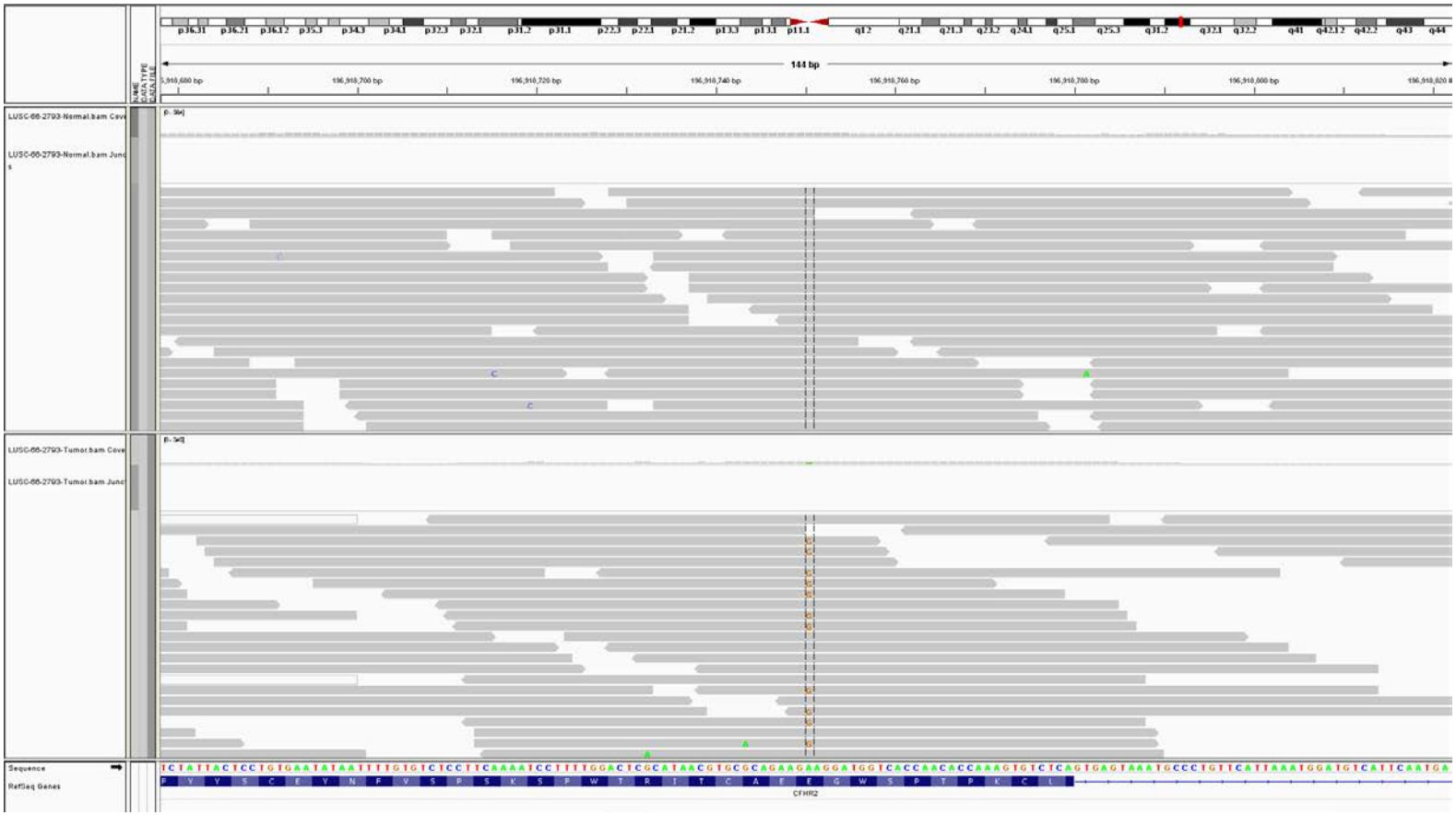
Comparison and Validation of Somatic Mutation Callers

