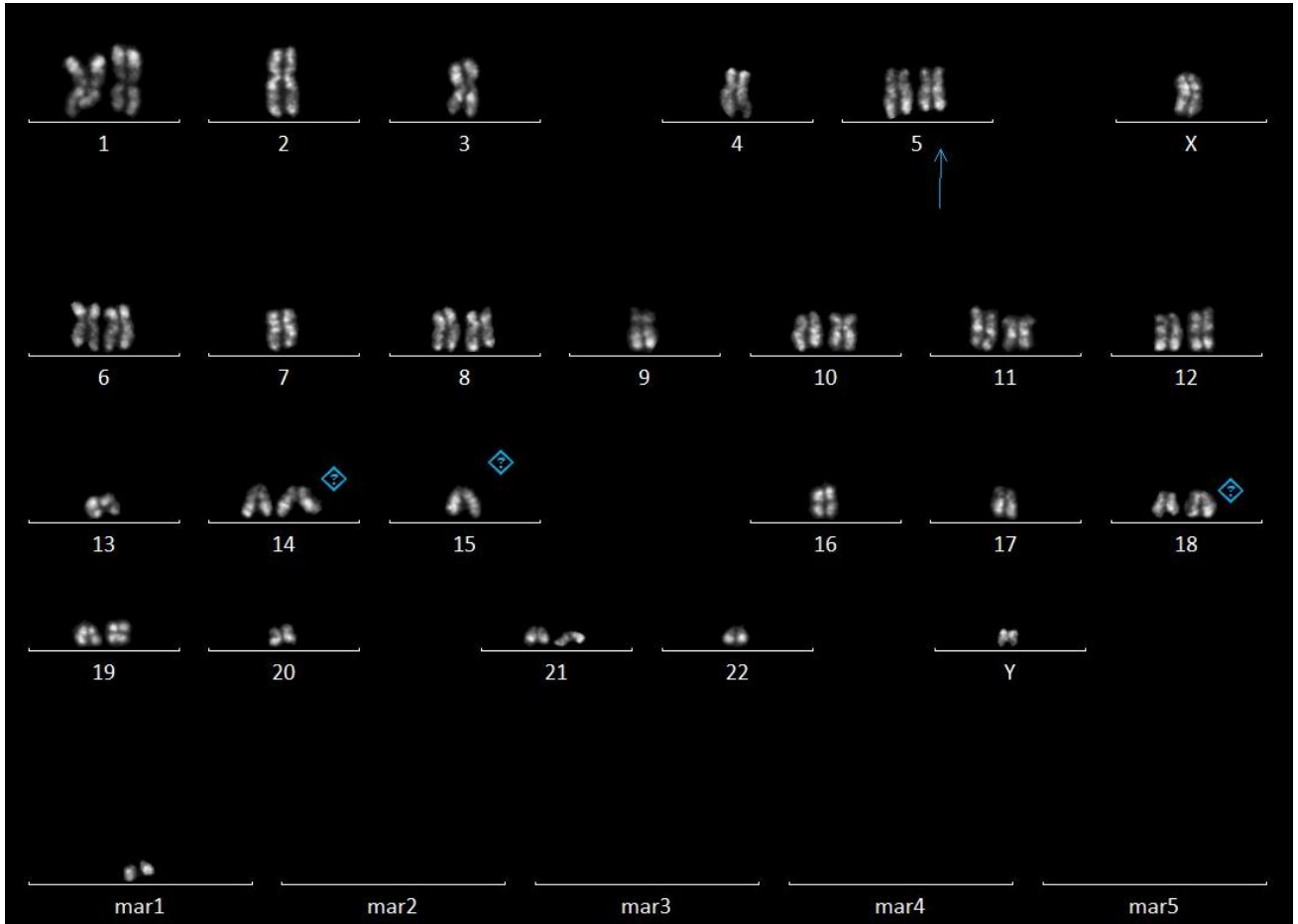
=> low hypodiploid karyotype

Example 2: 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



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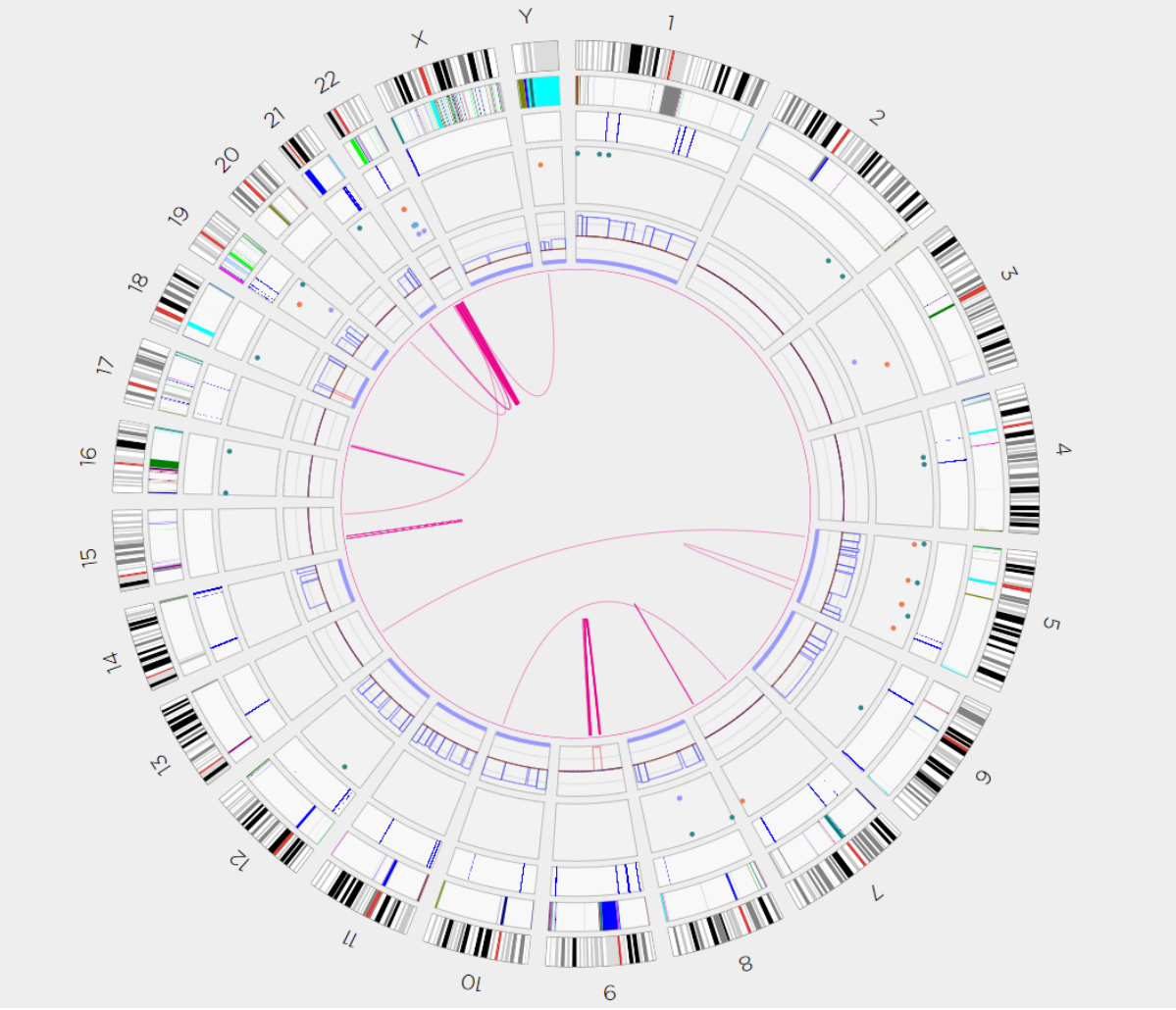

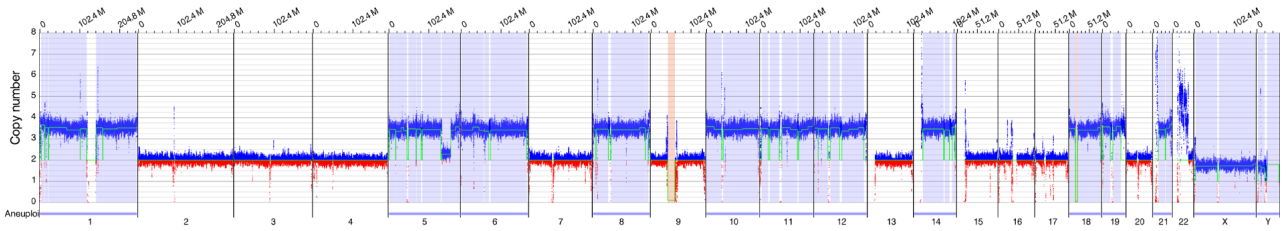

If you only run the RVA:
Seems like hyperdiploidy: gain of multiple chromosomes: gain of #1, gain of #8, etc

Example 2: correction baseline based on zygosity of structural variants

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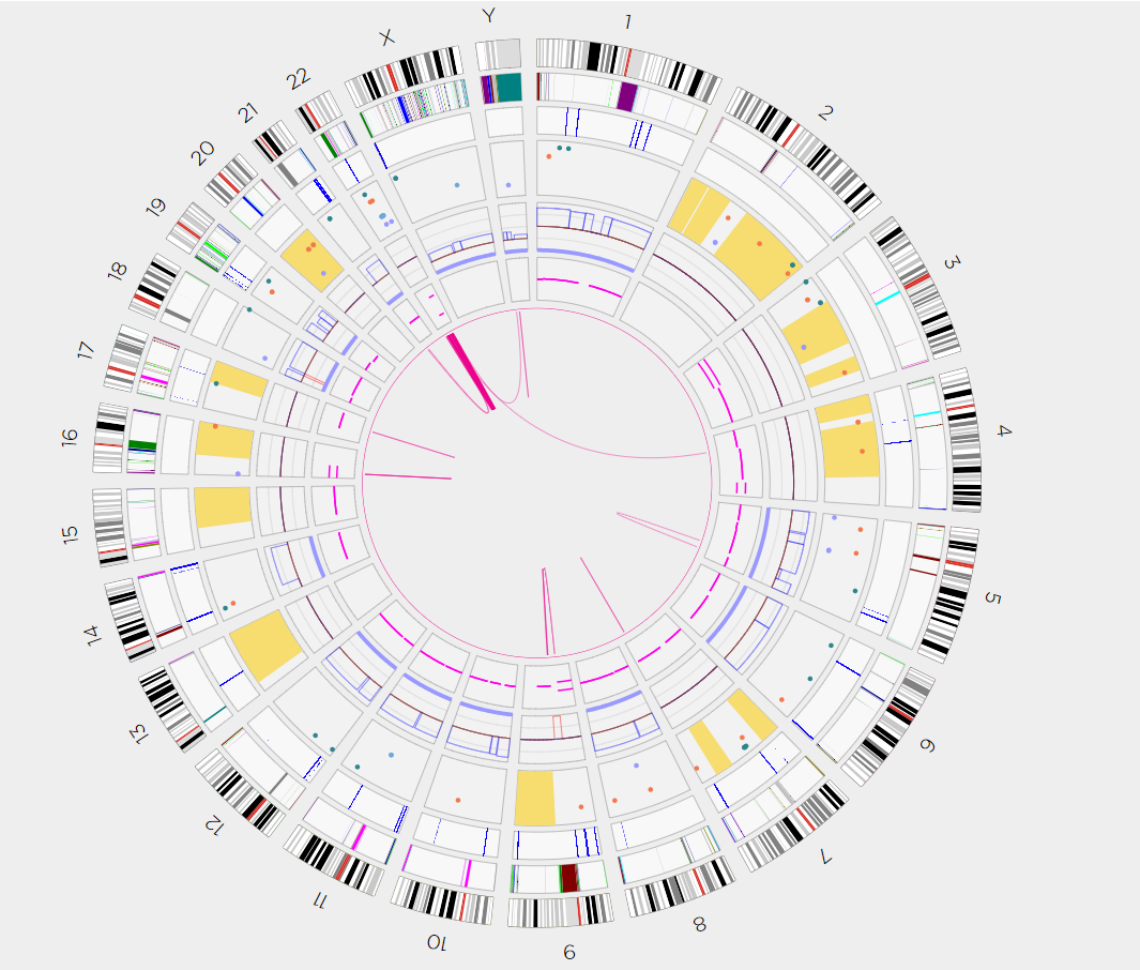
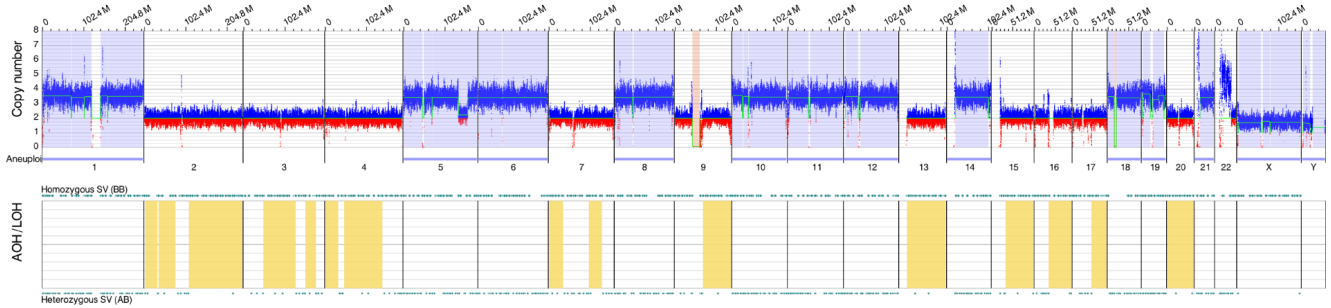
- Flow: pre-B-ALL



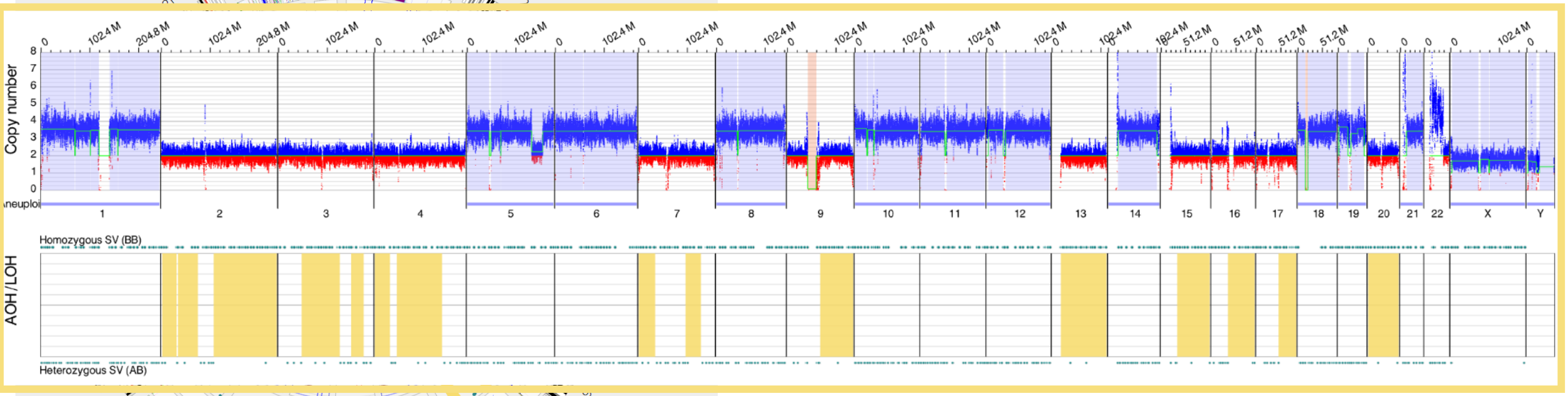


If you run the De Novo:

