Considerations for Optimizing Non-Viral Transfections – Suspension Growing Cells

The efficiency of all transfection reagents depends on cell type to be modified, nature of polynucleotide to be delivered and cell culture conditions. The conditions for each cell type should be optimized to ensure maximal gene expression (or silencing) while minimizing cellular toxicity due to treatment. Non-viral protocols are attractive to induce transient changes with minimal chance of genotoxicity. Non-viral transfections are performed after complexing a polynucleotide with a transfection reagent in aqueous buffers. Here are some factors to consider for optimum transfection efficacy;

Considerations for Complex Formation

- Transfecting reagents, buffers and nucleic acids must be sterile and free from any contamination. If the solutions are to be filter-sterilized, final concentrations should be assessed for any loss during sterilization.
- All necessary materials should be warmed to room temperature before proceeding with experiments. Routine storage of reagents at 4 °C is recommended, while storage at –20 °C may be considered.
- All solutions must be well-mixed before formulating the complexes. Pipetting up and down is recommended for sensitive solutions, especially for long polynucleotides that might be shear-sensitive.
- Serum-free medium is recommended for formulation of complexes. The complexes are expected to be functional in serum-containing medium.
- The composition of complexes, specifically the ratio of transfection reagent to polynucleotide, need to be optimized for each application. The typical ratios range from 1:1 (w/w) for relatively toxic reagents to 5-20:1 (w/w) for biocompatible reagents. The nitrogen:phosphate (N/P; corresponding to N mole in transfection reagent to P mole in polynucleotides) ratio is another parameter that can be used to formulate complexes, but with proprietary reagents, this ratio is not readily available.
- Concentration of plasmids and mRNA in culture medium should be 0.5-2.0 µg/mL and 0.1-0.5 µg/mL, respectively, while siRNA concentration can be 20-80 nM. To account for non-specific effects, cells should be treated with a non-expressing (blank) plasmid or a scrambled (inactive) siRNA under identical conditions. It is typical to obtain non-specific effects even if no treatment toxicity is observed.
- The volume used to make transfection complexes could affect transfection efficiency. With smaller volumes (e.g., 20 µL), the complexes will have an increased chance for contact, resulting in aggregate formation and large complexes. With larger volumes (e.g., 200 µL), the complexes could be separated and remain in smaller sizes. There is no universal agreement about the role of complex size and transfection efficiency (this is likely to depend on specific cell type and endocytosis mechanisms) but care should be taken related to this issue.

Considerations for Cell Seeding

- Cell density should be optimized. We recommend employing 4,000 to 10,000 cells/well (96 well-plate) in case of attachment-dependent cells. As a rule of thumb, the lower the cell numbers, the greater the efficiency of transfection, but toxicity is accordingly higher.
- It is critical to transfect low passage cells for better efficiency. Make sure that the cells are sufficiently active and healthy (>90% viable). Cells grown to plateau phase (>90% confluency) generally show lower metabolic activity and reduced growth rate which might be passed to subsequent generations.

Considerations for Transfection and Incubation

- It is typical to incubate polynucleotides with transfection reagents for 20-30 min at room temperature to form the complexes. Complexes exhibit a dynamic behavior during this time (and beyond) so that care should be taken to maintain a reproducible incubation time.
- Complexes do not have to be removed following transfections; cells can be incubated with complexes until the end-point analysis. If excessive toxicity is seen, complex can be removed between 4-28 hours after the initial contact, and medium replaced with fresh medium.
- The possibility of both forward (cells added first, followed by complexes) vs. reverse transfection (complexes added first, followed by cells) should be considered. Transfection efficiencies might vary significantly between these modes. Little information exists on the role of tissue culture substrate on the transfection efficiency, and one is recommended to explore and identify a plasticware optimal for transfection purposes. This might be critical especially for reverse transfection protocols.
- Use of centrifugation may be considered. Centrifugation at a suitable speed (force) will enhance contact of complexes with cells and transfection efficiency. Care should be taken to ensure that the toxicity of complexes is minimized. This could be achieved by using reduced incubation times (e.g., 2-4 hours vs. 24 hours without centrifugation) when centrifugation is employed in the protocol.
- The time to end-point is critical to fully reveal differences among the treatments. Cells should be actively growing during the assay period without reaching a 'plateau' phase. This provides a strong 'dynamic range' for analysis of transfection outcome.