Andrey Sivachenko

TCGA Symposium, November 17-18, 2011

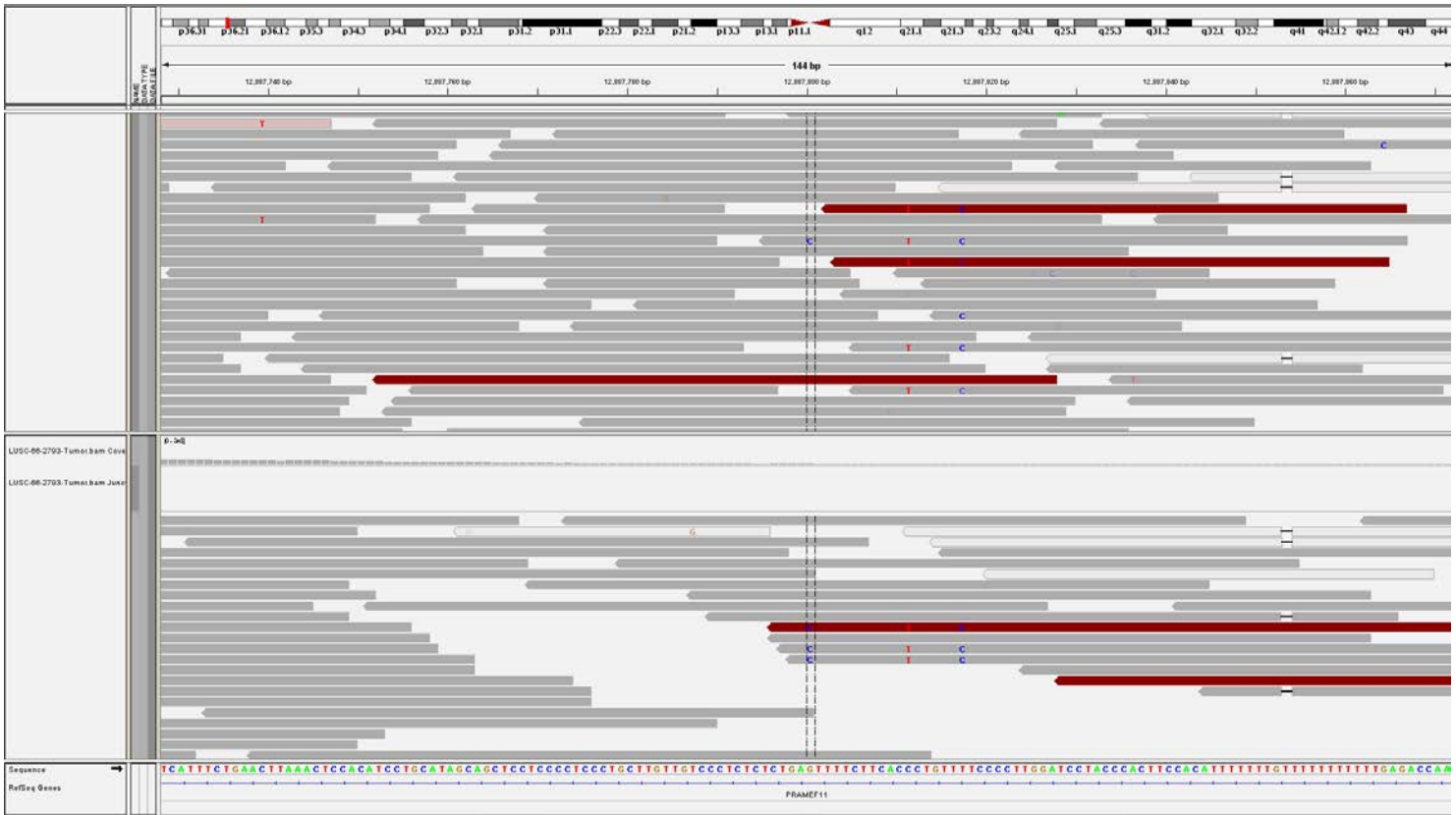
SNVs are Defined Simply…

- (single nucleotide) differences from the reference
- Ideally: resequence and read the results out
- If only everything looked as in the example below



… but SNVs Can be Hard to Call

- Multiple issues in library preparation, sequencing and data processing (base calling, alignments) can result in a spectrum of SNV-like events, from good to terrible
- Need to watch for:
  - Alignment quality around the event
  - "Strandness" – orientation of supporting reads
  - Position in read
  - Sufficient coverage (both in tumor *and* normal)
  - Sequence context
  - Potential tumor contamination in normal
  - …



Specificity → Need to protect against two types of errors

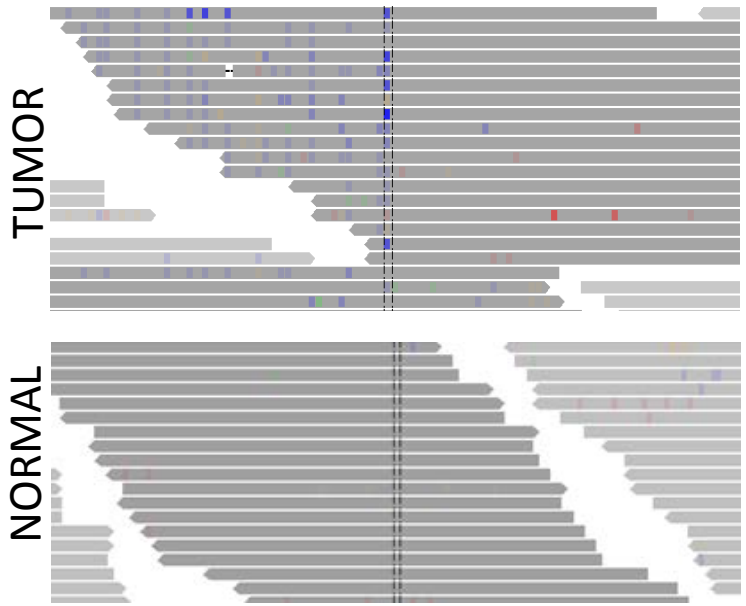
Signal: ~1 somatic mutation per Mb

Goal: >95% validation rate and ideally approach 100%

➔ Need error rate to be ≤ 0.05 errors/Mb! 99.9999% is not good enough

Noise: Two types of false positives

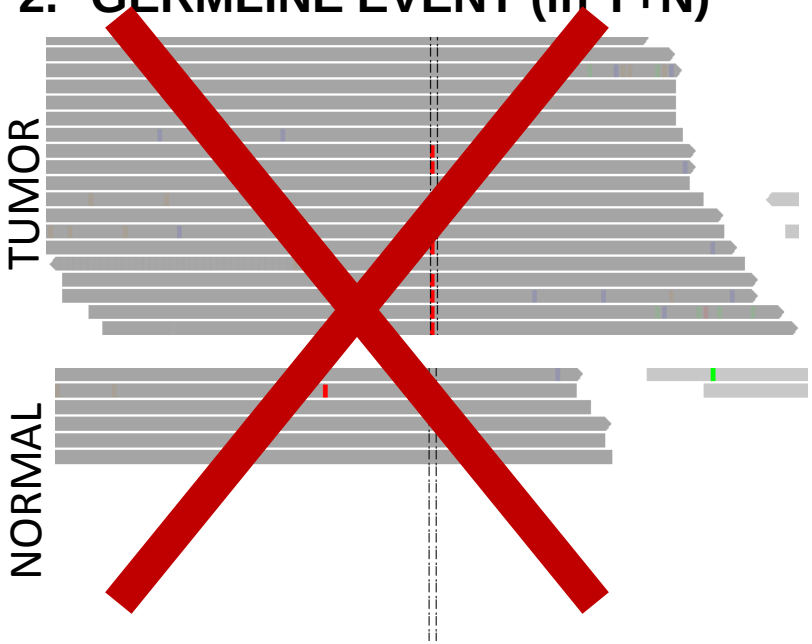
1. NO EVENT



At risk: Every base

Source: Misread bases
Sequencing artifacts
Misaligned reads

2. GERMLINE EVENT (in T+N)



At risk: ~1000 germline / Mb (known)
10-20 rare germline / Mb (novel)

Source: Low coverage in normal

Cross-Center Comparison

- The project initiated with the goal of comparing, evaluating, and improving mutation calling algorithms
  - Select a set of reference samples
  - Call mutations using different algorithms & compare
- Comparison alone allows only to contrast the callers against each other
  - If caller A makes a call and caller B does not, it is helpful to characterize the difference
  - Is there a difference in heuristics involved?
  - Is there a difference is some statistics of such caller-specific SNVs
  - Ultimately, one needs the ground truth (validation data)

