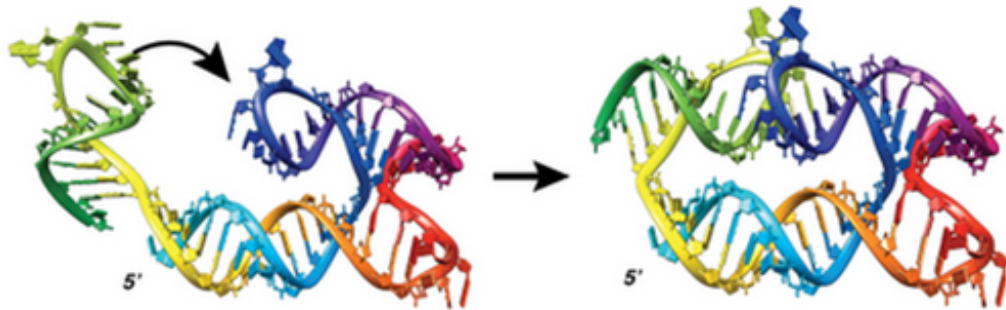
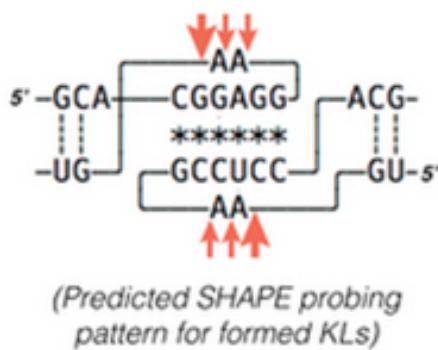


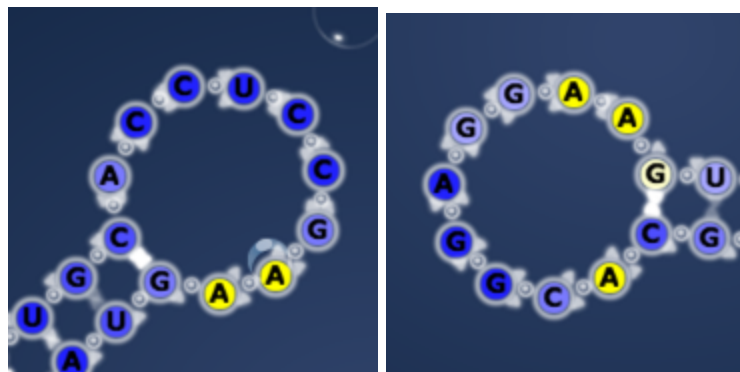
Kissing Loop Lab



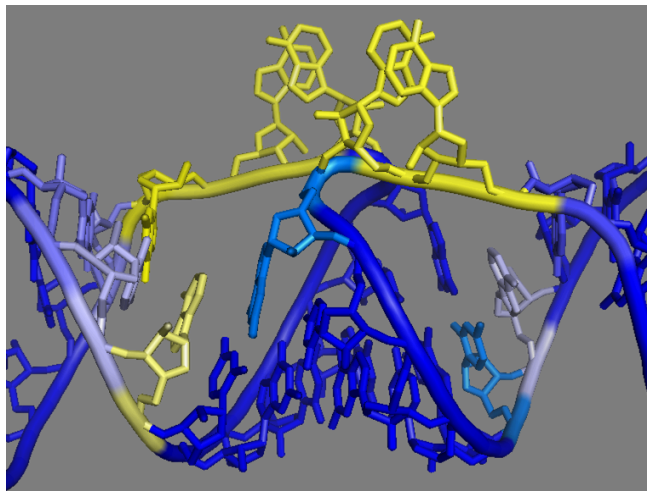
A series of RNA constructs were designed to test the SHAPE probing of kissing loops that form 6bp loop-loop interactions. For sequences where the kissing loops form properly, a protection pattern is expected to be observed. In the cartoons (above), the loops each have a two-nt bulge (AA) that protrudes out into solution when the loops link up. These hot-spots are expected to be strongly probed by SHAPE compared to the 6bps of the loop regions that pair up.



