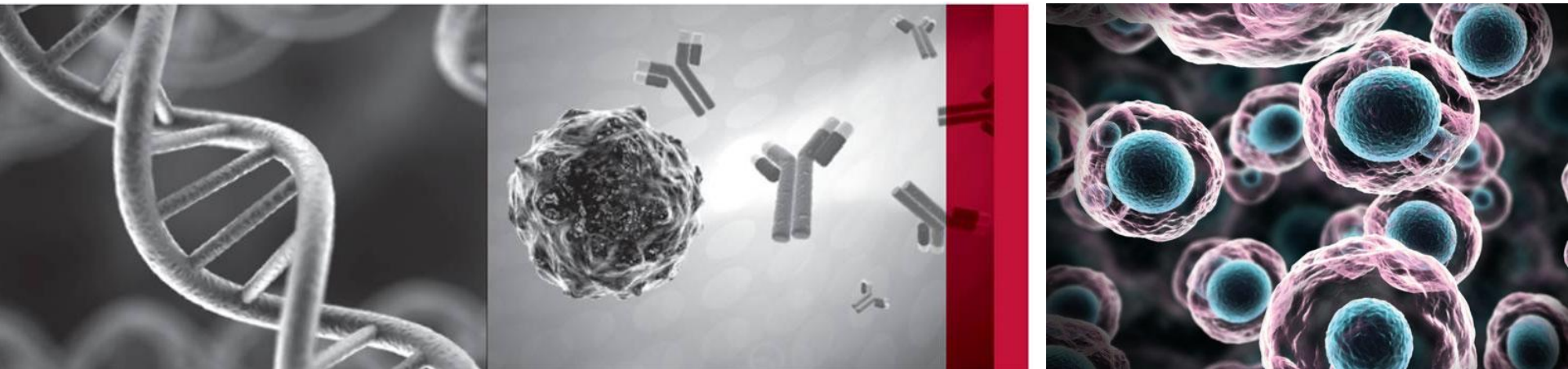


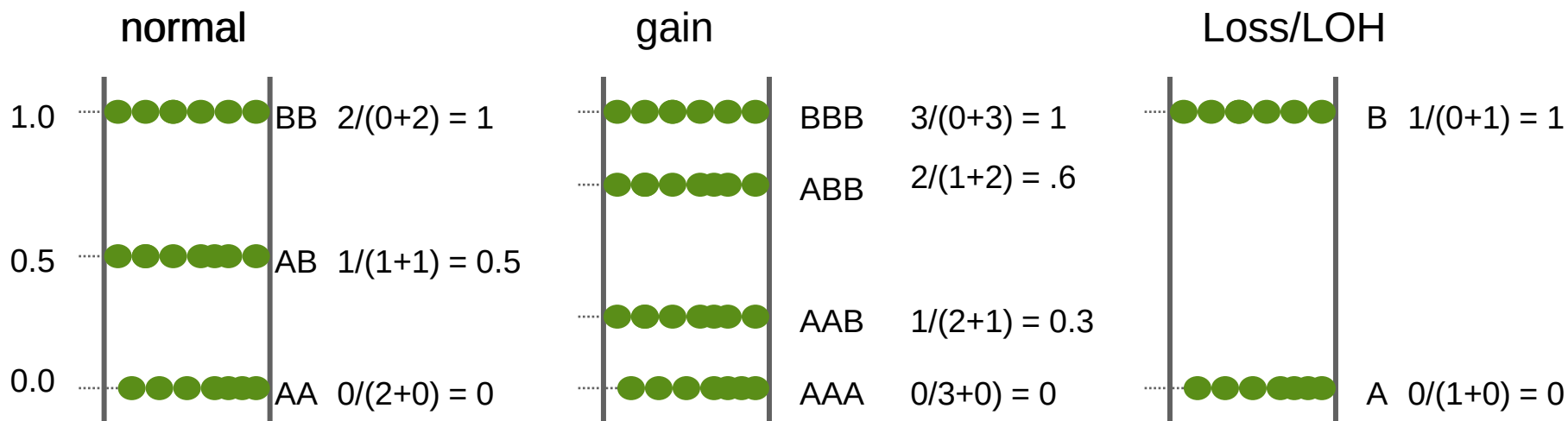


Examples of Different aberration types on the OncoScan™ FFPE Assay

Graph Interpretation

- Log ratios for each marker are calculated relative to the reference signal profile. The log ratio is simply $\log(\text{sampleZ}) - \log(\text{referenceZ})$, for each marker, "Z". For 2 copies of alleles the log graph should be centered around 0 on the Y axis.

