

Bioinformatic and empirical tools for screening and validating siRNA target sites with respect to sequence-based off-target activity

December 16, 2011

Julja Burchard, RNA Therapeutics Bioinformatics Lead
Merck Research Laboratories

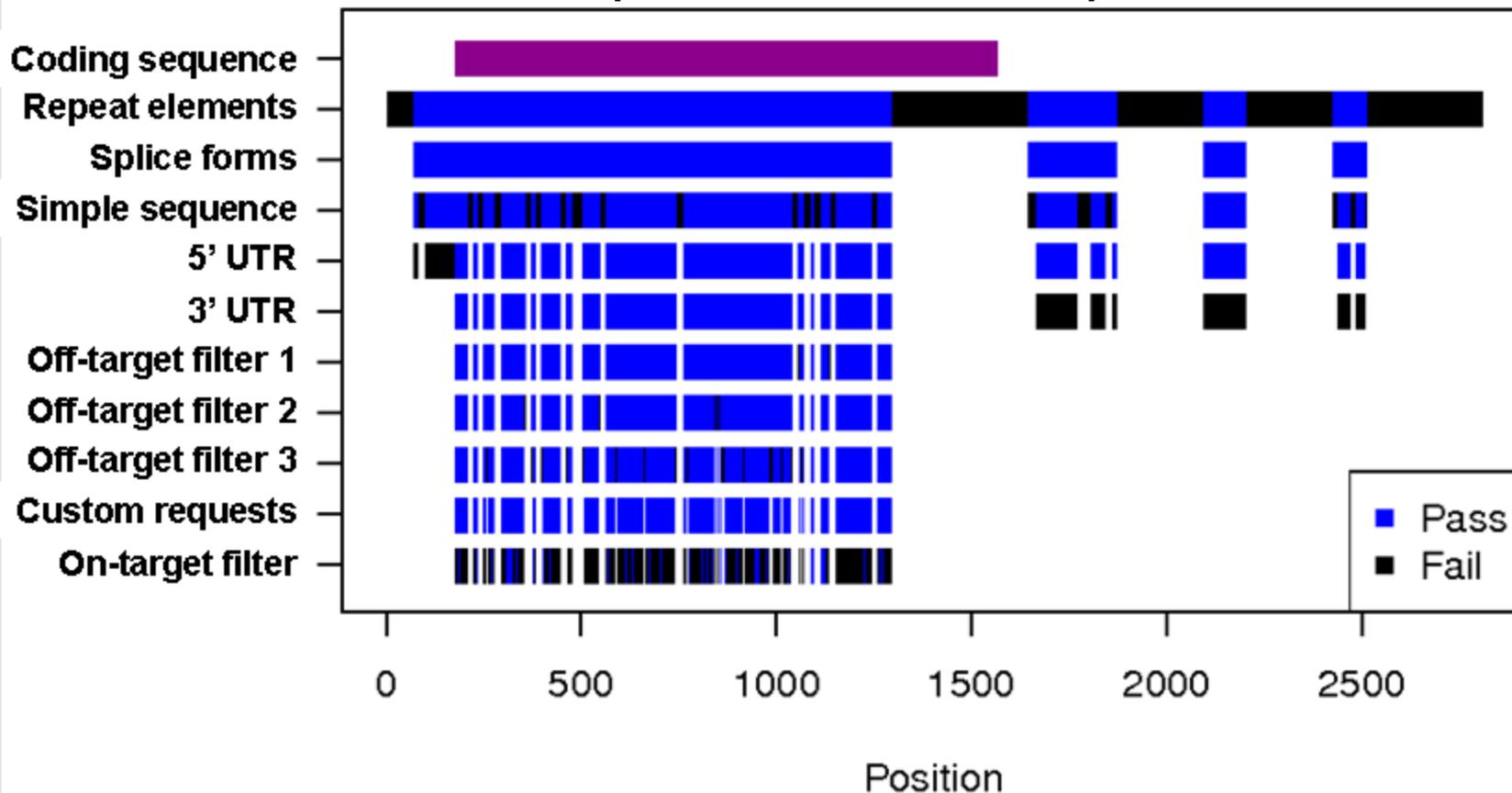


Topics

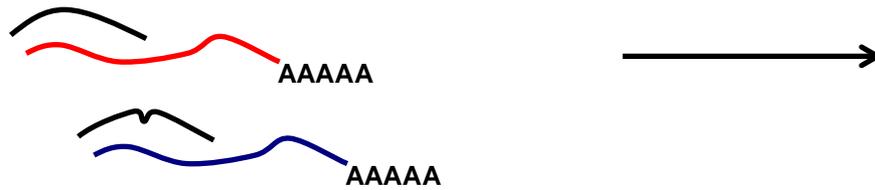
- **siRNAs can silence unintended targets by long matching or seed matching**
- Understanding siRNA long match silencing
- Understanding siRNA seed match silencing
- Off-target activity risks are best addressed through standard safety assessment measures in an appropriate safety species

