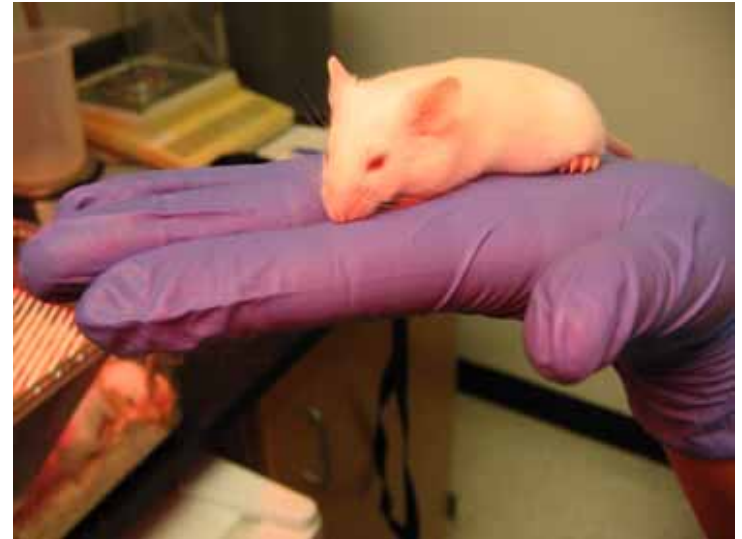
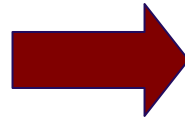
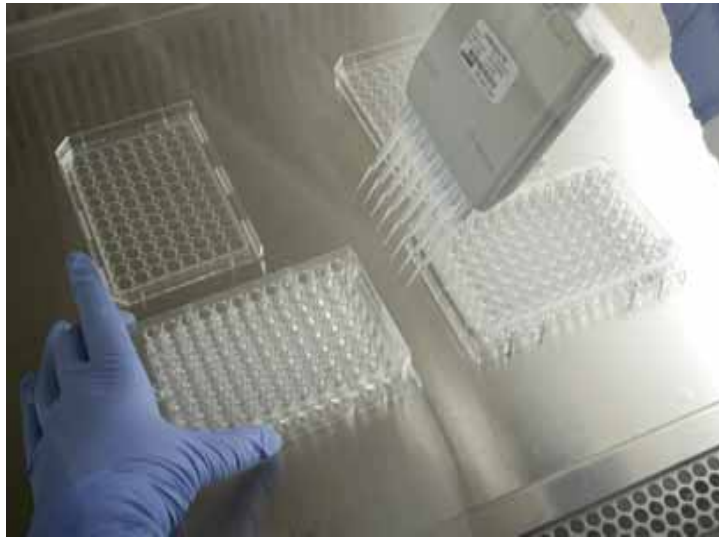


Next Generation siRNA: Combining SVM Algorithm Learning with Chemical Modification for More Consistent siRNA Performance