- B-allele frequency (BAF): Calculated as $B/A+B$



- loss of heterozygosity (LOH): lack of AB calls in the BAF graph can be distinguished from a loss by examining the logR data

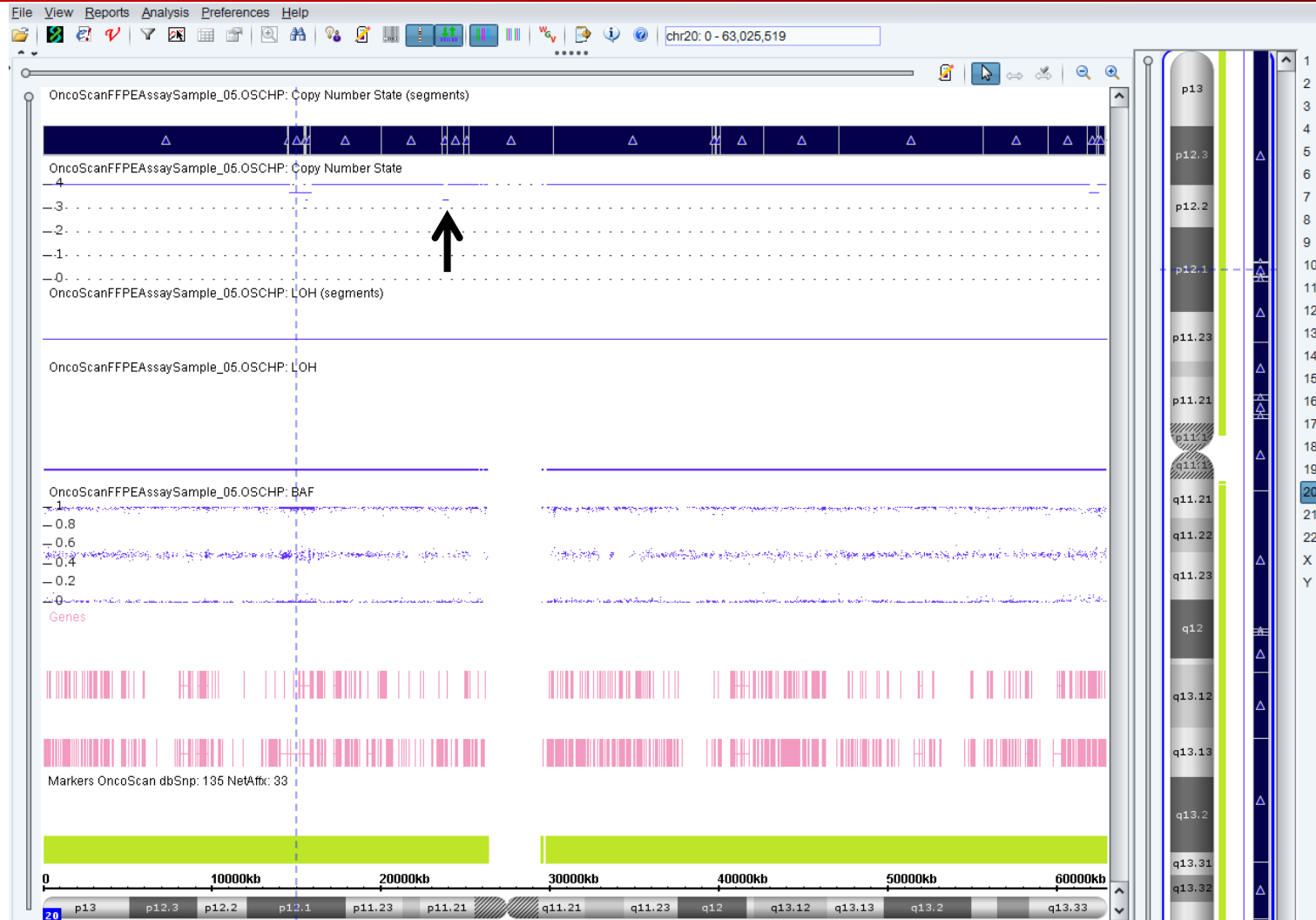
CHAS View: Example of a gain - FFPESample01

- Most of the Chromosome 1p arm is structurally neutral
 - Linear copy number of 2
 - 3 tracks in the BAF graph
- The rest Chromosome 1 has a gain
 - 4 tracks in the BAFs
 - Linear copy number call of 3 as seen in the mouseover



CHAS View: Balanced gain of Chromosome 20 - FFPESample05

- Linear copy number call of 4.
 - Small region of CN=3 is indicated by arrow in ideogram)
- 3 BAF tracks indicate this gain is balanced