Off-target prediction tools are a subset of multiple factors to be considered in siRNA design

Example schema for siRNA sequence selection

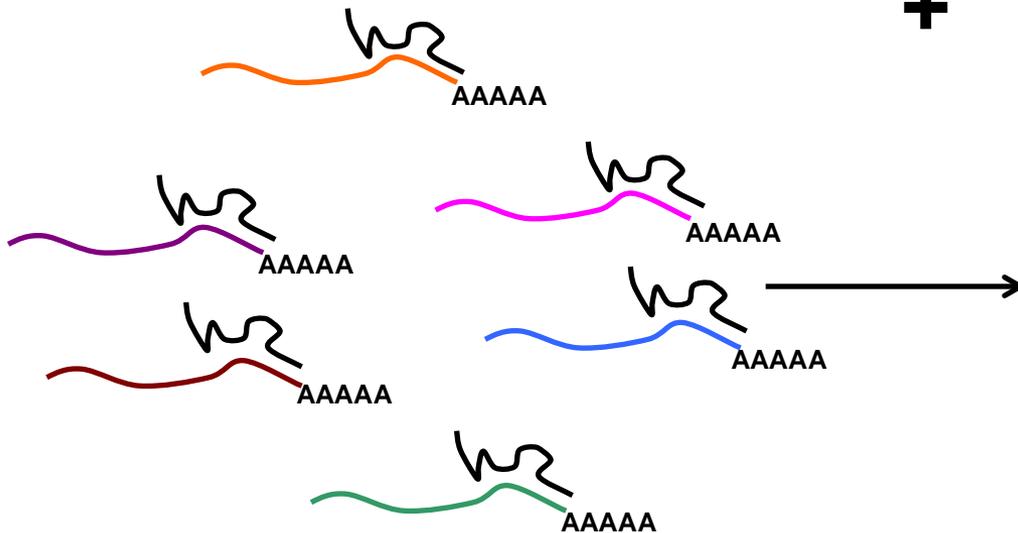
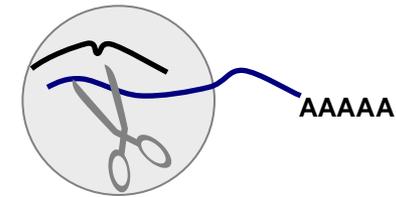


RNA duplexes show two modes of transcript regulation in animal cells



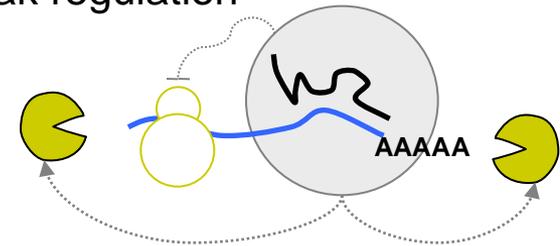
few long-matched targets

- similar to on-target activity
- extensive matches to guide strand (GS)
- strong regulation



