Mapping reads to the HIV genome

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Abstract This shows the results for mapping Mi-Seq (RNA-seq) reads taken from HIV to the HIV genome.

I. SUMMARY

Suppose we want to map Mi-Seq reads to a reference genome? To address this, I wrote a bash script that takes in a pair of Mi-Seq reads, a reference genome file, and a quality control parameter to accomplish this task. I then performed a small analysis to identify where mapping occurred, and whether this might be influenced by GC content.

The problem of mapping RNA to genome has been studied for a long time, with many proposed solutions. Which one should I choose? A study suggests that STAR is a well performing general solution to this sort of problem [1]. Additionally, it can handle introns, an important consideration for genomes.

To perform the mapping, I used trimmomamatic to filter read pairs with either pair having average phred scores less than a specified threshold, and then used STAR to map these filtered reads to a reference genome. I then used samtools to convert STAR's sam output to a sorted bam format. I used bedtools to summarize the read coverage from the sorted bam file, excluding introns from the calculation. Finally I wrote and ran Python2.7 script to summarize read coverage and its relationship to GC content. An example analysis using NCBI's SRR961514 mi-seq run and the K03455.1 HIV genome was performed (see fig 1). To speed up this first example, I used a high average phred score threshold (i.e. 38).

I next asked if different average phred score filters would affect the coverage. Except for counterproductively high filters (see fig 2), I saw little effect for 0, 10, 20, and 30 average phred thresholds (see fig 2). I next asked if there were any relationship between coverage and GC content with these different cutoffs, but found almost no difference (see fig 3).

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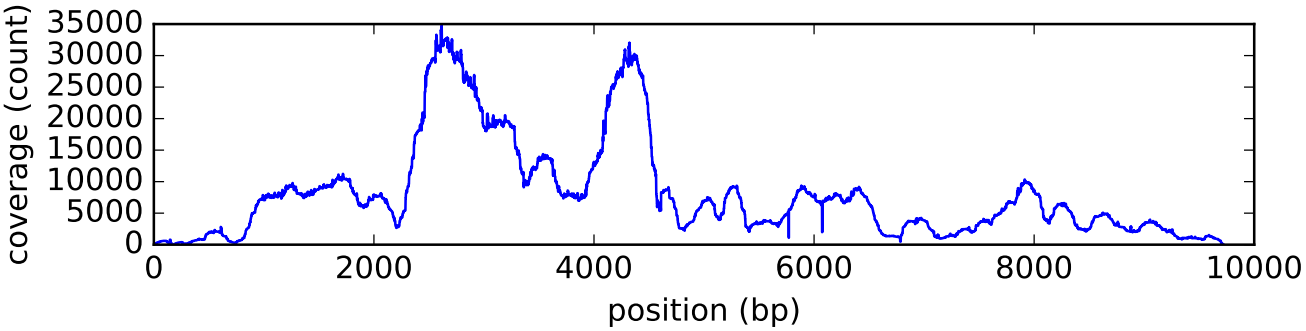
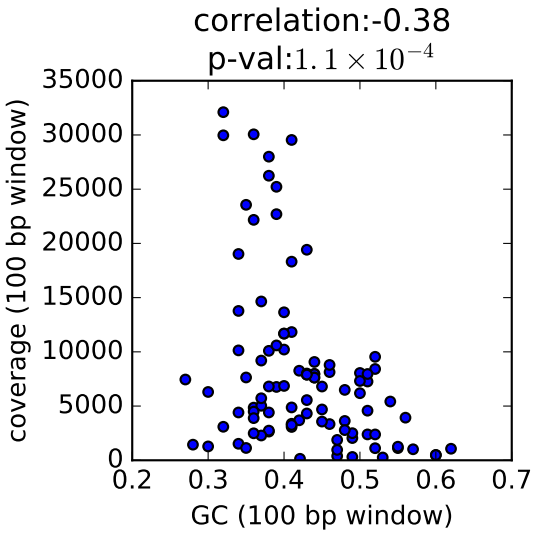
[1] Engstrm, P. G., Steijger, T., Sipos, B., Grant, G. R., Kahles, A., The RGASP Consortium, Rtsch, G., Goldman, N., Hubbard, T. J., Harrow, J., Guig, R., and Bertone, P. (2013). Systematic evaluation of spliced alignment programs for RNA-seq data. *Nature Methods*, 10(12):1185–1191. 00214.

