Improving the Chemical Synthesis and Performance of Extended-Length Guide RNAs for Genome Editing and Control of Gene Expression

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The landscape of CRISPR-based technologies for genome editing and control of gene expression is expanding rapidly and many of these technologies utilize extended-length guide RNAs that are challenging to produce by traditional RNA synthesis chemistries. Two examples include:

(i) the SAM CRISPR-activation system (Konermann, et. al., Nature, 2015) which employs guide RNAs around 160-nt long containing two MS2 RNA aptamers, and

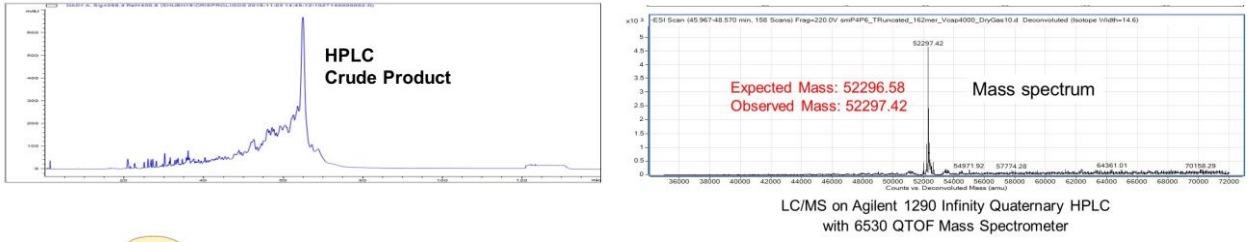
(ii) Prime Editing (Anzalone et. al., Nature, 2019) which employs extensions of the guide RNA to direct the replacement or insertion of new DNA sequences into the genome.

Using a novel RNA synthesis chemistry (Dellinger et. al. JACS, 2011) we find it straightforward to chemically synthesize long RNA oligos in the size range currently utilized for these applications. We are continuing to further develop synthesis and purification methodologies to enable effective synthesis of even longer RNA oligos.

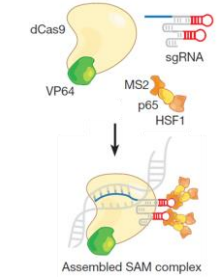
Studies of the stability and activity of 163mer guide RNAs designed for the SAM CRISPR-activation system demonstrate that different numbers and types of modifications incorporated at the 5' and 3' ends can enhance their stability in cells while maintaining guide RNA functionality.

Robust manufacturing of extended-length synthetic guide RNAs

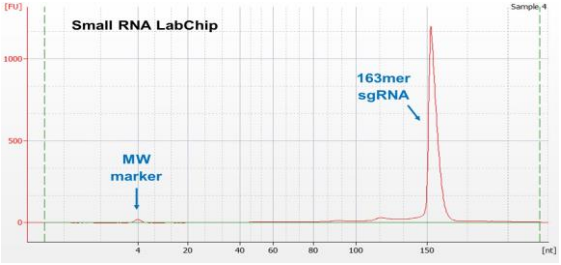
162mer single guide RNA



LC/MS on Agilent 1290 Infinity Quaternary HPLC with 6530 QTOF Mass Spectrometer

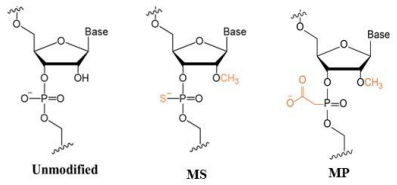


*SOURCE: Konermann et al. *Nature* 2015, *517*, 583-8 (*used by permission*)



Bioanalyzer analysis of synthetic 163mer sgRNA (post-HPLC purification)

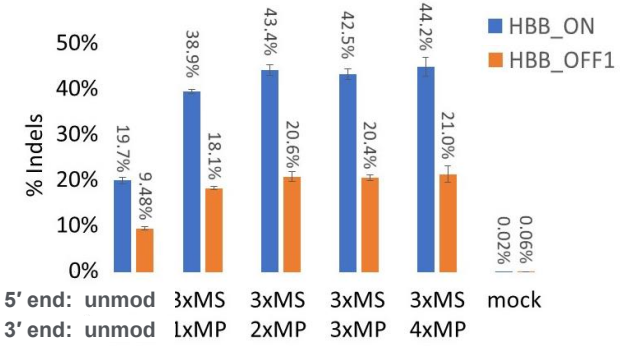
Alternative end-protection extends half-life of extended-length guide RNAs in cells

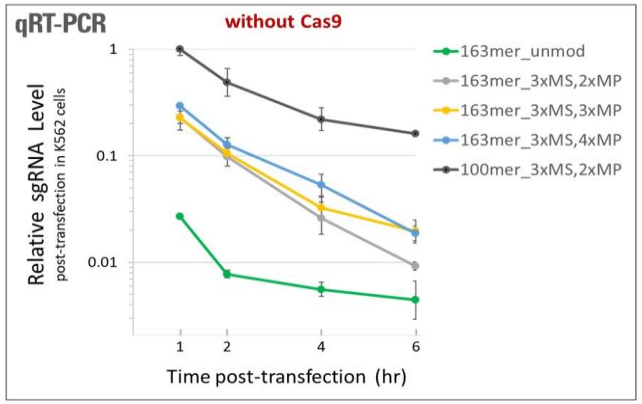


A 100-nt single guide RNA targeting the HBB gene was extended to 163 nt by adding two MS2 RNA aptamer sequences. 163mer HBB guide RNAs containing different numbers of MS or MP modifications at their 5' and 3' ends were nucleofected into mammalian cells with or without Cas9 protein.

Indel frequencies at the on-target site (HBB_ON) and a highly impacted off-target site (HBB_OFF1) were measured by amplicon deep sequencing of genomic DNA from HepG2 cells co-transfected with chemically-modified 163mer guide RNAs and Cas9 protein.

Quantitative RT-PCR was used to directly measure the relative amounts of guide RNAs transfected without Cas protein persisting over time post-nucleofection.





Conclusions

Robust chemical synthesis of extended length guide RNAs for emerging applications like CRISPR-activation and Prime Editing affords multiple advantages including:

- increased efficacy of guide RNAs
- robust and scalable production for biotechnological and therapeutic applications
- greater flexibility in the guide RNA design including the ability to incorporate chemical modifications site-specifically to enhance performance