CHAS View: High Amplification of FGFR1 - FFPESample03

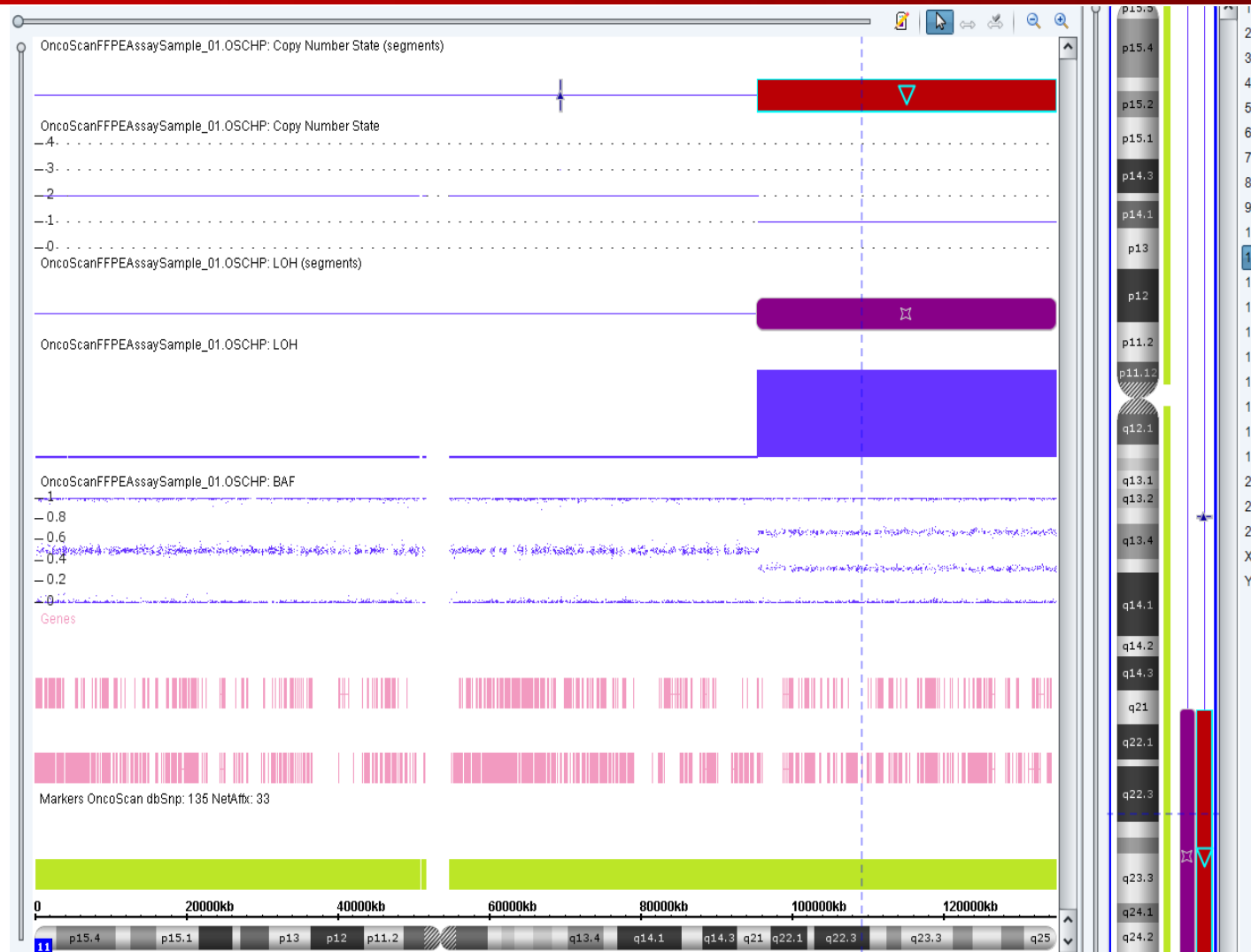
- Linear copy number call of 18 (triangle in dark blue bar)



CHAS View: Example of a loss - FFPESample01

- Most of Chromosome 11 is structurally neutral
 - Linear copy number of 2
 - 3 tracks in the BAF graph.
- The end of the Chromosome 11q arm has a loss
 - Linear copy number call of 1
 - 4 tracks in the BAF graph.

(Note: this sample is a mosaic resulting in 4 BAF tracks, revisit this slide after reviewing the section on mosaicism)



CHAS View: Homozygous deletion in 12p – FFPESample02

- Hemizygous deletion of most of 12p and 12q23.1-12q24.32 (red blocks)
 - Linear copy number of 1
 - 2 BAF tracks see mosaicism on slide 9 and 12
- 4.7MB homozygous deletion (left red block)
 - Log graph shifts down dramatically,
 - linear copy number = 0,
 - BAF has a dispersed heterozygous band indicative of a homozygous deletion.



