Low-pass sequencing for CNV detection in hemato/onco routine diagnostics?

Groupe Francophone

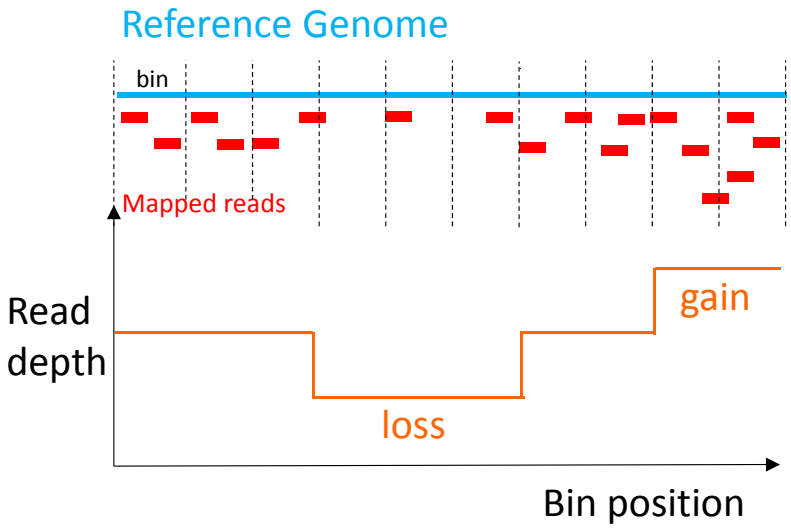
de Cytogénétique Hématologique

du 1er février 2018

Paris

Low-pass sequencing (LPS) or shallow sequencing

- Depth of coverage method: infers copy number from the observed sequence depth across the genome and does not require both ends of the molecule to be sequenced
- Divide the reference genome into bins and count the number of reads into each bin: copy number is inferred from the observed read counts across the genome.



QDNA seq pipeline

- No normal reference necessary (reduces cost by half, samples for which normal reference is not available can be analyzed, avoids measurement noise from the reference sample)
- Use of fixed-sized bins (=key feature of QDNAseq), allows relatively rapid analysis
- BAM files as input (produced by BWA algorithm)

Method: QDNA Seq

Genome Res. 2014 Dec;24(12):2022-32. doi: 10.1101/gr.175141.114. Epub 2014 Sep 18.

DNA copy number analysis of fresh and formalin-fixed specimens by shallow whole-genome sequencing with identification and exclusion of problematic regions in the genome assembly.

Scheinin I[1], Sie D[2], Bengtsson H[3], van de Wiel MA[4], Olshen AB[3], van Thuijl HF[5], van Essen HF[2], Eijk PP[2], Rustenburg F[2], Meijer GA[2], Reijneveld JC[6], Wesseling P[7], Pinkel D[8], Albertson DG[9], Ylstra B[10].

Author information

Abstract

Detection of DNA copy number aberrations by shallow whole-genome sequencing (WGS) faces many challenges, including lack of completion and errors in the human reference genome, repetitive sequences, polymorphisms, variable sample quality, and biases in the sequencing procedures. Formalin-fixed paraffin-embedded (FFPE) archival material, the analysis of which is important for studies of cancer, presents particular analytical difficulties due to degradation of the DNA and frequent lack of matched reference samples. We present a robust, cost-effective WGS method for DNA copy number analysis that addresses these challenges more successfully than currently available procedures. In practice, very useful profiles can be obtained with ~0.1× genome coverage. We improve on previous methods by first implementing a combined correction for sequence mappability and GC content, and second, by applying this procedure to sequence data from the 1000 Genomes Project in order to develop a blacklist of problematic genome regions. A small subset of these blacklisted regions was previously identified by ENCODE, but the vast majority are novel unappreciated problematic regions. Our procedures are implemented in a pipeline called QDNAseq. We have analyzed over 1000 samples, most of which were obtained from the fixed tissue archives of more than 25 institutions. We demonstrate that for most samples our sequencing and analysis procedures yield genome profiles with noise levels near the statistical limit imposed by read counting. The described procedures also provide better correction of artifacts introduced by low DNA quality than prior approaches and better copy number data than high-resolution microarrays at a substantially lower cost.

PMID: 25236618 PMCID: PMC4248318 DOI: 10.1101/gr.175141.114

HiSeq 4000

- 1 run yields 210–250 Gb of data for 1x50bp
- 8 lanes per run (26 250 000 000 bp per lane)

- One lane contained 14 to 18 samples
- Sequence 35 bp per read
- On average: 46,9 million total reads per sample
- (on average: human genome has 3,2 billion nucleotides)

Output of QDNAseq

- Read counts per bin
- Corrected for GC-content and sequence mappability
- Filtered (of problematic genomic regions)
- Median-normalized
- Log_2-transformed

- Picking up aberrations: calls starting from 500 à 600kb
- Mean coverage +/-0,17

  [(PF_READS_ALIGNED * Read length) / genome length; with genome 1-22 +X +Y hg19: 3.095.677.412 and read length 35 nt]

Noise in copy number profiles comes from the statistics of counting as well as from the many steps during the analysis of the sample

Noise sources:

- Sample acquisition and storage/transportation
- DNA extraction
- Array: DNA labeling, hybridization
- NGS: library prep, sequencing
- Computational processing

- The variances of all these independent sources of noise are additive
- Look at the variances of the profiles to investigate their noise characteristics
- Noise in LPS is dominated by counting statistics for most of the samples, so can be reduced by read depth

Compare signal-to-noise ratio of array versus shallow sequencing

Array-CGH Leuven

- Oxford Gene Technology's CytoSure Haematological Cancer + SNP array (8x60k format)
- 60.000 spots
- Targets regions important for CLL, MM, MPN and MDS
- Backbone: 1 probe every 117kb
- Average gene resolution: 1 probe every 68 kb
- LOH regions > 30 Mb
- Segmentation based normalisation

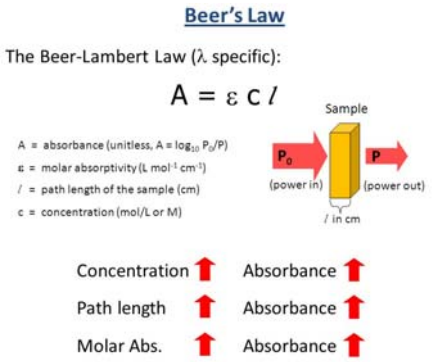
=> Dye Swap: every sample is analysed twice

CLL: current routine diagnostic methods

- Karyotype at diagnosis after 3 day culture stimulated with CpG/IL2
- FISH after culture
    - LSI ATM (SO) / CEP12 (SG), 11q22/centromere 12 (Vysis)
    - LSI13 (RB1) (SG) / D13S319 (SO), 13q14 (Vysis)
    - LSI TP53 (SO) / CEP17 (SG), 17p13.1/centromere 17 (Vysis)
- CLL FU: FISH for TP53 if the patient needs (new) treatment

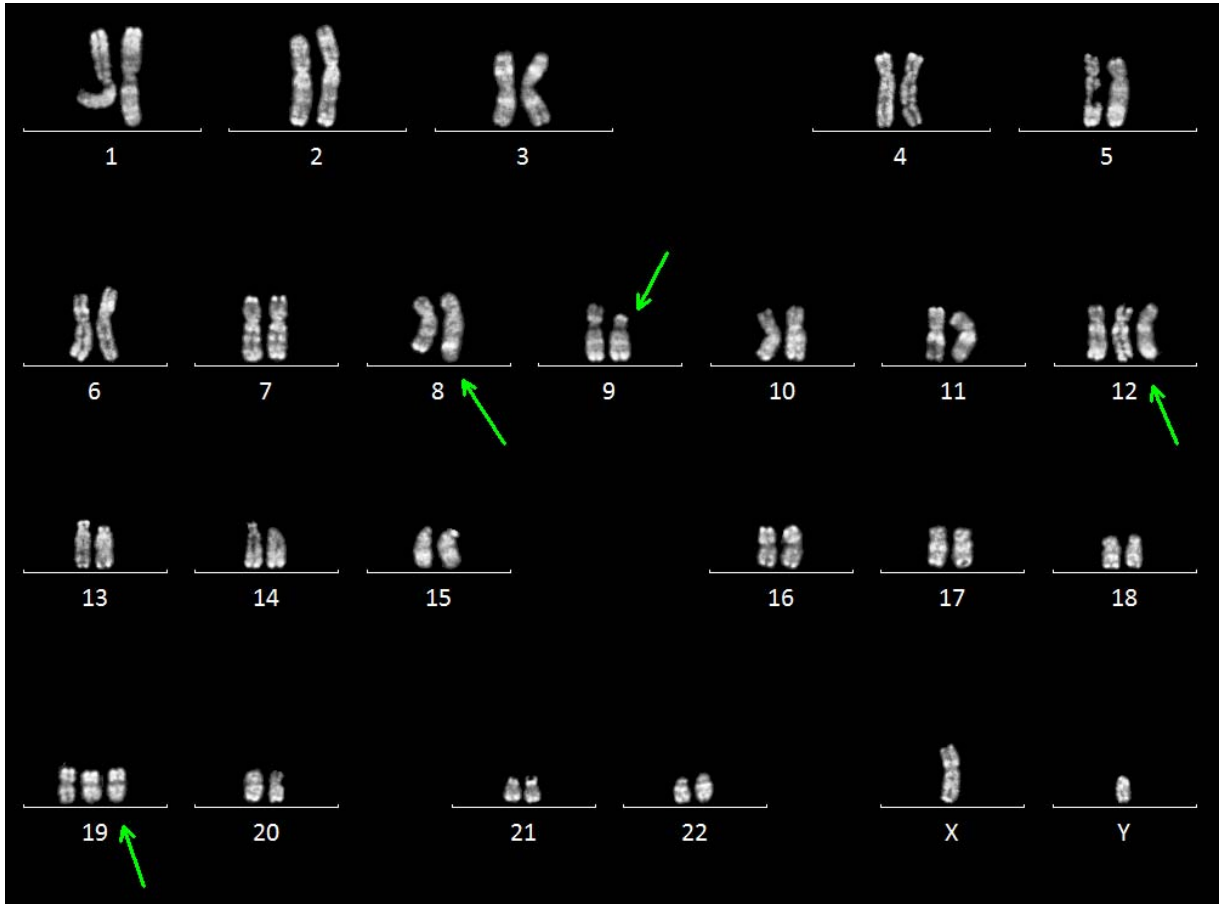
CLL: DNA for array-CGH and LPS

- DNA extracted from uncultured whole blood sample (<3ml; EDTA)
- Chemagen B-4K method
- Magnetic polyvinyl alcohol beads (M-PVA beads) which will bind the DNA after lysis of the blood
- DNA concentration and purity: measured by DropSense96 (UV-VIS absorption analysis) (Beer-Lambert law)



CLL case 8: karyotype and FISH

Caryotype: 48,XY,+12,+19[1]/48,sl,t(8;9)(q24;p12)[9]
Anomalies clonales complexes (3). Pronostic moins favorable.



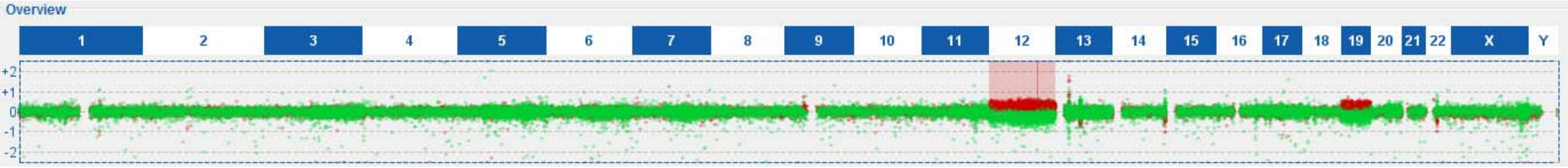
Loss of 13q14 not detected in karyotype; but only using karyotyping the t(8;9) is observed!

CLL case 8: karyotype and FISH

FISH results:

Probe	Result
LSI ATM (SO) / CEP12 (SG), 11q22/centromere 12 (Vysis)	gain 12 (51%)
LSI13 (RB1) (SG) / D13S319 (SO), 13q14 (Vysis)	loss 13q14 (51%)
17p13.1/centromere 17 (Vysis)	normal 17p13

CLL case 8: array-CGH profile, whole genome view



DLR Spread: 0.1318
Red Signal Intensity: 759.888
Green Signal Intensity: 521.413
Red Background Noise: 8.104
Green Background Noise: 7.5138
Red Signal-to-Noise Ratio: 93.7667
Green Signal-to-Noise Ratio: 69.394
Green Signal Reproducibility: 0.0589
Red Signal Reproducibility: 0.0616
Non-Uniform Features: 0%
Red Signal Intensity (SNPs): 173.6497
Green Signal Intensity (SNPs): 116.2103

Control of the quality parameters: OK

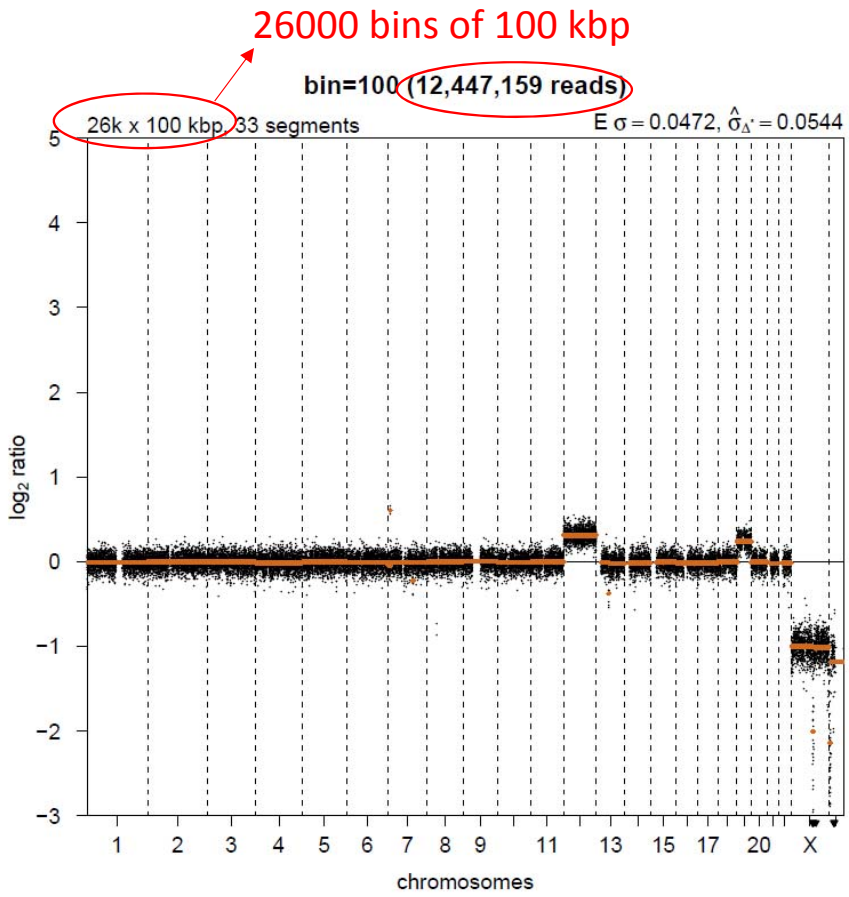
DLR (derivative log ratio): < 0,20
Red signal intensity: >300
Green signal intensity: >200
Red background noise: <15
Green background noise: <15
S/N (red/green signial to noise): > 30

FISH, array-CGH or LPS: same conclusion:

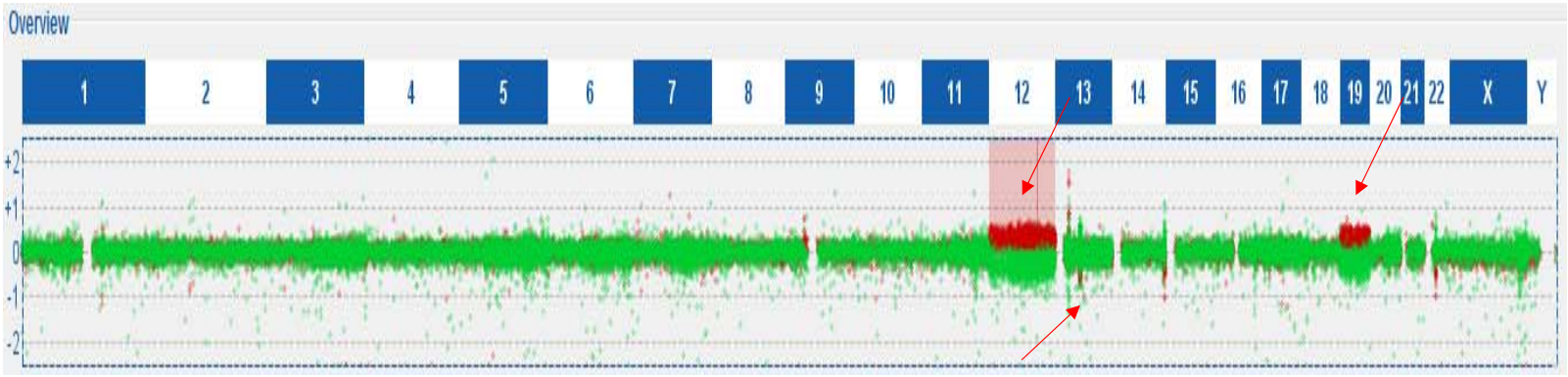
- 13q loss (containing *DLEU1, DLEU2, DLEU7, MIR15A and MIR16-1* and NOT *RB1*) and trisomy 12 are recurrent abnormalities in CLL.
- Trisomy 12 is associated with an intermediate prognosis.