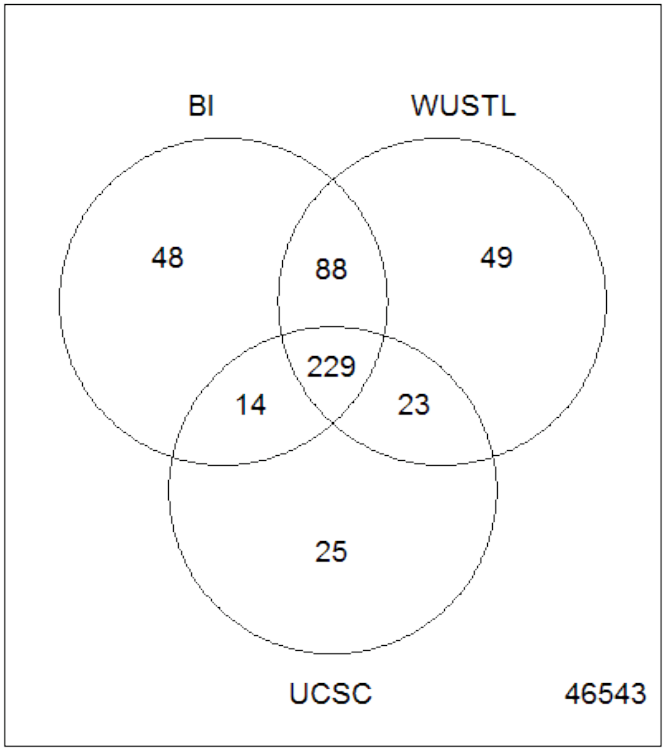
Data

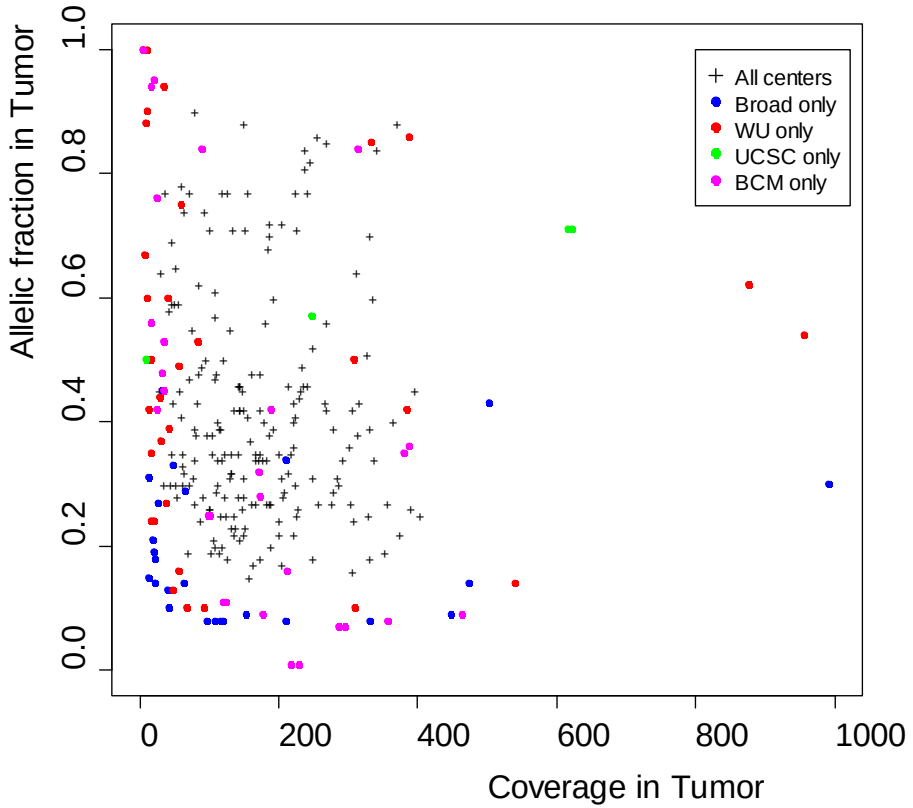
- For this round of the analysis, the subset of data from Phase III of the project was used
  - 20 Lung Squamous TCGA samples sequenced at Broad (whole-exome)
  - Same sequencing data (distributed between centers as aligned bam files) were called at 4 centers using different algorithms
    - Broad
    - Washington University, Saint Louis
    - UCSC
    - Baylor College of Medicine
  - Resulting callsets shared between the centers for comparison
- In addition, for this work we use RNA-Seq data as a validation dataset
  - Sequenced at UNC for TCGA

Simple Characterization of Mutation Callers

- Look at shared vs center-specific events
  - There is a large overlap, but there are still many calls made by each center alone
  - The center-specific calls have, in general, different properties
  - Are these specific false-positive modes of each caller or specific strength?

