Nucleic Acid Delivery Methods And Their Applications in RNAi

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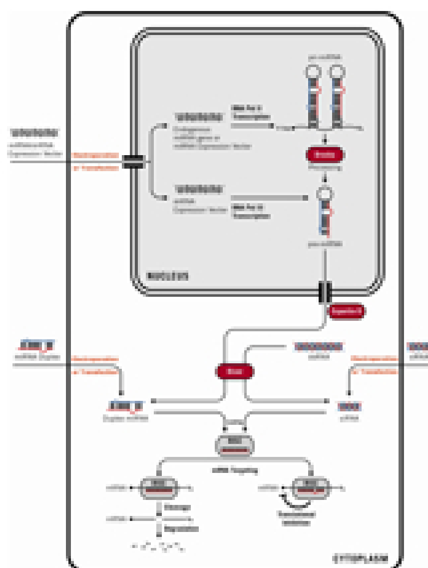
Abstract

RNAi technology based applications are invaluable tools used to elucidate cellular pathways and gene function. Small interfering RNA (siRNA) and microRNAs (miRNAs) are critically involved in RNAi pathways and inhibit target gene expression by either promoting mRNA cleavage or inhibiting translation. The targeted and effective inhibition of the gene expression makes RNA interference an important technique for gene function analyses.

Mirus Bio has developed a variety of transfection and electroporation reagents to deliver siRNA/miRNA into eukaryotic cells. TransIT-TKO® and TransIT-siQUEST® are low toxicity reagents that efficiently deliver siRNA/miRNA into cultured mammalian cells. The Ingenio™ electroporation solution has been designed to facilitate efficient and reliable delivery of nucleic acids to eukaryotic cells, including those that are traditionally resistant to chemical transfection. The reagent is compatible with multiple instruments including BioRad GenePulser® and the Amaxa Nucleofector® and facilitates a wide range of applications requiring nucleic acid delivery to cells.

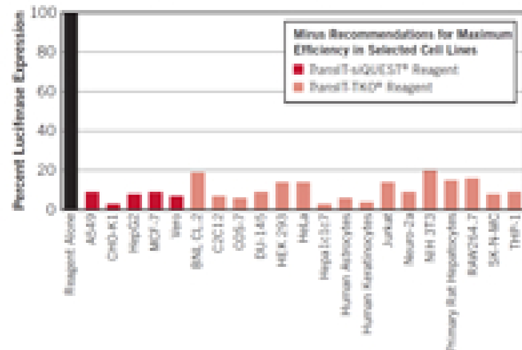
1. RNAi delivery and pathways



1. RNAi is triggered by small double-stranded RNAs (dsRNAs), either introduced into the cell by transfection or electroporation (siRNA or duplex miRNA), or arising from nuclear transcripts containing a stem-loop structure (pri-miRNAs). Pri-miRNAs are processed by the RNAse III-like enzymes Drosha in the nucleus and Dicer in the cytoplasm to yield mature miRNAs. siRNAs can also be generated by Dicer-dependent cleavage of long dsRNA. Both siRNAs and miRNAs can inhibit expression of target mRNAs by promoting mRNA cleavage or inhibiting translation.

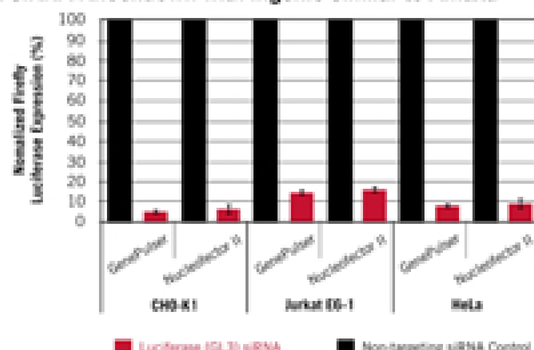
Results and Discussion

2. Transfection of siRNA with TransIT-siQUEST and TransIT-TKO



2. Firefly and sea pansy luciferase reporter vectors were co-transfected into various cell lines. Subsequently, firefly luciferase expression was knocked down by transfection of 25 nM anti-firefly luciferase siRNA using either the TransIT-siQUEST (red) or TransIT-TKO (pink) Transfection Reagent. Bars indicate the percent of normalized firefly luciferase expression as compared to the reagent alone control 24 hours post-transfection.

