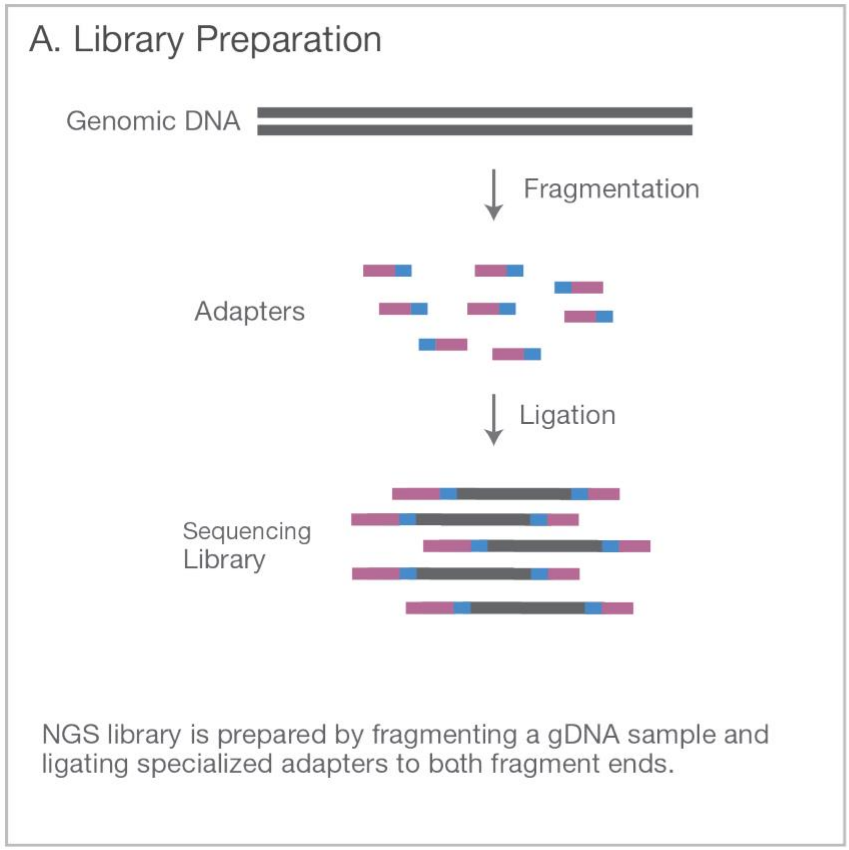
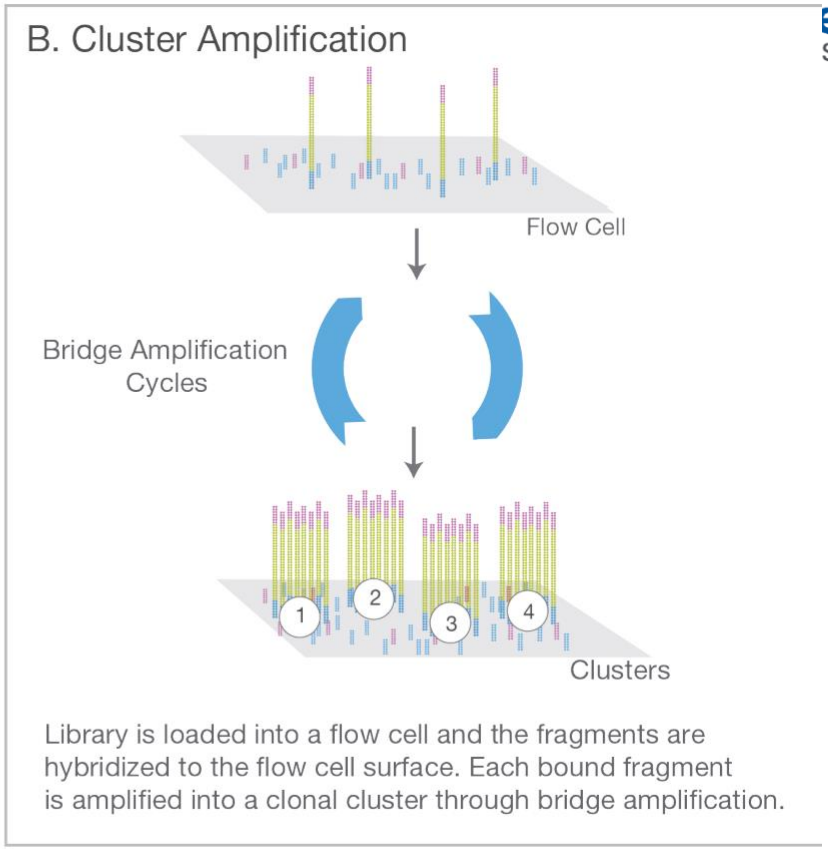
NGS (next generation sequencing) generates short reads, and usually the short reads need to be mapped (aligned) to a reference sequence. For genome sequencing, this is a reference genome.

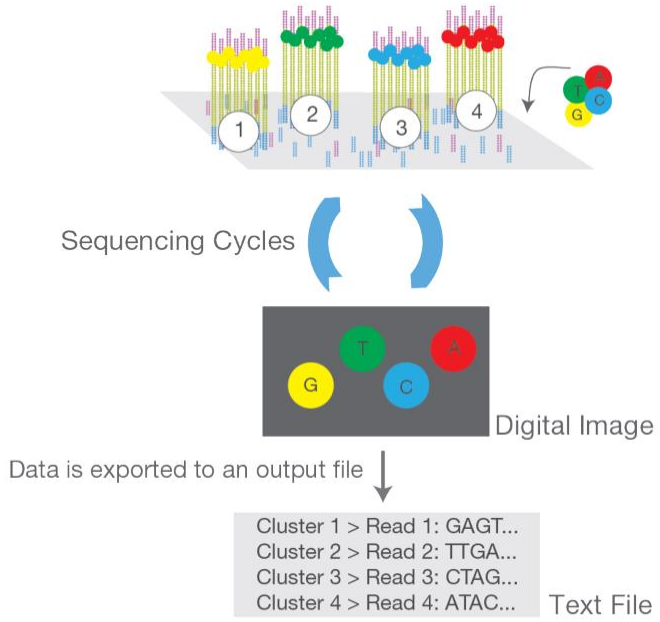
Why does the technology generate short reads?





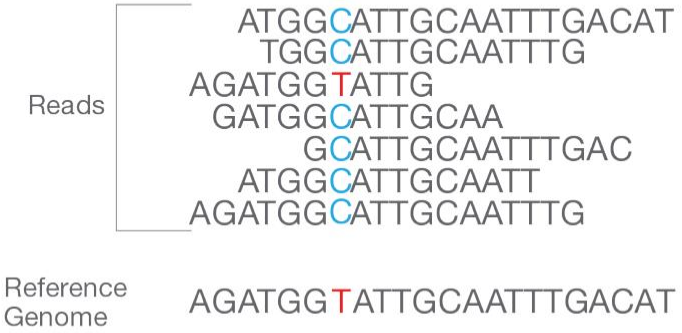
https://sapac.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

C. Sequencing



Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

D. Alignment and Data Anaylsis



Reads are aligned to a reference sequence with bioinformatics software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.

https://sapac.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

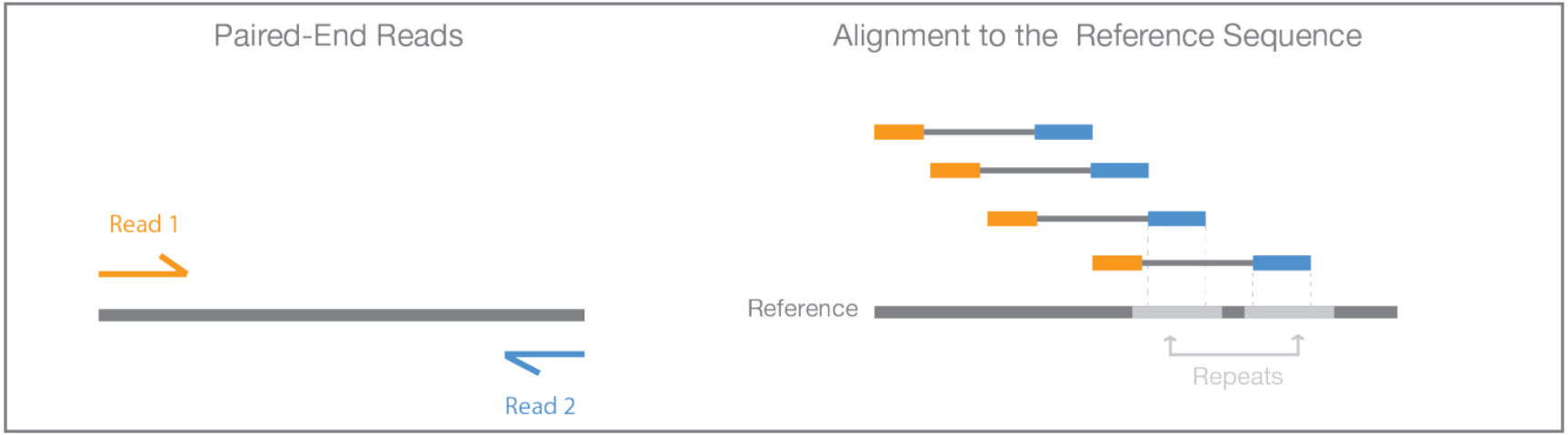


Figure 4: Paired-End Sequencing and Alignment—Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map the reads over repetitive regions more precisely. This results in better alignment of reads, especially across difficult-to-sequence, repetitive regions of the genome.

