(SD2021-089) Unbiased approach for identification of regulators of materials and molecular uptake into cells

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BACKGROUND

A major bottleneck in nanocarrier and macromolecule development for therapeutic delivery is our limited understanding of the processes involved in their uptake into target cells. This includes their active interactions with membrane transporters that co-ordinate cellular uptake and processing. Current strategies to elucidate the mechanism of uptake, such as painstaking manipulation of individual effectors with pharmacological inhibitors or specific genetic knockdowns, are limited in scope and biased towards previously studied pathways or the intuition of the investigators. Furthermore, each of these approaches present significant off-target effects, clouding the outcomes.

Methods for intracellular transport of nucleic acids are much sought after in the context of both in vitro delivery reagents and in vivo therapeutics. Recently, we found that micellar assemblies of hundreds of amphiphiles consisting of single-stranded DNA which has been covalently linked to a hydrophobic polymer, referred to as DNA-polymer amphiphile nanoparticles or DPANPs, can readily access the cytosol of cells where they modulate mRNA expression of target genomes without transfection or other helper reagents, making them potential therapeutic nucleic acid carriers. However, despite their effective uptake properties and efficacy in the cytosol, it was unknown how these polyanionic structures can enter cells. Indeed, generally, bottlenecks in understanding and achieving delivery and uptake remain a forefront issue in translatability of macromolecular and nanomaterials-based therapeutics generally, including with respect to nucleic acid therapies.

The nature of pooled screening requires amplifying a single ~200nt region per cell, leading to screens that require amplification from tens-to hundreds of micrograms of genomic DNA. Inhibitory effects of high DNA concentration per PCR have led to a variety of solutions, ranging from simply pooling hundreds of PCR reactions to utilizing restriction enzyme sites present in the lentiviral backbone constant regions flanking the sgRNA to perform DNA gel electrophoresis and size selection to remove undesired gDNA. However, these approaches can be both expensive and have significant handling challenges when scaled to large screens.

TECHNOLOGY DESCRIPTION

To address this challenge, researchers from UC San Diego developed a pulldown-based approach utilizing biotinylated RNA oligonucleotides complementary to the backbone constant regions flanking the sgRNA site. The researchers developed a simple, low-cost approach for targeted amplification of CRISPR/Cas9 and other pooled screens using biotin-coupled RNA oligonucleotides that shows near-complete recovery of desired sgRNA target regions while removing unwanted genomic DNA background, allowing low-amplification library preparation at scale and with decreased technical variability. The development of this approach enabled the researchers to perform a genome-scale CRISPR/Cas9 screen to discover modulators of DPANP uptake in HEK293T cells. Confirming the value of this unbiased approach to the study of material transport, they

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OTHER INFORMATION

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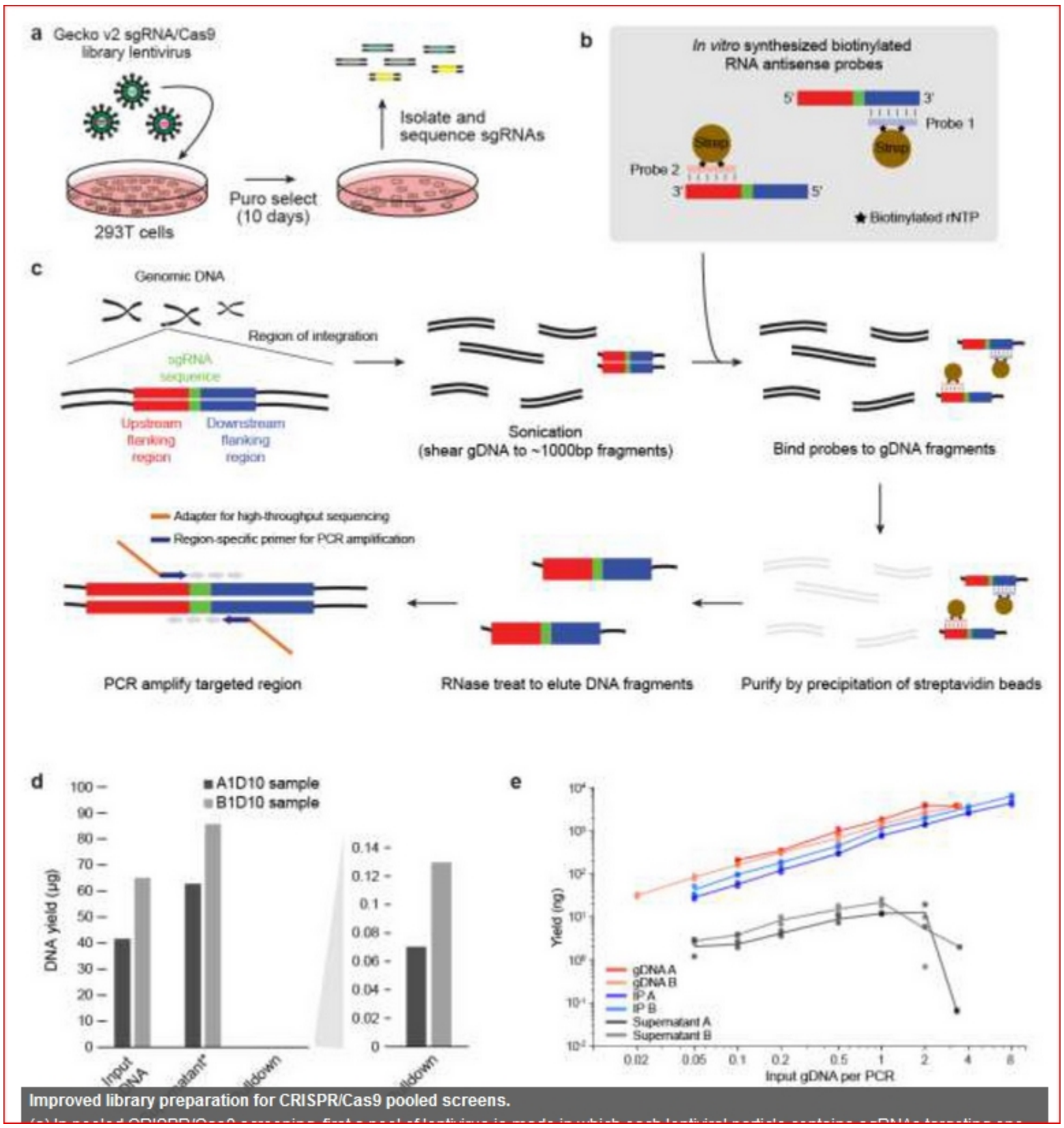
identified a solute carrier protein transporter (SLC18B1) as a novel effector of DPANP uptake. Thus, the use of pooled screening approaches can provide novel insights into as yet uncharacterized mechanisms of transport of therapeutically relevant materials and molecules, creating unique opportunities for future therapeutic development.

APPLICATIONS



Improved library preparation for CRISPR/Cas9 pooled screens.

RELATED MATERIALS

- Unbiased identification of nanoparticle cell uptake mechanism via a genome-wide CRISPR/Cas9 knockout screen Eric L. Van Nostrand, Sarah A. Barnhill, Alexander A. Shishkin, David A. Nelles, Eric Byeon, Thai Nguyen, Yiu Chueng Eric Wong, Nathan C. Gianneschi, Gene W. Yeo bioRxiv 2020.10.08.332510; doi: https://doi.org/10.1101/2020.10.08.332510 - 10/09/2020

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