When Good Experiments Go Bad

Chris Miller

However improbable we regard this event, or any of the steps which it involves, given enough time it will almost certainly happen at least once.

--George Wald

Anything that can go wrong,
will go wrong

--Murphy

Shit happens.

--Forrest Gump

Case #1

- Exome sequencing – Glioblastoma Tumor/Normal pairs
- Alignment, somatic variant calling, filtering

```
$ wc -l H_RL-01-0*/snvs.indels.annotated

  159 H_RL-01-0203-1412449/snvs.indels.annotated
  219 H_RL-01-0216-1412454/snvs.indels.annotated
10583 H_RL-01-0334-1412447/snvs.indels.annotated
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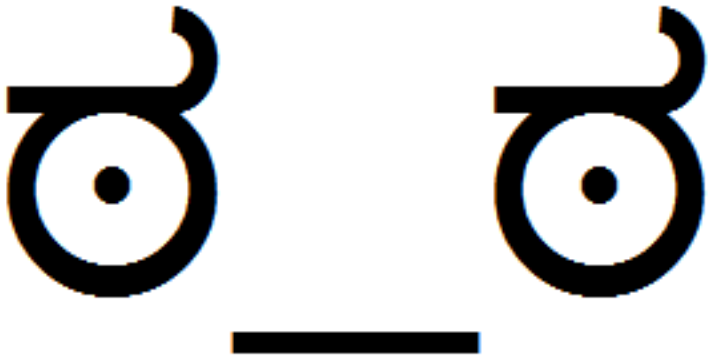

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- How many of these occur at known dbSNP sites?

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  ~85%

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- How many of these occur at known dbSNP sites?
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- What is their VAF?
  ~50% or 100%

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- How many of these occur at known dbSNP sites?
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dx: SAMPLE SWAP

Damage Control

- Check other samples in the cohort
  - May not be resolvable!

    S1 Tumor vs S1 Normal ##########
    S2 Tumor vs S2 Normal ##########

    S1 Tumor vs S2 Normal ##
    S2 Tumor vs S1 Normal ##

    are the tumors or normals swapped?

- Often need more information to resolve (RNAseq? Cytogenetics?)
- Check other lanes/indices on the same machine/batch

- Often, the resolution is to drop the samples

Case #2

- 2 projects, 3 patients
  - Patient 1 – Normal, CML, AML