https://sapac.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

Gritty details for reference, do not need to know: https://seekdeep.brown.edu/illumina_paired_info.html

Flow cell

5 minutes of much more detail from Illumina

- https://youtu.be/fCd6B5HRaZ8
- What you need to know
  - DNA (or cDNA) is fragmented
  - You can optionally select fragments (e.g. just from exons) by hybridization or PCR amplification)
  - Various adapters and index sequences are added on to the ends of the fragments
  - Illumina generates reads from both ends (“paired reads”) or just one end
  - Sometimes, if the fragments are short, paired reads overlap, or reads capture adapter or index sequence at the far end of the fragments
  - Reading is done by incorporating fluorescently labelled nucleotides
  - For most applications you have to map (align) the short reads to a reference; this could be a genome or a transcriptome (= the set of all transcripts in an organism)

Short read mappers

only a few in wide use

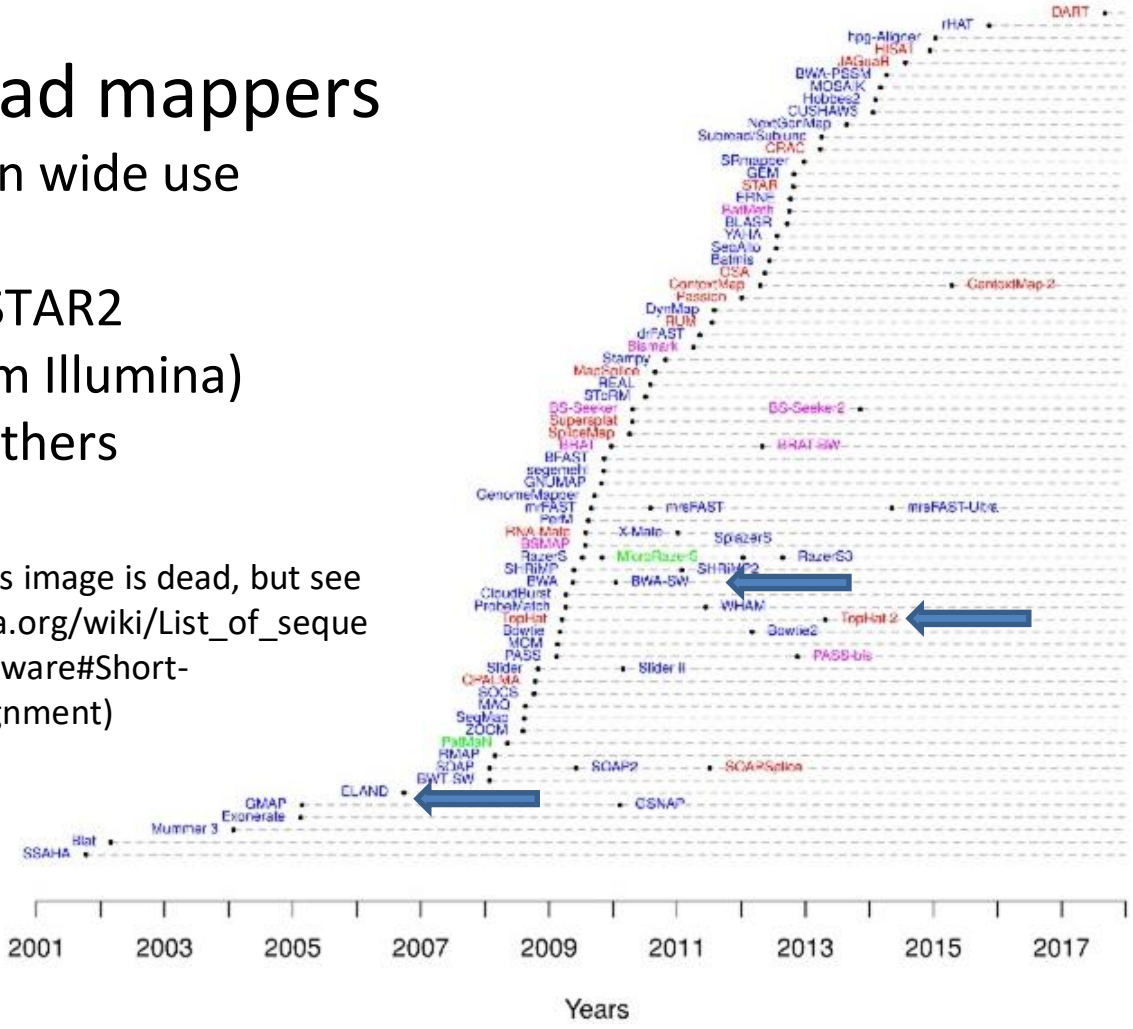
BWA-MEM

TOPHAT2, STAR2

ELAND (from Illumina)

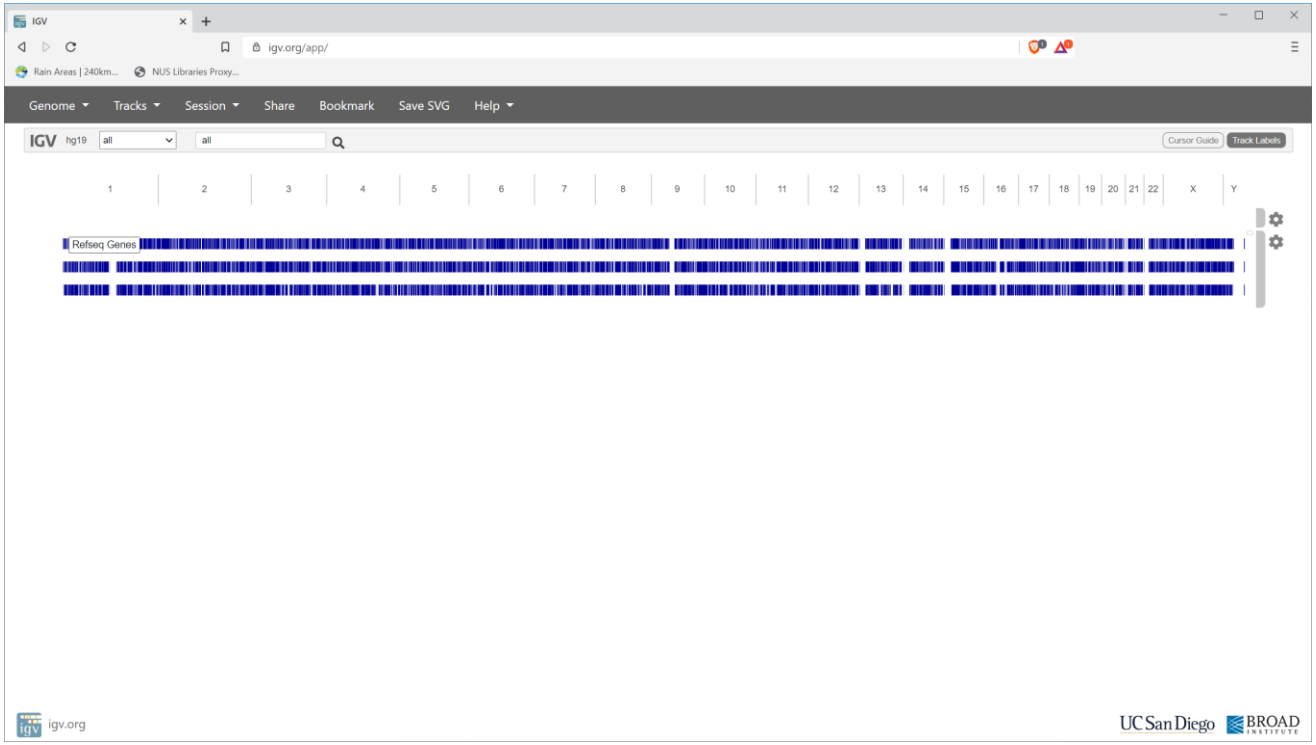
and a few others

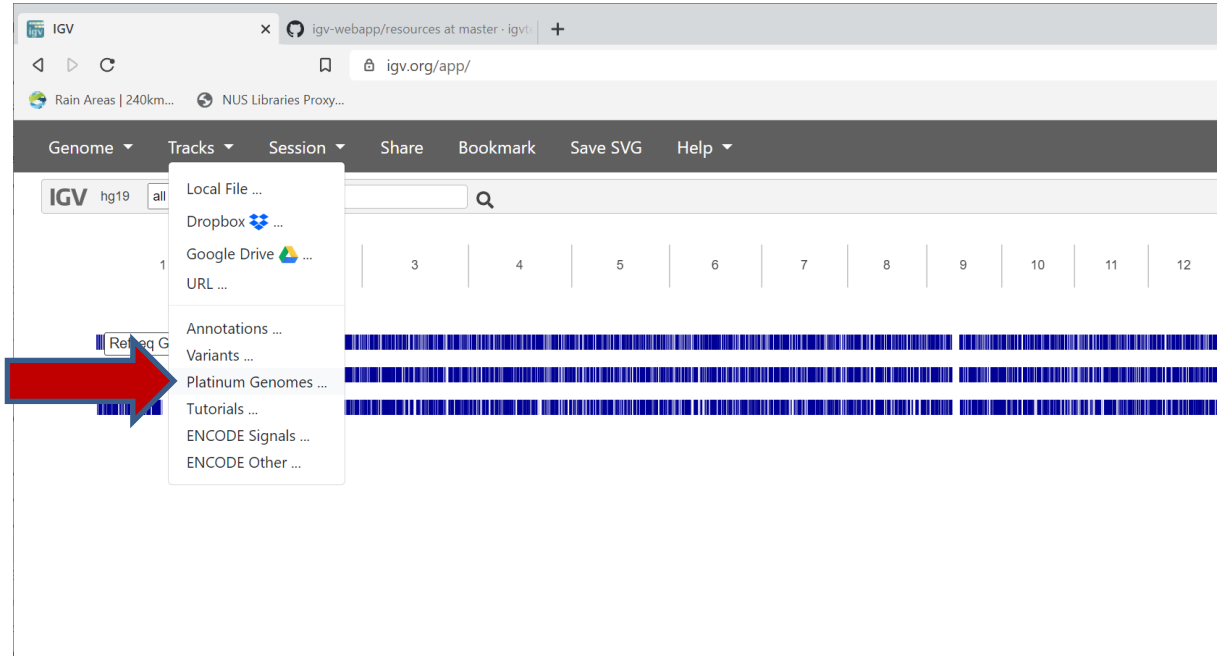
(Original URL for this image is dead, but see https://en.wikipedia.org/wiki/List_of_sequence_alignment_software#Short-read_sequence_alignment)



