siPOOL™: Fast, Reliable Gene Silencing With Exceptional Target Specificity Using Optimally-Designed High Complexity siRNA Pools

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RNA interference (RNAi) is widely used as a gene silencing tool to determine gene function. This is due to its ease of application, broad cell type applicability, drug-like properties and quick time to results. However, it is widely established that synthetic RNAi mediators, short interfering RNAs (siRNAs), can produce wide-spread off-target effects. This gives rise to highly variable results, necessitating time-intensive and costly validation efforts with multiple siRNA reagents. False positive results from siRNA off-targeting may also incur significant costs if left undetected. siPOOLs are complex pools of 30 optimally-designed siRNAs against a single gene. The high complexity pooling reduces the concentration of individual siRNAs, diluting siRNA-specific off-target effects. In contrast, target gene knock-down efficiency is increased due to the greater transcript coverage offered by a siPOOL. As a result, loss-of-function phenotypes become more robust and reproducible. siPOOL rescue constructs were also demonstrated to work efficiently to restore gene function. siPOOLs are ideal tools to ascertain gene function quickly and reliably and can be used in high-throughput RNAi-based screens to identify novel targets across various cell-based systems.

RNA Interference With Existing Reagents Produce Unreliable Results

Benefits of RNAi

- **Fast (Results in Days, Not Months)**
- **Dose-Dependent (Drug-Like)**
- **Transient (Avoids Adaptation)**
- **Simple to Apply (No Need For Engineered Cell Lines)**
- **Broadly Applicable in Various Biological Context**

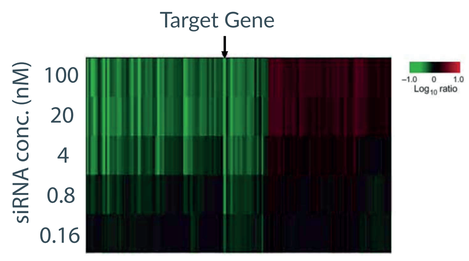
Drawbacks of RNAi (with Existing Reagents)

- **Variable Results Requiring Costly and Time-Consuming Validation Efforts**

siPOOLs Greatly Reduce Off-Target Effects and Improve Result Reliability

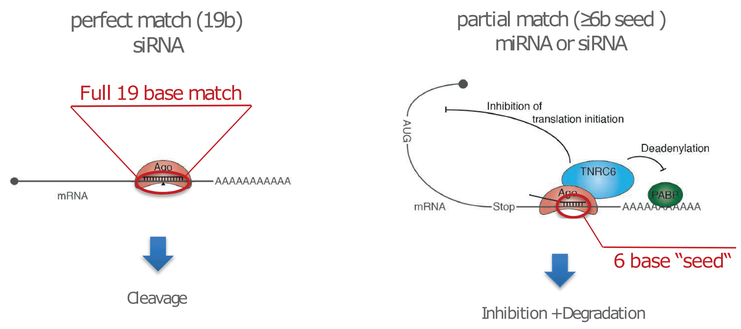
The Main Problem: siRNA-induced Off-Target Effects

Wide-Spread Gene Deregulation by siRNAs



An siRNA can have wide-spread concentration-dependent off-target effects (Jackson et al., 2003)

Mechanism of siRNA-induced Off-target Effects

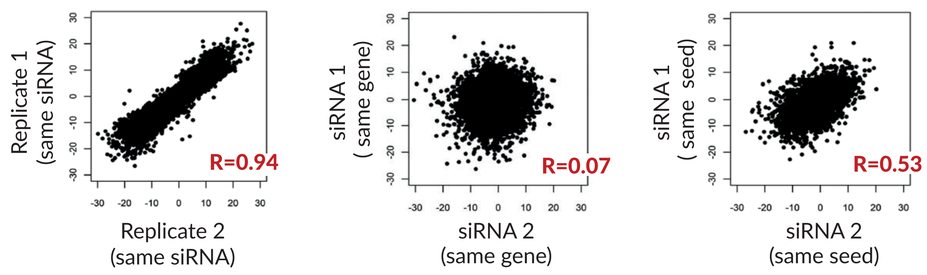


siRNA off-target effects are largely derived from siRNAs mimicking microRNAs, downregulating transcripts via a 6-base seed recognition sequence.

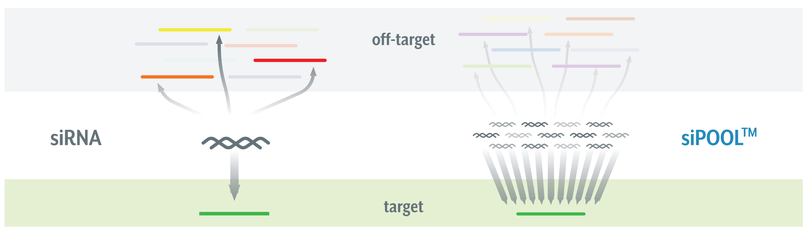
Dominance of siRNA Off-Targets Demonstrated By Correlation Between siRNA-induced Phenotypes

Many genome-wide RNAi screens such as this one show two single siRNAs that target the same gene often produce discrepant phenotypes.