many seed-matched targets

- similar to miRNA activity
- matching to first 8 bases of GS
- weak regulation



Topics

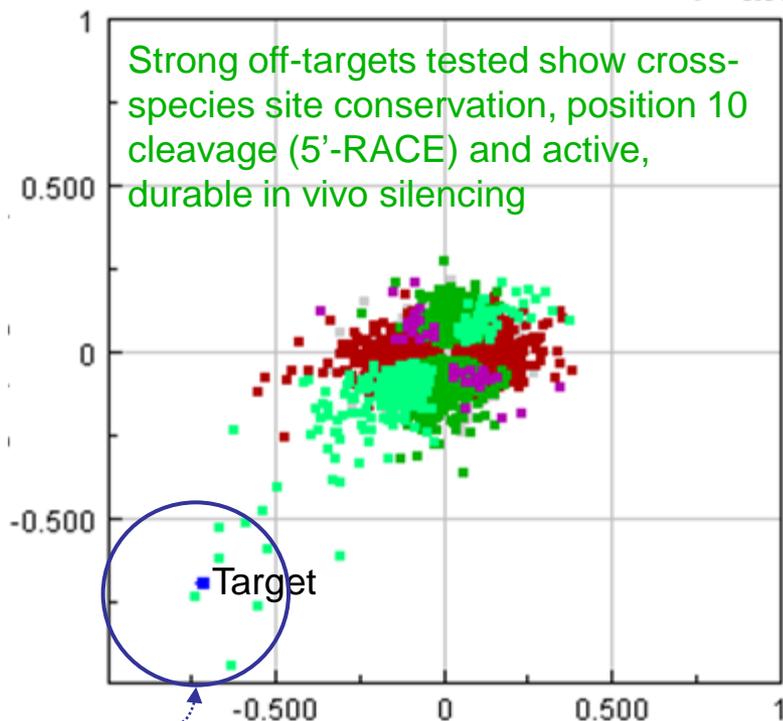
- siRNAs can silence unintended targets by long matching or seed matching
- **Understanding siRNA long match silencing**
- Understanding siRNA seed match silencing
- Off-target activity risks are best addressed through standard safety assessment measures in an appropriate safety species

A subset of siRNAs show strong *in vitro* off-target regulations conserved in human and rhesus cell lines

Log₁₀ expression ratio in human cell line

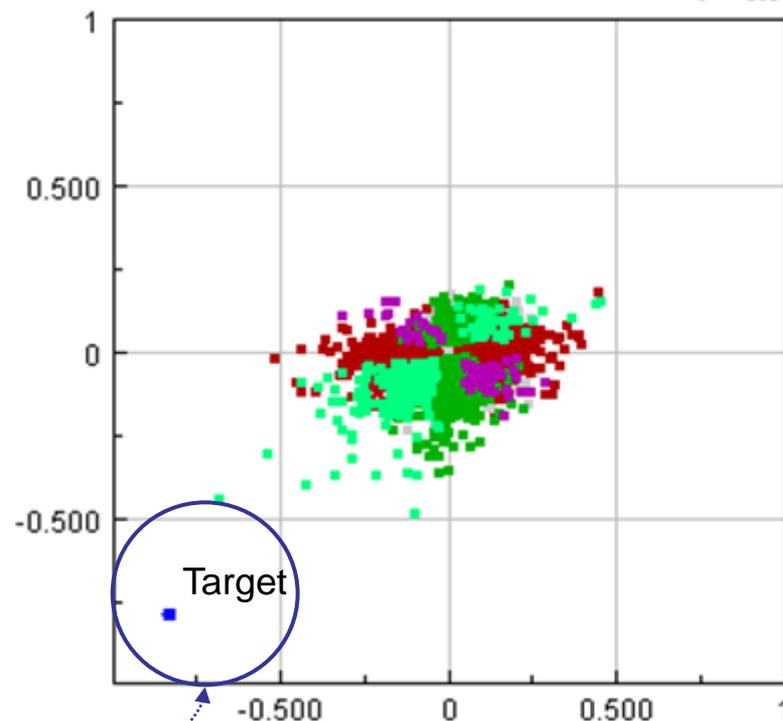
siRNA #1

P<=0.01



siRNA #2

P<=0.01



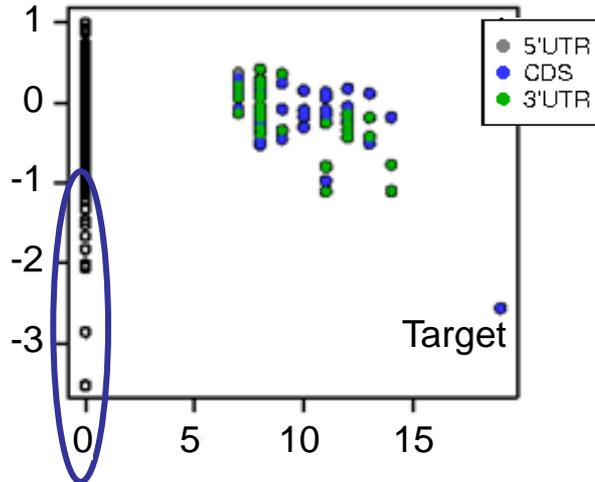
Log₁₀ expression ratio in rhesus cell line