  - Patient 2 – Normal, CML, AML
  - Patient 3 – Normal, Tumor

- Exome Sequencing
  - 1 lane of HiSeq2500 - 79-99X mean depth

Variant	Patient 1 CML	Patient 1 AML	Patient 2 CML	Patient 2 AML
SNVs	14430	669	5276	87
Indels	255	115	55	17

same normal

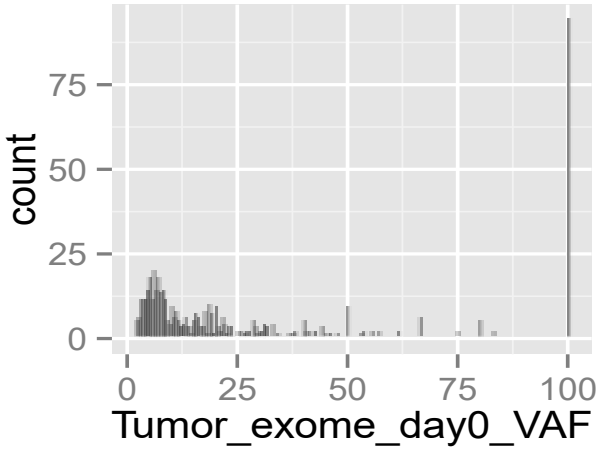
same normal

How many SNVs are common variants?

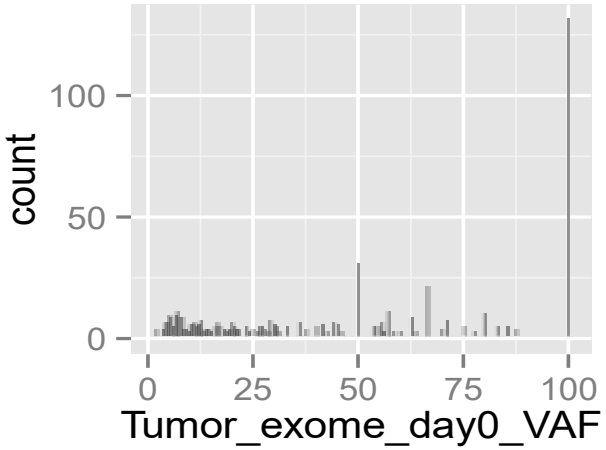
Variant	Patient 1 CML	Patient 1 AML	Patient 2 CML	Patient 2 AML
SNVs	14430	669	5276	87
Indels	255	115	55	17
in dbSNP	>90%	<5%	>90%	<5%

Coding variants with an rsID (dbSNP)

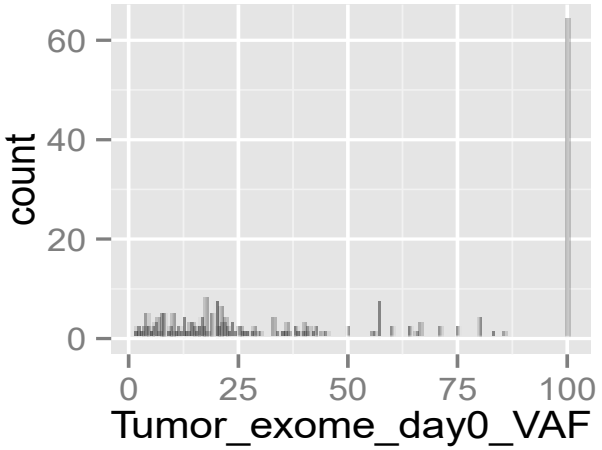
Patient 1 AML



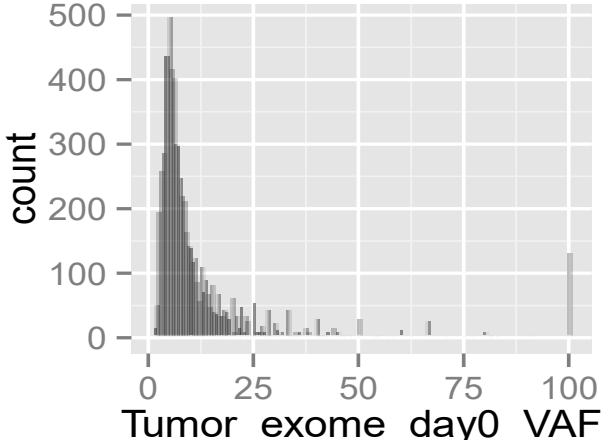
Patient 2 AML



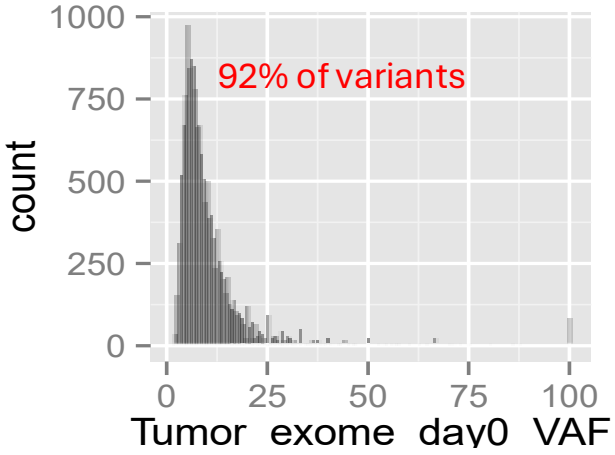
Patient 3 Tumor



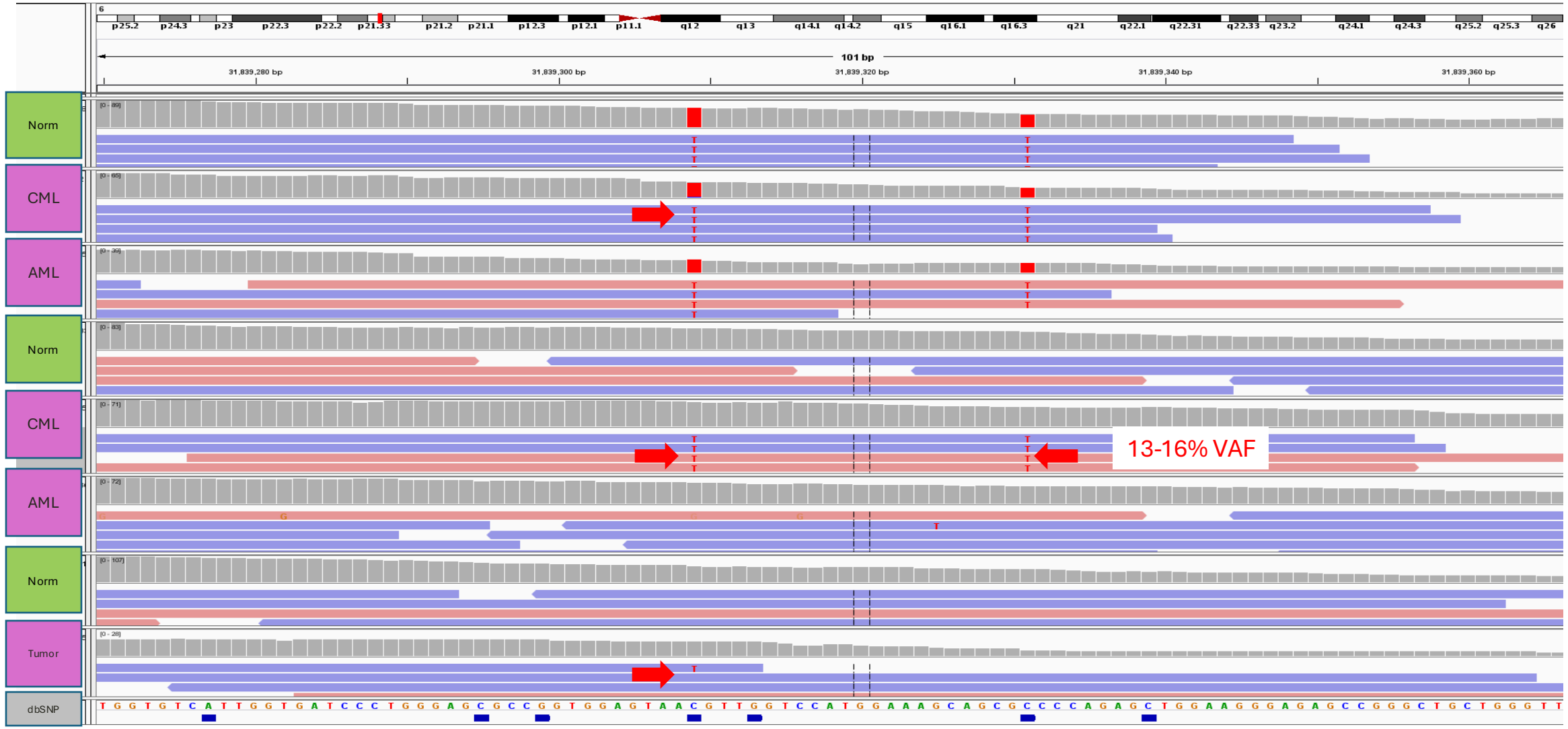
Patient 1 CML



Patient 2 CML



IGV inspection of variants/reads



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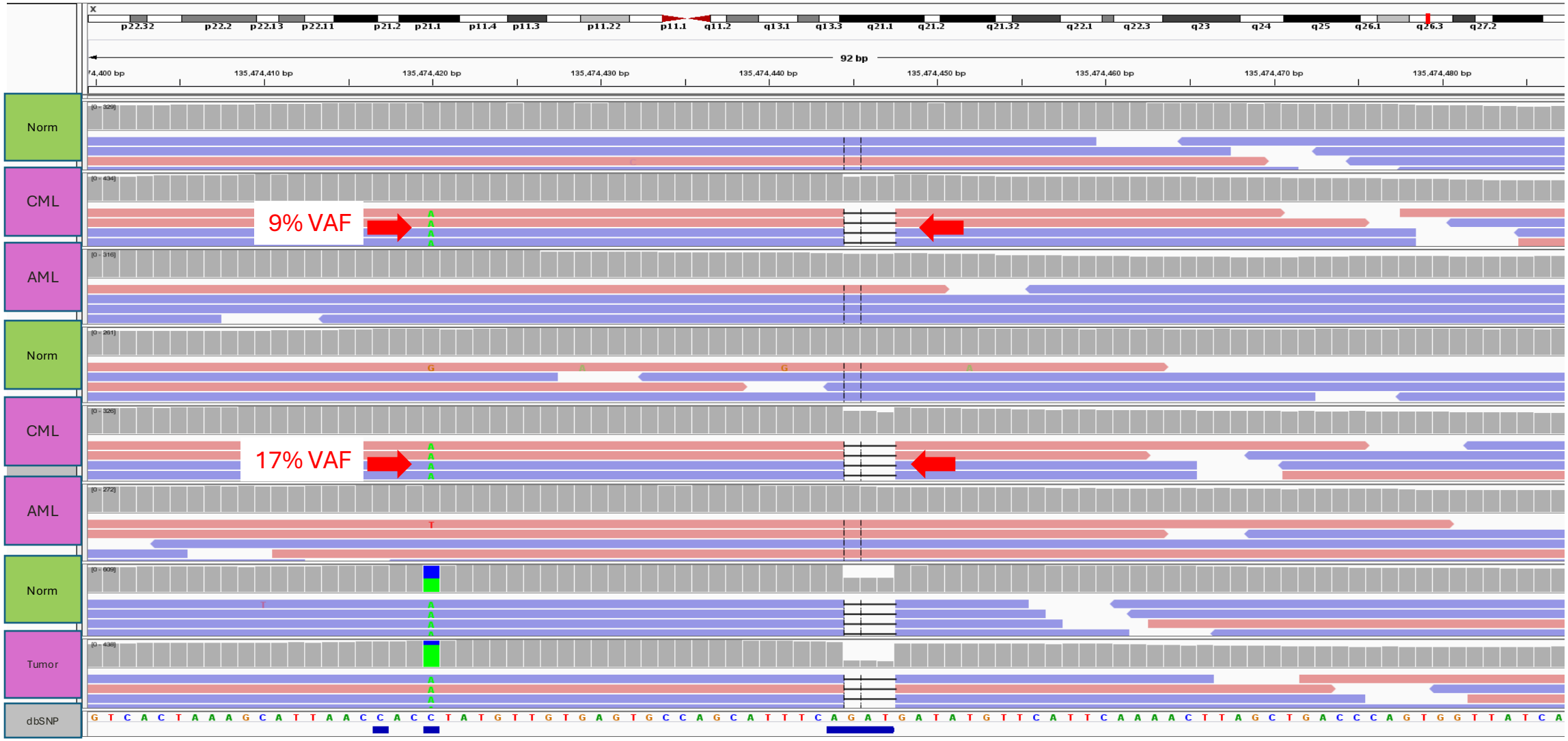


- How many of these occur at known dbSNP sites?
    most

- What is their VAF?
        NOT ~50% or 100%

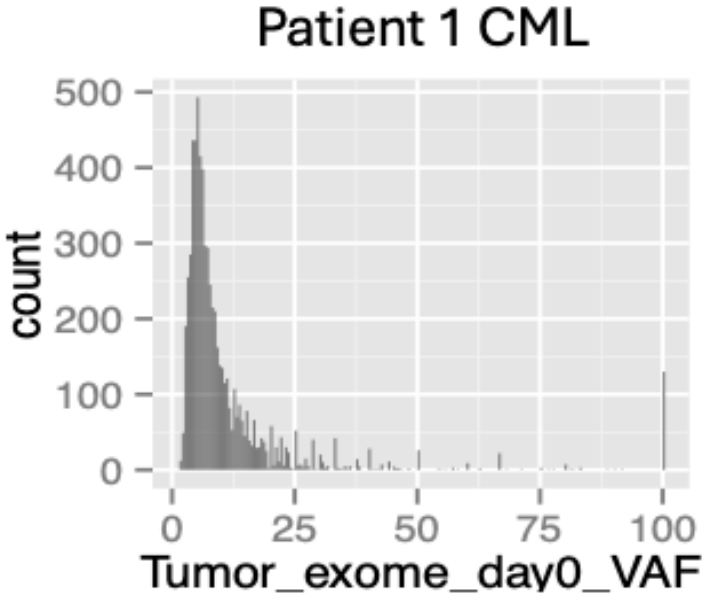
dx: SAMPLE CONTAMINATION

Damage Control

- Check other samples in the cohort to figure out source
  - May not be resolvable! (what if it's from someone else's samples?)

- If it's low-level enough, could apply filters
  - Only keep VAFs >30%
  - Downside: you may miss real events!

- Best solution is to make new libraries from the original source tissue



Case #3

- Single-cell RNA sequencing data
- Transcriptome alignment
  – we expect high fraction: 90%+
- Our data had ~10% alignment

This example is
good, ours was not!

Mapping

Reads Mapped to Genome	100.0%
Reads Mapped Confidently to Genome	21.4%
Reads Mapped Confidently to Intergenic Regions	2.6%
