RNA interference - The Transient Gene Silencing Method

www.sitoolsbiotech.com

siTOOLs BIOTECH

Overview

- siTOOLs Biotech: products and services
- Part 1: Why RNAi is so useful
- Part 2: Off-target effects are Seed-effects
- Part 3: Complex siRNA Pools
- Part 4: Using Seed effects

siTOOLs Biotech: Products and Services

siPOOL™ (RNAi)

High complexity pooled siRNA for reliable gene silencing

Custom siPOOLs

siPOOL libraries

raPOOL™ (Biochemistry)

High complexity pooled biotinylated probes for robust lncRNA capture

riboPOOL™ (RNAseq)

Complex biotinylated oligo pools for rRNA depletion probes

Services

Phenovault:
Seed base RNAi screen analysis

Experiment services

- Knock-down validation
- Transfection optimization
- Screening assay development
- High-content RNAi screening

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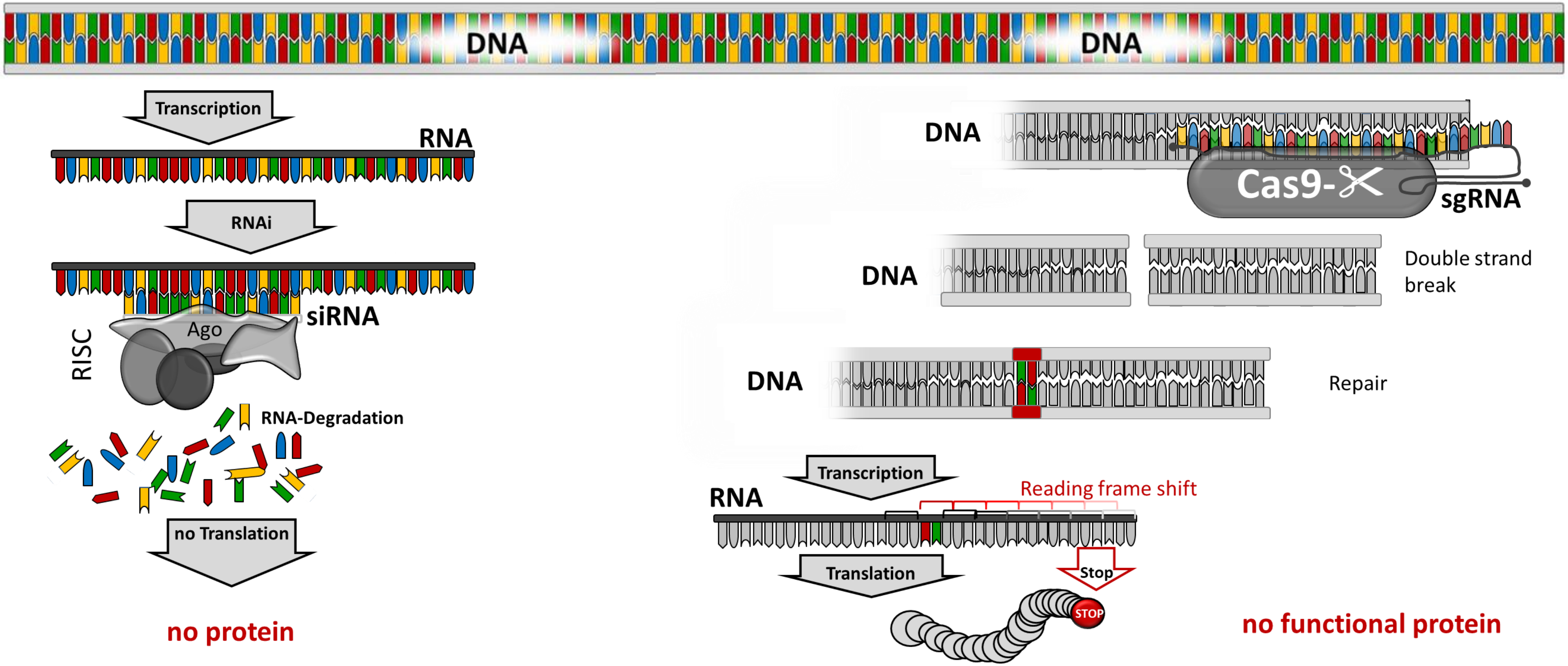
RNAi and CRISPR

RNAi

CRISPR/Cas

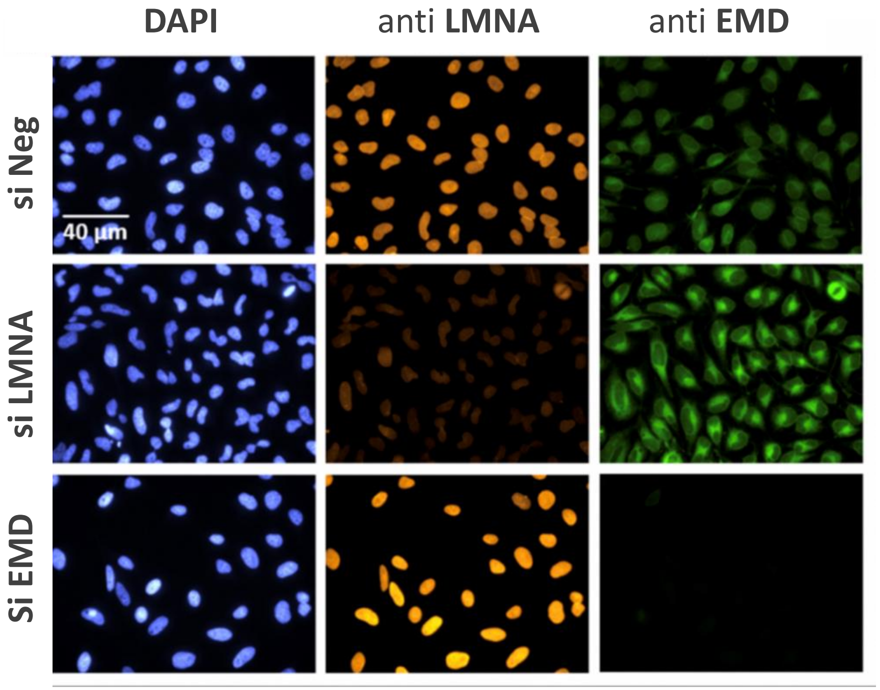


no protein

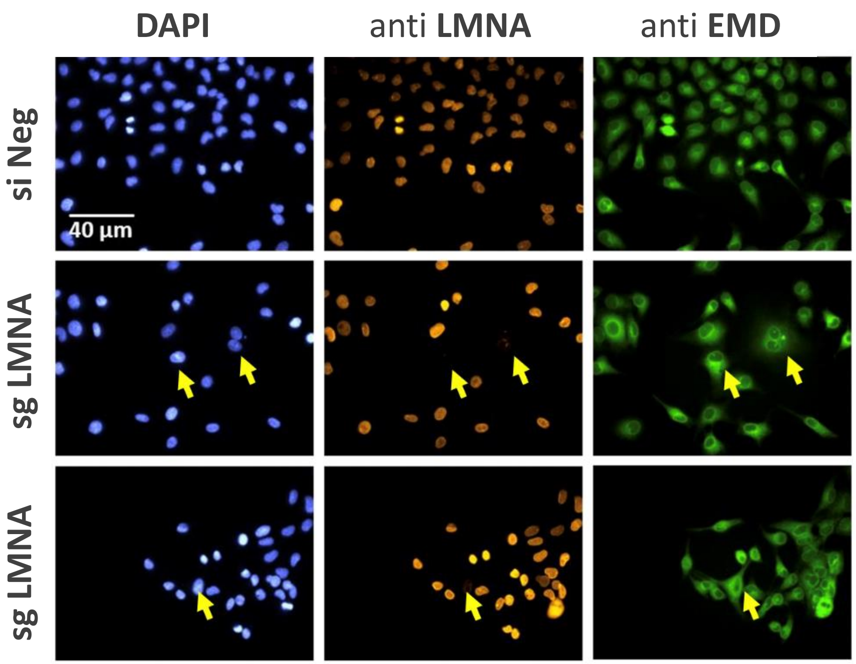
no functional protein

Case Study 1: RNAi is penetrant

RNAi (siPOOL)



CRISPR (sgRNA)