Alexander (Sasha) Vlassov

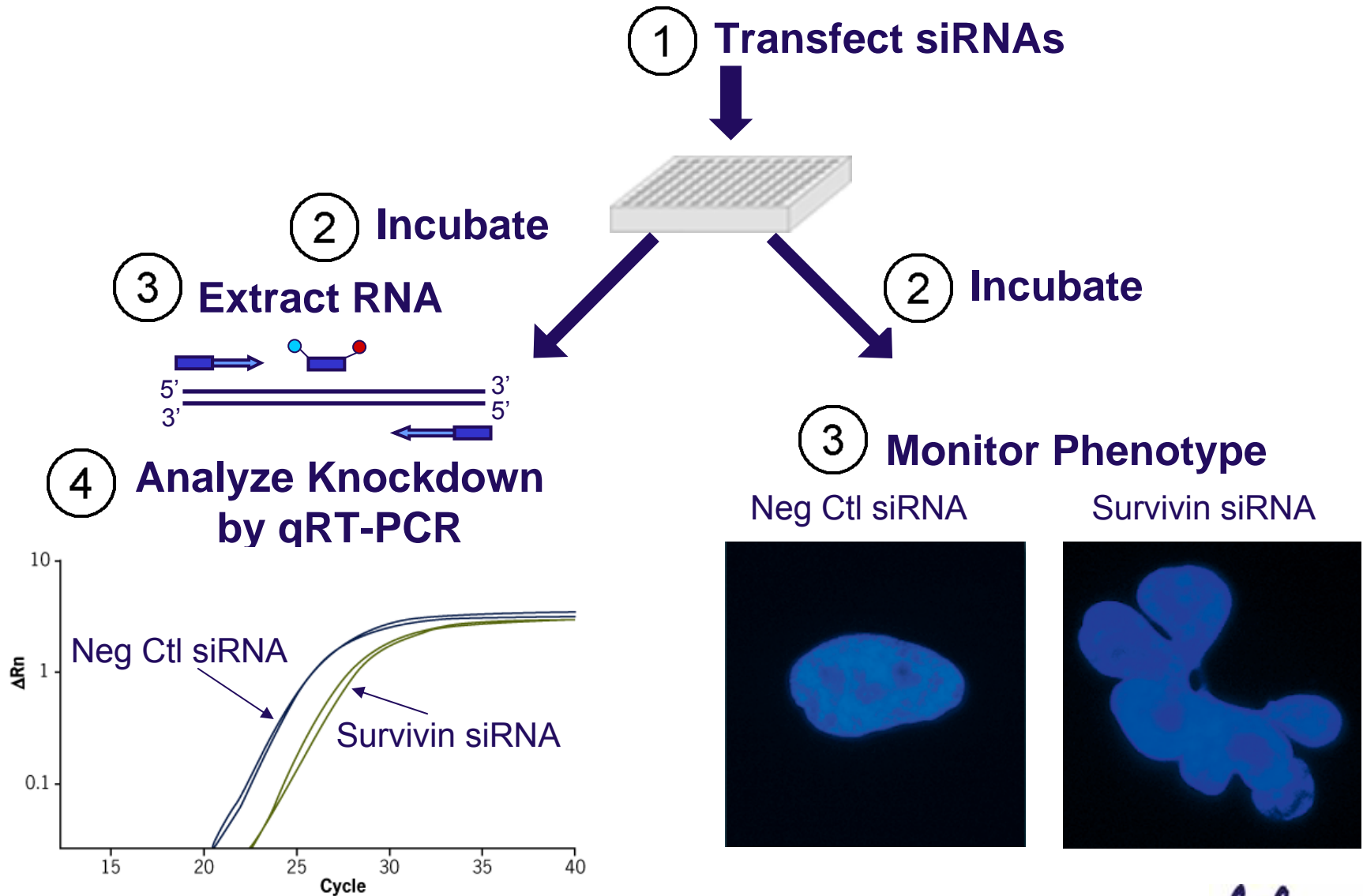
life
technologies™

RNA interference (RNAi) is a natural mechanism by which small interfering RNA (siRNA) can mediate degradation of a target mRNA molecule in a sequence-specific manner



- **Analyze Gene Function**
- **Target Validation**
- **Therapeutic Silencing**

siRNA experimental workflow

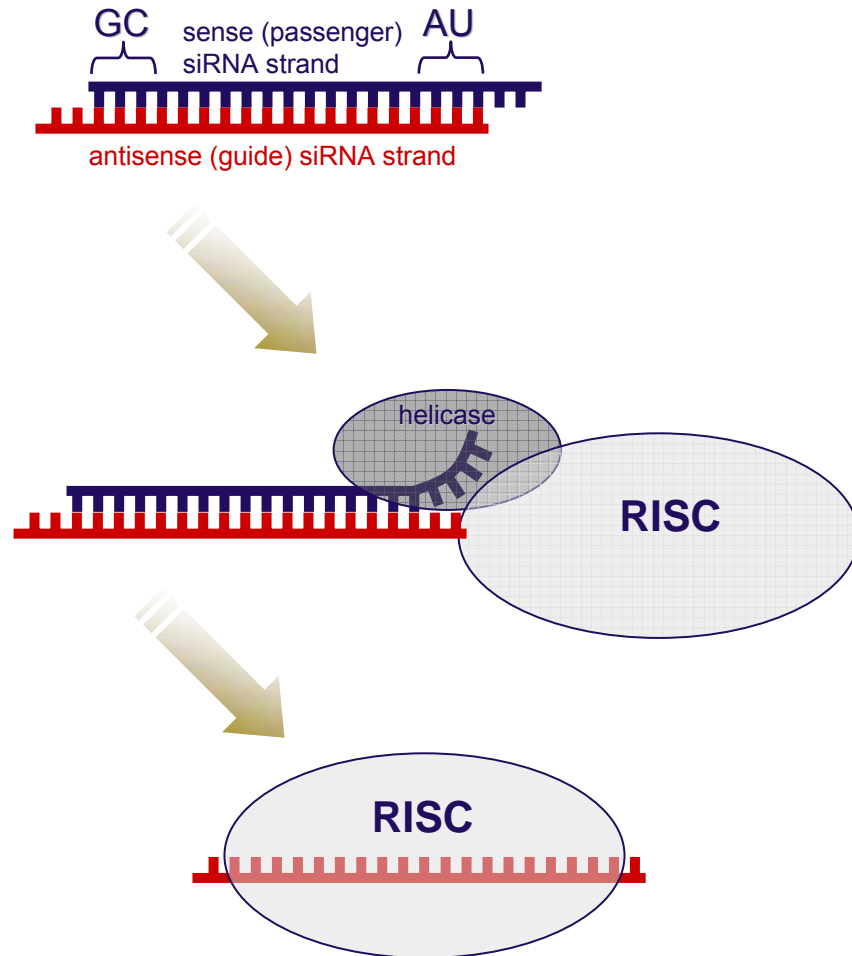


How to make a perfect siRNA?

