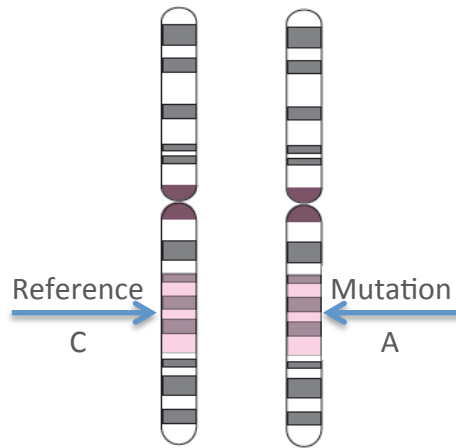


Mutation calling –  
wrap up

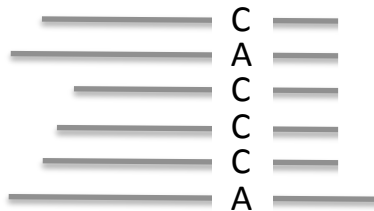
# Part one

Where do we find info about alternative allele frequencies based on read depths for a given mutation?

# Alternative Allele Frequency



Reference: ACGGCTAGCGTAGCAT



$$AF = \frac{\text{\# reads A}}{\text{\# reads A} + \text{\# reads C}} = 0.33$$

# Look in the mutect.vcf file

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	HCC1143.normal	HCC1143.tumor
17	1001315	.	C	T	.	REJECT	.	GT:AD:BQ:DP:FA	0:51,3:..:54:0.056	0/1:29,2:23:33:0.065
17	1001331	.	G	T	.	REJECT	.	GT:AD:BQ:DP:FA	0:30,3:..:33:0.091	0/1:15,2:34:17:0.118
17	1003390	.	G	A	.	REJECT	.	GT:AD:BQ:DP:FA	0:17,2:..:18:0.105	0/1:16,1:28:17:0.059
17	1004967	.	A	T	.	REJECT	.	GT:AD:BQ:DP:FA	0:28,1:..:29:0.034	0/1:16,4:15:20:0.200
17	1004974	.	C	T	.	REJECT	.	GT:AD:BQ:DP:FA	0:27,2:..:29:0.069	0/1:11,3:13:14:0.214
17	1024903	.	C	T	.	PASS	SOMATIC	GT:AD:BQ:DP:FA:SS	0:106,0:..:102:0.00:0	0/1:84,6:34:90:0.067:2
17	1277664	.	C	A	.	PASS	SOMATIC	GT:AD:BQ:DP:FA:SS	0:59,0:..:59:0.00:0	0/1:41,25:34:66:0.379:2
17	1527066	.	C	G	.	PASS	SOMATIC	GT:AD:BQ:DP:FA:SS	0:35,0:..:31:0.00:0	0/1:26,5:29:31:0.161:2

FORMAT (Each code is described in VCF header)

GT:AD:BQ:DP:FA

GT=Genotype

AD=Allelic depths for the ref and alt alleles in the order listed

BQ=Average base quality for reads supporting alleles

DP=Approximate read depth

FA=Allele fraction of the alternate allele with regard to reference

SS=Variant status

(0=wildtype,1=germline,2=somatic,3=LOH,4=post-transcriptional modification,5=unknown")

# Allelic depths in IGV



AD=Allelic depths for the ref and alt alleles in the order listed

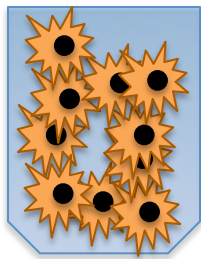
Reference = C Alternative = T

AD=13,13

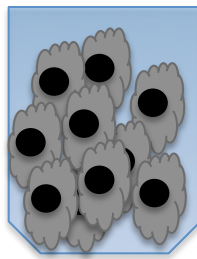
# Today's Practical

## part two

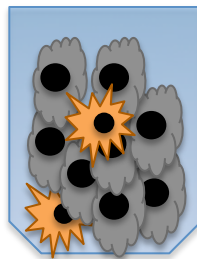
- Analyze how various degrees of normal contamination of the tumor sample affects allele frequencies
- Do the alternative allele frequency distributions for the different tumor/normal mixtures “fit” with what you would expect?



