Bundesministerium für Wirtschaft und Energie

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siTOOLs BIOTECH

siPOOLs: Complex and Defined siRNA Pools for Specific and Potent Gene Knock-down

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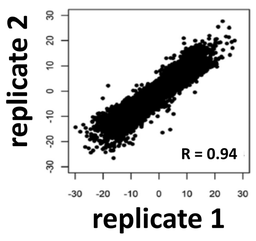
RNAi is an excellent method for gene function analysis: it is reversible, dose-dependent, versatile, fast and cost-efficient. These drug-like properties have made it the tool of choice for functional genomic screens. However, off-target effects dominate published RNAi screens and limit their yield to a small number of strongly responding, frequently well-established gene factors. siPOOLs are complex pools of 30 carefully selected siRNAs. The very low concentration of each individual siRNA dilutes off-target effects below detection. Target gene silencing becomes highly efficient and reliable due to the cooperative activity of the pooled siRNAs. siPOOLs therefore allow a far deeper interpretation of RNAi screening data, increasing the yield of novel targets and aiding the understanding of complex biology.

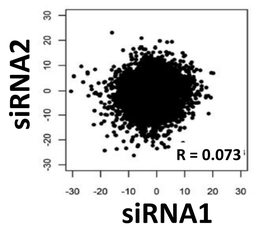
siRNAs: Noise from off-target effects

Correlation Analysis of Typical RNAi Screening Data



Single dot = **two replicate** values for **one siRNA**

- High correlation
- Great assay reproducibility

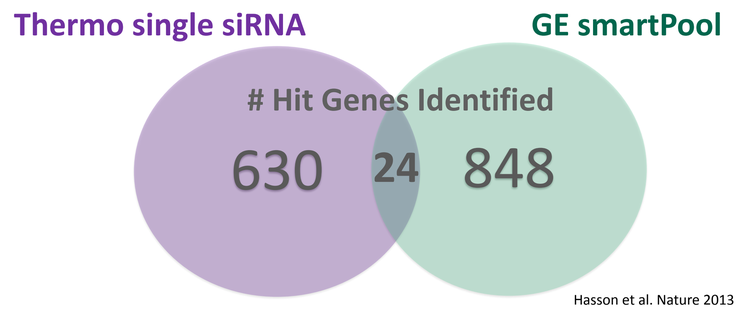


Marina et al., J. Biomol. Screen. 2012

Single dot = values of **two siRNAs** targeting **one gene**

- No correlation
- Non-interpretable results

RNAi Screening is Highly Inefficient



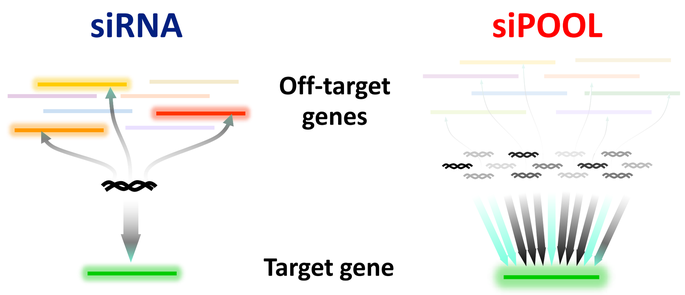
Hasson et al. Nature 2013

- Two siRNA libraries were screened in an identical assay by the same lab.
- Overlap between hits at noise level *(16 expected by chance).*
- Majority of hits expected to be false.

- Current RNAi reagents are plagued by off-target effects.
- RNAi screening with current reagents is highly inefficient and unreliable.

siPOOLs: Complex and defined siRNA pools

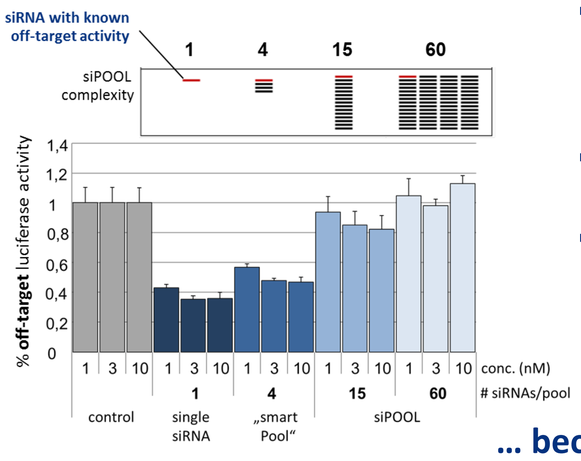
The siPOOL Concept



- An **siRNA** produces many off-target effects when administered alone ⇨ low specificity, unreliable results.
- A **siPOOL is 30 selected siRNAs**, each siRNA at picomolar concentrations ⇨ Minimal off-target effects, co-operative target knock-down, enhanced reliability.

