Altered gene expression by control unspecific dsRNAs: an inquiry

Sandra Grossi Gava[1,2]; Naiara Cristina Clemente Santos Tavares de Paula[1]; Anna Christina de Matos Salim[3]; Flávio Marcos Gomes Araújo[3]; Guilherme Oliveira[4], Marina de Moraes Mourão[1]

[1]Grupo de Helmintologia e Malacologia Médica, CPqRR; [2]Instituto de Ciências Biológicas, UFMG; [3]Plataforma de Sequenciamento Genômica e transcriptoma NGS, CPqRR; [4]Instituto Tecnológico Vale, ITV

RNA interference is long-serving and still the only reverse genetic tool available for gene function studies in trematodes. Most of de RNAi assays in trematodes have been performed in *Schistosoma*, especially in *S. mansoni*. Gene silencing generally use a nonrelevant dsRNA from another species as controls and quantitative real time PCR (qPCR) to measure the knockdown levels achieved. Despite the applicability of RNAi to study many genes in schistosomes and others helminthes parasites, several authors have noticed inconsistencies associated with this technique. To globally check if there are genes affected by unspecific dsRNA exposure, schistosomula (~500,000 larvae) were cultivated and exposed to 100 nM of unspecific dsRNA synthetized from the Green Fluorescent Protein (GFP) or mCherry, two sequences with no similarity with *Schistosoma* genome and widely used by the scientific community. After two days of culture, total RNA extraction was carried. RNA-Seq libraries were prepared according to the *Truseq stranded mRNA Library Prep* protocols and were sequenced on *Illumina HiSeq 2500* platform. We generated 10 paired-end libraries containing reads of 100 bp, ranging from 34 to 92 million reads per library, with GC content of 38-39%. The sequences were aligned to the *S. mansoni* reference genome using STAR with more than 87% of uniquely mapped reads. Counts of reads aligned were obtained with the sub-command multicov in BEDTools suite. To ascertain genes differentially regulated (DEGs) after non-specific dsRNA exposure, we compared the expression profiles with the untreated controls. RNA-seq analysis resulted in 6 DEGs in the GFP dsRNA treatment and 3 DEGs in the mCherry dsRNA treatment (edgeR P-value < 0.01, FDR <0.05). KEGG and GO databases were used to elucidate the functional classifications of these DEGS. The majority of DEGs coded for uncharacterized proteins and none of them are used as control in RT-qPCR experiments. We visualize the sample-to-sample distances in a heatmap and PCA analysis and found that the biological replicates are more prone to cluster than unspecific and untreated controls. Here, we conclude that there are more differences between biological replicates, than due to the treatment with GFP or mCherry unspecific dsRNAs. These observations may be relevant to other model systems applying RNA interference for gene function assessment.

Financial support: CAPES; FAPEMIG; CNPq, CPqRR-FIOCRUZ, European Comission 7th Framework, A-ParaDDisE