RNAi Screening Results You Can Trust with siPOOL (siRNA pool) libraries

Improve and economize your RNAi screening experiments

✓ Each gene within a siPOOL library is targeted by 30 pooled siRNAs

Why RNA interference?

RNA interference (RNAi) is widely used as a gene silencing tool to determine gene function.
This is due to its ease of use, wide applicability to cell types, drug-like properties, and quick results.
However, it is known that synthetic RNAi mediators, short interfering RNAs (siRNAs), can produce widespread off-target effects.
This leads to varying results, which require time-consuming and costly validation work with other siRNA reagents.
With highly complex siRNA pools, siPOOLs for short, off-target effects are minimized and RNAi becomes a reliable and fast method to determine gene functions.

How siPOOLs improve specificity

siPOOLs are complex pools of 30 optimally designed, distinct siRNAs.
The high complexity pooling approach leads to a reduction of the concentration of each individual siRNA, resulting in dilution of the siRNA-specific off-target effect.
In contrast, the efficiency of the gene knockdown is increased by a siPOOL due to the greater transcript coverage.
As a result, loss of function phenotypes become more robust and reproducible.

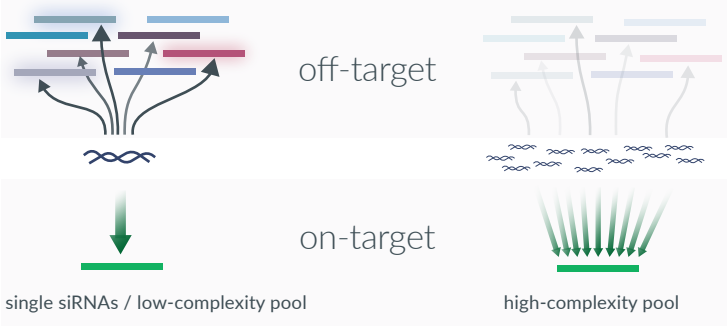


Figure 1: Left: single siRNAs and low-complexity pools of siRNAs are used at relatively high concentration to achieve an efficient knockdown. However, high concentration can lead to wide-spread off-target effects giving rise to highly variable results. **Right:** siPOOLs are a pool of 30 selected siRNAs with diverse seed sequences enabling maximal gene target coverage. The concentration of each siRNA is lowered due to the pooling approach. The low conentration leads to minimization of the off-target effect and improve silencing robustness.

Support & Expertise

We provide excellent and competent scientific support during RNAi assay development and screening.
Our team consists of experts with > 20 years of RNAi screening experience.
Our bioinformatics team has in-depth expertise in siRNA design and RNAi screening data analysis (both siRNAs and shRNAs-based screening).

Never screen with outdated siRNA libraries!

siPOOL libraries are up to date !

To ensure that siPOOLs target currently annotated genes, we continuously update siPOOL designs based on the latest RefSeq annotations.

Product	Species	Cat. No.*
E3 Ligase siPOOL library	human	si-L010-000E3L
Kinase siPOOL library	human	si-L010-000505
RNA-binding protein siPOOL library	human	si-L010-000RBP
GPCR siPOOL library	human	si-L010-00GPCR
Ubiquitinase siPOOL library	mouse	si-L010-000UBI

*Cat. No. shown for 1 nmol scale

Format Information

1. siPOOL Scales

0.1 nmol	0.25 nmol	0.5 nmol	1 nmol
Catalog-No. si-L001-XXXXXX	Catalog-No. si-L002-XXXXXX	Catalog-No. si-L005-XXXXXX	Catalog-No. si-L010-XXXXXX

2. siPOOL layout

2D barcoded tubes	96-well plates*	384-well plates*

*customizable plate layout possible

Finding true hits with RNAi

Off-target effects dominate RNAi screens

siRNAs exhibit high reproducibility of technical replicates. However, when comparing two different siRNAs targeting the same gene, the reproducibility is weak. Intriguingly, much better correlation can be seen with siRNAs having identical seed sequences but targeting different genes. Meaning, the seed sequence of siRNAs is crucial for targeting genes.