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

Example 2: 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 …
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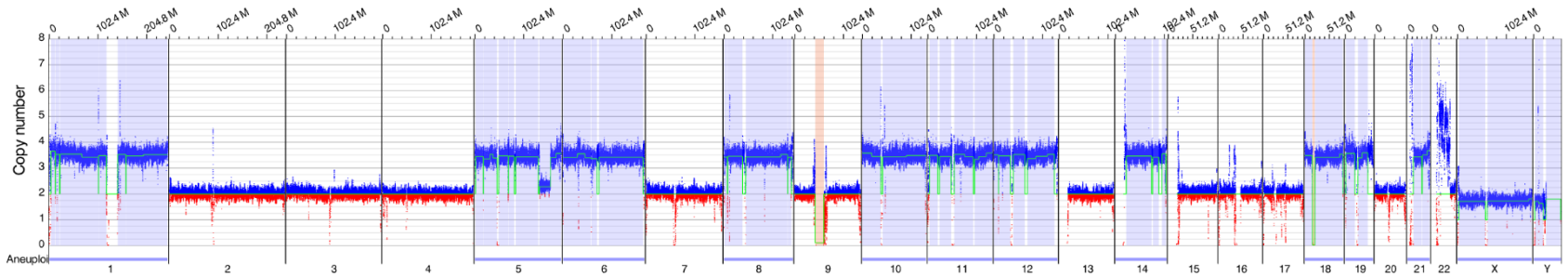
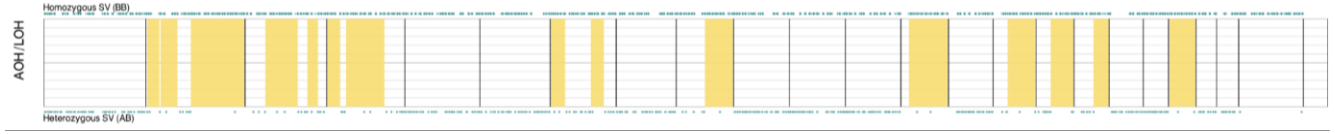
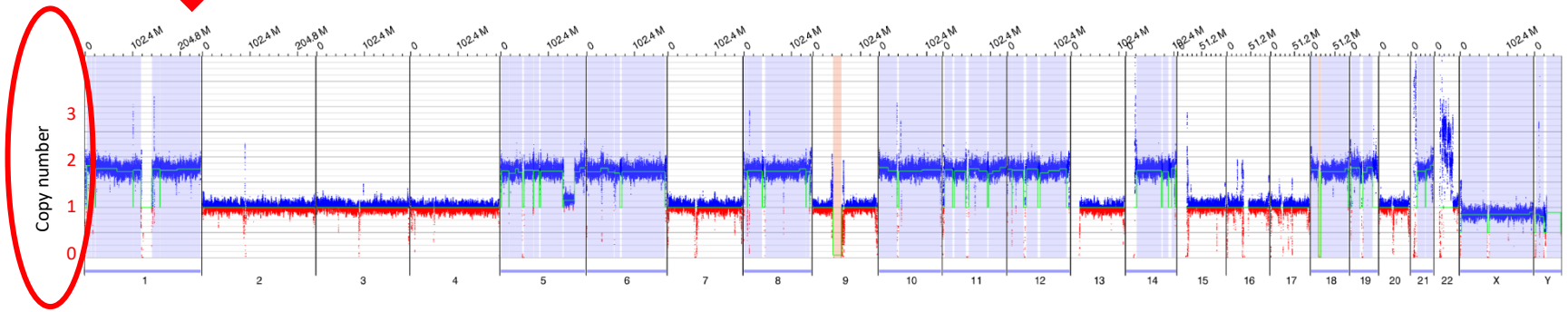
Example 2: correction baseline based on zygosity of structural variants





gains
losses

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



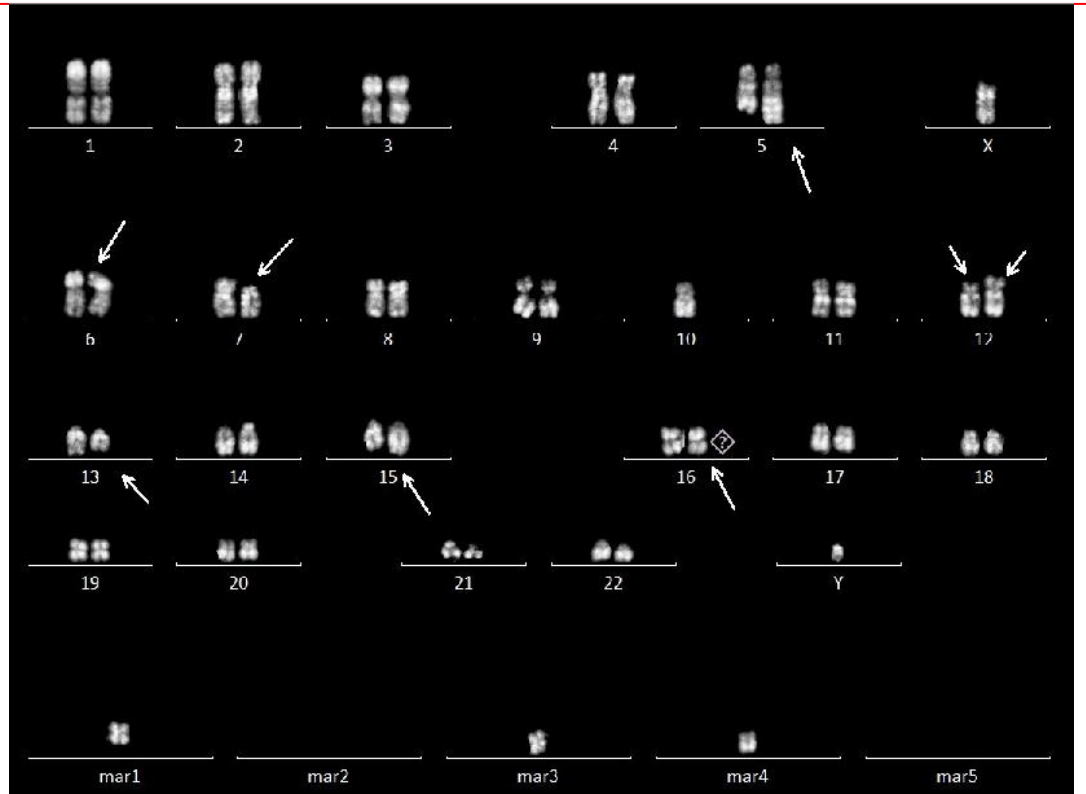
After correction (baseline reset):
Karyotype according to OGM: 36,XY,-2,-3,-4,del(5)(q31.1q33.3),-7,-9,-13,-15,-16,-17,-20,(22p11.2q13.1)cth,del(22)(q13.1q13.33)
WHO: "B-lymphoblastic leukaemia/lymphoma with low-hypodiploidy".

Example 3: 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:

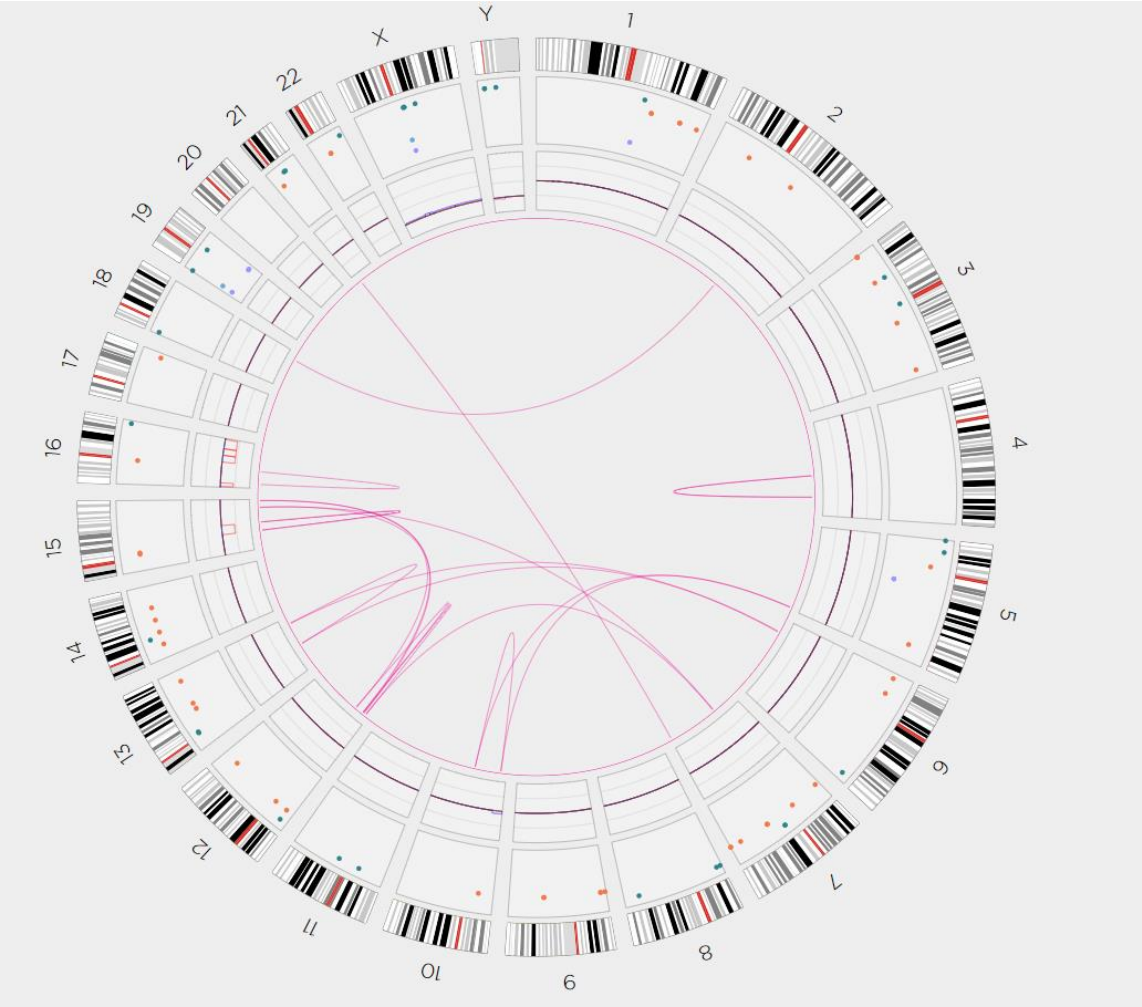
39-48,XY,der(5)t(5;?10)(q3?;q?),?t(6;13)(p21;q14),del(7)(p11) or der(7)t(7;15)(p11;q26),add(12)(p13),del(12)(p12),add(15)(q26) or der(15)t(7;15)(p11;q26),-16[3],?add(16)(p13)[7],+mar1,+mar2[3],inc[cp10]//46,XX[4]
In total: 4/80 mitoses with donor hematopoiesis