2 regions of Copy Neutral LOH on Chromosome 7 – FFPE Sample 05

- The 2 regions shaded in purple indicate copy neutral loss of heterozygosity.
 - The linear copy number indicates 2 copies.
 - The BAF graph has 4 tracks indicating CN LOH.
 - The BAF tracks hit at ~0.8 and ~0.2 indicating there is approximately 70% of the sample with this aberration (see slide 9 to 15).



- Cancer samples contain a mixture of cells with different copy number states
 - Normal cells
 - Tumor cells
- Tumor heterogeneity
 - Not all “normal contamination” from “normal” cells
 - Not all tumor cells have the same CN changes
 - Different groups of cells have different collections of CN changes

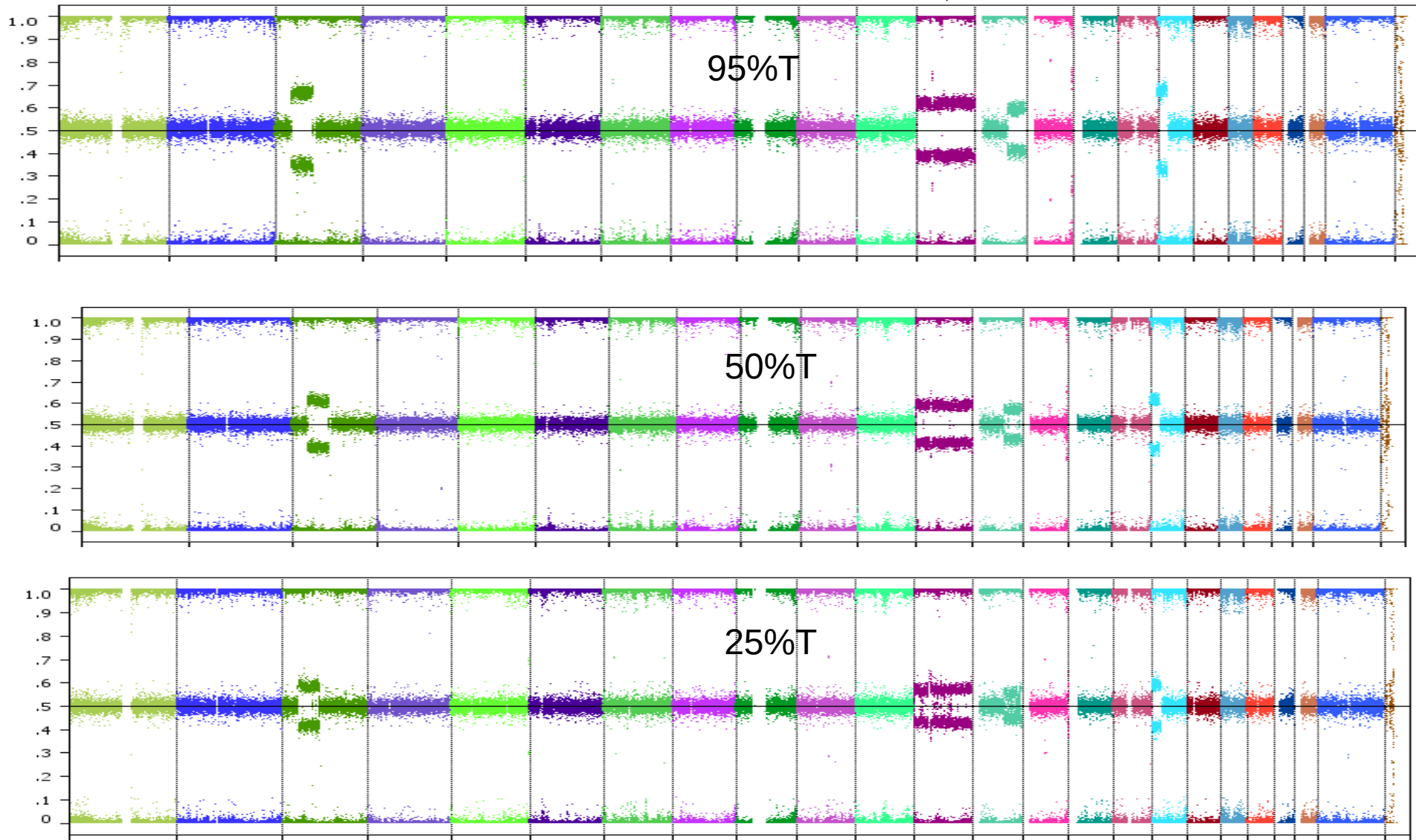
Q. How does this effect my graph interpretation?