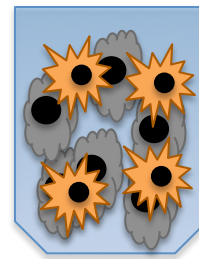
Normal



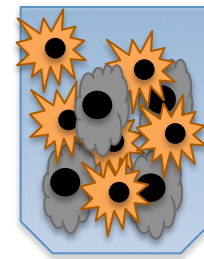
tumor



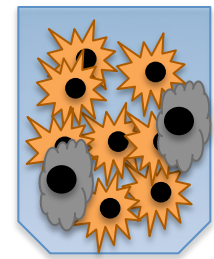
20/80



40/60

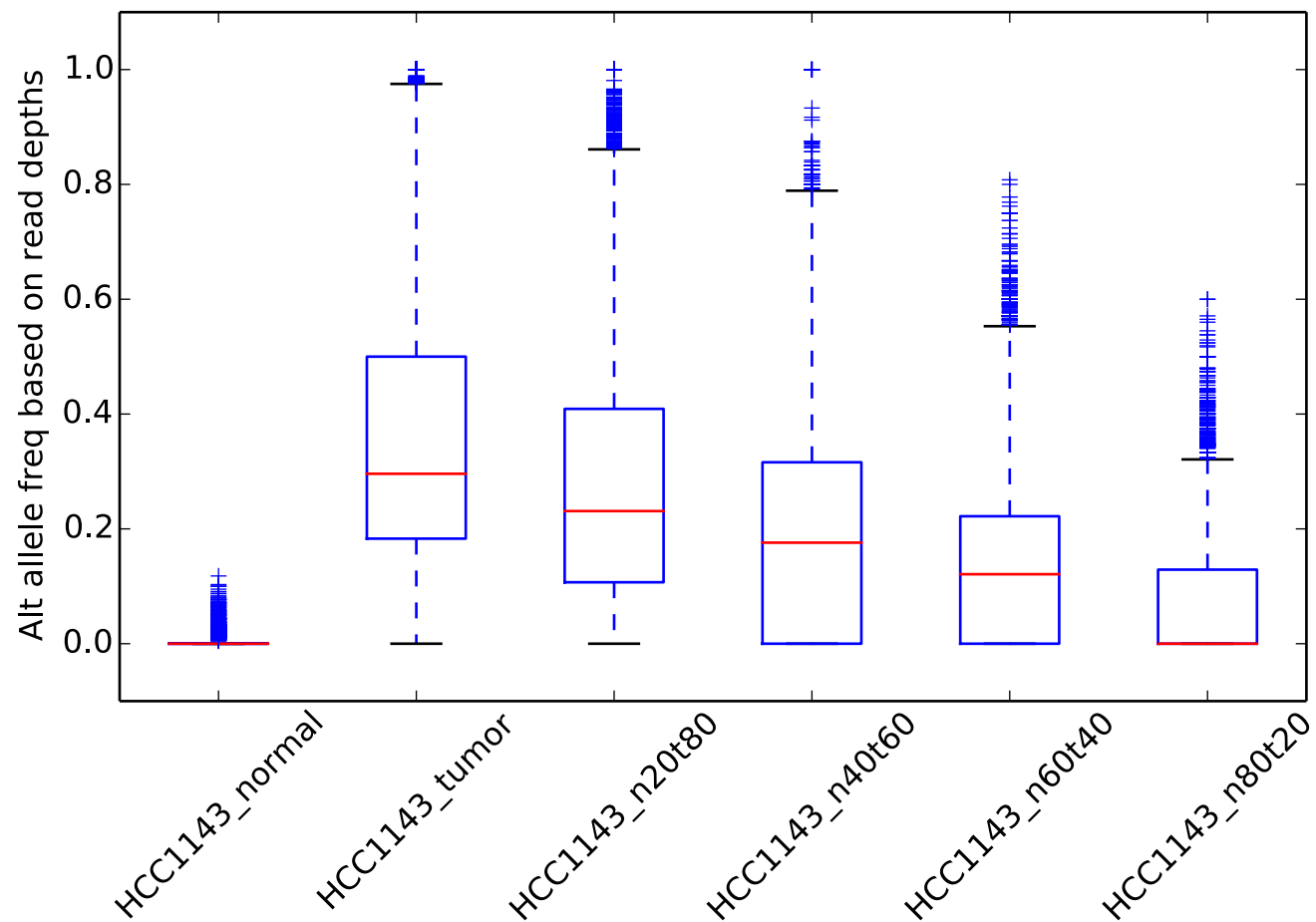


60/40



80/20

Alt allele frequency distributions in HCC1143.final.vcf



Alt allele frequency distributions in HCC1954.final.vcf