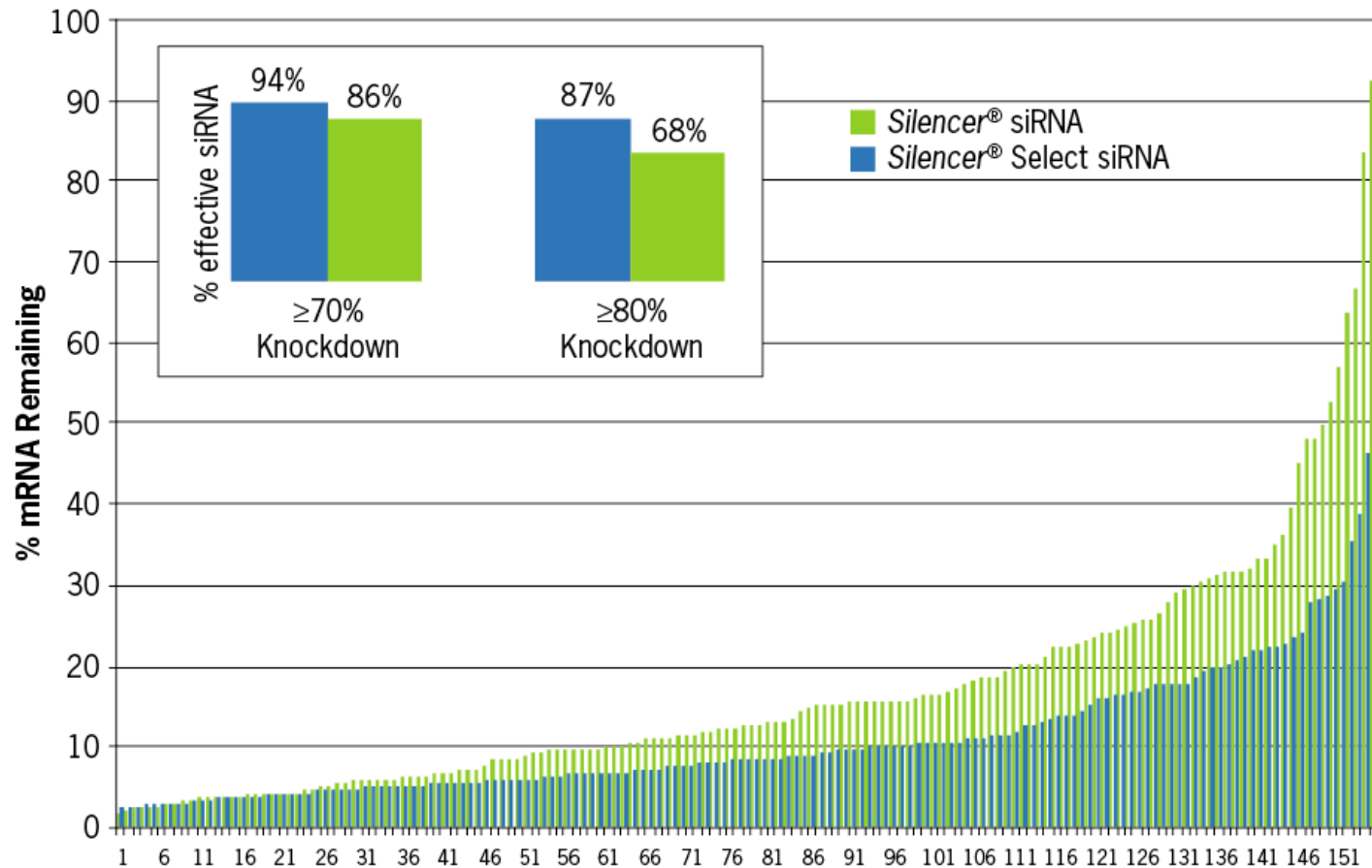
BIOINFORMATICS

CHEMICAL
MODIFICATIONS

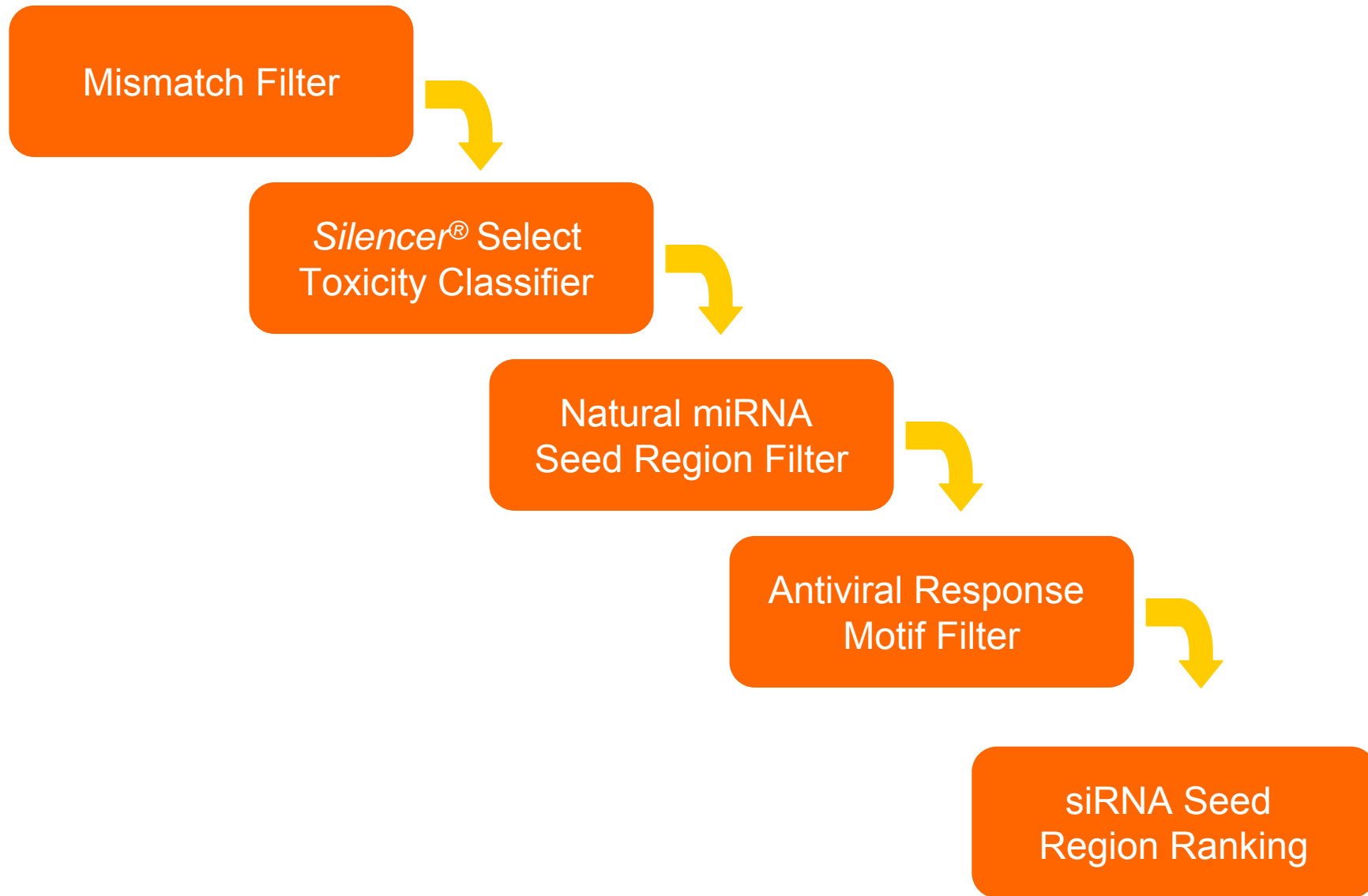
SYNTHESIS



Silencer[®] Select siRNA design algorithm significantly improves effective siRNA prediction accuracy



