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the accuracy of gene counts from the rna-seq analysis can be impacted by many factors, such as the presence of sequencing errors, gene structure, intron-exon overlap, and sequencing depth. in order to validate the gene expression data generated by rabema, we determined the extent of agreement between gene expression estimates from the ion torrent and illumina platforms. as shown in additional file 8 : figure s4 and additional file 9 : figure s5, the correlation coefficients calculated using the raw gene counts were 0.9411 and 0.9196 for the raw counts from the two platforms, respectively. for counts expressed as fpkm, the results were 0.8789 and 0.9184, respectively. the higher correlation coefficient between the two platforms for the fpkm counts is in agreement with the previous studies where fpkm was used [8]. in fact, we are currently developing a new method to quantify gene expression levels from rna-seq data that is based on the fpkm model, which will likely result in higher correlations between the two platforms (see below). gene expression is one of the most challenging areas in which to compare rna-seq technologies. there are many different levels to this comparison: counting reads, mapping reads, quantifying gene expression, and comparing the quantification of gene expression from different studies. this benchmark was designed to focus on the first three of those levels. the first step is to count the number of reads mapped to each gene in each sample. the number of reads mapped to each gene ranged from 2,014 to 40,858 (additional file 8 : figure s6). the average number of reads mapped to each gene in the two platforms was 15,000 and 32,000, respectively, for the rna-seq data from the 5 biopsies. for the illumina data, the reads aligned to a gene was dependent on the number of mapped reads in the corresponding sample (i.e., the number of reads mapped to a gene does not necessarily reflect the number of reads in the sample). for example, a gene with a read count of 60 could have been represented by two, three, or more reads in a sample. the mean number of reads per gene was similar between the two platforms. for the ion torrent data, the reads aligned to a gene was dependent on the number of mapped reads in the corresponding sample (i., the number of reads in a sample does not necessarily reflect the number of reads in the sample). however, the read counts were not dependent on the number of mapped reads in the corresponding sample (i., a read count of 60 does not necessarily reflect a 60% of mapped reads).

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here, we evaluated the performance of five aligners to align rna-seq data obtained with different sequencing platforms. we tested the performance of the aligners using two sets of sequencing data from a rna-seq project carried out on mouse embryonic stem cells and mouse embryonic fibroblasts (mefs) (see table 3 for details). for the mouse embryonic stem cells, we aligned the rna-seq data to the mouse genome mm10 and to the human genome hg38 using the five aligners. we also aligned the rna-seq data from mefs to the mouse genome mm10 and to the human genome hg38 using the five aligners. we found that the alignment performances were not correlated with the differences in the total number of reads and their length. specifically, star [17] and tophat2 [18] had the highest number of mapped reads and were able to map a larger number of reads to the mouse genome than the other aligners (table 4). from a biological standpoint, the effect of the alignment algorithm choice on gene expression would be best characterized by a direct comparison of the two sequencing platforms. however, this is challenging due to the fact that the data produced by the two platforms have different library preparation protocols, and thus, data normalization is significantly more difficult. we therefore chose to compare the platforms in the context of a more general rna-seq analysis pipeline (section 5.1 in additional file 1). for all sequencing data we used the port pipeline to normalize the reads and to quantify gene expression (see additional file 1 for details). in the absence of a cost-effective solution, we performed this analysis using the default parameters of the port pipeline, as we had little evidence that this would significantly alter the observed results. despite this approach, we observed a consistent, yet modest, increase in the overall level of gene expression (f-measure decreases by 1.7 and 1.8% for the 100- and 400-base reads, respectively) when using the bowtie2 alignment algorithm compared to the other mappers (section 5.2 in additional file 1). this decrease was particularly notable for the 400-base reads with bowtie2 aligning around 66% of the reads (fig. 3a). since bowtie2 is a seed-based aligner, this is not surprising. however, these effects were not significant in the direction of increased expression, suggesting that the differences observed between the two sequencing platforms do not arise from a bias introduced by the alignment algorithm. 5ec8ef588b

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