Genome-wide RNAi screen for human 60S subunit biogenesis factors

Genome-wide RNAi screen identifies novel players in human 60S subunit biogenesis including key enzymes of polyamine metabolism

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Dataset subtitle

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Dataset abstract

Ribosome assembly is an essential process that is linked to human congenital diseases and tumorigenesis. While great progress has been made in deciphering mechanisms governing ribosome biogenesis in eukaryotes, an inventory of factors that support ribosome synthesis in human cells is still missing, in particular regarding the maturation of the large 60S subunit. Here, we performed a genome-wide RNAi screen using an imaging-based, single cell assay to unravel the cellular machinery promoting 60S subunit assembly in human cells. Two genome wide libraries from different vendors (Qiagen and Ambion) were screened in HeLa cells expressing RPL29-GFP under a tetracycline inducible promoter. Screening plates were imaged by automated microscopy (9 sites per well). Images were subjected to an automated pipeline for illimitation correction, segmentation and feature extraction. Phenotypes of individual cells were then classified using a supervised machine learning algorithm and the rate of cells displaying 60S ribosome biogenesis defects (hitrate) was calculated. Hitrates of individual siRNAs were combined by the redundant siRNA analysis (RSA) algorithm. Genes which ranked high in the genome-wide screen, but were previously not associated with ribosome assembly, were validated using a custom-made library containing pools of 30 siRNAs per gene.

This dataset includes all raw images obtained in the genome-wide screening approach and in the validation screen.

Dataset collection methods

Qiagen and Ambion libraries as well as the were screened in 384 well plates using HeLa RPL29-GFP reporter cells as a readout. Plates were imaged on a Molecular Devices ImageXpress microscope equipped with a Thermo CRS plate loader. Images were acquired with a 10X Plan Fluor 0.3 objective using Laser-based autofocus. Total number of images: roughly 3’415’000 (Qiagen library: 296x384 wells, Ambion Library 192x384 wells and Validation screen 3x2x384 wells; per well: 9 images, 2 channels).

Dataset file structure

- Annotation files: Annotation of the position of controls and siRNAs in the individual screening plates of the genome wide screens using the Qiagen and Ambion library and for the Validation screen using siPools.

    * Ambion_assay well annotation.xlsx
    * Ambion_control_annotation.xlsx
    * Qiagen_controls_annotation.xlsx
    * Qiagen_library_information_qiagen_assay_wells.xlsx
    * ValidationScreen_annotation_assaywells_controls.xlsx

- image files: zip compressed by plate in the respective folders. Illumination corrected images of Qiagen library screen show composite images of GFP and DAPI channel which can be easily split e.g. using ImageJ/Fiji. Raw images of the Ambion library and validation screen only show GFP channel.