IGV (genome assembly browser)

- https://igv.org/app

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Variants ...
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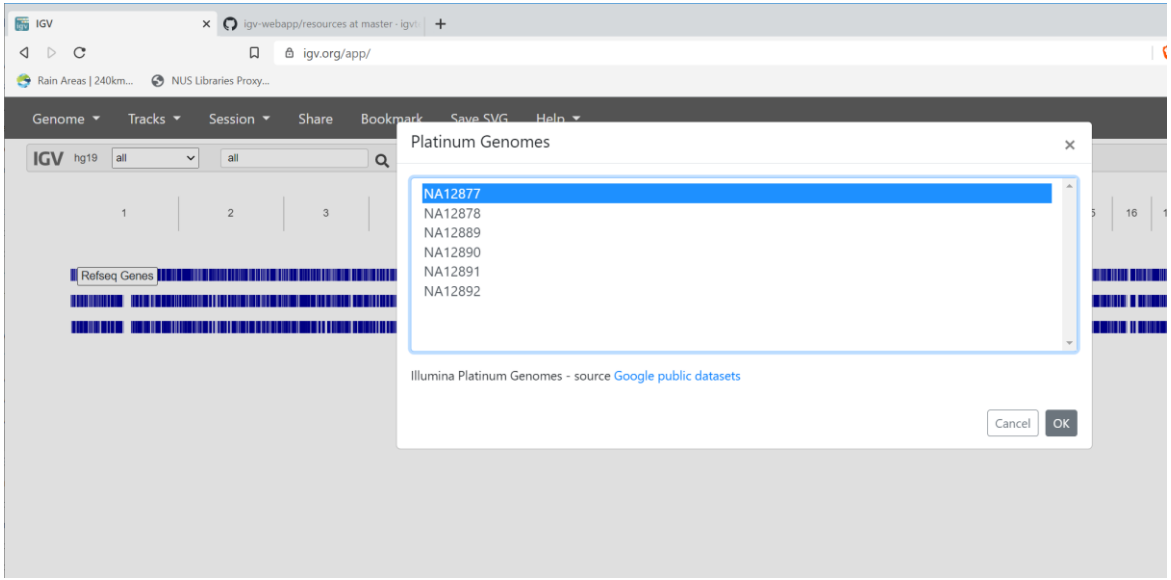
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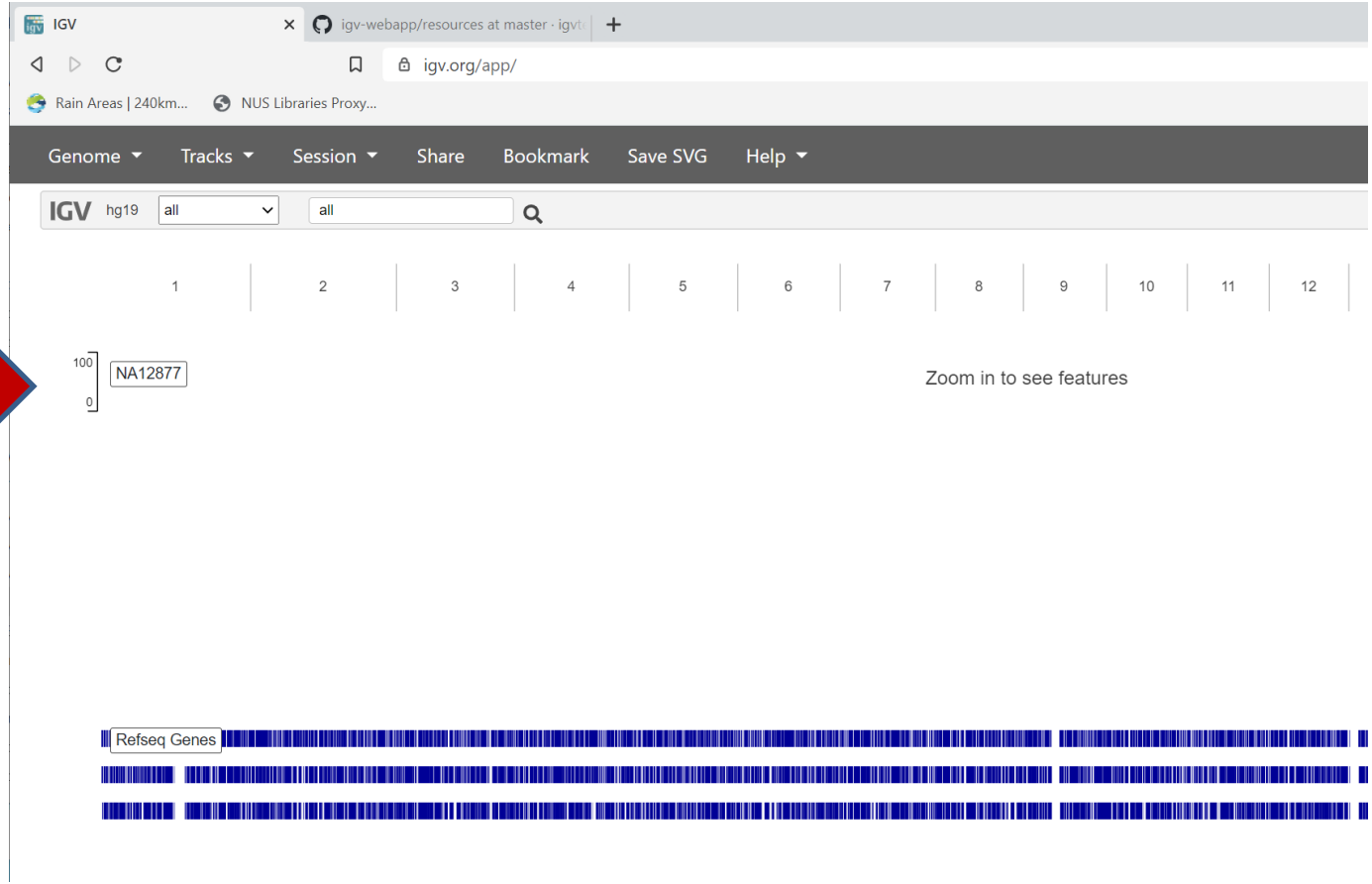
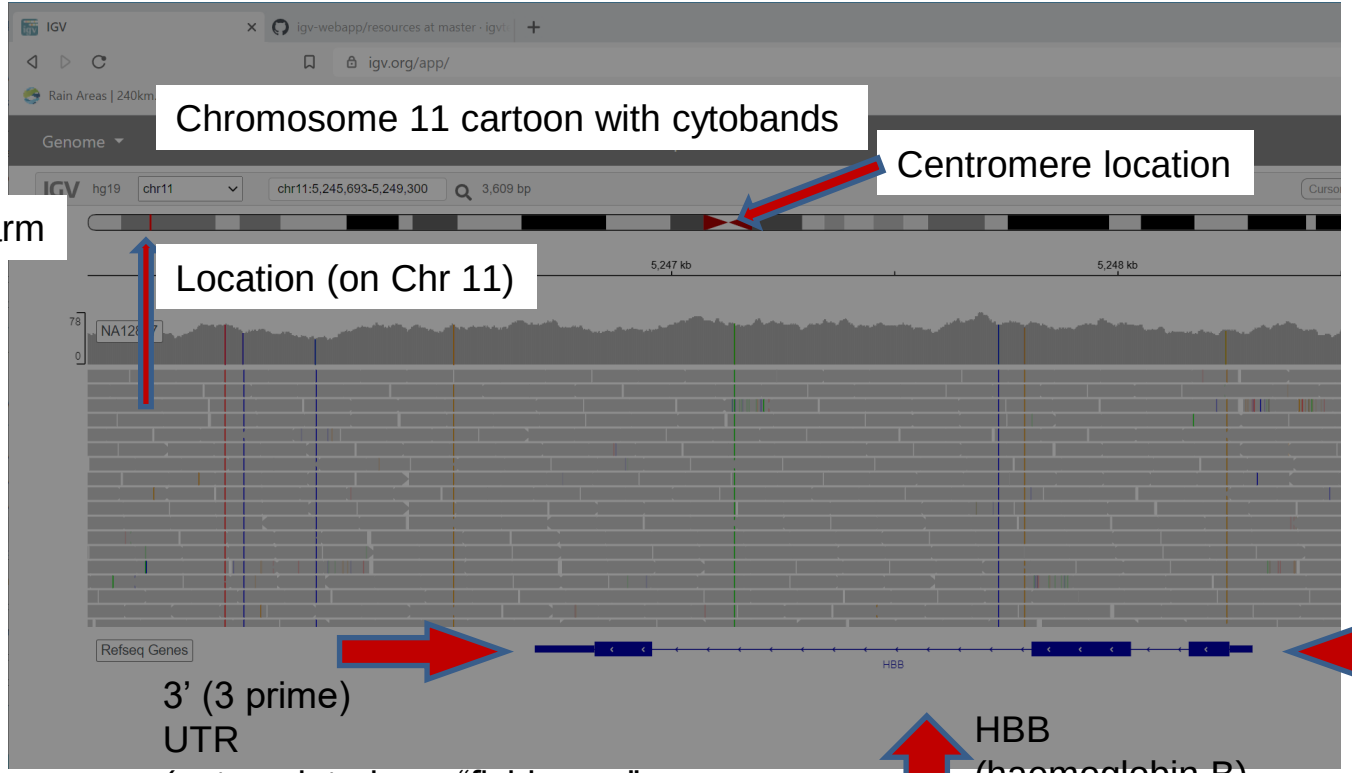