- Conclusión: persisting aberrations with clonal evolution

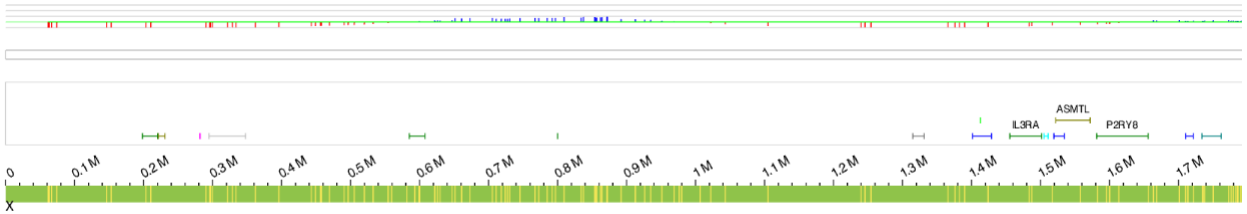


Example 3: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

- 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



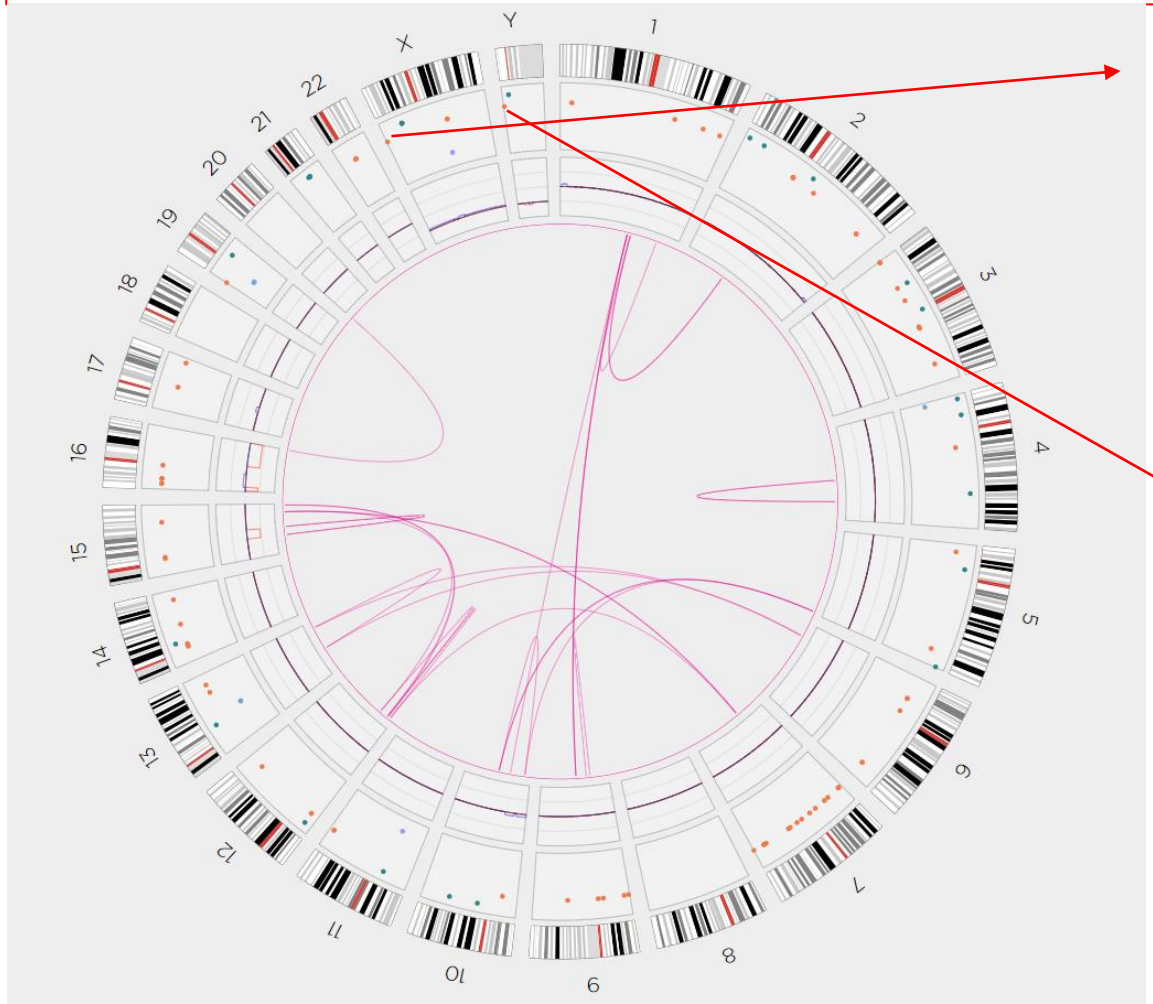
Xp22.33: completely normal



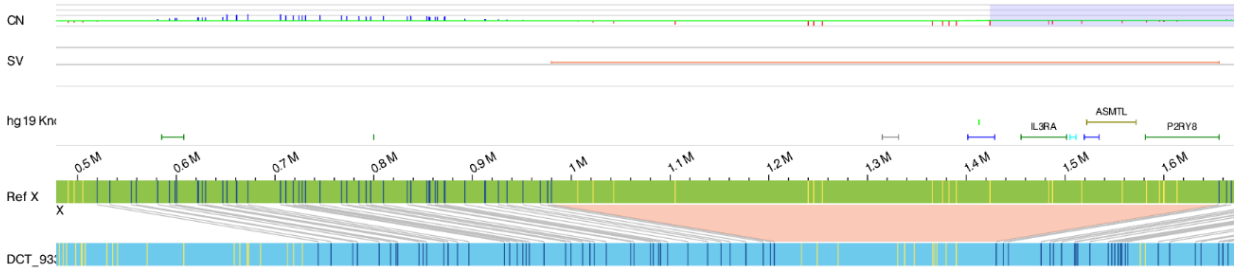
Yp11.32: completely normal

Example 3: detection of small deletions: e.g. the one leading to the *CRLF2::P2RY8* fusion

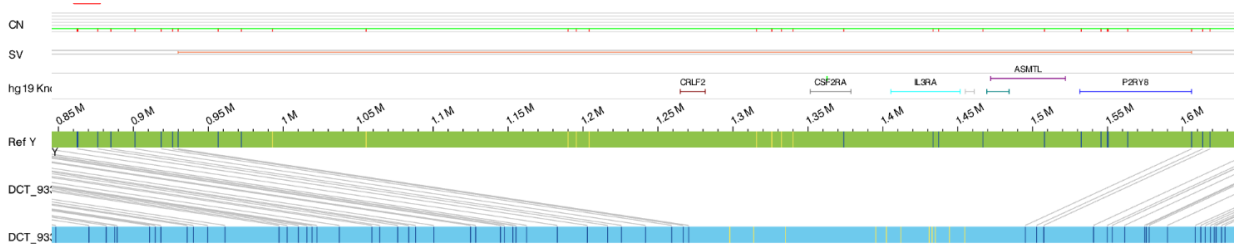
- Male, 13 years old
- 90% blasts in bone marrow
- Flow: B-ALL relapse
- OGM/Bionano: **De Novo Assembly pipeline**: very complex pseudodiploid karyotype comparable to the conventional karyotype
- De Novo Assembly detects a deletion on Xp22.33 and Yp11.32: resulting in **the *CRLF2::P2RY8* fusion gene**!



Xp22.33: *CRLF2::P2RY8*

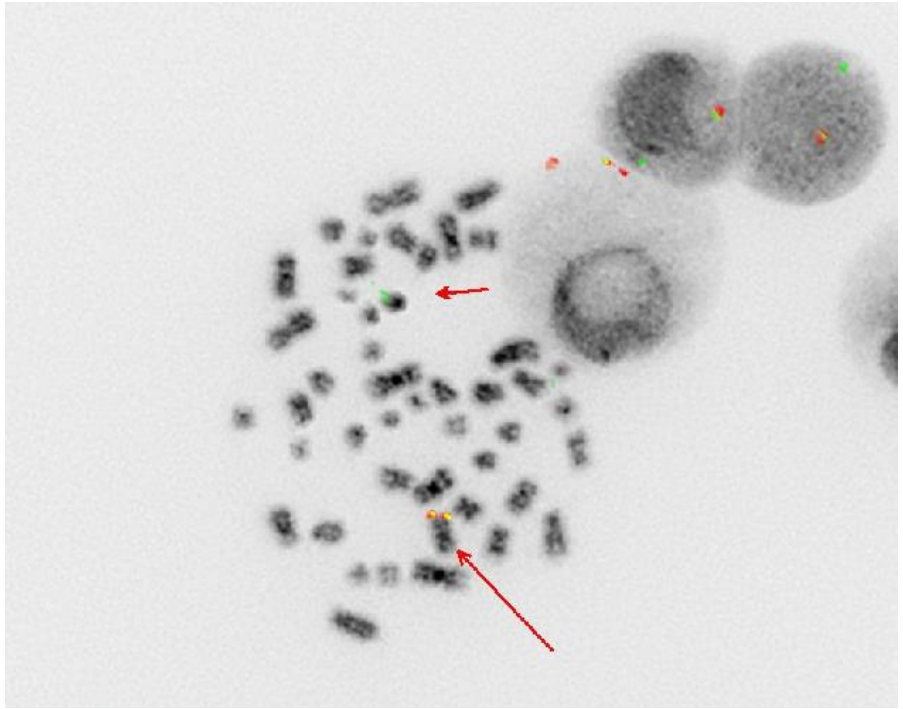


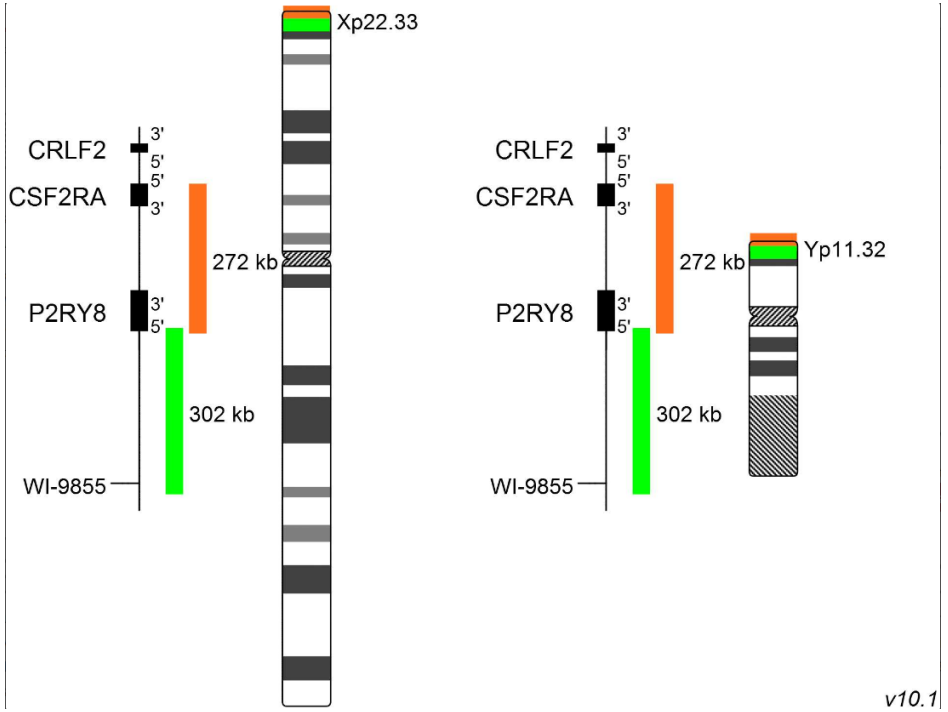
Yp11.32: *CRLF2::P2RY8*



Example 3: 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 3: 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

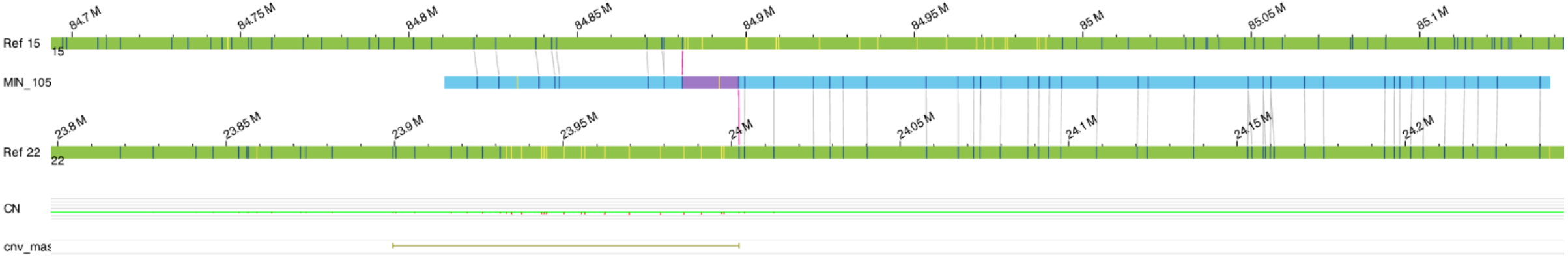
Criteria for manual review:

- 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:



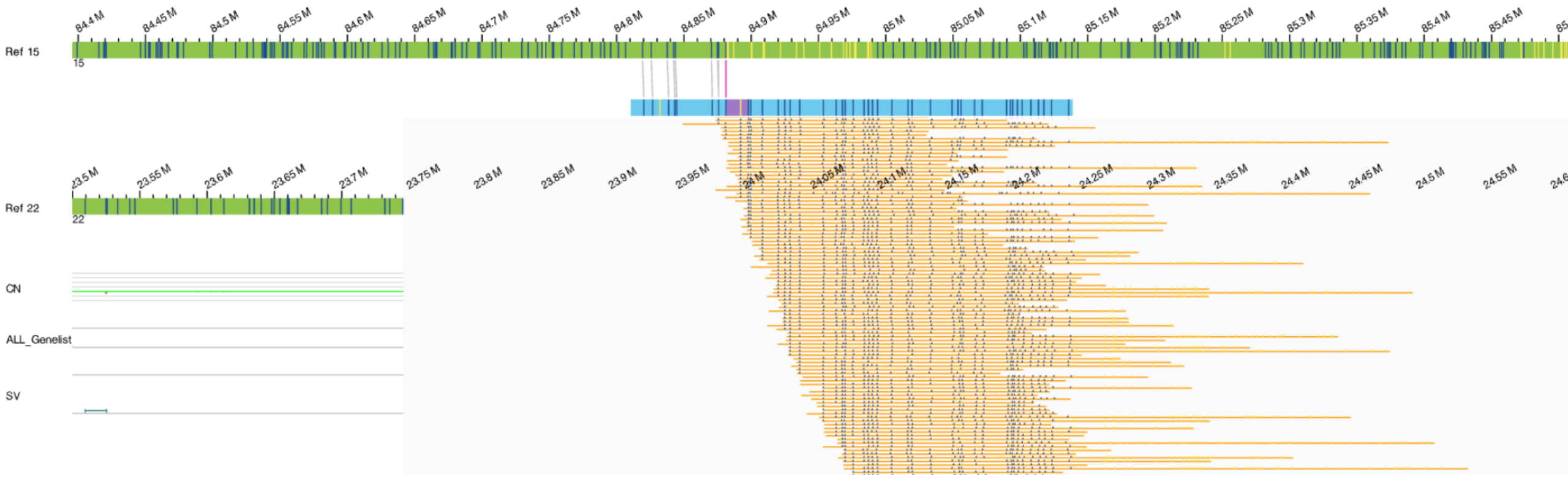
translocation_interchr: t(15;22)(q25.3;q11.23): example of translocation I would discard

=> 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

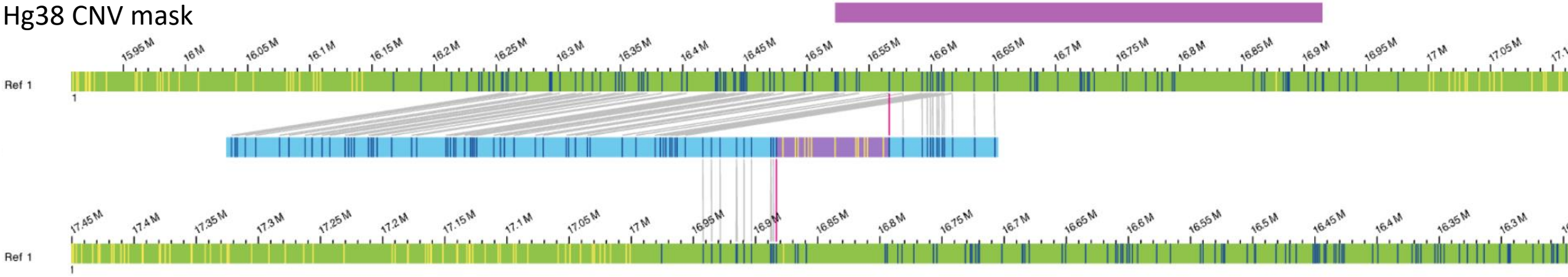
Analysis: variant review: fail assembly chimeric score

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:

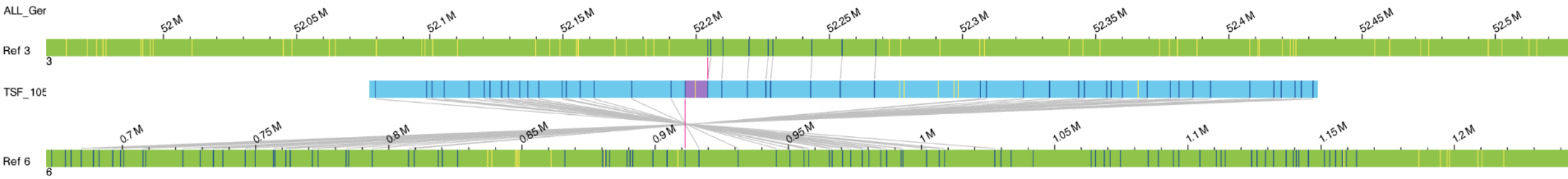


translocation_intrachr: ogm[GRCh38] t(1;1)(p36.13;p36.13): example of translocation I would discard

