Identification and analysis of RNA bridges. RNA bridges were predicted around every annotated internal exon using RNAhybird (with parameters: -e = -40, -chi = -20, theta= 10, n=70) (*12*). If upstream or downstream intronic sequences were >500nt long, they were sub-divided into exon-proximal (500nt) and exon-distal (>500nt) regions. We next attempted to "pair" sequences in exon-proximal region (using a 70nt sliding window overlapping by 28nt) to complementary regions distal from the exon (using a 10kb sliding window overlapping by 1kb). The minimum free energy (mfe) of hybridization (kcal/mole) was determined for each predicted RNA-RNA duplex i.e. RNA bridge, discounting the loop. To identify evolutionarily conserved RNA bridges we required that the exon-proximal and distal regions were overlapping piSCRs and diSCRs respectively. To determine the enrichment of RNA bridges nearby cassette (skipped) or constitutive exons, we first used a permissive cutoff of -45kcal/mole. As the absolute valued mfe cutoff for defining an RNA bridge was increased, only conserved RNA bridges whose mfe of binding satisfied at least that cutoff were retained. RNA bridges where the piSCR was neighboring a cassette or constitutive exon were associated with that cassette or constitutive exon and we calculated the fraction of exons with at least one bridge surpassing each cutoff, relative to the total number of exons around which we attempted to predict RNA bridges. To generate an even more stringent set, we retained RNA bridges where the mfe value from each of at least four of the five species (human, chimp, rat, dog and mouse) was lower than -65 kcal/mole. The location of either the piSCR or diSCR that constituted the RNA bridge were moved to at least 10 random positions within the same pre-mRNA, but restricted to the original region (distal or proximal) from which it originated. The mfe cutoff of -65 kcal/mole resulted in 15% of the "random" RNA bridges generated from random locations satisfying the cutoff.

Python code used to perform this analysis is located here:
https://github.com/YeoLab/gscripts/tree/master/gscripts/structure

References

1. M. Rehmsmeier, P. Steffen, M. Hochsmann, R. Giegerich, Fast and effective prediction of microRNA/target duplexes. *RNA* **10**, 1507 (Oct, 2004).