Strikingly, two siRNAs that share the same seed sequence but target different genes tend to produce more similar phenotypes. This indicates that seed-based off-target effects dominate most RNAi-based assays (Marine et al., 2006).



The siPOOL Concept

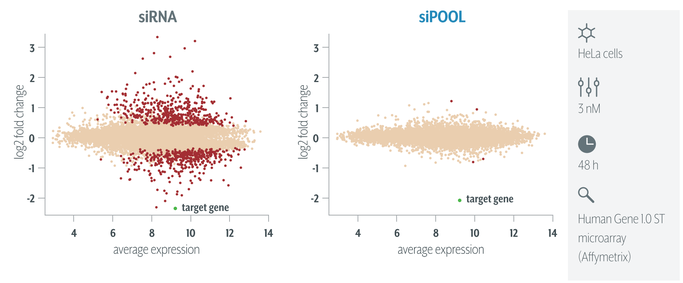


1 siRNA
[on-target] = # nM
[off-target] = # nM

30 optimally-designed siRNAs
[on-target] = # nM
[off-target] = (# / 30) nM

- **Multiple Off-Targets**
- **Variable Target Knock-down**
- **Greatly Reduced Off-Targets**
- **Co-operative Target Knock-down**

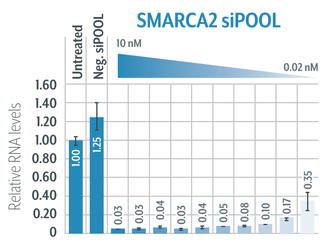
Exceptional Targeting Specificity with siPOOLs

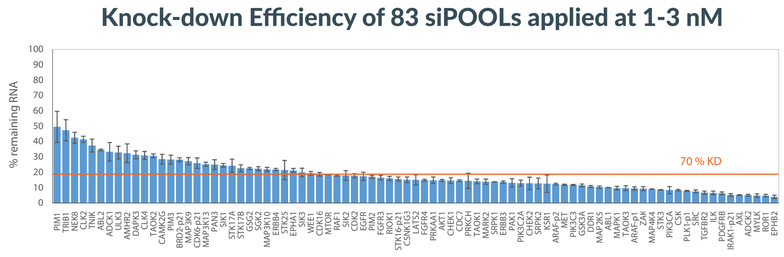


Transcriptome-wide profiling revealed a single siRNA can deregulate numerous off-target genes (red dots) while a siPOOL against the same target gene (green dot) and **containing the non-specific siRNA** had greatly reduced off-target effects (Hannus et al., 2014).

Efficient Gene Silencing at Low Concentrations with siPOOLs

Efficient at very low concentrations, siPOOLs can be multiplexed with other treatments or siPOOLs with minimal risk of side-effects.



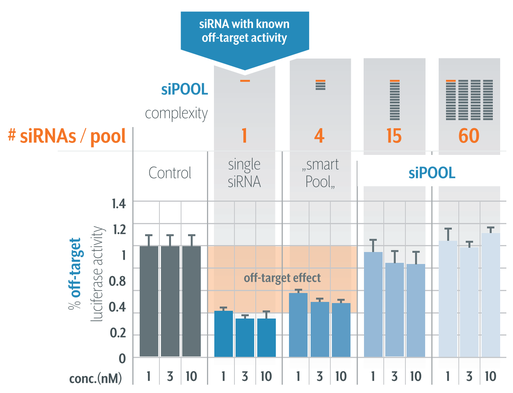


Why 30? Because 4 is not enough!

An siRNA with a known off-target was spiked into pools of increasing siRNA complexity. A luciferase reporter assay for off-target gene expression demonstrates a high complexity > 15 siRNAs was necessary for complete removal of off-target gene downregulation.

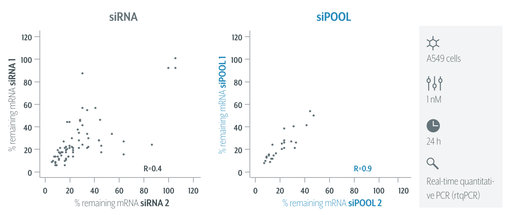
Similar results were obtained with other read-outs: Off-target protein expression & functional mitotic escape assay (Hannus et. al., 2014).

High Complexity siRNA Pools Required to Completely Remove Off-target Effect

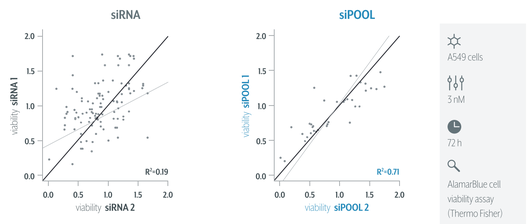


Reliable, Reproducible Phenotypes with siPOOLs

Correlation of Knock-down Efficiencies (rtqPCR)



Correlation of Phenotypes (Cell Viability)



References **Jackson et al.** (2003) Expression profiling reveals off-target gene regulation by RNAi. Nat. Biotechnol. 21, 635–637; **Marine et al.** (2012) Common seed analysis to identify off-target effects in siRNA screens. J. Biomol. Screen. 17, 370–8; **Hannus et al.** (2014) siPools: highly complex but accurately defined siRNA pools eliminate off-target effects. Nucleic Acids Res. 42, 8049–61