Five bioinformatic filters for enhancing siRNA specificity



Strategy for identifying best siRNA chemical modifications and patterns

5'-CGAUGAGUGCACGUUCAUAdTdT-3'
3'-dTdTGCUACUCACGUGCAAGUAU-5'

5'-CGAUGAGUGCACGUUCAUAdTdT-3'
3'-dTdTGCUACUCACGUGCAAGUAU-5'

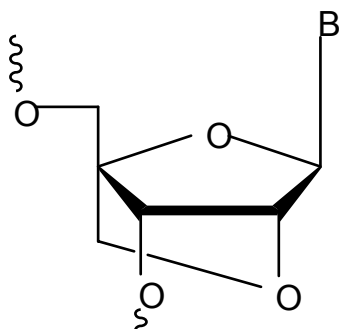
5'-CGAUGAGUGCACGUUCAUAdTdT-3'
3'-dTdTGCUACUCACGUGCAAGUAU-5'

5'-CGAUGAGUGCACGUUCAUAdTdT-3'
3'-dTdTGCUACUCACGUGCAAGUAU-5'

5'-CGAUGAGUGCACGUUCAUAdTdT-3'
3'-dTdTGCUACUCACGUGCAAGUAU-5'



Assays: Potency, Strand Bias, Microarray, Cell Assays

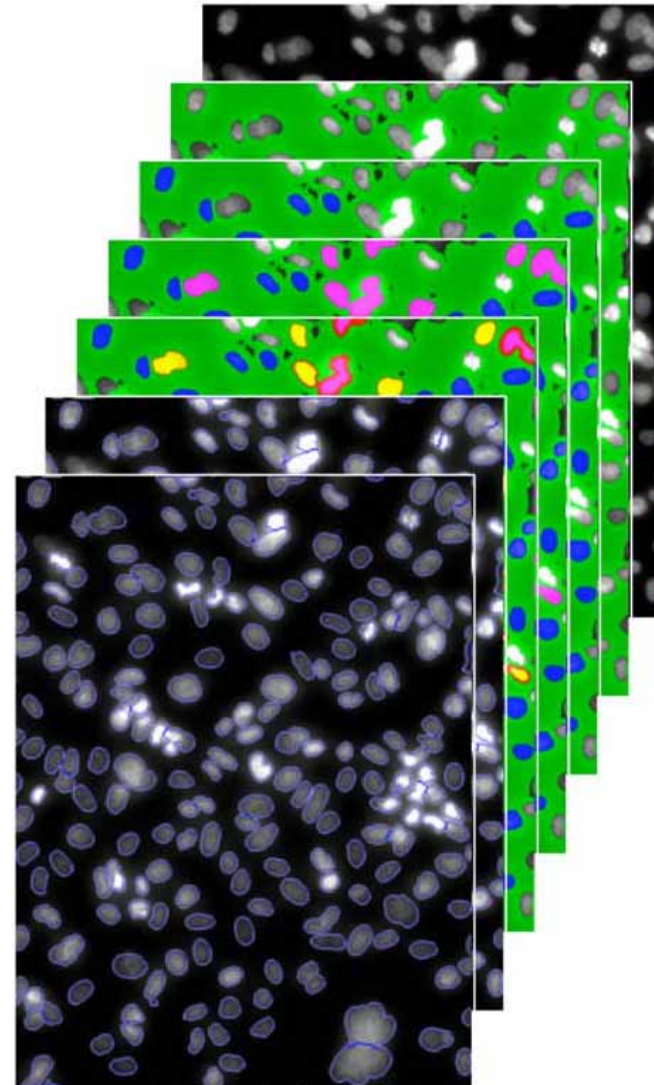


LNA[®]

Lead format:
Silencer[®] Select siRNA

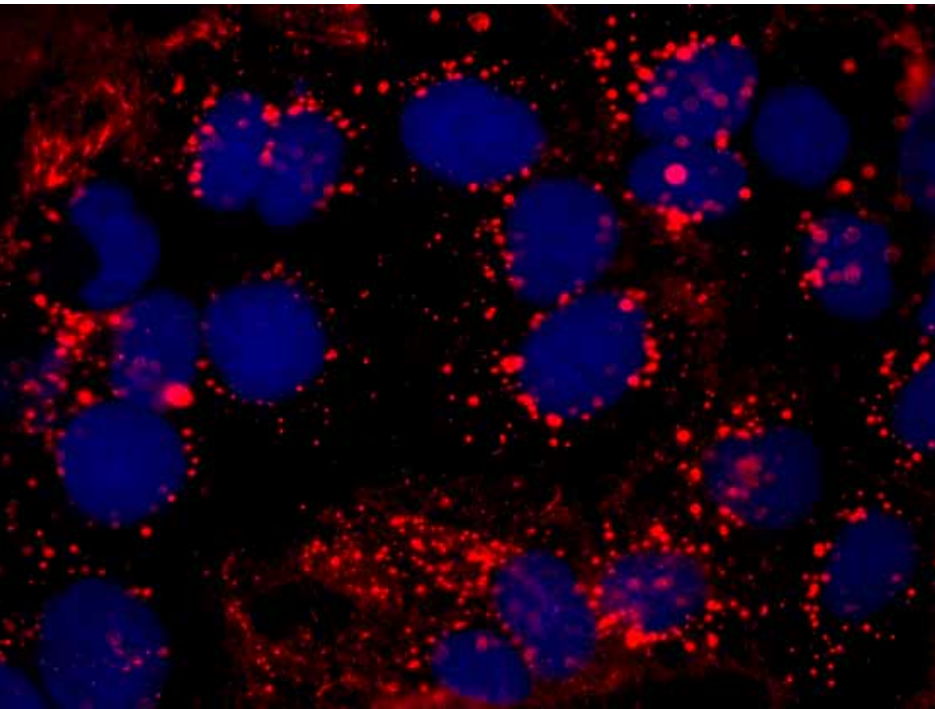
High content microscopy assays for siRNA performance