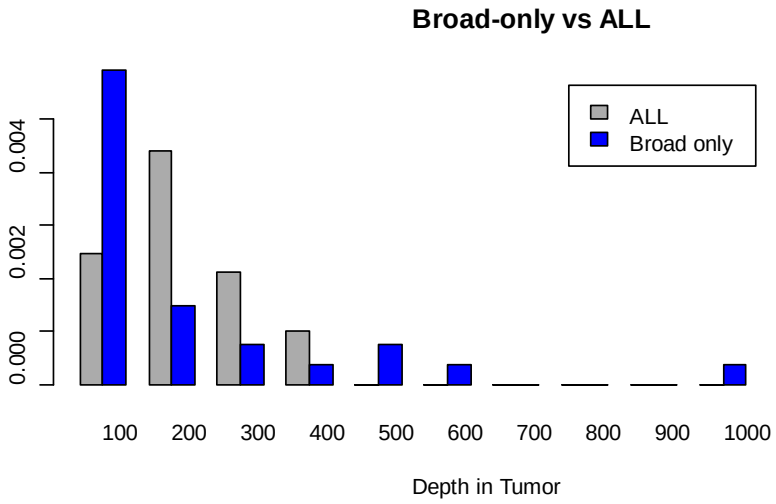
TCGA-33-4532

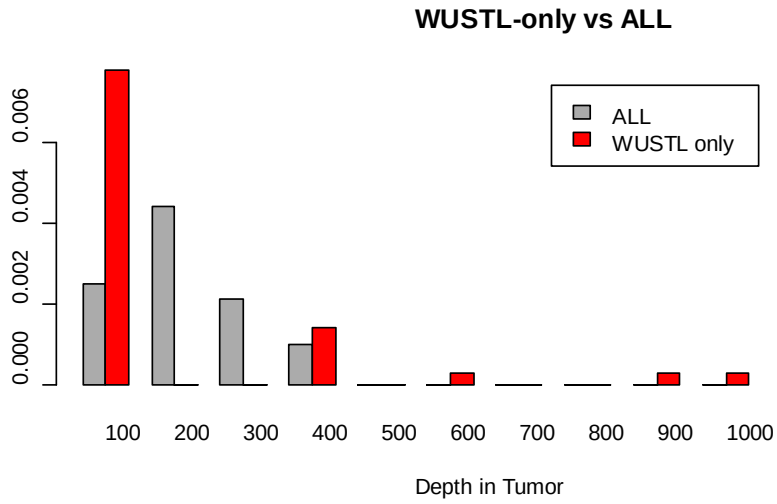


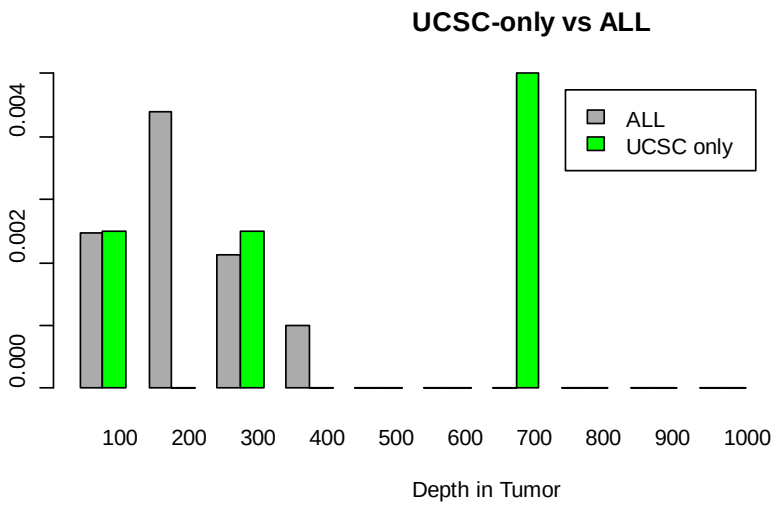


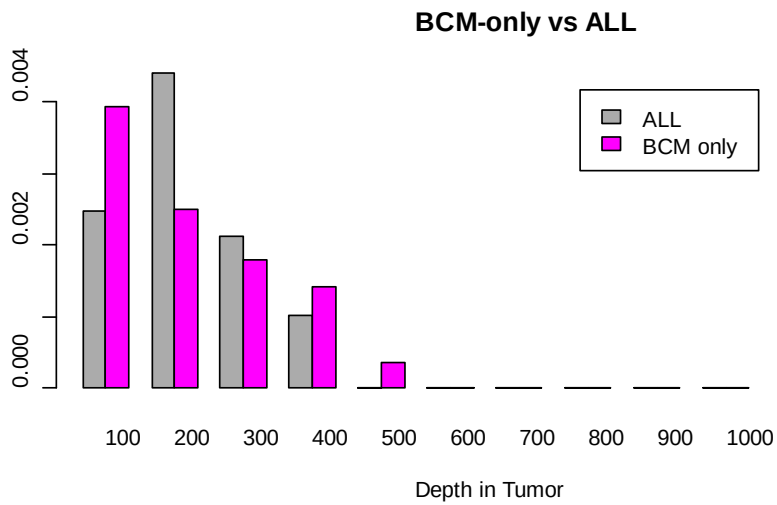
Calls vs Coverage

- Tendency to call center-specific events at coverages different from where shared events are located