Off-target genes with similar regulation to intended target in both rhesus and human cells

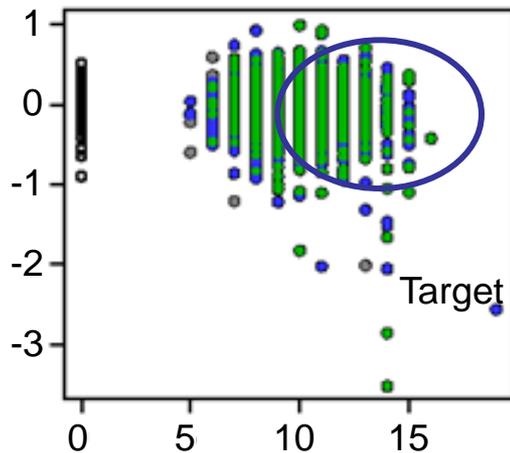
BLAST under-predicts and FASTA over-predicts long-match off-targets

siRNA #1

BLAST

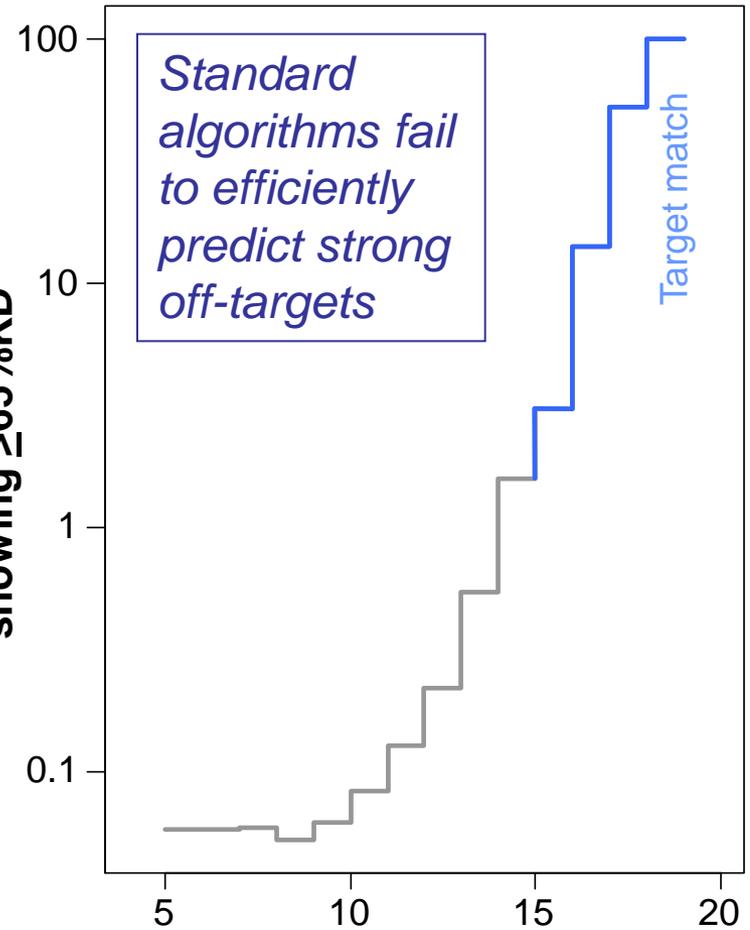


FASTA



Log₂ fold change in human cell line transcript expression

Percent of genes with $\geq x$ FASTA Matches showing $\geq 65\%KD$