Chromosome 11 cartoon with cytobands

Centromere location

p arm

q arm

Location (on Chr 11)

Read depth plot

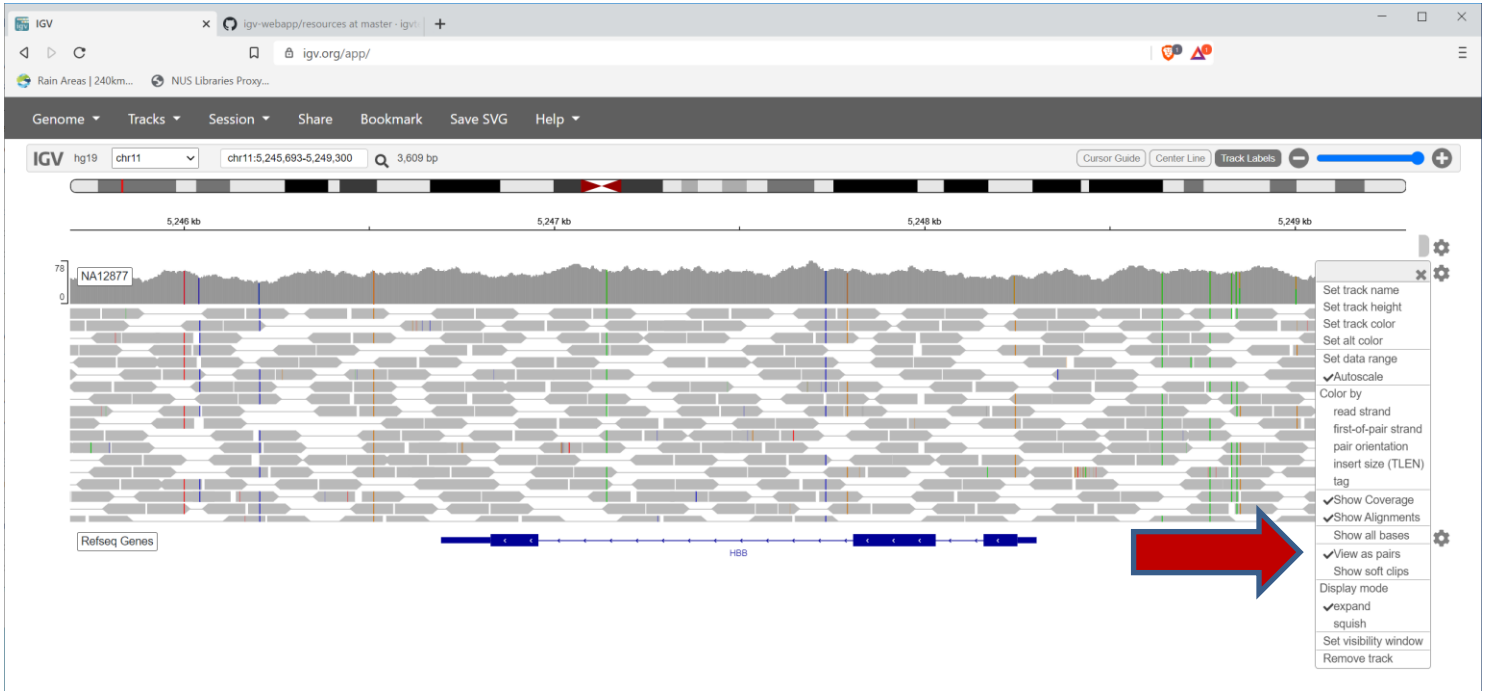
Reads

5’ (5 prime) UTR (untranslated region)

3’ (3 prime) UTR (untranslated region)