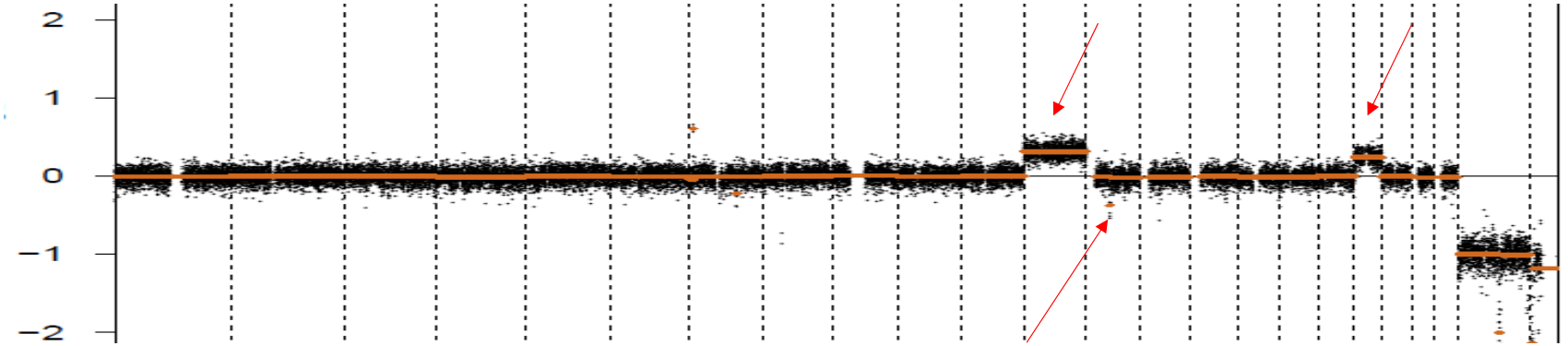
Note: chromosome 19 not analysed by FISH …

CLL case 8: array-CGH profile, whole genome view



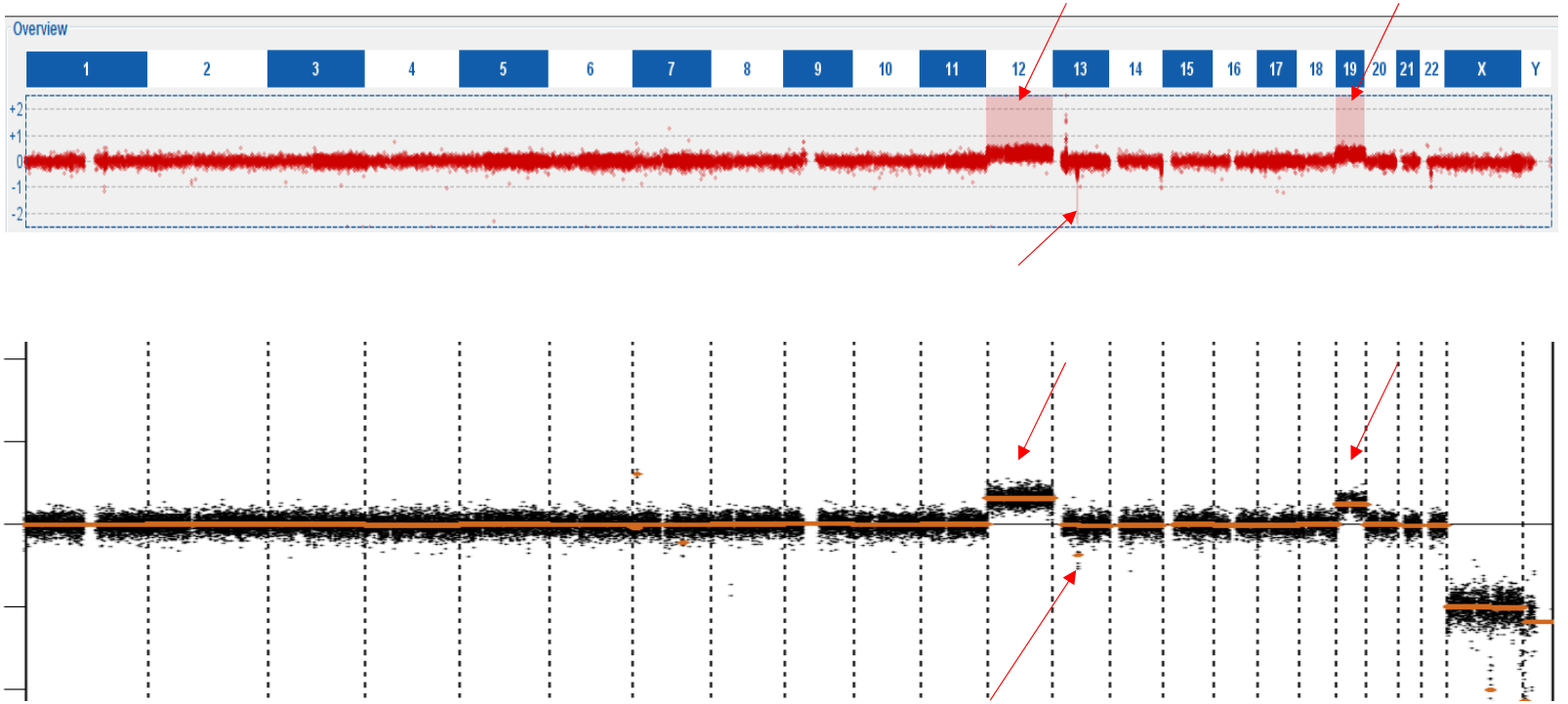
CLL case 8: more noise with array than LPS





Dye Swap
(2 experiments visualized)

CLL case 8: more noise with array than LPS

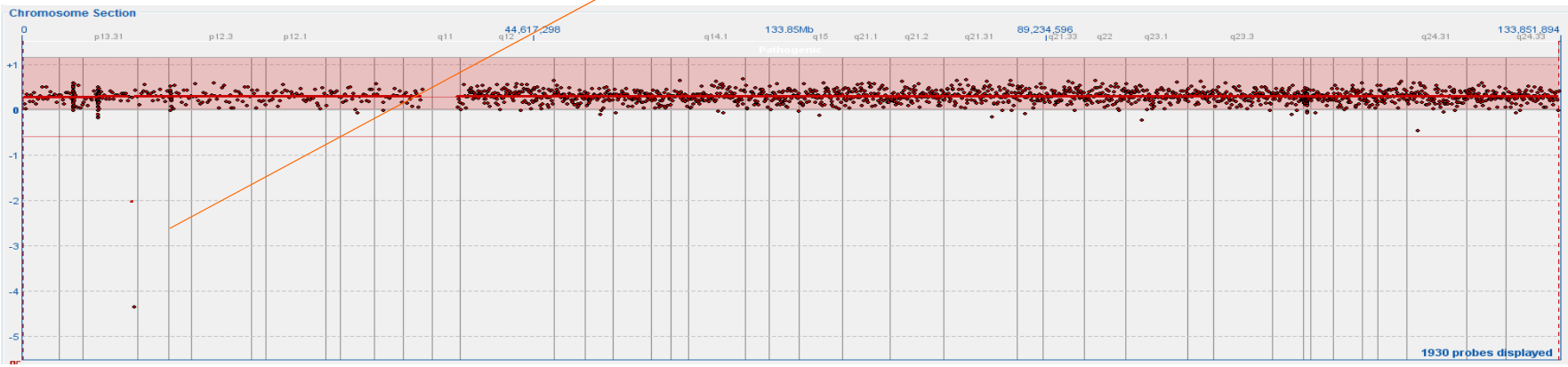


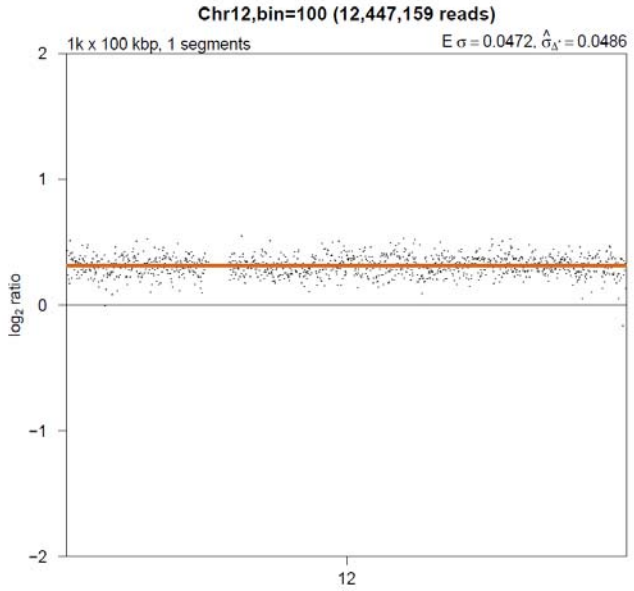
Visualisation without Dye Swap

Chromosome 12: array vs LPS

Outliers are frequently observed, all over the genome

Log2 ratio: 0,31

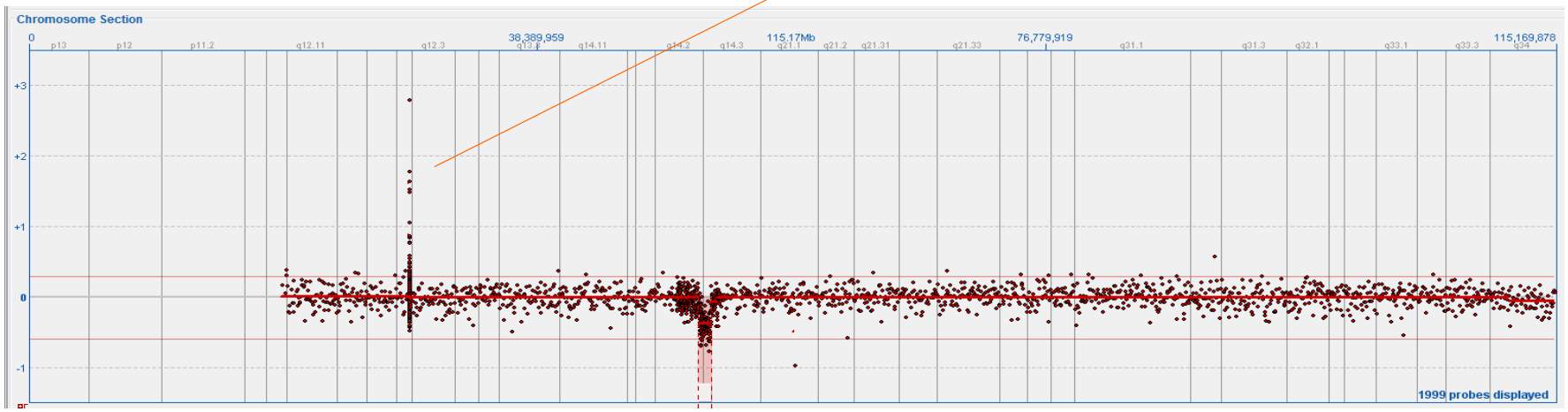




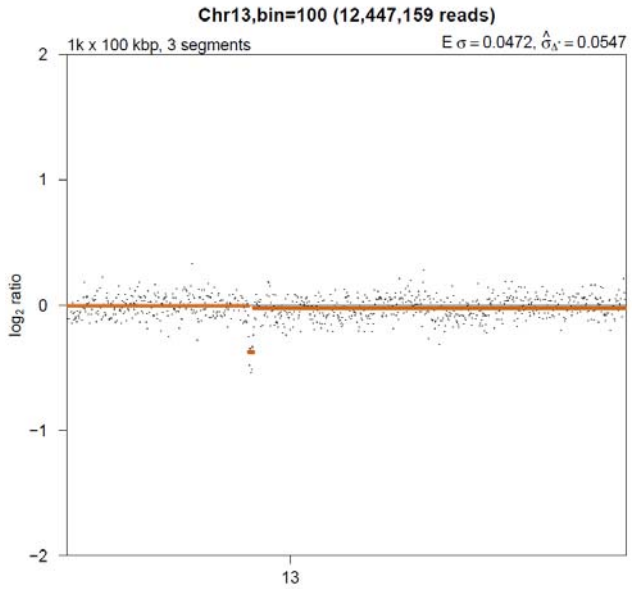
- The trisomy 12 is very clear
- Clean profile

Chromosome 13: array vs LPS

error in OGT array design



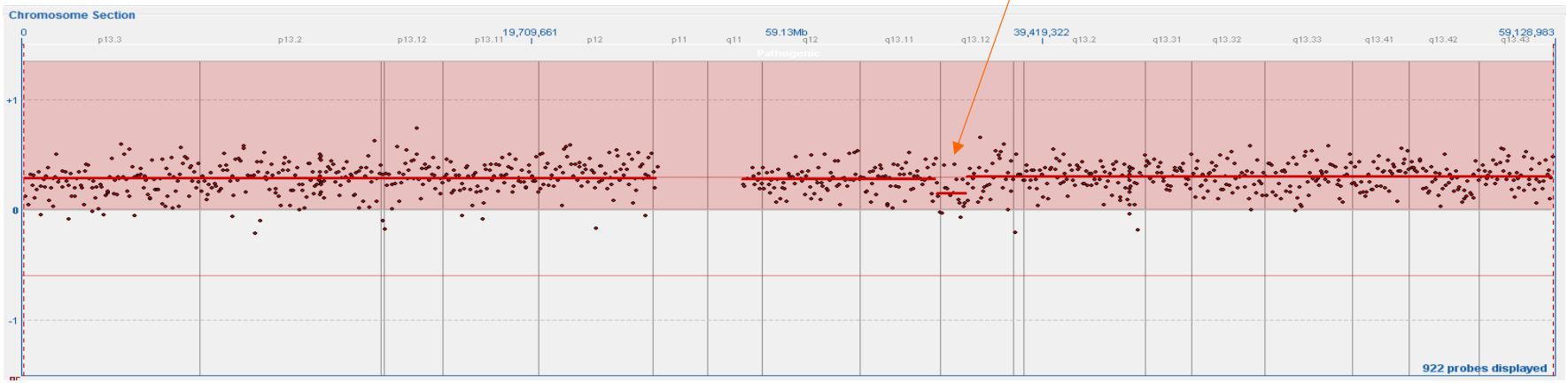
Log2 ratio:
-0,37

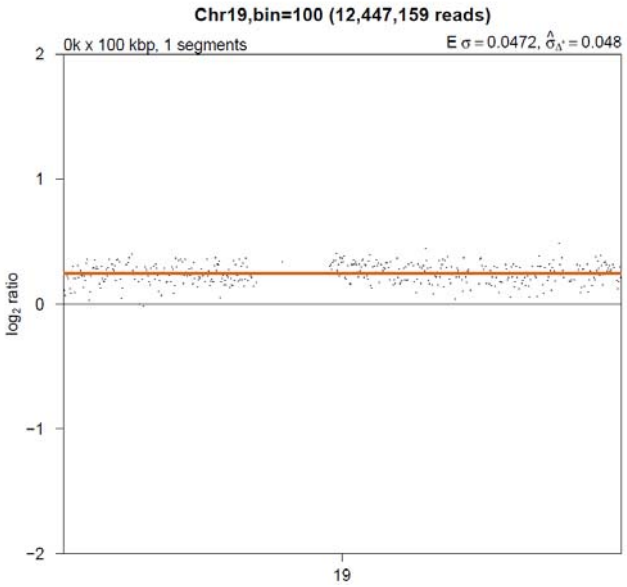


Chromosome 19: array vs LPS

Fluctuations like this are common using these arrays, all over the genome

Log2 ratio: 0,30





Output LPS data in Excel

chr	start	end	Size	sample	AVG_score	regcount	DupDel	ISCN_notation
12	400000	131000000	130,6 Mb	GC042604_100	0,41509009		GAIN	arr[hg19]12p13.33q24,33(400,000-131,000,000)x3
13	50600000	51100000	0,5 Mb	GC042604_100	-0,49875		LOSS	arr[hg19]13q14.2q14.3(50,600,000-51,100,000)x1
19	7500000	56900000	49,4 Mb	GC042604_100	0,399923077		GAIN	arr[hg19]19p13.2q13,43(7,500,000-56,900,000)x3

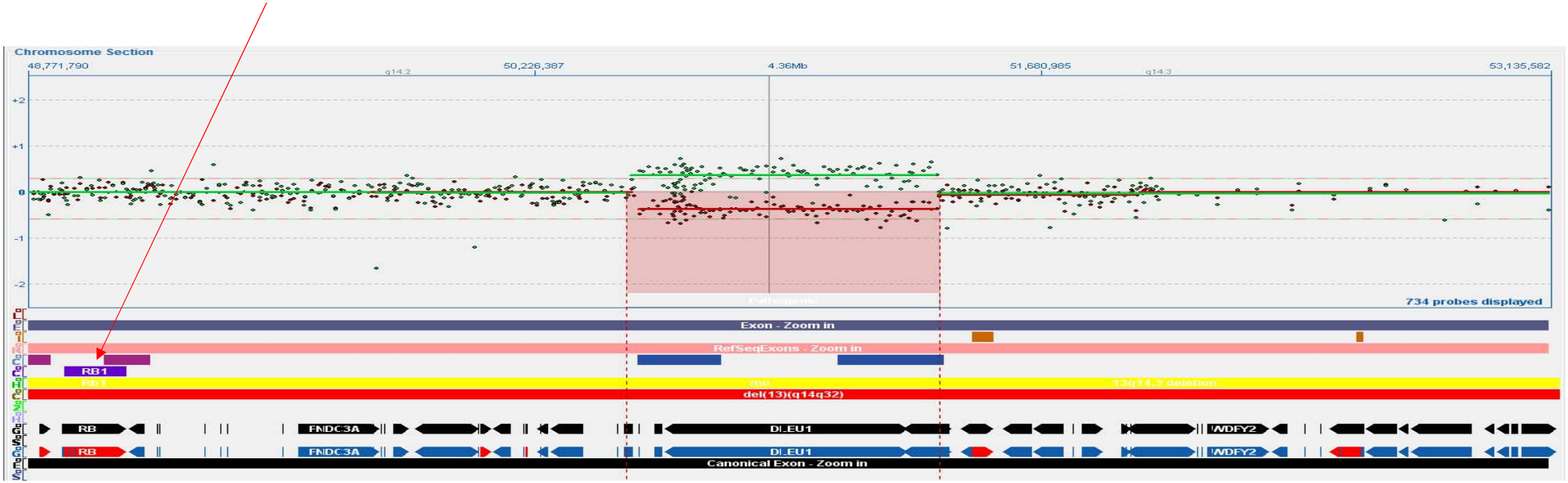
Visualisation software?