All cells show similar knock down

=> Uniform & penetrant effect

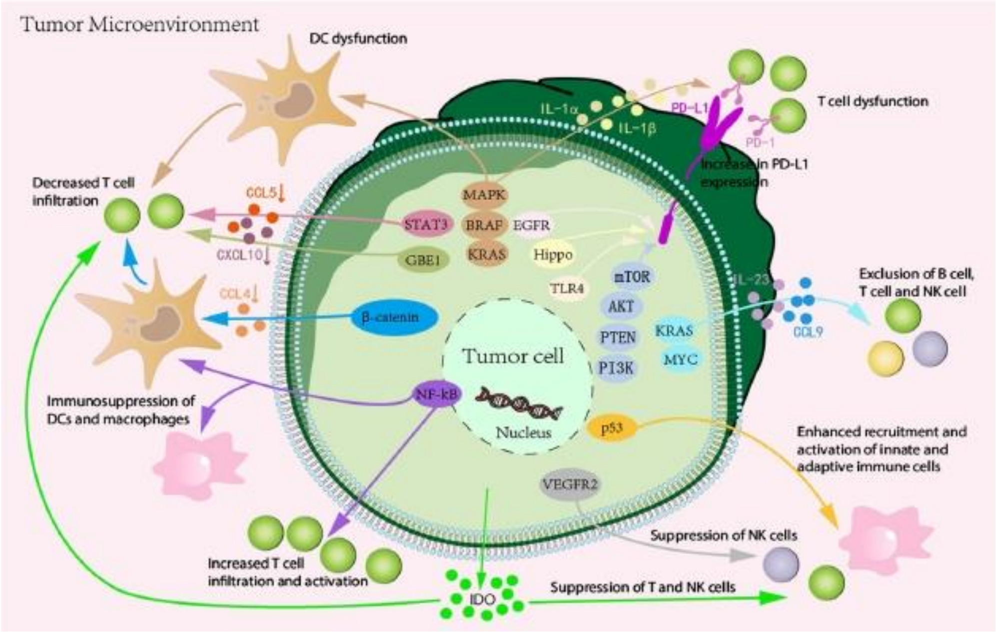
only few cells show gene knock-out

=> Very sensitive assay or cloning

Supported by the Bayerische Forschungsstiftung

Case Study 2: silencing multiple targets

How does cancer avoid T-cell infiltration?



Complicated assay setup:

- ➢ Two cell types:
  - T-cells
  - cancer cells
- ➢ Automation
  - Transfection
  - Live cell washing
- ➢ Maximize assay window
  - Suitable cancer cell line
  - Strong positive controls

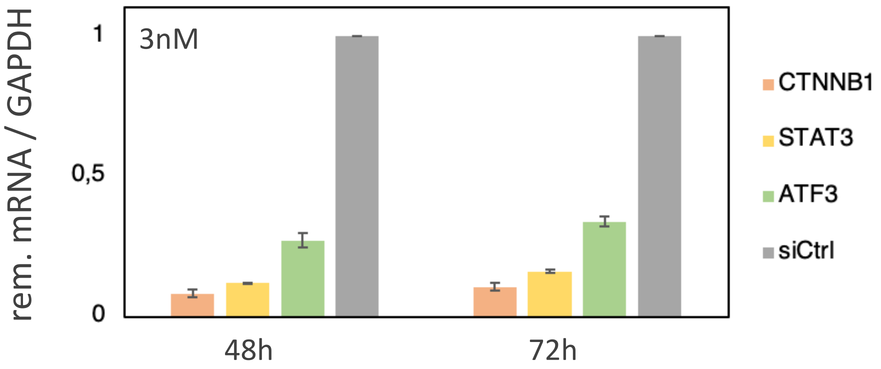
Additional Problem: Redundant escape mechanisms in cancer cells

⇒ co-silencing of multiple, essential genes

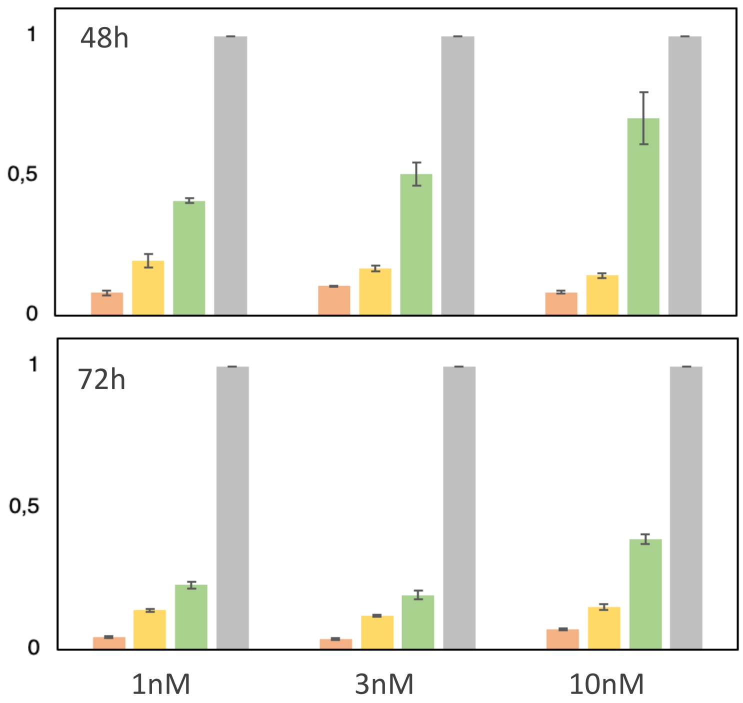
data by **Michaela Bauer**, AG **Gunter Meister**, University of Regensburg, Germany

Case Study 2: triple target knock-down

single target knock down



triple target knock down



⇒ Triple target KD similar to single target KD

⇒ Even with triple target, 1nM saturating

data by **Michaela Bauer**, AG **Gunter Meister**, University of Regensburg, Germany

Case Study 3: knocking down a virus

Silencing a virus: SARS-CoV-2 in Vero Cells

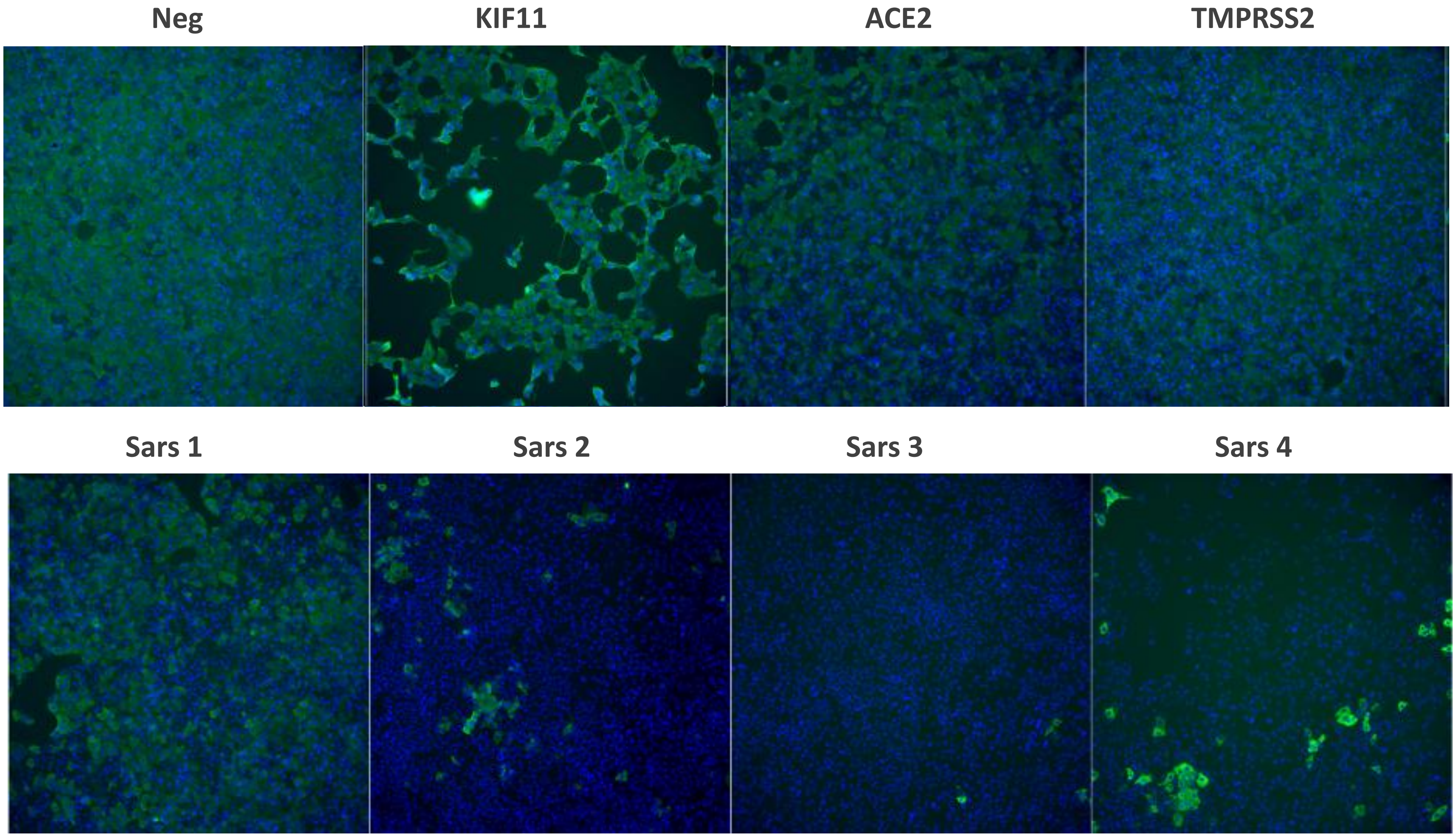


Image data by Stefan Finke, FLI Riems

Why RNAi

fast	=> days not weeks
simple	=> only 1 component
penetrant & homogenous	=> no cell cloning
Knock-down	=> essential genes
transient	=> no adaptation

