APPLICATION BRIEF

Applied Genomics

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Simultaneous Sequencing of mRNA and MicroRNA in IPSC Derived Cortical Neurons

Abstract

MicroRNAs (miRNA) are promising biomarkers for different health outcomes[1-3]. However, correlation between miRNA profiles and their target gene is not sufficiently understood in many cases. There is a need of performing integrated analysis of miRNA and mRNA expression levels, but due to limitations of traditional workflows is not possible to address this in a single preparation[4-7]. Here, we show that the NEXTFLEX® Combo-Seq™ mRNA/miRNA kit can be used for simultaneous sequencing of miRNAs and mRNAs obtained from cell cultures.

Methods

iPSC-derived cortical neurons were generated from skin cells, peripheral blood or fibroblasts of patients. These iPSC-derived cortical neurons were exposed to various compounds to see whether these compounds could induce cognitive diseases. The NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit was used to construct libraries for combined mRNA and miRNA sequencing. The samples were sequenced on 100 cycle S2 flowcells in paired-end mode on a NovaSeq® 6000 sequencer (Illumina). The obtained data was pre-processed using the excerpt pipeline (https://hub.docker.com/r/rkitchen/excerpt) according to PerkinElmer's recommendations. Statistical analysis was done according to the R-ODAF best practice sequencing pipeline (https://github.com/MCTverheijen/R-ODAF) to filter out low read counts and spurious spikes.

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Results

Sequence Quality

The reads were sequenced with high quality. All bases of all reads depicted Q-score above 30 and most bases even above 35. The length distribution indicated the NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit (Figure 1) generated data from miRNAs (24nt) and mRNAs (>80 nt) simultaneously and detected other kind of RNAs such as tRNAs (50-75 nt).

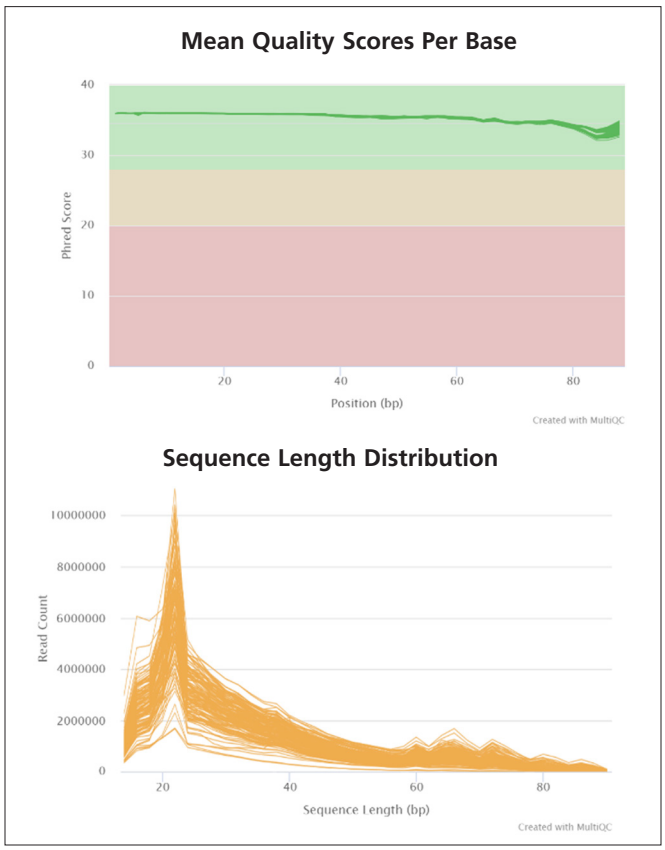

Figure 1. FastQC-MultiQC quality control.

Sequence Depth and Mapping

For the *in vitro* analysis, 128 samples were divided into six groups based on their treatment (con= control, exp= experimental treatment). The NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit generated on average 48.5 M reads per sample (sd: 10.9 M). Between 78.7% and 90.4% of the reads were mapped to the genome (except for a single outlier of which only 14.8% mapped) (Figure 2).

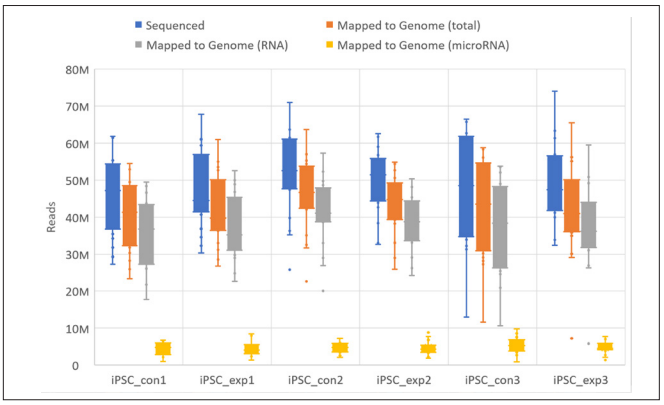

Figure 2. Sequenced reads and mapped reads.

Most of the mapped reads were gained from RNAs, on average 77.8% of the total read count (sd: 7.1%). For miRNAs, 9.95% of the total read count was mapped (sd: 3.5%), which resulted in the detection of 525 to 994 miRNAs which can be investigated for their potential application as biomarkers for cognitive diseases. An example of the biotypes found for each sample are listed in Figure 3.

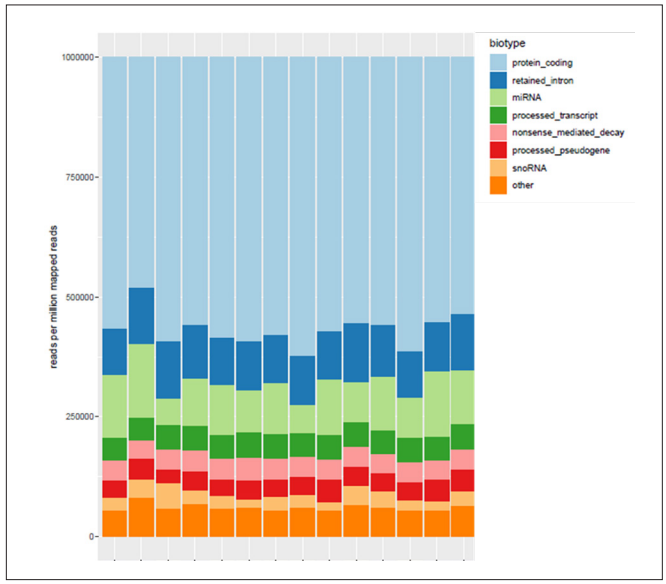

Figure 3. Biotypes per sample, normalised.

Conclusions

We show the NEXTFLEX® Combo-Seq™ mRNA/miRNA kit can be used for simultaneous sequencing of miRNAs and mRNAs in total RNA obtained from *in vitro* cell cultures. Differential expression profiles of mRNA and miRNA in control and treated iPSC-derived cortical neurons will be studied using this data.

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