Output Array data from CytoSure in pdf

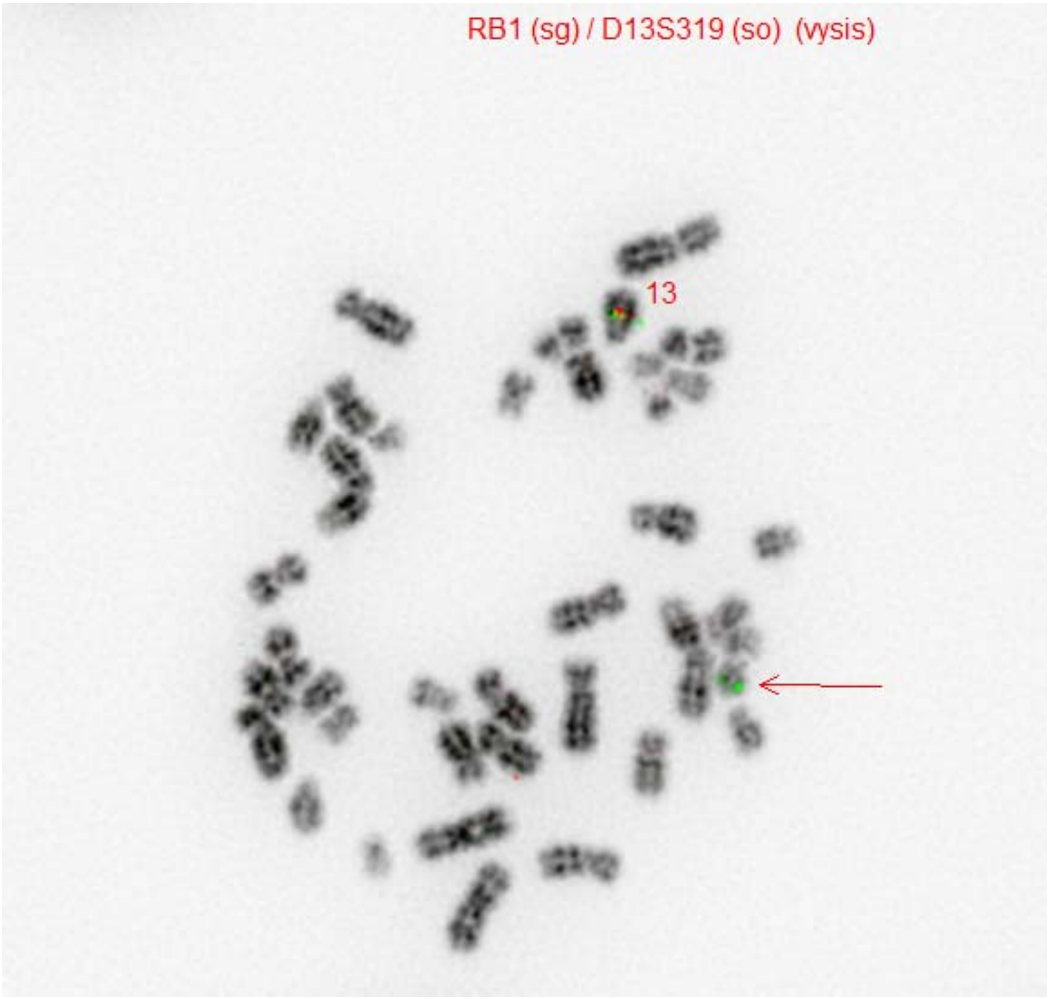
ISCN Notation : *arr[hg19] 12p13.33q24.33(151,196-133,773,393)x3*
Gain/Loss : *Gain*
 # Probes : *1930*
Mean Log Ratio : *0.3148*
Size : *133.62Mb*
Classification (Initial) : *Unclassified*
Classification (Final) : *Unclassified*

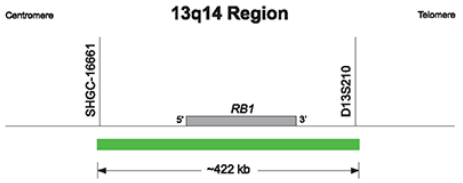
ISCN Notation : *arr[hg19] 13q14.2q14.3(50,500,242-51,374,099)x1*
Gain/Loss : *Loss*
 # Probes : *108*
Mean Log Ratio : *-0.3672*
Size : *873.86Kb*
Classification (Initial) : *Unclassified*
Classification (Final) : *Pathogenic*

With CytoSure analysis/visualisation software for arrays: you can quickly SEE that *RB1* is not in the deletion

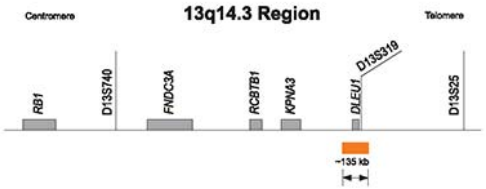


CLL case 5





LSI 13 SpectrumGreen Probe



LSI D13S319 SpectrumOrange Probe

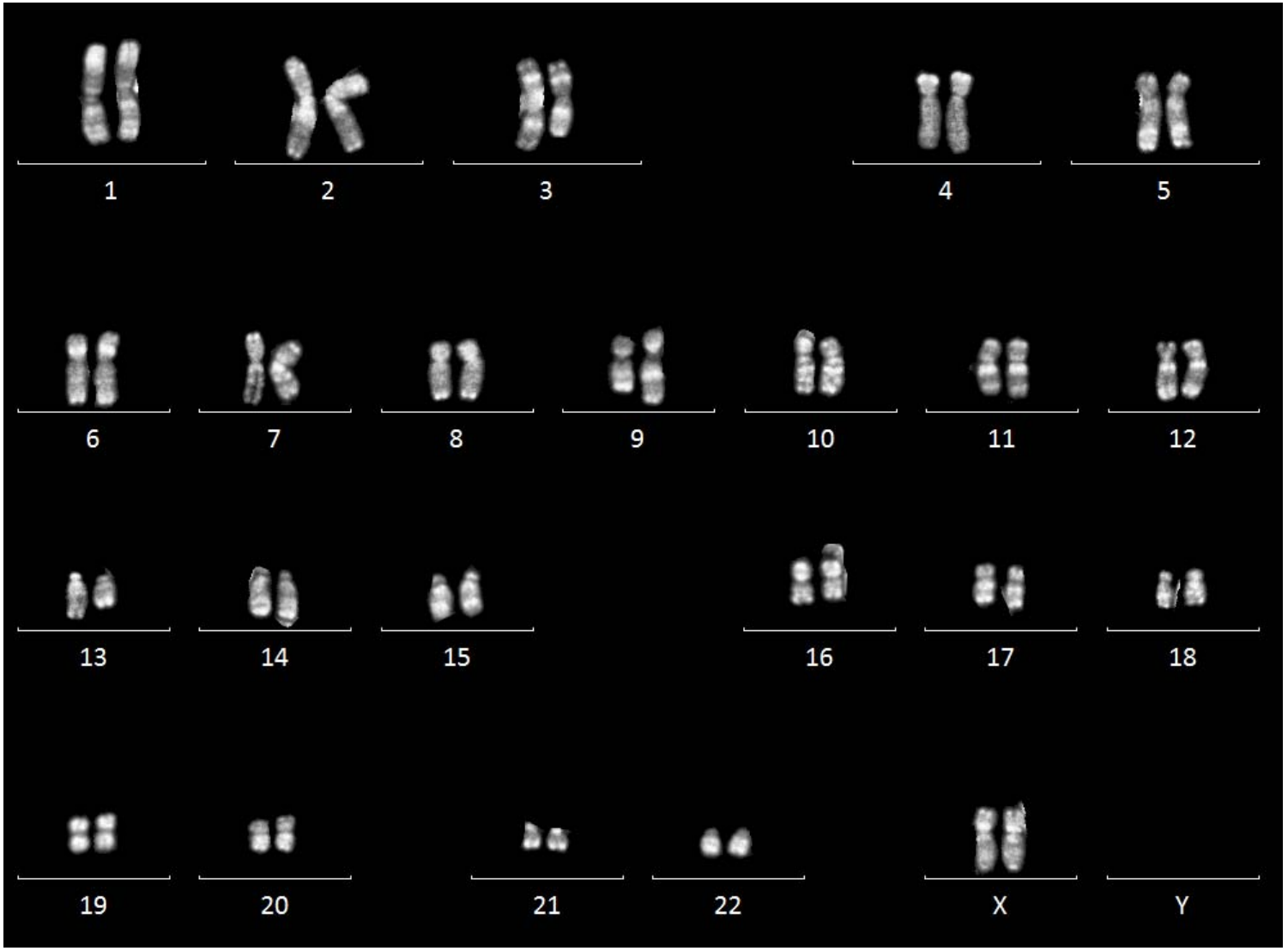
Probe	Result
LSI ATM (SO) / CEP12 (SG), 11q22/centromere 12 (Vysis)	normal 12, normal 11q22
LSI13 (RB1) (SG) / D13S319 (SO), 13q14 (Vysis)	loss 13q14 (11,5%), without loss of RB1
17p13.1/centromere 17 (Vysis)	normal 17p13

FISH on peripheral blood after culture (on 10 metaphases and 200 interphase cells):

Loss of 13q14 (D13S319), without loss of RB1 locus in 4/10 metaphases [loss of D13S319 on the der(13)t(9;13)] and in 11,5% of the interphase cells.
Good prognosis in CLL.

CLL case 5

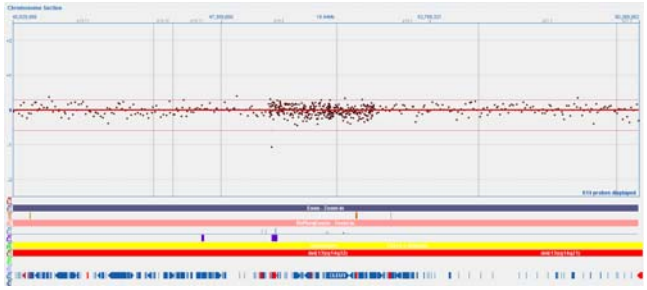
Karyotype: 46,XX,t(9;13)(q13;q12)[4]/46,XX[11]



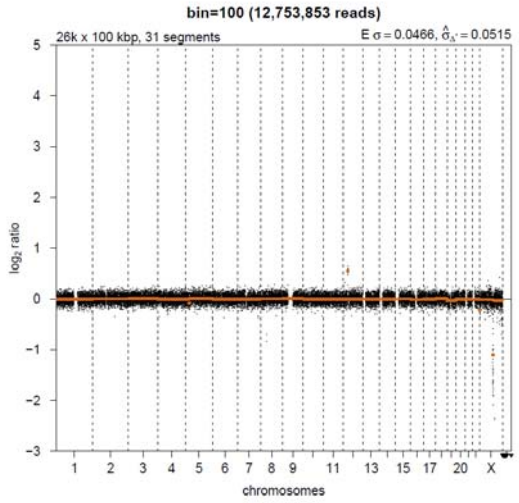
CLL case 5: 13q14 loss not found by array nor LPS

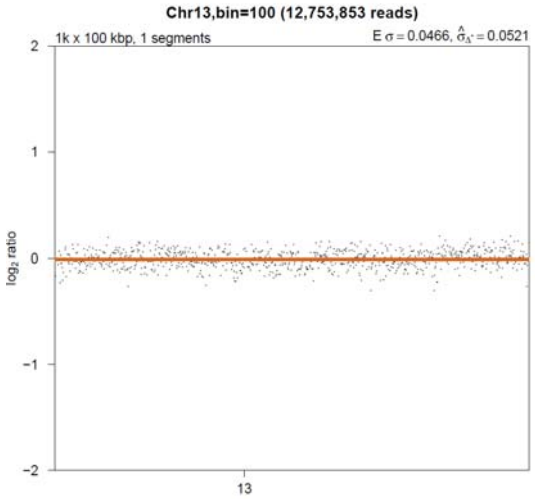
Whole genome view

Chromosome 13 overview



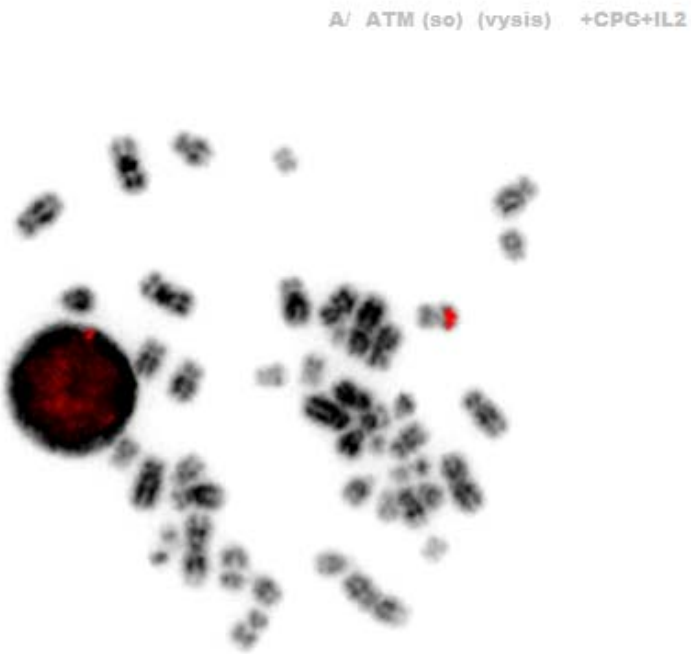
array





LPS

CLL case 6: aberrations in +/- 20-30% of cells are detected by FISH and karyotyping



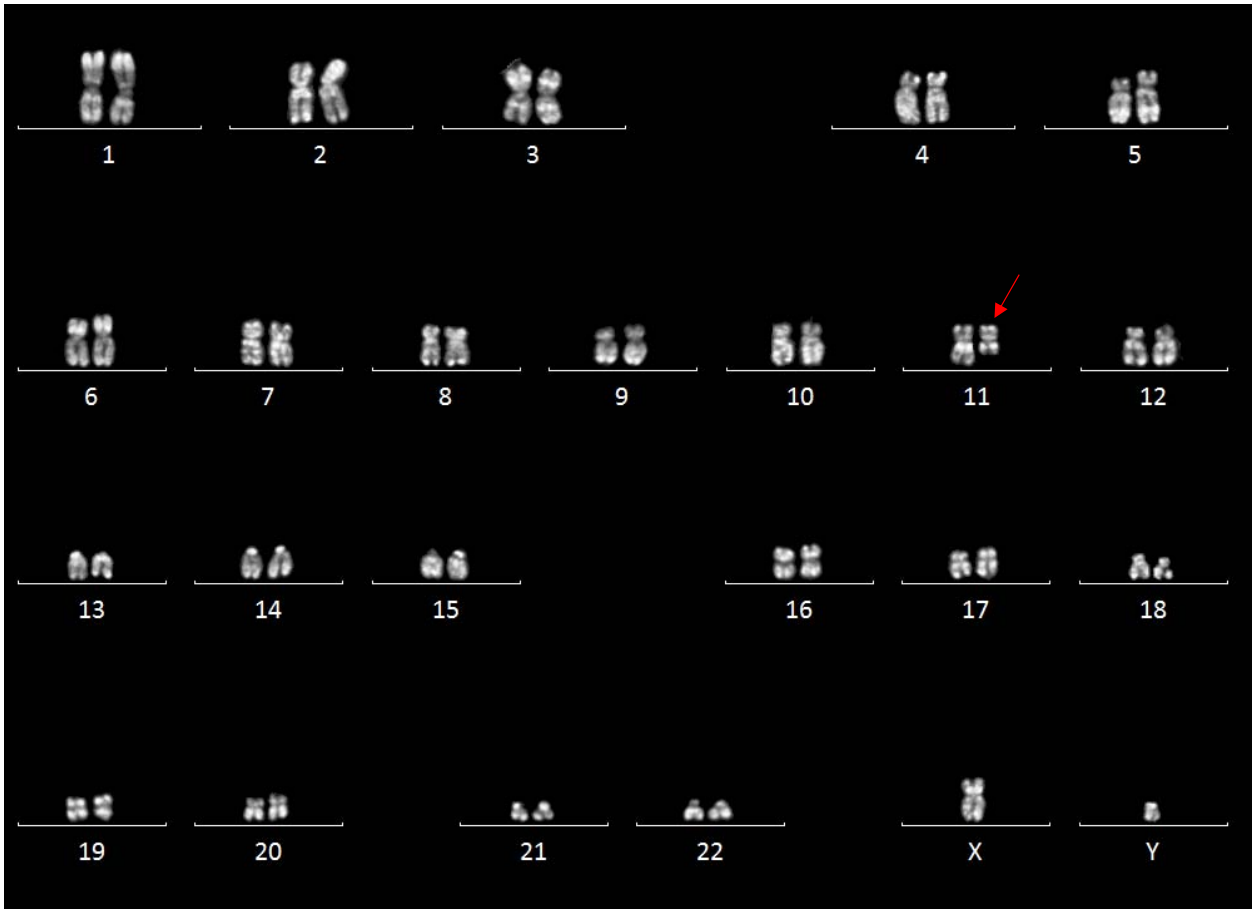
FISH results:

Probe	Result
LSI ATM (SO) / CEP12 (SG), 11q22/centromere 12 (Vysis)	normal 12, loss of 11q22 in 7/10 metaphases and 23% of interphase cells
LSI13 (RB1) (SG) / D13S319 (SO), 13q14 (Vysis)	loss 13q14 (28%), without loss of RB1
17p13.1/centromere 17 (Vysis)	normal 17p13

FISH on peripheral blood after culture (on 10 metaphases and 200 interphase cells):

Loss of 11q22 (ATM) in 7/10 metaphases and in 23% of the interphase cells.
Loss of 13q14 in 28% of the interphase cells.
Worse prognosis in CLL.

