When Good Experiments Go Bad

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Applied Computational Genomics II
BFX Workshop - Week 18

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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- 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?

  ~85%

- What is their VAF?

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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
Tier 1 SNVs	14430	669	5276	87
Tier 1 indels	255	115	55	17

How many SNVs are population variants?

Patient 1 AML - < 5% dbSNP

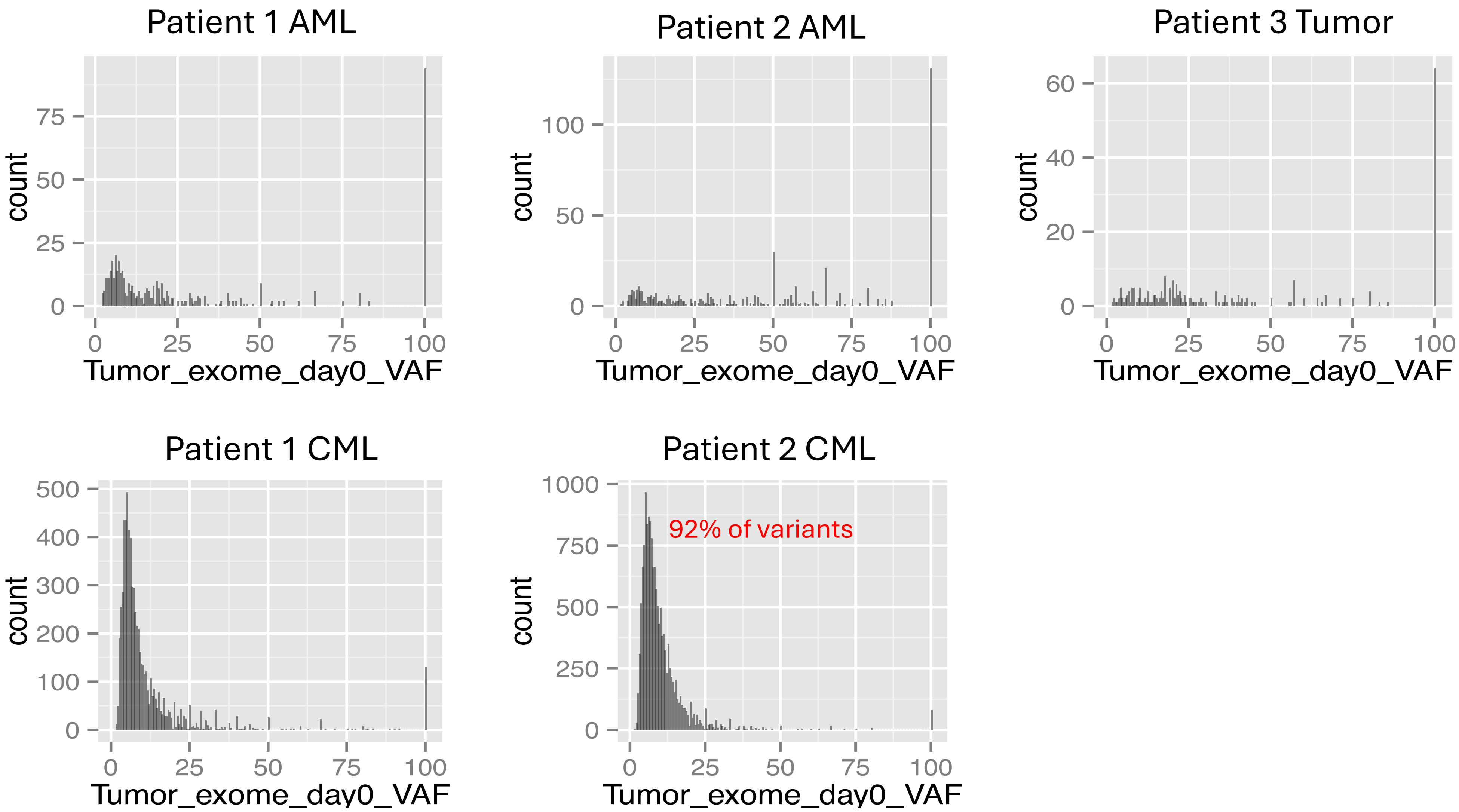
Patient 1 CML - > 90% dbSNP

Patient 2 AML - < 5% dbSNP

Patient 2 CML - > 90% dbSNP

Patient 3 AML - < 5% dbSNP

Coding variants with an rsID (dbSNP)