Why 30 siRNAs?

Off-target spiking experiment



- An **siRNA** with known off-target gene (MAD2) was spiked into pools of increasing complexity.
- Luciferase assay detects activity of off-target gene.
- Efficient off-target dilution requires pools of > 15 siRNAs.

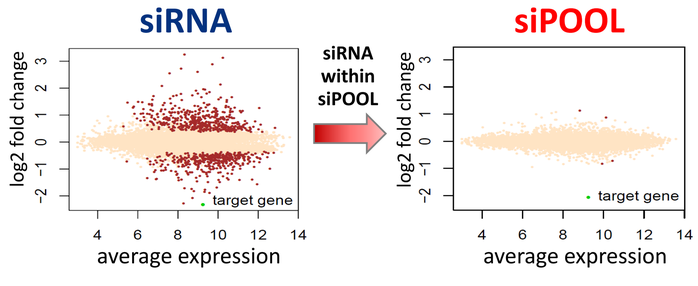
… because 4 are not enough

- Complex pools of more than 15 siRNAs are required to prevent off-target effects.

siPOOLs: Specific and potent gene knock-down

Specificity

Whole genome expression analysis

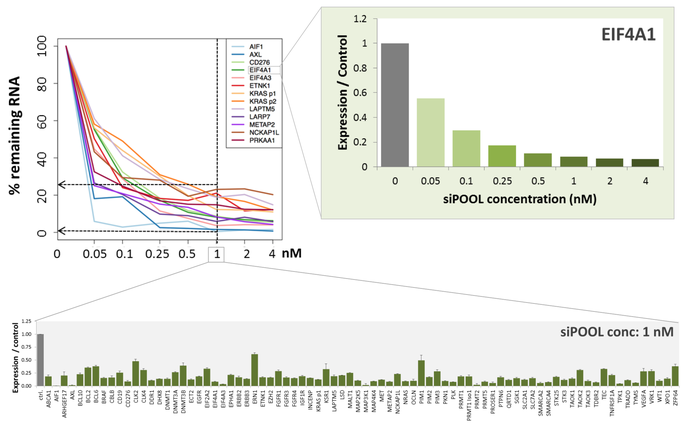


Hannus et al., Nucleic Acids Res. 2014

- An **siRNA** (left) induces massive off-target gene deregulation.
- The same siRNA incorporated into a **siPOOL** (right) shows dramatically reduced off-targets.

Potency

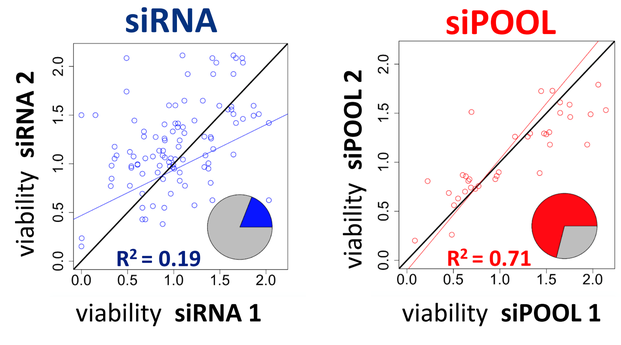
siPOOL-mediated gene knockdown: 1 nM is sufficient



- Most **siPOOLs** achieve 75-98% target gene knockdown at less than **1 nM**.

Reproducible Phenotypes

Phenotypic correlation study



- 36 genes were silenced with **3 siRNAs/gene** (left) or **2 siPOOLs/gene** (right).
- Cell viability was measured after 72h.
- Phenotypic correlation was greatly improved with siPOOLs.

- Specific and potent gene knock-down with siPOOLs ensures reproducible and reliable phenotypes.
- RNAi screening with siPOOLs produces high-quality hits.

References

1. Marine S, Bahl A, Ferrer M, Buehler E (2012). Common seed analysis to identify off-target effects in siRNA screens. *Journal of Biomolecular Screening*, *17*(3), 370–8.
2. Hasson SA, Kane LA, Yamano K, Huang CH, Sliter DA, Buehler E, Wang C, Heman-Ackah SM, Hessa T, Guha R, Martin SE, Youle RJ (2013). High content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy. *Nature*, 504(7479), 291-5.
3. Hannus M, Beitzinger M, Engelmann JC, Weickert MT, Spang R, Hannus S, Meister G (2014). siPools: highly complex but accurately defined siRNA pools eliminate off-target effects. *Nucleic Acids Research*, *42*(12), 8049–61.