Affinity purification of T7 RNA transcripts with homogeneous ends using ARiBo and CRISPR tags.

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Supplementary Material

Supplementary Figures

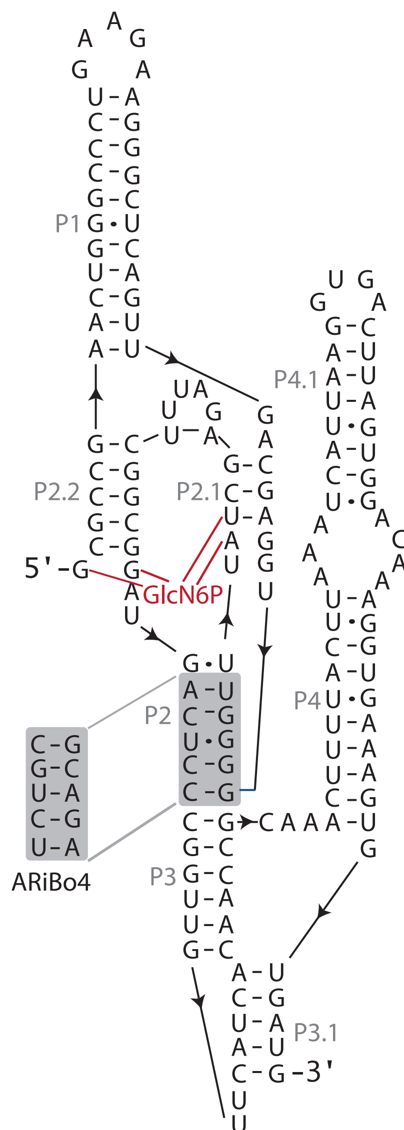


Figure S1. The ARiBo4 tag used in this study is a P2 helix mutant of the ARiBo1 tag previously employed for affinity purification (Di Tomasso et al. 2011; Di Tomasso et al. 2012). The primary and proposed secondary structures of the ARiBo1 tag are shown with the original P2 region and the corresponding region in ARiBo4, both boxed in grey, as indicated.

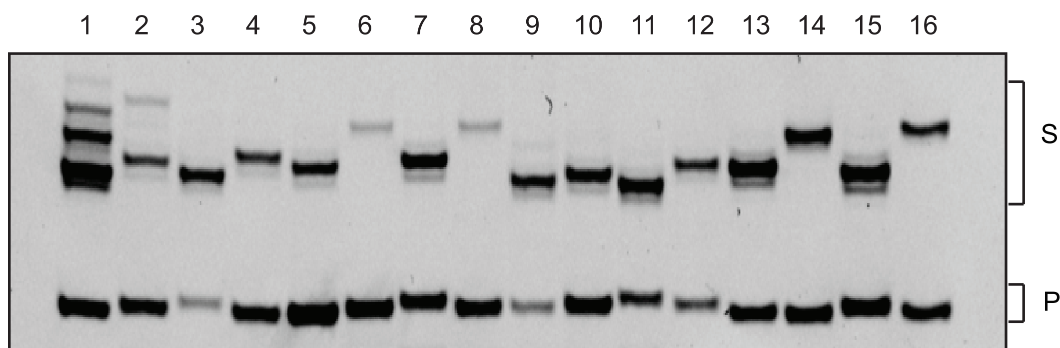


Figure S2. Evidence of 5'-heterogeneity from VS ribozyme cleavage of affinity-purified SLI RNAs transcribed as SLI-ARiBo precursors with the wild-type T7 RNAP from the T7 class III promoter. The E1 elution fractions of each of the 16 small-scale affinity purifications are analyzed on a 20% denaturing polyacrylamide gel stained with SYBR Gold. Prior to elution, the bound ARiBo-fusion SLI RNAs were incubated with the VS ribozyme. The 29-nucleotide SLI substrates (S) and 24-nucleotide products (P) are detected on the gel. Gel lanes match the SLI numbering given in Fig. 2A. From the 400-μL elution volumes, 2 μL aliquots were loaded on the gel.

