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Quantitative Multiparametric Image Analysis for Estimation of siRNA Induced Off-target Effect

Small Interfering RNA (siRNA) and automated high-throughput high-resolution microscopy provides technological platform for systematic genome-wide survey of individual gene knockdown phenotype. Quantitative multi-parametric description of knockdown phenotype can be used for gene functions elucidation and establishing mechanistic models of cellular processes in which genes participate. However, the large degree of morphological variation between cells in repetitions of biological experiment as well as variation between phenotypes of different siRNAs, which are targeting the same gene, represents a major challenge to the reliable identification of gene silencing phenotypes. We have developed a system for the high content analysis of automatically acquired high-resolution images, which describes the endosomal organelles in quantitative terms (gene silencing profile) (Collinet et al, Nature 2010). The stability of individual parameters of phenotypic profiles between different imaging sessions and experimental replicates were tested. The analysis showed that different parameters reveal a wide variation of stabilities which dependent on biological variability, typical automatic imaging problems and parameter calculation details. Analysis of multi-parametric phenotype profiles produced by independent siRNAs, which are targeting the same gene, reveals the mean level of off-target effect, its dependence on siRNA concentration and chemical modification. The estimation of the minimum number of independent siRNAs which are required to infer the gene knockdown phenotype with given confidence was done. Quantitative estimation of off-target effect gives an objective feedback for no off-target siRNA selection, for the new generation siRNA development and could provide insight for deeper understanding of siRNA-mediated gene silencing mechanism.