Drug like

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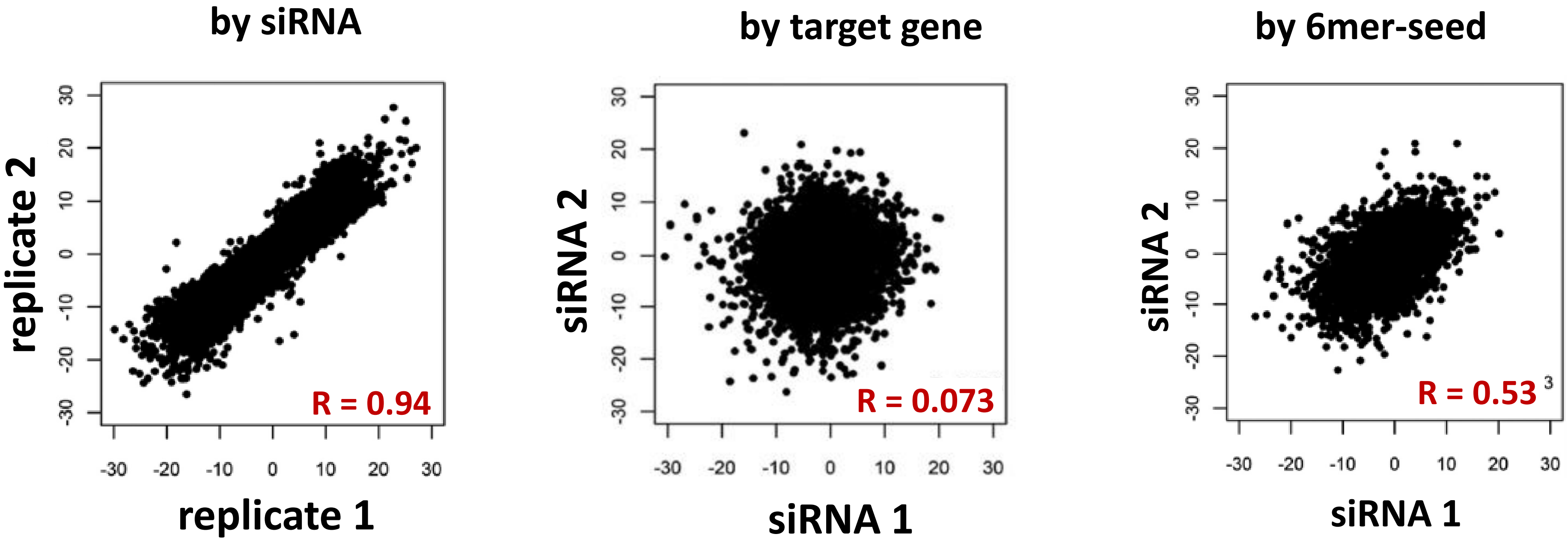
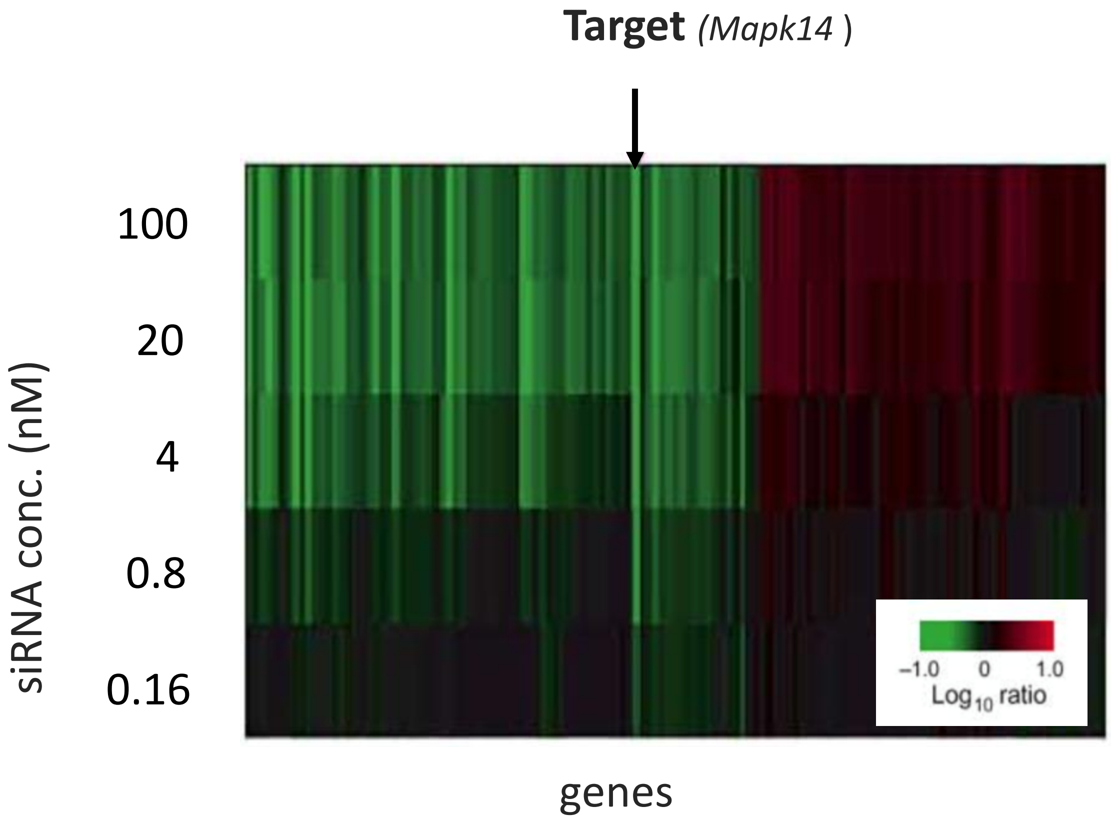
Off-target effects dominate RNAi screens



Marine et al., J.Biomol. Screening. 2012

siRNAs and miRNAs affect multiple genes

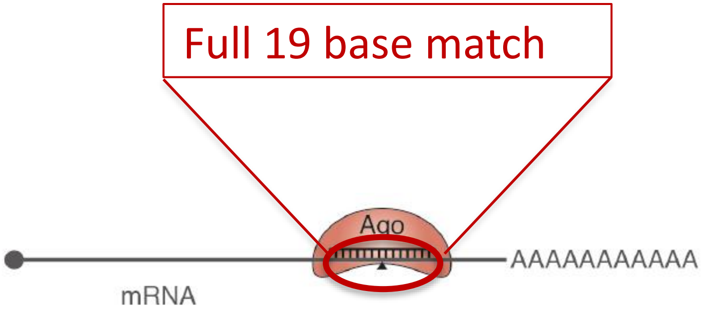


Jackson et al., Nat Biotechnol. 2003

The two faces of RNAi

perfect match (19b)

siRNA

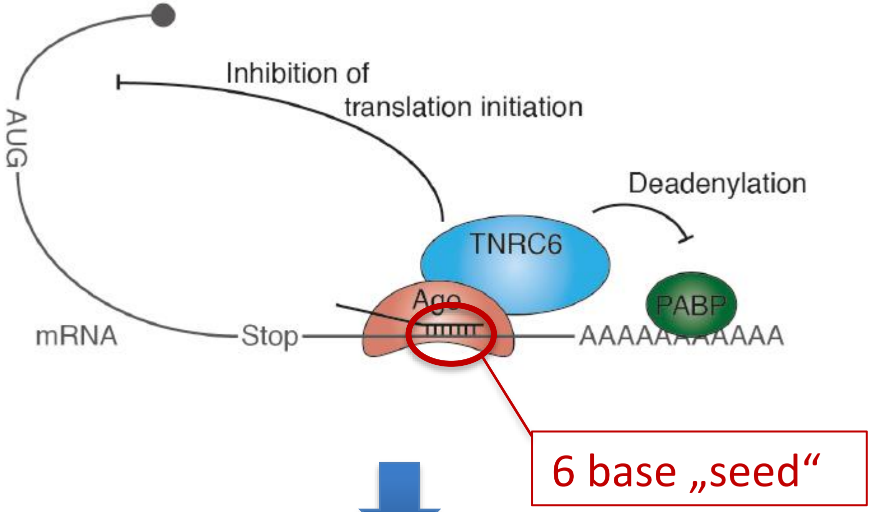


Full 19 base match

Cleavage

partial match (≥6b seed)