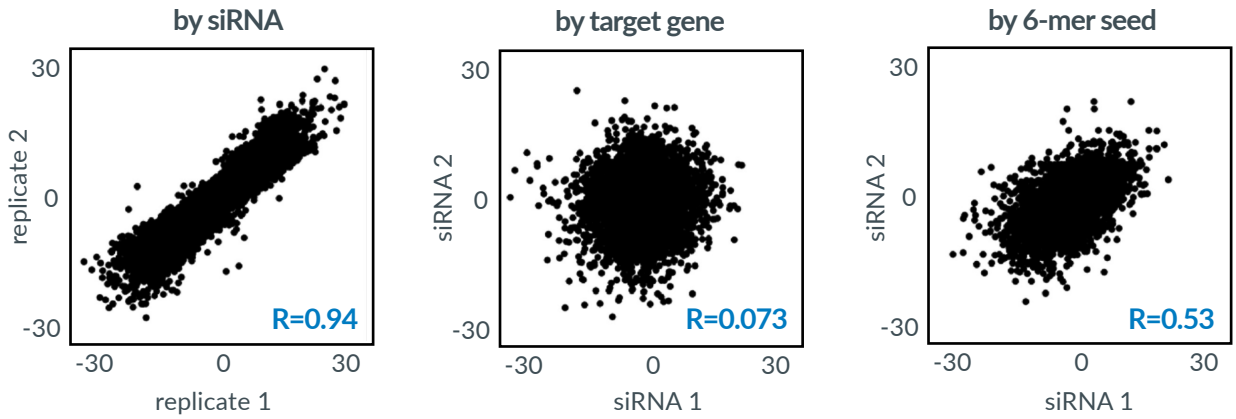


Figure 2: Seed-based off-target effects dominate RNAi screens. Sources of RNAi screening variance in RNAi screening data are shown. Published by Marine et al., J.Biomol. Screening. 2012. **Left:** Correlation of technical replicates (replicate 1 and 2). **Middle:** Correlation between different single siRNAs designed against the same genes. **Right:** Correlation of single siRNAs with same seed sequence but are designed to target different genes.

Complexity is key

Seed-based off-target effects are a known flaw of RNAi experiments. The strong off-target effects of siRNAs result in weak reliability and false-positive phenotypes. The off-target effect can be minimized by pooling siRNAs in high-complexity pools. The high-complexity pooling approach enables reliable RNAi experiments.

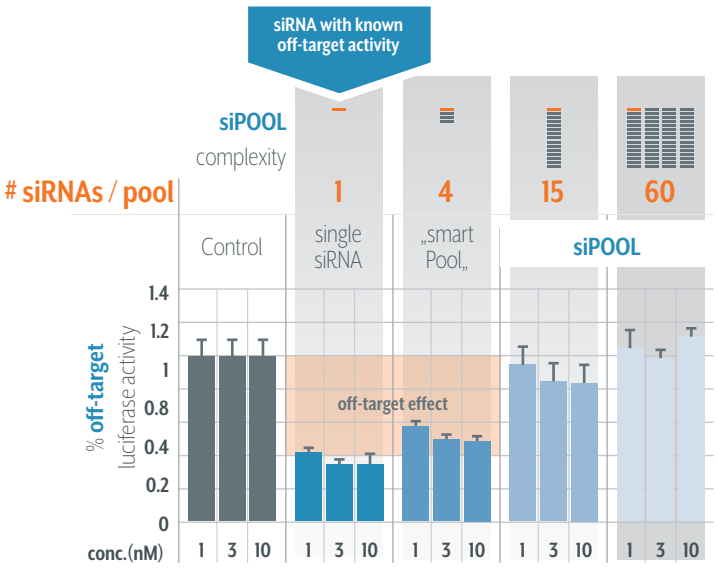


Figure 3: Complexity is key – 4 siRNAs may not be enough. We show here that an siRNA with known off-target activity against MAD2 gene required high complexity pools of > 15 siRNAs to sufficiently reduce off-target effects. Similar results were obtained when off-target activity was assayed by a MAD2 3'UTR-linked luciferase reporter, MAD2 protien expression and MAD2 functional assay (mitotic escape).