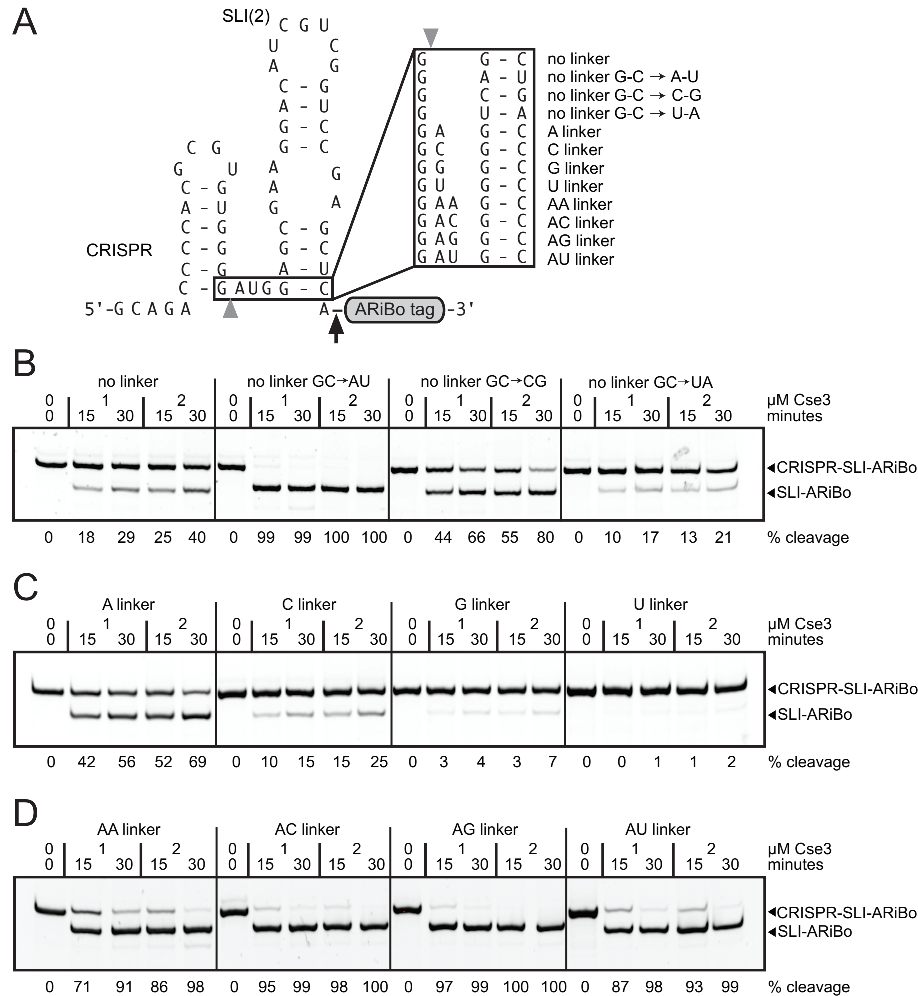


Figure S3. Effect of CRISPR-RNA junction sequence on Cse3 cleavage at 37˚C. (*A*) Schematic representation of CRISPR-SLI(2)-ARiBo double-fusion RNAs with the original CRISPR sequence (AUG linker) or related variants with sequence changes at the CRISPR-RNA junction (boxed area). The grey arrowhead points to the Cse3 cleavage site, whereas the black arrow points to the *glmS* cleavage site. (*B*), (*C*) and (*D*) Cse3 cleavage of CRISPR-SLI-ARiBo RNAs analyzed on 10% denaturing polyacrylamide gels stained with SYBR Gold. Cse3 cleavage was performed at 37˚C using aliquots from the transcription reactions (~1 µM RNA), 20 mM HEPES pH=7.5, 150 mM KCl, either 1 or 2 µM Cse3 and different incubation times, as indicated above each lane. The gel mobility of the RNA precursor (CRISPR-SLI-ARiBo) and the Cse3 cleavage product (SLI-ARiBo) are indicated with arrows on the right side of the gel. The percentages of Cse3 cleavage are given below the gels.