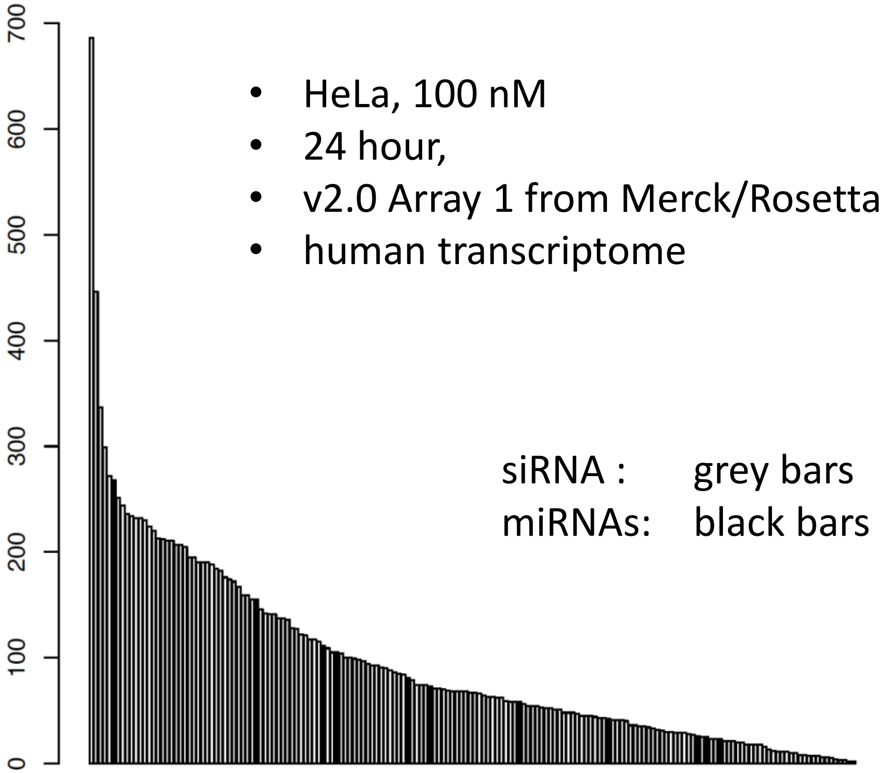
miRNA or siRNA



6 base „seed“

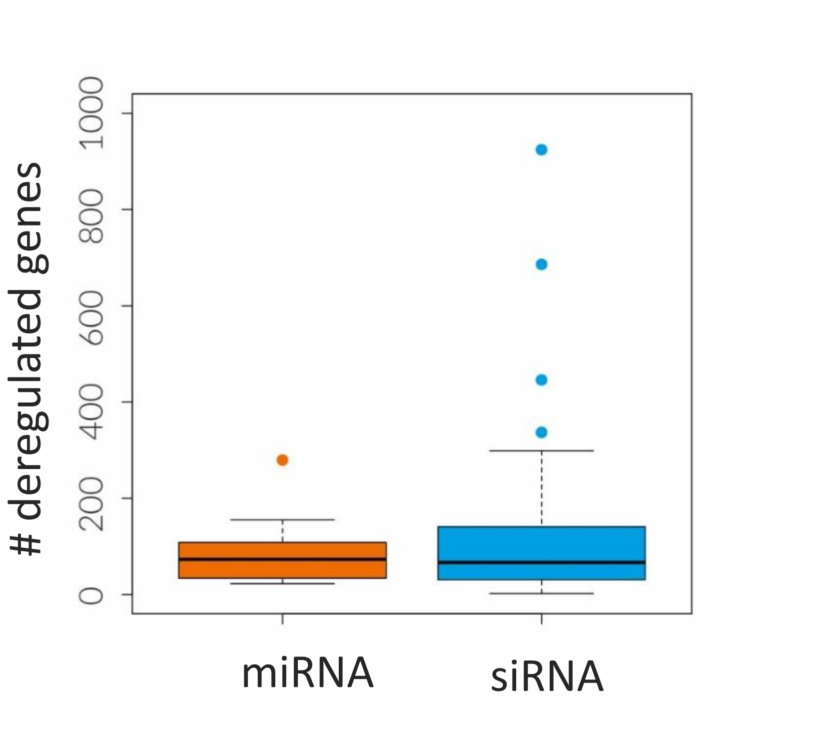
Inhibition + Degradation

siRNAs are artificial microRNAs



- HeLa, 100 nM
- 24 hour,
- v2.0 Array 1 from Merck/Rosetta
- human transcriptome

siRNA : grey bars
miRNAs: black bars

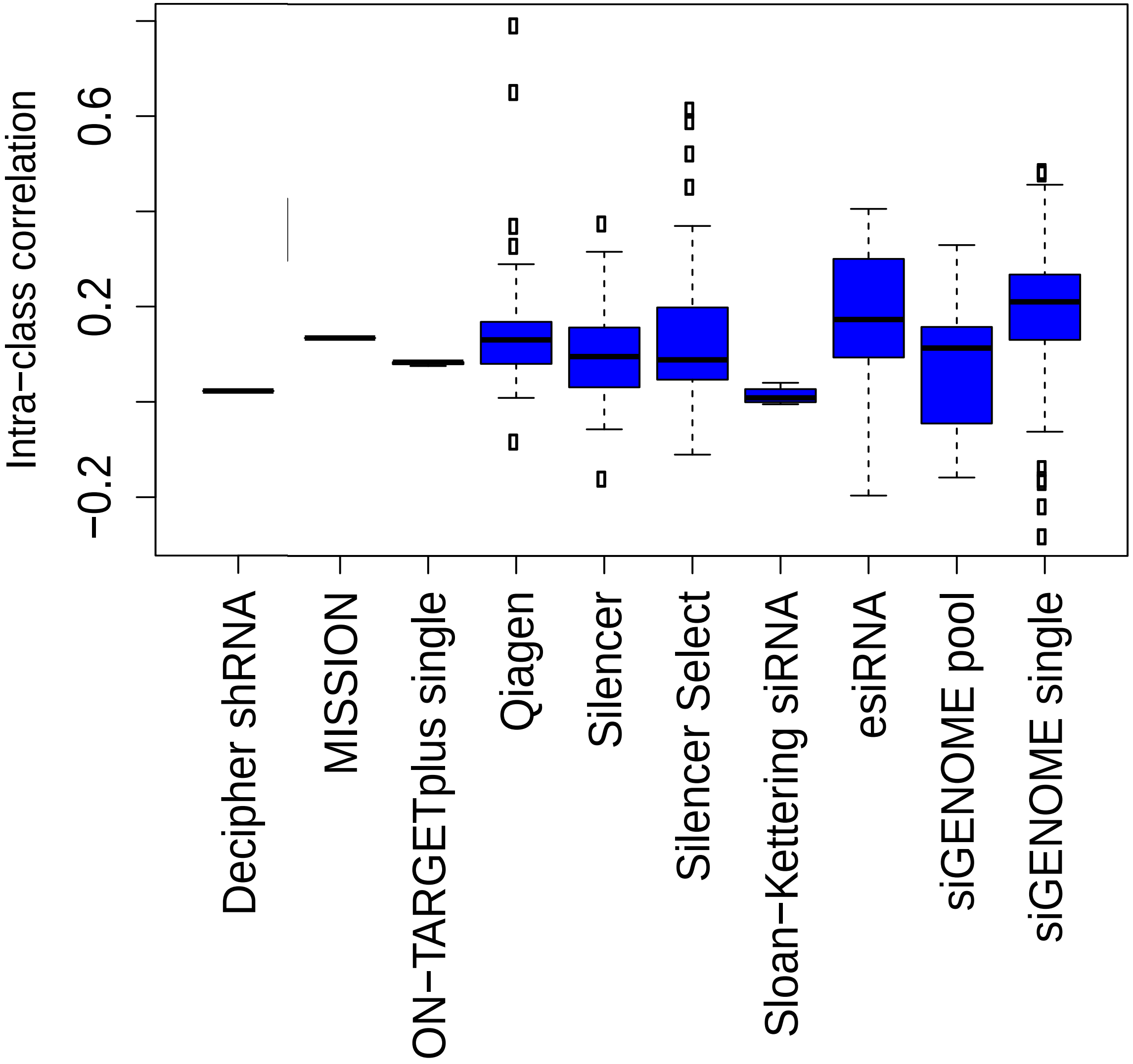


Garcia et al., Nat. Struct. Mol. Biol . 2011

⇒ siRNAs hit **multiple** off-target genes (median >60, average >100)