- **20 genes growth assay**
 - Apoptosis – cleaved lamin A
 - Mitosis – Phospho-histone H3
 - Cell size/shape – tubulin
 - Nucleus size/shape – Hoechst
 - U-2 OS osteosarcoma cells
 - WEE1, PLK1, AURKA
- **20 genes endocytosis assay**
 - LDL uptake
 - Transferrin uptake
 - Dextran uptake
 - Cell number – Hoechst
 - HUH 7 hepatoma cells
 - HMGR, FDFT1, LDLR, TFRC

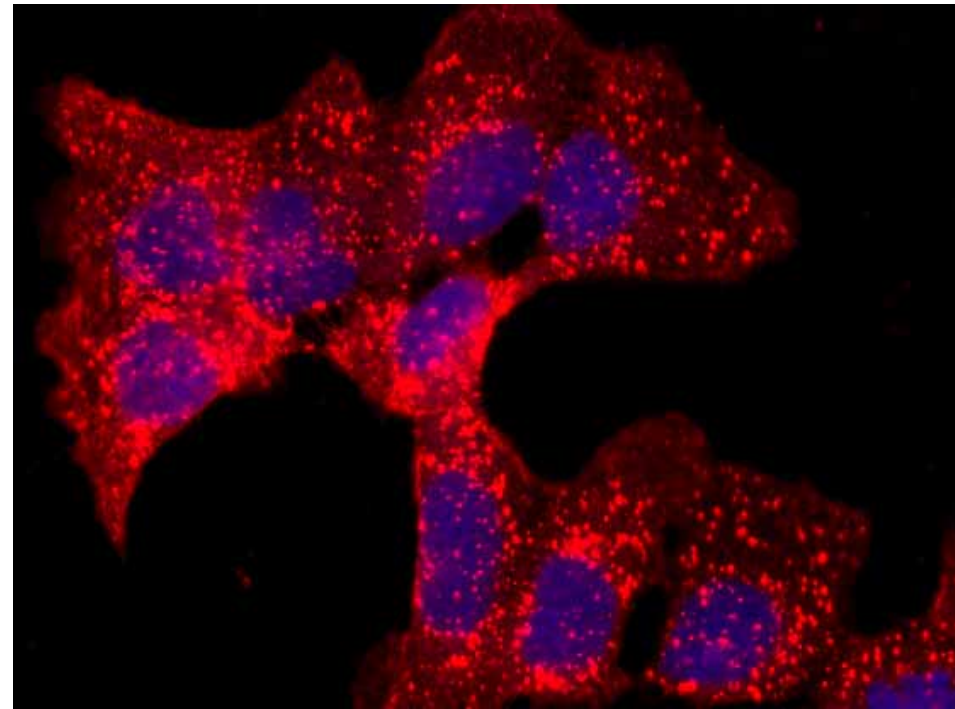


ImageXpress^{micro}
Cenix Bioscience GmbH

DiI-LDL Uptake Assay

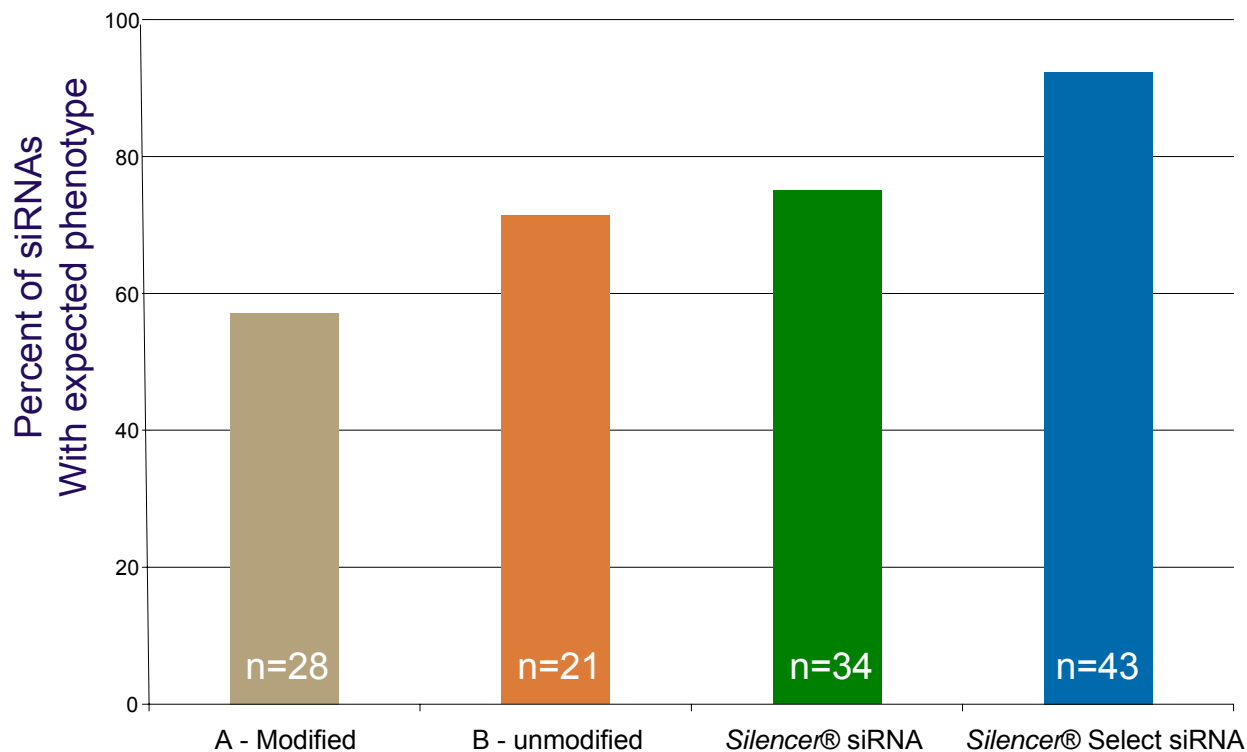


Neg control

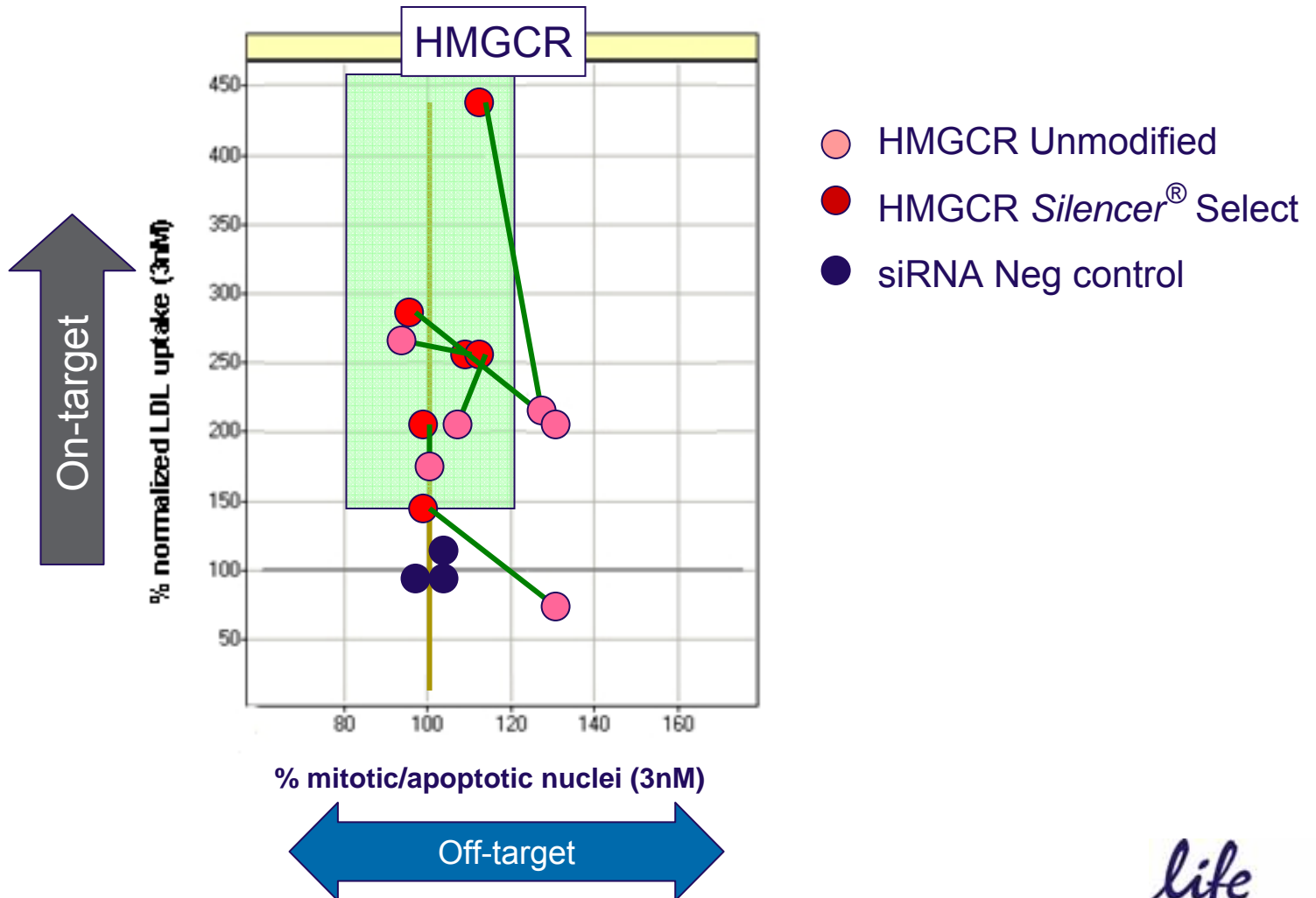


siRNA HMGCR

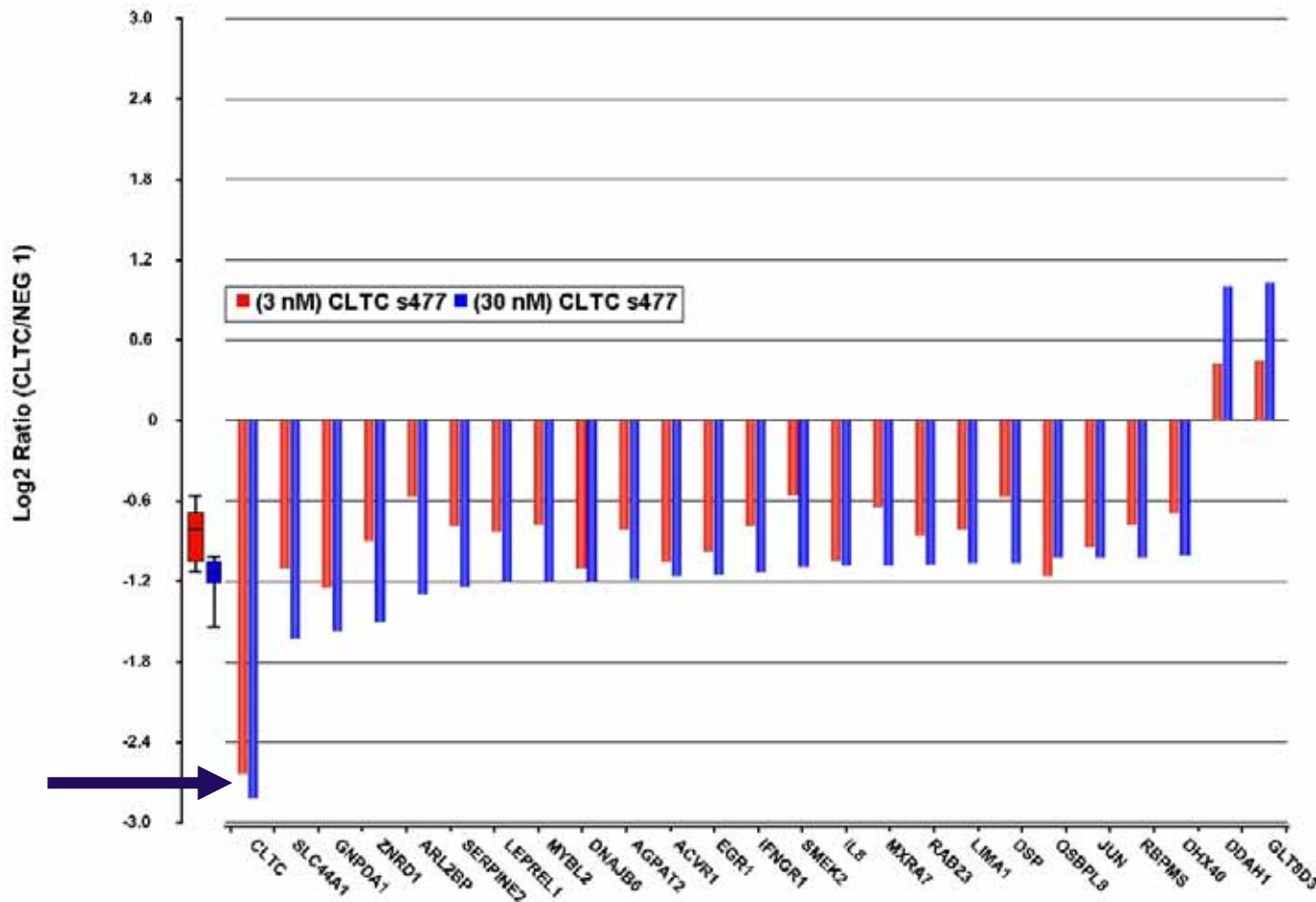
siRNAs designed with a new algorithm elicit expected phenotype at a higher rate than other siRNAs