(Expectation)



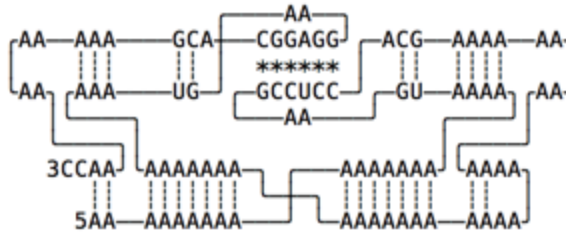
(Reality)



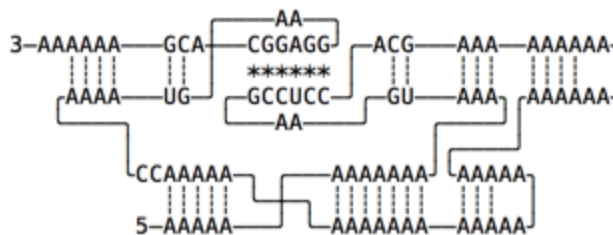
Here, the above SHAPE data is mapped to the 3D fold of the kissing loop (shown in Pymol)

Two different RNA scaffold shapes were chosen (S1 and S2), to ensure robust data collection.

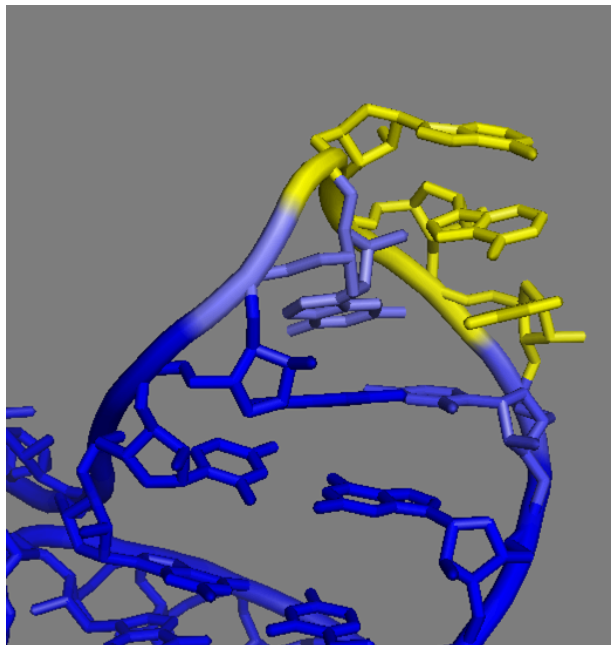
S1:



S2:



Both scaffold designs performed well, resulting in clean SHAPE data for the test KLs. However, in S1 the stems were short compared to S2, and the 4nt loops in S1 were not well-protected compared to those in S2.



GAAA terminal loop in design S2. Notice the 5'G of the loop is protected from SHAPE. This is because the 2'OH of the G in GAAA tetraloops forms an H-bond with the neighboring residue and points inwards on the loop. By contrast, the AAA all point their 2'OH out towards solution (and so are strongly probed)

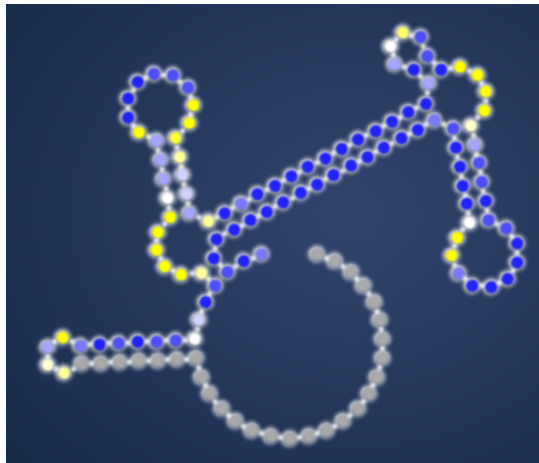
S1-KL1 / S2-KL1

Positive controls for “Kissing Loops” (KL).