A. The following slides show how BAF tracks are effected based on the tumor/normal ratio in the sample.

Expected BAF tracks based on various % mosaicism with 20 cells: 1 CN Gain

	Possible genotypes	# tumor cells	# normal cells	# of each Genotype	# A alleles	# B alleles	BAF formula	BAF
100% tumor	ABB	20	0	20 ABB	20	40	40/60	0.67
	AAB	20	0	20 AAB	40	20	20/60	0.33
75% tumor	AB and ABB	15	5	15 ABB, 5 AB	20	35	35/55	0.64
	AB and AAB	15	5	15 AAB, 5 AB	35	20	20/55	0.36
50% tumor	AB and ABB	10	10	10 ABB, 10 AB	20	30	30/50	0.60
	AB and AAB	10	10	10 AAB, 10AB	30	20	20/50	0.40
25% tumor	AB and ABB	5	15	5 ABB 15 AB	20	25	25/45	0.56
	AB and AAB	5	15	5 AAB 15 AB	25	20	20/45	0.44
Normal	AB	0	20	20 AB	20	20	20/40	0.50

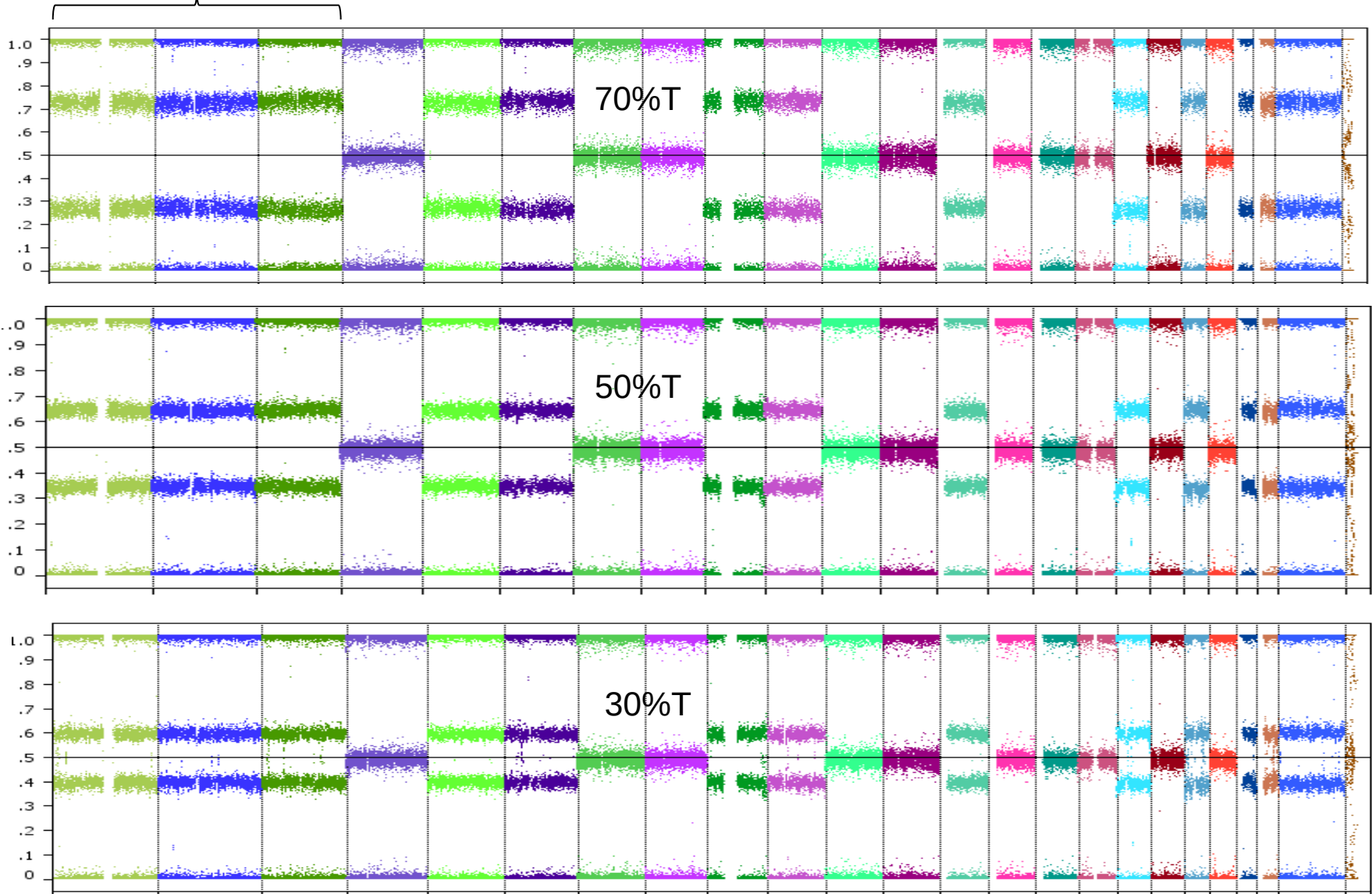
Het tracks move toward 0.5 when % normal is increased
Gain on Chromosome 12