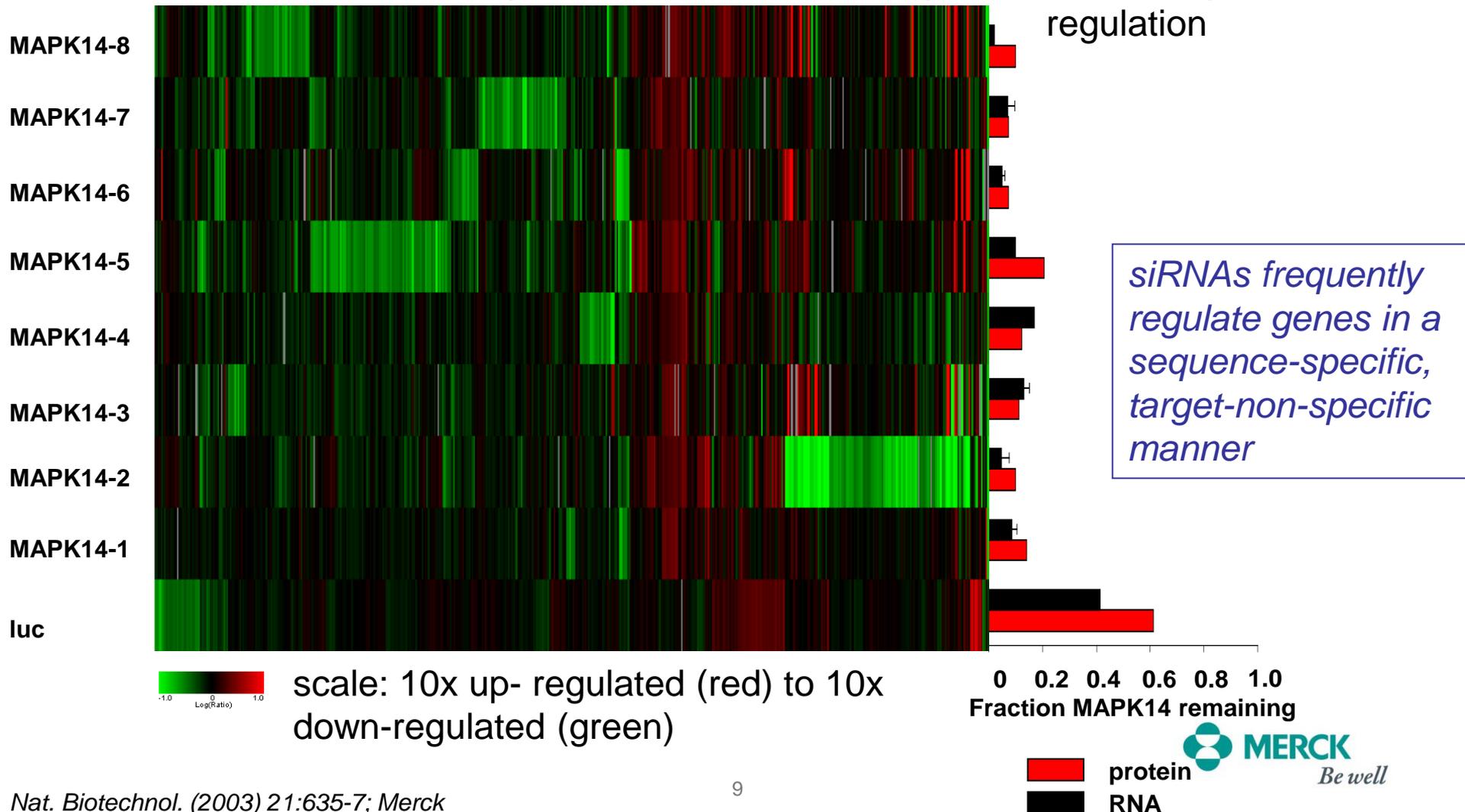
FASTA Alignment Matches

Topics

- siRNAs can silence unintended targets by long matching or seed matching
- Understanding siRNA long match silencing
- **Understanding siRNA seed match silencing**
- Off-target activity risks are best addressed through standard safety assessment measures in an appropriate safety species

