PRODUCT BULLETIN

Stability testing of synthetic RNA oligos

Summary

The functionality of three synthetic RNA oligos was tested under various storage conditions to model suboptimal temperatures which may be encountered during shipment. Functionality of the oligos was measured by CRISPR-Cas9 gene editing in a standard T7 Endonuclease I (T7EI) mismatch detection assay. While storage of the dried RNA at the recommended temperature (-20°C) is encouraged, none of the tested conditions adversely impacted the ability of the oligos to induce gene editing.

Experimental details

5 tubes each of: Edit-R PPIB synthetic crRNA control, Edit-R CRISPR-Cas9 synthetic tracrRNA, and Edit-R Human PPIB synthetic sgRNA positive control were stored dry in each of the following conditions:

1. -20°C for four weeks
2. 24°C for two weeks
3. 37°C for two weeks
4. 24°C for four weeks
5. 37°C for four weeks

Samples were resuspended in 10mM Tris-HCl pH 7.4 and tested for gene editing efficiency in U2OS-CAG-Cas9 stable cells using the following conditions: 10,000 cells per well, 0.2 µL DharmaFECT 4 per well. A T7E1 mismatch detection assay conducted 72-hours after transfection (results shown in Figure 1). High-temperature-stored crRNA was paired with tracrRNA stored at matching conditions to test both RNAs at consistent storage conditions.

Conclusion

None of the tested conditions reduced the gene editing activity of the RNA. Therefore, the synthetic RNA oligos are stable under recommended storage conditions as well as those that deviate from the recommended conditions for the durations examined.

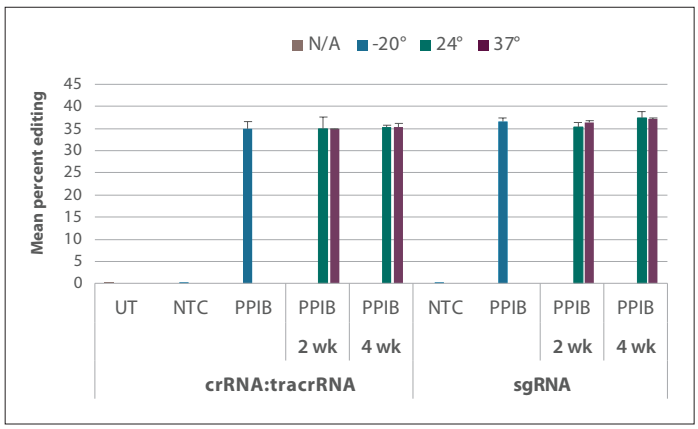

Figure 1. Synthetic RNA oligos retain functionality following storage at suboptimal conditions. Percent editing was measured by T7E1 mismatch repair assay 72-hours after transfection for the oligos stored at 24° or 37° for 2 or 4 weeks, compared to the recommended -20°C. Untransfected (UT) cells and a non-targeting control guide (NTC) served as experimental controls.

For more information

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