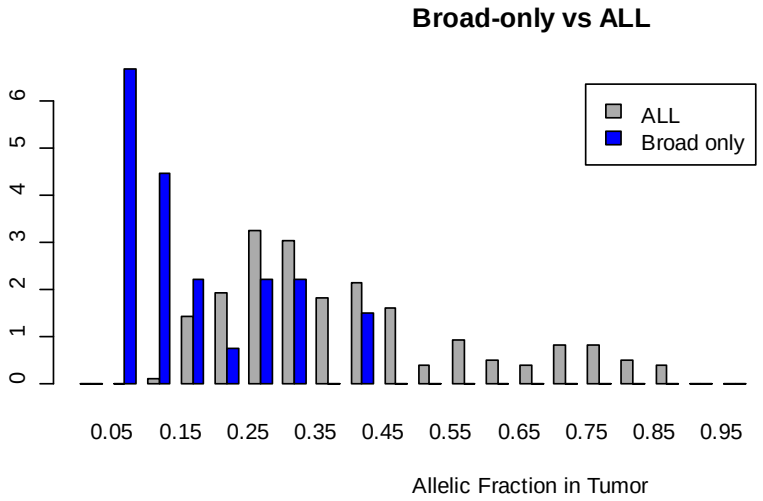


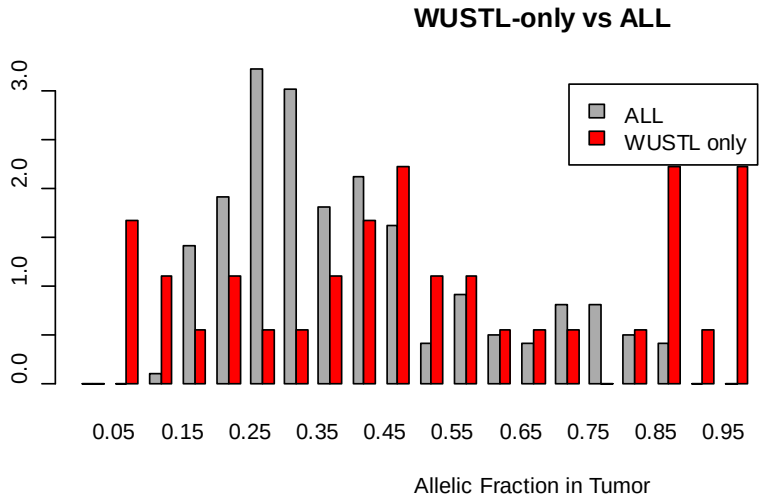


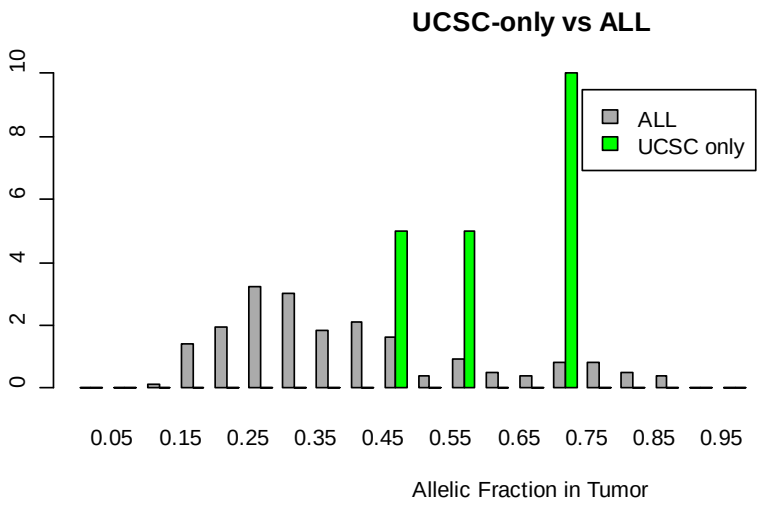


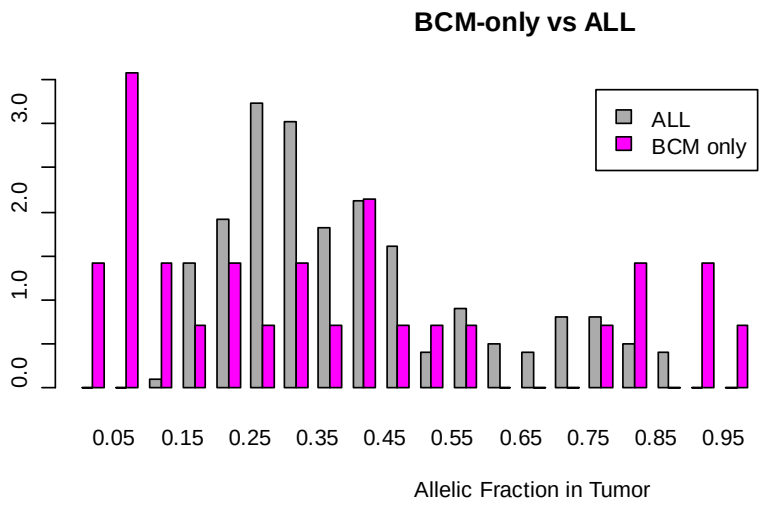
Calls vs Allelic Fraction

- Allelic fraction distribution of center-specific calls differs from that of shared calls



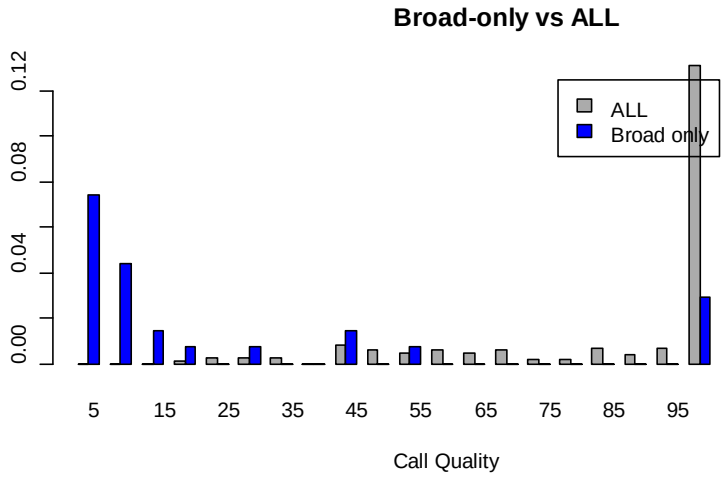


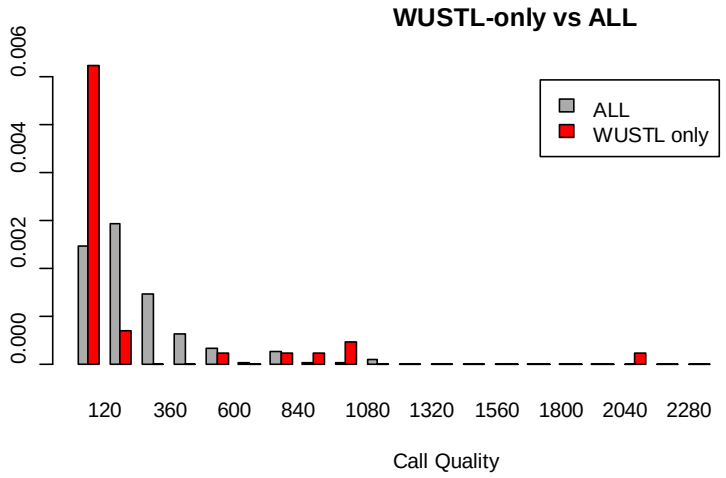


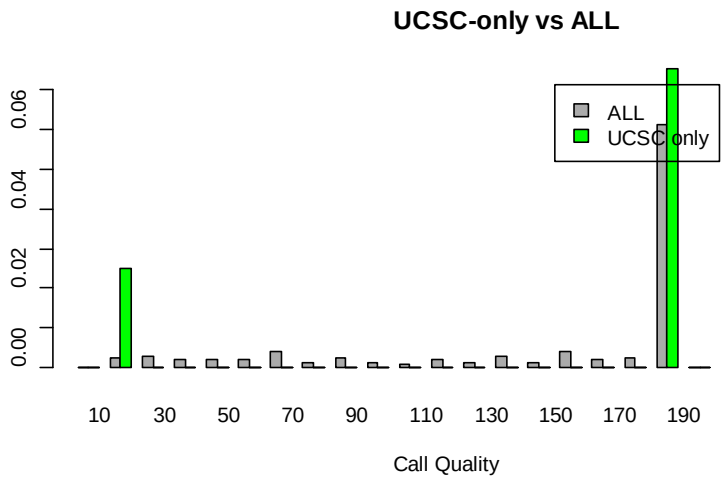


Calls vs Call Quality

- How do callers qualify their own unique calls – are reported qualities meaningful/reliable?