Reads Mapped Confidently to Intronic Regions	12.5%
Reads Mapped Confidently to Exonic Regions	6.3%
Reads Mapped Confidently to Transcriptome	16.3%
Reads Mapped Antisense to Gene	2.0%

Case #3

- Checked the kit - 3’ vs 5’ (matched)
- Checked the data – blatted a read at random

https://genome.ucsc.edu/cgi-bin/hgBlat

Human BLAT Search

BLAT Search Genome

Genome: ☐ Search all genomes — Human

Assembly: Dec. 2013 (GRCh38/hg38)

Query type: BLAT's guess

Sort output: query,score

Output type: hyperlink

☐ All Results (no minimum matches)

Submit | I'm feeling lucky | Clear

Paste in a query sequence to find its location in the the genome. Multiple sequences may be searched if separated by lines starting with '>' followed by the sequence name.

Human (hg38) BLAT Results

BLAT Search Results

Custom track name: blat YourSeq

Custom track description: blat on YourSeq

Build a custom track with these results

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHROM	STRAND	START	END	SPAN
browser details	YourSeq	146	1	146	146	100.0%	chr2	-	25229049	25229194	146
browser details	YourSeq	21	81	102	146	100.0%	chr12	-	18672011	18672033	23

Case #3

- Checked the kit - 3’ vs 5’ (matched)
- Checked the data – blatted a read at random (matched to human)
- Checked 10x indices – do they appear in the whitelist (yes)
- Pulled our hair out, contacted production

Case #3

- Retraced our steps double checking all of our work

- Blatted a few more reads
  - They all matched poorly to the human genome
  - They all matched well to the mouse genome

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dx: SPECIES MIXUP

- Just by chance, the first read we checked was from a very highly conserved gene!

