

Metatranscriptomics in the NGS Era

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In situ analysis of gene expression patterns in natural microbial communities has long captured the scientific community interest. Recently microbial gene expression has been monitored in both human [1,2] and non-human animal [3-5] environments, including the mouth, skin and the gut. Microbial communities have been studied using several different analysis approaches, including the analysis of 16S ribosomal variable regions and direct sequencing of the “metagenome”. However, metatranscriptomics is a more powerful tool for understanding microbial processes within communities and consortia as it not only reveals the organisms present, but also provides information on their function and how this is influenced in different environments.

Until recently the limitations in available molecular technologies such as qPCR and microarrays, which were only able to analyse a limited number of genes at a time, required prior sequence information to design probes and primers, and the needed large mRNA quantity to perform experiments, have meant that the full potential of metatranscriptomics has not been realised [6,7]. However, with the rapid advances in “next generation sequencing” (NGS) technology many constraints of these constraints have been removed. The direct sequencing of the metatranscriptome to determine variations in expression profile e.g. in response to cytotoxic or antiviral compounds [8,9] will contribute to knowledge of microorganism interactions, and may be used to discover new bioactive compounds.

Data on sequences from environmental microorganisms is stored in publicly available databases such as EBI metagenomics and transcriptomics (<https://www.ebi.ac.uk/metagenomics/>, <https://www.ebi.ac.uk/arrayexpress/>) and CAMERA (<http://camera.calit2.net/>). Nevertheless, there are still a limited number of sequences available and the annotation of the sequences available, is the biggest bottleneck and is a challenge that needs to be solved in order to fully exploit data obtained from metatranscriptome studies. In 2009 only 33% [10] of putative protein-encoding sequences had matches with annotated proteins in the NCBI RefSeq database (<http://www.ncbi.nlm.nih.gov/RefSeq/>). During the last three years the percentage has increased, but a large number of sequences remain unannotated. Leaving sequences assigned to gene families with no known function and inaccessible biochemical pathways. In addition, the large number of uncultivable microorganisms in environmental samples (e.g. from the rumen or in marine samples [11]), leaves a large gap in knowledge regarding the diversity of genes present. The interpretation of metatranscriptomic data will be greatly improved and more valuable as better gene annotations become available. To fill this gap in knowledge linking sequence data with gene function, many metatranscriptomic studies are underway under controlled experimental conditions, in which microbial gene expression can be quantified in direct response to a defined set of parameters.

Interpretation of metatranscriptomic data from “field” samples is made more difficult because of the natural variability between independent samples which has to be accounted for in the statistical analysis to determine the robustness of differential expression data. In addition, field samples can be difficult to deal with because of the low abundance of the microorganisms present and as a consequence, the low quantity of RNA available for analysis. This means that low abundance transcripts are poorly represented and are poorly quantified or may be missed.

Current metatranscriptomics studies are providing microbial expression profiles from different ecosystems (e.g. ocean, soil, human microbiome) and obtaining valuable information on ecologically important microbes whose biology is poorly known. Understanding the human microbiome and the relationship with health is receiving considerable attention. Connections between microbiota and human health may identify signatures that can be used as early markers of health status and an early warning of problems e.g. obesity.

Recently, metatranscriptomic analysis using next generation sequencing approaches has been used to study the impact of food microbiota on the human gut microbiome [12,13]. Interestingly, only a few studies of NGS metatranscriptomics in food (e.g. Kimchi cheese) [14], are available in literature. Metatranscriptomic analysis of the microorganisms naturally present in food is a technical challenge, not only because of the lack of basic sequence data and annotation, but also because food samples can contain fats, polysaccharides and inhibitors. This makes it very difficult to extract microorganisms from the food matrix and my result in poor RNA quality. In addition degraded RNA from the food matrix (e.g. plant, meat, fish) may be mixed with RNA from the microorganisms present, which may swap the information from the microorganisms, or confuse the interpretation of the data. Protocols for effectively separating the microorganisms from food matrix without distorting the abundance and composition and which are rapid enough to avoid any changing the metatranscriptomic still need to be developed. In addition, sequence information available in public databases is limited to the most studied bacteria strains, such as lactobacilli, staphylococci and common food pathogens.

With all these constraints, the study of food metatranscriptomic is an exciting challenge. The rewards will be a better understanding of the interactions between human gut and food microbiota. This knowledge may create a solid background for nutrigenomics, creating products that have a real benefit for human health. In addition understanding the interactions between food microorganisms may identify bioactive molecules that can be used as new kinds of antibiotics (lantibiotics) [15].

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Received January 31, 2014; Accepted February 03, 2014; Published February 06, 2014

Citation: Gorni C (2014) Metatranscriptomics in the NGS Era. *Transcriptomics* 2: e107. doi:10.4172/2329-8936.1000e107

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