Discussion

In this study we wanted to investigate how qPCR technology using individuals corroborated with RNA-seq analysis. Overall, two general trends were observed. First, directionality of expression was congruent for a majority of the assayed genes. For those targets that were not in agreement, the difference in expression between the samples was within 2 fold. This observation is consistent with previous studies examining the correlation between RNA-seq and qPCR (e.g. Marioni et al., 2008, Beane et al., 2011). Second, the fold difference between samples was generally larger by RNA-seq analysis. For example, for all 4 genes determined to be significantly different by both analyses (DPGN, GSPA, GP17A and HMG2) the fold difference was larger for the RNA-seq analysis than for qPCR. Previous studies have also indicated that RNA-seq analysis reports larger fold differences than qPCR or microarray analysis (Hoen et al., 2008). The genes identified as not significantly different (CALL, GNRR2 and TIMP3) using RNA-seq had the lowest number of mapped reads. Aside from these general trends, there were differences observed between between these orthologous methods. There could be multiple explanations for these discrepancies, which are described below.

A major difference between these analyses is while RNA-seq analysis was performed using pooled tissue of 8 individuals, qPCR was performed on 8 individual samples. It is therefore easy to conceive that some individuals may be contributing a large number of transcripts to the pooled library but that individual responses may be highly varied so that differences in means may be undetectable by qPCR analysis. Another potential reason for differences between the analyses may be due to alternative splicing. The RNA-seq analysis is based on total reads that map to a particular contig. It is possible, as a result of alternative splicing, that qPCR analysis may target an alternatively transcribed exon. As a result, differential expression could be observed by qPCR, whereas the total number of reads averaged over the entire contig may mask the difference in expression. This bias between the methods is actually seen as a benefit of using an RNA-seq approach and alternative splicing is another important area of investigation using deep sequencing approaches. If a large number of reads map to a particular exonic region, it may suggest that this region would be a good target for follow-up isoform analysis. Whereas examination of individual transcripts is outside the scope of this study, it is important to note the advantage of this approach when considering experimental design. Finally, differences could arise from PCR bias. Oyster genomes are known to have a large number of sequence polymorphisms and different individuals may show different primer efficiencies (Sauvage et al., 2008; Taris et al., 2008).

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