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Top 10 Tips For Gene Silencing & Delivery

1. **Determine the RNAi tools best suited to your application.**
   With the popularity of siRNA-mediated gene silencing techniques, a wide variety of commercial siRNA kits and reagents have become available. These range from chemically generated siRNA oligos, to shRNA-generation plasmids, to kits for *in vitro* generation of siRNAs, to shRNA viral vectors. To choose the best reagents for your application, determine whether you need to obtain short-term gene suppression (~3-7 days) or longer-term gene suppression (e.g., >3-7 days). Also, consider the cells with which you will be working and how difficult they are to transfect, since siRNA oligos and shRNA viral vectors are easier to deliver into cells than are shRNA plasmids.

2. **Perform appropriate positive and negative controls**
   Some researchers fail to perform appropriate positive and negative controls for RNAi-mediated gene silencing experiments. These should include scrambled siRNA sequences and transfection reagent alone for negative controls, and validated siRNA's against unrelated genes for positive controls. Studies lacking such controls will typically not be acceptable for publication.

3. **Minimize exposure to RNases.**
   RNases are everywhere, and they can easily ruin your study. Therefore, extra precautions need to be taken to avoid RNase contamination of precious siRNA reagents. Typical precautions include pretreatment of all glass and plastic ware with a validated RNase inhibitor, and the utilization of a designated RNase-free work area.

4. **Use a transfection reagent optimized for siRNA delivery.**
   Most available transfection reagents are optimized for plasmid DNA, not siRNA. Therefore, transfection efficiencies for siRNA can be suboptimal, especially when transfecting primary cells. Use of a siRNA-specific transfection reagent, such as the **GeneSilencer® siRNA Transfection Reagent**, is the best way to ensure efficient siRNA delivery in a wide range of cells, with minimal cytotoxicity.

5. **Use proper cell culture technique for obtaining optimal transfection efficiency.**
   Just as with plasmid transfection, siRNA transfection is generally optimal when cells are at their healthiest. This means maintain optimal culture conditions, minimize stress, avoid contamination, and transfect at mid-log phase (typically 50-70% confluency for adherent dividing cells).

6. **Use previously validated siRNA sequences when possible.**

Don't reinvent the wheel! Numerous siRNA sequences for a wide variety of genes from many species have been validated and published in the literature. Also, there are libraries of validated siRNA sequences available from several companies. The internet is a valuable resource for identifying pre-validated siRNA sequences.

7. **If a validated siRNA sequence for your target gene has not been identified, use siRNA generated against the entire target gene ORF.**
   Typically, identifying a 22 bp siRNA sequence that provides significant target gene knockdown (e.g., >70%) requires testing 2-4 different chemically synthesized siRNA oligos.
   This can be a serious drain on your lab's financial resources. That is why it is a good idea to use a system such as the **Dicer siRNA Generation Kit**, to produce a heterogeneous population of siRNAs against your entire target gene ORF (or a large portion thereof). This method is quicker and less expensive and it allows generation of multiple siRNA pools from one kit.

8. **BLAST your siRNA sequence to confirm only one gene is targeted.**
   It is important to confirm that your siRNA sequence is unique, so that you do not unintentionally knock down any closely related genes. This is easily done by using NCBI's free BLAST query available at: http://www.ncbi.nlm.nih.gov/BLAST/. Since siRNAs are exquisitely sensitive, only a single bp mismatch with the target sequence could significantly reduce, if not eliminate, target gene suppression and produce non-specific gene silencing.

9. **If you are unsure how much siRNA to use for a given experiment, start with a transfection concentration of 10-50 nM.**
   The amount of siRNA required to effectively silence a given gene varies significantly with the gene, the siRNA sequence, and the cell type. Therefore, optimization of siRNA concentration is usually necessary. Again, this assumes that you cannot find experimental details for your gene / cell type already worked out in the published literature or in manufacturers' supplied literature.

10. **If the initial application of siRNA to your cells does not reduce the level of your target protein sufficiently, consider repeating the transfection 2-3 times on the same cells over several days.**
    Some proteins are abundant and long-lived in the cell. Thus, it may be necessary to apply siRNA to your cells two or three times to effectively reduce the amount of ambient target protein. Simply repeat the transfection 24-48 hours after the initial transfection, and then repeat after an additional 24-48 hours if necessary.