RNA-seq-based Nucleic Acid Therapeutics lead optimization

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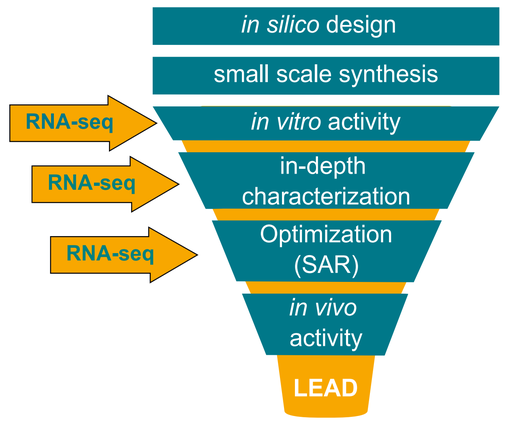
Nucleic Acid Therapeutics by LGC

Introduction

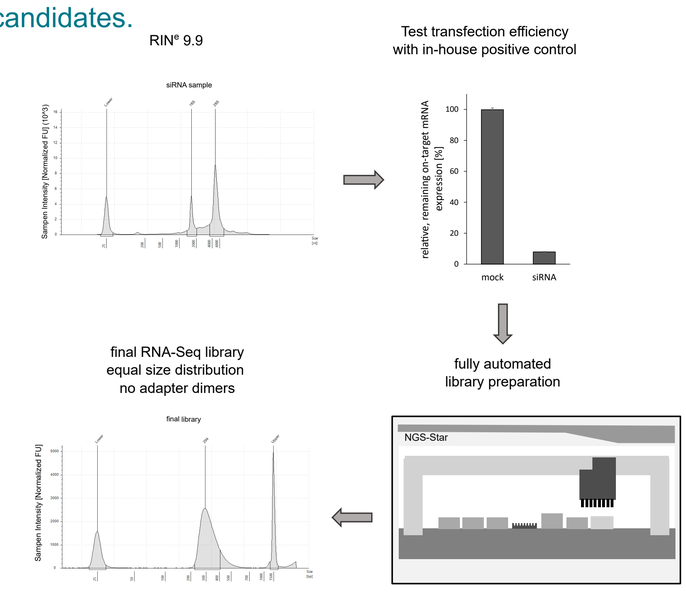
Axolabs is the leading service provider for the discovery, development, and manufacturing of gene expression modifying Nucleic Acid Therapeutics (NATs). Potency-, stability- and safety-optimized lead candidates are the basis for the development of new clinically relevant NATs. At Axolabs, lead identification is a well-established and proven process. At multiple stages of the lead identification process, RNA-seq analysis can provide additional information important for the decision, which candidates to pick for further development. By sorting out the least promising candidates already in an early stage, RNA-seq analysis provides a cost-efficient speed-up of the lead identification process.



Scheme of the lead identification process at Axolabs

Experimental Design

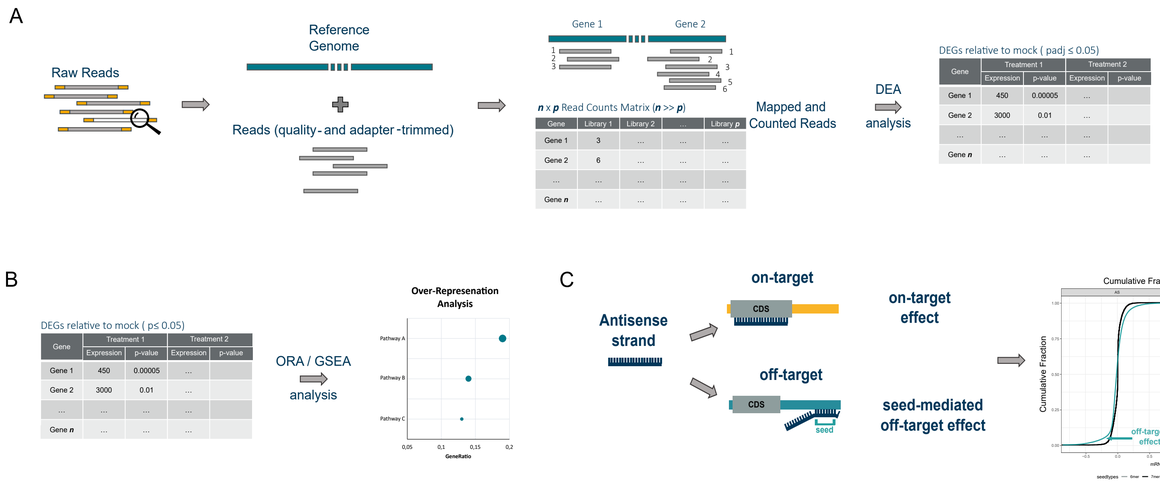
Together with your team, we design an RNA-seq experiment that is tailored to your needs. Regardless of whether you would be interested in an off-target profile of candidates in a big screening set or a more in-depth transcriptomic analysis with detailed off-target characterization of a smaller number of possible lead candidates.



Scheme of quality assessment for each RNA-seq experiment

Bioinformatic Analysis

Analysis of the RNA-seq experiment will be performed with our in-house data analysis pipeline facilitating a comprehensive RNA-seq analysis. For each transcriptomic analyses, we routinely perform a differential gene expression (DGE) analysis. Furthermore, a more detailed off-target characterization can be offered. This includes functional analysis, like Over-Representation Analysis (ORA) and Gene Set Enrichment Analysis (GSEA). For RNA-seq experiments with siRNAs, we also offer a seed-mediated off-target analysis.



