Data Sheet

Agilent
Trusted Answers

SureGuide Chemically Synthesized CRISPR Guide RNA

Introduction

Agilent SureGuide chemically synthesized CRISPR single guide RNAs (sgRNAs) are the industry standard for quality RNA oligonucleotides. Using our patented, proprietary synthesis chemistry[1] and proprietary "SureGuide Purified" purification method makes the custom synthesis of long RNA oligonucleotides robust and efficient. This combination of chemistry and purification methods allows for reliable, high-quality production of high-fidelity, long sgRNAs with a variety of available chemical modifications. These include modifications such as 3xMS, that have become widely used to improve editing efficiency, as well as other modifications that can further enhance stability and specificity of guide RNAs in a variety of cell types.[2,3] Produced with stringent quality control under ISO 13485, fully customizable Agilent SureGuide Purified sgRNAs give you quick and easy gene editing, high indel frequency, and reproducible results. With SureGuide Purified sgRNAs for research applications and Agilent ClinGuide GMP sgRNAs for clinical applications, we can support your development needs from research through clinical applications.

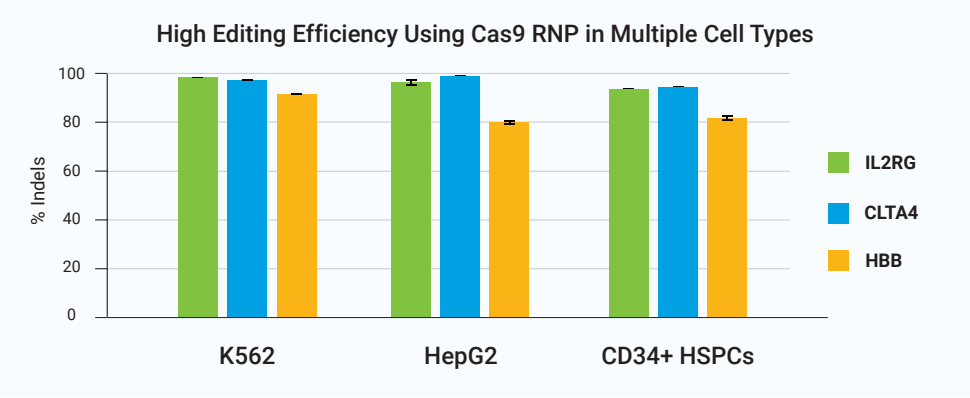

Figure 1. High levels of on-target editing are observed across multiple cell lines including K562 and HepG2 cells and in sensitive human primary cells such as C34+ HSPCs. SureGuide Purified sgRNAs containing 3xMS modifications (175 pmol) were precomplexed with *Streptococcus pyogene* Cas9 (SpCas9) protein (50 pmol) and transfected into 0.2 million cells by electroporation. Three sgRNAs were tested in each cell line to perform editing of IL2RG, CLTA4, and HBB gene targets. Editing yields were measured by deep sequencing of PCR amplicons of the target locus. Bar charts display the mean of triplicate transfections with standard deviation represented as error bars.

Key Features of Agilent SureGuide Purified sgRNA

- High editing efficiencies across guide designs, cell types, and Cas enzymes
- Guides are manufactured using our proprietary chemistry and purification method under ISO 13485 to ensure high quality and high reproducibility across sgRNA sequences and orders
- Fully custom, de novo synthesis of every guide, including the tracrRNA sequence, to facilitate a wide variety of applications
- SureGuide Purified sgRNAs enable 90-120nt lengths and up to 10 chemical modifications, including 2'O-methyl (M), 2'-O-methyl 3'-phosphorothioate (MS), Deoxy (D), and 2'-ribo 3'-phosphorothioate (S) (additional modifications and lengths available as HPLC purified custom requests)
- SureGuide Purified sgRNAs can include our patented 3xMS chemical modifications at the 5' and 3' ends for sgRNA stability and higher editing efficiency (Figure 2)
- Combine ribonucleotides and deoxyribonucleotides in SureGuides for broader applications
- Easy-to-use CRISPR design and sequence upload software, and eCommerce checkout

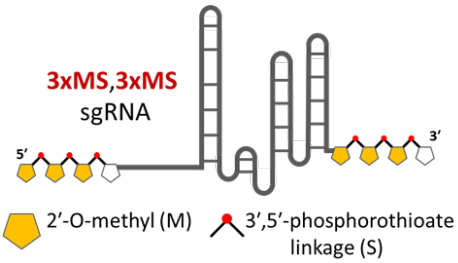

Figure 2. Agilent 3xMS Chemical Modification.