Expected BAF tracks based on various % mosaicism with 20 cells: 1 CN Loss

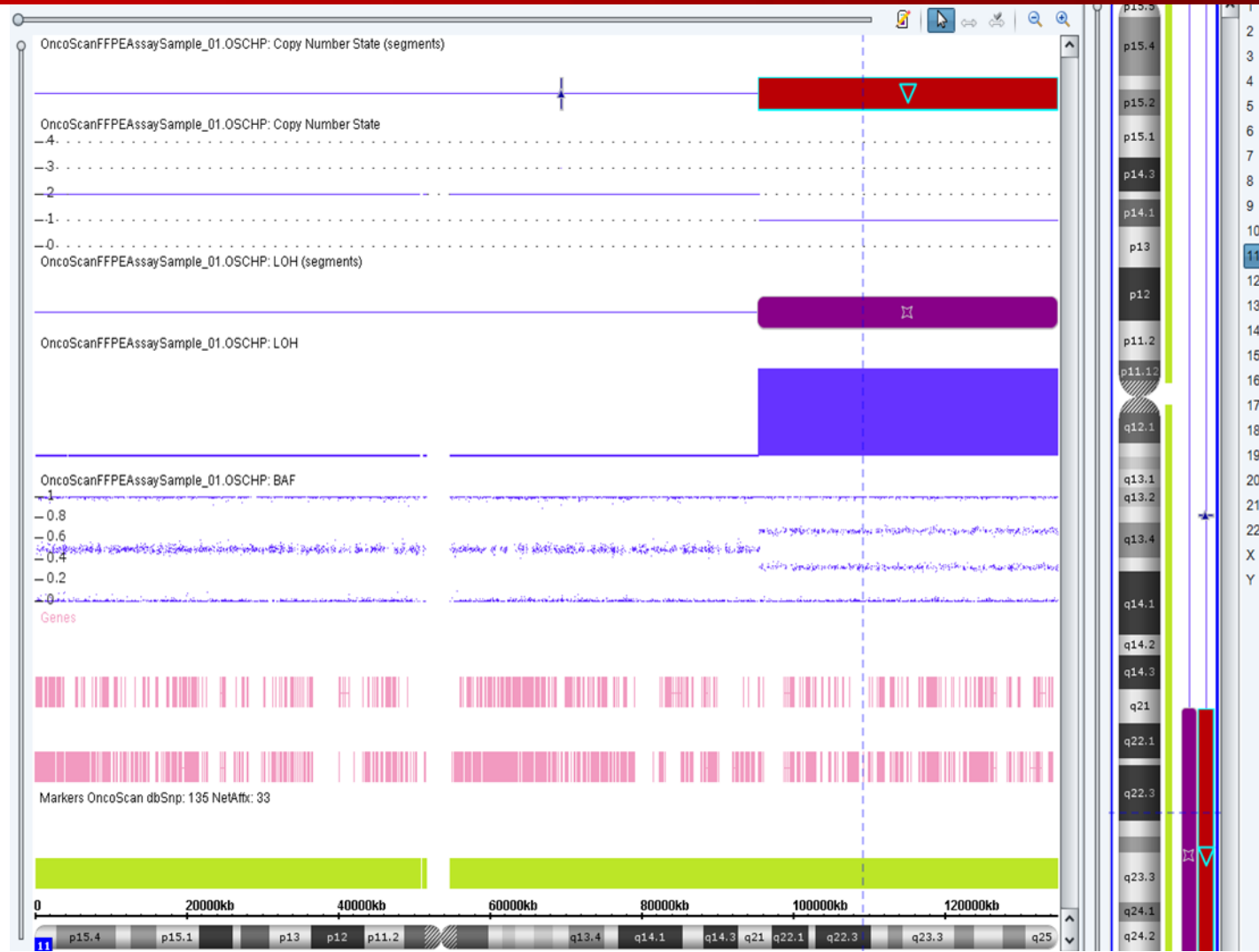
	Possible genotypes	# tumor cells	# normal cells	# of each Genotype	# A alleles	# B alleles	BAF formula	BAF
100%tumor	B	20	0	20 B	0	20	20/20	1.00
	A	20	0	20 A	20	0	0/20	0.00
75% tumor	B and AB	15	5	15 B, 5 AB	5	20	20/25	0.80
	A and AB	15	5	15 A, 5 AB	20	5	5/25	0.20
50% tumor	B and AB	10	10	10 B, 10 AB	10	20	20/30	0.67
	A and AB	10	10	10 A, 10 AB	20	10	10/30	0.33
25% tumor	B and AB	5	15	5 B, 15 AB	15	20	20/35	0.57
	A and AB	5	15	5 A, 15 AB	20	15	15/35	0.43
Normal	AB	0	20	20 AB	10	10	10/20	0.50

