



### Examples of Different aberration types on the OncoScan<sup>™</sup> FFPE Assay

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### **Graph Interpretation**

- Log ratios for each marker are calculated relative to the reference signal profile. The log ratio is simply log(sampleZ) – log(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

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### CHAS View: Balanced gain of Chromosome 20 - FFPESample05

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- 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



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![](_page_3_Picture_5.jpeg)

#### CHAS View: High Amplification of FGFR1 - FFPESample03

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

![](_page_4_Figure_2.jpeg)

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![](_page_4_Picture_3.jpeg)

### 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)

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![](_page_5_Picture_9.jpeg)

### 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 and12
- 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.

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![](_page_6_Picture_9.jpeg)

#### 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 (see slide 9 to 15).

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![](_page_7_Figure_5.jpeg)

**USB** 

![](_page_7_Picture_6.jpeg)

![](_page_8_Picture_0.jpeg)

- 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.

![](_page_8_Picture_11.jpeg)

# 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

![](_page_9_Picture_2.jpeg)

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

![](_page_10_Figure_1.jpeg)

![](_page_10_Figure_2.jpeg)

![](_page_10_Figure_3.jpeg)

# 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	В	20	0	20 B	0	20	20/20	1.00
	А	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	٨R	0	20	20 48	10	10	10/20	0.50
Normal	AD	0	20	ZUAD	10	10	10/20	0.50

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![](_page_11_Picture_2.jpeg)

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

![](_page_12_Figure_1.jpeg)

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### Revisiting the loss - FFPESample01

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- 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

![](_page_13_Figure_8.jpeg)

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![](_page_13_Picture_9.jpeg)

# 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

![](_page_14_Picture_2.jpeg)

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#### 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

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![](_page_15_Figure_5.jpeg)

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![](_page_15_Picture_6.jpeg)

#### Mode 1 = % Aberrant Cell Population Determined -FFPESample05

OS-MAPD		0.20
OS-ndSNPQC		38.25
OS-CelPairCheck	Status	Pass
OS-ndWavinessS	d [	0.15
OS-% Aberr. Cell	s	80
OS-Ploidy		2.00
OS-Low Diploid Fl	ag	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.

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#### 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

![](_page_17_Figure_6.jpeg)

# 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 (normal copies) X (100%- Tumor burden%) + Y (copies in aberrant cell) X Tumor burden = TuScan non-integer value.

Example:

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

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$$(2 \times 30\%) + (Y \times 70\%) = 6.33$$
  
 $0.6 + 0.7Y = 6.33$   
 $0.7Y = 5.73$   
 $Y = 8.18$  or ~8 copies

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