Damage Control

- Realign to the correct species
  - Gave expected high alignment rate

- Still have to resolve what happened with the sample naming
  - Was the species designation just wrong?
  - Is the entire sample named wrong? (swap)

Xenograft contamination

- Related topic is dealing with Xenograft data
  - e.g. human tumors implanted in a mouse
- Mouse reads with homology to human genome
- One solution is Xenosplit – alignment-based read filtering
  - Human, mouse, ambiguous

Global alignment/mismatch issues

- Sample swaps
  - check SNP concordance
  - Somalier is a tool for rapid sample identity checking
- Contamination
  - VAFs, IGV inspection are your friends
- Species swaps
  - Check a few reads, some concordance is expected!

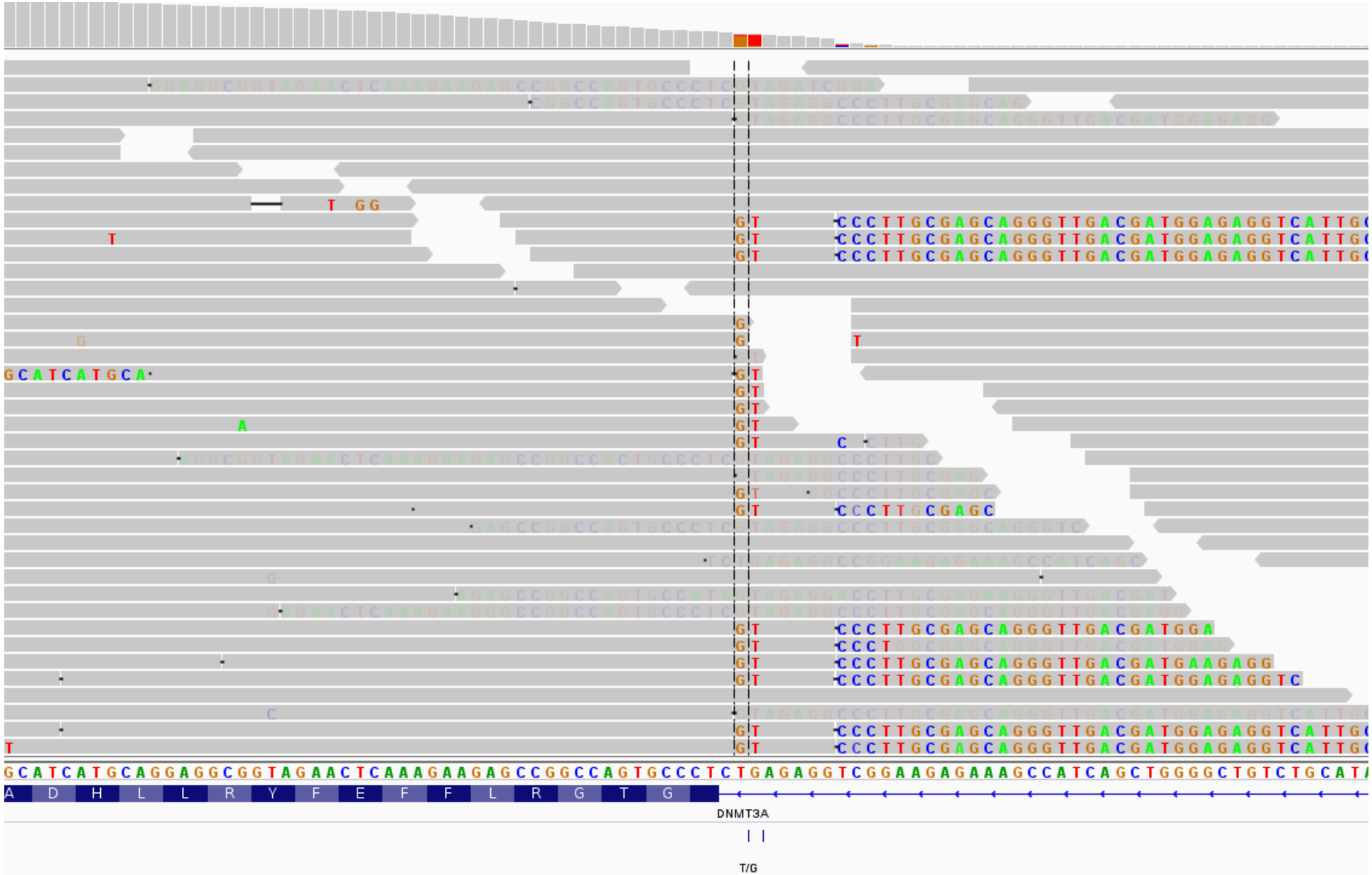
Case #4