Het tracks move toward 0.5 when % normal is increased
Chromosomes 1,2,3



Revisiting the loss - FFPESample01

- Most of Chromosome 11 is structurally neutral
 - Linear copy number of 2
 - 3 tracks in the BAF graph.
- The end of the Chromosome 11q arm has a loss
 - linear copy number call of 1
 - 4 tracks in the BAF graph.
- This sample is a mosaic. The BAF het tracks kit ~0.7 and ~0.4 indicating ~55-60% of the sample has this aberration



Expected BAF tracks based on various % mosaicism with 20 cells: CN LOH

	Possible genotypes	# tumor cells	# normal cells	# of each Genotype	# A alleles	# B alleles	BAF formula	BAF
100%tumor	BB	20	0	20 BB	0	40	40/40	1.00
	AA	20	0	20 AA	40	0	0/40	0.00
75% tumor	BB and AB	15	5	15BB, 5 AB	5	35	35/40	0.88
	AA and AB	15	5	15 AA, 5 AB	35	5	5/40	0.13
50% tumor	BB and AB	10	10	10 BB, 10 AB	10	30	30/40	0.75
	AA and AB	10	10	10 AA, 10 AB	30	10	10/40	0.25
25% tumor	BB and AB	5	15	5 BB, 15 AB	15	25	25/40	0.63
	AA and AB	5	15	5 AA, 15 AB	25	15	15/40	0.38
Normal	AB	0	20	20 AB	20	20	20/40	0.50

2 regions of Copy Neutral LOH on Chromosome 7 - FFPESample05

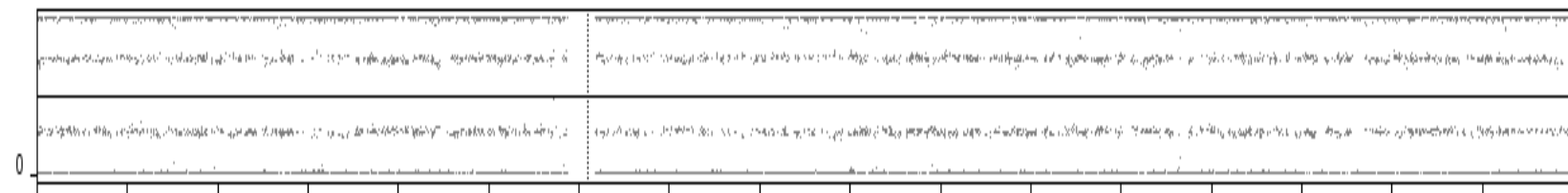
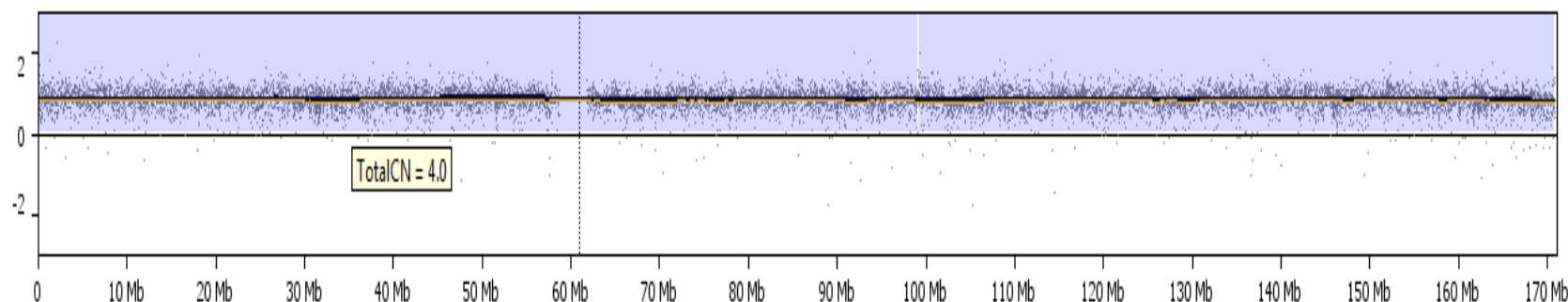
- The 2 regions shaded in purple indicate copy neutral loss of heterozygosity.
 - The linear copy number indicates 2 copies.
 - The BAF graph has 4 tracks indicating CN LOH.
 - The BAF tracks hit at ~ 0.8 and ~ 0.2 indicating there is approximately 70% of the sample with this aberration