Silencer[®] Select siRNAs produce coherent phenotypes



Silencer® Select siRNA induce potent knockdown at low concentration, leading to reduced off-target effects

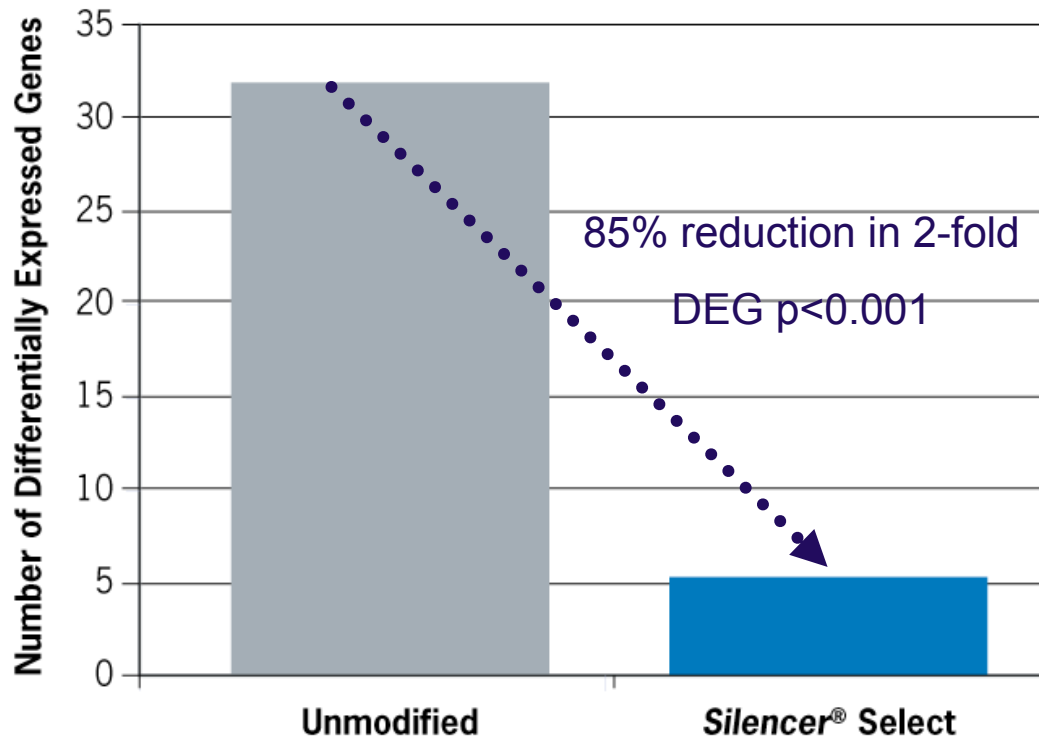


| [siRNA] conc | # of DEGs |
|--------------|-----------|
| 30 nM | 24 |
| 3 nM | 4 |