Minimal off-target effects with siPOOLs

Single siRNAs and low complexity pools of siRNAs show high off-target effects. Off-target effects result in variable RNAi knockdown efficiency and phenotypes.
siPOOLs overcome the off-target effect enabling clean RNAi knockdown experiments.

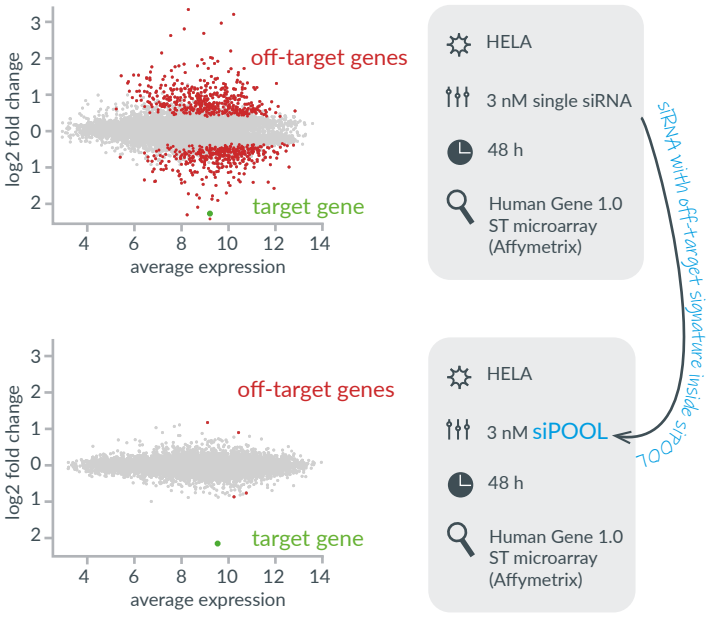


Figure 4: Minimal off-target effect. Transcriptome-wide profiling revealed a single siRNA can induce numerous off-target genes (red dots) while a siPOOL against the same target gene (green dot), and containing the non-specific siRNA, had greatly reduced off-target effects.

Use high-complexity pools of siRNAs for RNAi screens!

✓ Potent & specific RNAi Knockdown ✓ 30 siRNAs in one siPOOL ✓ Reliable phenotypes

Minimal-effort RNAi

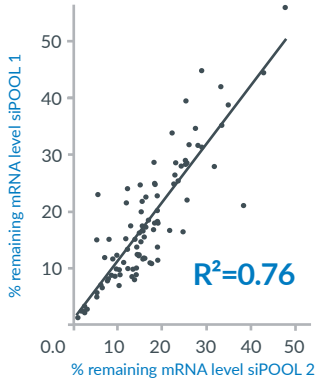
siPOOLs result in efficient gene silencing at low nanomolar concentrations

Efficient at very low concentrations, siPOOLs can be multiplexed with other treatments or siPOOLs without risk of side-effects (applicable for synthetic lethality screens). siPOOLs are highly likely to knockdown target gene at mRNA level by 70% when used under optimized transfection conditions.

Figure 5: siPOOLs result in efficient gene silencing at low nanomolar concentrations. 91% of human RBP siPOOLs tested (92 out of 101) produced ≥ 70% gene knockdown at 0,2 - 1 nM in standard cell lines (A549, RPE1, HeLa) as measured by rt-qPCR.

Best RNAi knockdown with minimal effort

A single siPOOL is sufficient to reliably silence the target gene.

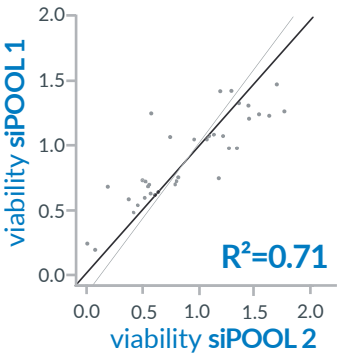


A549 HEK293 HUH7 MCF7 SW48
1 nM (96 genes)
24 h
rt-qPCR

Figure 6: Best RNAi knockdown with minimal effort. Two siPOOLs against the same gene (96 tested) give similar KD (R^2=0.76, compared to R^2=0.4 of single siRNAs). No need for screening multiple siRNAs; one siPOOL is sufficient.