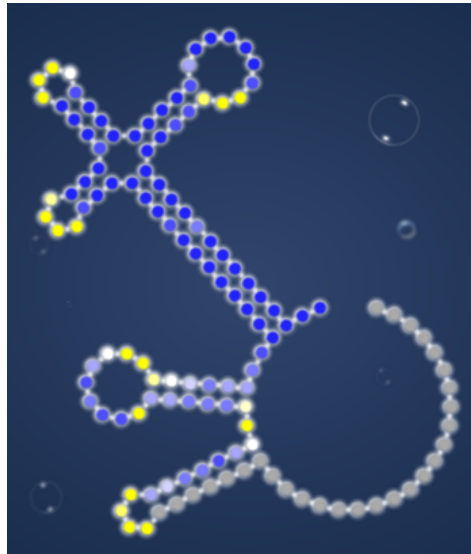
The protection pattern for the 6bp interaction is clearly seen on both of the 9nt terminal loops, indicating that KLs are forming well under the conditions of the probing experiment.

Both scaffold designs (S1 and S2) probed well, although the longer stems in the S2 design results in more protected positions overall.

General notes: The tighter 3D constraints of the 4-way junction in design S2 compared to 3-way multi in S1 might also have an effect on the probing of the KL, but differences in probing between S1 and S2 were not substantial. Embedding the 5'GG into the structure seemed to work well, although it did confuse some lab participants because it requires the user to override the target shape when submitting the sequence.



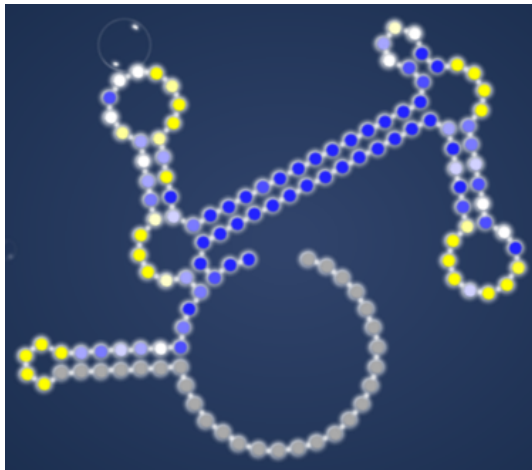
(left) S1-KL1 (*Kiss the Looop*, by Hyphema)



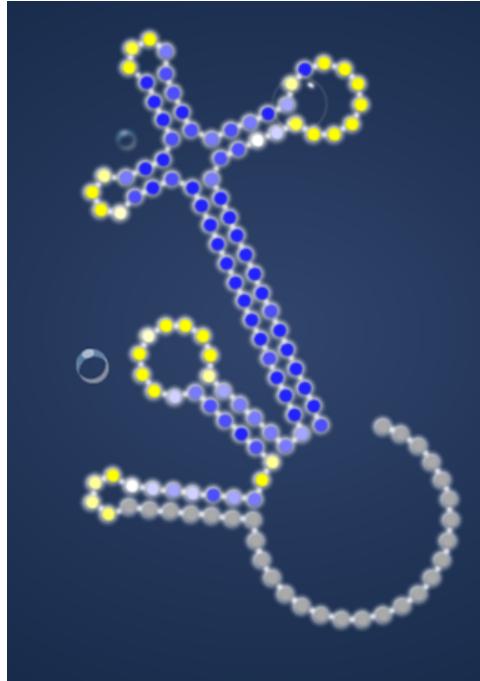
(right) S2-KL1 (*Fisker D1*, by Eli Fisker)

S1-KL1_negative / S2-KL1_negative

In this negative control, kissing-loop sequences were taken from two ‘orthogonal’ kissing-loop interactions. In all designs, both of the 9nt loops were substantially more cleaved compared to the KL1 positive control group. Interesting are some single-nt positions that seem to be protected, indicating either an interaction between the two loops, or otherwise interactions occurring within the loops.