Commercial siRNA libraries

On-target siRNA correlation



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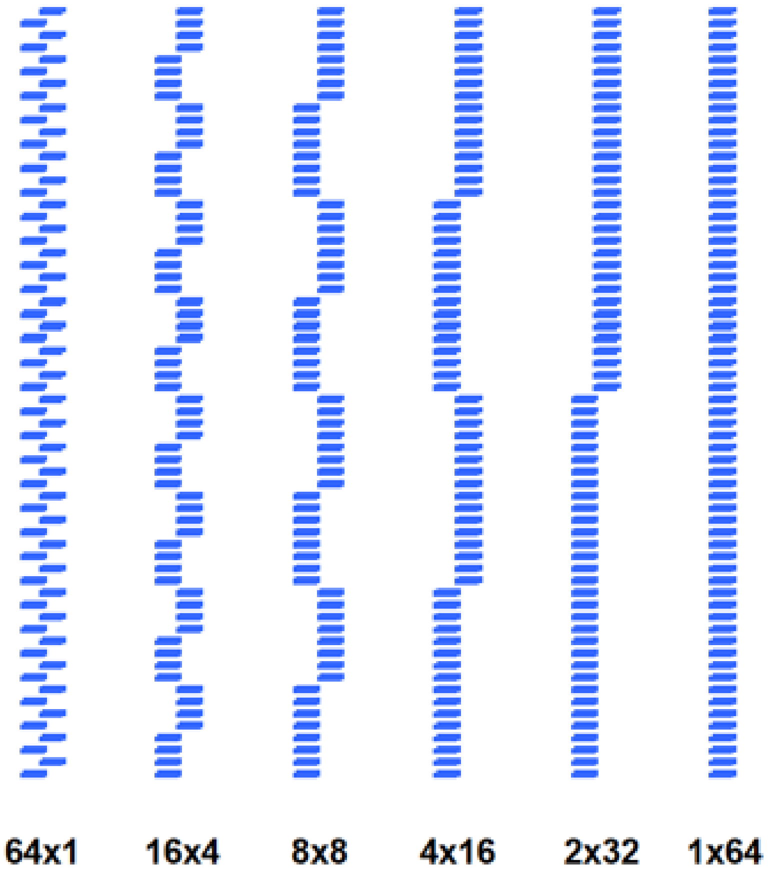
2006, The Vision: Complex siRNA Pools

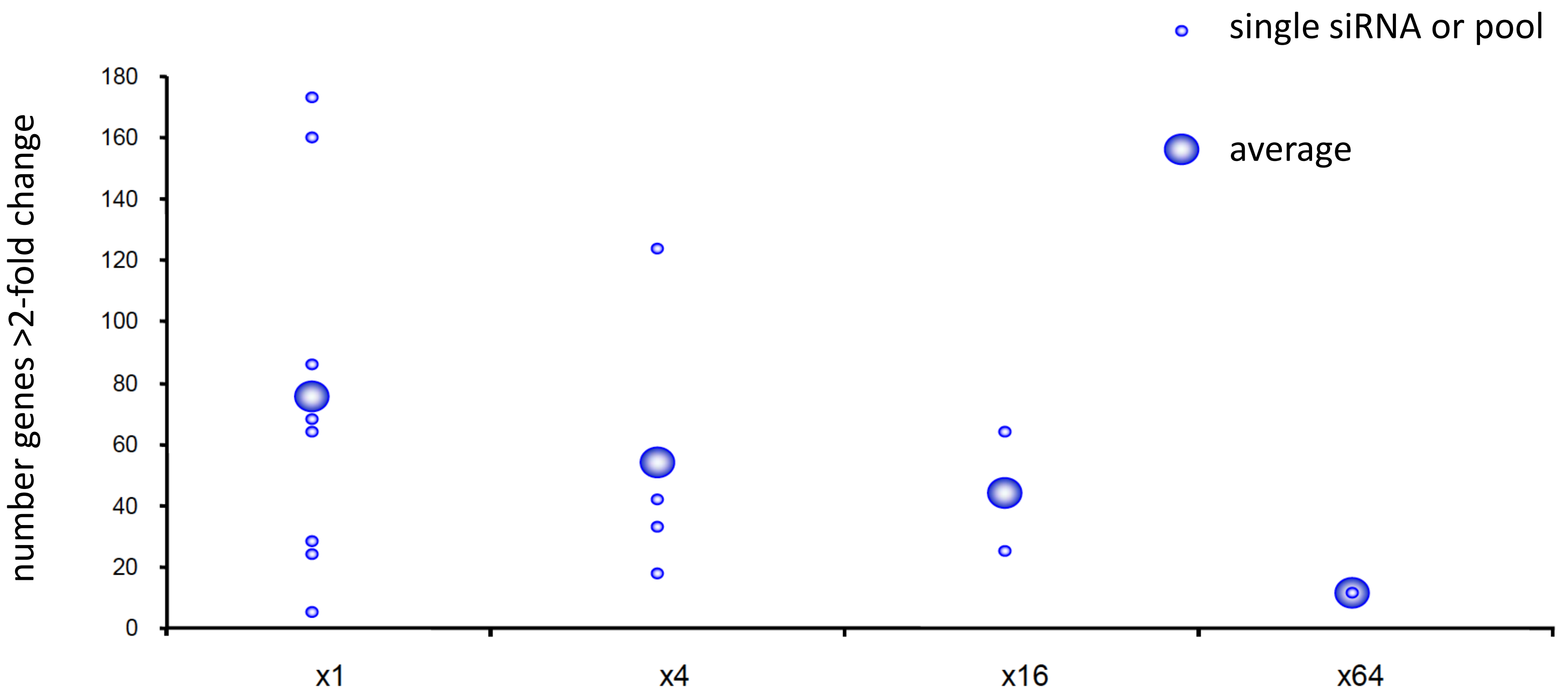
Dharmacon 2006:

Combining siRNAs to pools



Specificity improves with Pool Complexity

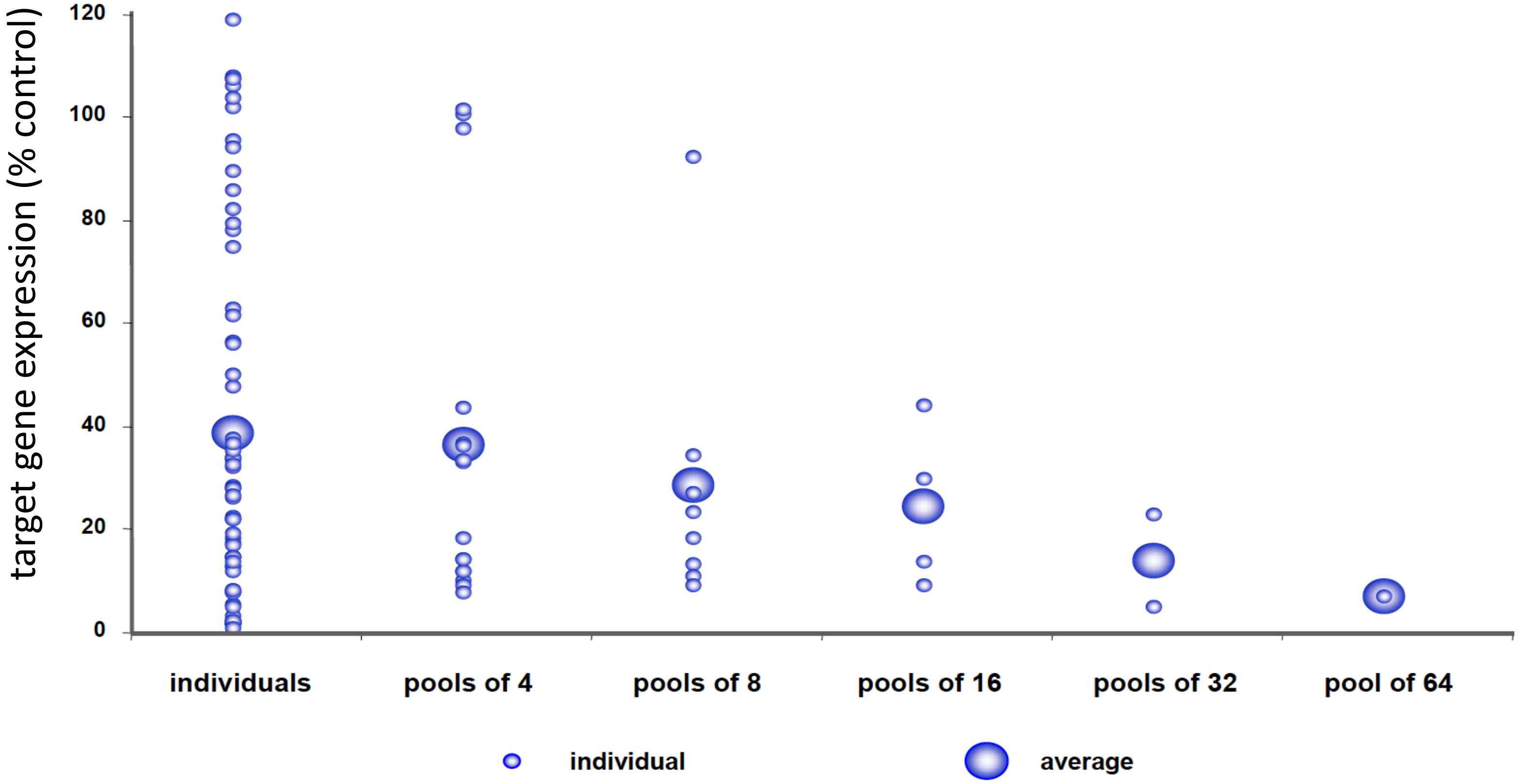
off-target analysis with expression arrays