Bioinformatics analysis: From Read counts to DEGs (A), from DEGs to Functional Insights (B) and seed-mediated off-target analysis (C).

Further Questions?

Visit us on www.axolabs.com

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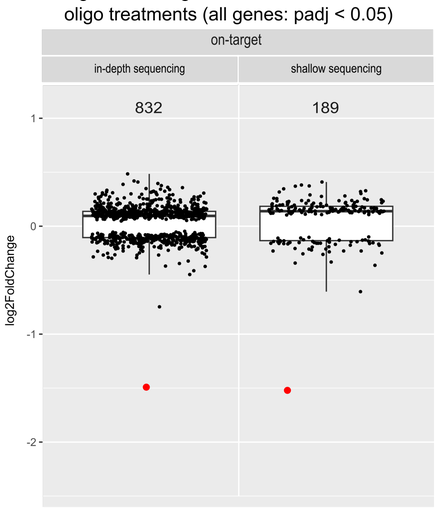
Implementing RNA-Seq analyses at different stages of the lead identification process

Should an in depth RNA-seq analysis or a screening RNA-seq be conducted?

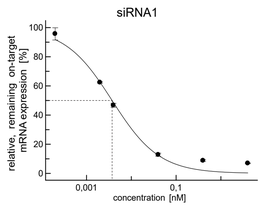
RNA-seq analysis with a higher sequencing depth offers the possibility to characterize the lead candidates further, while the shallow sequencing depth used in a screening RNA-seq will speed-up the decision-making process.

log2FoldChange Distributions across all oligo treatments (all genes: padj < 0.05)

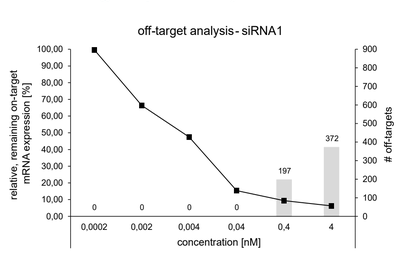


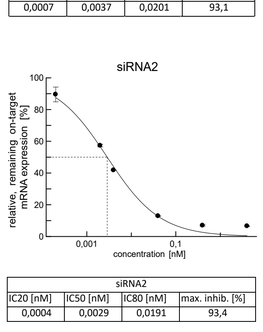
Which lead candidates to pick for further development when the candidates are equally well performing in the dose-response curve analysis?

Transcriptomic analysis in the early lead identification process in a screening approach does provide information regarding the off-target profile of the candidates in addition to the on-target potency.

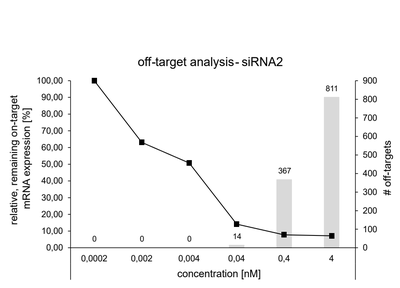


siRNA1			
IC20 [nM]	IC50 [nM]	IC80 [nM]	max. inhib. [%]
0,0007	0,0037	0,0201	93,1



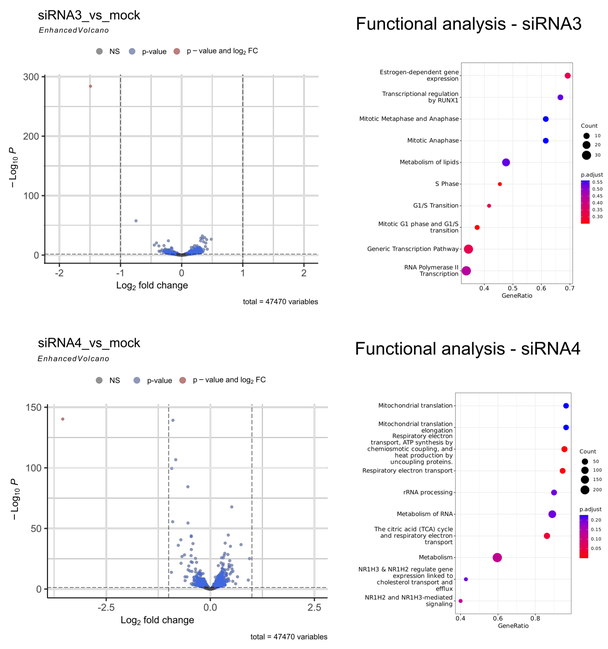


siRNA2			
IC20 [nM]	IC50 [nM]	IC80 [nM]	max. inhib. [%]
0,0004	0,0029	0,0191	93,4



How safe is my lead candidate?

In the advanced experimental stage, a more in-depth transcriptomic analysis can offer detailed characterization of the lead candidates. Following the DEA analysis, a functional analysis of all DEGs that overlap between different treatments, or individually for each of the treatments relative to mock can be conducted.



Are off-targets of my siRNA lead candidate seed-mediated?

We specifically offer a seed-mediated off-target analysis. In this analysis, we generate all relevant seed matches for sense- and antisense strand. With an in-house curated 3’UTR database, seed matches are counted, combined with the DEA results and plotted in a Cumulative Distribution Fraction (CDF) plot.