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eXIST Existenzgründungen aus der Wissenschaft

Universität Regensburg

siTOOLs BIOTECH

siPOOLs: highly complex but accurately defined siRNA pools eliminate off-target effects

Abstract: Short interfering RNAs (siRNA) are widely used as tool for gene inactivation in basic research and therapeutic applications. One of the major shortcomings of siRNA experiments are sequence-specific off-target effects. Such effects are largely unpredictable because siRNAs can affect partially complementary sequences and function like microRNAs (miRNAs), which inhibit gene expression on mRNA stability or translational levels. Here we demonstrate that novel, complex siRNA pools - referred to as siPOOLs - containing up to 60 selected siRNAs eliminate off-target effects while maintaining highly efficient and reliable target gene silencing. This is achieved by the low concentration of each individual siRNA diluting sequence-specific off-target effects below detection limits. In fact, whole transcriptome analysis reveal that single siRNA transfections can severely affect global gene expression. However, when complex siRNA pools are transfected, almost no transcriptome alterations are observed.

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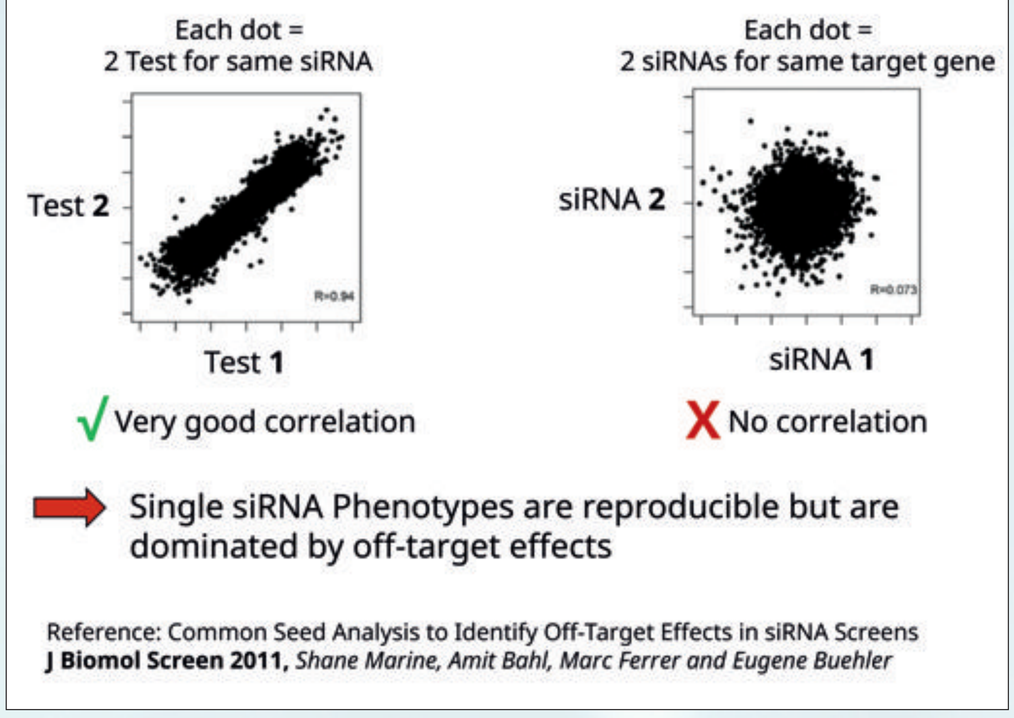
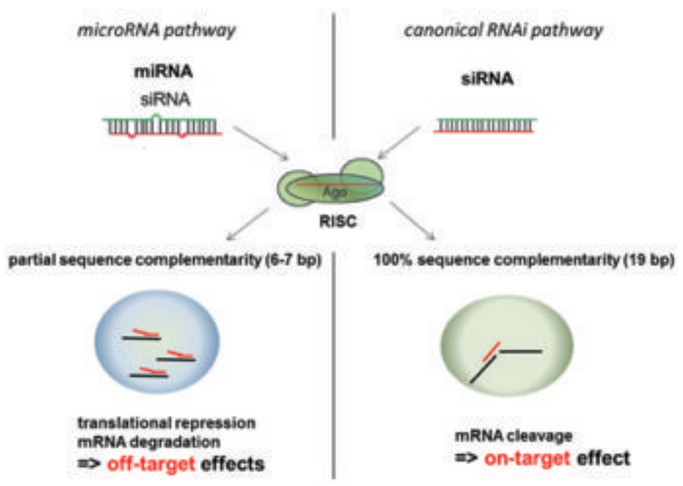
Correlation analysis of siRNA screening results