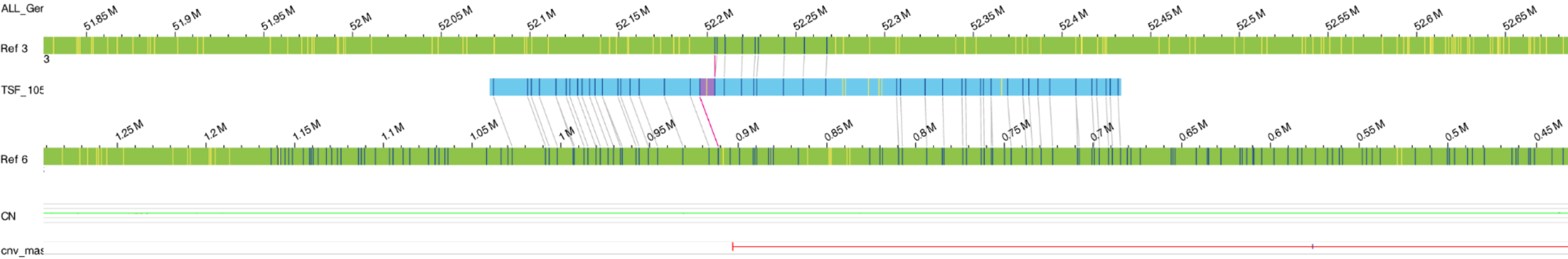
=> in region of CNV mask (purple)

=> seen in many samples

Analysis: variant review: example of a false translocation:



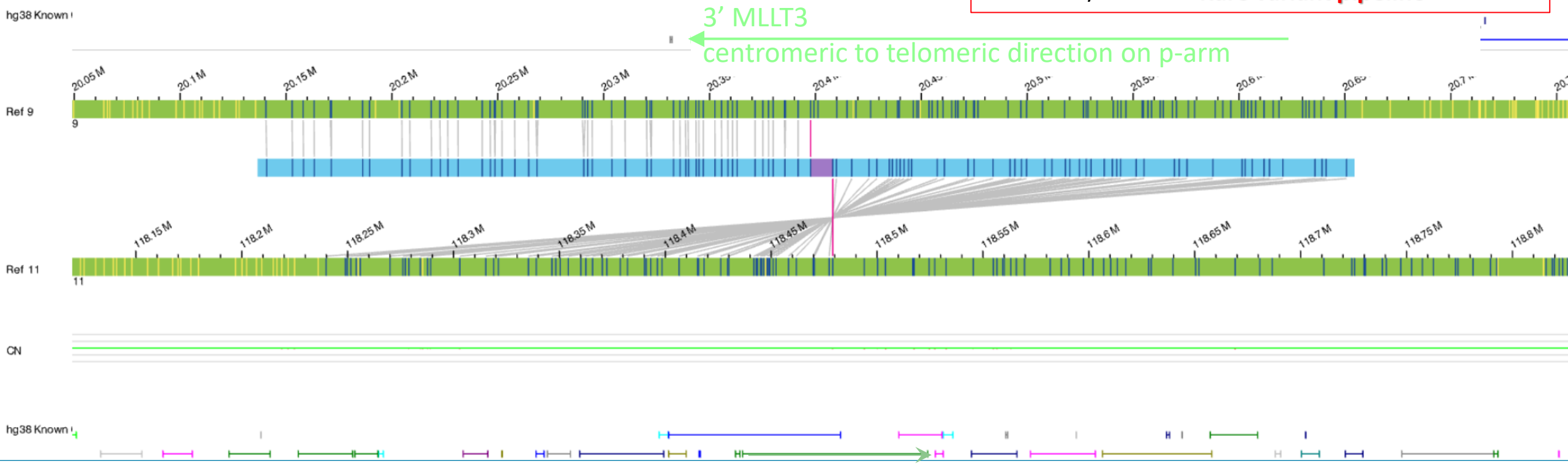
Same as above, but inverted, so that you can better perform a visual inspection:



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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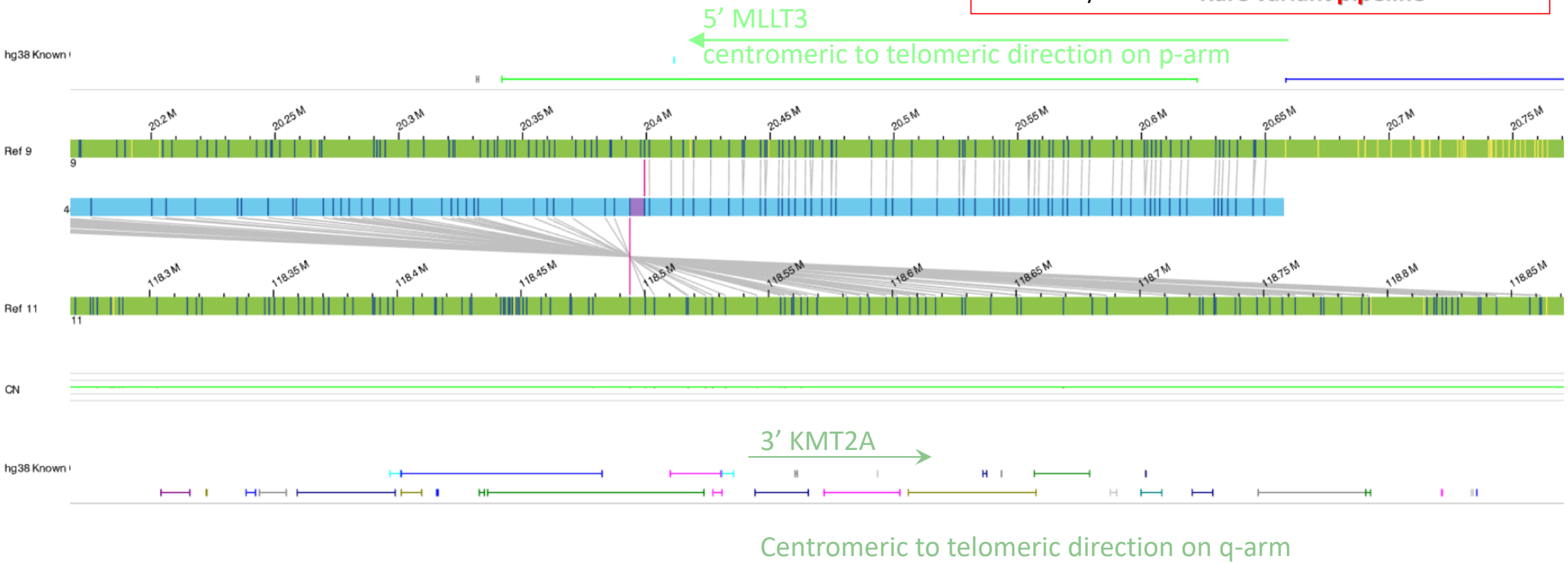
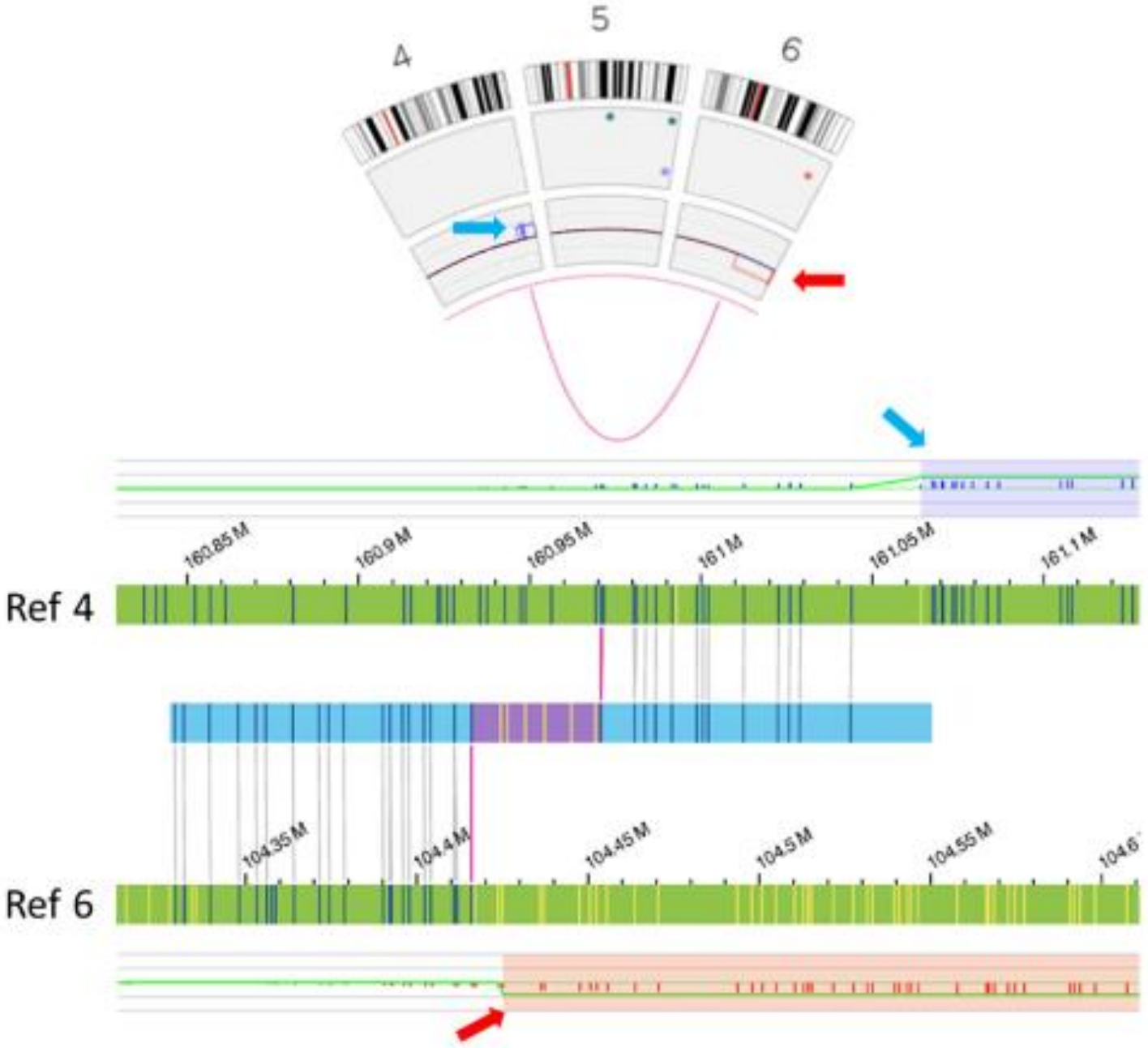
- Flow: AML
- OGM/Bionano: **Rare variant pipeline**

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

Analysis: variant review: example of a deletion

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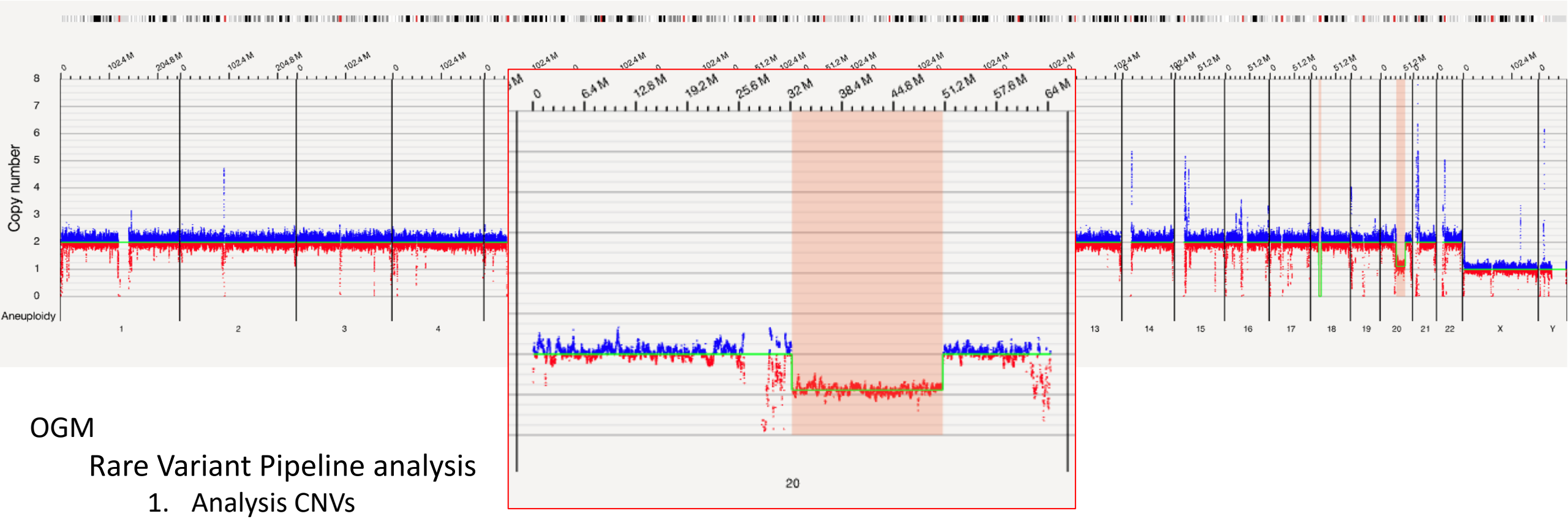