“fishbones” indicate direction of transcription

HBB (haemoglobin B) gene 3 exons



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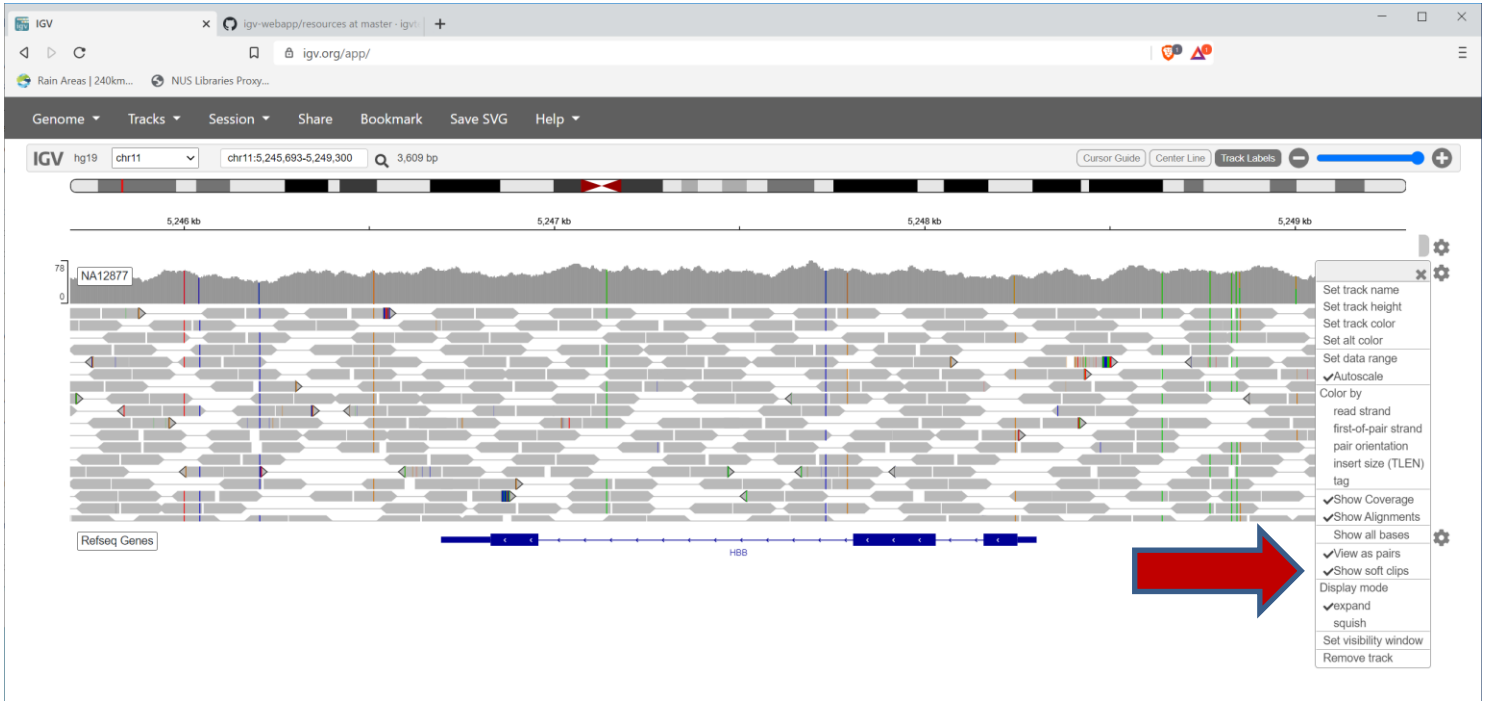
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Help
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hg19
chr11
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3,609 bp
Cursor Guide
Center Line
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HBB
Set track name
Set track height
Set track color
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Show soft clips
Display mode
expand
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Set visibility window
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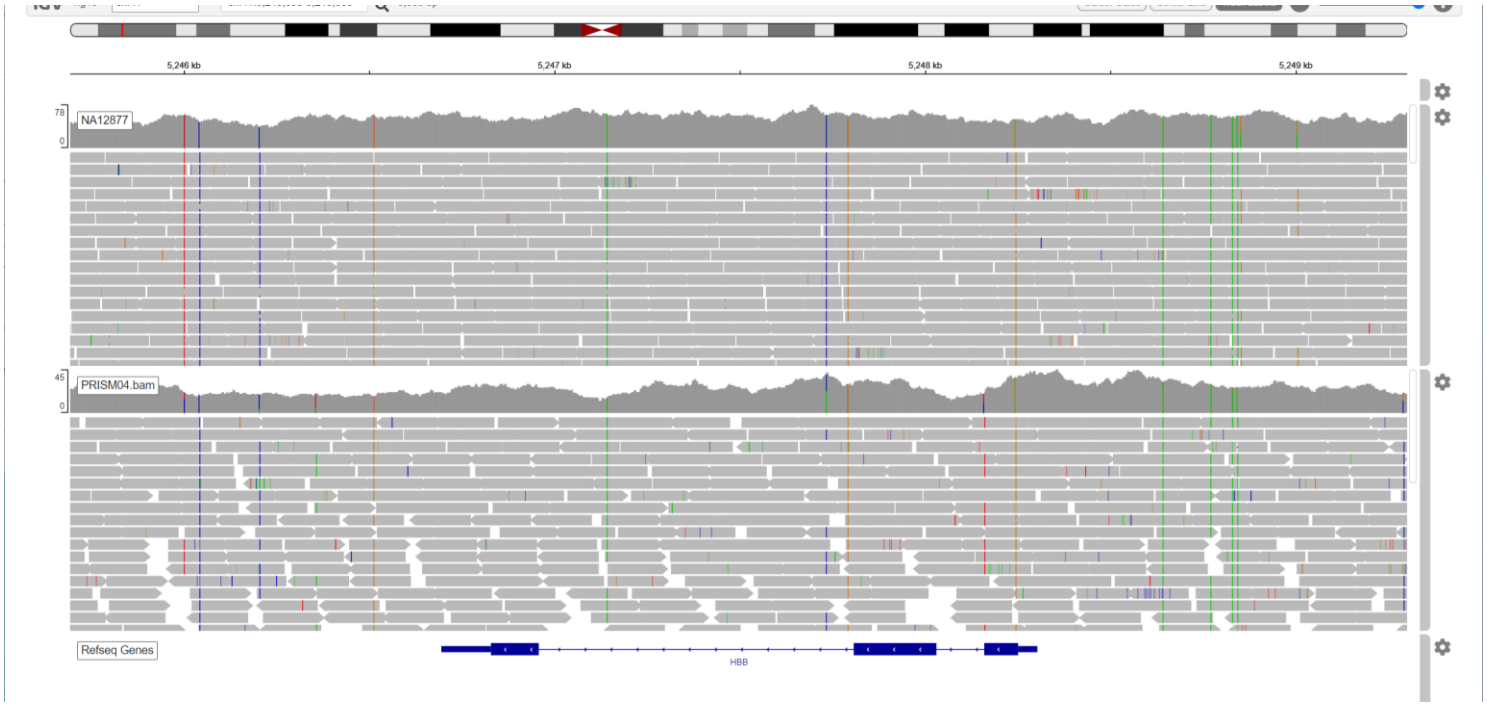
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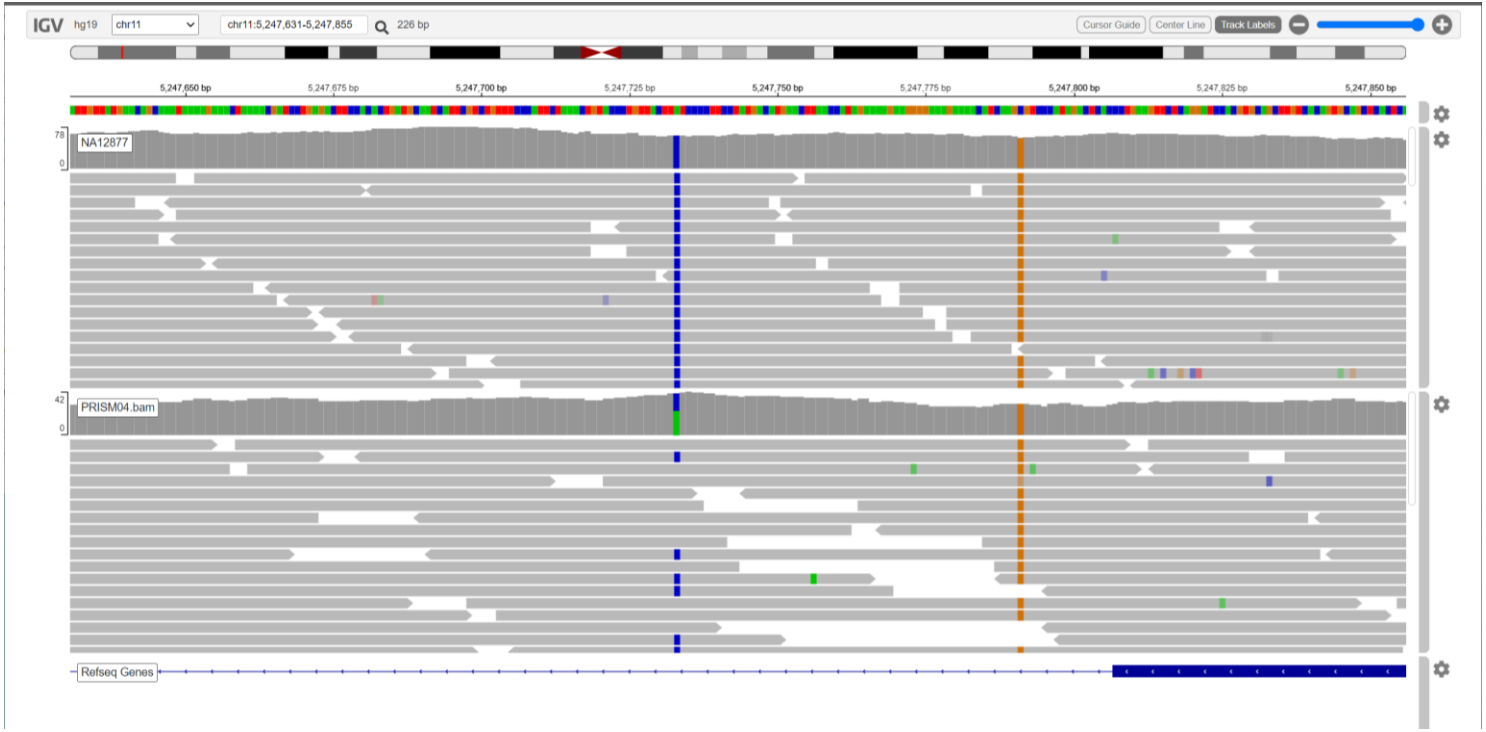
Refseq Genes
HBB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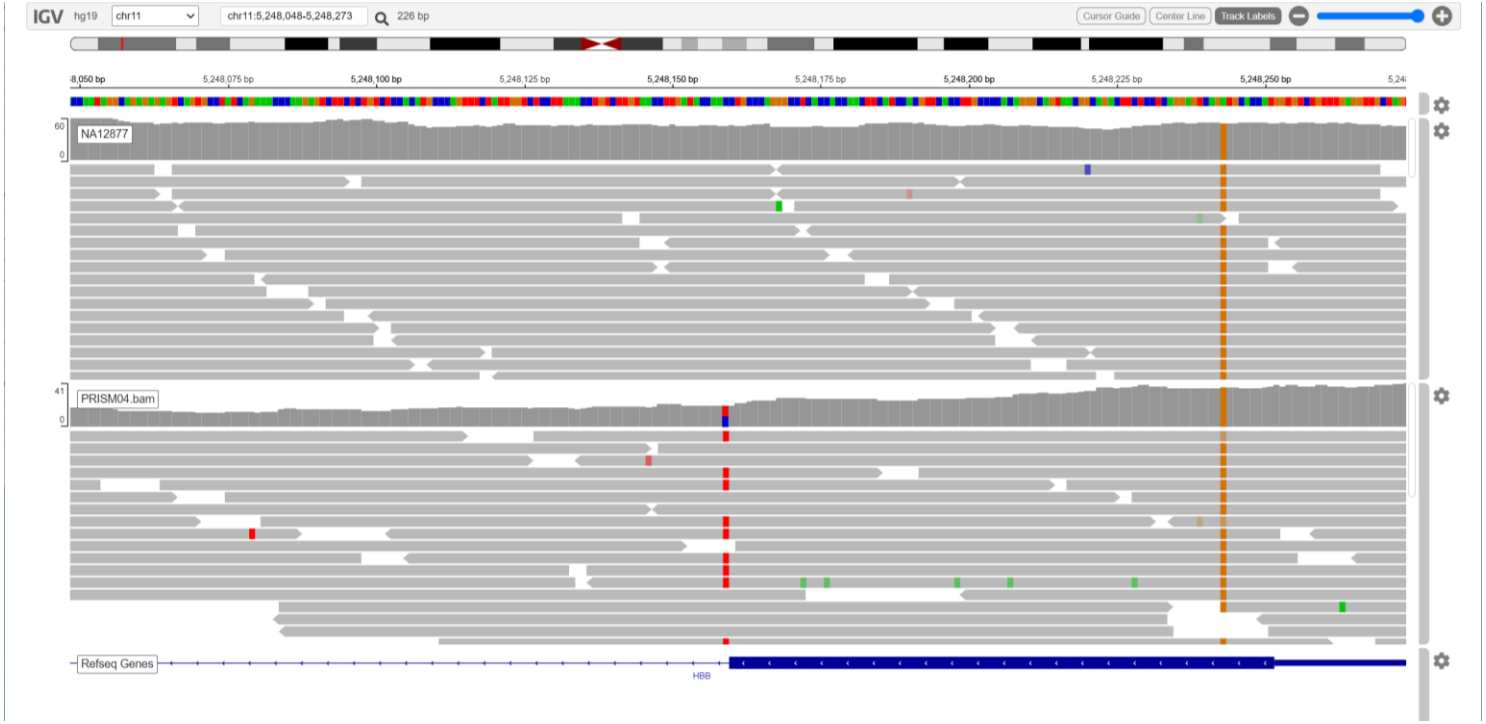
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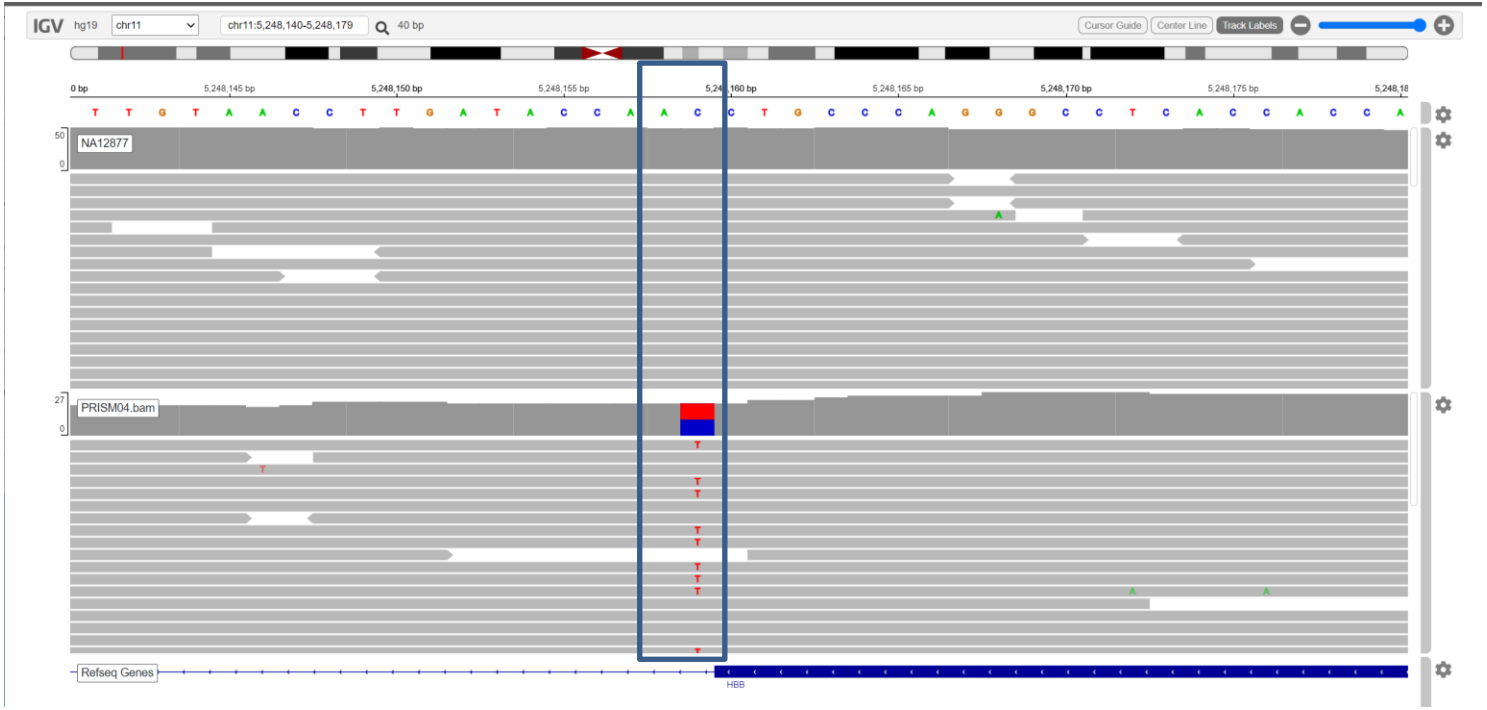
Refseq Genes
HBB