Patient 1 AML

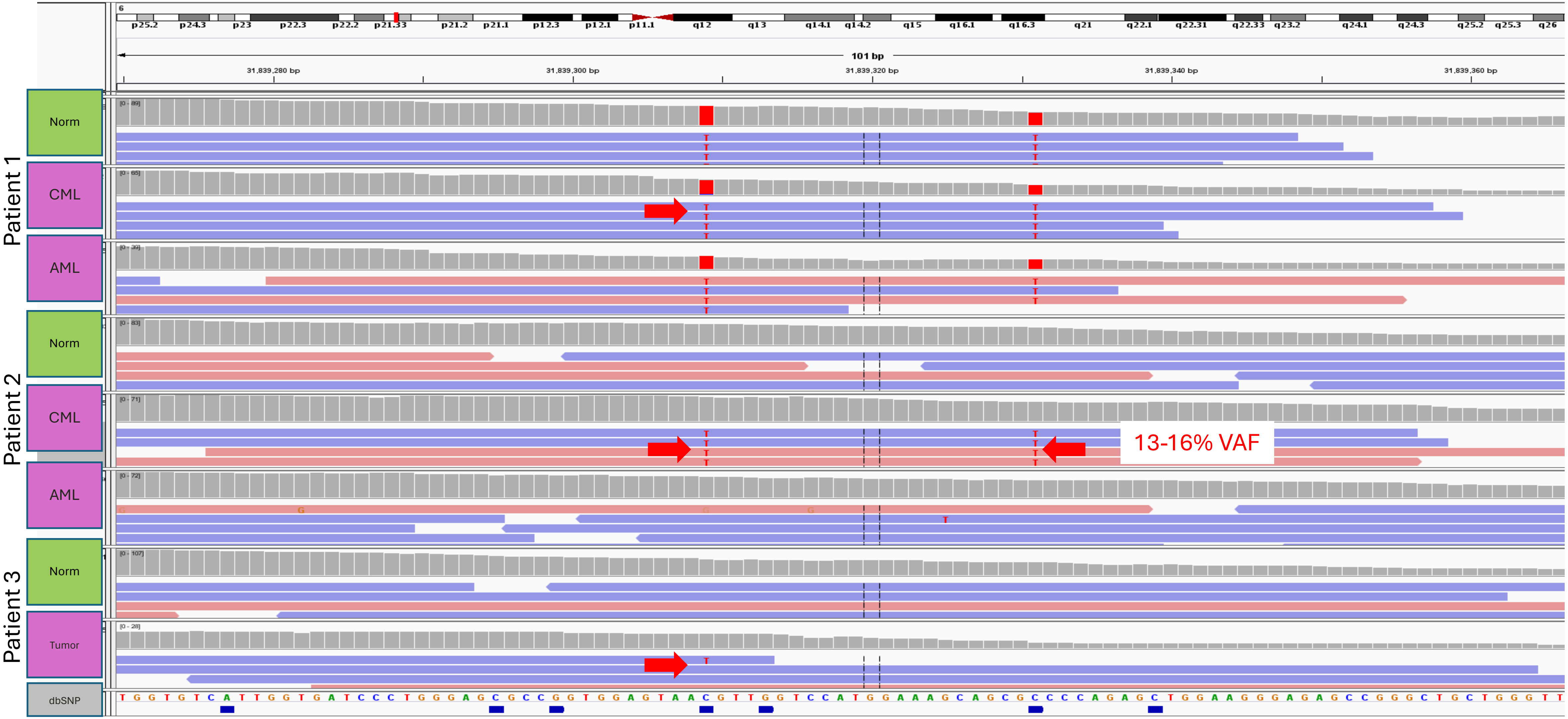
Patient 2 AML

Patient 3 Tumor

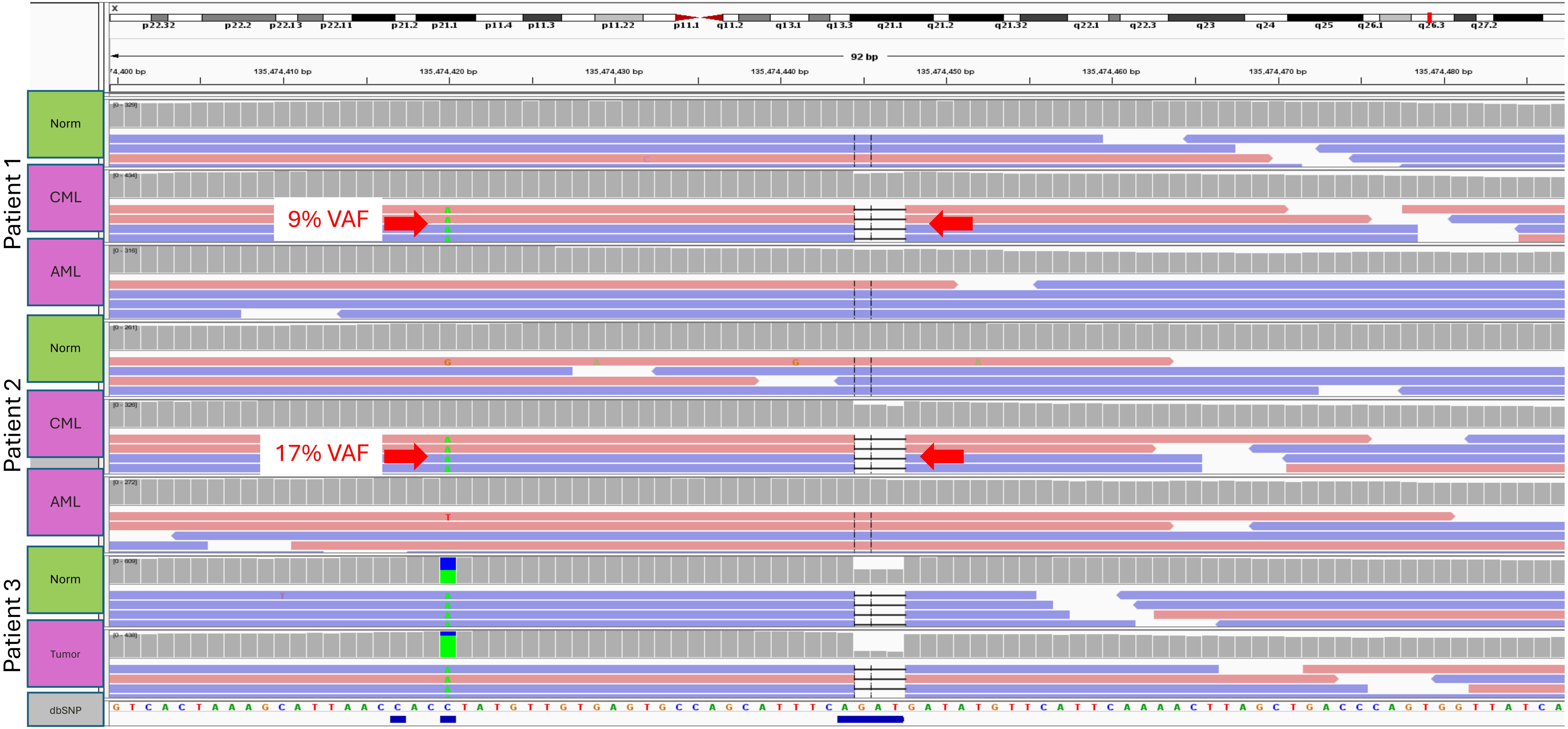
Patient 1 CML

Patient 2 CML

IGV inspection of variants/reads



IGV inspection of variants/reads



Malachi Griffith & Obi L. Griffith

- How many of these occur at known dbSNP sites?

  most

- What is their VAF?

  NOT ~50% or 100%

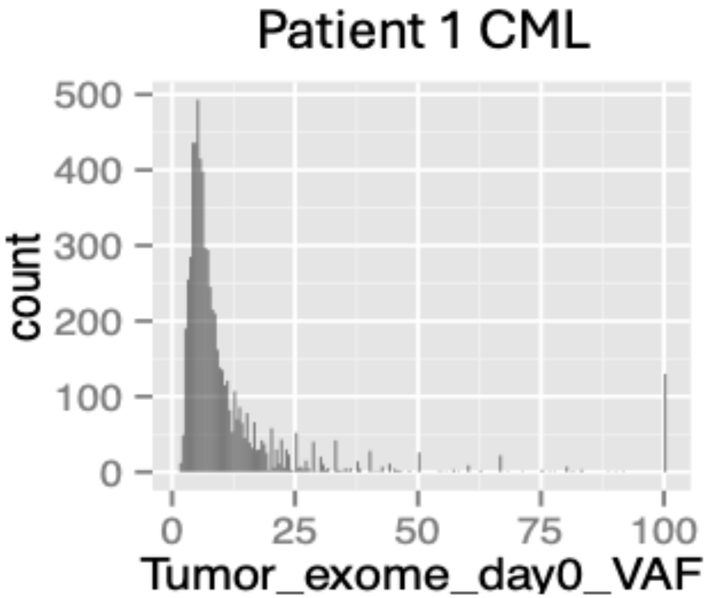
dx: SAMPLE CONTAMINATION

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!