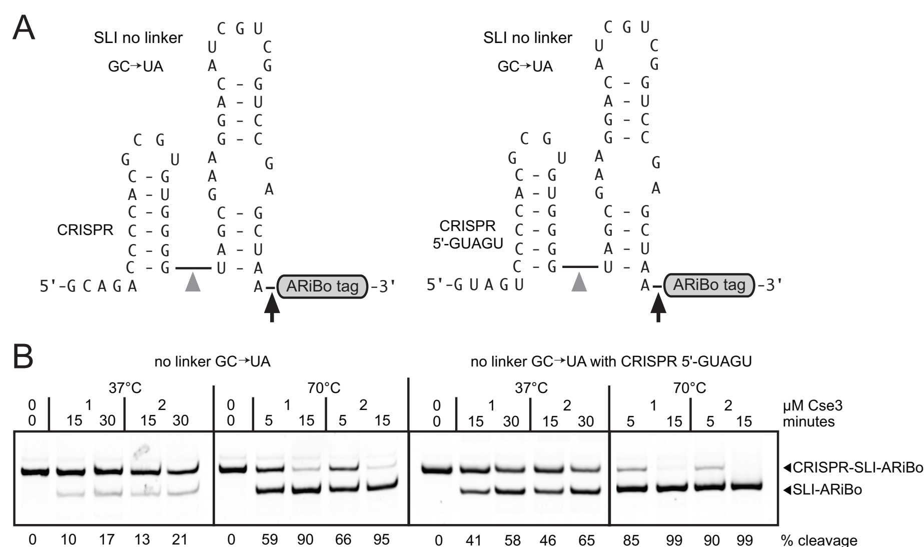


Figure S4. Reversion of the CRISPR 5'-tail back to the wild-type sequence favors Cse3 cleavage of a CRISPR-SLI-ARiBo double-fusion RNA with a U-A base pair closing the SLI stem. (*A*) Schematic representation of CRISPR-SLI-ARiBo double-fusion variant RNAs with no linker between the CRISPR and the SLI RNA and a G-C to U-A base-pair change at the bottom of the SLI stem (no linker GC→UA). The original sequence with the 5'-GCAGA CRISPR tail (no linker GC→UA) was changed to a 5'-GUAGU tail (no linker GC→UA with CRISPR 5'-GUAGU). The grey arrowhead points to the Cse3 cleavage site, whereas the black arrow points to the *glmS* cleavage site. (*B*) Cse3 cleavage of CRISPR-SLI-ARiBo RNAs analyzed on 10% denaturing polyacrylamide gels stained with SYBR Gold. Cse3 cleavage was performed at 37˚C or 70˚C using aliquots from the transcription reactions (~1 µM RNA), 20 mM HEPES pH=7.5, 150 mM KCl, either 1 or 2 µM Cse3 and different incubation times, as indicated above each lane. The gel mobility of the RNA precursor (CRISPR-SLI-ARiBo) and the Cse3 cleavage product (SLI-ARiBo) are indicated with arrows on the right side of the gel. The percentages of Cse3 cleavage are given below the gels.

