siPOOLs and CRISPR: Partnering RNAi with gene editing

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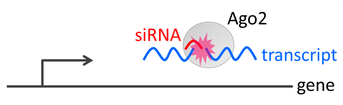
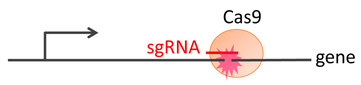
With the discovery of CRISPR, gene editing has gained tremendous traction in the scientific community due to its ease of application and low cost. The rise in popularity mirrors that of the use of RNA interference (RNAi) in the early 2000s. Although RNAi is still widely used today for functional genomics, screening efforts are hampered by the lack of specificity of individual siRNAs, giving rise to wide-ranging off-target effects and hence, unreliable results. Many are therefore now turning to CRISPR which promises higher specificity and clearer phenotypes due to complete depletion of gene activity.

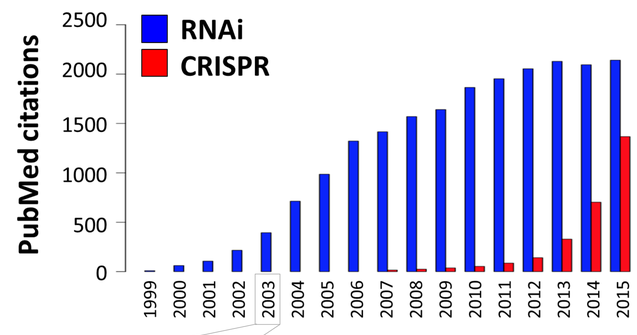
Here we compare key features of both technologies, bringing to attention that complete gene knock-out also comes with its own set of challenges. Of particular importance is the phenomenon of adaptation, which has now been shown to occur in several gene knock-out models. The complete knock-out of a gene can incur compensatory effects not seen when a gene is transiently knocked-down. With siPOOLs, transient knock-down by RNAi can be specific and potent. The dose-dependent nature of RNAi-mediated gene knock-down also mimics pharmacological inhibition. In view of their complementary strengths and challenges it seems highly commendable to use both RNAi and CRISPR for a thorough investigation and understanding of gene function. With siPOOLs, a new and extremely specific RNAi reagent has now become available that will allow RNAi screening with dramatically reduced off-target effects.

RNAi and CRISPR

A Comparison

Technique	RNAi (siPOOLs)	CRISPR
Mechanism		
Nature of phenotype	Transient (knock-down)	Permanent (knock-out)
Extent of phenotype	Partial (dose-dependent)	Complete
Time to phenotype	24 h	48 h – 14 days
Variability of effect	Low (homogenous knock-down across cells)	High (heterogenous recombination and clonal artefacts)
Cost	Low	Low
Off-targeting	Low (only with **siPOOLs**!)	Varies with sgRNA (single guide RNA)
Site of action	Largely in cytoplasm	Nucleus
Efficiency	Good	Poor to moderate (all copies of the gene have to be edited)

Hype Dynamics



Nat Biotechnol. 2003 Jun
Expression profiling reveals off-target gene regulation by RNAi

- RNAi was immensely popular despite knowledge of off-target effects.
- A similar hype is now seen with CRISPR though reports on its drawbacks have recently been made known.

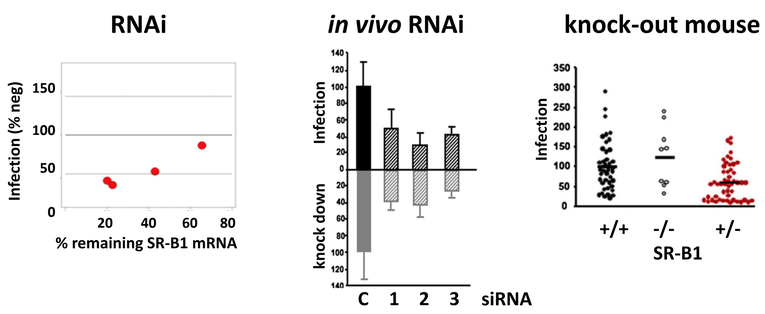
Potential Issues with CRISPR

With Knock-out comes Adaptation

Case Study 1:

There once was a perfect RNAi hit… until it met its knock-out mouse

Rodrigues, Hannus et al. **Cell Host Microbe**. 2008

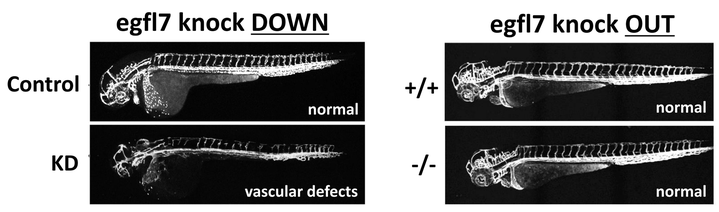


- RNAi screen identified Scavenger Receptor B1 (SR-B1) as an essential Malaria liver stage host factor gene.
- Despite siRNAs, antibody and compound against SR-B1 decreasing malaria infection rate, **the knock-out mouse was no different to wildtype**. Why? **=> Adaptation!**

Case Study 2:

Genetic compensation in knock-OUTs but not knock-DOWNS

Rossi et al. **Nature**. 2015



- Knock-**DOWN** of egfl7, a gene involved in angiogenesis, caused severe vascular defects (bottom left).
- No phenotypic effect for egfl7 knock-**OUT** (bottom right).
- Upregulation of Emilin genes compensated in egfl7 knock-out phenotype.

=> Compensatory mechanisms induced in knock-outs mask loss-of-function phenotype.

Clonal Heterogeneity

- Genome sequencing revealed many more SNVs (single nucleotide variants) than indels between clonal cell lines derived from gene editing.

Study	Smith et al., 2014	Veres et al., 2014
Cell type	Human induced pluripotent stem cells (iPSC)	Human pluripotent stem cells (PSC)
# clones	4	9
Sequencing coverage	30X	60X
# indels/clone	7-12	2-7
# SNVs/clone	217-281	64-142

=> SNVs hinder generation of truly isogeneic cell lines.

- A germline SNV "corrects" a target site, decreasing mismatches with gRNA and increasing indel frequency to **36.7%.**

gRNA sequence		A	A	G	C	T	C	A	A	C	C	A	T	G	G	G	G	A	C	T	P	A	M
TAZ1	Chr5_OT$_{ref}$	C	A	G	C	A	C	A	A	G	C	A	T	G	G	G	G	A	C	T	T	G	G
	Chr5_OT$_{var}$	C	A	G	C	A	C	A	A	C	C	A	T	–	–	–	G	A	C	T	T	G	G
TAZ2	Chr5_OT$_{ref}$	C	A	G	C	A	C	A	A	G	C	A	T	G	G	G	G	A	C	T	T	G	G
	Chr5_OT$_{var}$	C	A	G	C	A	C	A	A	C	C	A	–	–	–	–	–	–	–	–	T	G	G

Yang et al., Nat Comm, 2014

=> SNVs can produce high-effiency off-target sites.

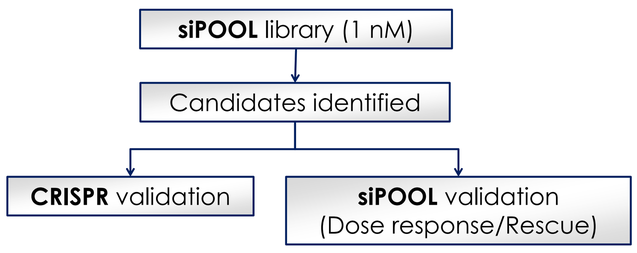
Off-targets

- CRISPR off-targeting varies widely with sgRNA (0 to > 150 off-targets in Guide-seq study) and is not readily predicted by computational methods.
- Other factors: transfection conditions, chromosome structure, cell line etc.
- To reduce off-targeting: Cas9 nickase, mutant Cas9.

=> Off-targets still exist!

Screening Approach with siPOOLs and CRISPR

Suggested screening approach:



- Advantages of **siPOOLs** as a **first-pass screen**:
  - ✓ High-quality hits
  - ✓ Simple and fast to use (days, not weeks)
  - ✓ Dose-dependent (drug-like)
  - ✓ Transient (avoids adaptation)
  - ✓ Broadly applicable
  - ✓ Low cost
- Advantages of **CRISPR** in **down-stream validation**:
  - ✓ Ability to evaluate complete loss-of-function phenotype
  - ✓ Confirm hits in various models
  - ✓ Gain-of-function assays with CRISPRa

References

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