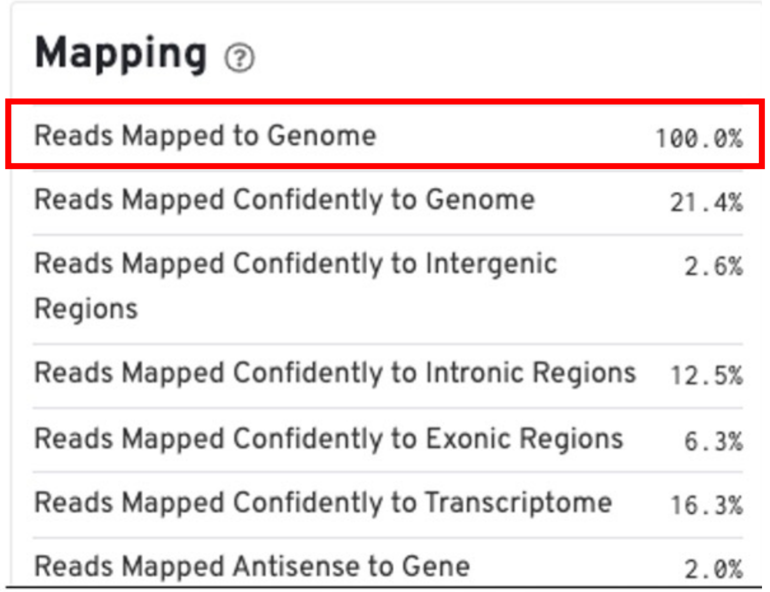
- Best solution is to make new libraries from the original source tissue



Case #3

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

This example is
good, ours was not!



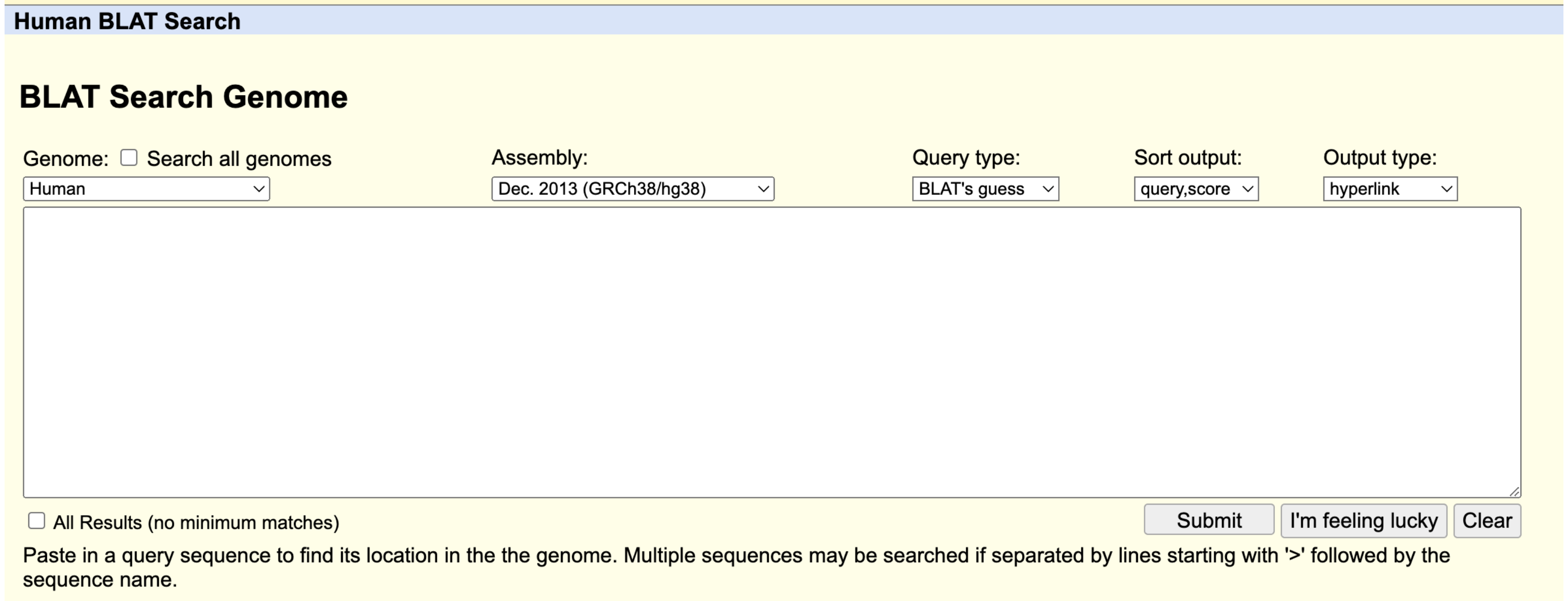
Mapping

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)

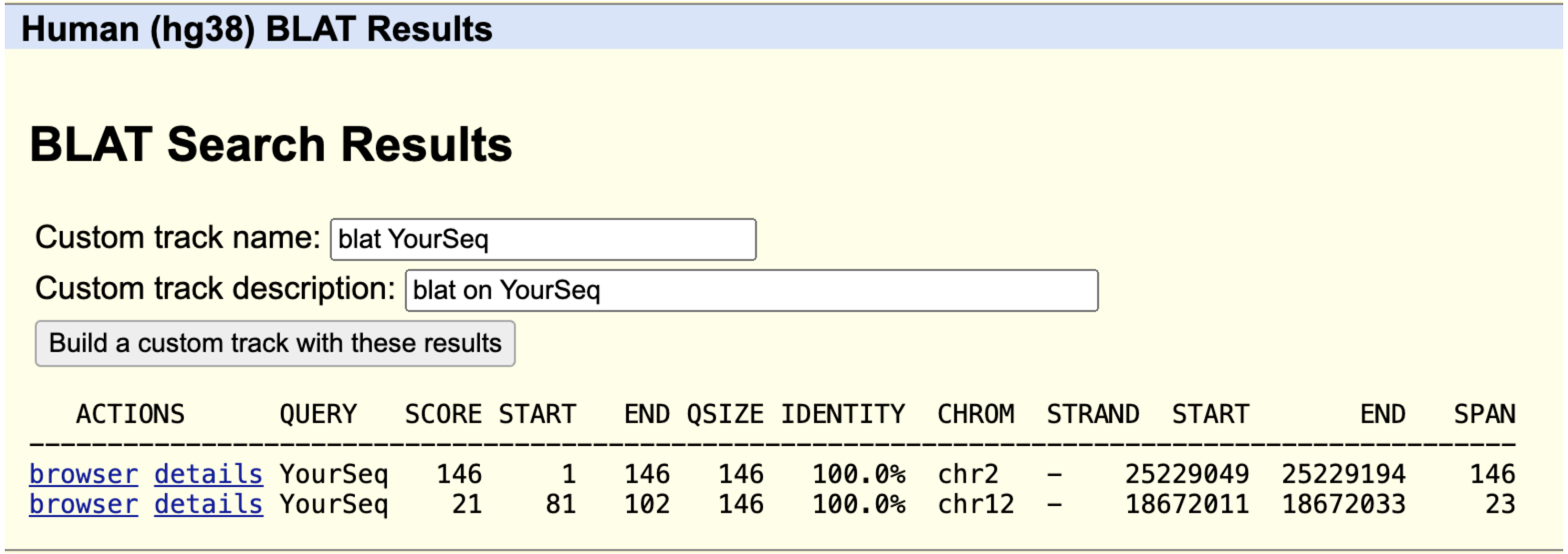
Query type: BLAT's guess

Sort output: query,score

Output type: hyperlink

□ All Results (no minimum matches)