Mode 1 = % Aberrant Cell Population Determined - FFPE Sample 05

OS-MAPD	0.20
OS-ndSNPQC	38.25
OS-CellPairCheck Status	Pass
OS-ndWavinessSd	0.15
OS-% Aberr. Cells	80
OS-Ploidy	2.00
OS-Low Diploid Flag	No
OS-Y gender call	female
OS-ndCount	6120

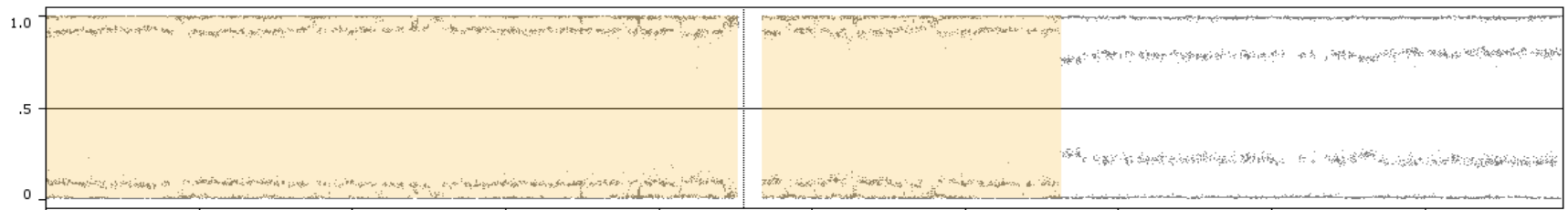
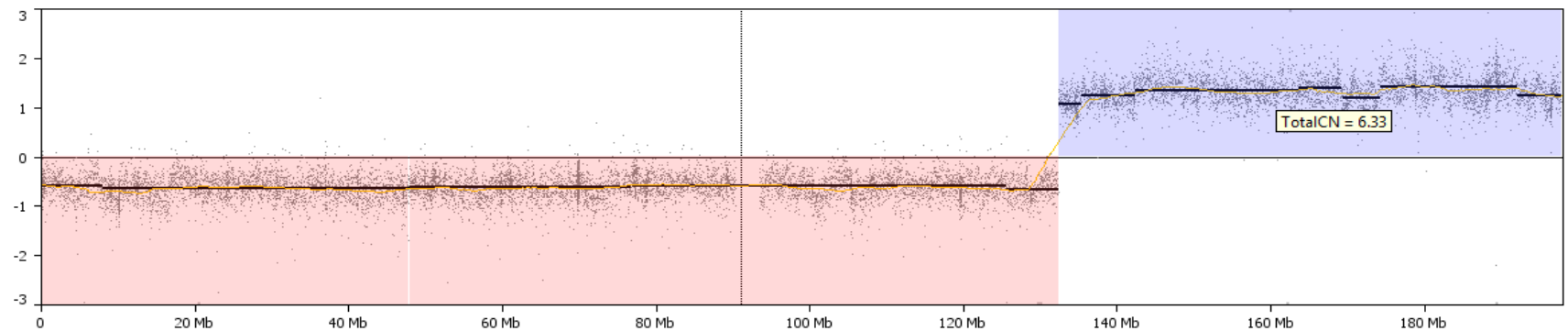
- The % Aberrant Cells for this sample = 80, meaning this sample has an estimated 80% aberrant cell population.
- The copy number 4 shown below corresponds to the copy number in the **aberrant cell population**.



Mode 2 = % Aberrant Cell assigned NA

Quality	0.08
OS-MAPD	0.19
OS-ndSNPQC	53.52
OS-CelPairCheck Status	Pass
OS-ndWavinessSd	0.14
OS-% Aberr. Cells	NA
OS-Ploidy	NaN
OS-Low Diploid Flag	No
OS-Y gender call	male
OS-ndCount	32015

- The % Aberrant Cells for this sample =NA.
 - Due to sample heterogeneity or low % aberrant cell fraction, the % Aberrant Cells can not be determined (NA),
- In this case the copy number displayed corresponds to the **average** copy number for the **whole sample**.
- This is what is given by most algorithms



How do I calculate linear copy number manually?

Q. Can I calculate linear copy number in aberrant cells when TuScan reports non-integer values (mode 2)?

A. If you have tumor burden information you can **estimate** this using the following equation:

$2 \text{ (normal copies)} \times (100\% - \text{Tumor burden}\%) + Y \text{ (copies in aberrant cell)} \times \text{Tumor burden} = \text{TuScan non-integer value.}$

Example:

The pathologist reports a tumor burden=70% and TuScan non-integer value is 6.33.

$$(2 \times 30\%) + (Y \times 70\%) = 6.33$$

$$0.6 + 0.7Y = 6.33$$

$$0.7Y = 5.73$$

$$Y = 8.18 \text{ or } \sim 8 \text{ copies}$$