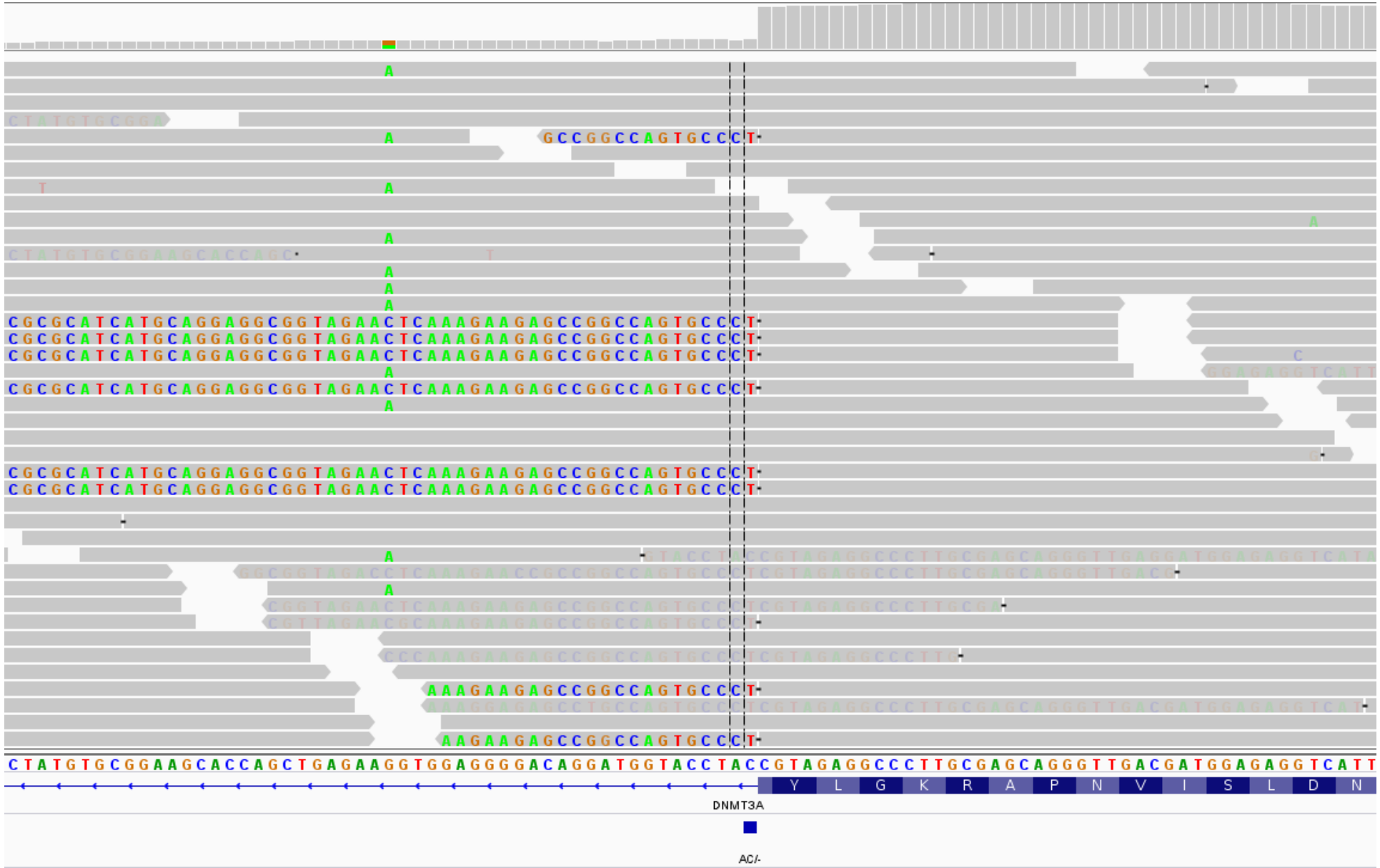
Exome sequencing of a tumor sample
Mutations sorted by the number of times a single gene is hit

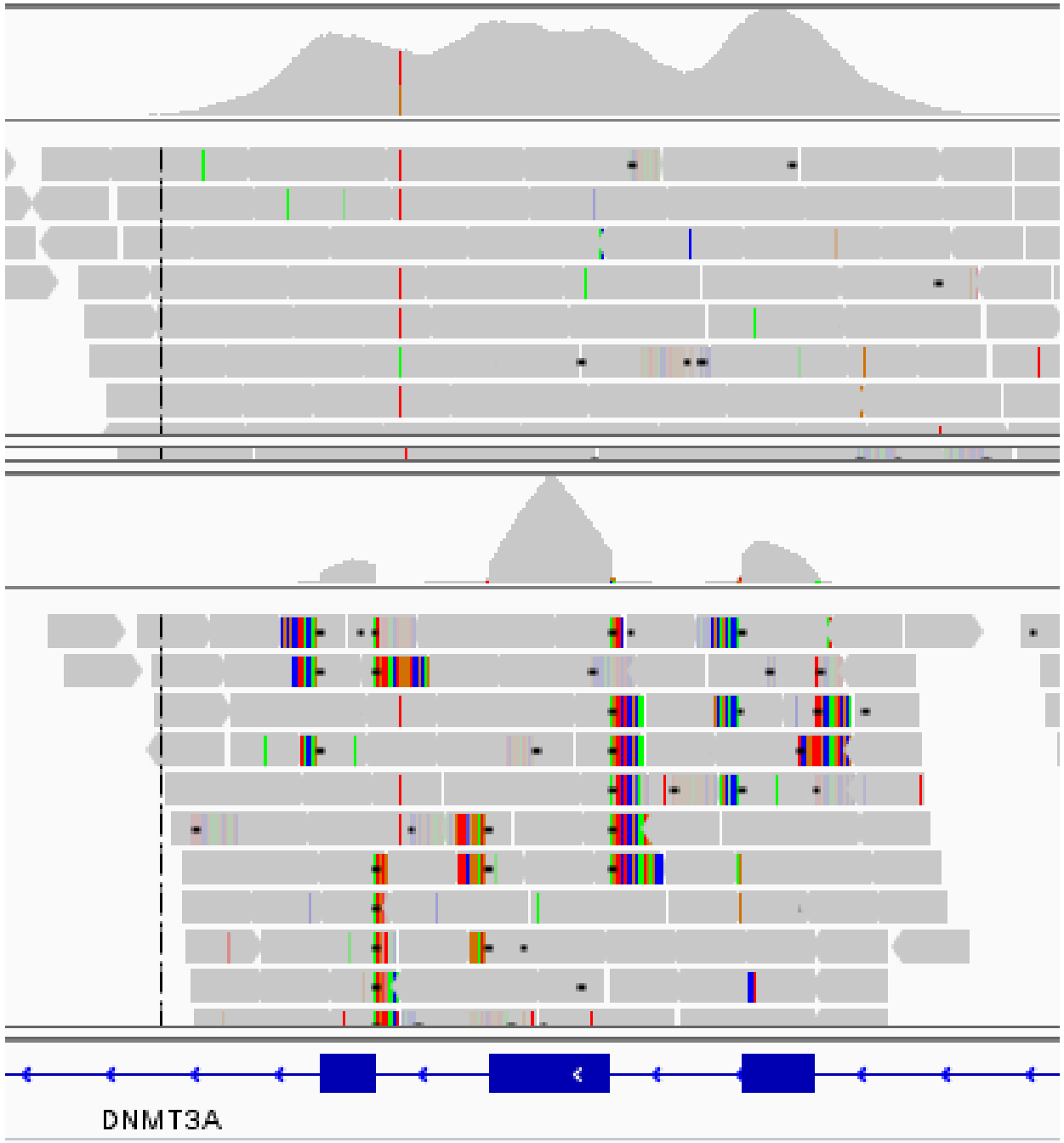
```
43 DNMT3A
 2 WT1
 2 SLC35F3
 1 UNC93B5
 1 TSLP
 1 TRPS1
 1 TARDBP
 1 SUN3
 1 SREBF1
 1 SPTBN2
 1 SPAST
 1 SNX1
 1 SNRNP40
 1 SLC17A3
 1 SELK
 1 RUNX1
 1 RCC1
 1 PTPN11
```

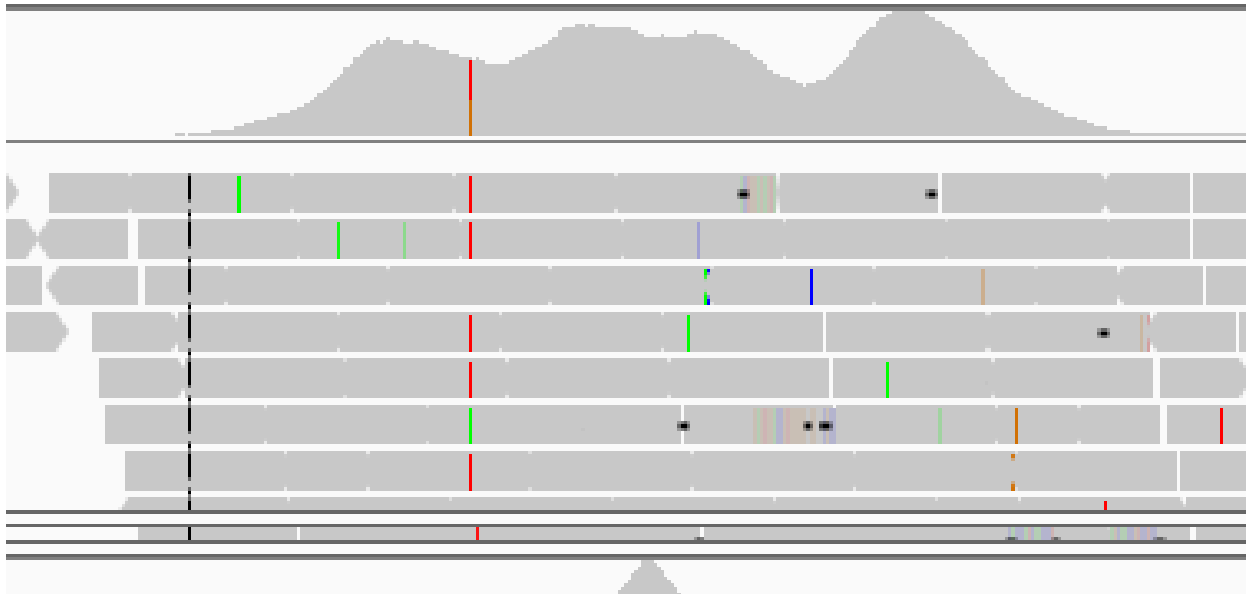
Variants called in DNMT3A

2	25457242	C	T	DNMT3A	missense	p.R882H
2	25458572	G	A	DNMT3A	splice_region	e21+4
2	25463169	A	G	DNMT3A	splice_site	e18+2
2	25463170	C	A	DNMT3A	splice_site	e18+1
2	25463321	T	G	DNMT3A	splice_site	e18-2
2	25463322	G	T	DNMT3A	splice_region	e18-3
2	25463507	AC	-	DNMT3A	splice_site_del	e17+1
2	25463600	C	A	DNMT3A	splice_site	e17-1
2	25464428	T	G	DNMT3A	splice_region	e16+3
2	25464430	C	T	DNMT3A	splice_site	e16+1
