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for Microscopy; 2.7 Genome Wide Screens; 2.7.1 C. elegans RNAi Library; 2.8 Selected Literature on C. elegans Research; 2.9 Useful C. elegans webpages; 2.10 References; 3 RNAi in Drosophila; 3.1 Introduction; 3.2 Application of RNAi in Drosophila; 3.2.1 dsRNA from Linear DNA Templates; 3.2.2 dsRNA from Inverted Repeat DNA; 3.2.3 Inducible Expression in Drosophila Cell Lines; 3.2.4 Limitations; 3.3 dsRNA Synthesis; 3.3.1 In-vitro dsRNA Transcription 3.3.2 Inverted Repeat DNA 3.4 Injections; 3.4.1 Injection Services; 3.4.2 Injection Method; 3.4.3 DsRNA or Inverted Repeat DNA Preparation; 3.4.4 Embryo Collection; 3.5 Cell Lines; 3.6 Protocols; 3.6.1 Thawing and Maintenance of S2 Cells; 3.6.2 Freezing Protocol; 3.7 RNAi in S2 Cells; 3.7.1 dsRNA Transfection Using the Calcium Phosphate Method; 3.7.2 dsRNA Soaking of S2 Cells; 3.8 High-Throughput Screens; 3.8.1 Drosophila RNAi Library; 3.9 Useful Webpages for Drosophila Research; 3.10 Books and Literature on Drosophila; 3.11 References; 4 RNAi in Mammals; 4.1 Introduction 4.2 Transient RNAi in Cell Culture 4.2.1 Chemical Synthesis and Modifications of siRNAs; 4.2.1.1 Advantages; 4.2.1.2 Limitations; 4.2.2 Custom Synthesis of siRNA Oligos; 4.2.3 siRNA Design Rules; 4.2.3.1 siRNA Strand Bias and Off-Targeting; 4.2.3.2 Improvements in siRNA Stability; 4.2.3.3 siRNA Design: Novel Modifications of the "Tuschl Rules"; 4.2.3.4 Homology Search by BLAST, FASTA, or Smith-Waterman Algorithm; 4.2.3.5 Troubleshooting; 4.2.3.6 siRNA Design Programs and Algorithms; 4.2.3.7 Preparation of siRNA Duplexes; 4.2.4 Enzymatic Synthesis of siRNAs 4.2.4.1 Designing DNA Oligonucleotides

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## Sommario/riassunto

This hands-on guide to RNA interference brings the power of targeted gene silencing to any laboratory with the basic equipment for handling nucleic acids. In easy-to-follow, step-by-step protocols you will learn\* how RNAi works in worms, flies and mammals,\* how to design the most efficient RNAi constructs,\* how to achieve transient, stable and conditional RNAi in cell cultures,\* how to determine the efficiency of an RNAi experiment,\* and how to use RNAi for gene therapy. All the protocols have been thoroughly tested in the author's own laboratory, and she provides exa

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