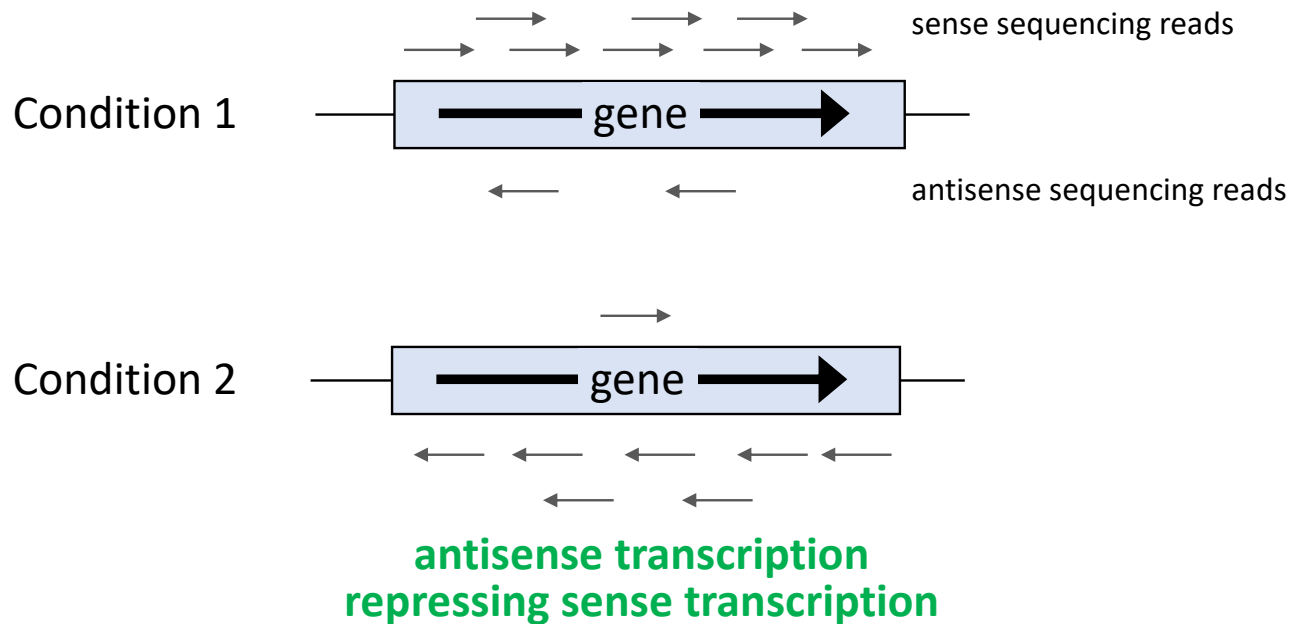
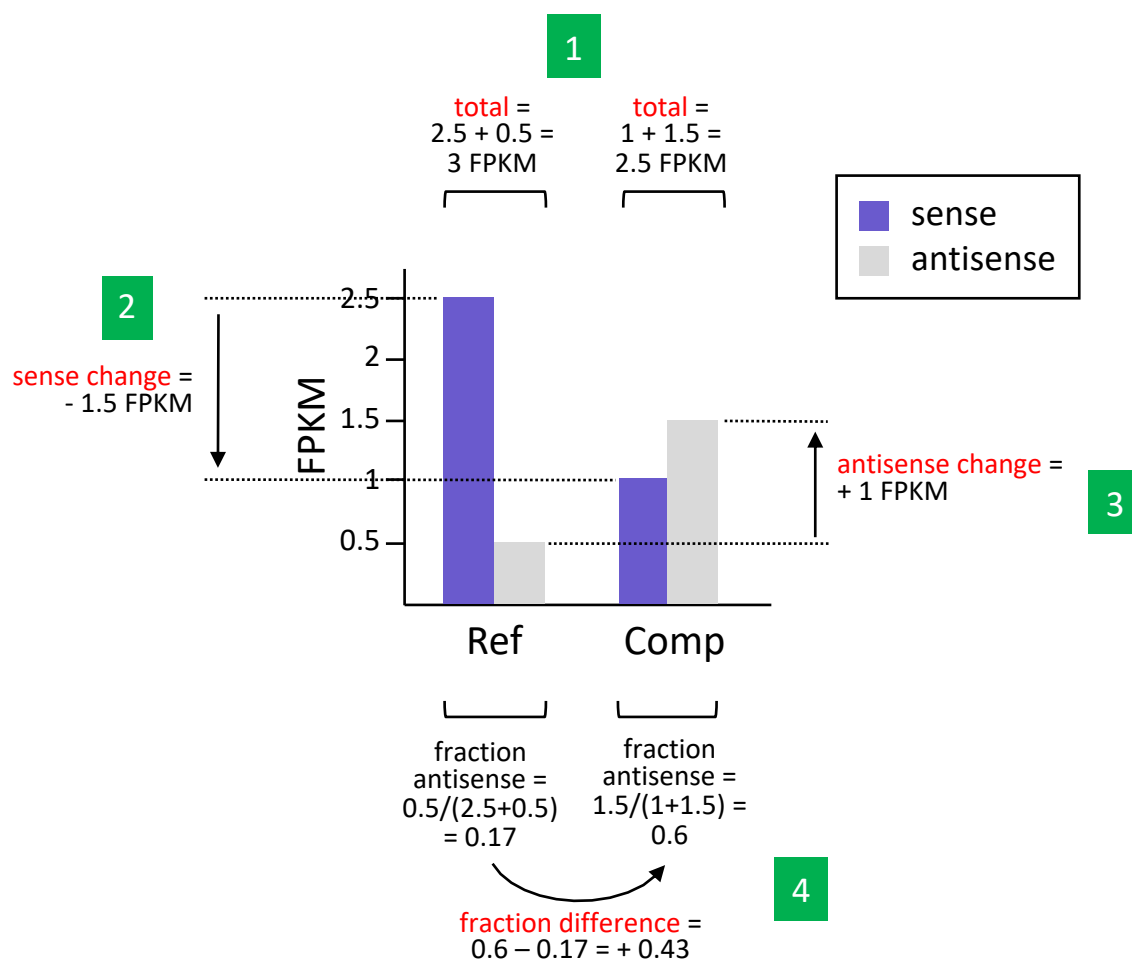