OGM

Rare Variant Pipeline analysis

1. Analysis CNVs

Analysis: variant review: example of a deletion

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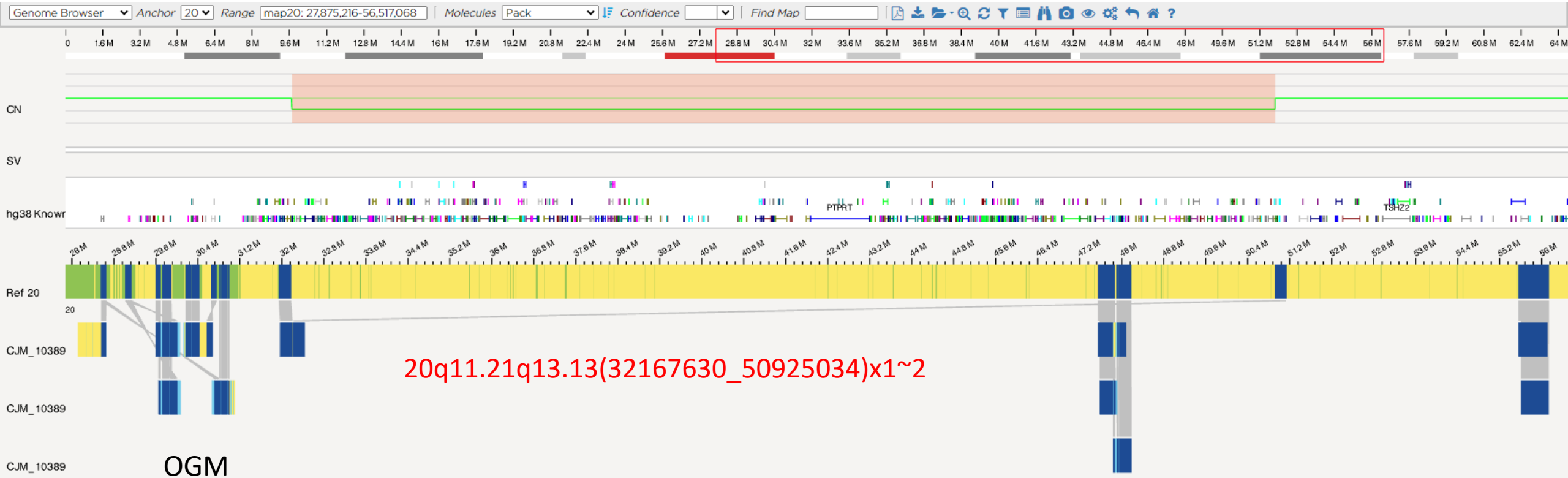
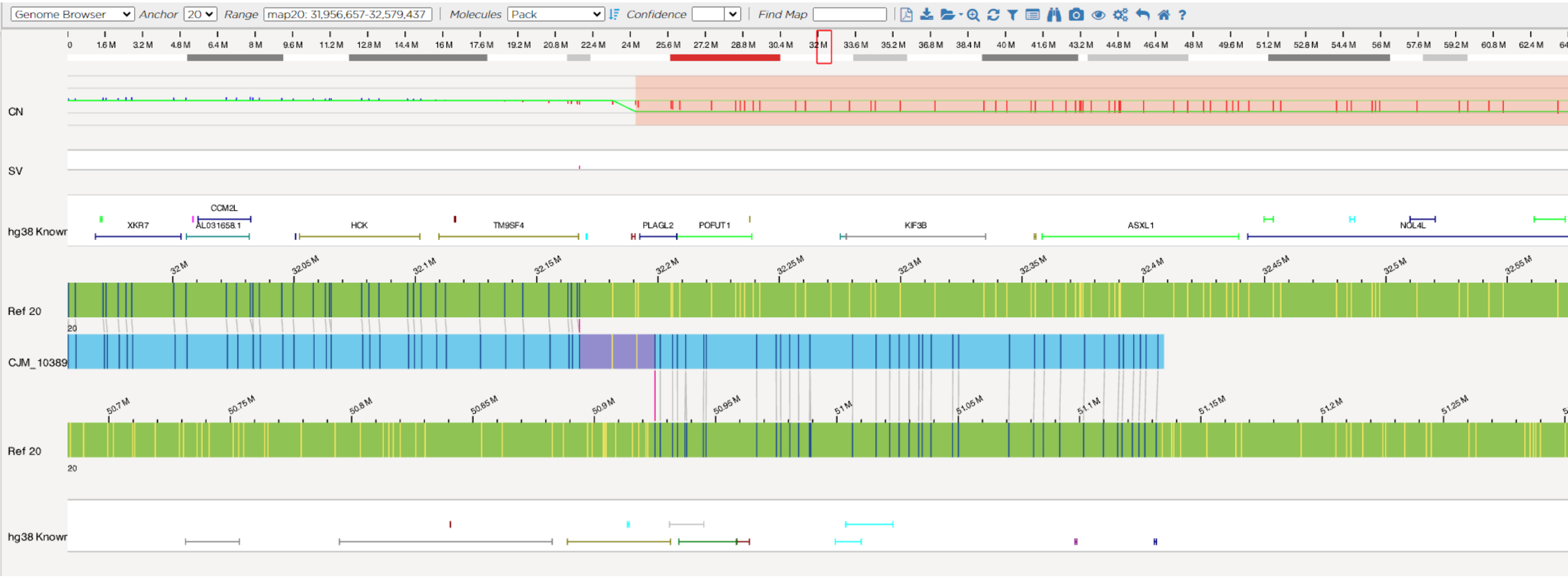


OGM

Rare Variant Pipeline analysis

1. Analysis CNVs

Analysis: variant review: example of a deletion

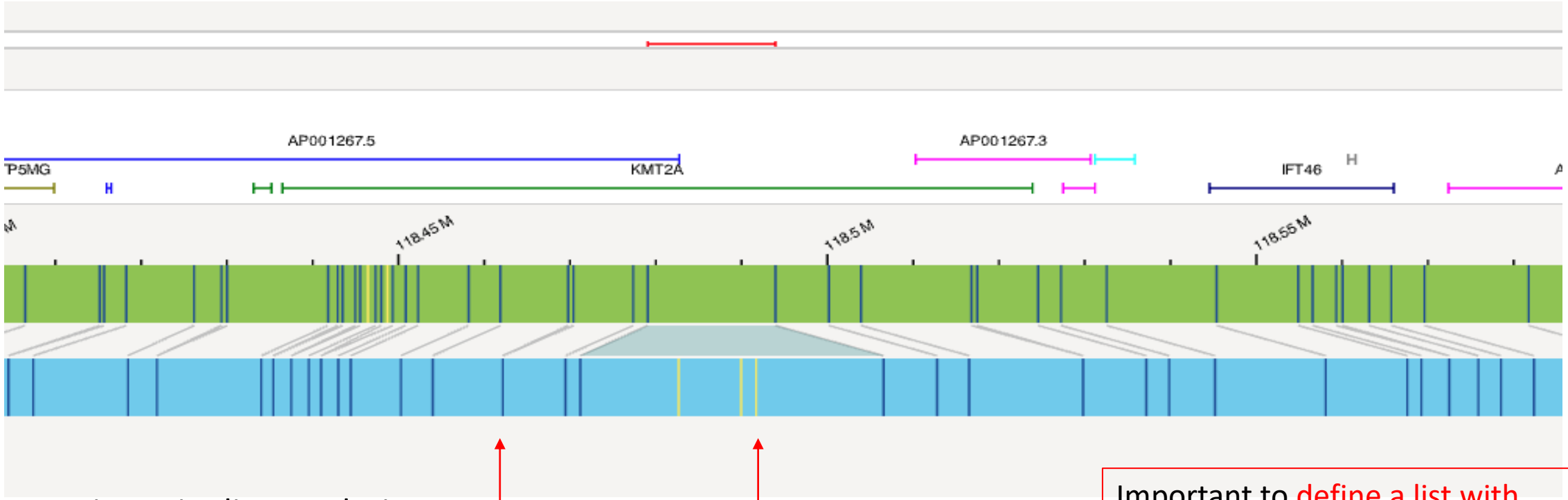


3685	11132	16	16	248524.8	275128.2	85.615.102,00	85.622.085,00	0.99	insertion	19620.4	0.42	CJM_1038	19621	3	GSE1	AC092127.1	19383.0	-	yes	65	-	ogm[GRCh38] ins(16;?)(q24.1;?)
4073	17852	20	20	223519.4	254658.4	32.167.630,00	50.925.034,00	0.99	translocation_intrachr	-1.0	0.44	CJM_1038	-1	-1	ADNP	TM9SF4	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

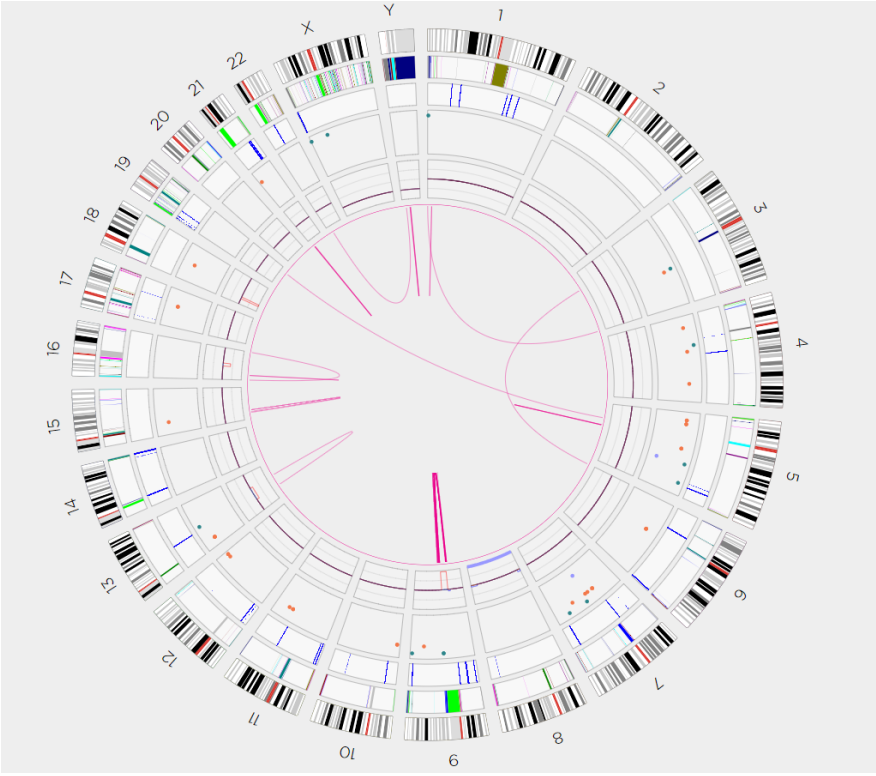
11q23.3q23.3(118470405_118479068)x3 [KMT2A-PTD]

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

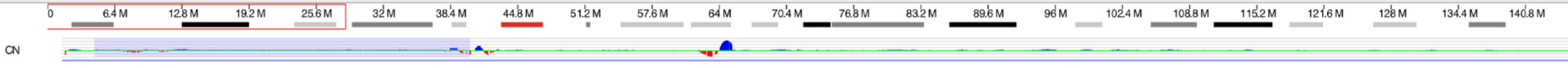
Analysis: allways check the “Whole Genome” view

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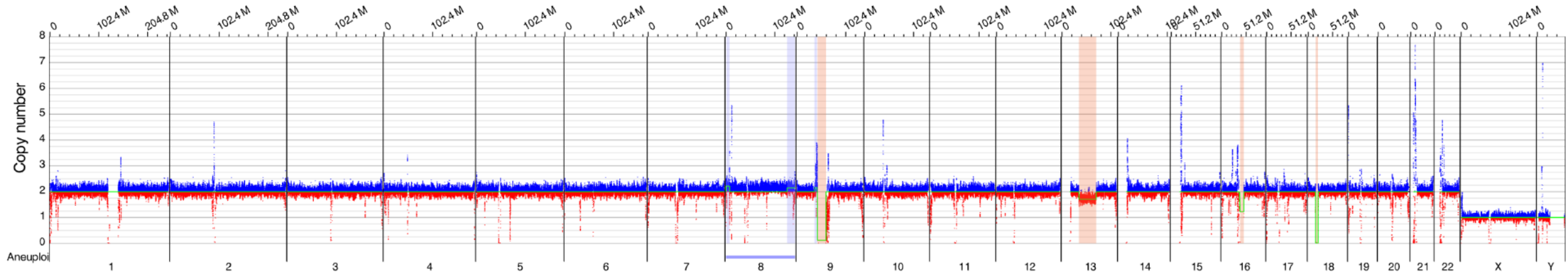
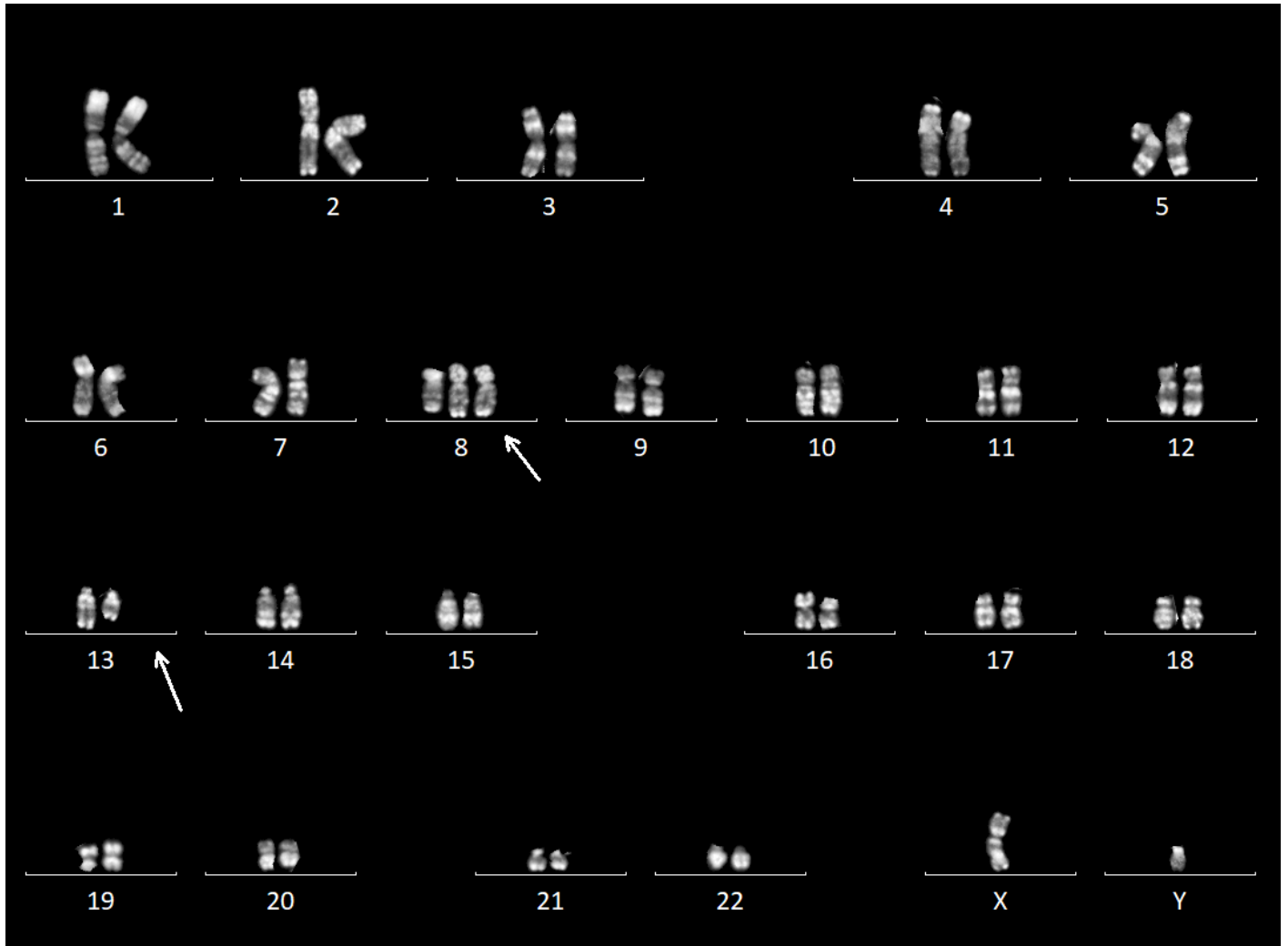
Example of case with AML
Trisomy 8 and deletion of 13q is much clearer in “whole genome” view than in circos plot



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.