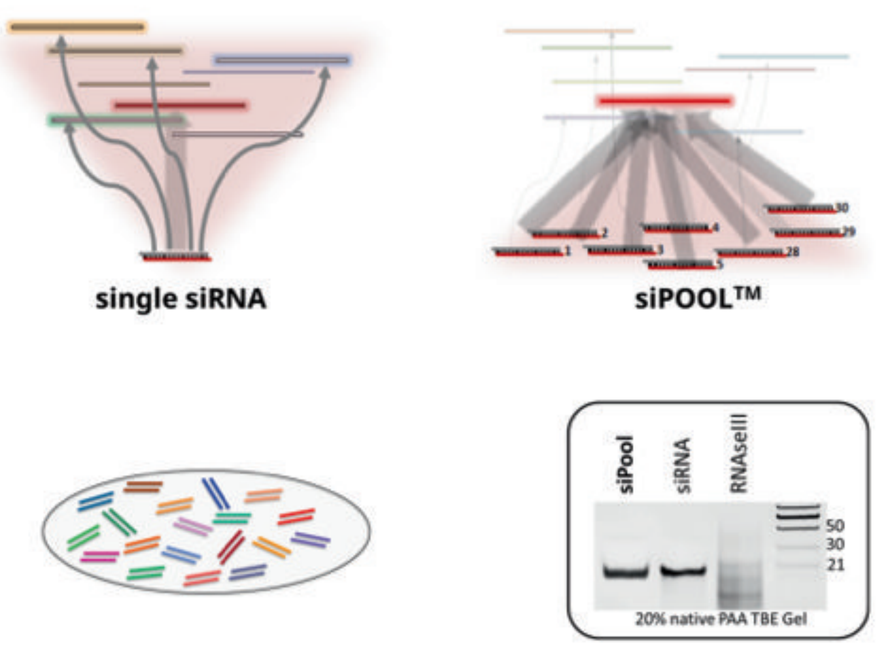


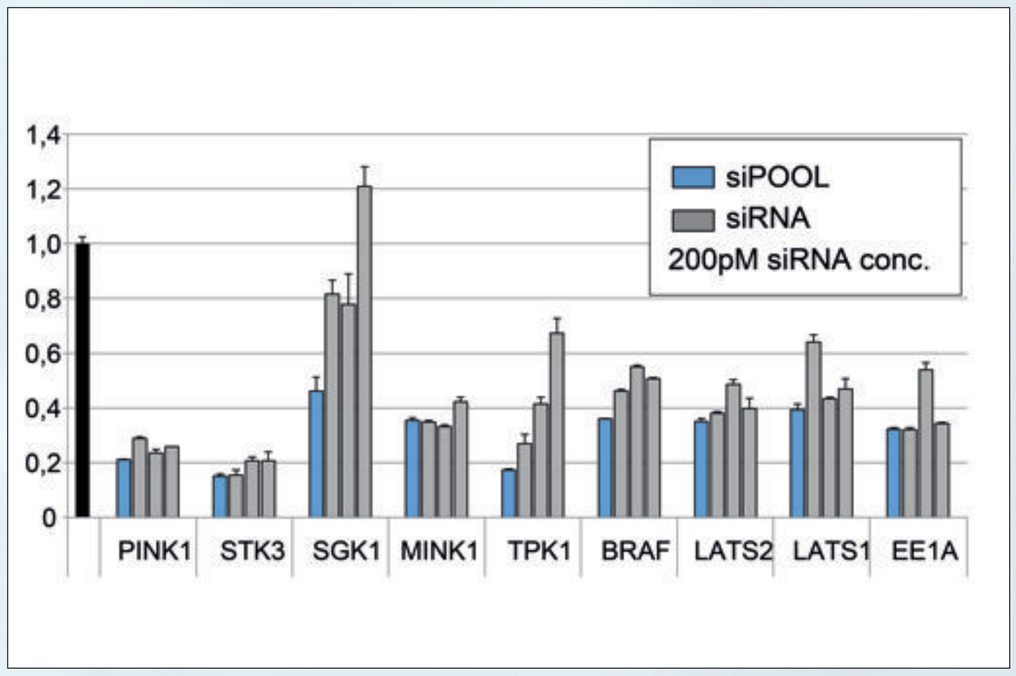
Reference: Common Seed Analysis to Identify Off-Target Effects in siRNA Screens
J Biomol Screen 2011, *Shane Marine, Amit Bahl, Marc Ferrer and Eugene Buehler*

siRNAs trigger off-target effects acting like microRNAs



siPools: Concept and Technology



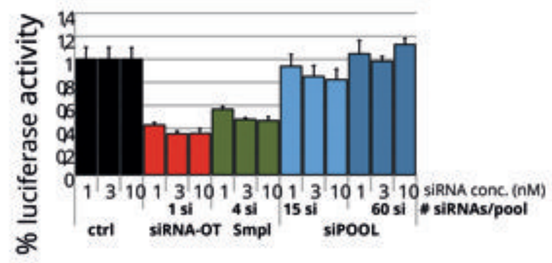


>15 siRNAs are required for efficient off-target dilution

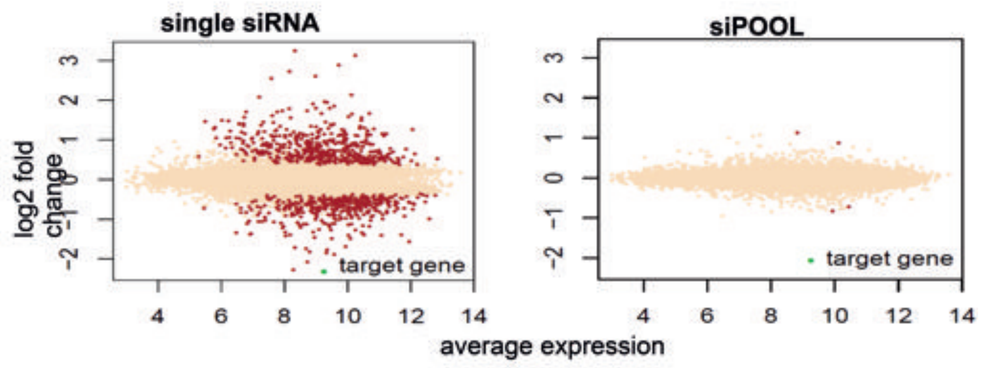
1: siRNAs with known strong off-target (King et al. 2012) for pool spiking

2: Luciferase off-target effect reporter

3: Results



siPOOLs do not affect global gene expression



Affymetrix array: 24h post transfection, 10nM, Hela