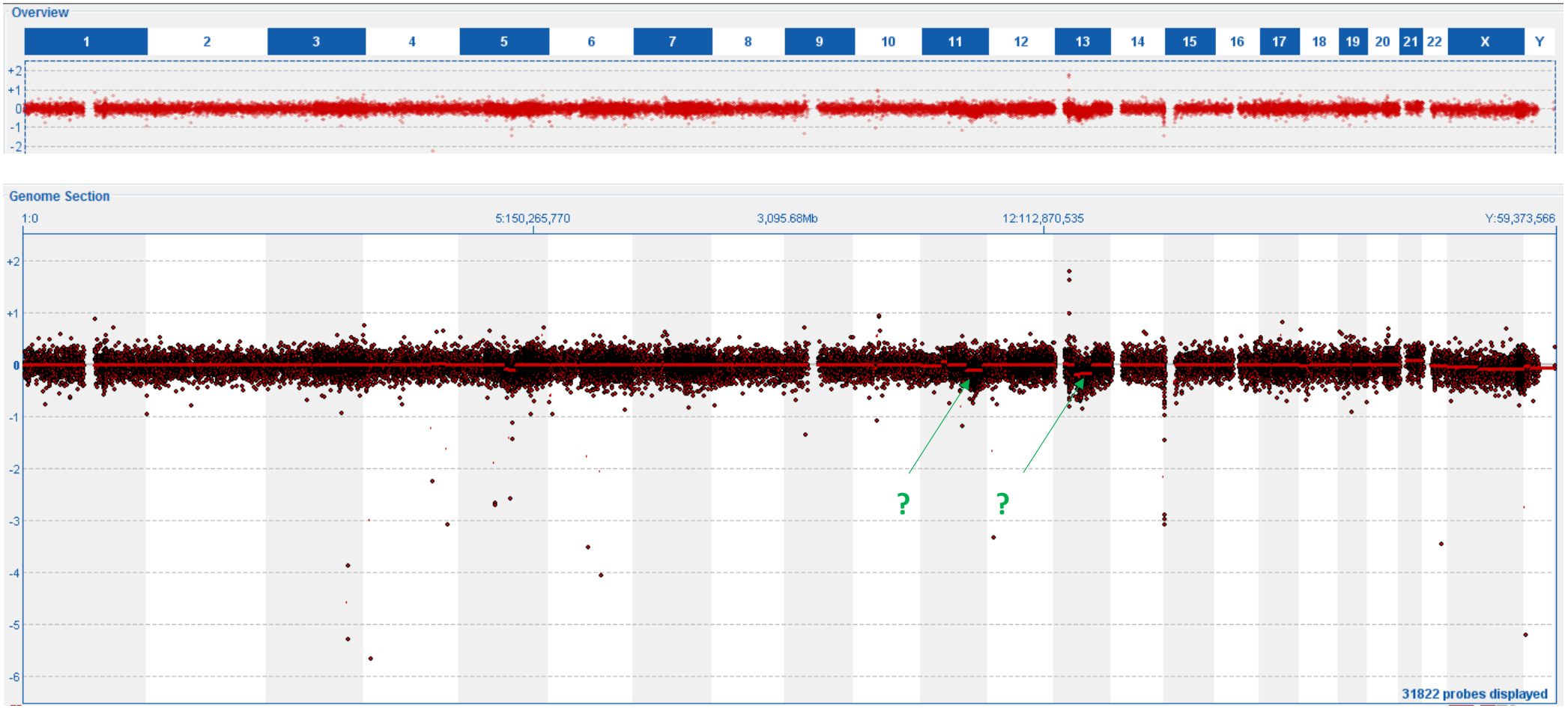
CLL case 6: aberrations in +/- 20-30% of cells are detected by FISH and karyotyping



Karyotype:

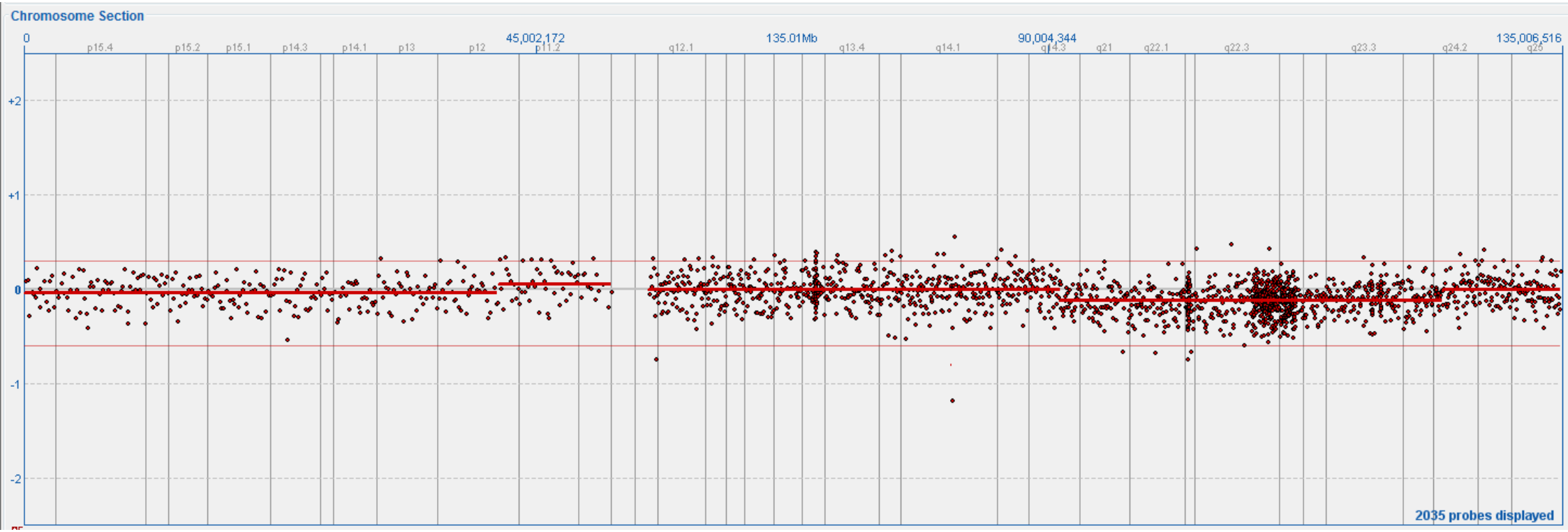
46,XY,del(11)(q22)[9]/46,XY[1]

CLL case 6: aberrations in +/- 20-30% of cells are NOT always detected by array (unclear, below cut-off)



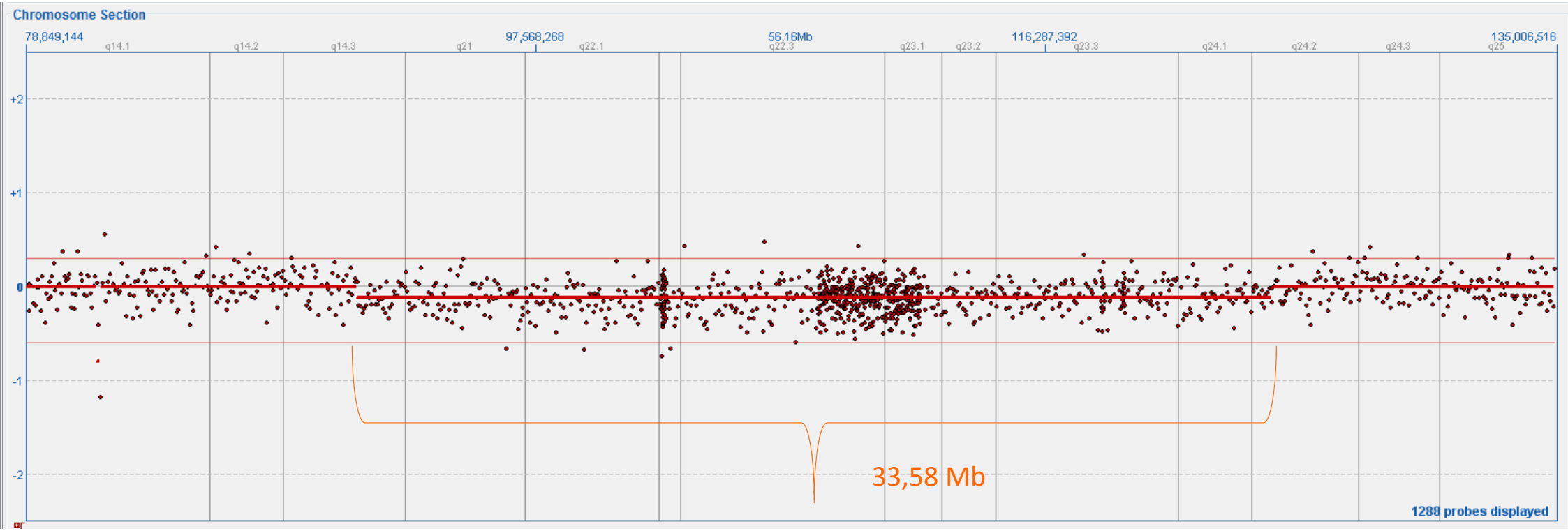
CLL case 6: aberrations in +/- 20-30% of cells are NOT always detected by array (unclear, below cut-off)

Chromosome 11



CLL case 6: aberrations in +/- 20-30% of cells are NOT always detected by array (unclear, below cut-off)

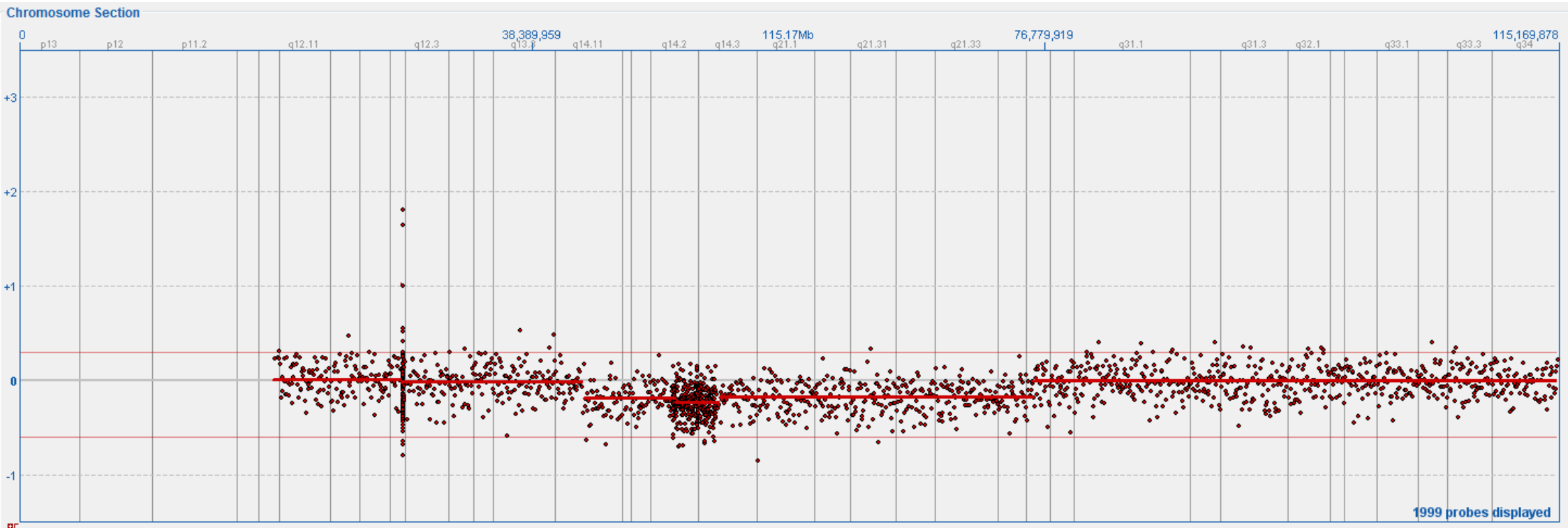
Chromosome 11 => zoomed in on 11q14.1q26



Deletion?? Unclear and below cut-off, log2=-0,1222

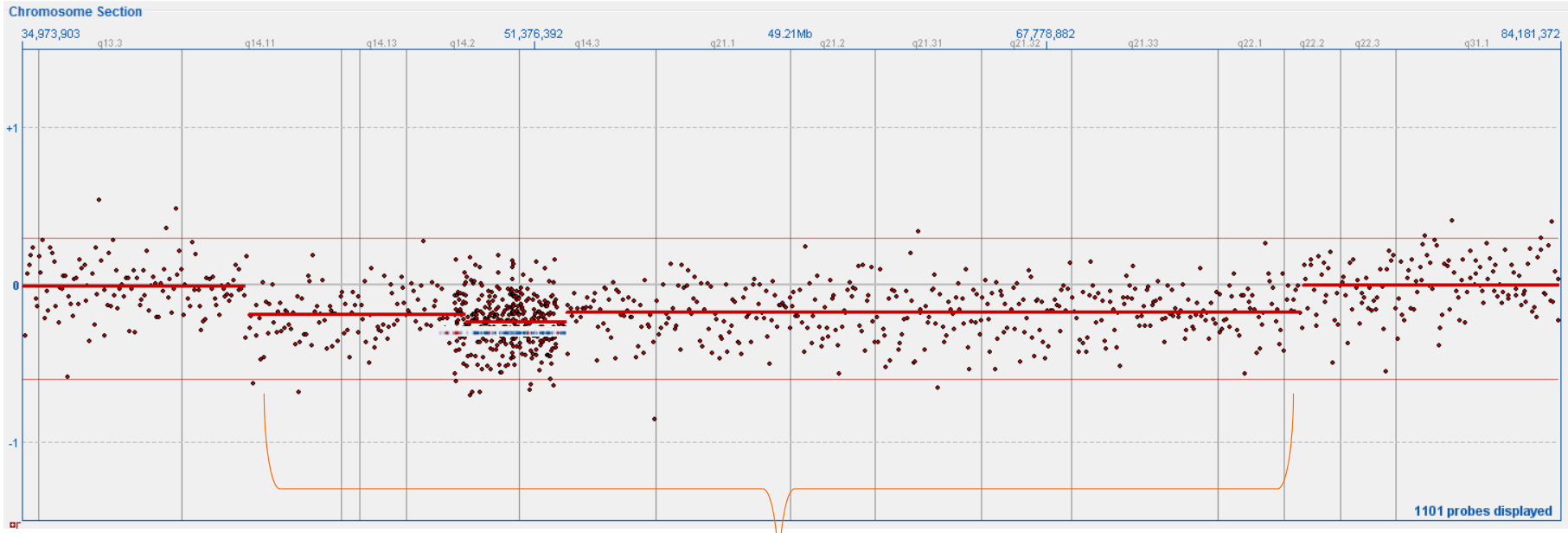
CLL case 6: aberrations in +/- 20-30% of cells are NOT always detected by array (unclear, below cut-off)

Chromosome 13



CLL case 6: aberrations in +/- 20-30% of cells are NOT always detected by array (unclear, below cut-off)

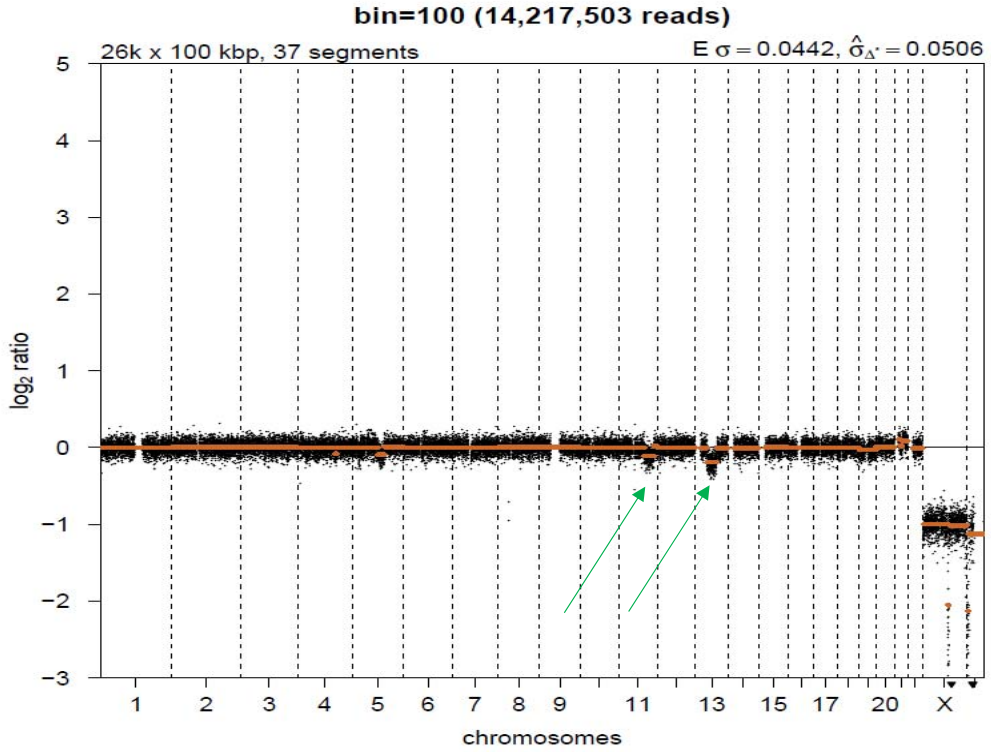
Chromosome 13 => zoomed in on 13q13.3q31,1