Prospective diagnostic AML cases

253 routine AML cases

Considered different cytogenetic groups (CBA)

Normal karyotypes	[108]
Fail karyotypes	[13]
Recurrent fusions	[30]
Simple karyotypes	[55]
Complex karyotypes	[47]

Complex karyotypes [46]

Recurrent trisomies [4]
- Good concordance
- Discordant subclone

Low complexity: ≥3<5 [3]
- Good concordance
- Discordant subclone

High complexity :≥5 [39] karyotype includes markers, rings, adds etc

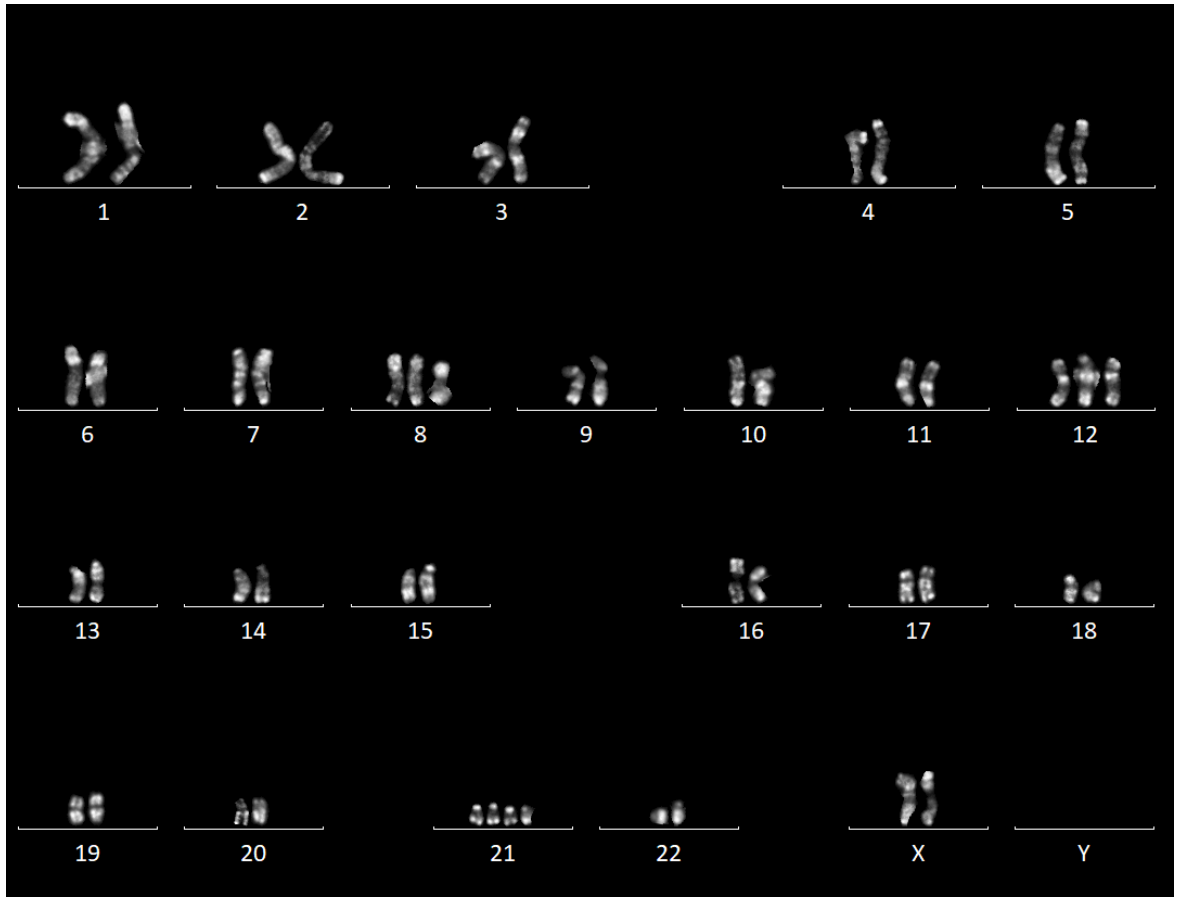
- Overall good concordance but with higher number of abnormalities identified by OGM
- In addition, OGM identified
  - Recurrent SOC rearrangements [5]
  - Potential rearrangement [5]

Low complexity: OGM did not detect	1 subclone in recurrent trisomies 1 subclone in low complexity group
High complexity OGM identified recurrent rearrangements [5]	*ETV6::ACSL6* *RUNX1::MECOM* *ZNF385B::ERBB4* *KMT2A::MLLT10* *KMT2A-PTD*
OGM identified potential rearrangements [5]	*FGFR1?* *RUNX1? [2]* *PICALM?* *DLC1::RUNX1*

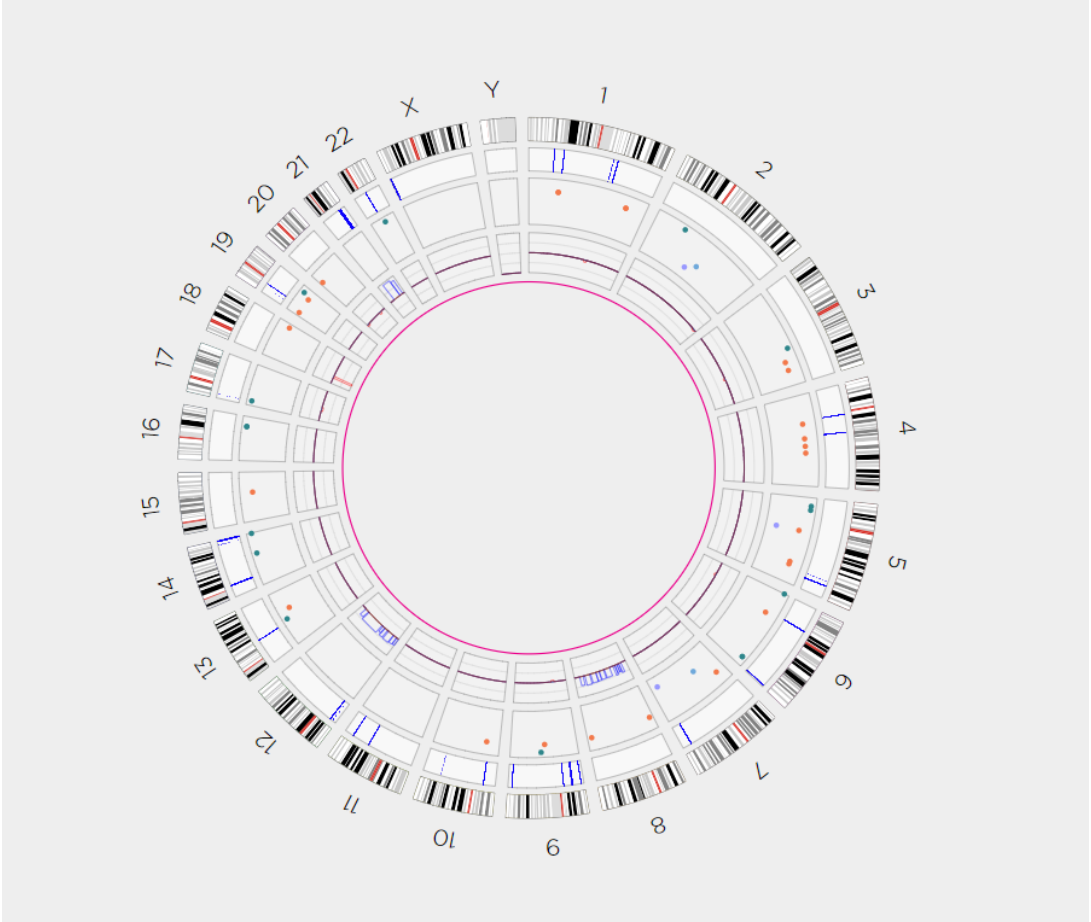
OGM identifies different levels of genomic complexity in complex karyotypes

Take home message: OGM can identify different levels of genomic complexity

Complex karyotype but not considered part of poor prognostic ‘complex’ sub-group (ELN 2022) excludes 3 or more trisomies without structural rearrangement



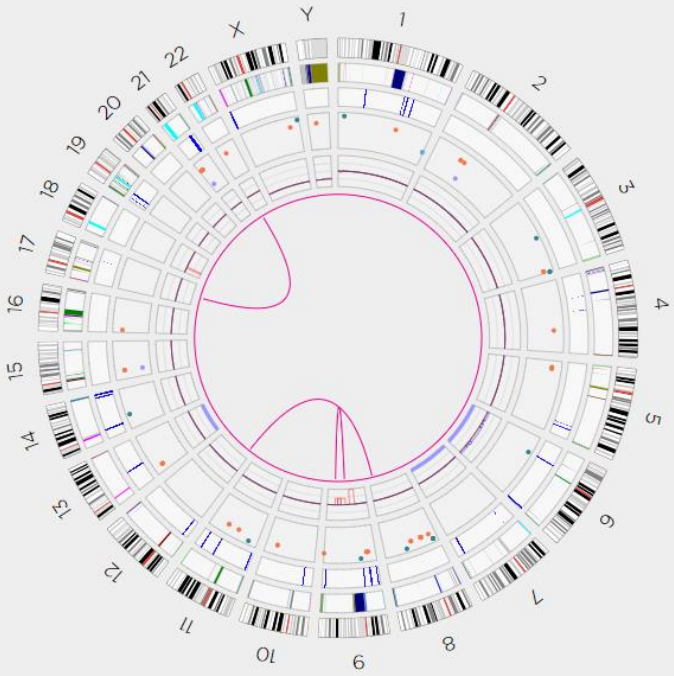
50,XX,+8,+12,+21,+21



Non-complex OGM genomic profile

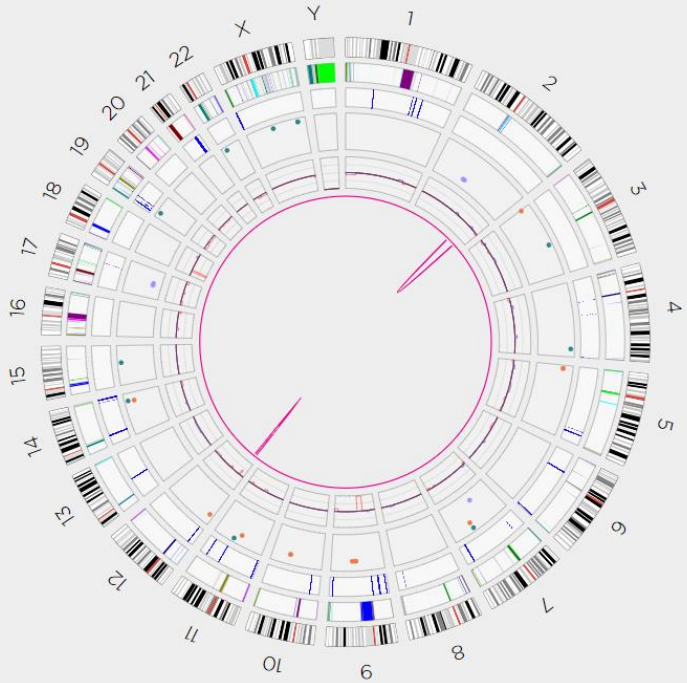
Take home message: OGM can identify different levels of genomic complexity

Trisomy and structural rearrangement - 4 aberrations
46,XY,del(9)(q21q33)[2]/47,sl,+6[2]/48,sdl,+7[3]/48,sl,+13[4]

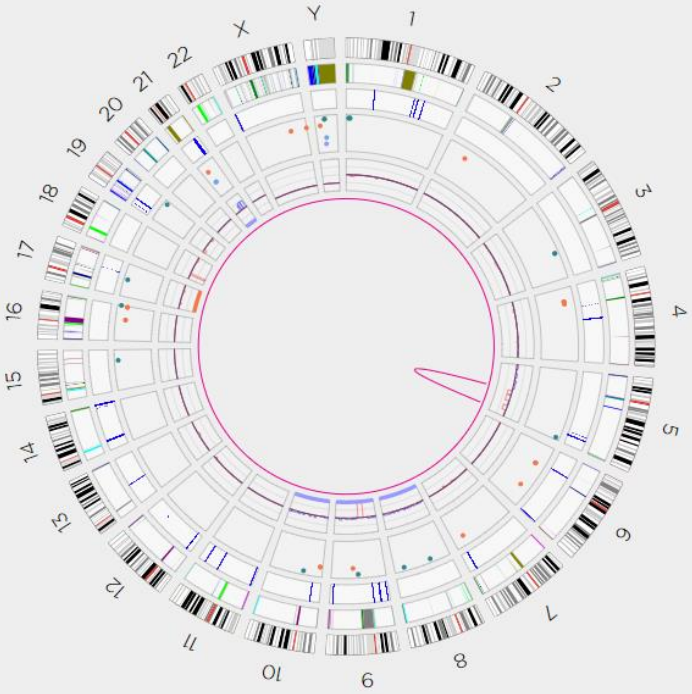


3 structural aberrations

46,XX,inv(2)(q32q34)[3]/46,sl,del(12)(p13p12)[5]/46,sdl,del(12)(p13p12)x2[2]



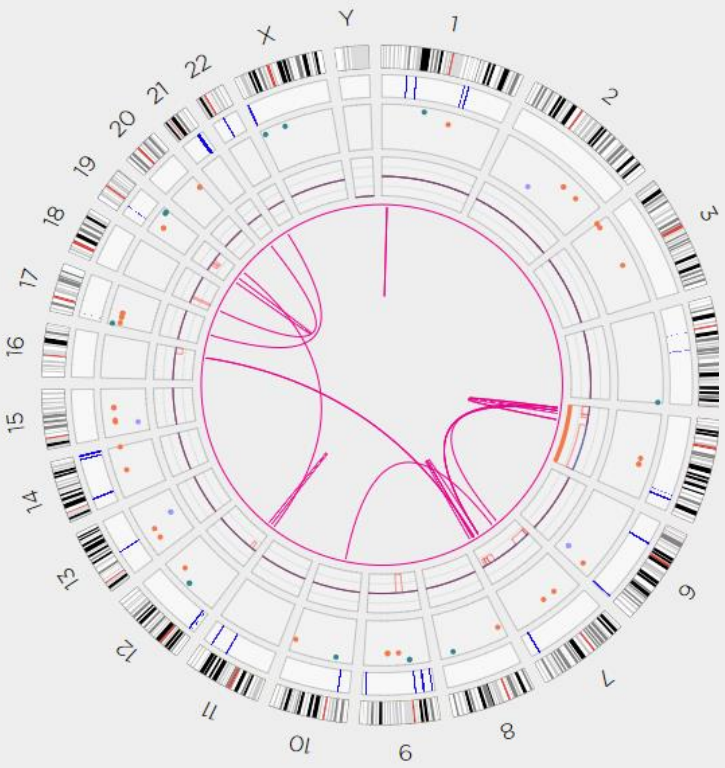
Trisomy and structural rearrangement: 7 aberrations
47,XY,del(5)(q14q34),+21[3]/48,sl,+21[4]/51,sdl,+5,+8,+9,+10[3]