High Editing Efficiency Across Targets, Cell Types, and Cas Enzymes

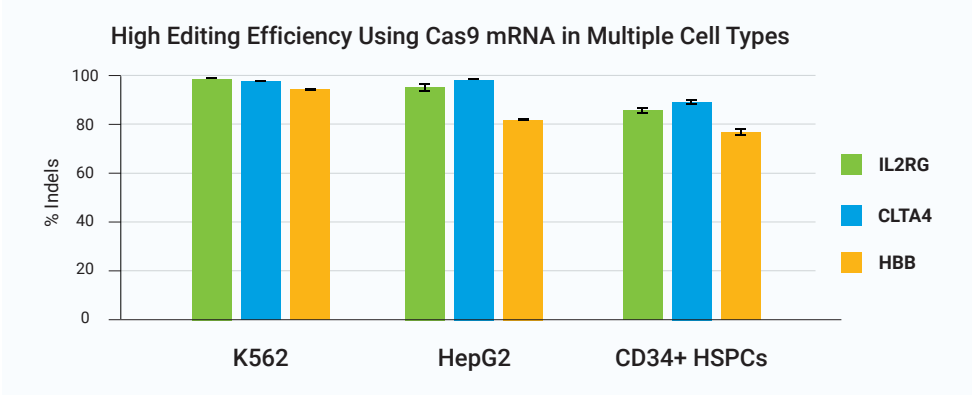

Figure 3. High levels of on-target editing are observed across multiple cell lines including K562 and HepG2 cells and in sensitive human primary cells such as C34+ HSPCs. SureGuide Purified sgRNAs containing 3xMS modifications (125 pmol) were mixed with SpCas9 mRNA (75 µg) and transfected into 0.2 million cells by electroporation. Three sgRNAs were tested in each cell line to perform editing of IL2RG, CLTA4, and HBB gene targets. Editing yields were measured by deep sequencing of PCR amplicons of the target locus. Bar charts display the mean of triplicate transfections with standard deviation represented as error bars.

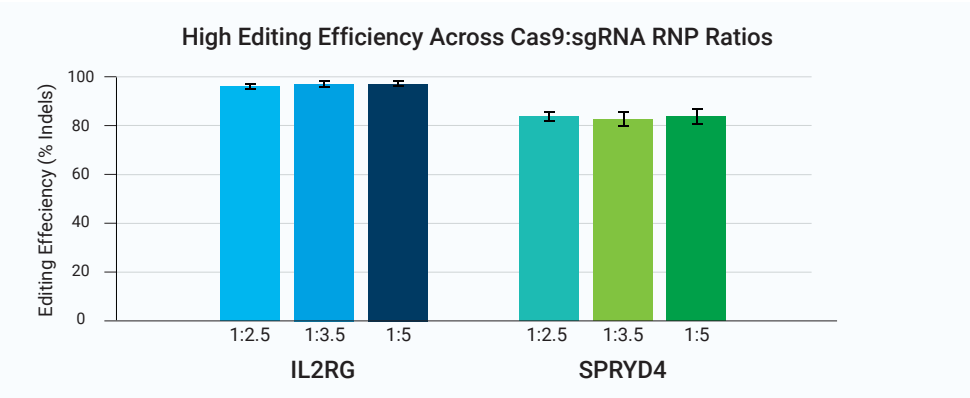

Figure 4. High levels of on-target editing are observed across multiple SureGuide designs when tested at RNP ratios ranging from more limiting to higher excess input of sgRNA. A constant amount of SpCas9 protein (10 pmol) was precomplexed with increasing amounts of two different SureGuide Purified sgRNAs containing 3xMS modifications targeting IL2RG and SPRYD4 genes at 25 pmol, 35 pmol, and 50 pmol to generate ratios of 1:2.5, 1:3.5 and 1:5, respectively. K562 cells were transfected with the different RNP mixtures using electroporation. Bar charts display the mean of quadruplicate transfections with standard deviation represented as error bars.