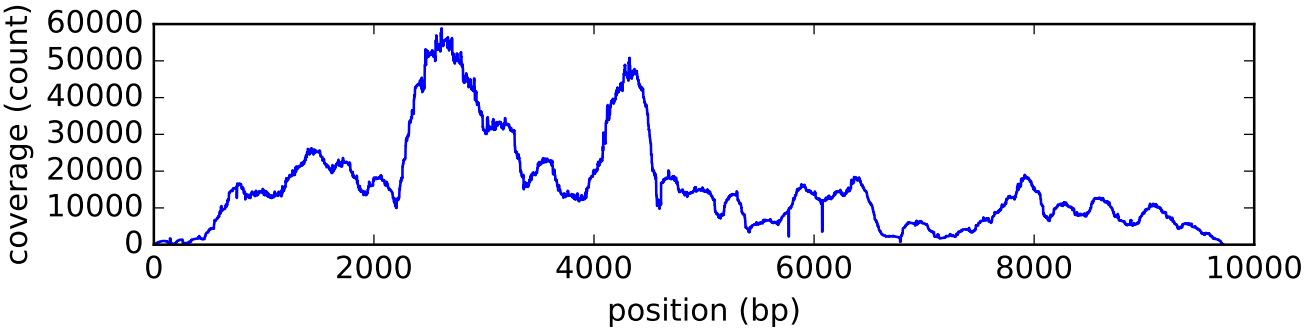


(a)

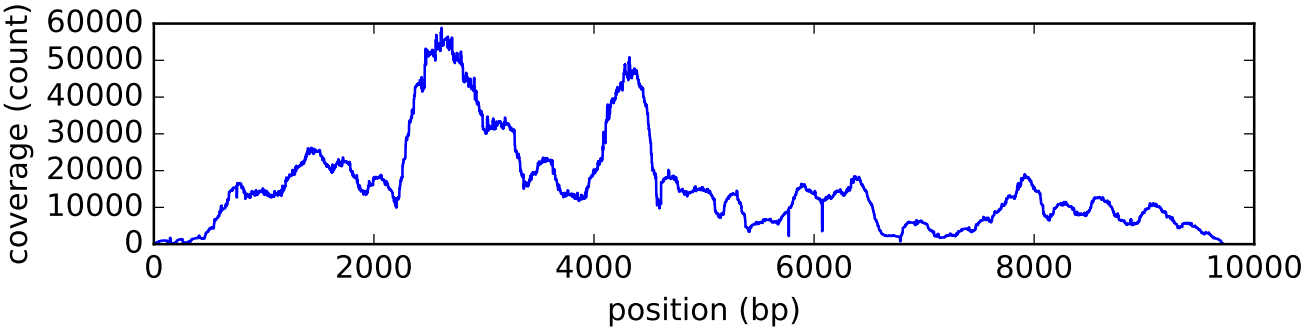


(b)

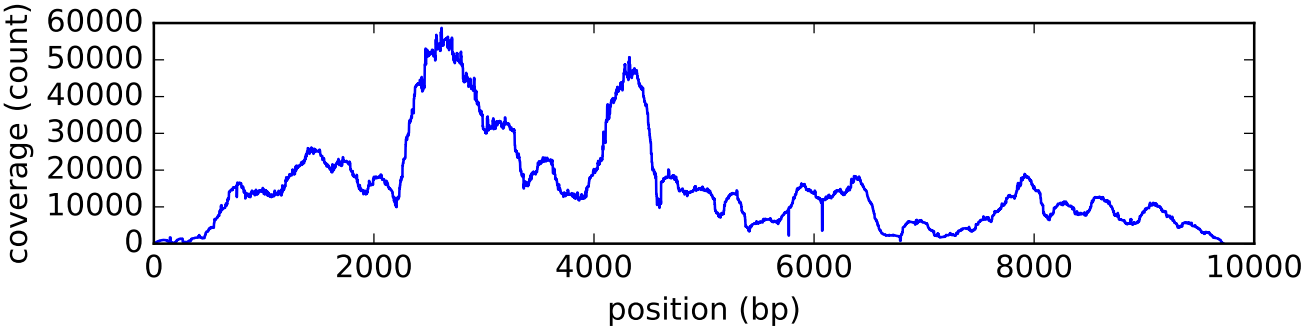
FIG. 1: a) Mapping coverage of mi-seq reads, excluding introns, taken from RNA → cDNA to the HIV genome (K03455.1). I filtered out reads with average quality scores less than 38. b) Over each 100 bp window, I averaged the read coverage and GC content, and compared the two. I found a negative correlation between GC content and read coverage. Further analysis will need to be performed to determine if this negative correlation is caused by a particular gene or cluster of positions.



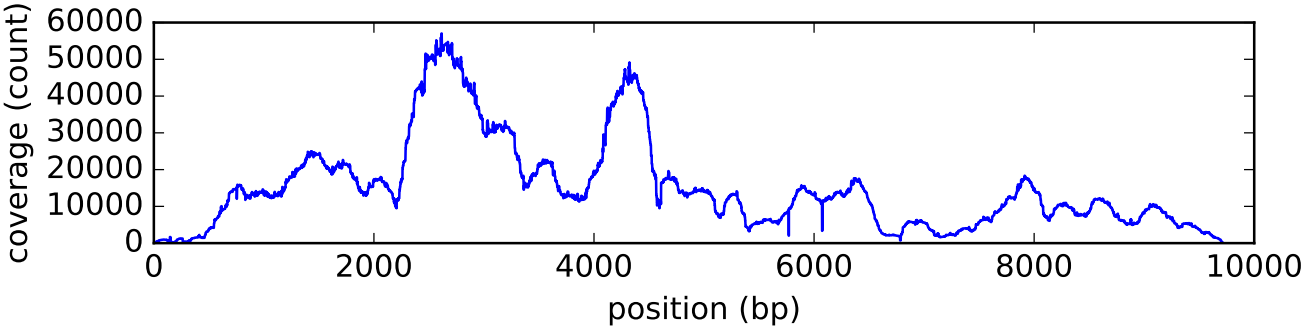
(a)