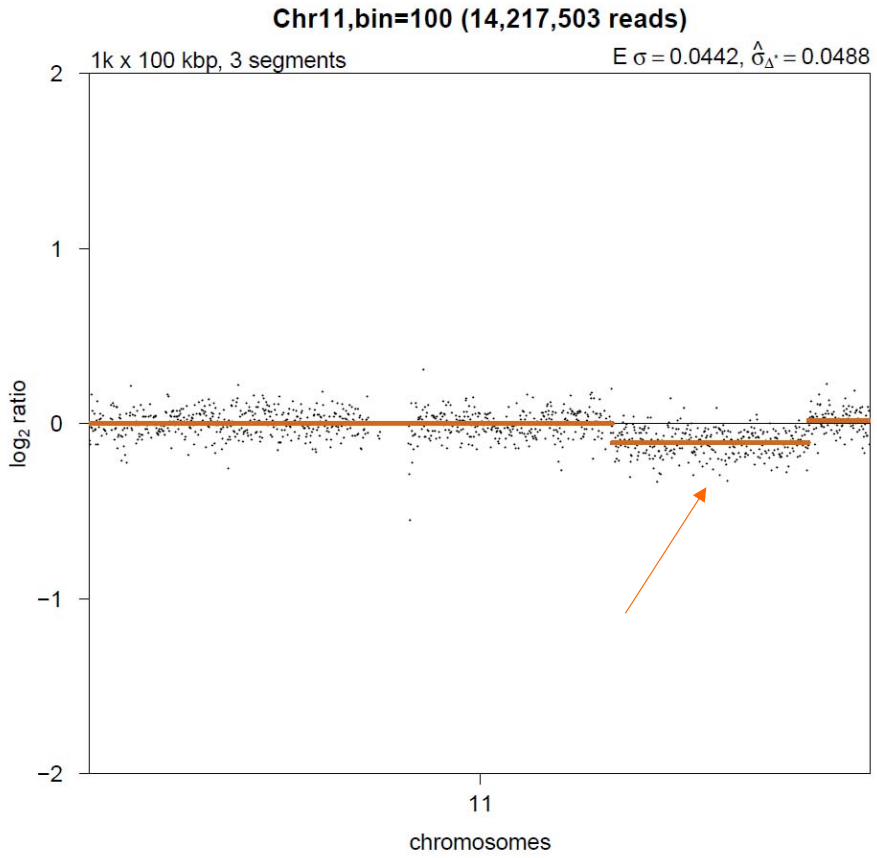
33,07 Mb

Deletion?? Unclear and below cut-off (-0,36); log2 ratio= -0,2001

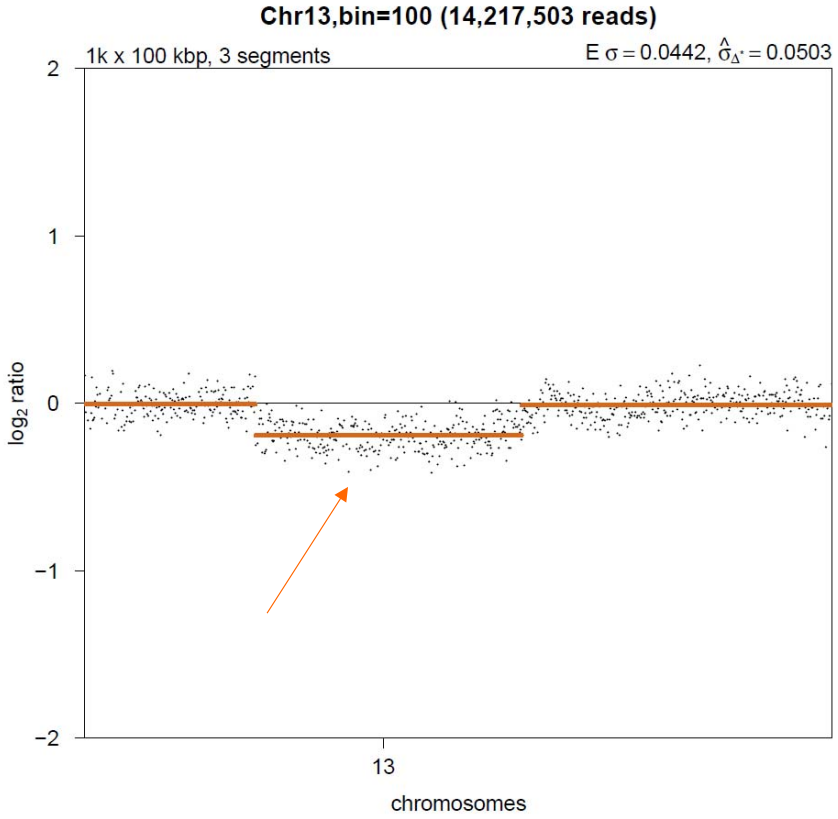
CLL case 6: aberrations in +/- 20-30% of cells are detected by low pass sequencing



CLL case 6: aberrations in +/- 20-30% of cells are detected by low pass sequencing



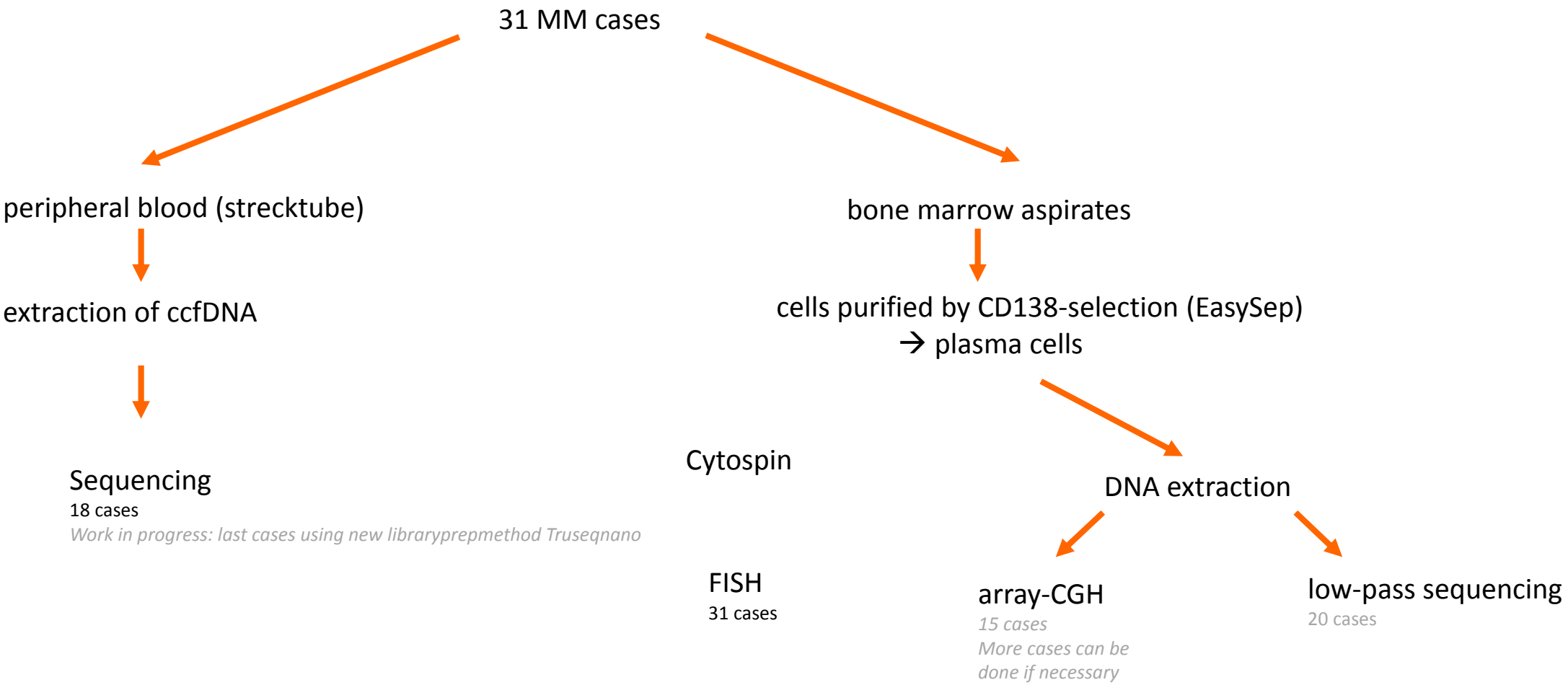
CLL case 6: aberrations in +/- 20-30% of cells are detected by low pass sequencing



Multiple Myeloma (MM): current routine diagnostic method

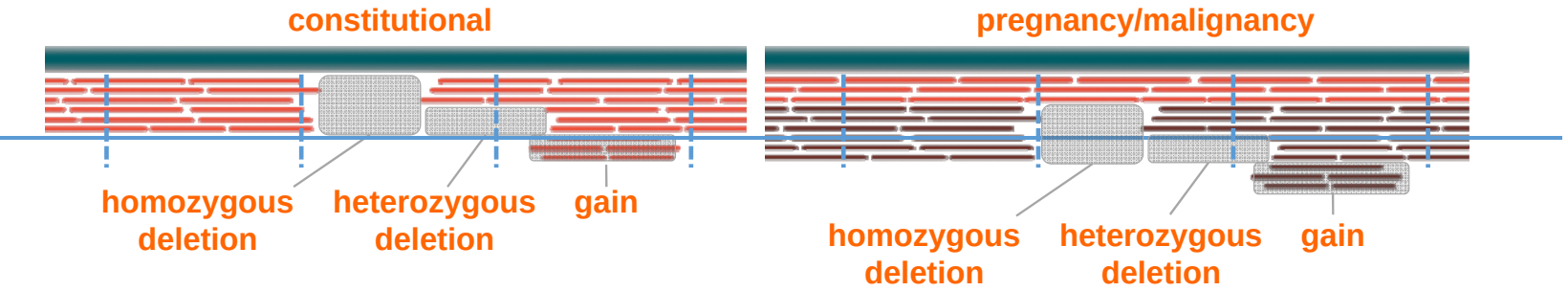
- Direct FISH on cytospin:
  - LSI TP53 (SO) / CEP17 (SG), 17p13.1/centromere 17 (Vysis)
  - LSI IGH DC BA, 14q32 (Vysis)
  - CEP9 (SO), centromere 9 (Vysis)
  - CKS1B (TR) / CDKN2C (SG) [1q21.3/1p32.3, Cytocell] / Chromosome 1 Control Probe [1p11.1-1q12, Empire Genomics]
  - further work-up of IGH if split in IGH (IGH/CCND1 – IGH/FGFR3 – IGH/MAF – MYC BA –IGH/MAFB – IGH/MYC/CEP8)
- FU MM:
  - LSI TP53 (SO) / CEP17 (SG), 17p13.1/centromere 17 (Vysis)
  - LSI MYC DC BA, 8q24.2 (Vysis)
  - CKS1B (TR) / CDKN2C (SG) [1q21.3/1p32.3, Cytocell] / Chromosome 1 Control Probe [1p11.1-1q12, Empire Genomics]
  - probes most important aberration at time of diagnosis

Low-pass sequencing (cfDNA and DNA CD138+ cells) for the detection of copy number aberrations in Multiple Myeloma



Genomic Representation (GR) Profiling

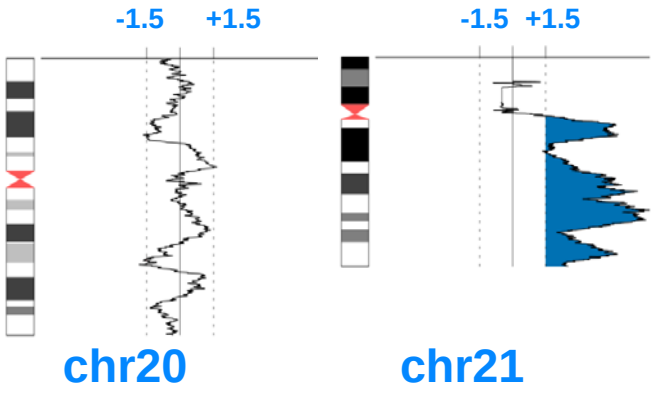
1. filtering and aligning of reads

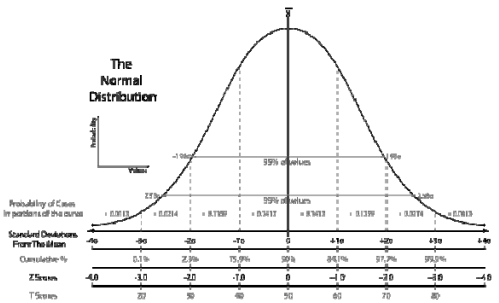


2. GR and z score

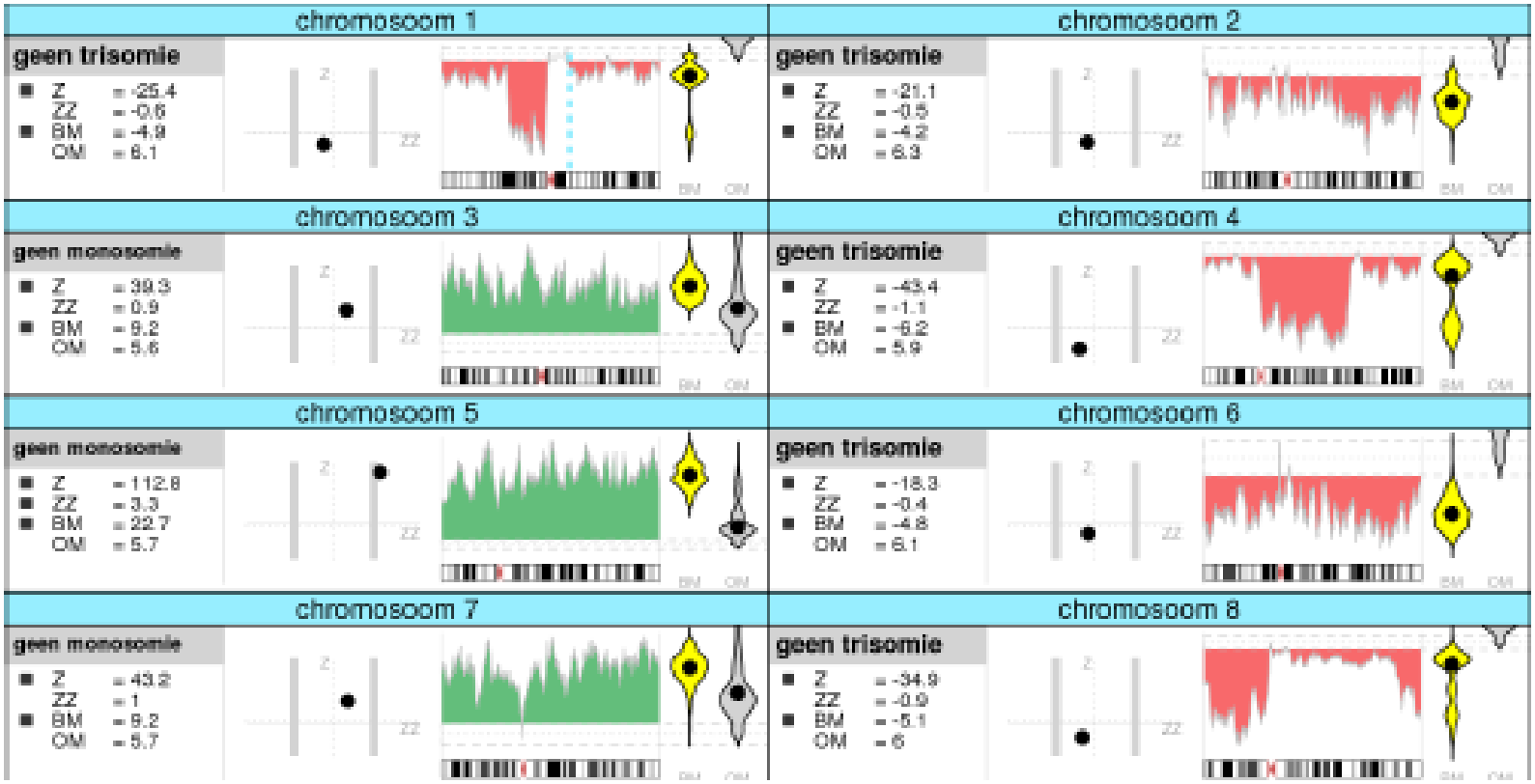
$$GR = \frac{\text{number of reads in a chromosome/partition/bin}}{\text{total of autosomal reads}}$$