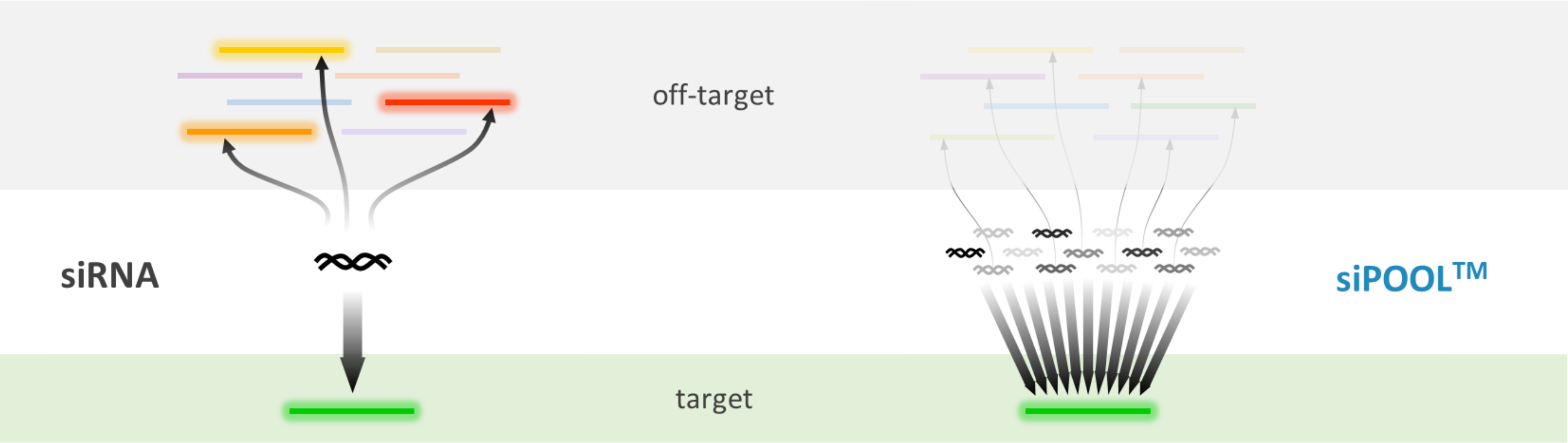
=> Specificity improves with complexity

Efficiency improves with Pool Complexity

on-target knock-down by RT-PCR



The siPOOL Concept



1 siRNA

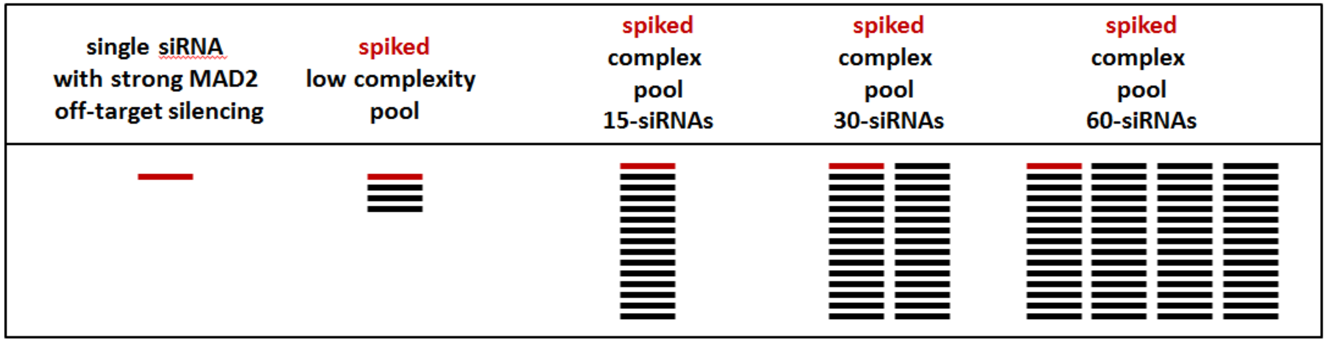
siPOOL = 30 siRNAs

off-target spiking experiment

1: Choose siRNAs with known strong off-target (King et al. 2012)