High Reproducibility for Confidence in Quality

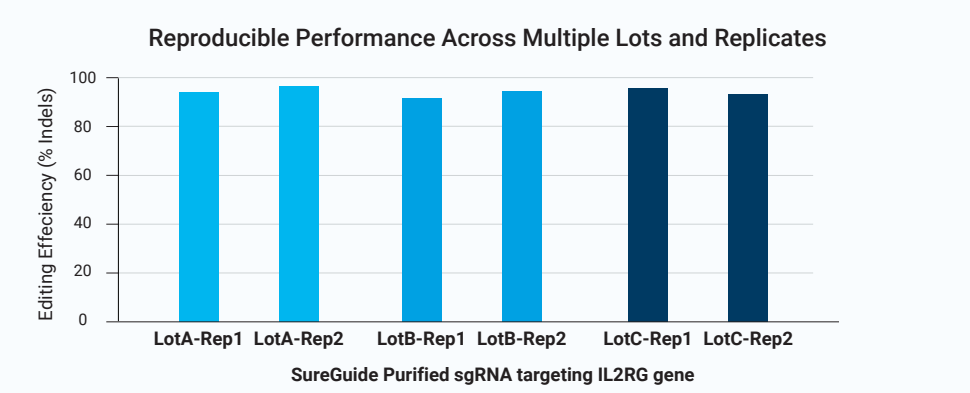

Figure 5. High levels of on-target editing are observed across multiple replicate syntheses within a lot and across multiple lots of SureGuide Purified sgRNA produced by high quality Agilent manufacturing processes. SureGuide Purified sgRNAs with 3xMS modifications targeting the IL2RG gene were produced in 3 lots with 2 replicates within each lot and transfected at 2.5x molar ratio of sgRNA (125 pmol) to SpCas9 protein (50 pmol) in K562 cells. Bar charts display the mean of quadruplicate transfections.

Easy to Use CRISPR Design and Sequence Upload Software

Use the online Agilent CRISPR gRNA Design Software to upload your guide sequences or choose from one in the Agilent Design Catalog to place your order or quote request. Start using the software today at:
https://crispr-tool.agilent.com/home

Delivering Research to GMP sgRNA Solutions

One of the advantages of partnering with Agilent is the ability to transition from research grade to GMP guide RNAs. Extensive knowledge gained over decades of experience enable Agilent nucleic acid experts to provide high quality R&D-grade RNA oligos for your preliminary research assays and development phases. When you are ready, experts are here to help you scale-up to larger quantity GMP material.

References

1. Dellinger, D. J., *et al.* Streamlined Process for the Chemical Synthesis of RNA Using 2'-O-Thionocarbamate-Protected Nucleoside Phosphoramidites in the Solid Phase. J. Am. Chem. Soc. **2011**, 133 (30), 11540–11556. https://doi.org/10.1021/ja201561z.
2. Hendel, A., *et al.* Chemically Modified Guide RNAs Enhance CRISPR-Cas Genome Editing in Human Primary Cells. Nat. Biotechnol. **2015,** 33 (9), 985–989. https://doi.org/10.1038/nbt.3290.
3. Ryan, D. E., *et al.* Improving CRISPR-Cas Specificity with Chemical Modifications in Single-Guide RNAs. Nucleic Acids Res. **2018,** 46 (2), 792–803. https://doi.org/10.1093/nar/gkx1199.

Ordering Information

To learn more about Agilent's CRISPR gRNA portfolio, online design software, and user manual, visit:
http://www.agilent.com/chem/crispr-sgrna-info

Part Number	Product Name
G7250B	SureGuide sgRNA, 100µg, 90-120nt
G7250C	SureGuide sgRNA, 200µg, 90-120nt
G7250D	SureGuide sgRNA, 500µg, 90-120nt
G7250E	SureGuide sgRNA, 1mg, 90-120nt

For Agilent patent and license information please visit:
http://www.agilent.com/chem/sgrna-patent-info

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