$$z\text{-score} = \frac{GR_{\text{measured}} - GR_{\text{normal}}}{SD}$$



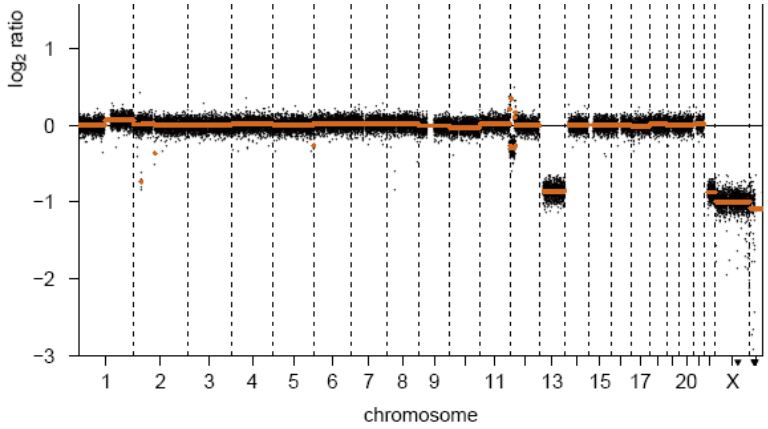


Analysis of low-pass sequencing data of cfDNA: GR and z score

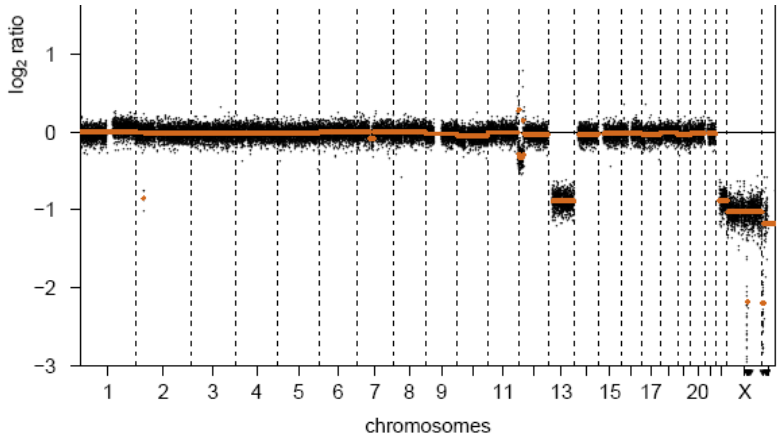


DNA concentration: 500 ng standard, but 50 ng or lower is possible

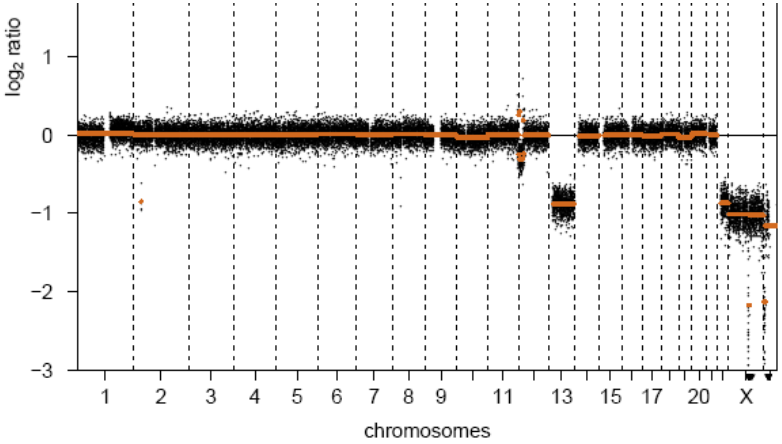
LPS op Hiseq4000 Paired-end DNA input: 500 ng
Run 2 GC037717



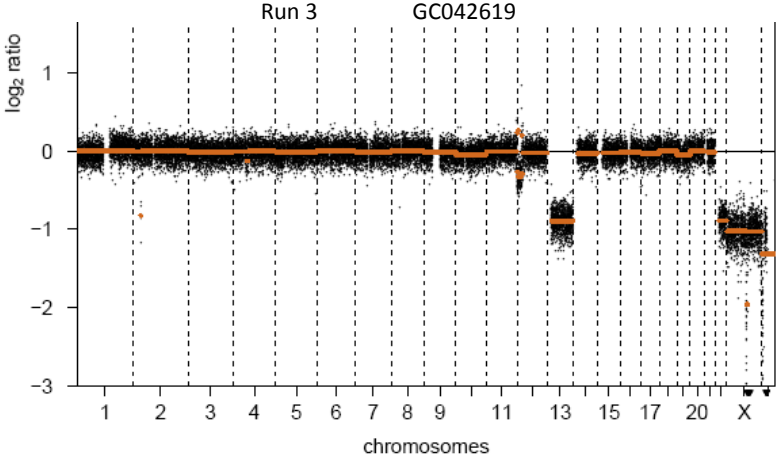
LPS op Hiseq4000 Single-end DNA input: 500 ng
Run 3 GC042617



LPS op Hiseq4000 Single-end DNA input: 100 ng
Run 3 GC042618



LPS op Hiseq4000 Single-end DNA input: 50 ng
Run 3 GC042619

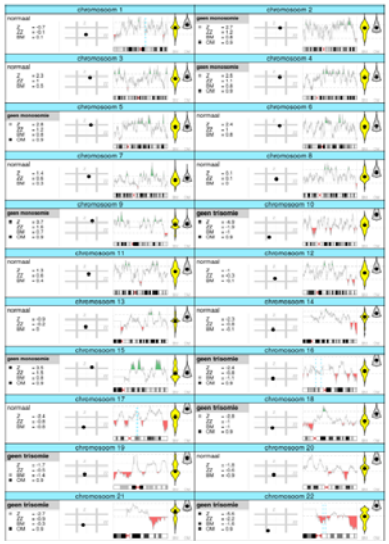


Result FISH: Trisomy 1q (zonder trisomie van centromeer) in 12% of the interphase cells ~ close to cut-off.
Balanced t(4;14)(p16;q32) in 98% of the interphase cells

FISH
Matrix: bone marrow plasma cells

Per sonde werden 48 tot 100 interfasekernen onderzocht.
Dit onderzoek toont:
- trisomie 4p16 in 53% van de kernen.
- trisomie 6p21 in 31-43% van de kernen.
- tri/tetrasomie 9 in 29% van de kernen.
- trisomie 11q13 in 45% van de kernen.
- trisomie 20q12 in 32-43% van de kernen.
- een afwijking van de regio 14q32: partieel verlies van één VH signaal in 37% van de kernen. Bijkomend onderzoek met specifieke probes (IgH/CCND1, FGFR3, CMAF, CCND3, MAFB) toont noch t(11;14), t(4;14), t(14;16), t(6;14), t(14;20).
- de andere regio's lijken normaal. (chromosoom 8 is normaal)
=> Klonale afwijkingen.
Hyperdiploïdie, prognostisch gunstig (standard risico) bij MM.

Low-pass sequencing
Matrix: cfDNA C1D1 (22/03/2016)

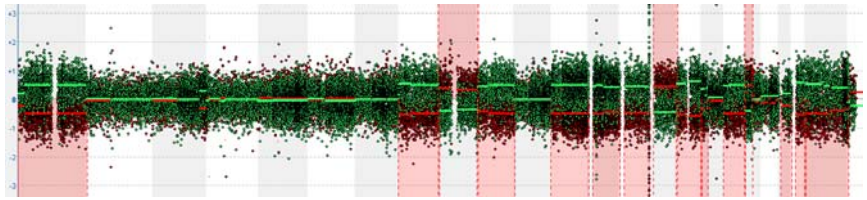


MM case 2
Plasma cells: 13,3 %

Karyotype
bone marrow after 5d culture:

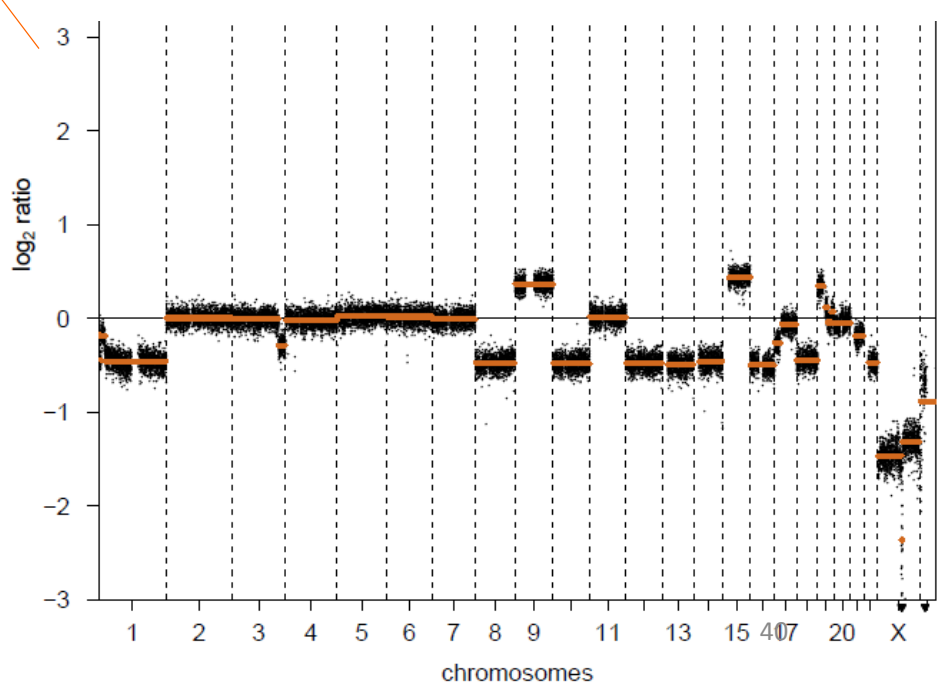
46,XY[10]

Array-CGH
Matrix: bone marrow plasma cells



Hyperdiploid case:
both array and LPS are
NOT correct!

Low-pass sequencing
Matrix: bone marrow plasma cells

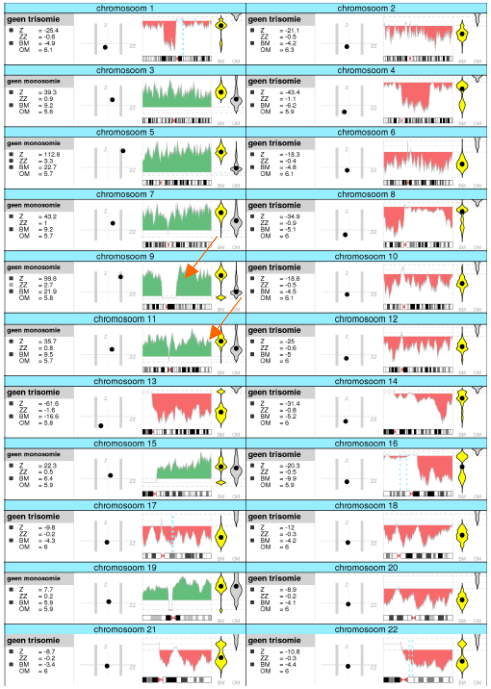


FISH (Matrix: bone marrow plasma cells)
Per sonde werden 100 interfasekernen onderzocht.

Dit onderzoek toont:
- tri/tetrasomie 9 in 89% van de kernen.
- trisomie 11q13 in 88% van de kernen.
- een afwijking van de regio 14q32: één gesplitst CH/VH signaal in 94% van de kernen. Bijkomend onderzoek met specifieke probes (IgH/CCND1, FGFR3, CMAF, CCND3, MAFB, CMYC) toont een niet-gebalanceerde t(8;14)(q24.3;q32) in 100% van de kernen.
- de andere regio's lijken normaal.

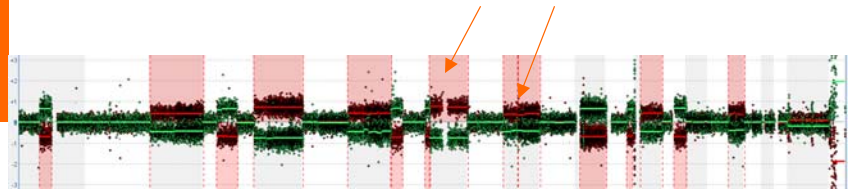
=> Klonale afwijkingen, o.a. t(8;14) (Burkitt translocatie). CMYC translocaties zijn laattijdige of "secundaire" afwijkingen in MM (prognostisch eerder ongunstig).

Low-pass sequencing
Matrix: cfDNA C1D1(29/07/2016)

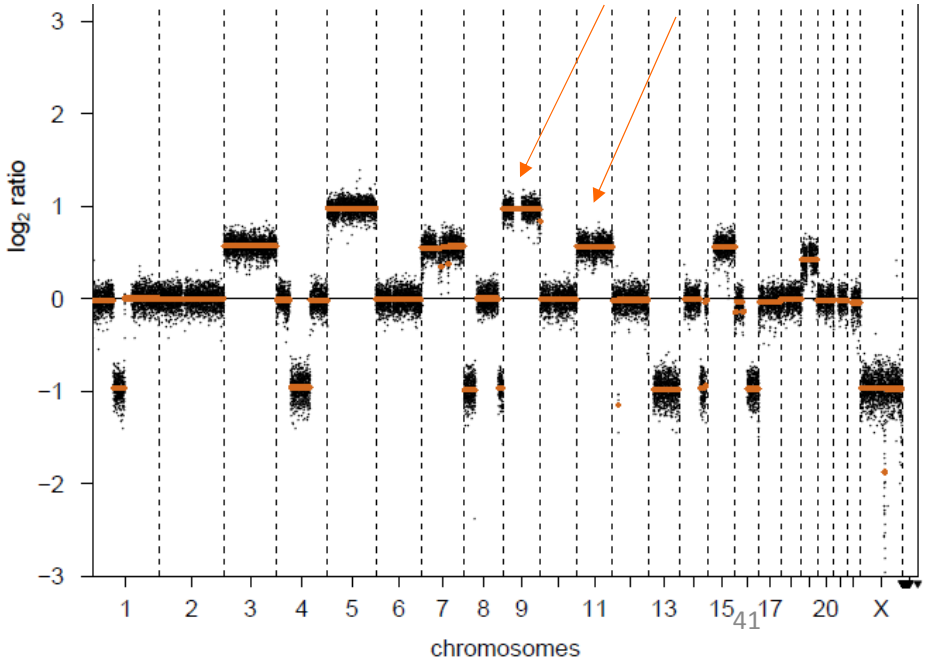


MM17
Plasma cell percentage : 67 %
(28/07/2016)

Array-CGH
Matrix: bone marrow plasma cells (28/07/2016)



Low-pass sequencing
Matrix: bone marrow plasma cells (28/07/2016)



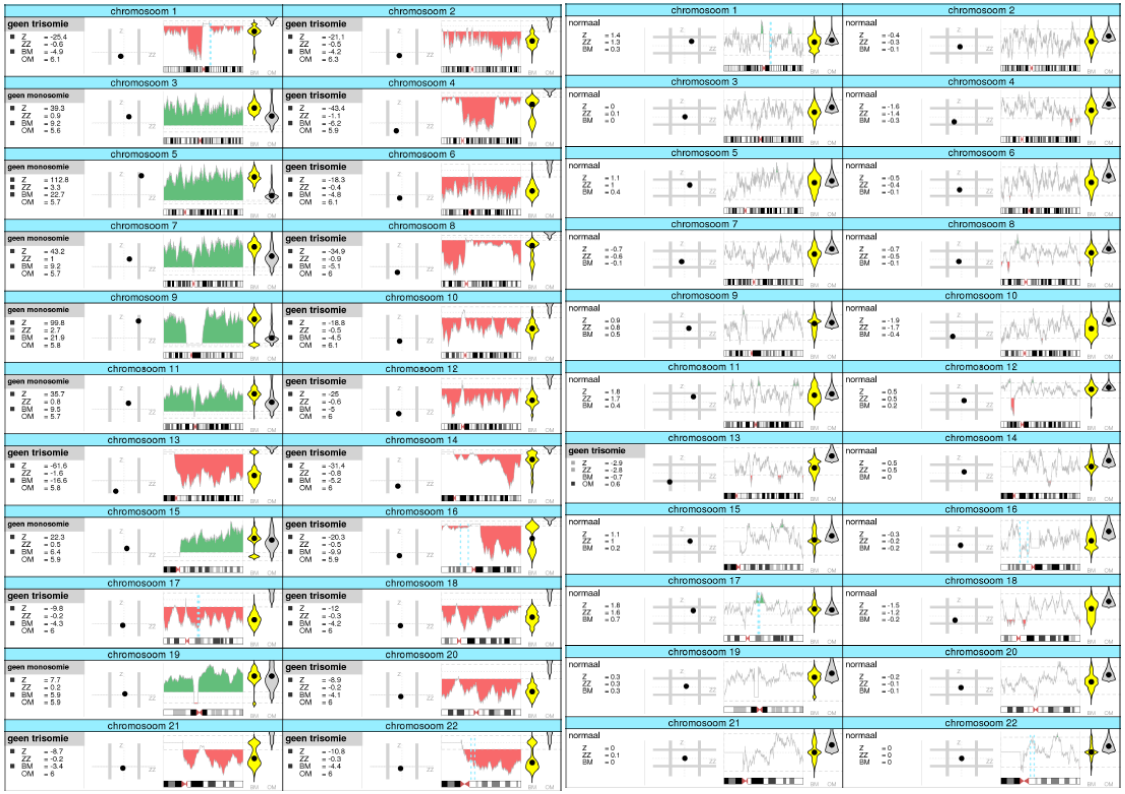
MM17
Plasma cell percentage : 67 % (28/07/2016)

Profile cfDNA normalises rapidly during treatment

Low-pass sequencing

Matrix: cfDNA C1D1 (29/07/2016)

cfDNA C2D1 (9/09/2016)



M-piek in gammafractie (bloed): 28/06/2016: 47,4 g/ L

M-piek in gammafractie (bloed): 9/09/2016: 11,8 g/ L

Laagste Z-score C1D1: 7,7

hoogste Z-score C2D1: 2,9

C3D1 en C4D1 werden ook afgenomen, maar profiel al genormaliseerd daarom niet meer gesequenced.