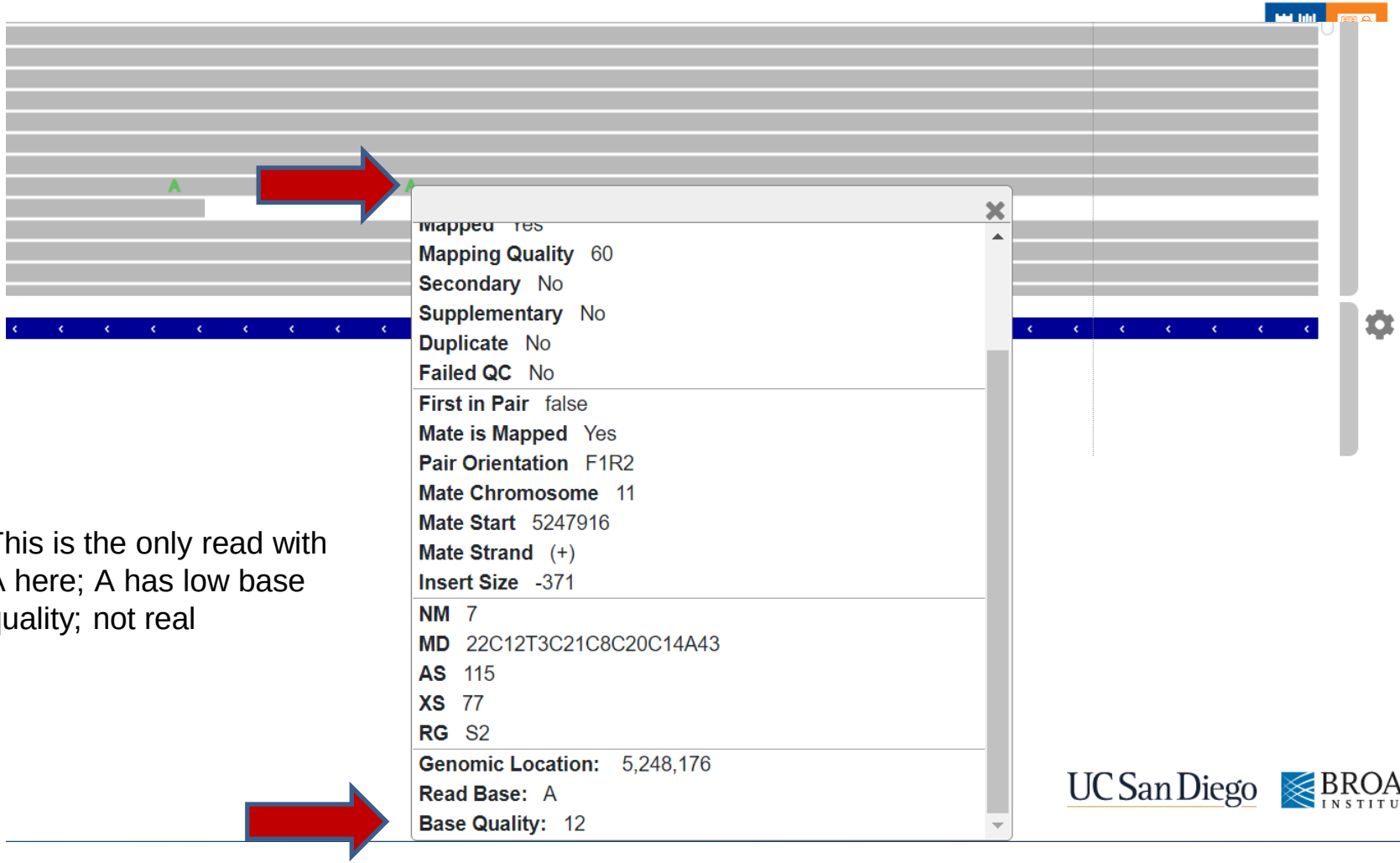
Heterozygous mutation at 3’ splice site of intron 2 of HBB (beta haemoglobin), “beta-thalassemia trait”



Sequence at splice sites very conserved: 5’ end of intron usually 5’-GT-3’, 3’ end is 5’-AG-3’; this variant takes