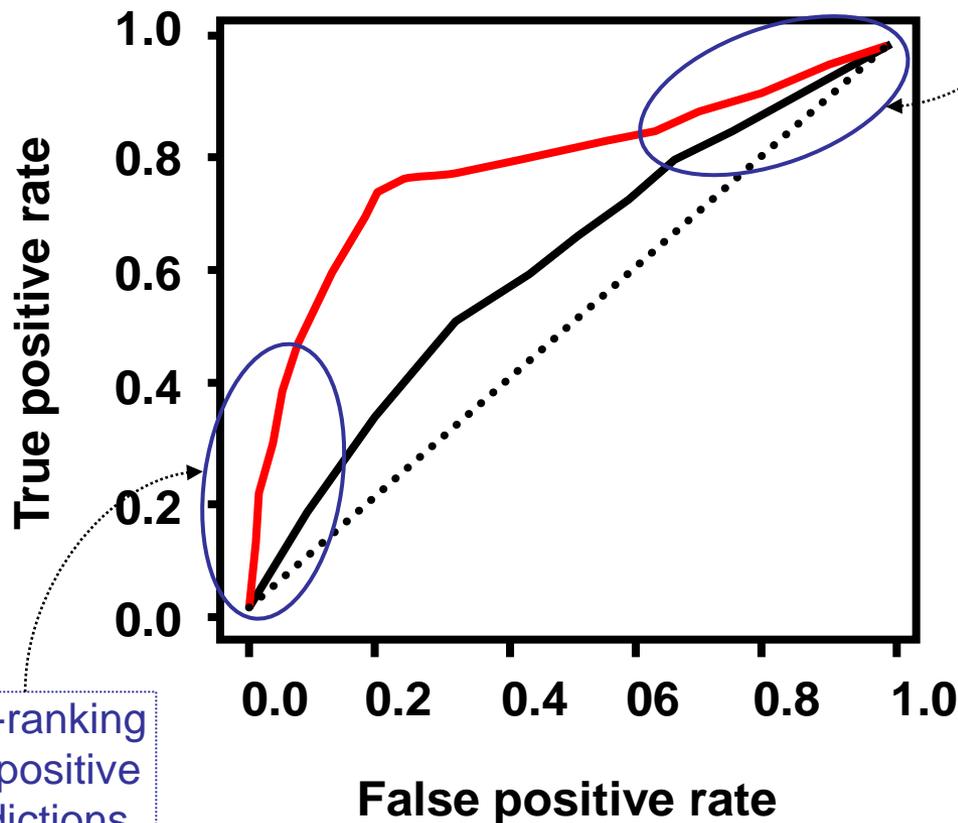
Microarrays reveal siRNA sequence-specific gene expression profiles

sequence-specific regulation, not related to target MAPK14 target regulation



Counting seed matches improves prediction of siRNA off-target regulation vs. FASTA

Predicting siRNA regulation of transcripts in microarray data



High-ranking true positive predictions

Low-ranking false negative predictions

- Weighted seed match count score
- Weighted FASTA alignment count score
- Random score

Algorithms show correlation to observed regulation but are not 100% accurate. Thus assessment in animal models is the ultimate arbiter of off-target toxicity.



Topics

- siRNAs can silence unintended targets by long matching or seed matching
- Understanding siRNA long match silencing
- Understanding siRNA seed match silencing
- **Off-target activity risks are best addressed through standard safety assessment measures in an appropriate safety species**

Genome-wide tools for target site validation remain exploratory

May use tools up front in selection of target site and siRNA

- Seed match silencing prediction
- Long match silencing prediction
- Expression profiling in vitro or in vivo

However, bioinformatics tools have limitations

- Prediction algorithms may miss important off-target genes
- Expression profiling is limited by choice of time point, tissue, organ etc.
- A potential off-target gene identified *in silico* or *in vitro* may not show toxicity *in vivo* as it may be a poor target, not expressed at the time or in the tissue where the drug is present, or non-critical in function

At present, *in silico* predictions and molecular profiling are exploratory tools not yet qualified as predictors of animal or human efficacy or toxicity

For assessment of off-target effects, GLP safety studies are the gold standard

For both small molecule and oligonucleotide therapeutics, only GLP safety studies assess responses by the whole organism to the effects of the drug.

No matter how well implemented, bioinformatics tools can never address the whole organism, all tissues and their interactions over time.

Acknowledgements

Cancer Biology group, Informatics, Computational Biology and Gene Expression Lab at Merck's Rosetta site

RNA Sciences and Lead Development groups at Merck's Sirna site

Applied Computer Science & Mathematics, Safety Assessment and RNA Biology and Chemistry groups at Merck's West Point site

Bibliography

- Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, Linsley PS. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol.* 2003 21:635
- Jackson AL, Burchard J, Leake D, Reynolds A, Schelter J, Guo J, Johnson JM, Lim L, Karpilow J, Nichols K, Marshall W, Khvorova A, Linsley PS. Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. *RNA.* 2006 12:1197-205.
- Jackson AL, Burchard J, Schelter J, Chau BN, Cleary M, Lim L, Linsley PS. Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *RNA.* 2006 12:1179-87.
- Burchard J, Jackson AL, Malkov V, Needham RH, Tan Y, Bartz SR, Dai H, Sachs AB, Linsley PS. MicroRNA-like off-target transcript regulation by siRNAs is species specific. *RNA* 2009 15:308-15. 2011 manuscripts submitted and/or in preparation.