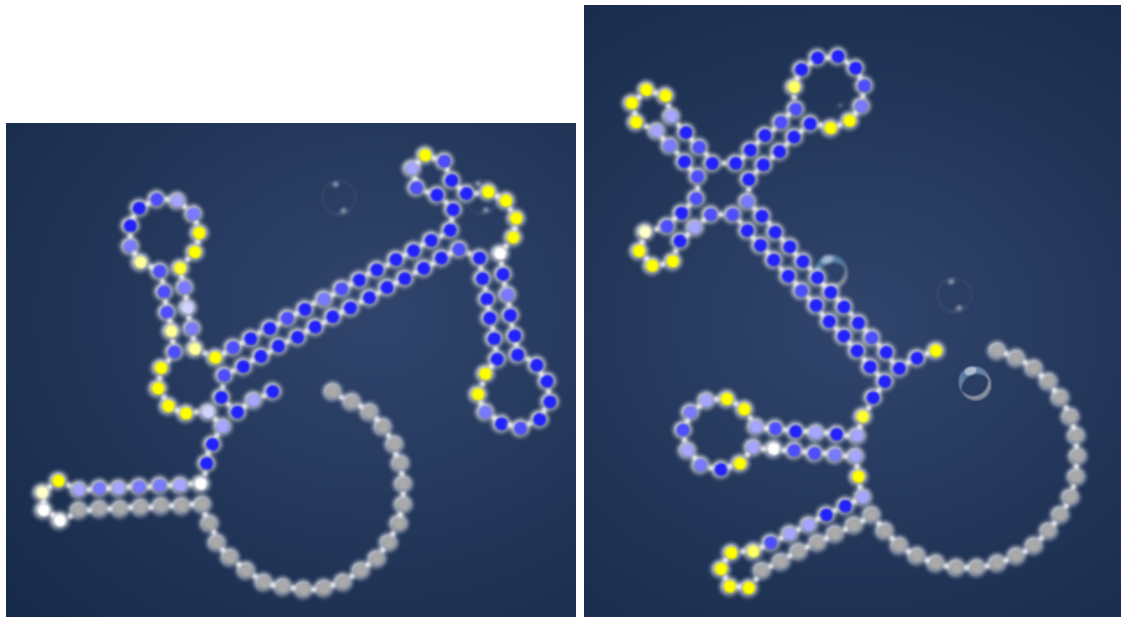
(left) S1 (*Negative 3, by Mayanne*)



(right) S2 (*Fisker D1, by Eli Fisker*)

S1-KL1_noGU / S2-KL1_noGU

In previous works the Kissing Loop sequences have always included a GU wobble just 1bp away from the terminal loop. This experiment tests the effect of removing the GU wobble requirement. The strong protection pattern observed in both of the 9nt loops indicates that the KLs are pairing correctly without the GU wobble part of the motif. In future probing experiments with KLs using short stems it might be worth considering that the GU is not required (although it's still unknown how removing the GU will affect the 3D shape).

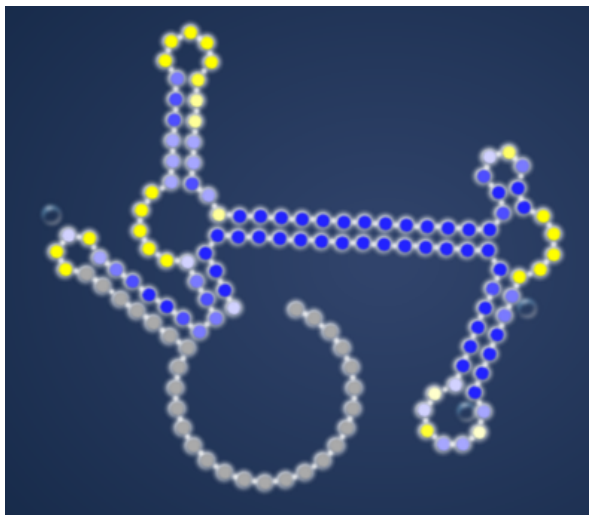


(left) S1 (*Kiss the loop without wobbling, by Hyphema*) **(right)** S2 (*Berry, by Hoglahoo*)