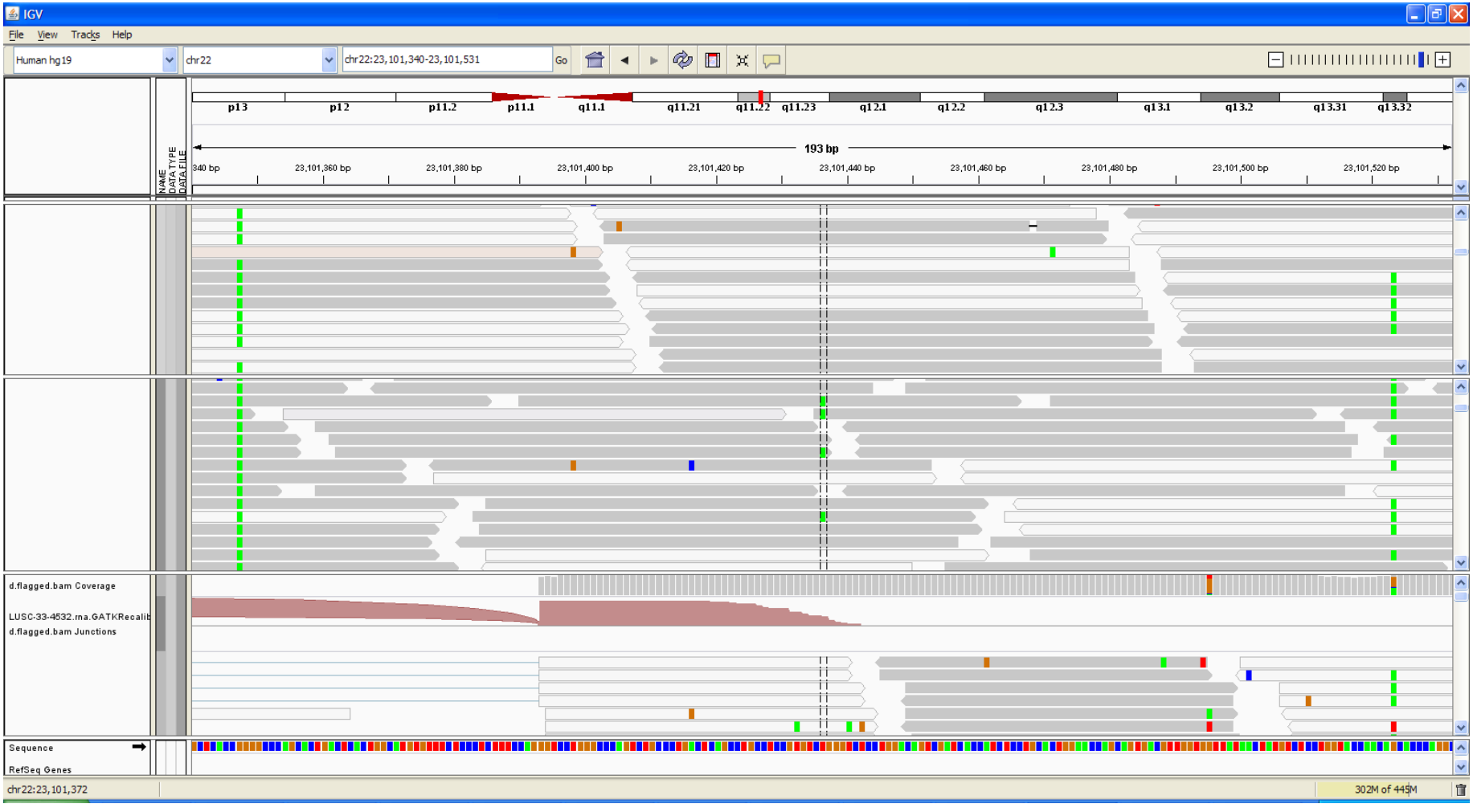




- Some center-specific calls are questionable upon “manual review” (examples follow)
- Many, however, are convincing

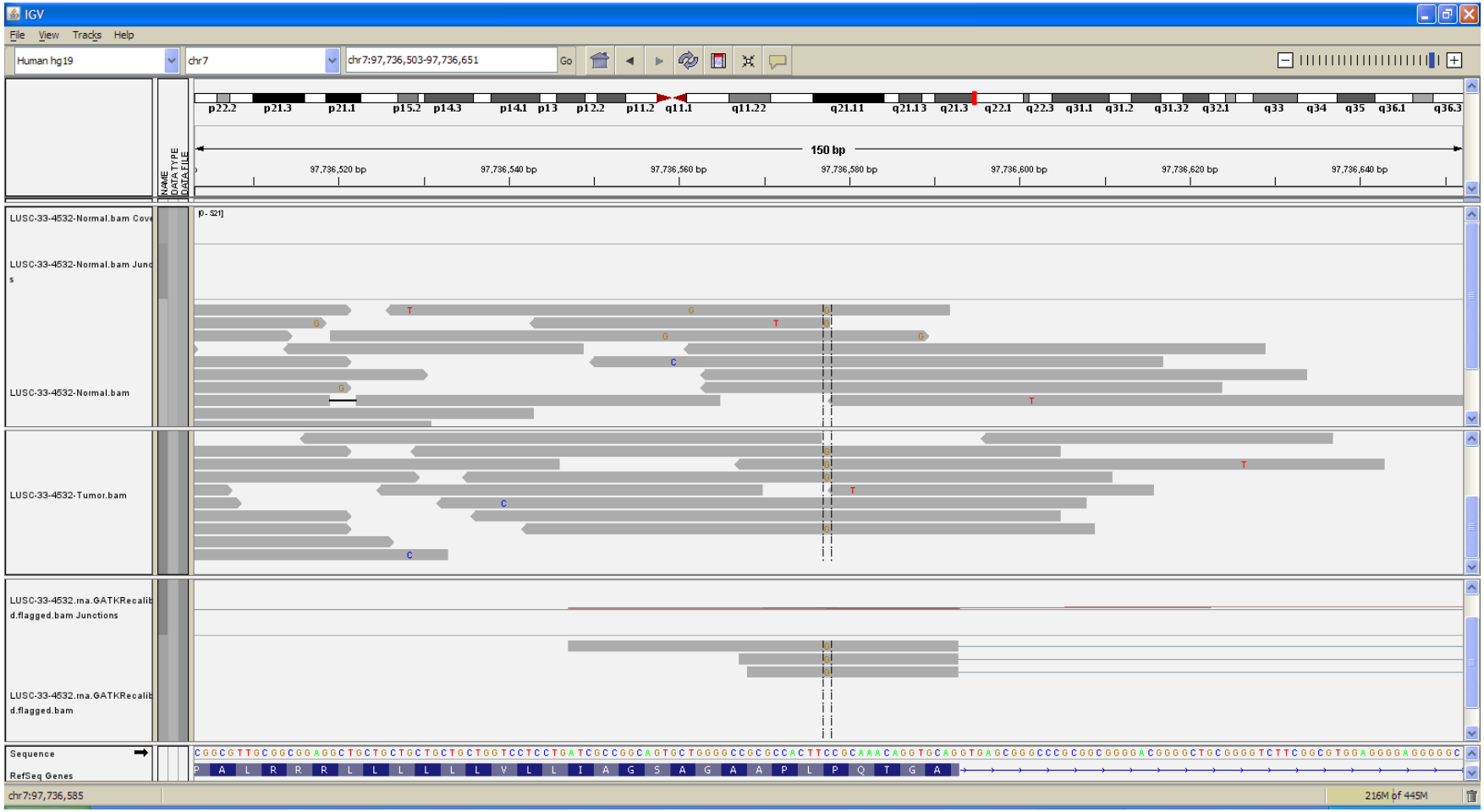
Center-specific call, questionable

- Broad-only, single event at coverage ~1000
  - Questionable alignments in the region; no support in RNA-Seq (all RNA-Seq reads are 0 mapping quality)