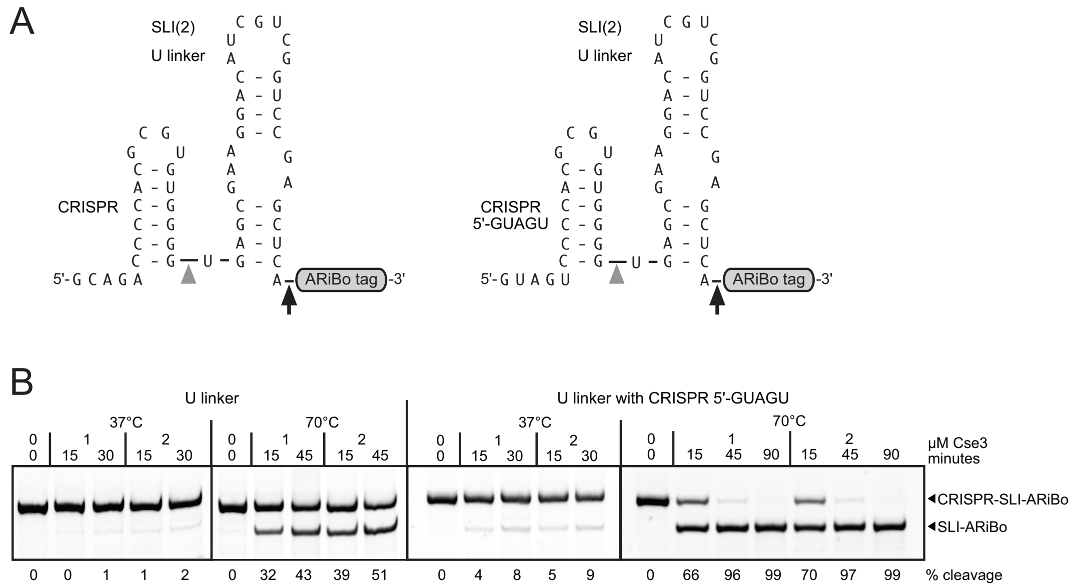
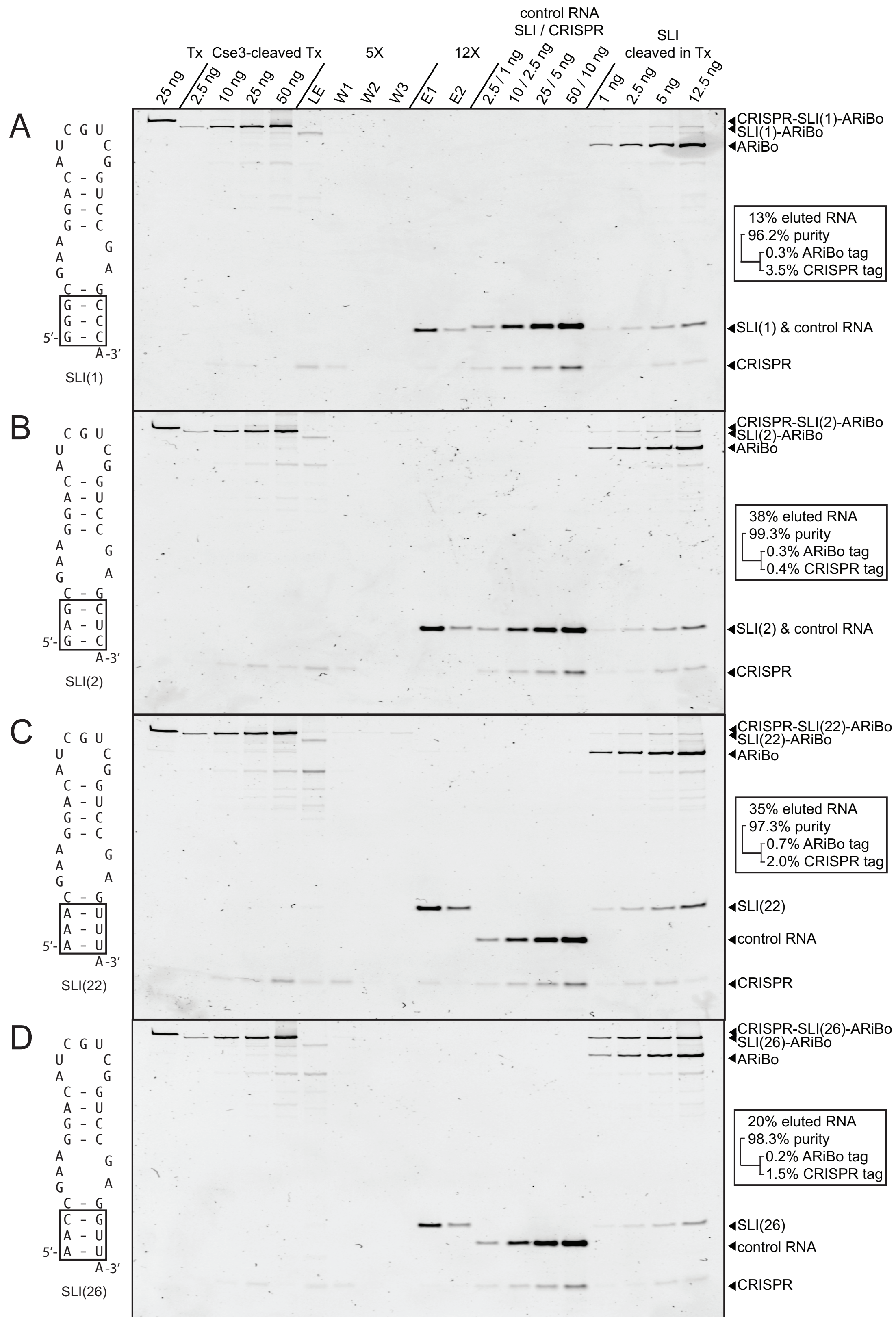