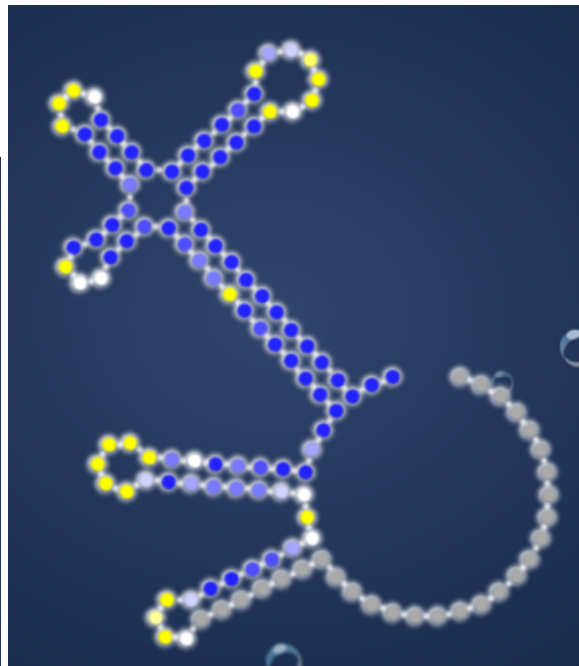
S1-KL_1S72 / S2-KL_1S72

This kissing loop comes from a ribosomal structure (PDB 1S72, resi 423).

In previous RNA experiments it had performed poorly for forming long-range interactions. However, it had never been tested again in the context of a pseudoknot-forming interaction on a continuous strand. In the context of an intramolecular interaction the KL might form more strongly. In most cases the probing data revealed no protection of the loops. However, weak protection of the loop was observed in the 3' loop in some cases, where the correct 2D structure was also strongly observed (better signal to noise ratio?).



(left) S1 (*D2*, by Eli Fisker)

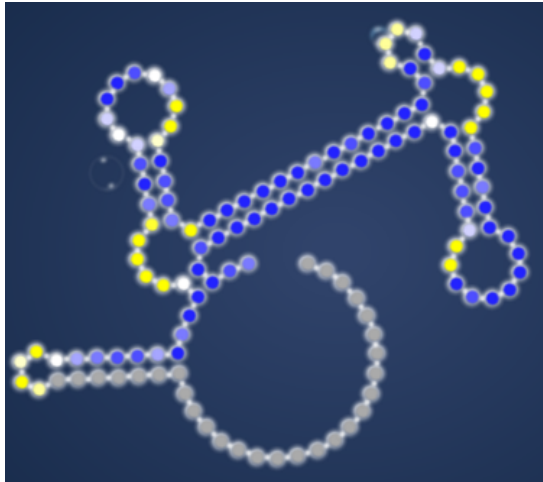


(right) S2 (*Reason to Live*, by Jieux)

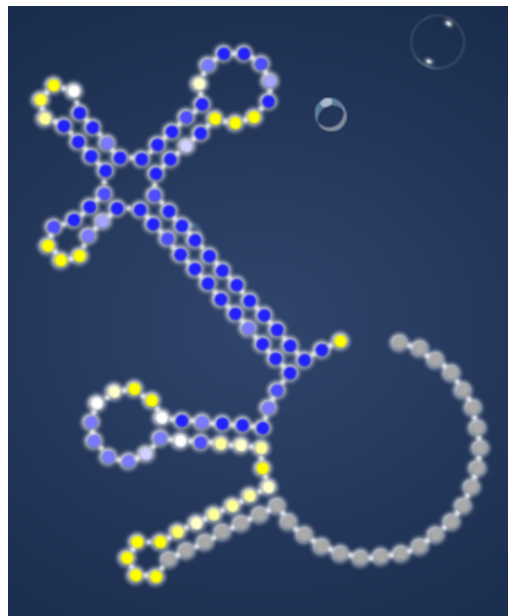
S1-KL_newA / S2-KL_newA

Trying out a new kissing-loop sequence. Kissing loops are notoriously tricky to predict, so testing them is the only way to know if they link up correctly. In particular, since these loops are G and C -rich they don't behave nicely like the poly-A loops of EteRNA, and can make stabilizing the adjoining stems difficult.

For the “newA” KL, the news is good. It has a strong protection pattern, which suggests that the two loops are pairing up as desired.