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

```
   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!

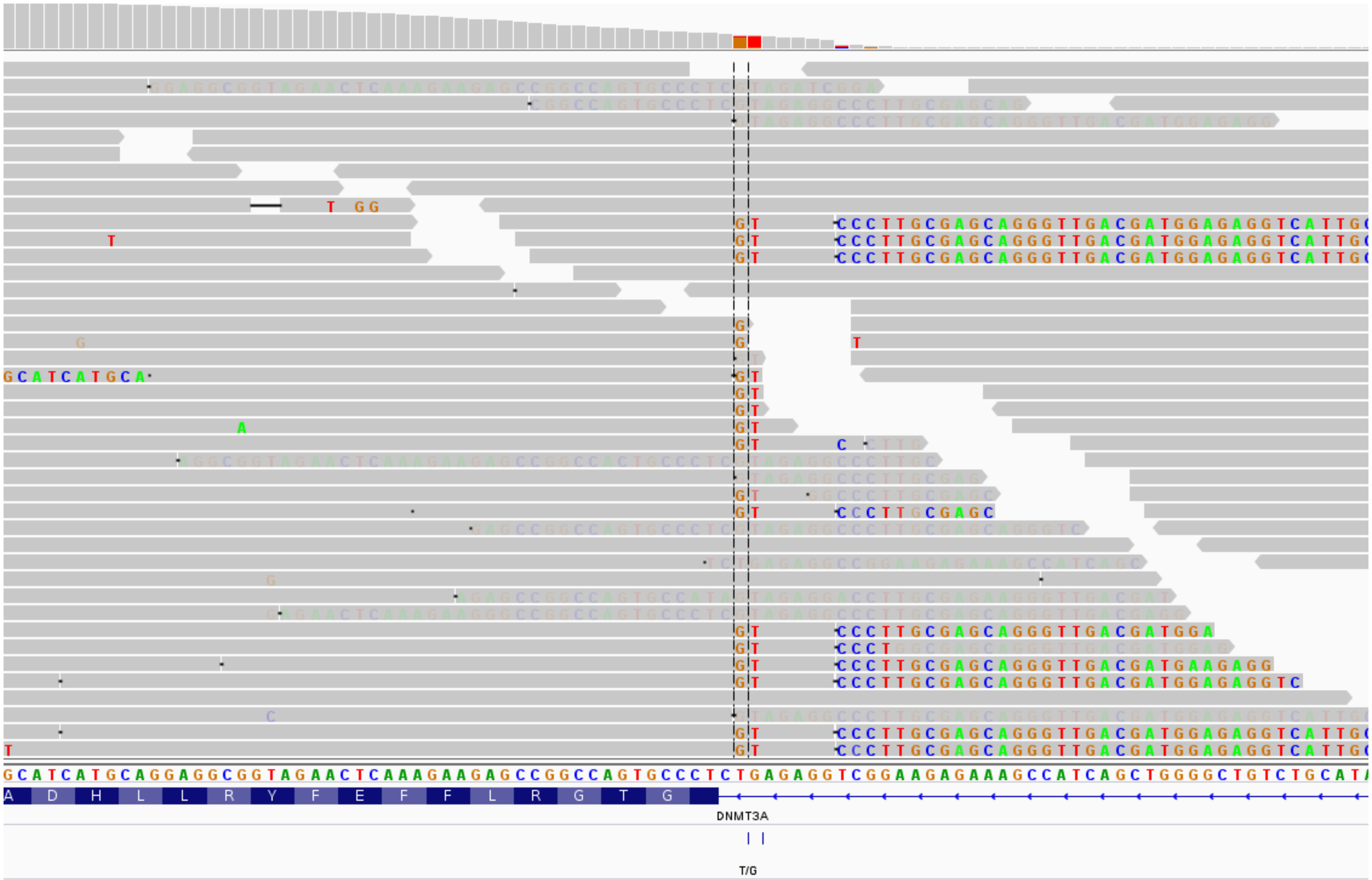
Case #4

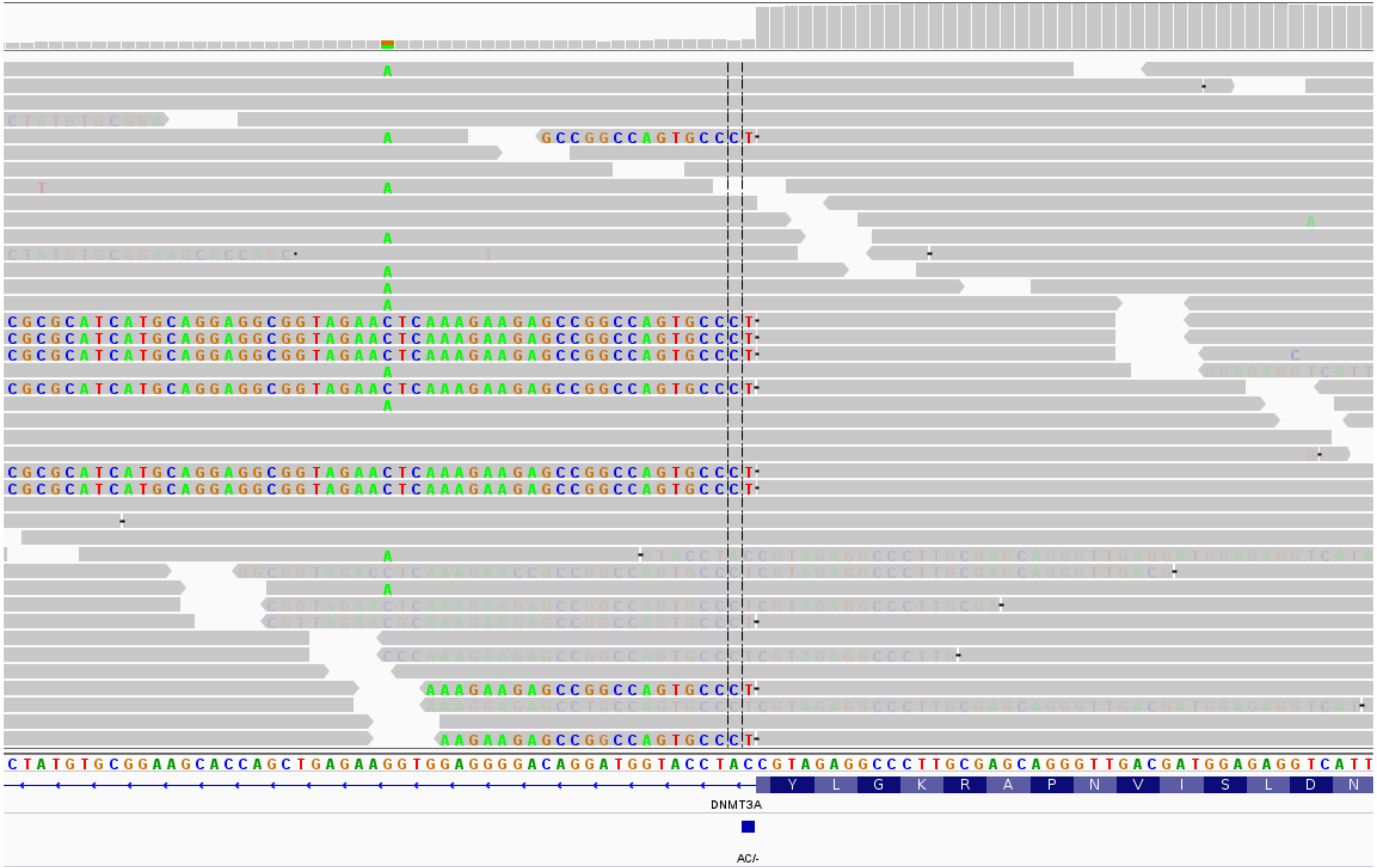
Mutations from a single AML sample, sorted by the number of times a single gene is hit