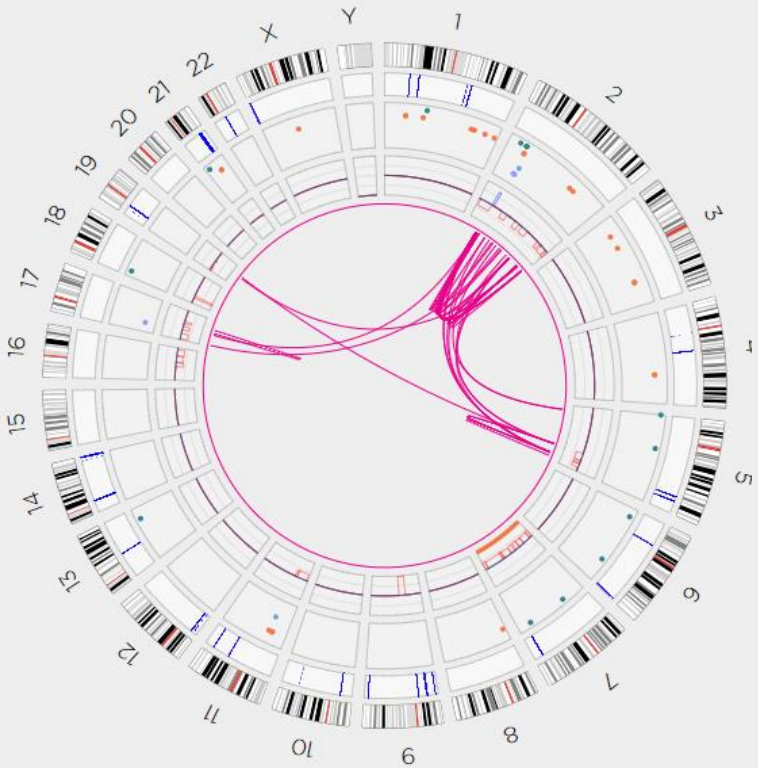


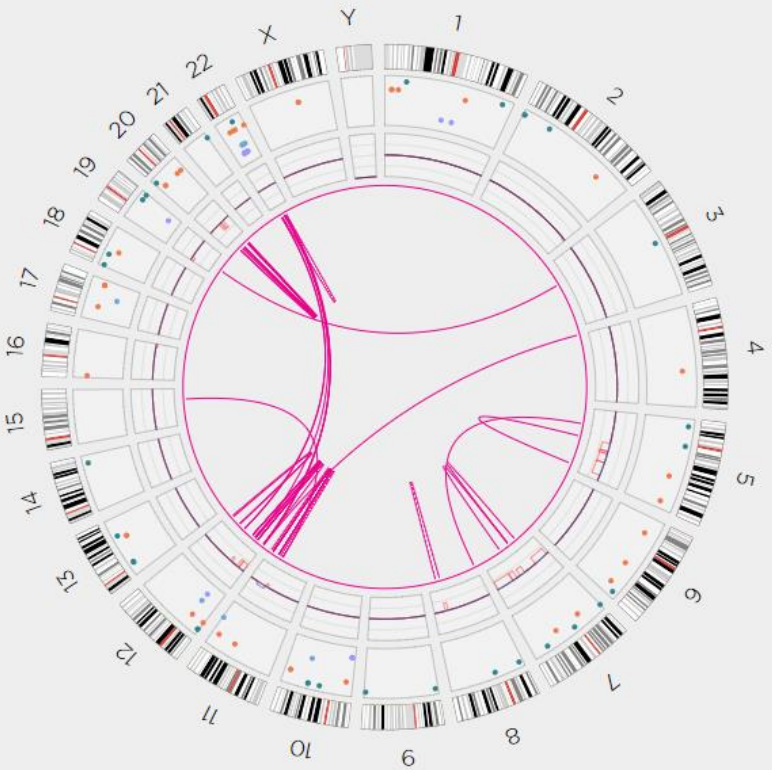
No or low genomic complexity

Take home message: OGM may help sub classify complex genomes

Complex karyotypes with multiple ill-defined aberrations – add, der, mar…







Medium or high complexity – definition?

Take home message: different types of chromoanagenesis

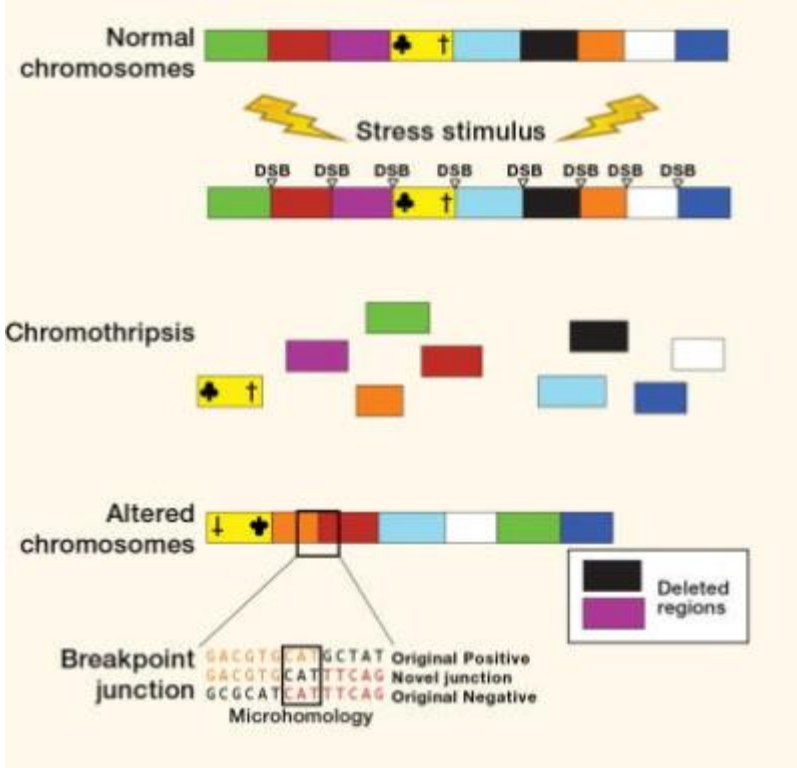
Chromoanagenesis: 3 types:

- Chromothripsis
- Chromoanasynthesis
- Chromoplexy

=> how to make the distinction:
subject of a more advanced course

Chromothripsis

- Neologism: the Greek words *chromo* which means color (represents chromosomes) and *thripsis* which means 'shattering into pieces‘
- What? phenomenon whereby tens to hundreds of chromosomal rearrangements localized to a limited number of genomic regions can be acquired in a single catastrophic event
- How? the simultaneous fragmentation of distinct chromosomal regions (breakpoints show a non-random distribution) and then subsequent imperfect reassembly by DNA repair pathways or aberrant DNA replication mechanisms (NHEJ)
- When? early in tumour development
- Described first in CLL in 2011
- Result? loss of tumor suppressor genes, amplification of oncogenes
- Predisposition? *TP53* mutations



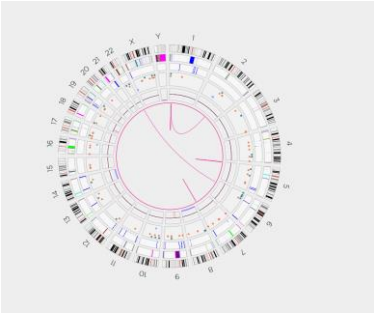
Maher & Wilson; Cell. 2012 Jan 20; 148(0): 29–32.

Take home message: Identification of small clones

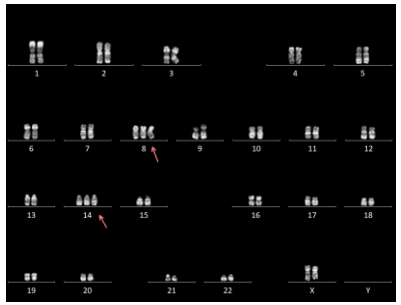
CBA misses small clone

Original karyotype 46,XX [20]

ogm[GRCh38](8)x2~3,(14)x2~3

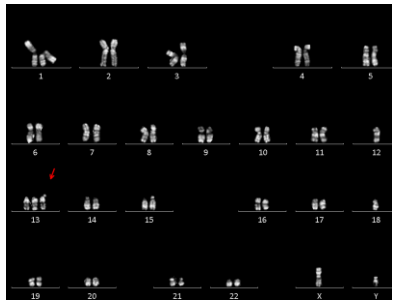


On review 48,XX,+8,+14[2]/46,XX [28]

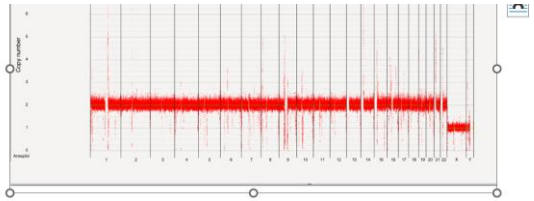


OGM misses small clone

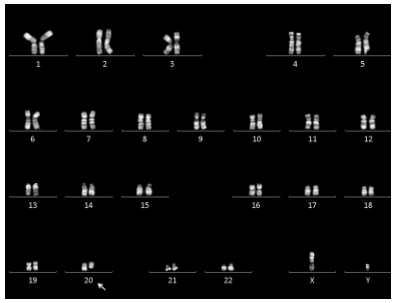
47,XY, +13[2]/14,XY[19]



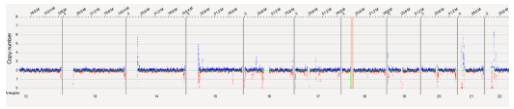
OGM normal



46,XY,del(20)(q12q13)[4]/46,XY[17]



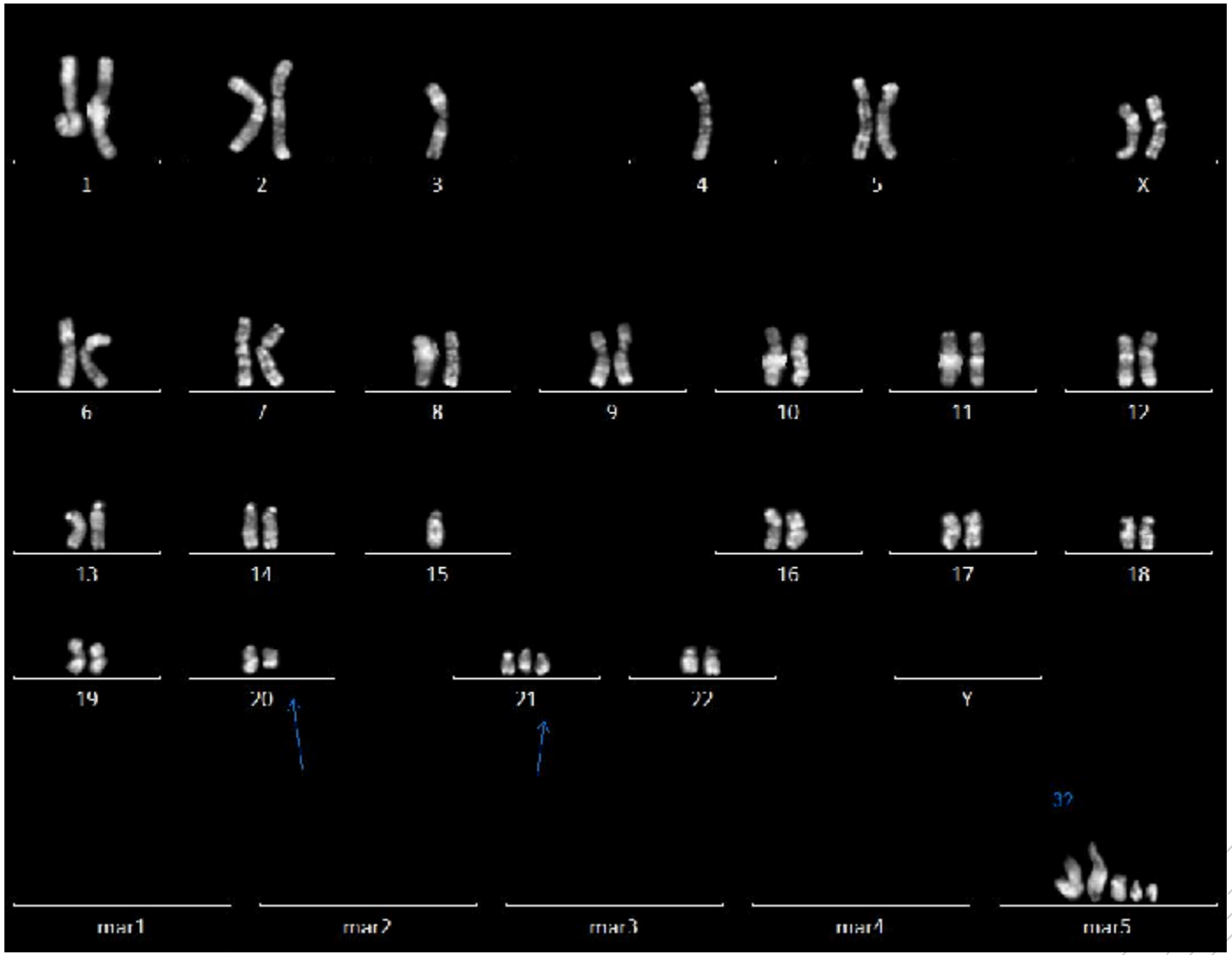
OGM normal



CASE REPORT

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

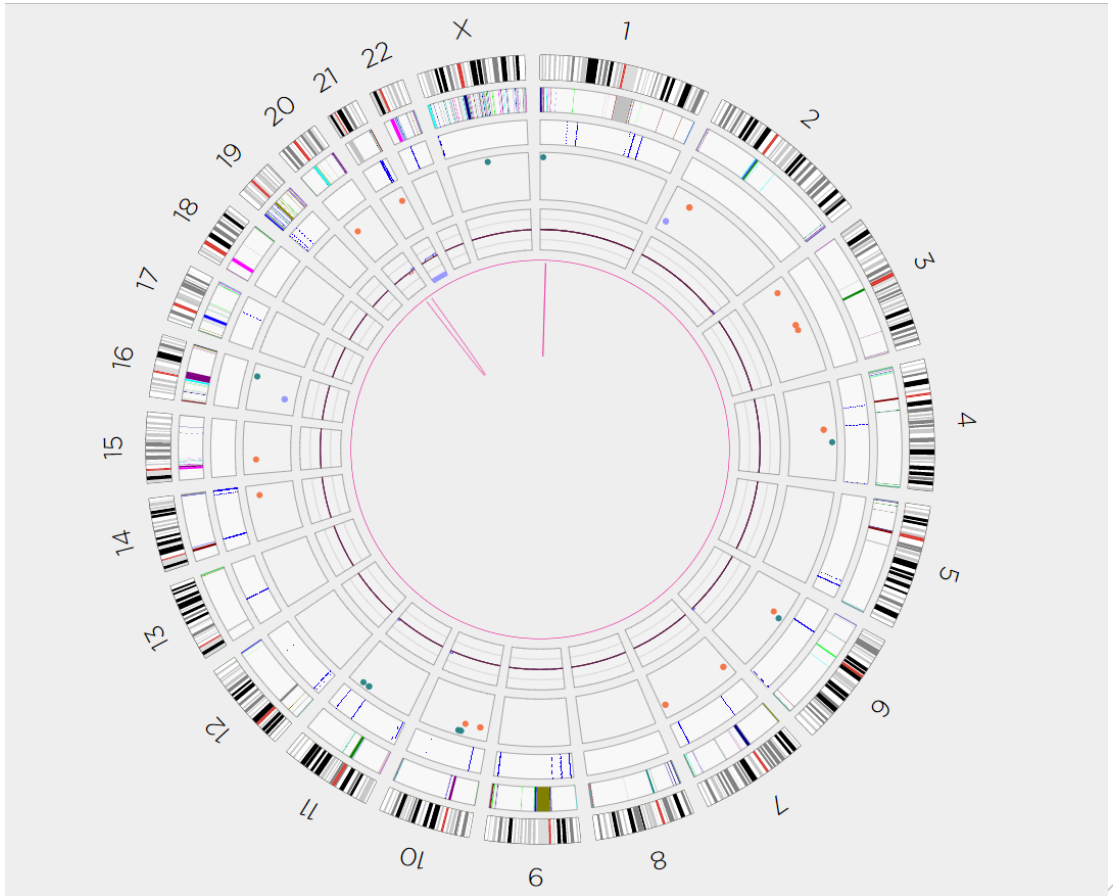
Conventional karyotype:

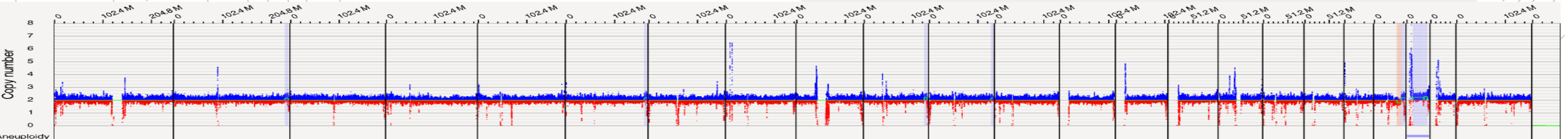


CASE REPORT

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

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



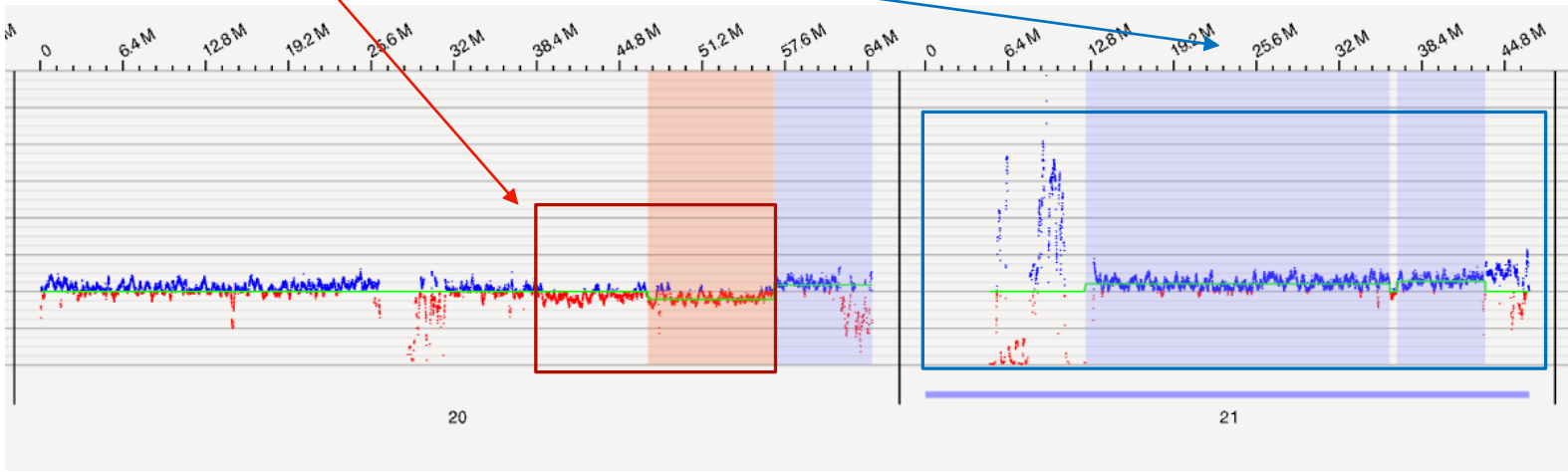


CASE REPORT

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

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

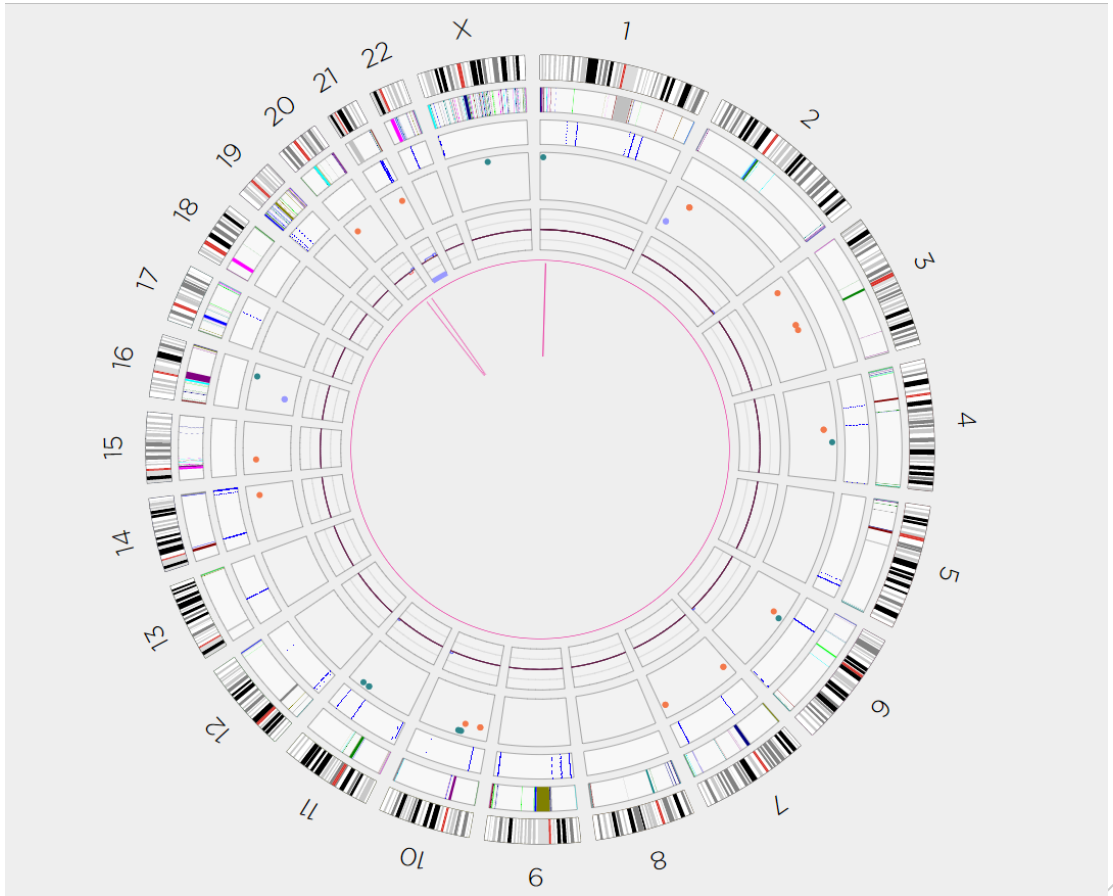
ogm[GRCh38]
20q11.23q13.31(38709036_56550158)x1~2,
(21)x2~3,



CASE REPORT

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

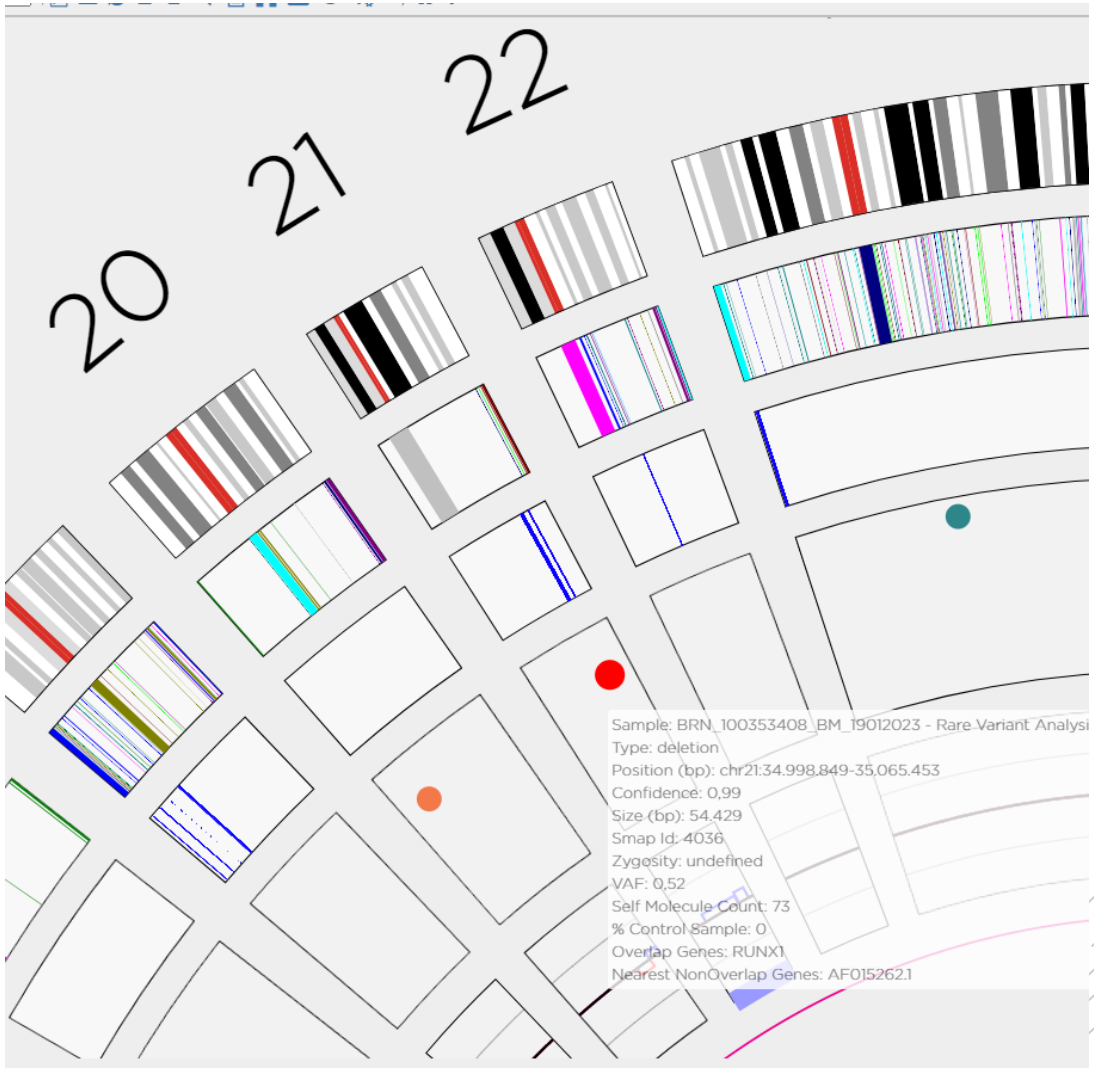
- **Conventional karyotype:** 47-49,XX,inc[2]
- OGM:



CASE REPORT

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

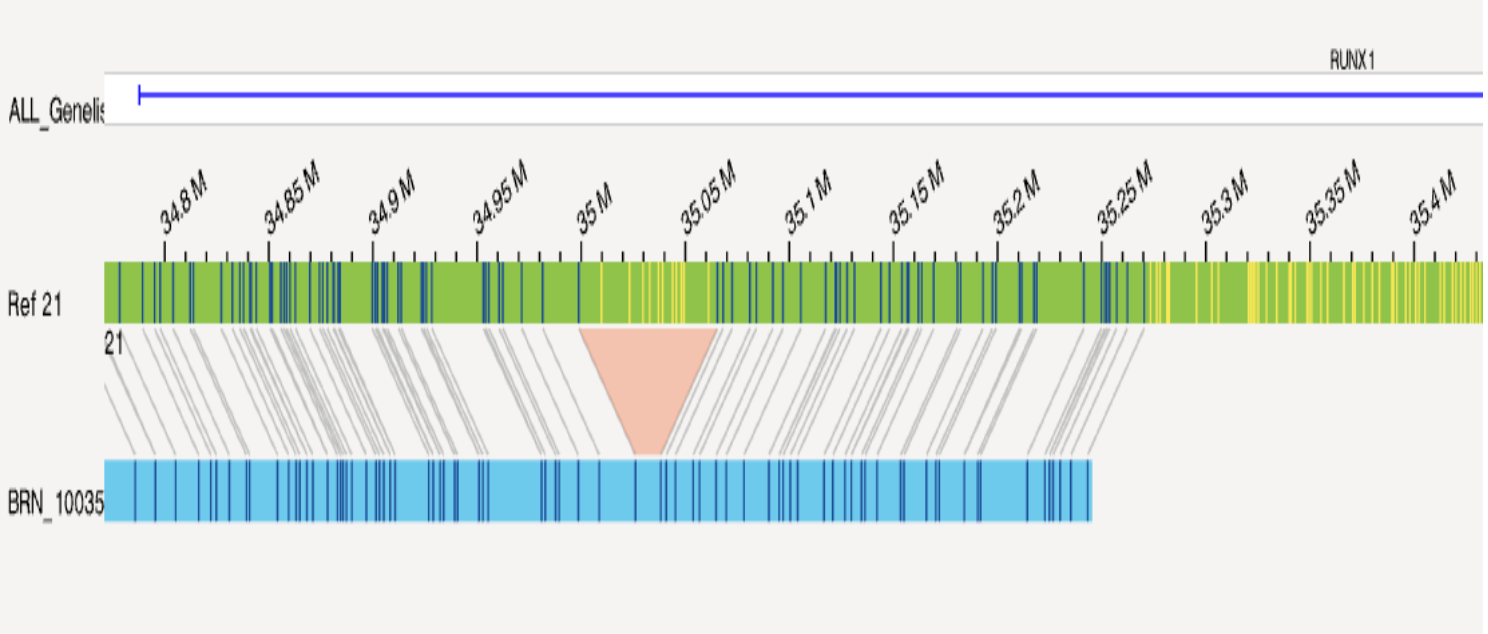
- **Conventional karyotype:** 47-49,XX,inc[2]
- OGM:



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,
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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Adam Smith *et al.*

FrOGG French-speaking OGM Group-GFCH

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