(left) S1 (*Forever*, by Jieux)



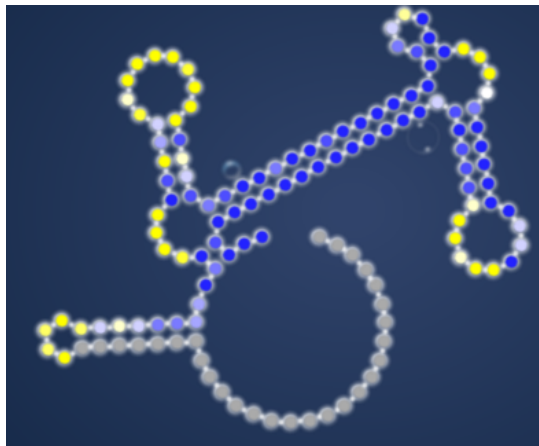
(right) S2 (*Reason to Live*, by Jieux)

S1-KL_newB / S2-KL_newB

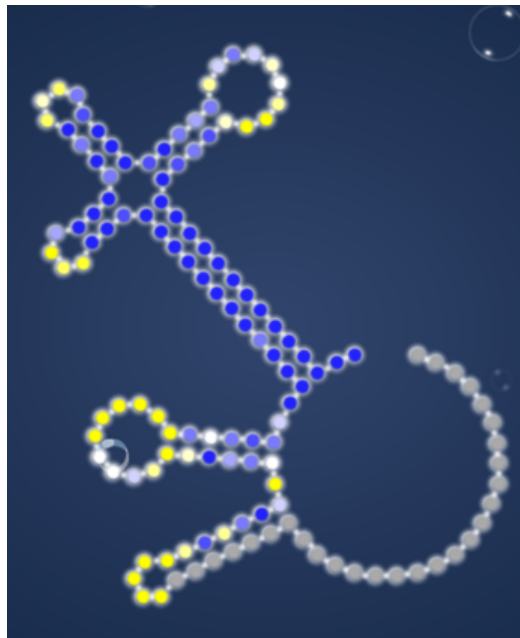
A second, new kissing loop sequence.

For the “newB” KL, the news is not so good.. It has a very weak protection pattern, which suggests that the two loops are not pairing up strongly. A more careful analysis of the design reveals that the two loops only have 4bps complementary between them! This was a design mistake, but now functions as a second control. For the loops (-GAAUGG- and -GGAUUC-), some protection was observed where the loops were still complementary. (GAA with UUC, or UGAA with UUCA). This data suggests that we may be able to form KL interactions with as few as 4bps! Also, looking at where the interaction apparently starts, it seems that pairing at the 3' end of the loops is favored (also consistent with base stacking on the 3' end of stems having greater stability).

ACUGAAGAAUGGACGGUUGUGAAGGAUUCACGCA



(left) S1 (*Strategy*, by Hoglahoo)



(right) S2 (*New B 1*, by Mayanne)

Kissing Loop Protection Patterns, in depth comparison

Data is sorted by increasing SHAPE threshold value. The best 6 are shown in each case, the 5'KL is shown on left and the 3'KL is shown on the right.

Note: In this data-set sorting by SHAPE-score did not work well since the paired-up loop sequences subtract from the SHAPE score. I used the SHAPE threshold value instead, with the hope that lower SHAPE threshold might be an indicator for data with better signal-to-noise ratio (ie lower minimum signal for a SHAPE pattern indicates less noise, but please correct me if I have interpreted this wrongly!).

S2-KL1

UCUG	A	AGCCU	CCAC	CGGA	ACUG	A	AGGA	GGCA	C	GGU
UCUG	A	AGCCU	CCAC	CGGA	CAUG	A	AGGA	GGCA	C	GUG
CGUG	A	AGCCU	CCAC	CGCG	AGUG	A	AGGA	GGCA	C	CGCU
CUUG	A	AGCCU	CCAC	CGAG	CAUG	A	AGGA	GGCA	C	GUG
UAUG	A	AGCCU	CCAC	CGUA	ACUG	A	AGGA	GGCA	C	GGU
UCUG	A	AGCCU	CCAC	CGGA	ACUG	A	AGGA	GGCA	C	GGU

S2-KL1

UCUG	A	AGCCU	CCAC	CGGA	UAUG	A	AGGA	GGCA	C	GUA
UCUG	A	AGCCU	CCAC	CGGA	UAUG	A	AGGA	GGCA	C	GUA
UCUG	A	AGCCU	CCAC	CGGA	UAUG	A	AGGA	GGCA	C	GUA
UCUG	A	AGCCU	CCAC	CGGA	AAUG	A	AGGA	GGCA	C	GUU
UGUG	A	AGCCU	CCAC	CGCA	AUUG	A	AGGA	GGCA	C	GAU
CUUG	A	AGCCU	CCAC	CGAG	UUUG	A	AGGA	GGCA	C	GAA

