3.2 Assay Performance, Hit Identification, and Statistics in Plate-Based Screening

In order to assess the quality of an arrayed screening campaign and to identify hits, several statistical measures can be used. First, data has to be normalized to remove systematic errors and to allow comparison of data points that were generated on different plates. Many normalization methods have been used in RNAi screening including Z-Scores and B-Scores. The Z-Score normalization provides information on the strength of each siRNA relative to the rest of the sample distribution and is defined as the number of standard deviations an observation is away from the mean of the plate (or screen). More robust methods, which account better for outliers and systematic plate effects, include the robust Z-Score (number of median absolute deviations) and B-Score, respectively (Brideau et al. 2003). Birmingham et al. (2009) comprehensively summarized and compared various suitable statistical methods to be used for high-throughput RNAi screens. The cellHTS web application (Pelz et al. 2010) is a tool that guides the user through the various normalization steps and facilitates the analysis of high-throughput data sets and is accessible at http://web-cellHTS2.dkfz.de. Hits of an RNAi screen are usually identified by comparison to a negative control or to all samples on the plate (assuming the genes to be targeted in the library are randomly distributed across the plates, i.e., in unbiased screens targeting the druggable (~10,000 genes) or the whole genome). The strictly standardized mean difference (SSMD) is another measure of effect size, which can be used to select hits in an HTS. It is the ratio of the mean to the standard deviation of the difference between two random values each from one of the two groups. For reading out pooled shRNA screens, similar methods as described above can be used. Moreover the pooled approach allows for assessing shRNA dropout or enrichment at multiple timepoints and in several cell lines in parallel.

4 Addressing Off-Target Effects in RNAi Screens

Unintended effects, both false-positive and false-negative screening results, are often intrinsic to high-throughput approaches. They have to be taken into consideration while interpreting the outcome of such screens (Mohr et al. 2014). Unlike genetic approaches that act on the DNA, RNAi decreases mRNA levels or interferes with the translation of the mRNA. Thereby, RNAi-based experiments can suffer from a lack of sensitivity due to incomplete suppression of gene expression or a lack of specificity due to suppression of unintended genes (Sigoillot and King 2011).

Off-target effects (OTEs) can arise from a range of mechanisms comprising both sequence-independent and sequence-dependent effects. Effects that are independently of the siRNA sequence might even be considered avoidable by careful assay development. For instance, can expression of exogenous shRNA disrupt the endogenous processing of miRNA or compete with endogenous miRNA entering the RISC, which causes alteration of the normal gene expression pattern (Sigoillot and King 2011; Khan et al. 2009)? Other unspecific effects can of course be induced by toxic transfection conditions or other stress-causing treatments. Another source for