```
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
```

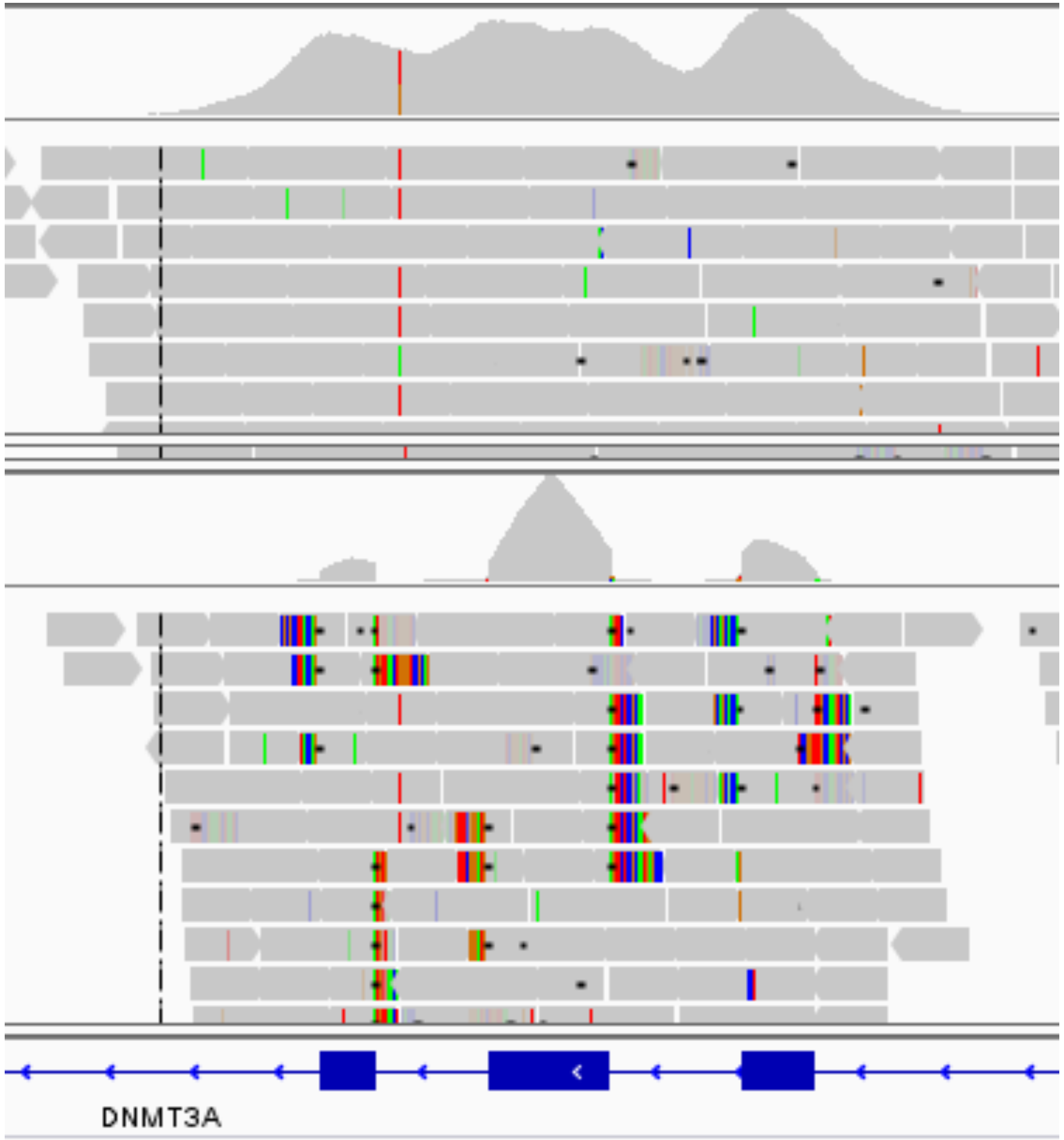
Variants called in DNMT3A

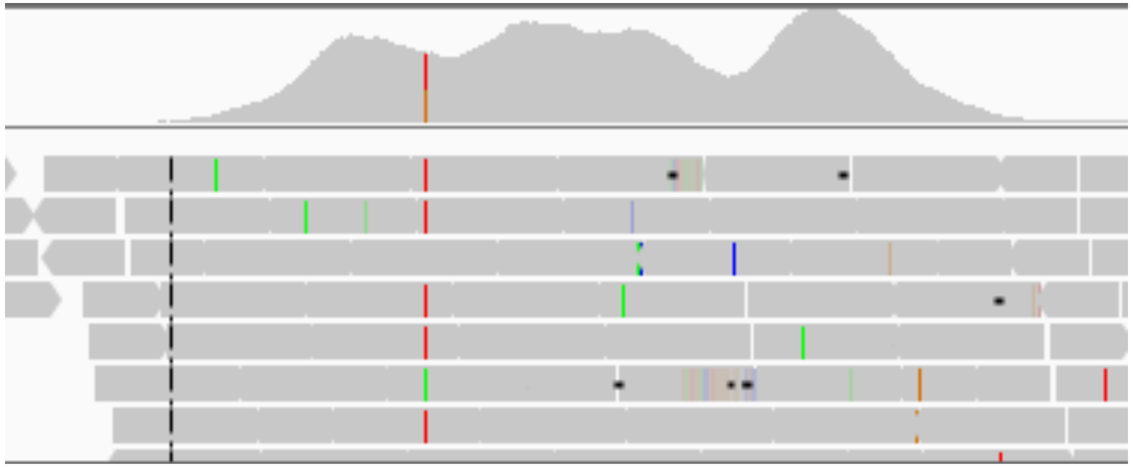
```
2  25457242  C    T  DNMT3A  missense           p.R882H
2  25458572  G    A  DNMT3A  splice_region      e21+4
2  25463169  A    G  DNMT3A  splice_site        e18+2
2  25463170  C    A  DNMT3A  splice_site        e18+1
2  25463321  T    G  DNMT3A  splice_site        e18-2
2  25463322  G    T  DNMT3A  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
```


GCATCATGCAGGAGGCGGTAGAACTCAAAGAAGAGCCGGCCAGTGCCCTCTGAGAGGTCGGAAGAGAAAGCCATCAGCTGGGGCTGTCTGCATA
A D H L L R Y F E F F L R G T G
DNMT3A
T/G

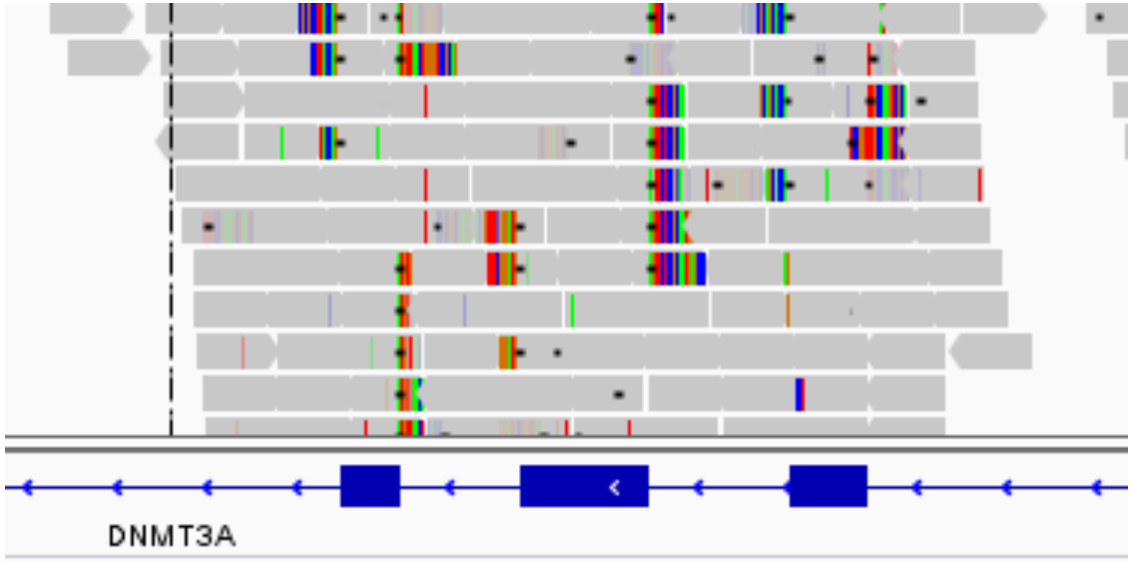


CTATGTGCGGAAGCACCAGCTGAGAAGGTGGAGGGGACAGGATGGTACCTACCGTAGAGGCCCTTGCGAGCAGGGTTGACGATGGAGAGGTCATT
Y L G K R A P N V I S L D N
DNMT3A
AC/-


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 AML30_final_filtered_clean_b20_q10.hq.txt

  10114  AML30_final_filtered_clean_b20_q10.hq.txt
```