## Search for genes that exhibit Antisense Regulation

This search allows you to search for genes that exhibit antisense regulation of transcript expression, using strand-specific RNA-seq data. Below is an example of this regulation. In Condition 1, the sense strand of the gene is expressed; thus, there is a greater amount of sense sequencing reads. In Condition 2, there is an increase in antisense transcripts which causes decreased expression of the sense transcripts.



On the next page is an example of RNA-seq data for a gene that exhibits signs of antisense regulation in the Comp sample, when contrasted to the Ref sample. In order to detect this regulation, there are four parameters to consider and we have allowed you to modify these parameters.



1. Floor: This parameter is important in eliminating genes that are low expressed or don't have a sufficient number of reads to calculate an antisense fraction. For a sample to be considered, the sense FPKM plus the antisense FPKM must be  $\geq$  floor. The default is based on the depth of the sequencing for the study and the average length of the gene in organism, so that the floor represents 10 uniquely mapped reads. You can raise this floor to eliminate genes with low expression. In this example, any floor of 2.5 FPKM or below would allow this gene to be kept. A floor of 5 FPKM would eliminate this gene.
2. Sense change: If there is antisense regulation in the Comp sample, it is expected that the number of sense transcripts (measured by FPKM) will decrease. You can select the required magnitude of the decrease. The default is that there is any level of decrease. In this example, the decrease is 1.5 FPKM.
3. Antisense change: If there is antisense regulation in the Comp sample, it is expected that the number of antisense transcripts (measured by FPKM) will increase. You can select the required magnitude of the increase. The default is that there is any level of increase. In this example, the increase is 1 FPKM.
4. Fraction difference: To be selected as having antisense transcripts in the Comp sample, a gene must exhibit an increase in the fraction of antisense transcripts. The fraction is equal to antisense FPKM / (sense FPKM + antisense FPKM). In this example, the fraction difference is 0.43. You can choose any fraction between 0 and 1. If you use the default of 0.6, then this example gene would be eliminated. Raising this parameter will result in fewer genes, but the genes will have a greater change in the fraction of antisense transcripts.