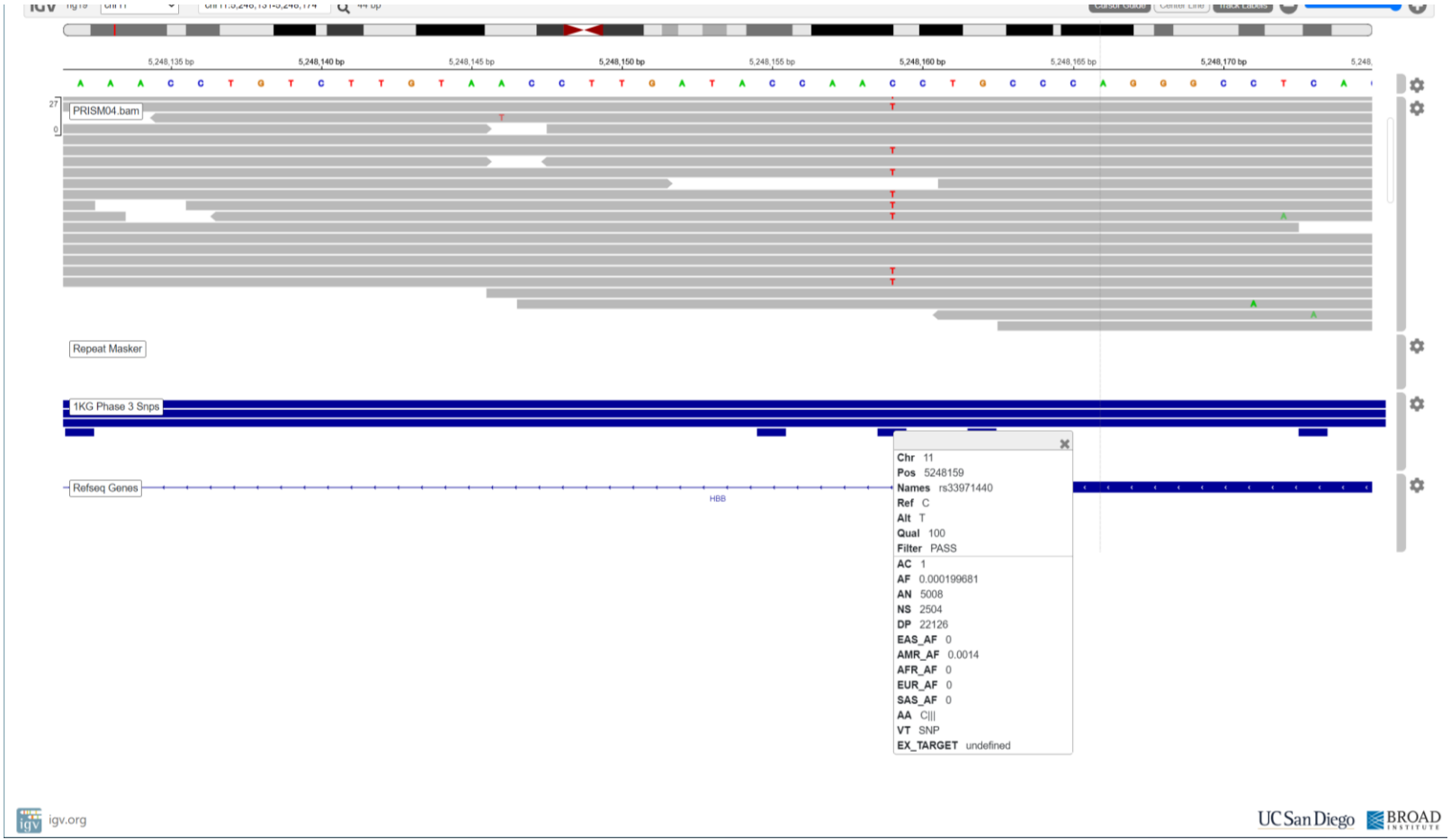
3′-TG-5′ → 3′-TA-5′
5′-AC-3′ → 5′-AT-3′

Info on one read and one base in that read
High base quality



This is the only read with A here; A has low base quality; not real

UC San Diego BROAD INSTITUTE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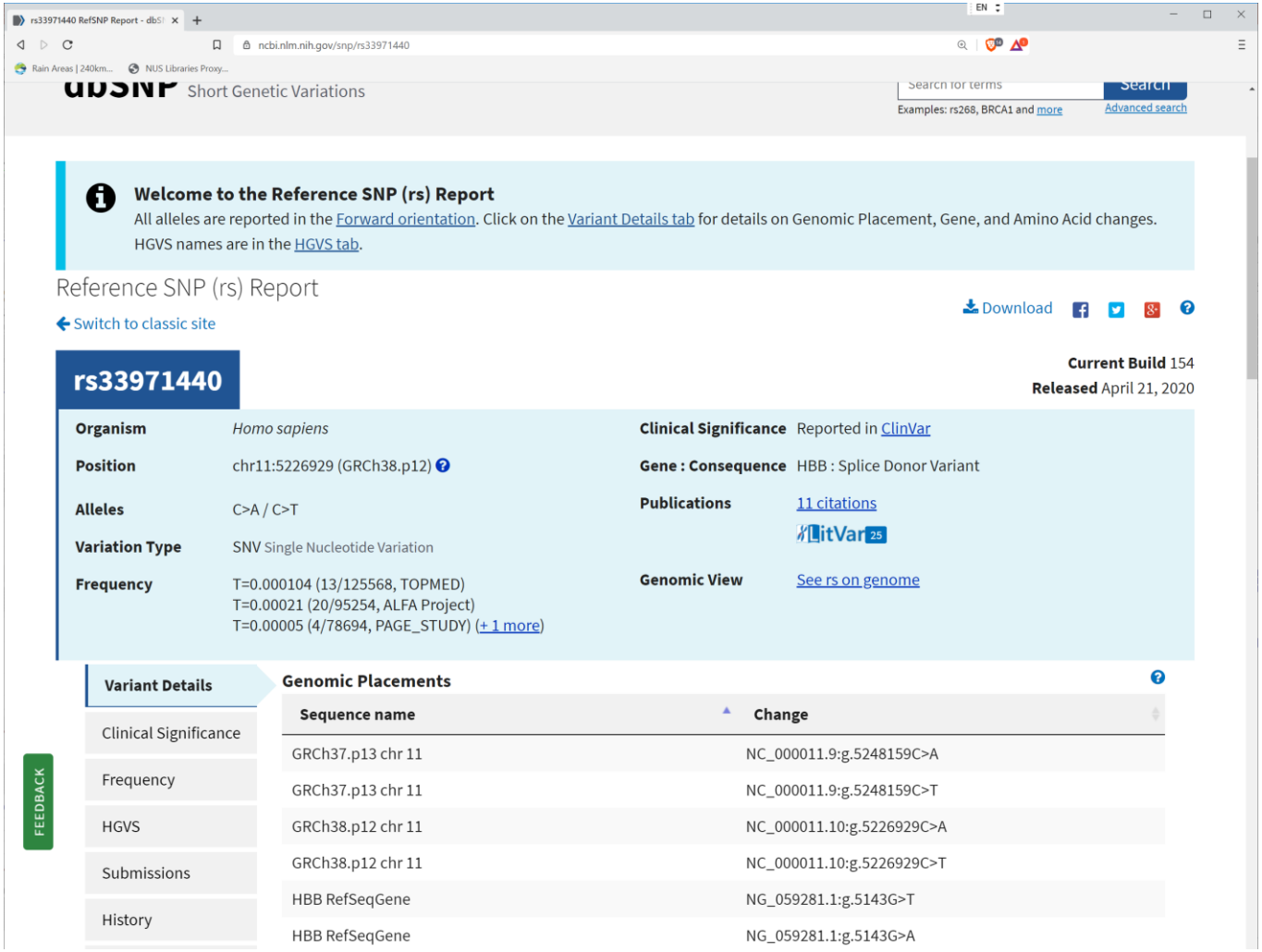
rs33971440 RefSNP Report - dbSNP
ncbi.nlm.nih.gov/snp/rs33971440
dbSNP Short Genetic Variations
Search for terms
Search
Examples: rs268, BRCA1 and more
Advanced search
Welcome to the Reference SNP (rs) Report
All alleles are reported in the Forward orientation. Click on the Variant Details tab for details on Genomic Placement, Gene, and Amino Acid changes.
HGVS names are in the HGVS tab.
Reference SNP (rs) Report
Download
Switch to classic site
rs33971440
Current Build 154
Released April 21, 2020
Organism Homo sapiens
Position chr11:5226929 (GRCh38.p12)
Alleles C>A / C>T
Variation Type SNV Single Nucleotide Variation
Frequency T=0.000104 (13/125568, TOPMED)
T=0.00021 (20/95254, ALFA Project)
T=0.00005 (4/78694, PAGE_STUDY) (+ 1 more)
Clinical Significance Reported in ClinVar
Gene : Consequence HBB : Splice Donor Variant
Publications 11 citations
LitVar 25
Genomic View See rs on genome
Variant Details
Clinical Significance
Frequency
HGVS
Submissions
History
Genomic Placements
Sequence name
Change
GRCh37.p13 chr 11 NC_000011.9:g.5248159C>A
GRCh37.p13 chr 11 NC_000011.9:g.5248159C>T
GRCh38.p12 chr 11 NC_000011.10:g.5226929C>A
GRCh38.p12 chr 11 NC_000011.10:g.5226929C>T
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HBB RefSeqGene NG_059281.1:g.5143G>A
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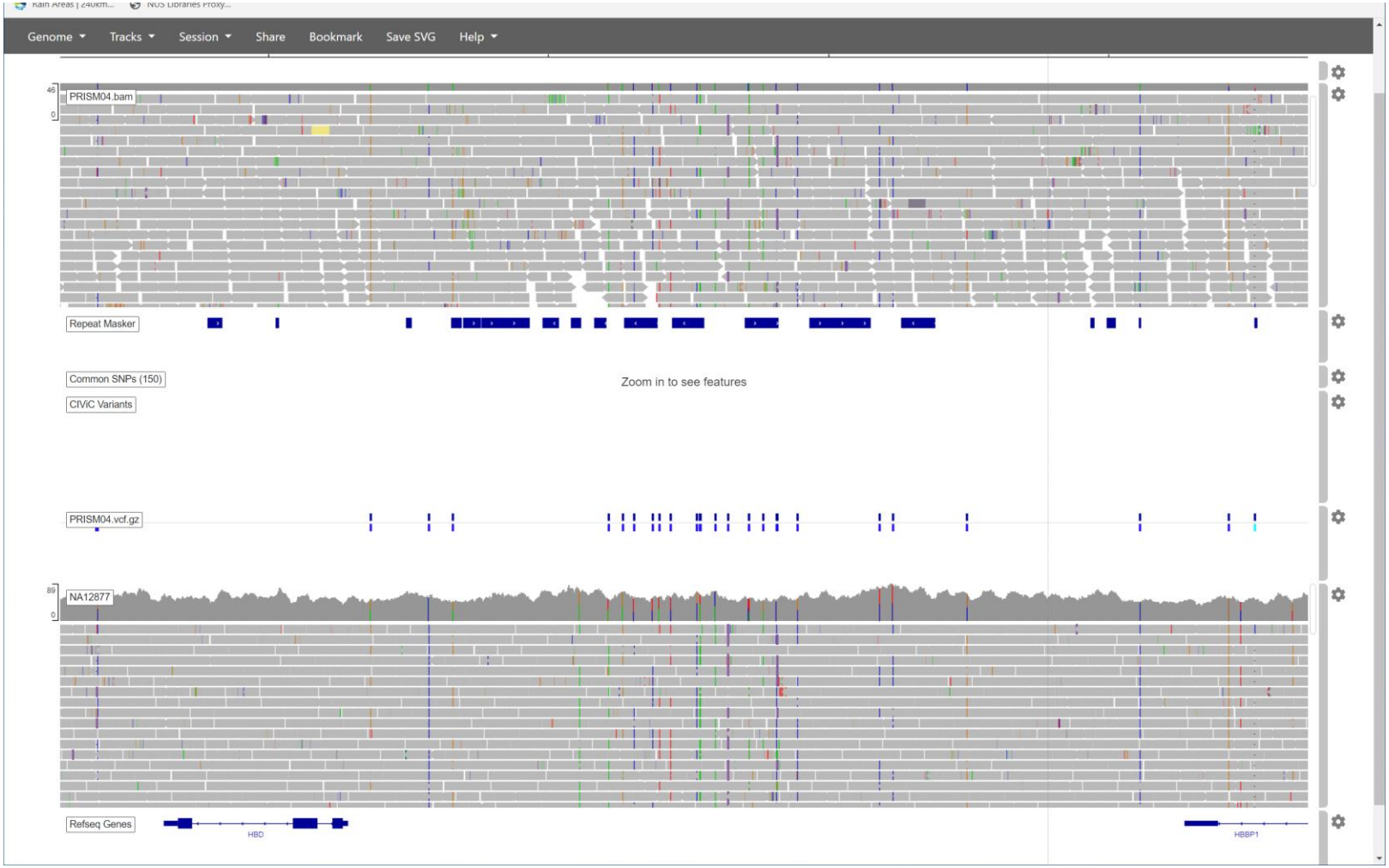