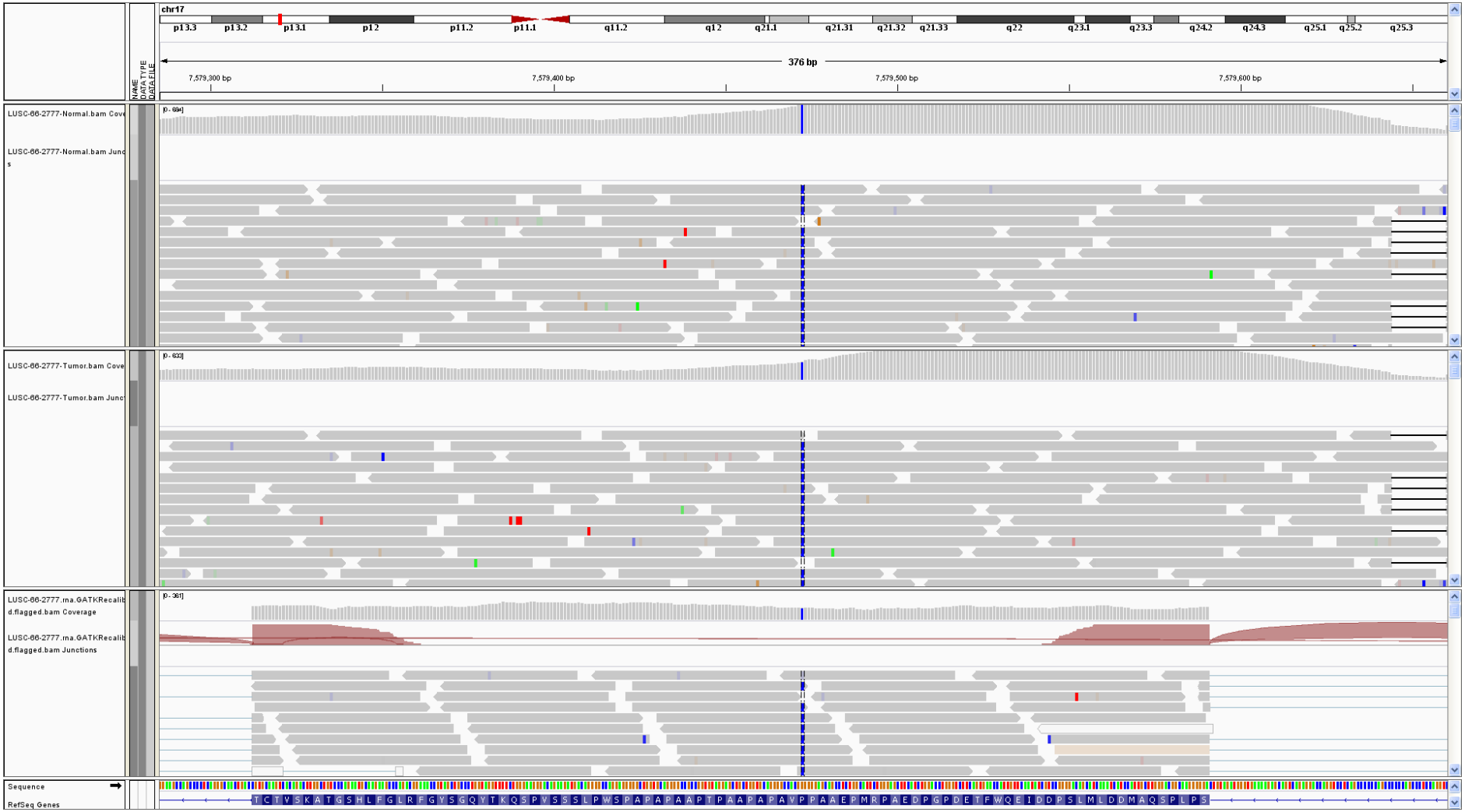
Center-Specific Call, questionable

- WUSTL at coverage 5, allelic fraction 0.67
  - Likely, a germline event



Center-Specific Call, questionable

- BCM in TCGA-66-2777
  - Clearly a germline event

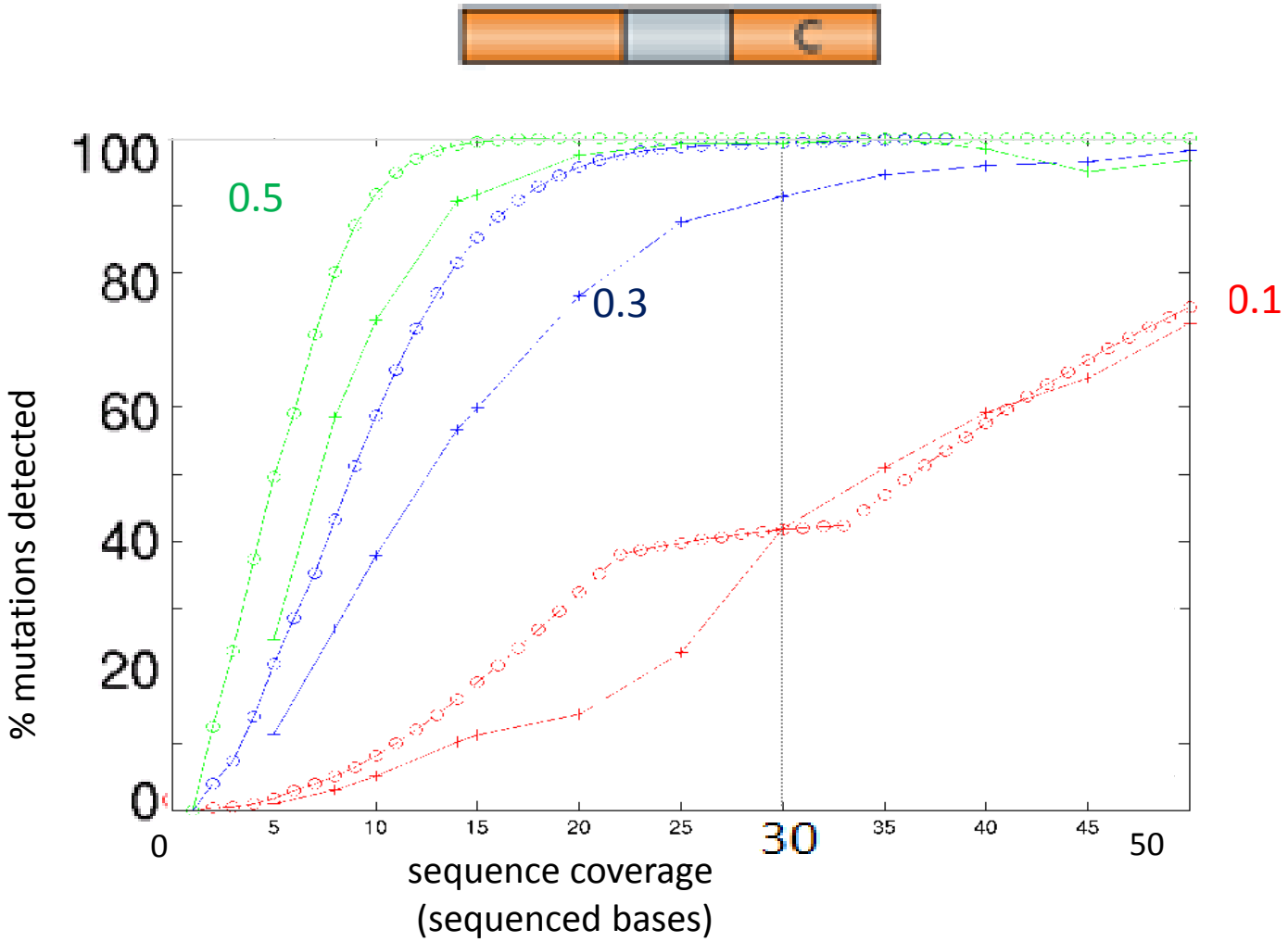


WE NEED A LOT OF VALIDATION DATA TO COMPARE THE TOOLS

Using RNA-Seq as Validation Set

- Independent library construction
- Different protocol
- Same sequencing technology
- It is possible to call mutations (de-novo) from aligned RNA-Seq data
  - Likely a too conservative approach
- Assume that de-novo DNA-Seq mutation calling is sufficiently conservative
  - Weaker evidence from RNA-Seq (than what would be required for a stand-alone de-novo call) can be considered as validation

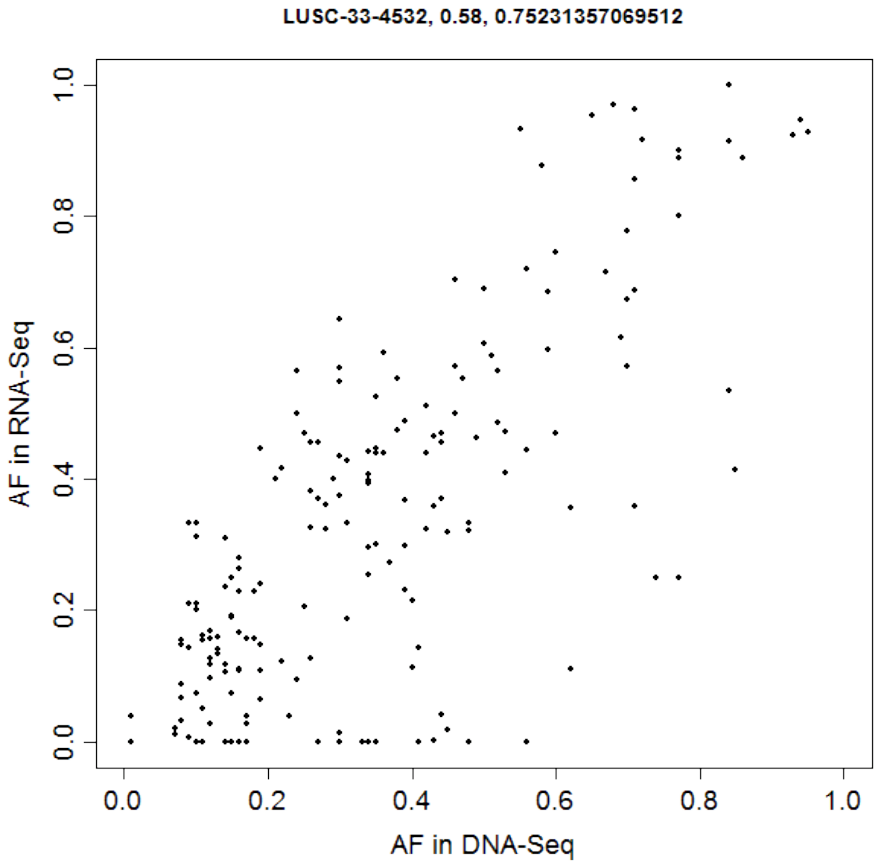
Sensitivity -- depends on coverage and allelic-fraction



Kristian Cibulskis

Is Allelic Fraction an Issue?

- Original calls have a range of allelic fractions
- Is it safe to ask for fixed (low) number of observations in RNA-Seq
  - In general, NO
  - However: AF in RNA-Seq and DNA-Seq strongly correlate



Looking for SNV in RNA-Seq