- **Scyl1** siRNA ⇨ **Mad2**
- **PolG** siRNA ⇨ **Mad2**

2: spike Off-target siRNA against PolG and Scyl1 into siPOOLs



single siRNA with strong MAD2 off-target silencing

spiked low complexity pool

spiked complex pool 15-siRNAs

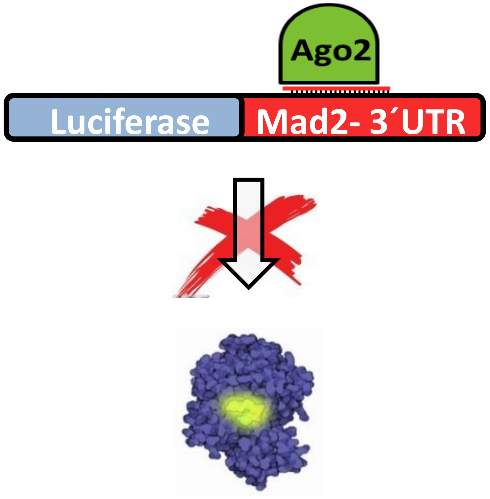
spiked complex pool 30-siRNAs

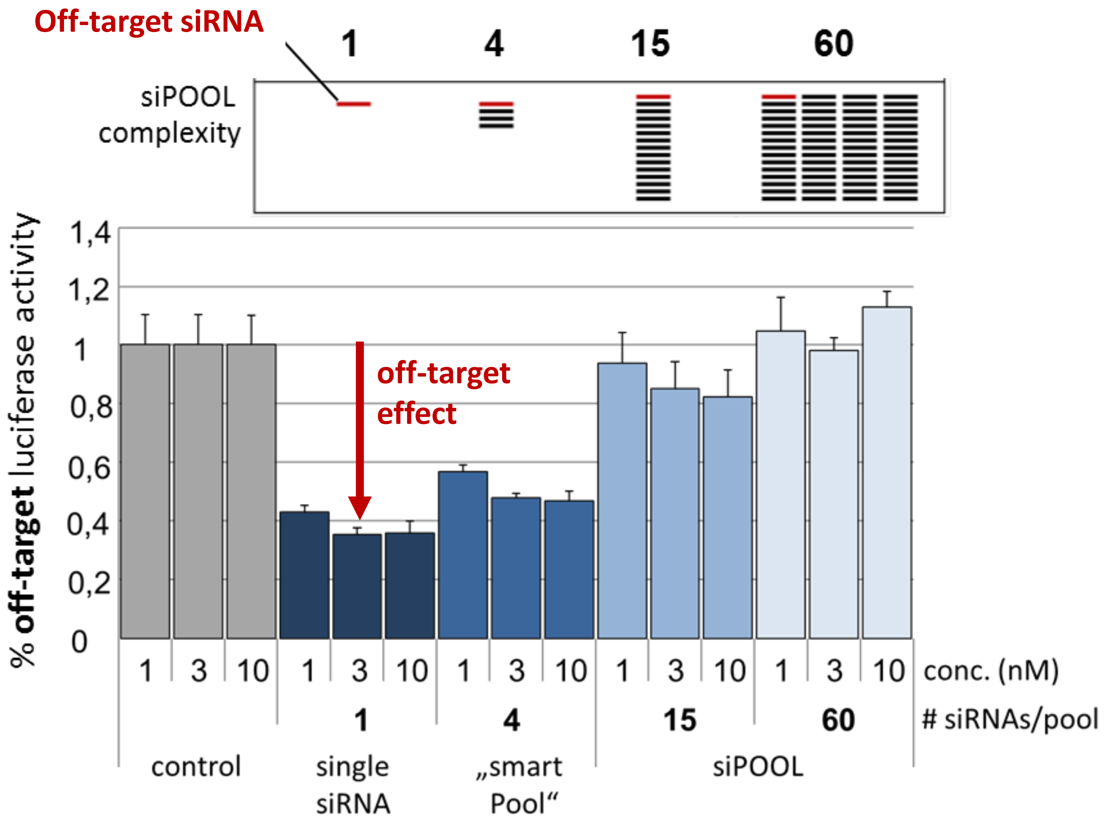
spiked complex pool 60-siRNAs

3: Read out: reporter assay, RT-PCR, Western, functional assay

complexity is key …and 4 may not be enough

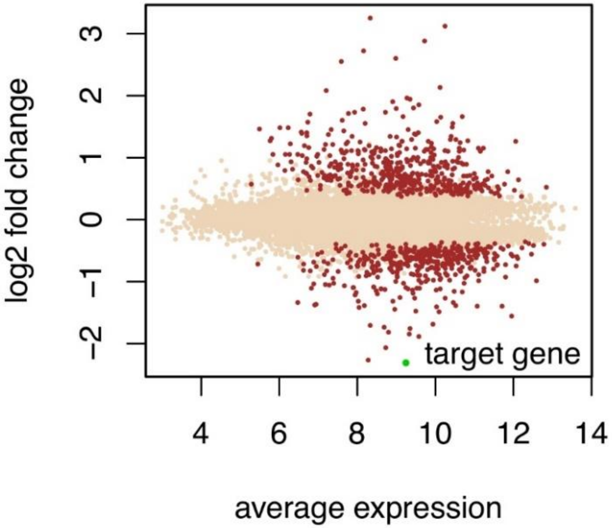
off-target
luciferase reporter





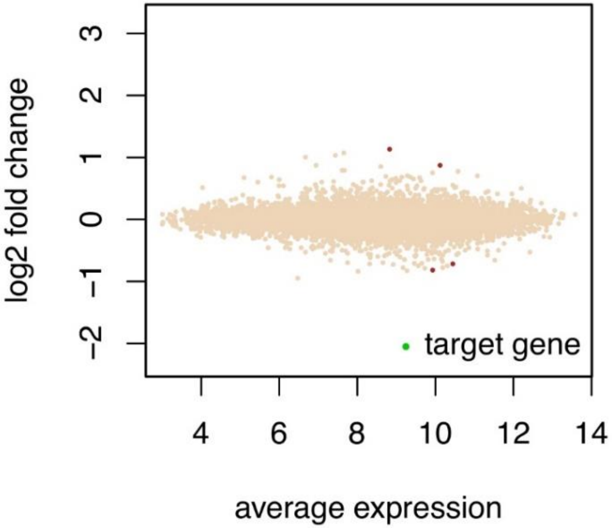
Efficient off-target dilution

siRNA



siRNA
within
siPOOL

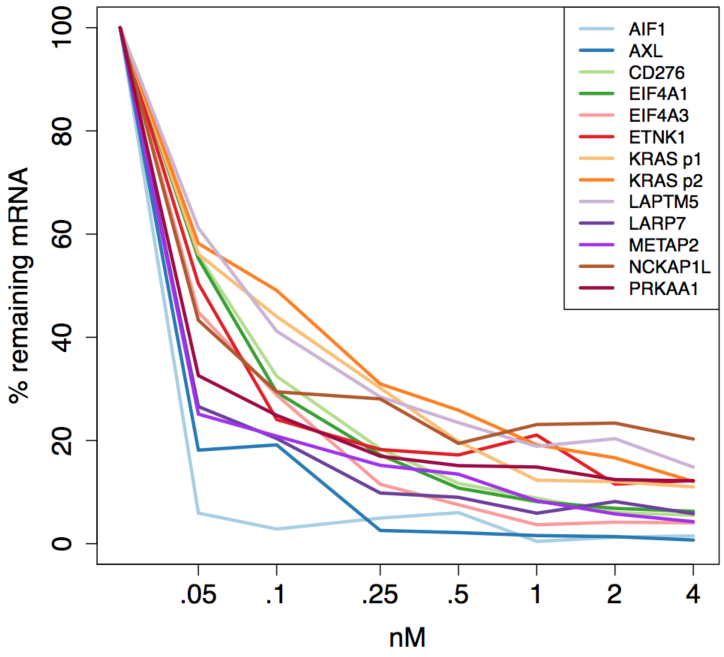
siPOOL

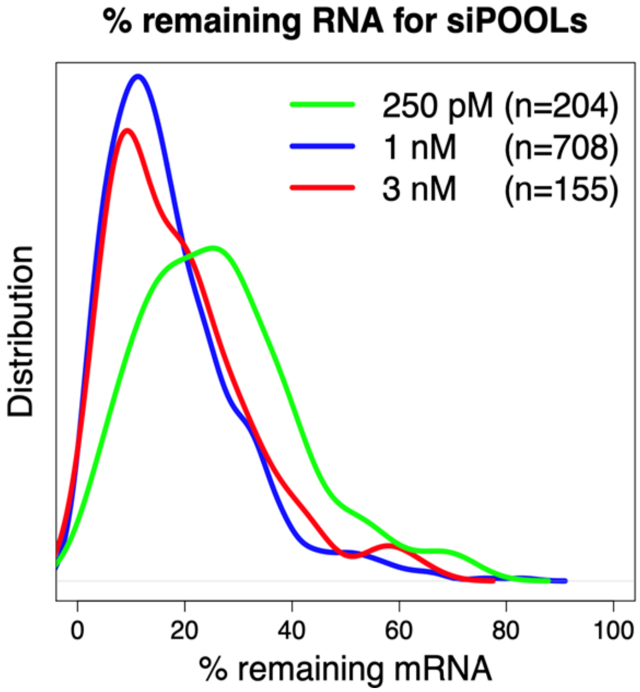


- *HeLa cells*
- *3 nM Scyl1 siRNA or siPOOL*
- *48 h*
- *whole transcriptome profiling by Affymetrix Microarray*

Hannus et al., Nucleic Acids Res, 2014

siPOOLs work at very low concentration





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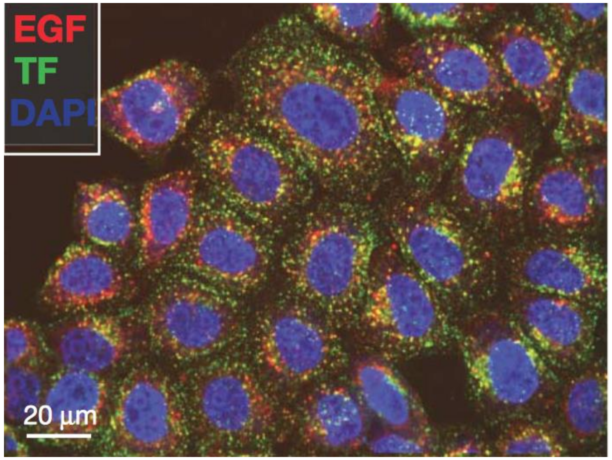
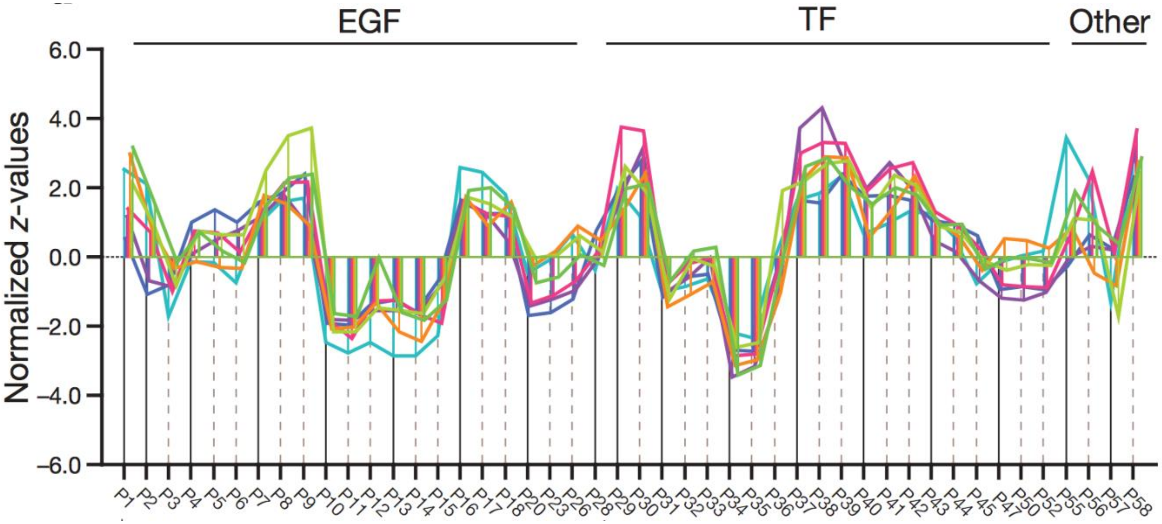
Case study: HIGH content endocytosis screen

Systems survey of endocytosis by multiparametric image analysis.