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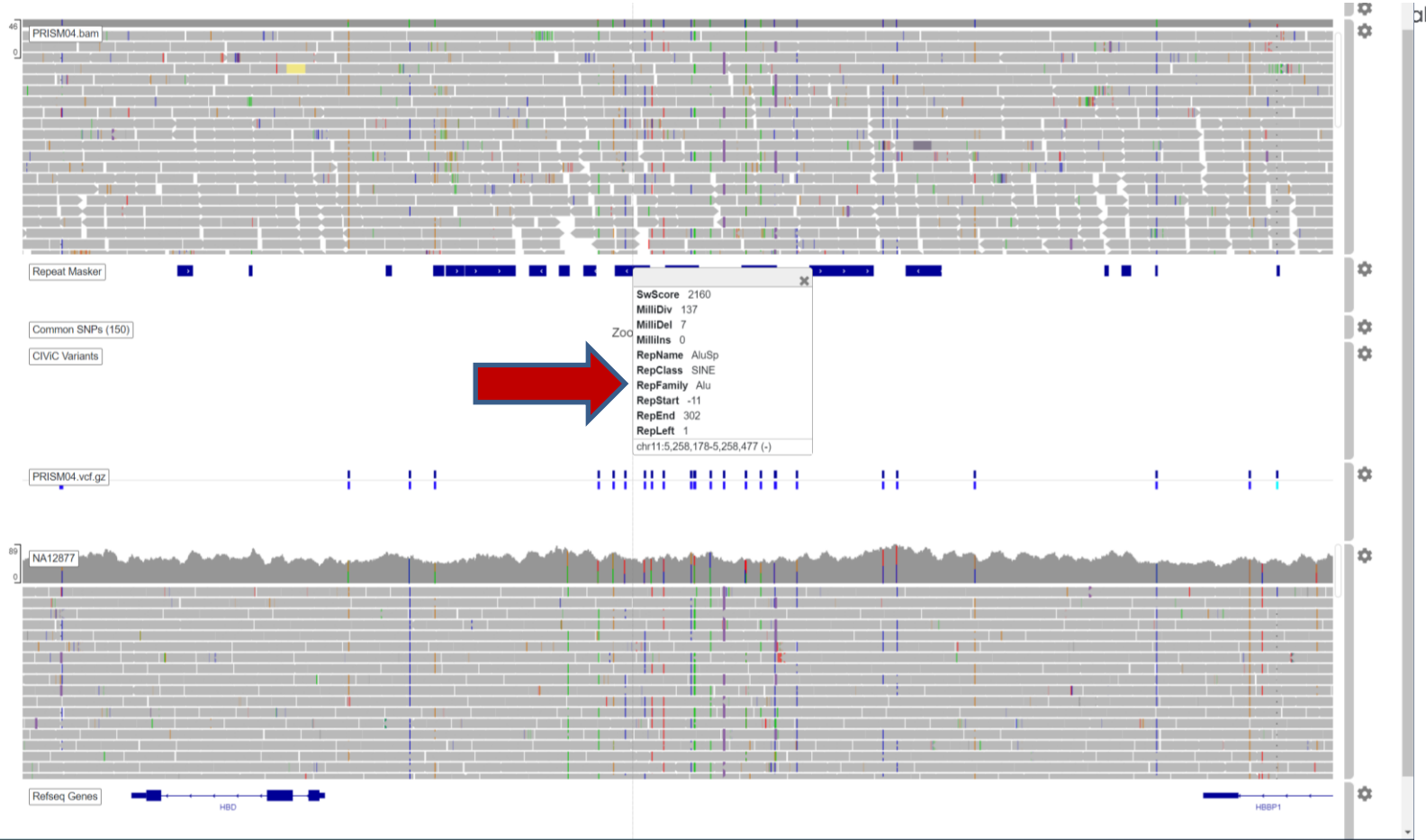
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RepFamily Alu
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RepEnd 302
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CIViC Variants
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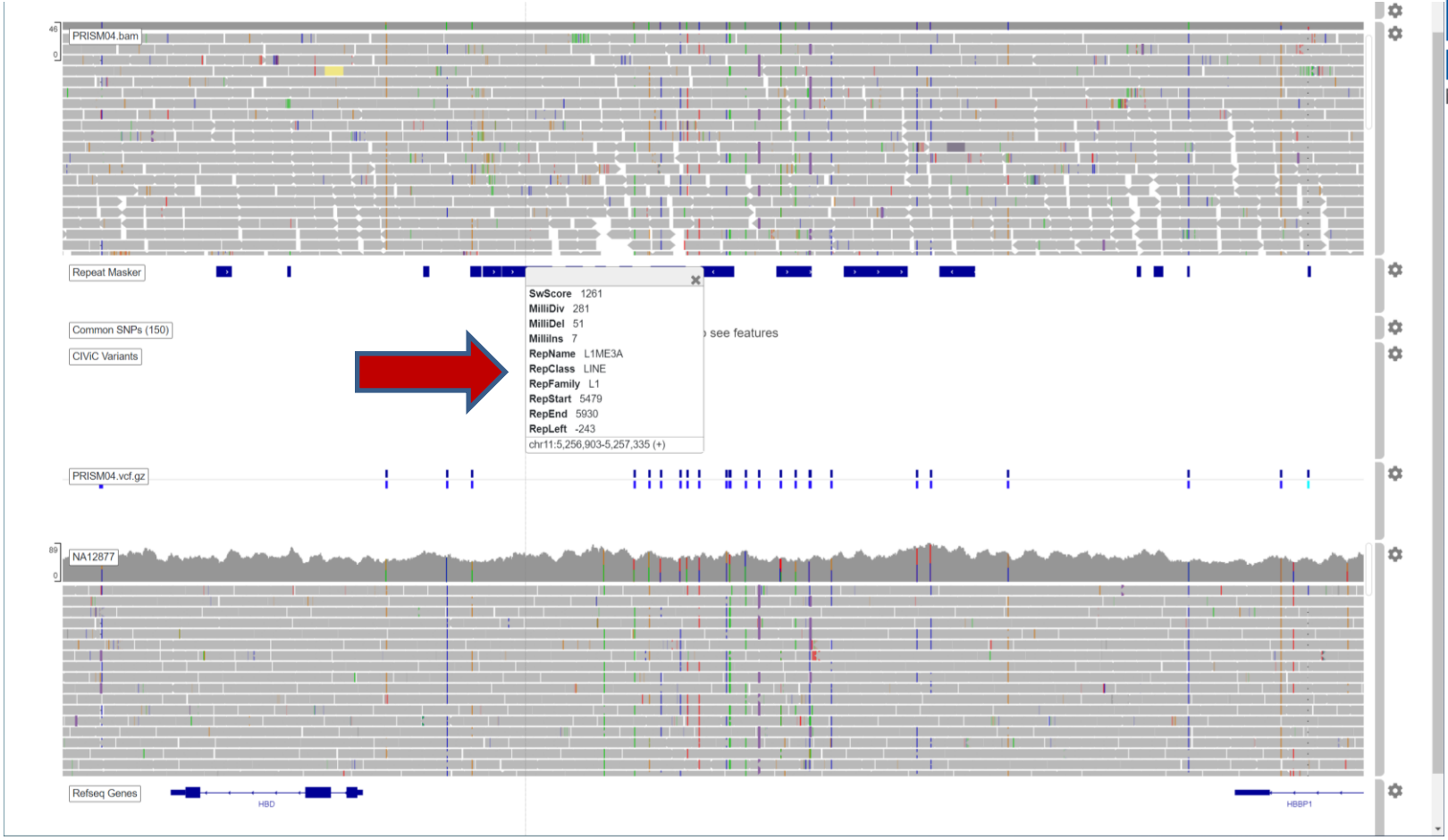
HBBP1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see features
Common SNPs (150)
CIViC Variants
PRISM04.vcf.gz
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Refseq Genes
HBD
HBBP1