- Consider every called mutation site with coverage in RNA-Seq above N as “covered”
- If covered site has at least two reads with alt. allele in RNA-Seq, consider it “validated”

center	n.calls	covered	validated	validated.pct.covered	
BI	405	186	152	81.7	RNA-Seq T cov. >=5

center	n.calls	covered	validated	validated.pct.covered	
BI	405	150	131	87.3	RNA-Seq T cov. >=10

center	n.calls	covered	validated	validated.pct.covered	
BI	405	109	102	93.6	RNA-Seq T cov. >=20

Conclusions

- A framework is established within TCGA for evaluating and improving mutation calling algorithms
- We are working on validating mutations:
  - Using additional experiments in the sequencing centers (but this may be only partial validation)
  - based on RNA-seq after correcting for the power to detect the mutation

Acknowledgments

Broad
Gad Getz
Kristian Cibulskis
Rui Jing
Alex Ramos
Carrie Sougnez
Peter Hammerman
Scott Carter

WUSTL
Li Ding
Mike McLellan
Ken Chen
Xian Fan

UCSC
David Haussler
Chris Wilks
Singer Ma
Zack Sanborn
Rachel Harte
Daniel Zerbino
Jing Zhu

Baylor
David Wheeler
Jennifer Drummond
Kyle Chang

UNC
Neil Hayes
Matthew Wilkerson

TCGA research Network