Reliable phenotypes

A single siPOOL per gene is sufficient as siPOOLs produce similar phenotypes.



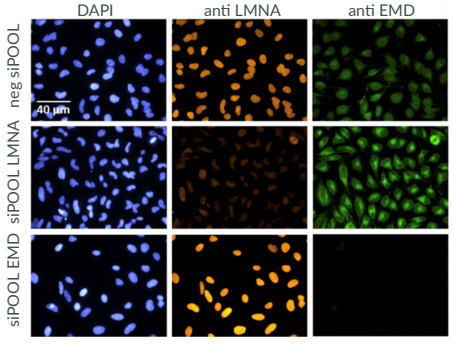
HELA
3 nM (36 genes)
72 h
cell viability image based read-out

Figure 7: Reliable phenotypes. Two siPOOLs against the same gene (36 tested) gave more reproducible phenotypes than single siRNAs (R^2=0.19).

Why RNAi? RNAi is penetrant, drug-like and quick - a CRISPR comparison

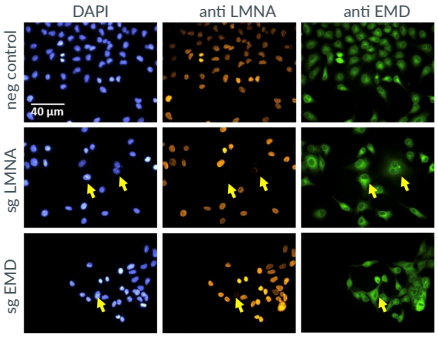
After only 48h the effects of RNAi are visible. Additionally, RNAi knockdown affects nearly all cells homogenously. CRISPR on the other hand is very time-intensive, shows rather low efficiency and off-target (compensatory) mutations are possible.

RNAi



A549
1 nM (siPOOLs)
48 h

CRISPR



A549
10nM (sgRNA)

Figure 8: Why RNAi? RNAi is penetrant, fast and quick. Left: RNAi experiment with siPOOLs targeting EMD, and LMNA. As control the siTOOLs standard neg control was used. Immunostaining: Lamin A (LAMNA) & Emerin (EMD) Hoechst staining for cell nuclei. Supported by the Bayerische Forschungsstiftung

Use highly complex siPOOLs for your RNAi screens!

- ✓ 30 siRNAs per target gene
- ✓ Potent & specific RNAi Knockdown
- ✓ Reliable phenotypes
- ✓ Ready-made & custom siPOOL libraries

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Follow us on social media

Please contact us or our distributors for pricing information.

siTOOLS BIOTECH

Shipping & Storage

siPOOL libraries are shipped at RT in solution. If preferred, siPOOL can be shipped as dry pellets. siPOOL libraries are stable to be shipped at RT for at least 4 weeks.

Upon arrival, siPOOL librares should be stored at -20°C to -80°C. Under these conditions siPOOL libraries are stable for at least two years.

If siPOOL libraries are shipped as dry pellets, siPOOLs should be resuspended in RNase-free water.

Upon resuspension (or arrival), we recommend to aliquot siPOOLs into small volumes and store at -20°C to -80°C. For best results, minimize freeze-thaw cyclesv. Under these conditions and under RNase-free handling, siPOOLs are stable for at least 2 years.

Literature

Hannus M. et. al.: siPools: highly complex but accurately defined siRNA pools eliminate off-target effects. Nucleic Acids Res 42(12): 8049-61(2014)

Marine S. et. al.: Common Seed Analysis to Identify Off-Target Effects in siRNA Screens. Journal of Biomolecular Screening 1-9 (2011)

Jackson A. et. al.: Expression Profiling reveals off-target gene regulation by RNAi. Nature Biotechnology (2003)