S1-KL1_noGU

CCUC	A	AGCCU	CCAG	GAGG	UGUC	A	AGGA	GGCAG	GACA
AUUC	A	AGCCU	CCAG	GAU	ACAC	A	AGGA	GGCAG	UGU
CUGC	A	AGCCU	CCAG	CAG	CAUC	A	AGGA	GGCAG	AUG
CCUC	A	AGCCU	CCAG	GAGG	UGUC	A	AGGA	GGCAG	GACA
UCUG	A	AGCCU	CCAC	AGA	CAUG	A	AGGA	GGCAG	CAUG
GUAC	A	AGCCU	CCAG	GUAC	AUGC	A	AGGA	GGCAG	GCAU

S2-KL1_noGU

UGAC	A	AGCCU	CCAG	GUCA	GGUC	A	AGGA	GGCAG	GACC
UCUG	A	AGCCU	CCAC	CAGA	AUAG	A	AGGA	GGCAG	CUAU
GUAC	A	AGCCU	CCAG	GUAC	CAUC	A	AGGA	GGCAG	AUG
GUGC	A	AGCCU	CCAG	CAC	CAUC	A	AGGA	GGCAG	AUG
GUGC	A	AGCCU	CCAG	CAC	CGAC	A	AGGA	GGCAG	UCG
UCUG	A	AGCCU	CCAC	CAGA	CUAG	A	AGGA	GGCAG	CUAG

S1-KL1_negative

U	G	U	G	A	A	G	U	G	G	A	C	A	C	G	C	A	A	G	U	G	A	A	G	G	A	G	G	C	A	C	G	C	U	
A	G	U	G	A	A	G	U	G	G	A	C	A	C	G	C	U	A	A	G	U	G	A	A	G	G	A	G	G	C	A	C	G	C	U
U	G	U	G	A	A	G	U	G	G	A	C	A	C	G	C	A	A	G	U	G	A	A	G	G	A	G	G	C	A	C	G	C	U	
A	G	U	G	A	A	G	U	G	G	A	C	A	C	G	C	U	A	A	G	U	G	A	A	G	G	A	G	G	C	A	C	G	C	U
U	C	U	G	A	A	G	U	G	G	A	C	A	C	G	G	A	C	A	U	G	A	A	G	G	A	G	G	C	A	C	G	U	G	
C	U	U	G	A	A	G	U	G	G	A	C	A	C	G	A	G	C	A	U	G	A	A	G	G	A	G	G	C	A	C	G	U	G	

S2-KL1_negative

U	C	U	G	A	A	G	U	G	G	A	C	A	C	G	G	A	A	C	U	G	A	A	G	G	A	G	G	C	A	C	G	G	U
U	C	U	G	A	A	G	U	G	G	A	C	A	C	G	G	A	C	A	U	G	A	A	G	G	A	G	G	C	A	C	G	U	G
U	C	U	G	A	A	G	U	G	G	A	C	A	C	G	G	A	A	C	U	G	A	A	G	G	A	G	G	C	A	C	G	G	U
C	A	U	G	A	A	G	U	G	G	A	C	A	C	G	U	G	G	C	U	G	A	A	G	G	A	G	G	C	A	C	G	G	C
G	A	U	G	A	A	G	U	G	G	A	C	A	C	G	U	C	U	G	U	G	A	A	G	G	A	G	G	C	A	C	G	C	A
C	C	U	G	A	A	G	U	G	G	A	C	A	C	G	G	G	U	A	U	G	A	A	G	G	A	G	G	C	A	C	G	U	A

S1-KL_newA

U	C	U	G	A	A	C	A	A	G	U	C	A	C	G	G	A	C	A	U	G	A	A	G	A	C	U	U	G	A	C	G	U	G	
G	A	U	G	A	A	C	A	A	G	U	C	A	C	G	U	C	G	A	U	G	A	A	G	A	C	U	U	G	A	C	G	U	C	
U	G	U	G	A	A	C	A	A	G	U	C	A	C	G	C	A	G	A	U	G	A	A	G	A	C	U	U	G	A	C	G	U	C	
G	A	U	G	A	A	C	A	A	G	U	C	A	C	G	U	C	G	A	U	G	A	A	G	A	C	U	U	G	A	C	G	U	C	
G	C	U	G	A	A	C	A	A	G	U	C	A	C	G	G	C	G	U	U	G	A	A	G	A	C	U	U	G	A	C	G	G	A	
G	A	U	G	A	A	C	A	A	G	U	C	A	C	G	U	C	G	A	U	G	U	A	A	G	A	C	U	U	G	A	C	G	C	A