(b)



(c)



(d)

FIG. 2: Mapping coverage of mi-seq reads, excluding introns, taken from RNA → cDNA to the HIV genome (K03455.1). I filtered out reads with average quality scores less than a) 0, b) 10, c) 20, and d)30. I found little difference between filterings, suggesting fairly consistent and high quality read maps.

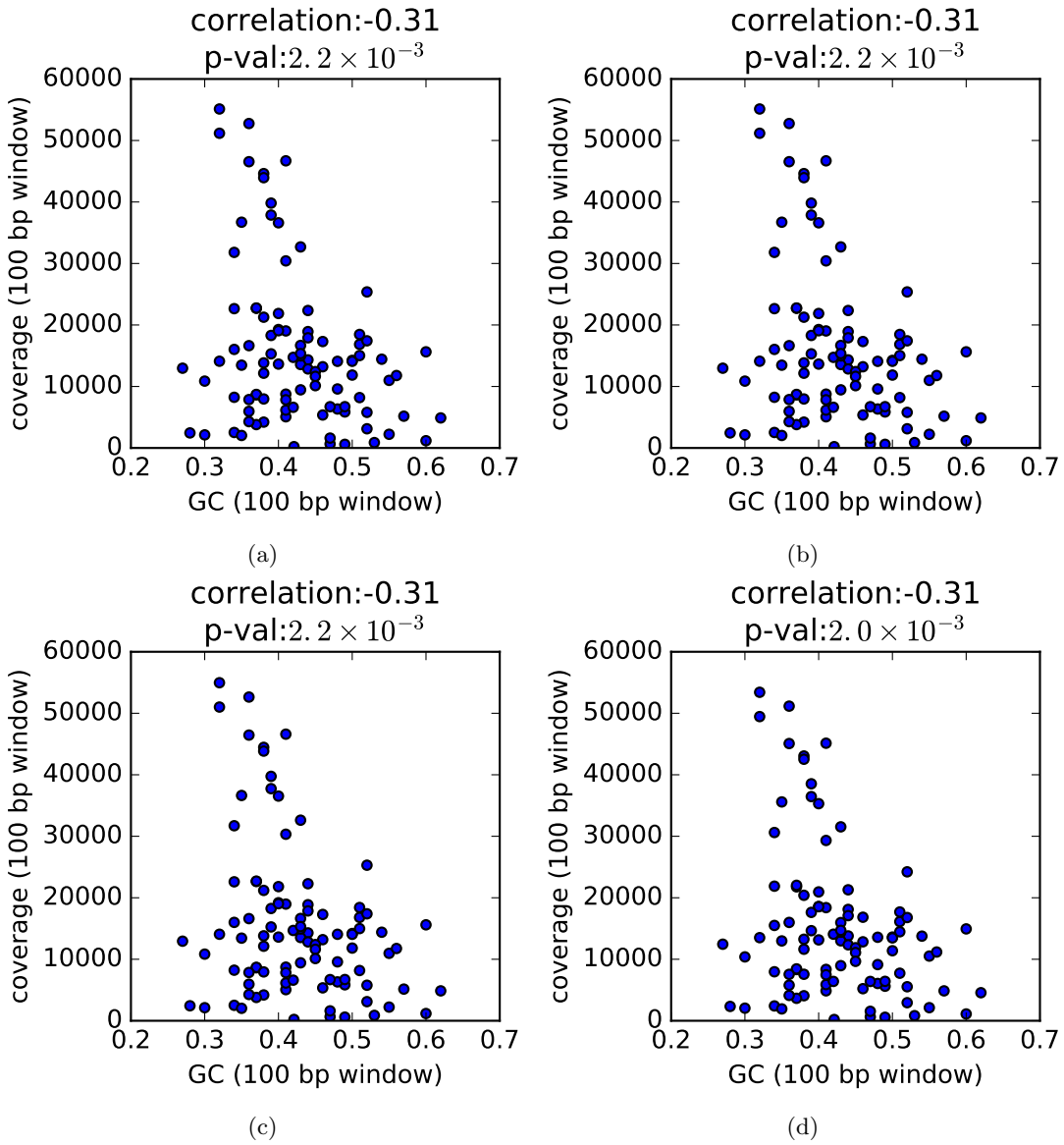


FIG. 3: Comparing coverage by position (excluding introns) to GC content yielded fairly consistent correlations for reads filtered by average quality scores less than a) 0, b) 10, c) 20, and d)30. p-values only drop a small fraction for QC control cutoff of 30.