Collinet C, et al.

Nature 2010 Mar 11;464(7286):243-9

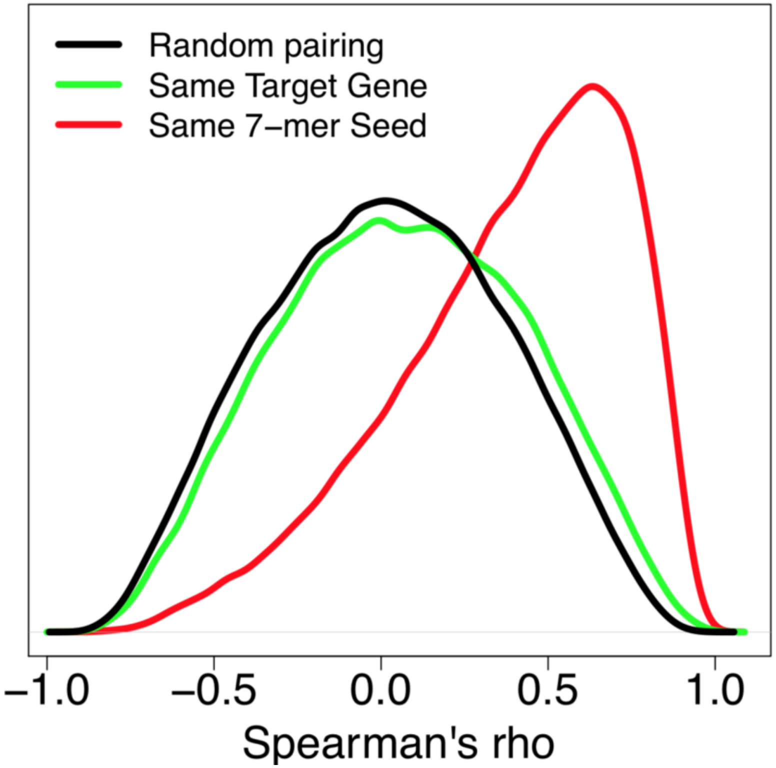




- >100.000 siRNAs for >15 000 genes
- > 40 features on EGF and TFN uptake
- 23,703 siRNAs for 4,609 hit genes

=> Phenotypic profiles allow correlation analysis between 2 siRNAs

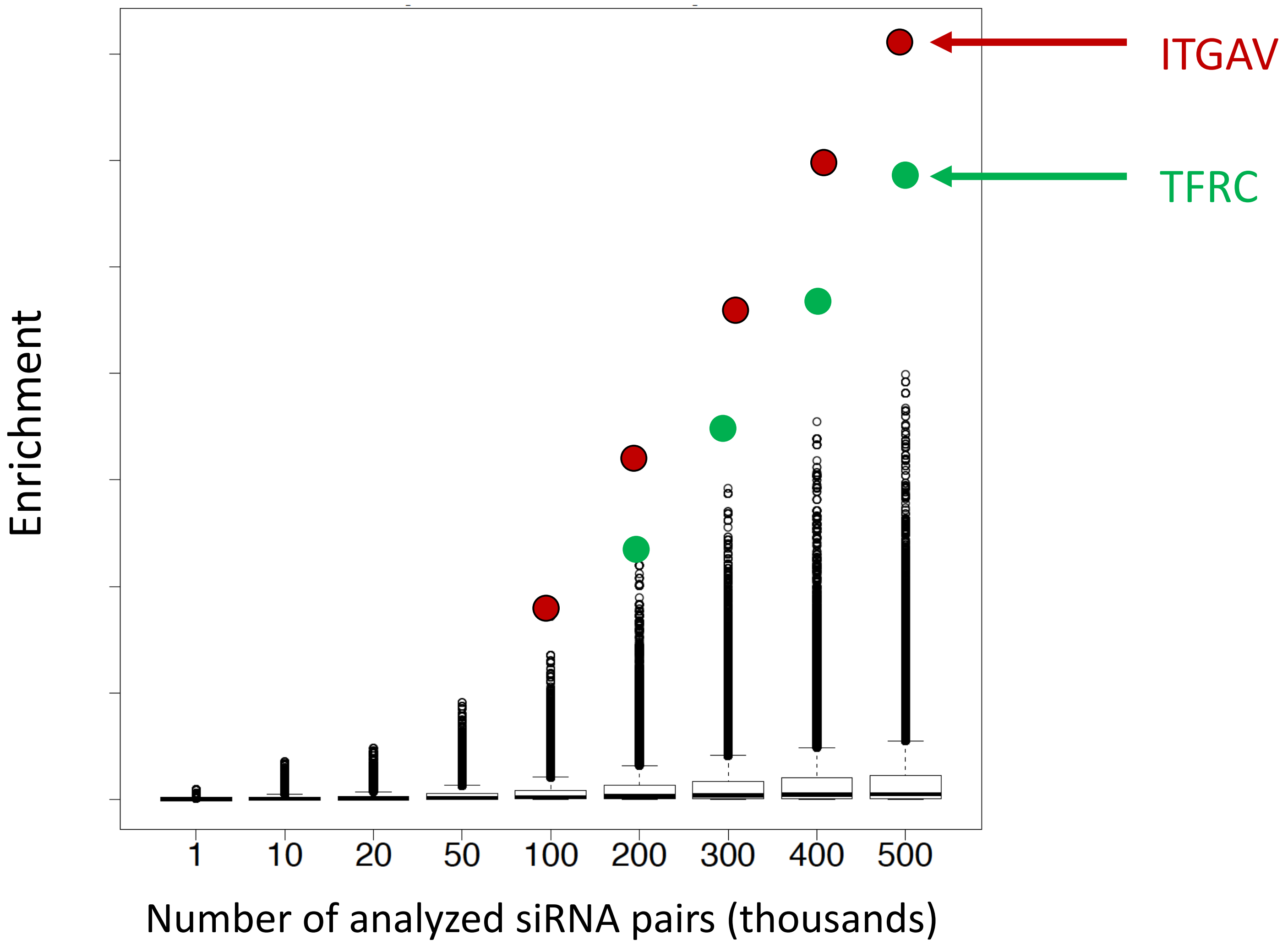
Pairwise correlation analysis of phenotypic profiles



- ~280 million correlations
- Plotted by:
  - Random pairing
  - Same target gene
  - Same 7-mer seed

⇒ Seed is by far strongest phenotypic determinant

Finding dominant off-target genes



Summary & Contact

Transient gene silencing has many advantages - and works best **RNAi**

Seed-based **off-target** effects are dominant in **single** siRNAs

Complex siRNA Pools minimize off-target effects by Seed dilution

Seed information can be used for alternative target discovery

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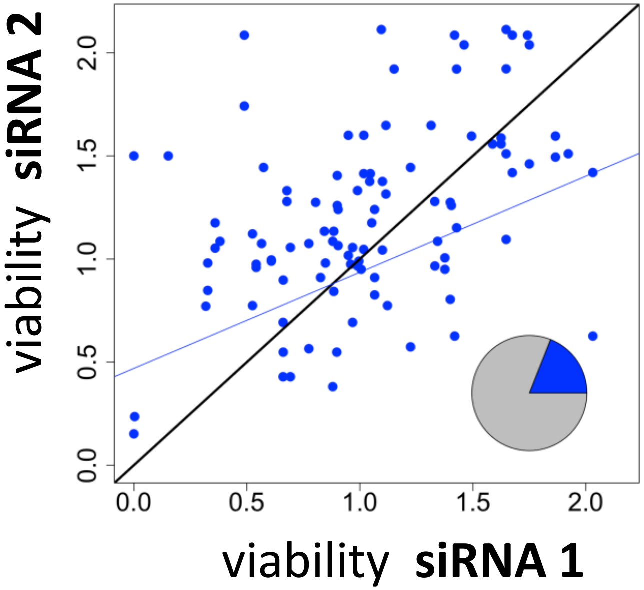
siPOOL Phenotypic Reliability

$R^2 = 0.19$



$R^2 = 0.71$