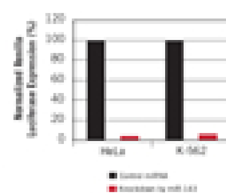
3A. siRNA Knockdown with Ingenio similar to Amaxa



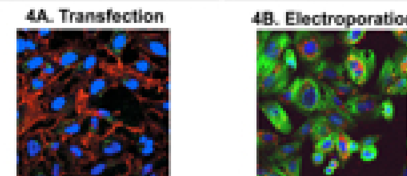
3A. siRNA and plasmid DNA were co-electroporated with Ingenio Electroporation Solution using either the Gene Pulser Xcell (Bio-Rad) or the Amaxa Nucleofector II (Lonza). Luciferase activity data from three different days were averaged and normalized to non-targeting siRNA control.

3B. miRNA Knockdown with Ingenio

psiCHECK2 plasmid (Promega) cloned with target sequence for miR-143 was co-electroporated with miR-143 microRNA (Ambion) or non-targeting control microRNA in Ingenio Electroporation Solution using the Gene Pulser Xcell. Cells were assayed using the Dual-Luciferase System (Promega). Activity is represented as a percentage of knockdown relative to the control.



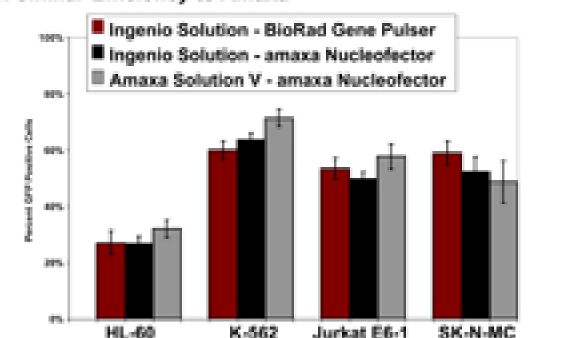
Localization of siRNA Delivered by Transfection or Electroporation



4A. Label IT® Fluorescein RNAi Delivery Control (green) was transfected using the TransIT-TKO Transfection Reagent. Cells were fixed then counterstained to locate the nuclei (blue) and actin (red).

4B. Cy3-labeled noncoding control siRNA (red) was electroporated into cells using Ingenio. Cells were fixed and counterstained for nuclei (blue) and actin (green).

High Efficiency Plasmid Delivery into Hard to Transfect Cells



5. Cells were electroporated in parallel with an EGFP reporter vector using either the Gene Pulser Xcell (Bio-Rad) or the amaxa Nucleofector™ II (Lonza). EGFP expressing cells were identified 24 hours post-electroporation by flow cytometry and presented as a percentage of the live cell population. Experiments were performed in triplicate on three separate days and the data averaged.

Conclusions

Mirus Bio LLC. provides a number of methods for delivery of many nucleic acids, including small RNAs, even in cells which are traditionally resistant to chemical transfection.

- **TransIT-siQUEST**
  - **Broad spectrum, high efficiency siRNA and microRNA transfection**
  - **Low toxicity**
- **TransIT-TKO**
  - **siRNA, microRNA, and plasmid DNA transfection**
  - **Can be used in standard or reverse transfections**
- **Ingenio Electroporation Solution**
  - **Compatible with any electroporation instrument including Gene Pulser Xcell, Amaxa Nucleofector, and BTX**
  - **One solution that delivers plasmid DNA, siRNA and microRNA**