2	25467021	C	G	DNMT3A	splice_region	e14+3
2	25467022	A	T	DNMT3A	splice_site	e14+2
2	25467211	G	C	DNMT3A	splice_region	e14-4
2	25467213	A	G	DNMT3A	splice_region	e14-6
2	25467403	CCT	-	DNMT3A	splice_region_del	e13+4
2	25467524	A	T	DNMT3A	splice_region	e13-3
2	25467526	A	C	DNMT3A	splice_region	e13-5
2	25469028	C	T	DNMT3A	splice_site	e10+1
2	25469181	G	T	DNMT3A	splice_region	e10-3

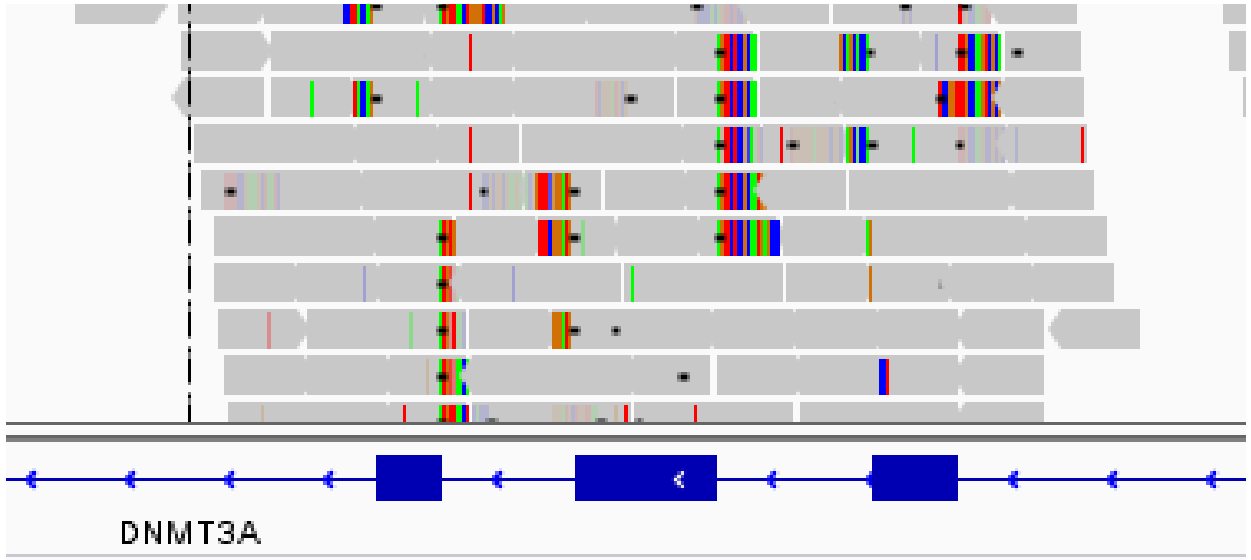

DNMT3A
T/G


CTATGTGCGGAAGCACCAGCTGAGAAGGTGGAGGGGACAGGATGGTACCTACCGTAGAGGCCCTTGCGAGCAGGGTTGACGATGGAGAGGTCATT
Y L G K R A P N V I S L D N
DNMT3A
AC/-


DNMT3A



dx: cDNA contamination



Damage Control

- If it is limited to a single gene (as in this case), could remove all splice-site adjacent mutations in that gene
  - If it's many genes/widespread, you might miss a lot of real events!