2-fold changers
p<0.001

Affymetrix U133 2.0 Plus

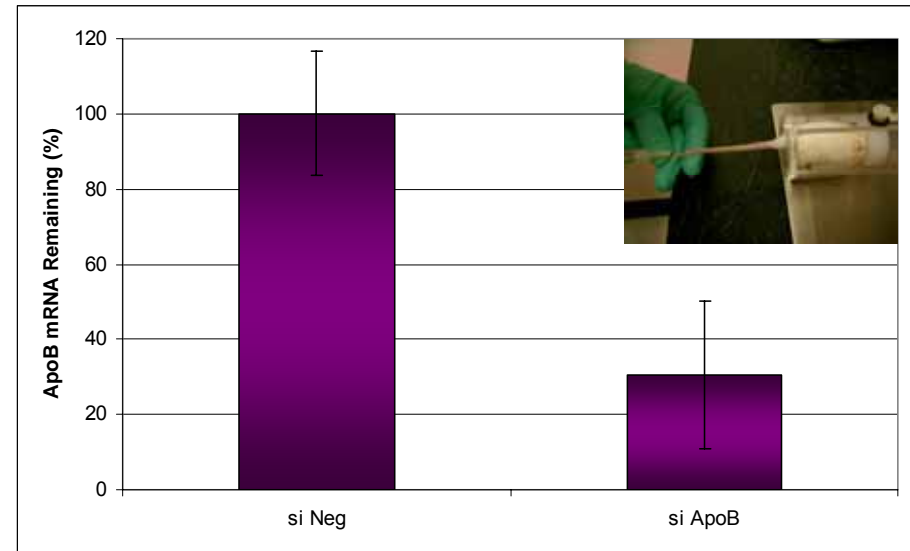
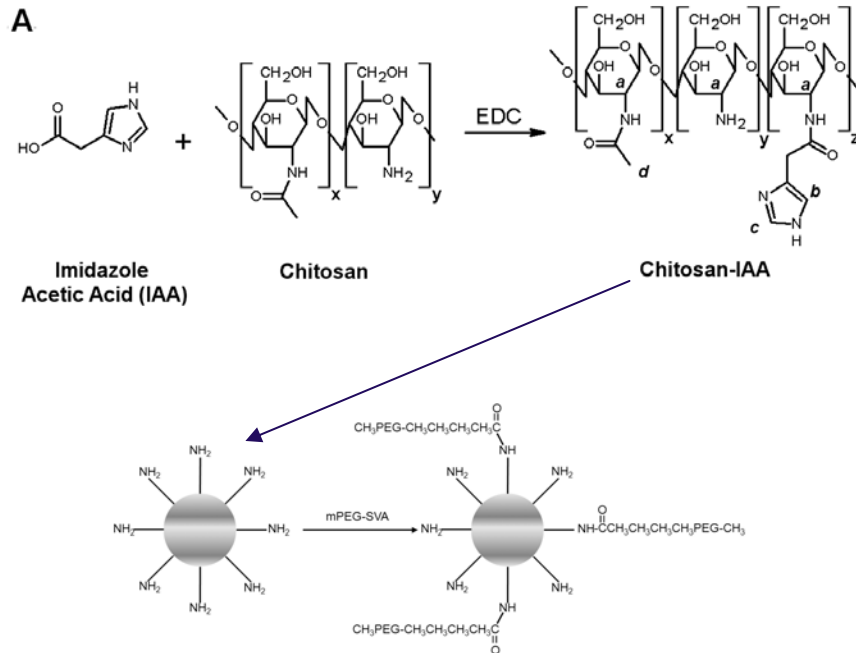
Silencer[®] Select siRNA modifications reduce the number of off-target, differentially expressed genes



Affy U133 2.0 plus

2-fold $p > 0.001$

Silencer® Select siRNA delivered with chitosan nanoparticles induce ApoB knockdown in the liver

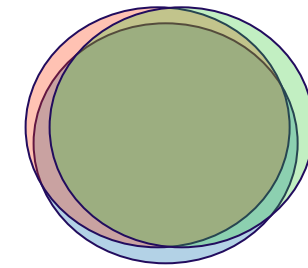
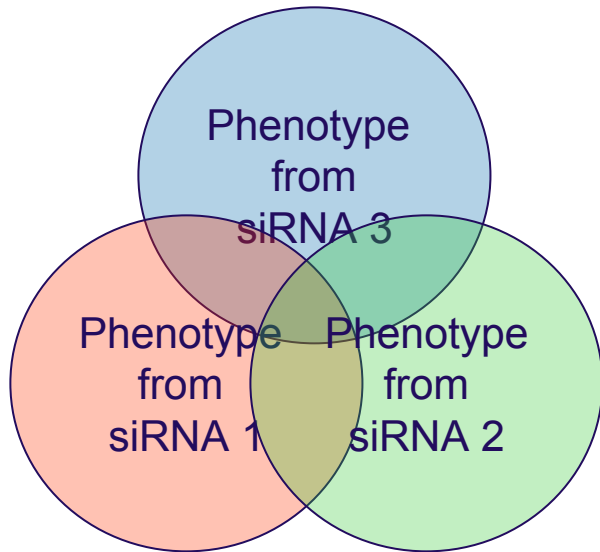


Schematic for the synthesis of imidazole-modified chitosan and subsequent PEG conjugation to the surface of chitosan-IAA/siRNA nanocomplexes.

Single low-pressure tail vein injection, of chitosan-IAA/siRNA nanocomplexes, 5 mg/kg siRNA.

Collaboration with Dr. Roy lab, UT Austin

Silencer® Select siRNA: the Next Generation of siRNA



Silencer® Select siRNAs

Save time and \$\$
Maximum knock-down of mRNA
Lower concentration to reduce off-targets
Fewer false hits, cleaner cell biology data

**Increased Confidence in Results
with Fewer siRNAs**