siPOOL-resistant rescue constructs

for further validation of gene knock-down

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To further validate that the loss-of-function phenotype produced by a siPOOL is specific to the targeted gene, we offer a **siPOOL-resistant rescue sequence**. When expressed in a plasmid construct, target gene function is restored which rescues/reverses the loss-of-function phenotype.

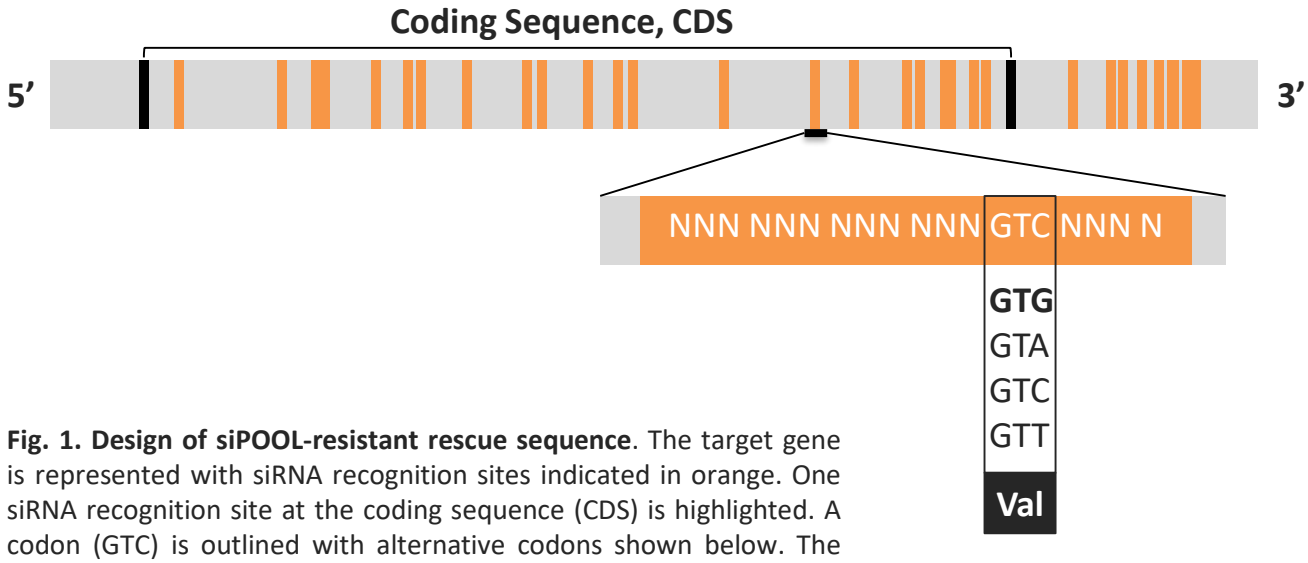

Fig. 1. Design of siPOOL-resistant rescue sequence. The target gene is represented with siRNA recognition sites indicated in orange. One siRNA recognition site at the coding sequence (CDS) is highlighted. A codon (GTC) is outlined with alternative codons shown below. The most abundant alternative codon in the transcriptome is shown in bold. Val: valine - encoded amino acid.

Design steps:

- select most abundant alternative codon at siRNA recognition site that alters mRNA sequence but not protein.
- Perform for **all codons** for **all CDS siRNA recognition sites** till sufficient mismatch reached.
- Remove common restriction enzyme sites / add flanking sequences for cloning siPOOL-resistant CDS into vector.

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Customer Data

Case 1: siPOOL reduced expression of kinase (X). Expression of siPOOL-resistant rescue construct restored kinase X expression and function, indicated by presence of phosphorylated substrate X (P-substrate X).

Data kindly provided by undisclosed customer

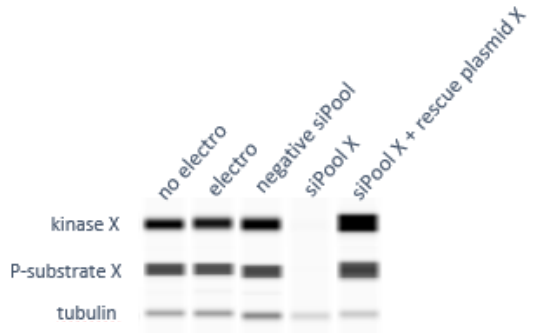

Fig. 2. Western blot of target kinase X and its phospho-substrate before and after siPOOL-mediated knock-down and rescue

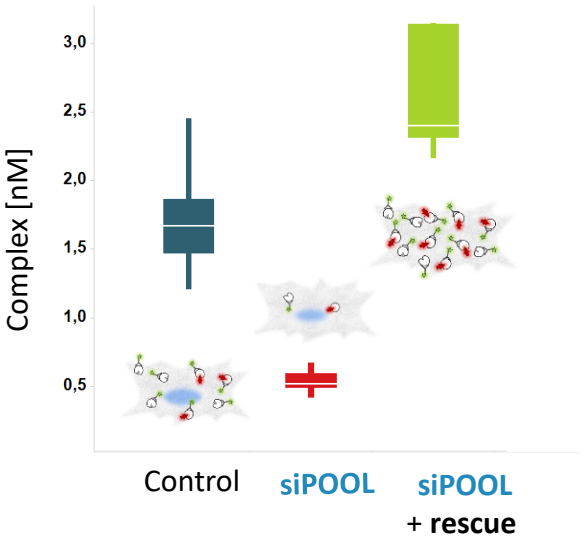

Fig. 3. Complex formation between two fluoro-labelled proteins as measured by FCCS in siPOOL-mediated knock-down and rescue conditions.

Case 2: Complex formation (measured by fluorescence cross-correlation spectroscopy, FCCS) was decreased on siPOOL-mediated knock-down of one labelled binding partner. Complex formation was restored upon expression of siPOOL-resistant rescue construct.

Data kindly provided by:

Intana Bioscience GmbH

siPOOL-resistant rescue sequences can be provided as a **sequence data file** or **cloned in a standard/custom construct**. Please contact us with your requests/enquiries.

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