- Remake the libraries, resequence the sample

Case #5 – WGS serial samples

```
$ wc -l relapse_mutations.txt

  10114  relapse_mutations.txt
```

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Max from TCGA AML cohort:
1298

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Max from TCGA AML cohort:
1298

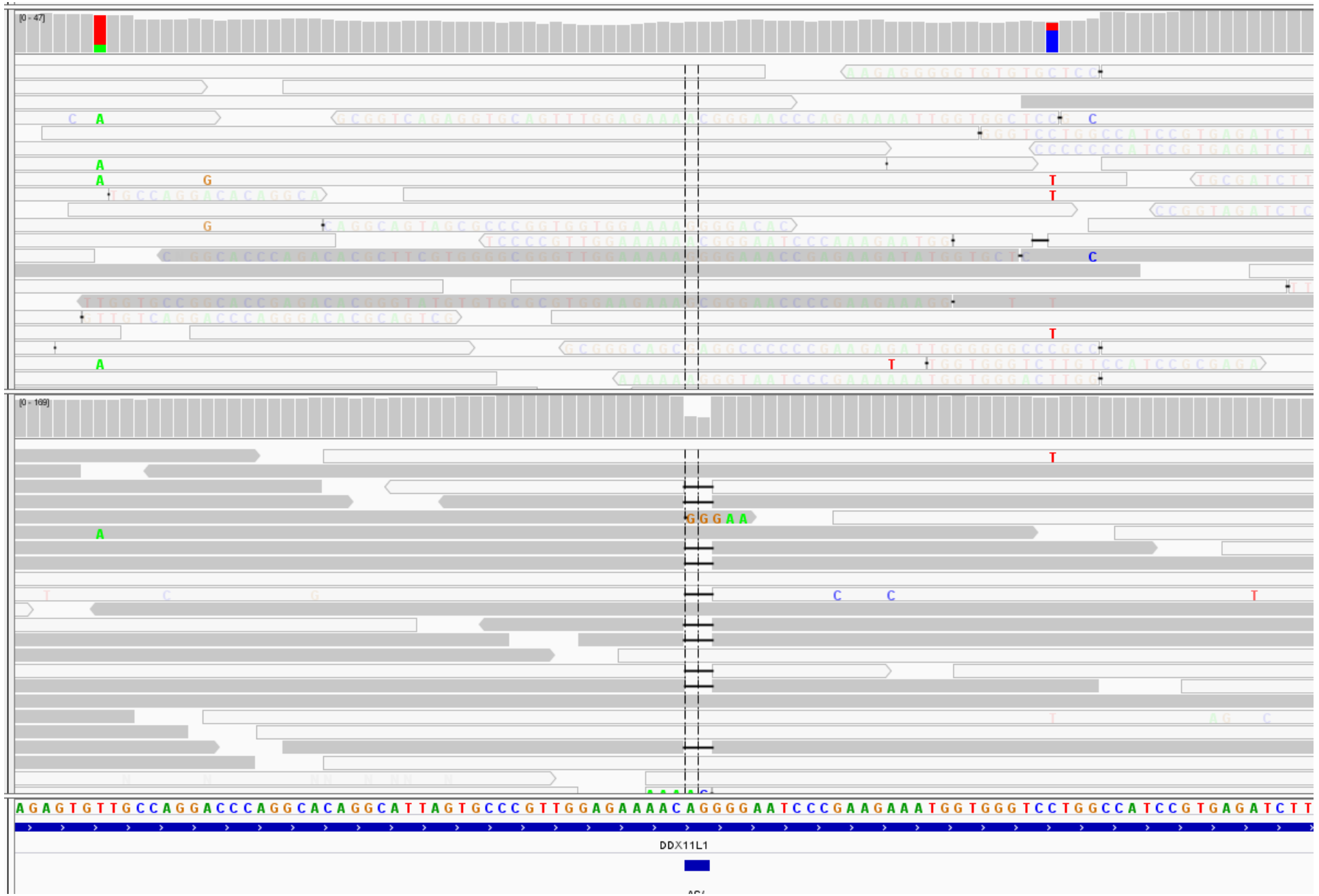
Primary tumor from this sample: 573

Case #5 – WGS serial samples

- Sample swap?
- Mismatch repair?
- Mutagenic therapy?

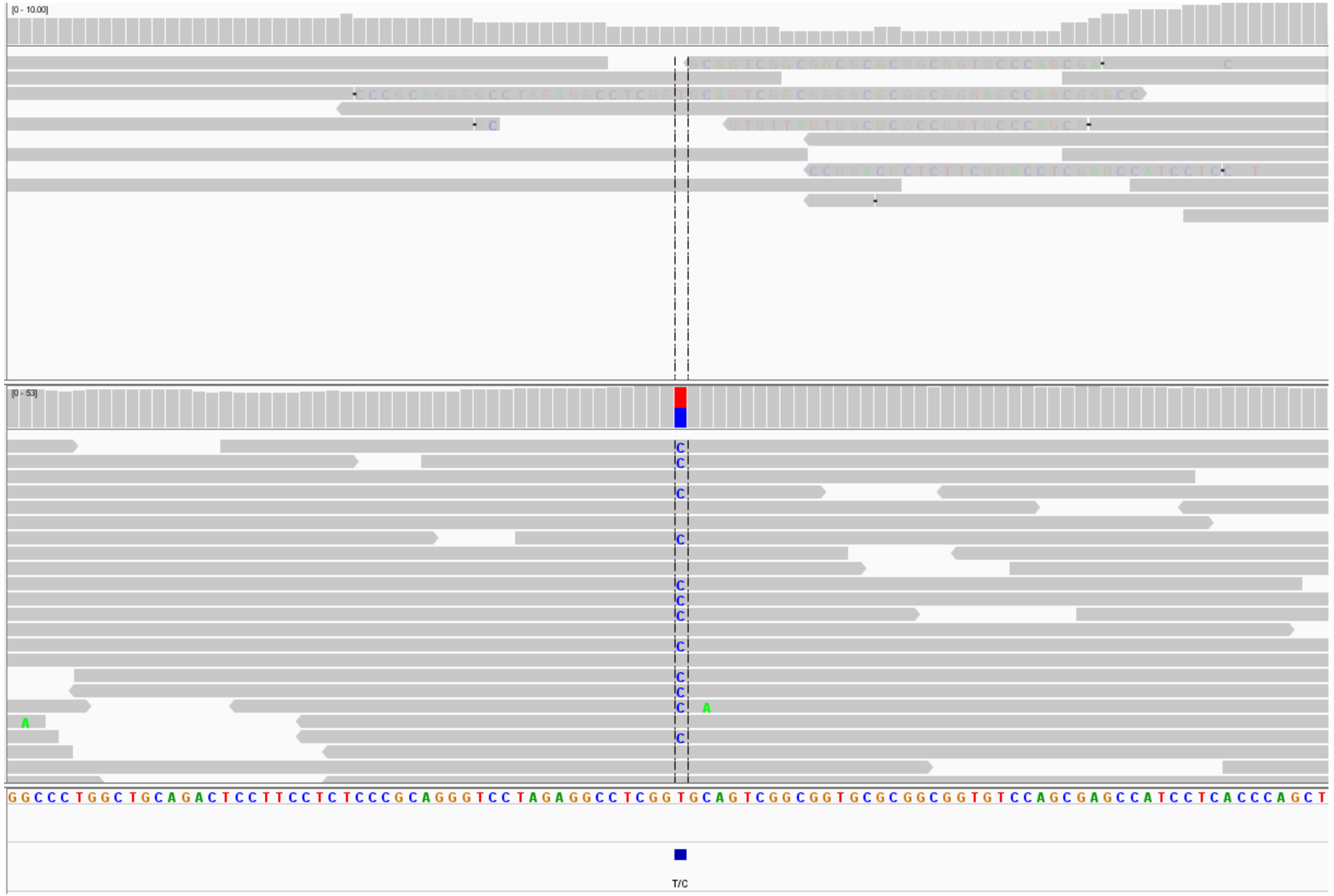
Normal

Tumor



Normal

Tumor

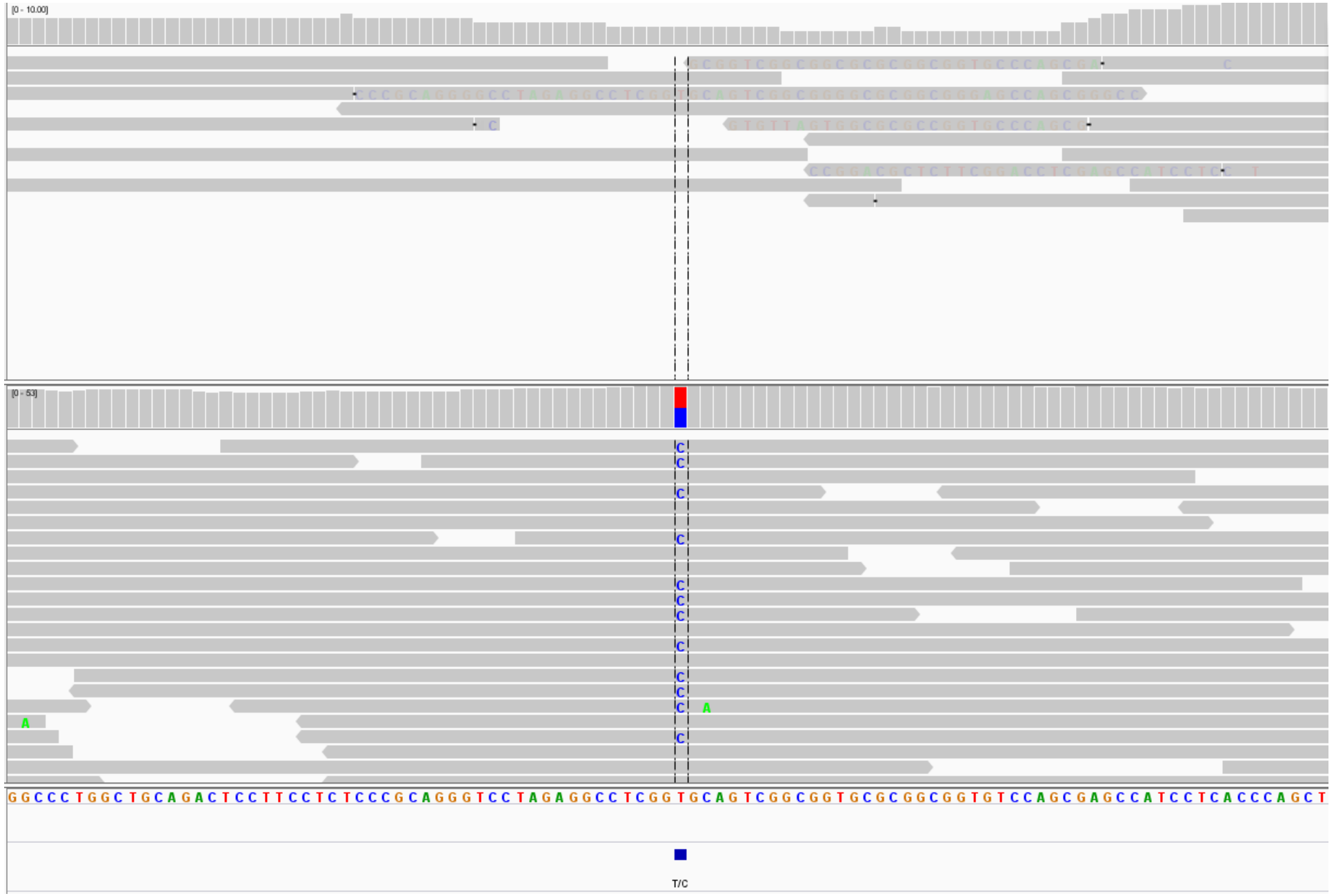


- Normal – sequenced with a mix of 75bp and 100bp PE reads
- Relapse – sequenced 6 years later with 125bp PE reads

dx: poorly matched controls

Normal

Tumor



Damage Control

- Resequence the normal with matching read lengths

- Match your data as closely as possible!
  - Read lengths
  - Capture kits
  - Sample preparation

General Tips

- Visualize your data
  - A picture is worth a thousand p-values
- Hone, and then trust your instincts
  - If something seems unusual, it's often either a big problem or a big finding
- Be relentless
  - don't stop digging until you convince yourself that nothing is wrong

An expert is a man who has made all the mistakes which can be made, in a narrow field.

--Niels Bohr