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A capture-based technique for enhancing RNA-Seq analysis of bacterial transcriptomes during *in vitro* and *in vivo* infections

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Bacterial pathogens must rapidly respond to changing conditions as they encounter different microenvironments within a particular host and infect different host cell types. This ability to quickly and appropriately respond to the challenges of infection requires rapid and global shifts in gene expression patterns. Analysis of bacterial gene expression should be possible using next-generation sequencing (NGS) technologies, however during an infection, bacterial transcripts make up an exceedingly small proportion of the total RNA in an infected sample. To address this issue, we have developed a method to separate bacterial transcripts from host transcripts prior to sequencing. Using this unbiased capture-based technique we are able to enrich for all possible bacterial transcripts leading to gene level analysis of the entire bacterial transcriptome. We previously used this technique to analyze the transcriptome of *F. tularensis* during phagosomal escape and cytosolic growth in murine macrophages. Here we present our recent work examining the transcriptome of *Y. enterocolitica* during the infection of murine macrophage cells. By focusing on four early time points during infection, we were able to observe the transcriptional shifts that occur as the bacteria move from log-phase growth in a nutrient rich medium at 26°C to an active infection of mammalian cells. We used growth in filter-sterilized spent tissue culture media as a control to focus our analysis on genes that are expressed upon contact with host cells. We also compared the transcriptomes of bacteria located on the surface of host cells to that of cells that have been internalized by the macrophage cells revealing numerous genes involved in intracellular survival. Finally, we present preliminary evidence of the efficacy of our enrichment strategy in the analysis of the *S*. Typhimurium transcriptome from the spleens and livers of infected mice where the bacterial transcripts initially make up less than 0.005% of the total RNA.

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