# Polysome profiling of oocytes, early development, and cell cycle in mammals.

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Abstract

Polysome profiling has provided a gold-standard method to precisely assay functional genome readout at a given time point. The wide-spread utilization of polysome profiling has nevertheless been historically hindered by its intrinsic complexity, time-consuming nature, limited capacity for high throughput adaptation and its requirement for relatively large initial sample sizes. Here, we present Scarce Sample Polysome Profiling (SSP-profiling); method that demonstrates a combination of the sucrose gradient ultracentrifugation in small SW55Ti tubes with the qRT-PCR-based quantification of 18S and 28S rRNAs in fractionated polysome profile. SSP-profiling is suitable for both scarce and conventional sample sizes and is compatible with downstream RNA-seq to identify polysome associated transcripts.

Using SSP-profiling we have assayed the translatome of mouse oocytes at the onset of nuclear envelope breakdown (NEBD). There is no active transcription during meiotic maturation of mammalian oocyte. Instead, maturation and successful meiotic progression relies on pre-synthesized maternal mRNA, the translation of which is highly coordinated in space and time. Our analyses identified 1847 transcripts with moderate to strong polysome occupancy, including abundantly represented mRNAs encoding mitochondrial and ribosomal proteins, proteasomal components, glycolytic and amino acids synthetic enzymes, proteins involved in cytoskeleton organization plus RNA-binding and translation initiation factors. In addition to transcripts encoding known players of meiotic progression, we also identified several mRNAs encoding proteins of unknown function.

We have confirmed SSP-profiling reproducibility and versatility by assaying several other types of scarce samples like mouse Mil oocytes, zygotes, 2-cell embryos, blastocysts, and more recently, human lymphoblastoid cell populations sorted according to their cell cycle phase by FACS.

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