bioinformatics tools. The analysis even led them to predict the biological function of gene.

However, to find exploitable susceptibilities in the disease process needs more than analyzing this empirically generated disease association data. To identify new vulnerabilities or opportunities to directly or indirectly influence cellular responses involved in the disease, it requires the knowledge of gene function: which gene products cause the changes in the biology and how they produce their effects (Diehl et al. 2014). For this, even today's vast reservoir of "big data" is not sufficient as there is still too much unknown about how most gene products work, what their protein interaction network is, and how they are activated – this requires more laboratory bench research to generate data about the roles different gene products play in driving or regulating various biological responses in different contexts. Therefore, for successful target-driven drug development, an understanding of the functional disease relevance of the genomic alterations is crucial. With the discovery of RNA interference (RNAi) in mammalian cells (Elbashir et al. 2001), a powerful technique became available to explore these disease-specific genotypic-phenotypic relations in high-throughput large-scale genome-wide fashion (Mohr et al. 2014).

The development of RNAi technology has used the endogenous microRNA (miRNA) system as a guide. Basically, short RNA sequences which are complementary to the target mRNA are used to charge the endogenous miRNA machinery to destroy the gene transcript (Fennell et al. 2014). By now, there are many different RNAi reagents available, both from commercial or academic resources to target every gene of the entire genome such as chemically synthesized short-interfering (si) RNAs or endonuclease-endoribonuclease-prepared (esi) siRNAs or plasmid-encoded short hairpin (sh) RNAs (Boutros and Ahringer 2008; Grimm 2004; Bernards et al. 2006; Surendranath et al. 2013). Assembled in so-called genome-wide libraries, they are used for assessing the effects of loss of function (LoF) for a given disease-relevant phenotype thereby combining the power of genetic screening with phenotypic readouts (Mohr et al. 2014).

Figure 1 summarizes the workflow of an RNAi-based screening project to identify novel drug targets including assay development and optimization, choice of screening in pooled or arrayed format in the primary screen, screen data analysis, and follow-up verification of the results. In this chapter, the strengths and weaknesses of this RNAi screening technology will be discussed. In addition, a short summary is provided of the next-generation functional screening method based on clustered regularly interspaced short palindromic repeats (CRISPR) genome editing.

## 2 Types of RNAi Used in Screens

RNA interference is an endogenous process used as a term for various gene regulatory mechanisms. It was observed for the first time in 1990 in transgene petunia when overexpression of the chalcone synthase gene resulted in an