S2-KL_newA

U	C	U	G	A	A	C	A	A	G	U	C	A	C	G	G	A	U	G	U	G	A	A	G	A	C	U	U	G	A	C	G	C	A	
U	C	U	G	A	A	C	A	A	G	U	C	A	C	G	G	A	A	C	U	G	A	A	G	A	C	U	U	G	A	C	G	G	U	
G	A	U	G	A	A	C	A	A	G	U	C	A	C	G	U	C	C	C	U	G	A	A	G	A	C	U	U	G	A	C	G	G	G	
A	C	U	G	A	A	C	A	A	G	U	C	A	C	G	G	U	C	C	C	U	G	A	A	G	A	C	U	U	G	A	C	G	G	G
A	C	U	G	A	A	C	A	A	G	U	C	A	C	G	G	U	C	C	C	U	G	A	A	G	A	C	U	U	G	A	C	G	G	G
G	A	U	G	A	A	C	A	A	G	U	C	A	C	G	U	C	C	A	U	G	A	A	G	A	C	U	U	G	A	C	G	U	G	

S1-KL_newB

UCUG	A	AGAAU	GGAC	CGGA	CAUG	AAGGA	UUCA	CGUG
UCUG	A	AGAAU	GGAC	CGGA	CAUG	AAGGA	UUCA	CGUG
CGUG	A	AGAAU	GGAC	CGCG	AGUG	AAGGA	UUCA	CGCU
CCUG	A	AGAAU	GGAC	CGGG	CAUG	AAGGA	UUCA	CGUG
AUUG	A	AGAAU	GGAC	CGAU	AUUG	AAGGA	UUCA	CGAU
GAUG	A	AGAAU	GGAC	CGUC	CAUG	AAGGA	UUCA	CGUG

S2-KL_newB

UGUG	A	AGAAU	GGAC	CGCA	GCUG	AAGGA	UUCA	CGGC
ACUG	A	AGAAU	GGAC	CGGU	UGUG	AAGGA	UUCA	CGCA
UGUG	A	AGAAU	GGAC	CGCA	CUUG	AAGGA	UUCA	CGAG
GAUG	A	AGAAU	GGAC	CGUC	AUUG	AAGGA	UUCA	CGAU
GUUG	A	AGAAU	GGAC	CGAC	UAUG	AAGGA	UUCA	CGUA
GAUG	A	AGAAU	GGAC	CGUC	AUUG	AAGGA	UUCA	CGAU

S1-KL_1S72

CUAG	GCAUC	GAC	CUAG	AUUG	GC	UUG	AU	GCG	AU
GAUG	GCAUC	GAC	CAUC	AGUG	GC	UUG	AU	GCG	CU
GUCG	GCAUC	GAC	CGAC	GCUG	GC	UUG	AU	GCG	GC
AUGC	GCAUC	GAC	GCAU	ACUG	GC	UUG	AU	GCG	GU
UGAC	GCAUC	GAC	GUCG	AUUG	GC	UUG	AU	GCG	AU
AUGC	GCAUC	GAC	GCAU	ACUG	GC	UUG	AU	GCG	GU

S2-KL_1S72

UCAG	GCAUC	GAC	CUGA	CAUG	GC	UUG	AU	GCG	UG
GUGC	GCAUC	GAC	GCAC	UAUG	GC	UUG	AU	GCG	UA
UCAG	GCAUC	GAC	CUGA	CAUG	GC	UUG	AU	GCG	UG
ACAG	GCAUC	GAC	CUGU	AUUG	GC	UUG	AU	GCG	AU
GUUG	GCAUC	GAC	CAAC	UAUG	GC	UUG	AU	GCG	UA
GUUG	GCAUC	GAC	CAAC	UAUG	GC	UUG	AU	GCG	UA