Case #5 – WGS serial samples

```
$ wc -l AML30_final_filtered_clean_b20_q10.hq.txt

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```

Max from TCGA AML cohort:
1298

Case #5 – WGS serial samples

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Max from TCGA AML cohort:
1298

Primary tumor from this sample: 573

Case #5 – WGS serial samples

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  10114  AML30_final_filtered_clean_b20_q10.hq.txt
```

Max from TCGA AML cohort:
1298

Primary tumor from this sample: 573

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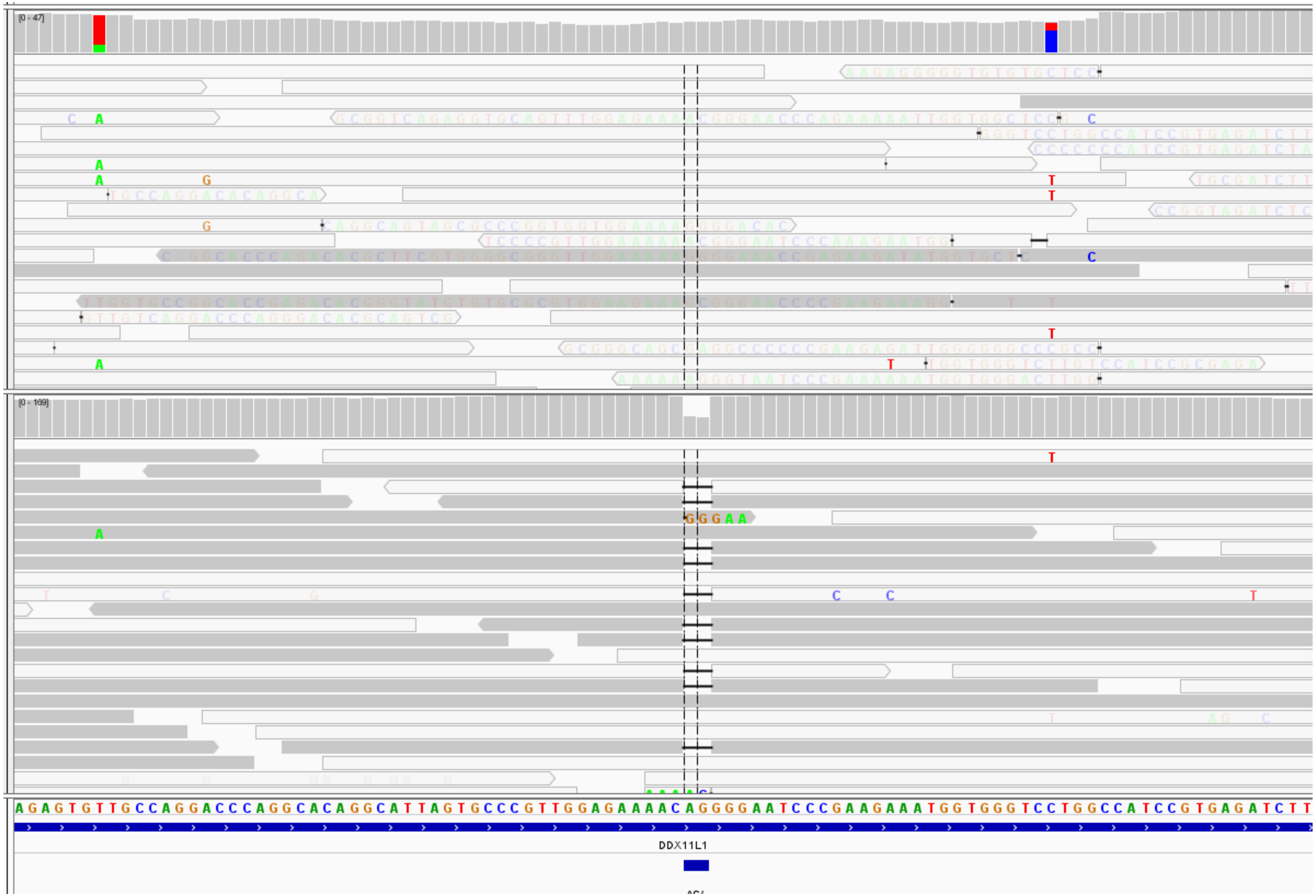
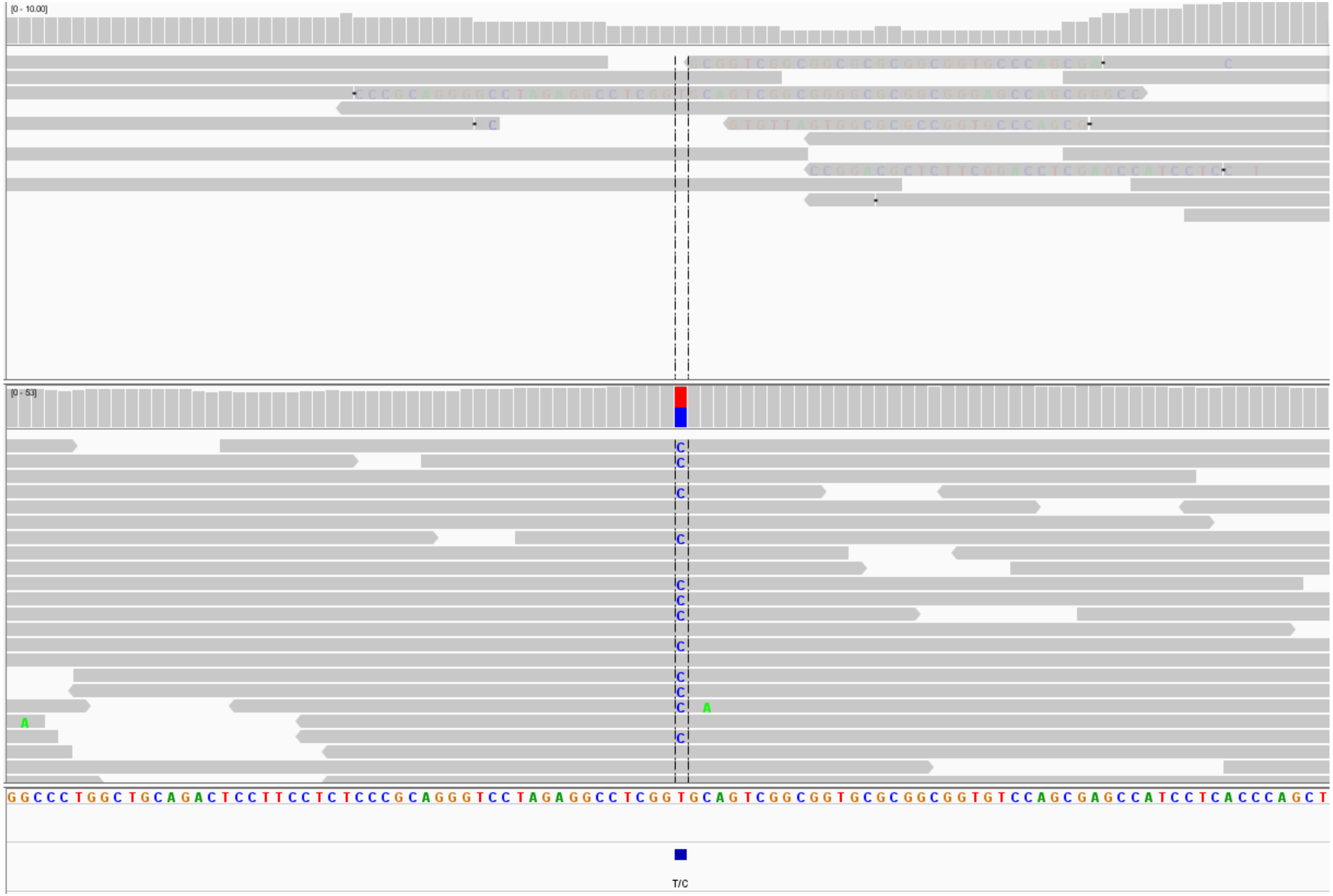
Case #5 – WGS serial samples

- Sample swap?

- Mismatch repair?

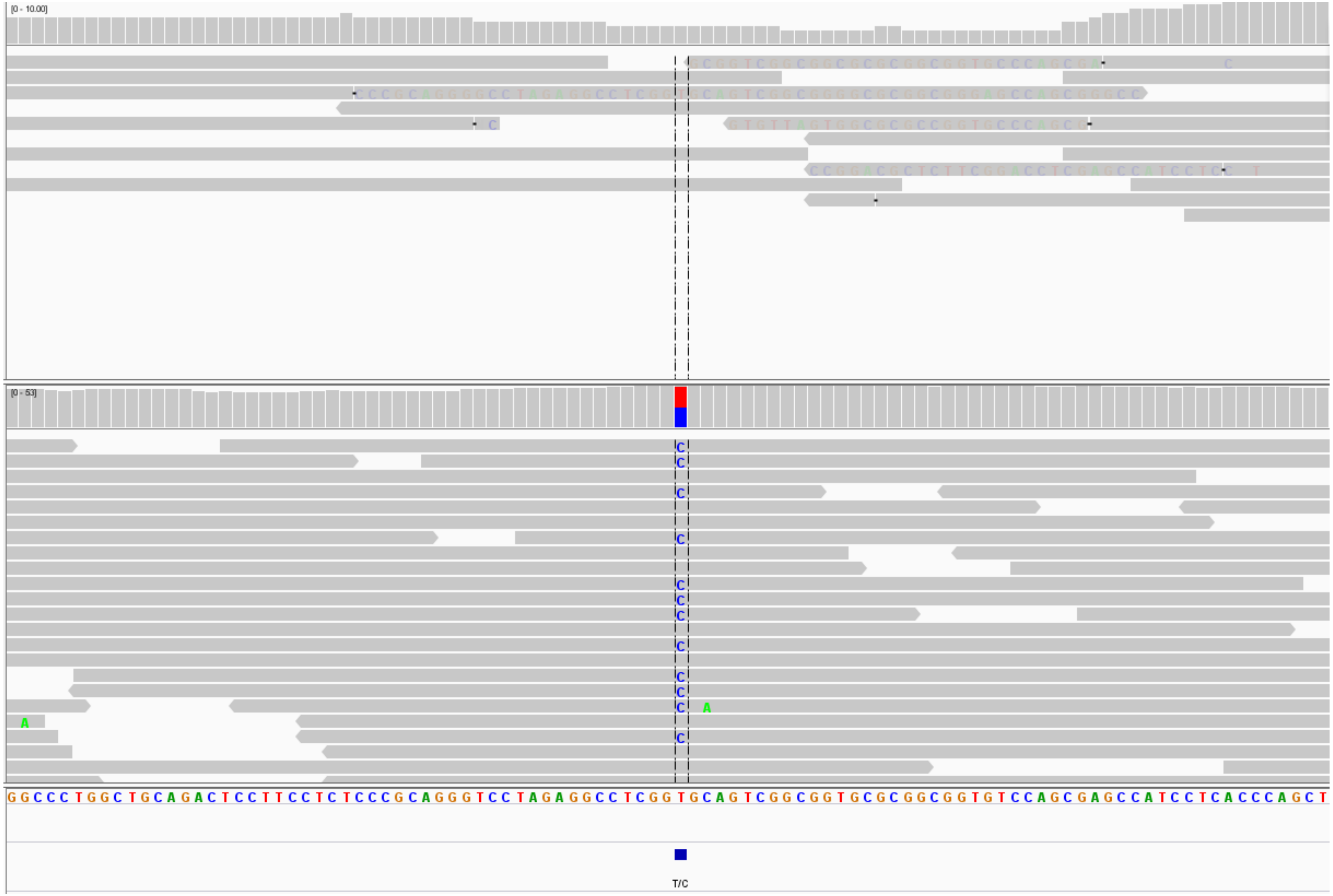
- Mutagenic therapy?

