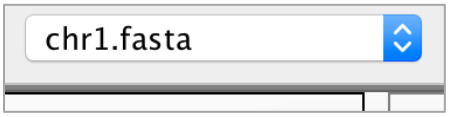
IGV Hands-on Exercise	Viewing SNPs

1. Reference Genome

First make sure the reference genome is set to the one you loaded in the previous exercise:

Select ***chr1.fasta*** from the genome dropdown menu



2. Load data

Click *File > Load from File*

Navigate to the folder that contains the workshop igvData folder, and then the **snps** subfolder. Open the following files

igvData / snps / ***snp_calls.bed***

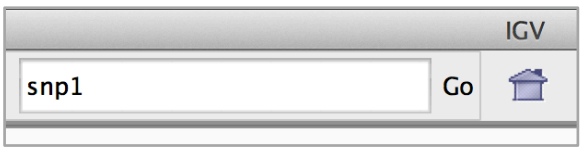
igvData / snps / ***NA12878.SLX.sample.bam***

Note: Do **not** load the .bai file

IGV automatically finds the index file – as long as it is named correctly and is in the same folder as the .bam file

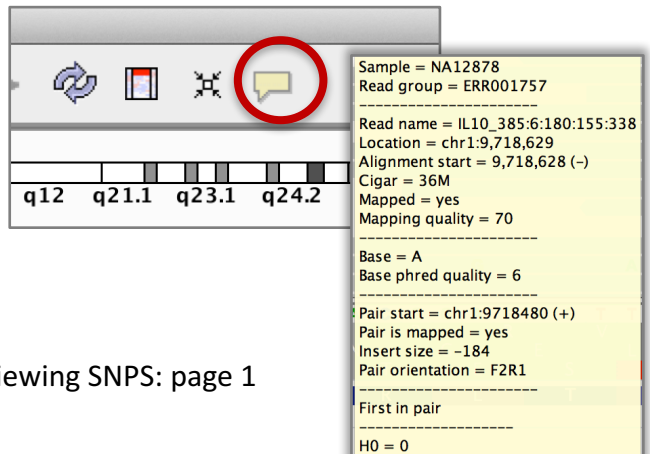
3. Navigate to first putative SNP locus

Type ***snp1*** in the search box and click **Go**

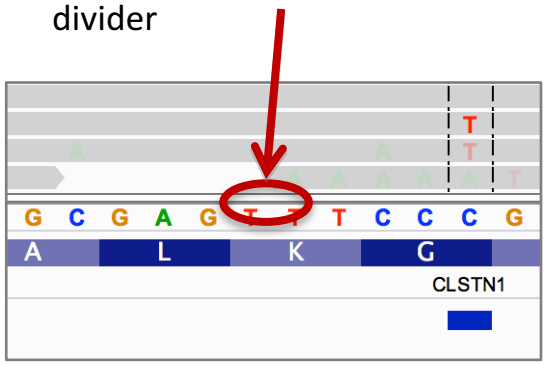


4. Optional

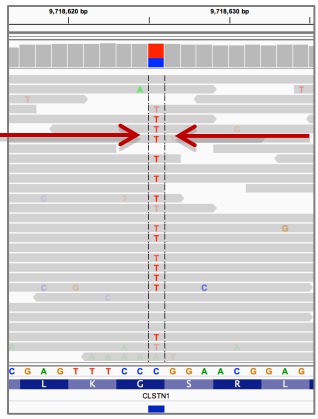
a) Modify the info popup behavior if you don't want the yellow info window visible all the time. Click on the yellow balloon icon, and select ***Show details on click***



b) For a larger data panel, click & drag the window divider

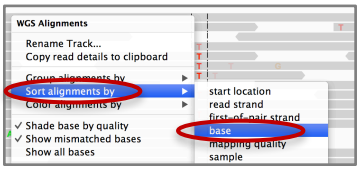


5. Sort the mismatched aligned reads by base

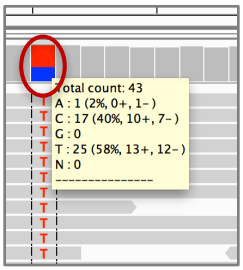


First, click and drag to position the mismatched bases between the center guidelines

Right-click (on Mac: control-click) anywhere in the aligned reads, and select *Sort alignments by > base*



6. See the allele counts and frequencies



Mouse over the red/blue bar in the coverage track (Or *click* on the bar, if you changed the information popup behavior to display on click only)

Observe the distribution of mismatches at that locus.
Observe the lack other mismatches in the region.

→This appears to be a heterozygous SNP.

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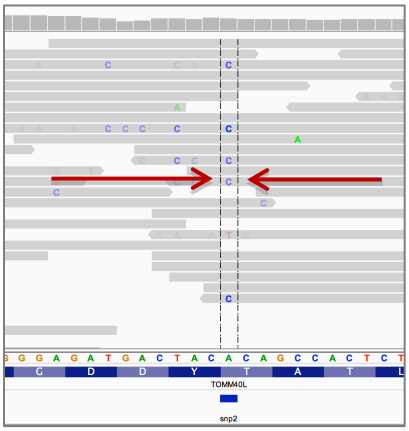
7. Go to the locus of the <u>second</u> putative SNP

Type ***snp2*** in the search box and click *Go*

Observe the mismatched bases and their apparent low qualities. (Mismatched bases are drawn in a fainter color if the base call is of low quality)

8. Disable shading by quality

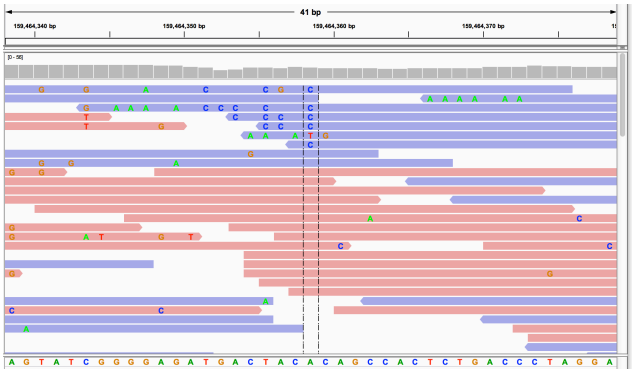


Click & drag to position the ***snp2*** locus (with the 5 blue Cs) between the vertical center guidelines.

Right-click (on Mac: control-click) anywhere in the aligned reads, and click ***Shade base by quality***

Observe the mismatched bases.

9. Sort and color the aligned reads by read strand



Right-click (on Mac: control-click) anywhere in the aligned reads, and select ***Sort alignments by > read strand***

Right-click (on Mac: control-click) anywhere in the aligned reads, and select ***Color alignments by > read strand***

Observe where the mismatches are.

Note: We know that this sequencing was not with a strand-preserving library, so the expected strand distribution is 50-50.

→ This is likely a false positive.

END OF EXERCISE