Figure S5. Reversion of the CRISPR 5'-tail back to the wild-type sequence favors Cse3 cleavage of a CRISPR-SLI-ARiBo double-fusion RNA with a U linker between the CRISPR and the SLI(2) RNA. (*A*) Schematic representation of CRISPR-SLI(2)-ARiBo double-fusion variant RNAs with a single-nucleotide U linker between the CRISPR and the SLI(2) RNA. The original sequence with the 5'-GCAGA CRISPR tail (U linker) was changed to a 5'-GCAGU tail (U linker with CRISPR 5'-GUAGU). The grey arrowhead points to the Cse3 cleavage site, whereas the black arrow points to the *glmS* cleavage site. (*B*) Cse3 cleavage of CRISPR-SLI-ARiBo RNAs analyzed on a 10% denaturing polyacrylamide gel stained with SYBR Gold. Cse3 cleavage was performed at 37˚C or 70˚C using aliquots from the transcription reactions (~1 µM RNA), 20 mM HEPES pH=7.5, 150 mM KCl, either 1 or 2 µM Cse3 and different incubation times, as indicated above each lane. The gel mobility of the RNA precursor (CRISPR-SLI-ARiBo) and the Cse3 cleavage product (SLI-ARiBo) are indicated with arrows on the right side of the gel. The percentages of Cse3 cleavage are given below the gels.



control RNA
SLI / CRISPR
SLI
cleaved in Tx
Tx
Cse3-cleaved Tx
5X
12X
25 ng
2.5 ng
10 ng
25 ng
50 ng
LE
W1
W2
W3
E1
E2
2.5 / 1 ng
10 / 2.5 ng
25 / 5 ng
50 / 10 ng
1 ng
2.5 ng
5 ng
12.5 ng
A
SLI(1)
CRISPR-SLI(1)-ARiBo
SLI(1)-ARiBo
ARiBo
13% eluted RNA
96.2% purity
0.3% ARiBo tag
3.5% CRISPR tag
SLI(1) & control RNA
CRISPR
B
SLI(2)
CRISPR-SLI(2)-ARiBo
SLI(2)-ARiBo
ARiBo
38% eluted RNA
99.3% purity
0.3% ARiBo tag
0.4% CRISPR tag
SLI(2) & control RNA
CRISPR
C
SLI(22)
CRISPR-SLI(22)-ARiBo
SLI(22)-ARiBo
ARiBo
35% eluted RNA
97.3% purity
0.7% ARiBo tag
2.0% CRISPR tag
SLI(22)
control RNA
CRISPR
D
SLI(26)
CRISPR-SLI(26)-ARiBo
SLI(26)-ARiBo
ARiBo
20% eluted RNA
98.3% purity
0.2% ARiBo tag
1.5% CRISPR tag
SLI(26)
control RNA
CRISPR

Figure S6. Small-scale affinity batch purifications of (*A*) SLI(1), (*B*) SLI(2), (*C*) SLI(22) and (*D*) SLI(26) RNAs analyzed on 15% denaturing polyacrylamide gels stained with SYBR Gold. The SLI RNAs (left panels) were transcribed as CRISPR-SLI-ARiBo double-fusion RNAs and purified by affinity as described in Fig. 5A. Aliquots from each purification step were loaded on the gel (right panels; LE: load eluate; W1–3: washes; and E1–2: elutions) in amounts shown, where 1X correspond to approximately 50 ng of ARiBo-fusion SLI present in the transcription reaction or the equivalent of 8.23 ng of SLI to be purified. In addition, standard quantities of CRISPR-SLI-ARiBo RNA from the transcription reaction (Tx), SLI-ARiBo RNA from the Cse3-cleavage transcription reaction (Cse3-cleaved Tx), a mixture containing known amounts of a control RNA (29 nt) and the CRISPR RNA (Control RNA/CRIPSR) as well as standard amounts of SLI from CRISPR-SLI-ARiBo cleaved with Cse3 and GlcN6P in the transcription reaction (SLI cleaved in Tx) were loaded as controls. Bands corresponding to the CRISPR-SLI-ARiBo RNA, the SLI-ARiBo, the ARiBo tag, the control RNA, SLI RNA [SLI(1), SLI(2), SLI(22) or SLI(26)] and CRISPR RNA are indicated on the right side of the gels.

REFERENCES

Di Tomasso G, Dagenais P, Desjardins A, Rompré-Brodeur A, Delfosse V, Legault P. 2012. Affinity purification of RNA using an ARiBo tag. In *Recombinant and in vitro RNA synthesis: Methods and Protocols*, Vol 941, p. 306. Humana Press.

Di Tomasso G, Lampron P, Dagenais P, Omichinski JG, Legault P. 2011. The ARiBo tag: a reliable tool for affinity purification of RNAs under native conditions. Nucleic Acids Res 39(3): e18.