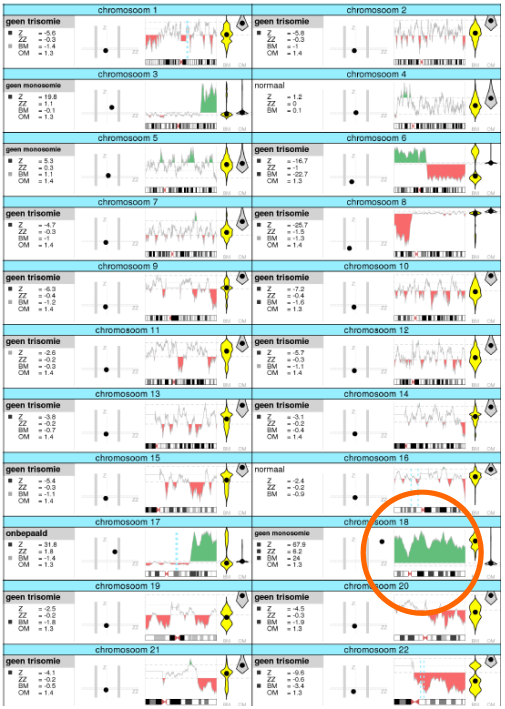
FISH Matrix: bone marrow plasma cells

Per sonde werden 100 interfasekernen onderzocht.
Dit onderzoek toont:

- een afwijking van de regio 14q32: één gesplitst CH/VH signaal in 82% van de kernen. Bijkomend onderzoek met specifieke probes (IgH/CCND1) toont een gebalanceerde t(11;14)(q13;q32) in 74% van de kernen.
- de andere regio's lijken normaal.

Klonale afwijking.
t(11;14), prognostisch gunstig (standard risico) bij MM

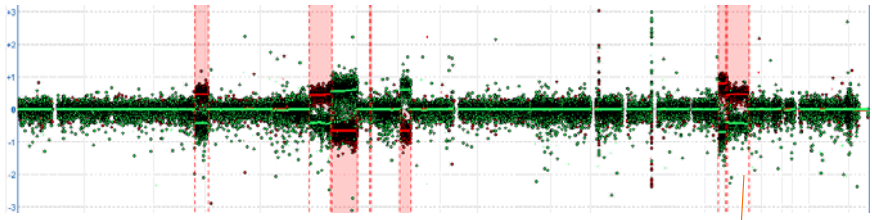
Low-pass sequencing
Matrix: cfDNA voor therapie (24/03/2017)



MM4
Plasma cell percentage (24/03/2016): 43 %

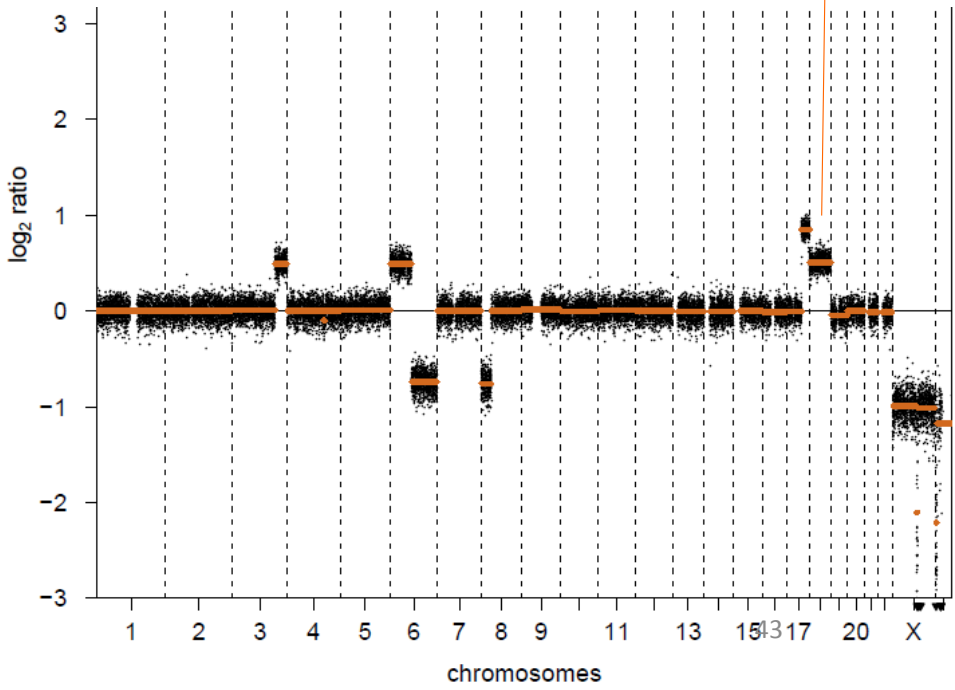
Karyotype
Matrix: bone marrow
48,XY,add(6)(q14),+add(6),-,add(9)(p13),t(11;14)(q13;q32),+18,+mar[6]

Array-CGH
Matrix: bone marrow (niet-opgezuiverd maar geprikt in plasmacytoom)



Cfr karyotype

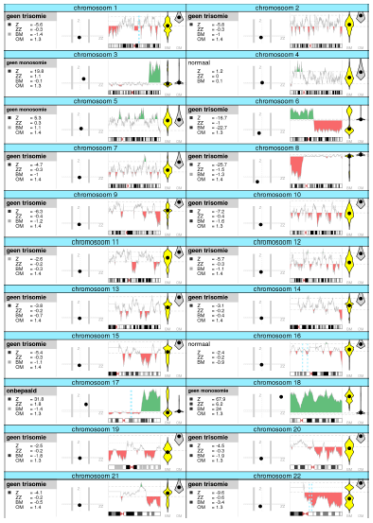
Low-pass sequencing
Matrix: bone marrow (niet-opgezuiverd maar geprikt in plasmacytoom)



MM4
Plasma cell percentage
(24/03/2016): 43 %

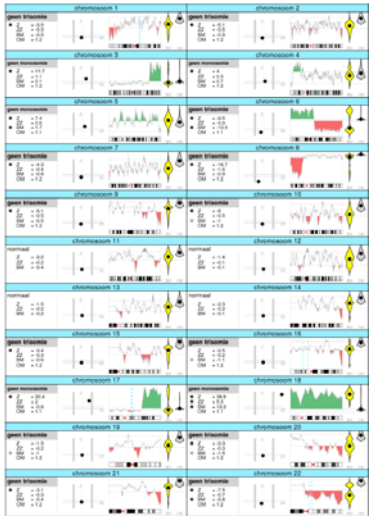
Extra aberration?

Voor C1D1 (24/03/2017)

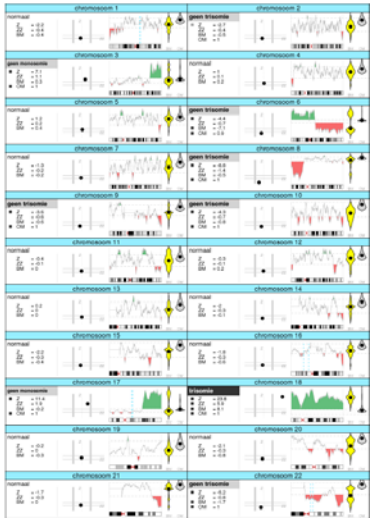


M-piek in gammafractie (bloed):
24/03/2016: 39,2 g/ L

C1D1 (22/04/2016)

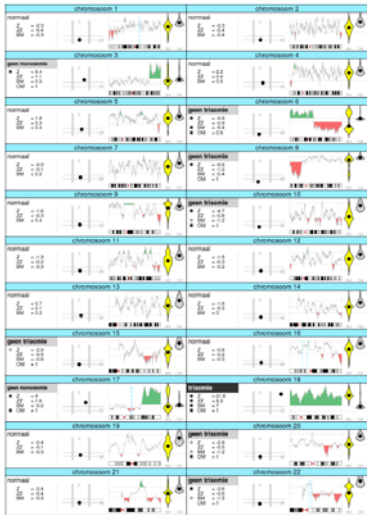


C2D1 (20/05/2016)



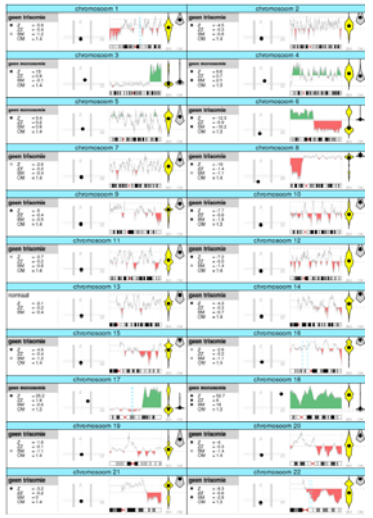
M-piek in gammafractie (bloed):
20/05/2016: 30,1 g/ L

C3D1 (17/06/2016)



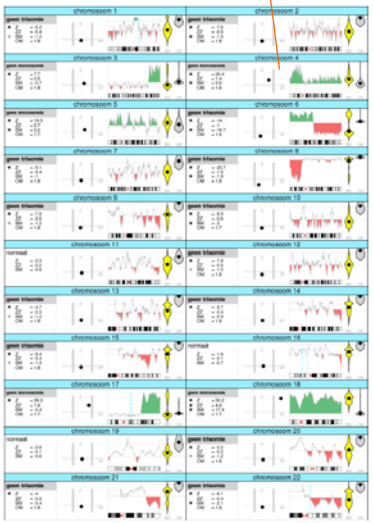
M-piek in gammafractie (bloed):
17/06/2016: 26,3 g/ L

C5D1 (9/09/2016)



M-piek in gammafractie (bloed):
9/09/2016: 20,3 g/ L

C9D1 (30/12/2016)

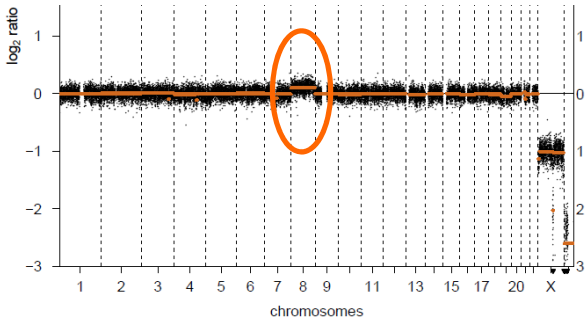


M-piek in gammafractie (bloed):
9/09/2016: 18,2 g/ L

CcfDNA sequencing profiles before and after treatment of MM patient: no respons to treatment.

MDS: several examples with +8: trisomie 8 is easily detected, even if present in low percentage of cells

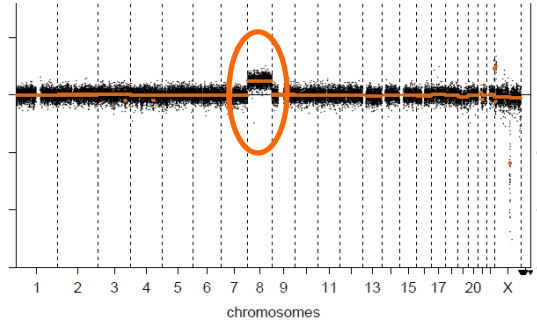
MDS17 GC042662
clonal B-cell population, pancytopenia progressive:
MDS, NHL. MDS TYPE RCMD



LPS: +8, -Y
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

Karyotype: 47,XY,+8[4]/45,X,-Y[6]
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

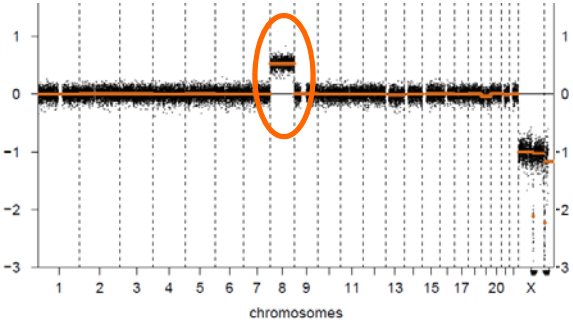
MDS23 GC042666
MDS, refractory anaemia



LPS: +8
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

Karyotype: 47,XX,+8[8]/46,XX [2]
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

MDS31 GC042623
RAEB-1 follow-up

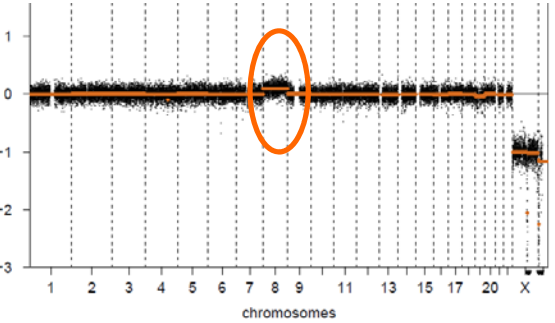


LPS: +8
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

Karyotype: 47,XY,+8[10]

IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

MDS42 GC042642
Pancytopenia, macrocytosis :
MDS, MDS-RAEB(2)?



LPS: +8
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

Karyotype: 47,XY,+8[2]/46,XY[8]
IPSS: Intermediate outcome
IPSS-R: 2 – intermediate prognosis

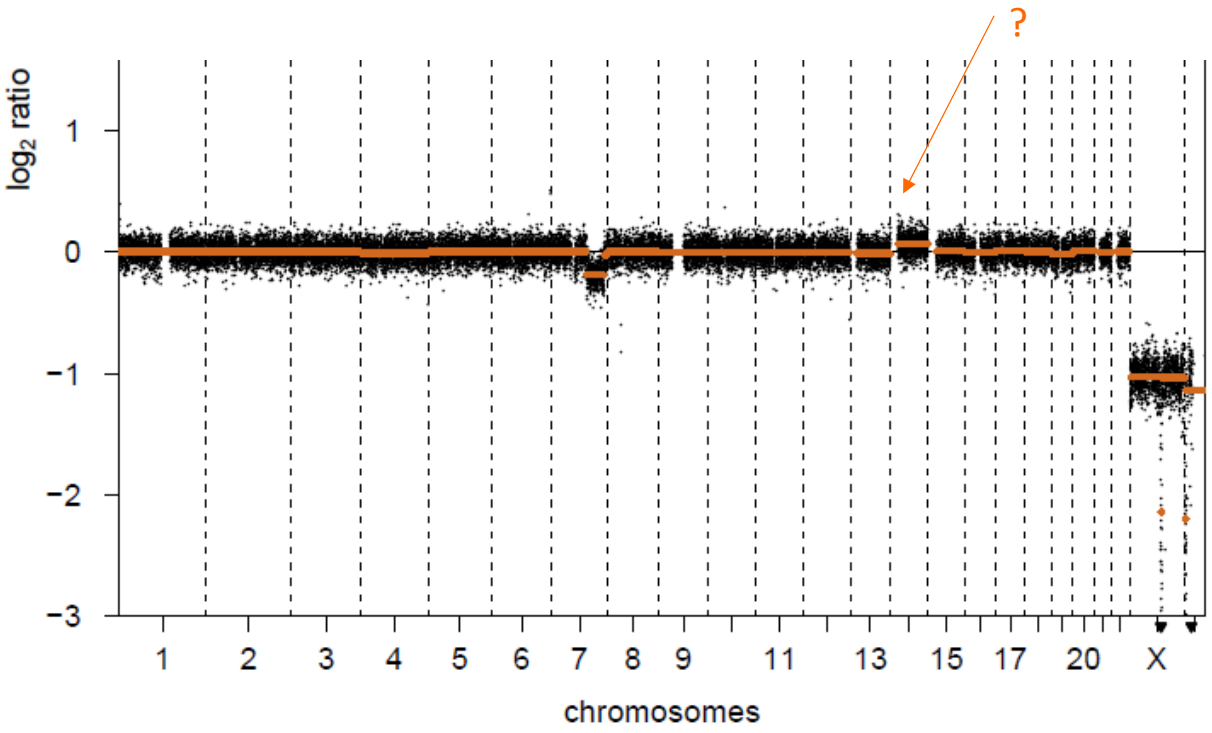
MDS: several examples with -7 in karyotype

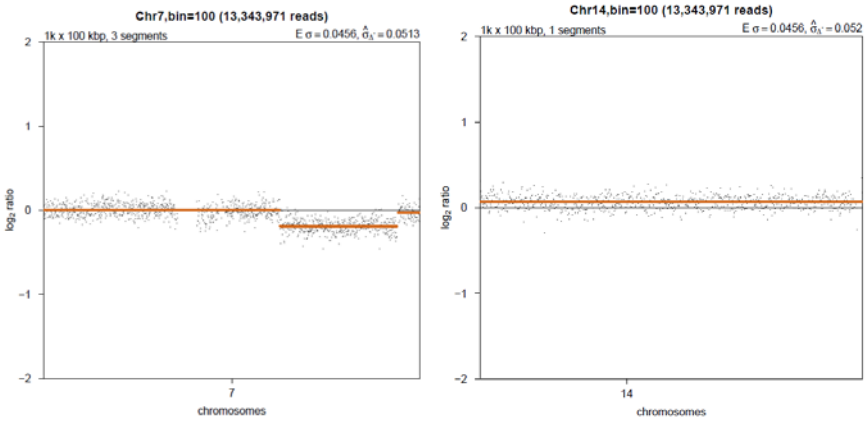
MDS21

Hypocellular marrow, dysplasia in the red lineage; MDS?

Karyotype: 46,XY,del(7)(q21q35)[3]/47,XY,+14[2]/46,XY[5]

IPSS-R: 3 – poor prognosis





All other chromosomes have a flat profile … trisomy 14?

LPS: del(7)(q21q35),+14?

