PROFILING LNC-RNA IN CHO CELLS USING NGS TECHNOLOGIES

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Key Words: Chinese Hamster Ovary (CHO), lncRNAs, RNA-seq, RIP-seq

Chinese Hamster Ovary (CHO) cells are the preferred host for protein production given their capability to perform human-like post-translational modifications. Nevertheless, limited yields and long term stability still represent an issue and are often pinpointed as the major drawbacks of CHO cells. The development of -omics technologies has allowed for a deeper understanding of the complexity of the cells and the factors that contribute to the unstable phenotype that CHO cells are often reported to have. A plethora of work has focused on engineering CHO cells at the transcriptional (e.g. transcription factors (TF), CRISPR tools, and epigenetic marks), post-transcriptional (e.g. miRNAs, siRNAs, and aptazymes), translational (e.g. uORFs, RNA-binding proteins, and unnatural amino acids) and post-translational (e.g. engineering of glycosyl/fucosyl-transferases, protein splicing) level leading to improved productivity, stability or modified glycosylation patterns.

In the complex picture of cell regulation, long non-coding RNAs (lncRNAs) represent a relatively new and yet largely unknown class of molecules that can operate at different subcellular compartments, making them interesting targets and potential tools to modulate the cell transcriptome and/or translatome.
Our work focuses on the functional characterization of lncRNAs and aims at improving the understanding of their basic biology and mechanism of action in CHO cells.

After overexpressing lncRNAs that were differentially expressed in different phases of a batch culture, we couldn't detect any major phenotypic change, in part because for many lncRNAs the precise sequence of the transcript is not known or does not match the annotated sequence. These preliminary results underlined that our knowledge on lncRNAs in CHO is still very limited and that we are in need of broader datasets to be able to set up more purposeful single molecule studies so that they can be added to the CHO synthetic biology toolbox.
We therefore decided to collect all publicly available and *in house* RNA-sequencing data to perform a transcriptome based lncRNA annotation, followed by differential expression (DE) analysis in CHO cells that aims at improving the current annotation, that would ultimately simplify the application of CRISPR and/or other synthetic biology tools to characterize such molecules. Moreover, the DE analysis will help with the identification of *house-keeping* and *condition-specific* lncRNAs and the differentiation between functional and non-functional lncRNAs, based on their expression levels in different cell lines and conditions.

Alongside with this approach we are performing an RNA immunoprecipitation (RIP) experiment under crosslinked conditions, followed by next generation sequencing to identify lncRNAs that are interacting with chromatin modifiers. We selected three proteins that have been reported to interact with lncRNAs and are involved in the regulation of gene expression, namely p300, Wdr5 and Ezh2. This approach generates a database consisting of interaction sites of lncRNAs with the selected regulatory proteins. As this experiment is performed on a global scale, it aims at establishing a catalogue of lncRNAs interacting with one or more protein(s) of interest. Firstly, this will help in elucidating the role of predicted lncRNAs. Secondly, having a list of lncRNAs interacting with a specific protein, will contribute to drawing general rules to predict their function(s).

Given the poor state of the art in CHO cells and the increasing interest of the scientific community in lncRNAs, we believe that our work will greatly help the general understanding of the regulation of gene expression and will therefore contribute to the development of more consistent, reliable and customized cell factories.