For each target, included summary file.

Original LE data is shown on the top, with variant sequences on the bottom. The x-axis shows the nucleotide number and identity. LE are indicated with a purple box, and the variant nucleotides are indicated below the box.

**SHAPE reactivity data:** black, yellow, and red columns represent SHAPE reactivity (level indicated on y-axis) for every measured nucleotide. Gray blocks represent nucleotides whose data are excluded from analysis, either because they were in the primer region (which do not incorporate mutations) or because their background mutation rate exceeded our threshold.

**Pairing probabilities:** yellow, blue, and green arcs represent the result of the SHAPE-informed partition function (RNAStructure partition and ProbabilityPlot), where pseudo-free energies (including SHAPE reactivity bonuses) are calculated for possible folding patterns. Arc colors represent the percent of structures where each indicated base pair are observed.

**Significantly different reactivities:** orange rectangles mark nucleotides whose reactivity is significantly different between the variants. This is calculated from z-scores generated from the “LOG relative reactivity difference” measure, the difference between the log transform of each sample’s modified rate/unmodified rate. See the methods section labeled “*Identification of in-cell changes in MUNC spliced SHAPE reactivity*” in this *Cell Reports* publication for more detail: <https://doi.org/10.1016/j.celrep.2022.110361>.

**Thoughts on net1:** alteration of sequence at the LE dramatically increases reactivity around the site, while overall reactivity throughout remains similar. In the unaltered sequence, high likelihood that this region is actually base-pairing with the shared sequence ends and potentially represents an artifact of native and non-native sequence pairing. The modeling programs tend to favor creating end pairings, so would have to follow-up with an adapter-based approach to get info on ends and be sure.

**Thoughts on trak2:** global profiles look very similar between unaltered and altered sequence, and significant changes are localized exactly at the LE. The sequence changes appear to dramatically alter which structures might dominate, but those structures again include pairings with the no data primer regions.

**Thoughts on trp52inp2:** this example appears to show a potentially true shift in structure upon sequence alteration that is entirely contained within the mapped sequence. (The altered sequence does make an extend structure into the primer region, but the important changes are irrespective to this). I included minimum free energy models (output of RNAStructure Fold) of each variant to compare.