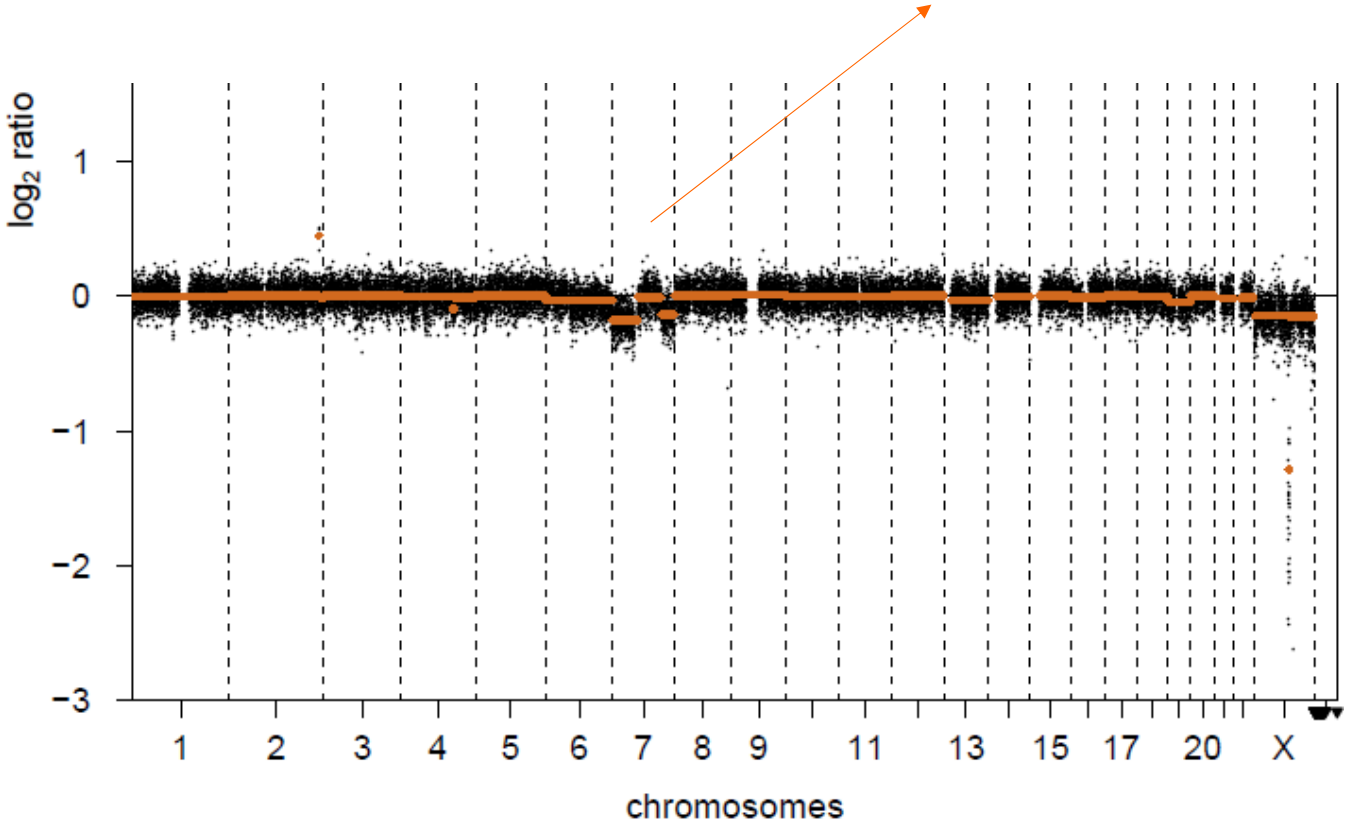
IPSS-R: 3 – poor prognosis

MDS: several examples with -7 in karyotype

MDS44

MDS-RCMD, Female 60y, status chimerism after transplantation with male donor

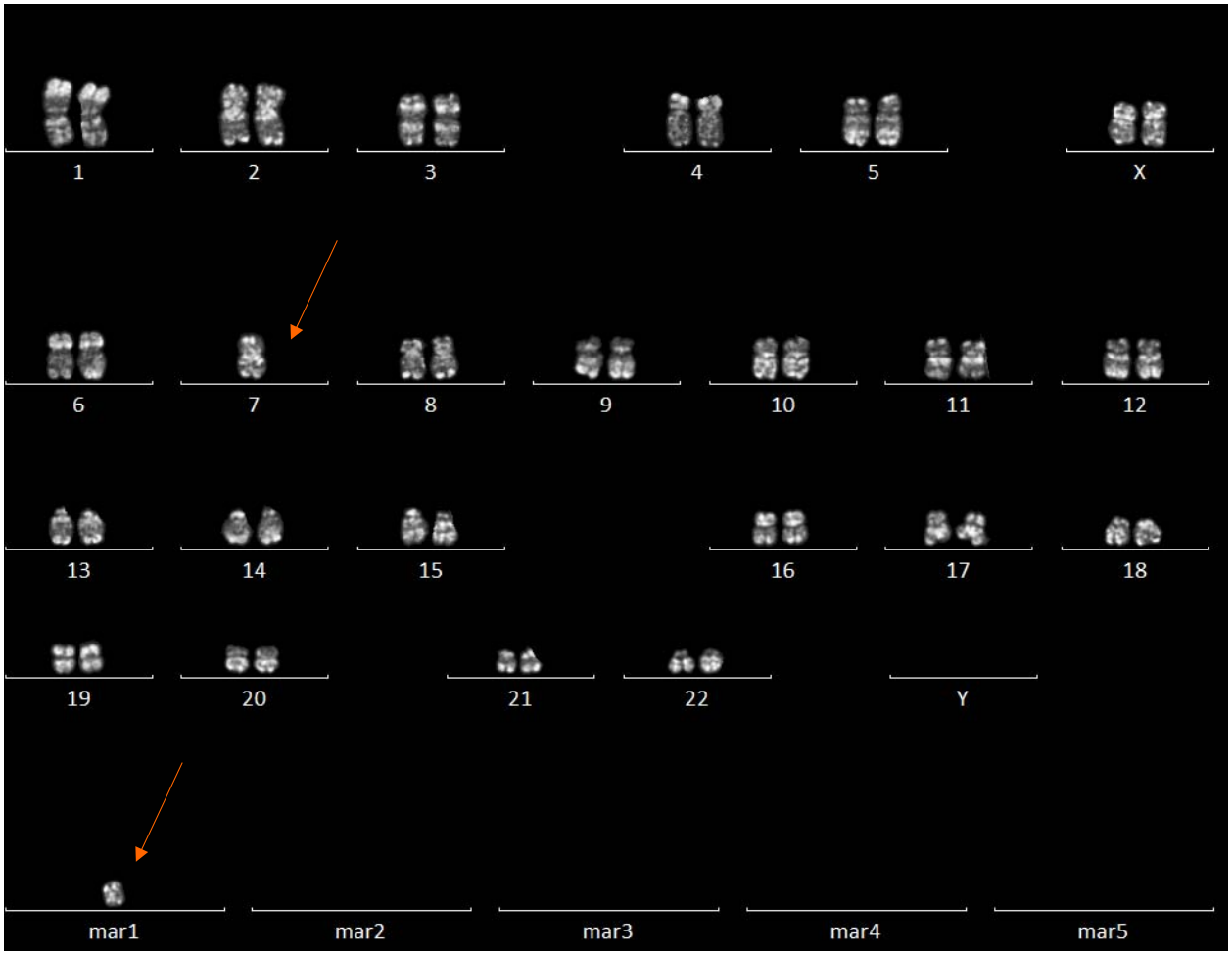
A quoi pensez-vous??



MDS: several examples with -7 in karyotype

MDS44;
MDS-RCMD, Female 60y, status chimerism after transplantation with male donor

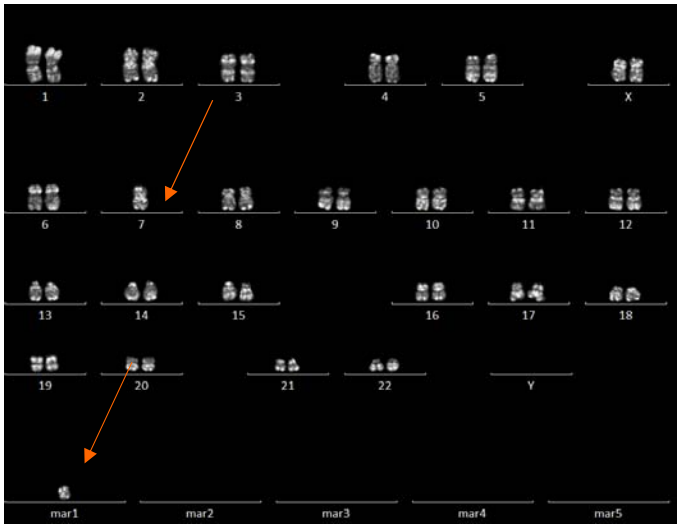
Karyotype: 46,XX,-7,+r[4]/46,XX[6]//46,XY[1]
IPSS-R: 3 – poor prognosis

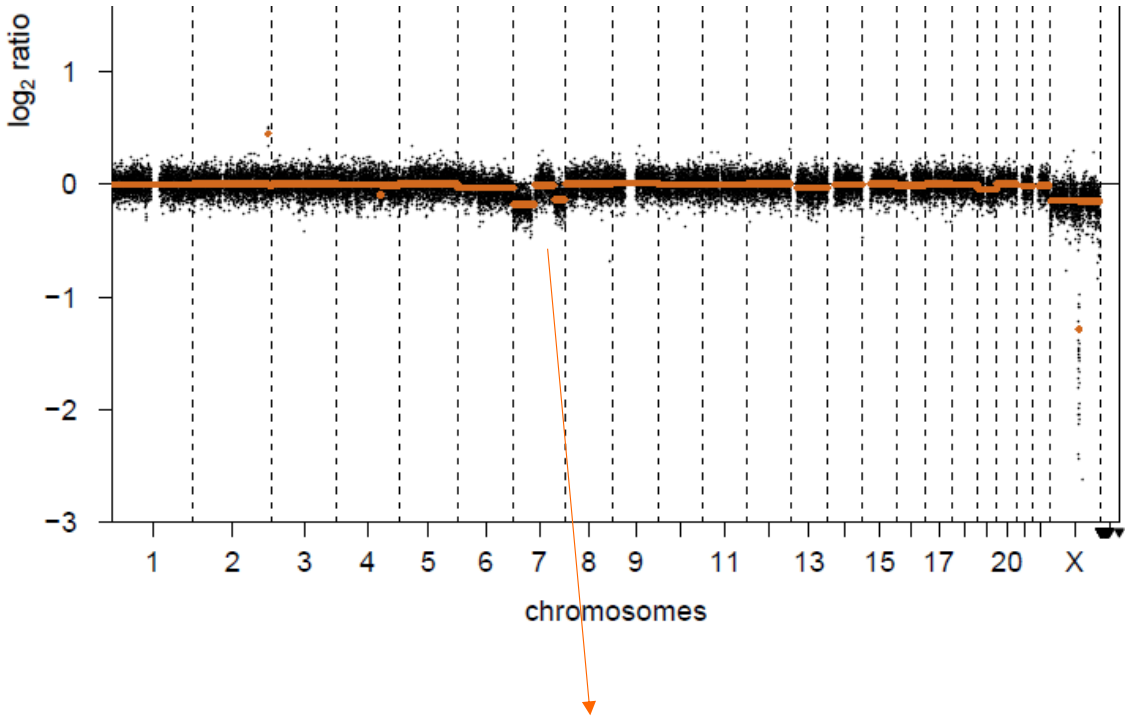


Conclusion karyotype:
90% receptor hematopoiesis.
Pseudodiploïd karyotype with (partial?) loss of chromosome 7.
Classic aberration in MDS with a bad prognosis (IPSS-R cytogenetic score 3).

MDS: several examples with -7 in karyotype

MDS44;
MDS-RCMD, Female 60y, status chimerism after transplantation with male donor





Karyotype: 46,XX,-7,+r[4]/46,XX[6]//46,XY[1]
IPSS-R: 3 – poor prognosis

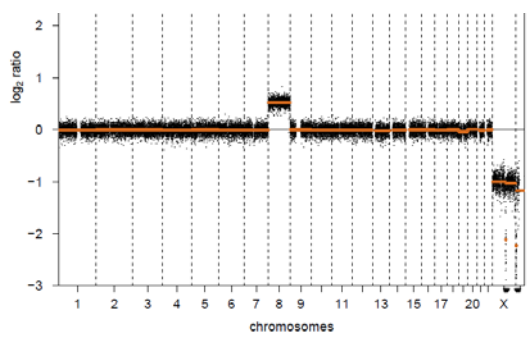
Conclusion karyotype:
90% receptorhematopoiesis. Pseudodiploïd karyotype with (partial?) loss of chromosome 7. Classic aberration in MDS with a bad prognosis (IPSS-R cytogenetic score 3).

Ring of chromosome 7
Other chromsomes: rather flat profile.

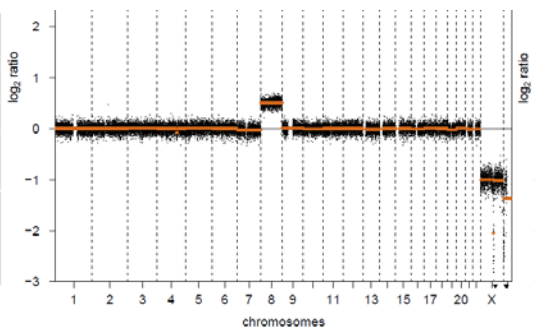
Serial dilution: samples +8/-7 in karyotype

Karyotype MDS31: 47,XY,+8[10]

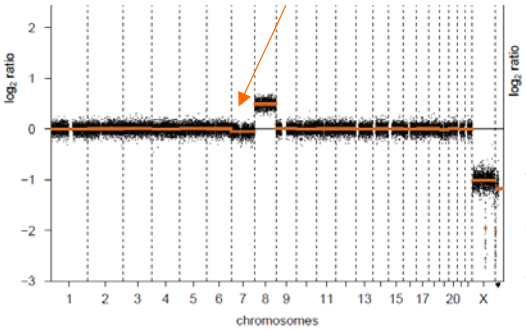
Karyotype MDS32: 45,XY,-7[10]



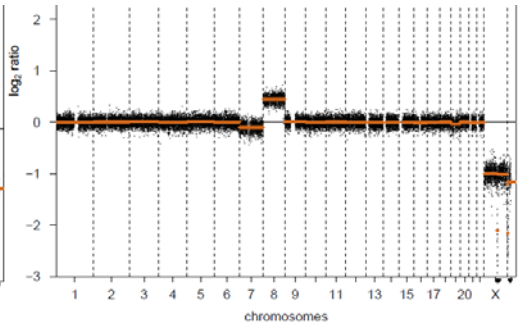
100% trisomy 8
GC042623



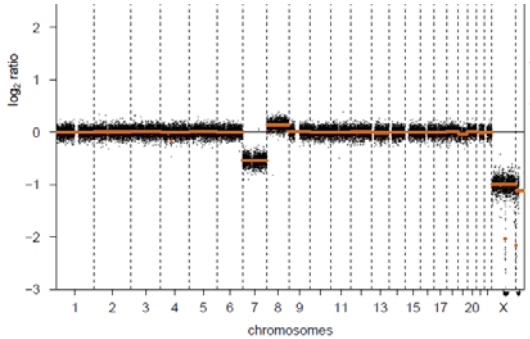
95% trisomy 8/ 5% monosomy 7
GC042624



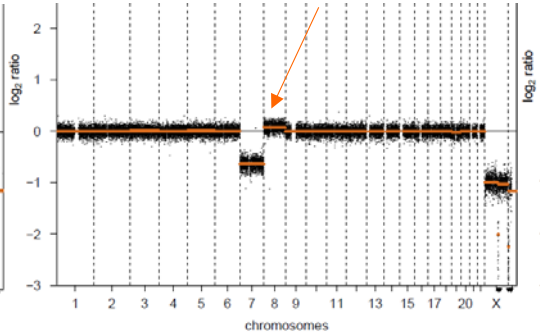
90% trisomy 8/ 10% monosomy 7
GC042625



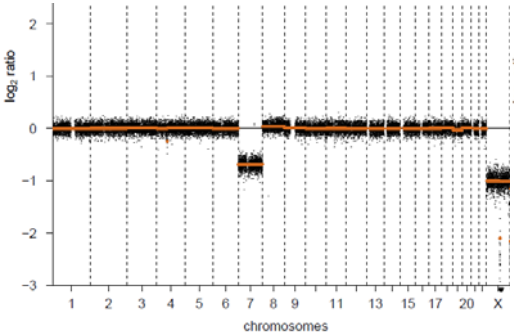
80% trisomy 8/ 20% monosomy 7
GC042626



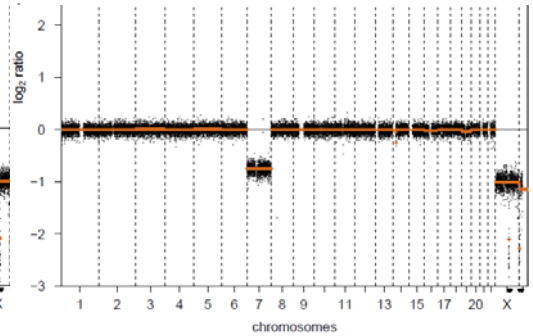
20% trisomy 8/ 80% monosomy 7
GC042629



10% trisomy 8/ 90% monosomy 7
GC042630



5% trisomy/ 95% monosomy 7
GC042631



100% monosomy 7
GC042632

Low-pass sequencing is very sensitive and can detect aberrations in low % of cells: 10% in normal background is reliably detected

Cost

- Plasmocyte purification (EasySep II): 34,25 euro/sample
- DNA extraction: 25 euro/sample

- Library Prep (KAPA HTP; automated): 115 euro (this cost can be reduced, KAPA Hyper prep)
- Sequencing cost: 1345/lane (75 à 96 euro/sample)

- Cost array (x2: Dye Swap): 285 euro
- Array is more labor-intensive

Conclusions

- Low-pass sequencing costs less, shows less outliers and yields a better signal-to-noise ratio than array

- Detection of CN-LOH with LPS: ongoing …