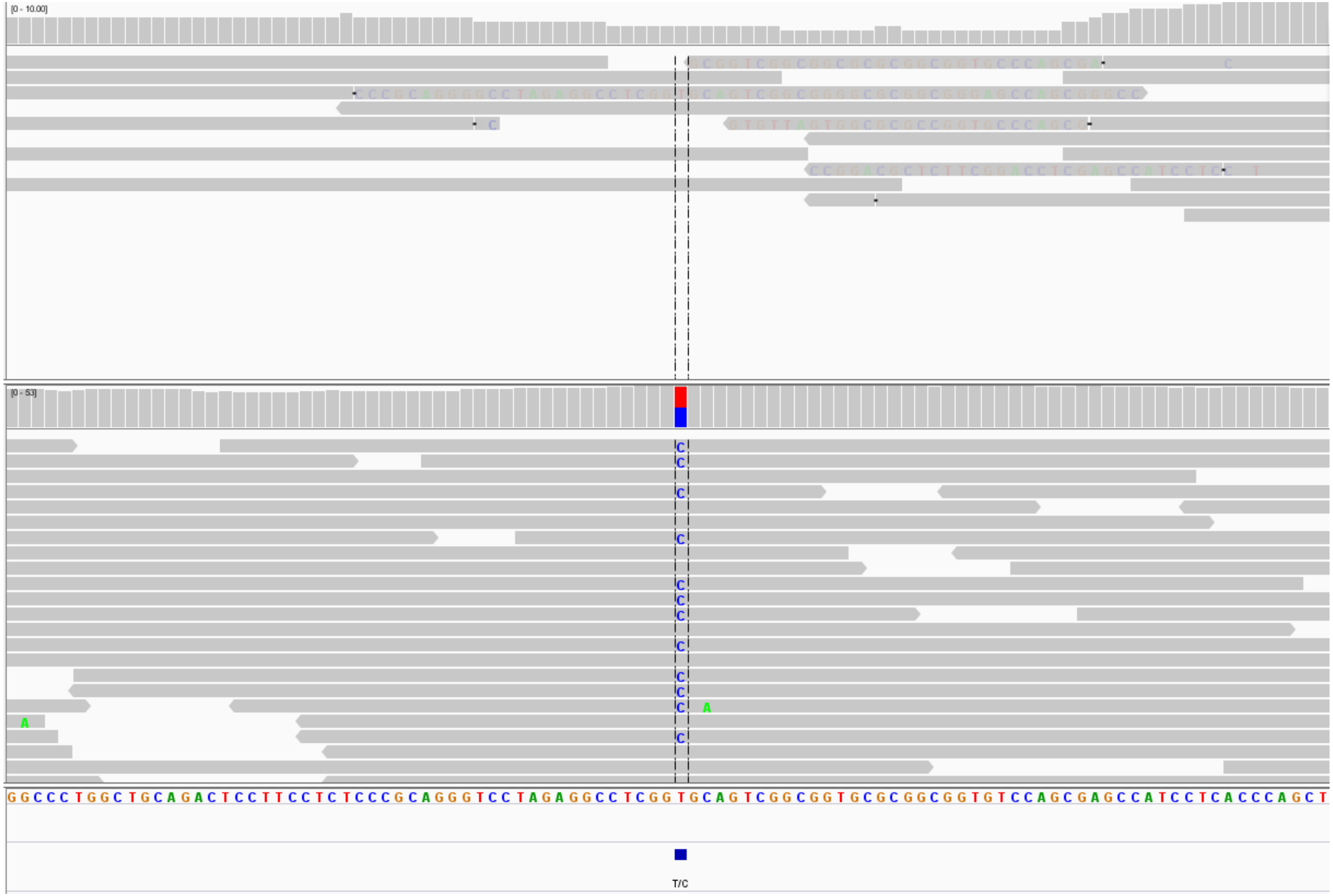

[0 - 47]
[0 - 169]
AGAGTGTTGCCAGGACCCAGGCACAGGCATTAGTGCCCGTTGGAGAAAACAGGGGAATCCCGAAGAAATGGTGGGTCCTGGCCATCCGTGAGATCTTT
DDX11L1


[0 - 10.00]
[0 - 53]
G G C C C T G G C T G C A G A C T C C T T C C T C T C C C G C A G G G T C C T A G A G G C C T C G G T G C A G T C G G C G G T G C G C G G C G G T G T C C A G C G A G C C A T C C T C A C C C A G C T
T/C

- Normal – sequenced in Aug 2009
  - Mix of 75bp and 100bp PE reads
- Tumor – sequenced in Nov 2015
  - 125bp PE reads

dx: poorly matched controls


[0 - 10.00]
[0 - 53]
GGCCCTGGCTGCAGACTCCTTCCTCTCCCGCAGGGTCCTAGAGGCCTCGGTGCAGTCGGCGGTGCGCGGCGGTGTCCAGCGAGCCATCCTCACCCAGCT
T/C


[0 - 10.00]
[0 - 53]
GGCCCTGGCTGCAGACTCCTTCCTCTCCCGCAGGGTCCTAGAGGCCTCGGTGCAGTCGGCGGTGCGCGGCGGTGTCCAGCGAGCCATCCTCACCCAGCT
T/C

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

Expertise

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