

Supplemental Figure S1

RNA nucleotide sequences of inverse-designed TLC1 alleles

Nucleotide substitution mutations were made in TLC1, without deletions or insertions, in non-essential regions previously deleted in Mini-T (Zappulla *et al.*, *NSMB* 2005) or stiffened in TSA-T (Lebo and Zappulla, *RNA* 2012). Mutated nucleotides are indicated in red. The terminal arm is highlighted in green (with 15 nucleotide substitutions), the Ku arm in orange (with 11–62 substitutions), the Est1 arm in blue (with 33 substitutions), and the template in gray.

(A) TLC1

(B) DA-TLC1

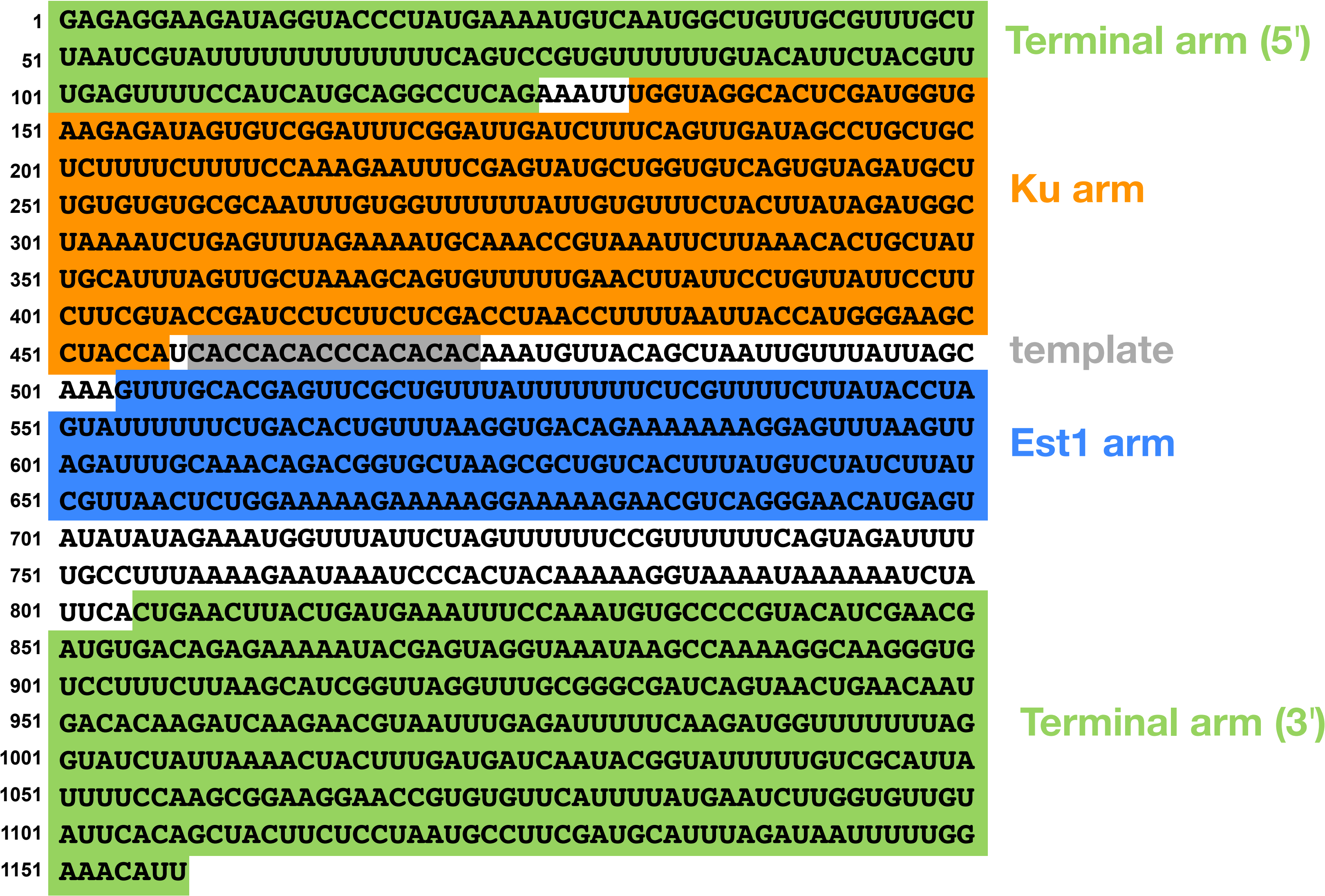
(C) DET-TLC1

(D) DK-TLC1

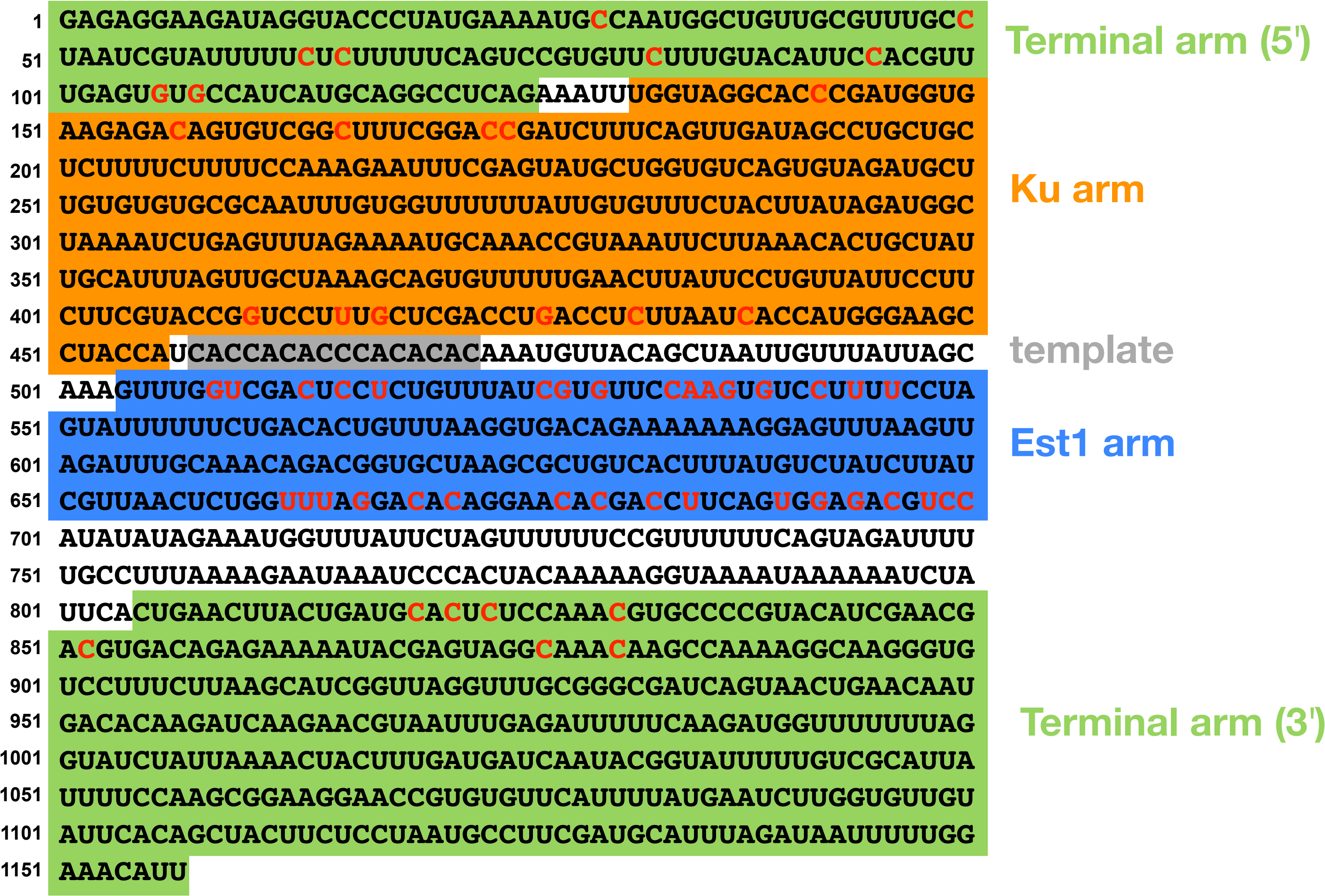
(E) DPhyK-DA-TLC1

(F) DPhyK-TLC1

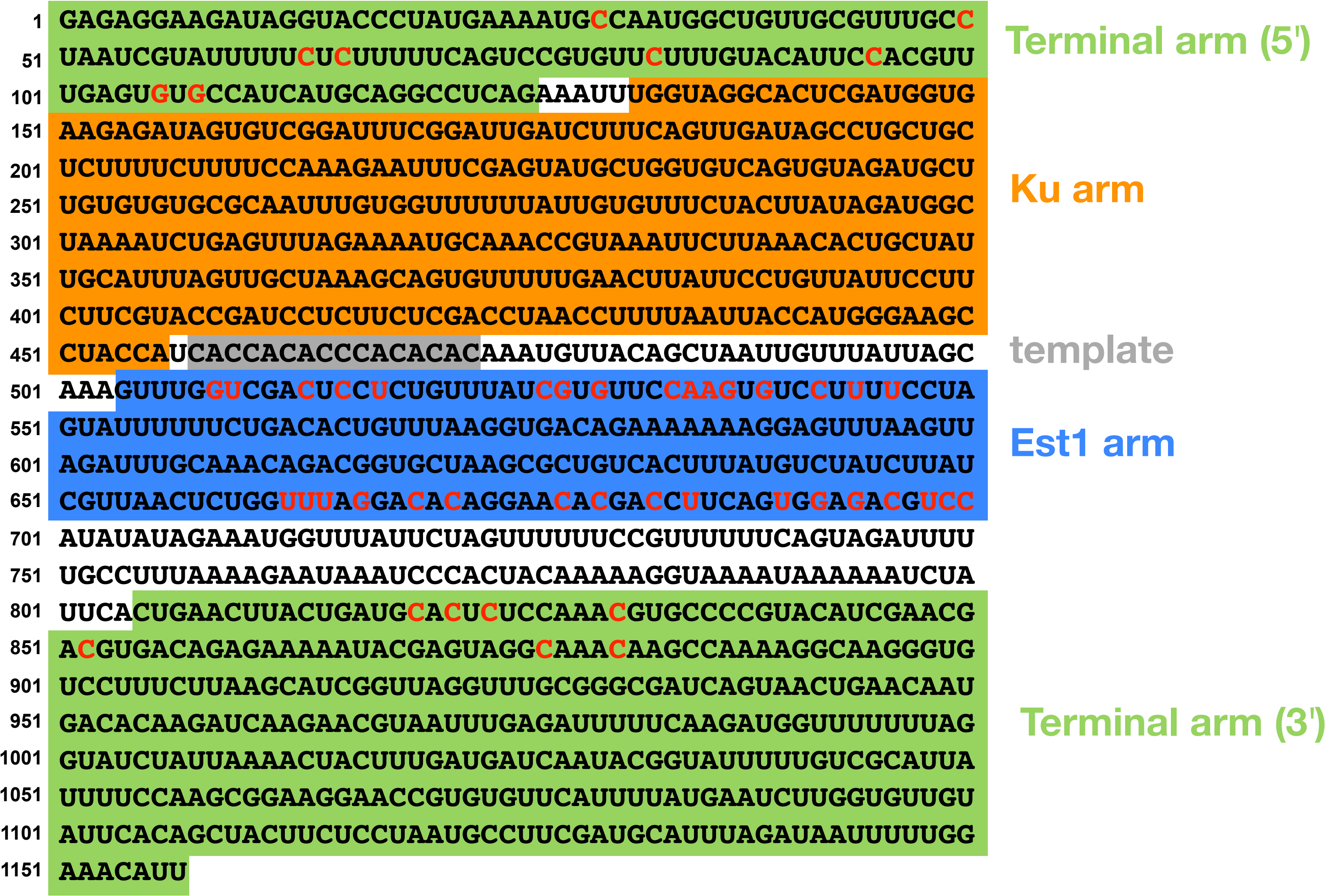
Supp. Fig. S1A: TLC1



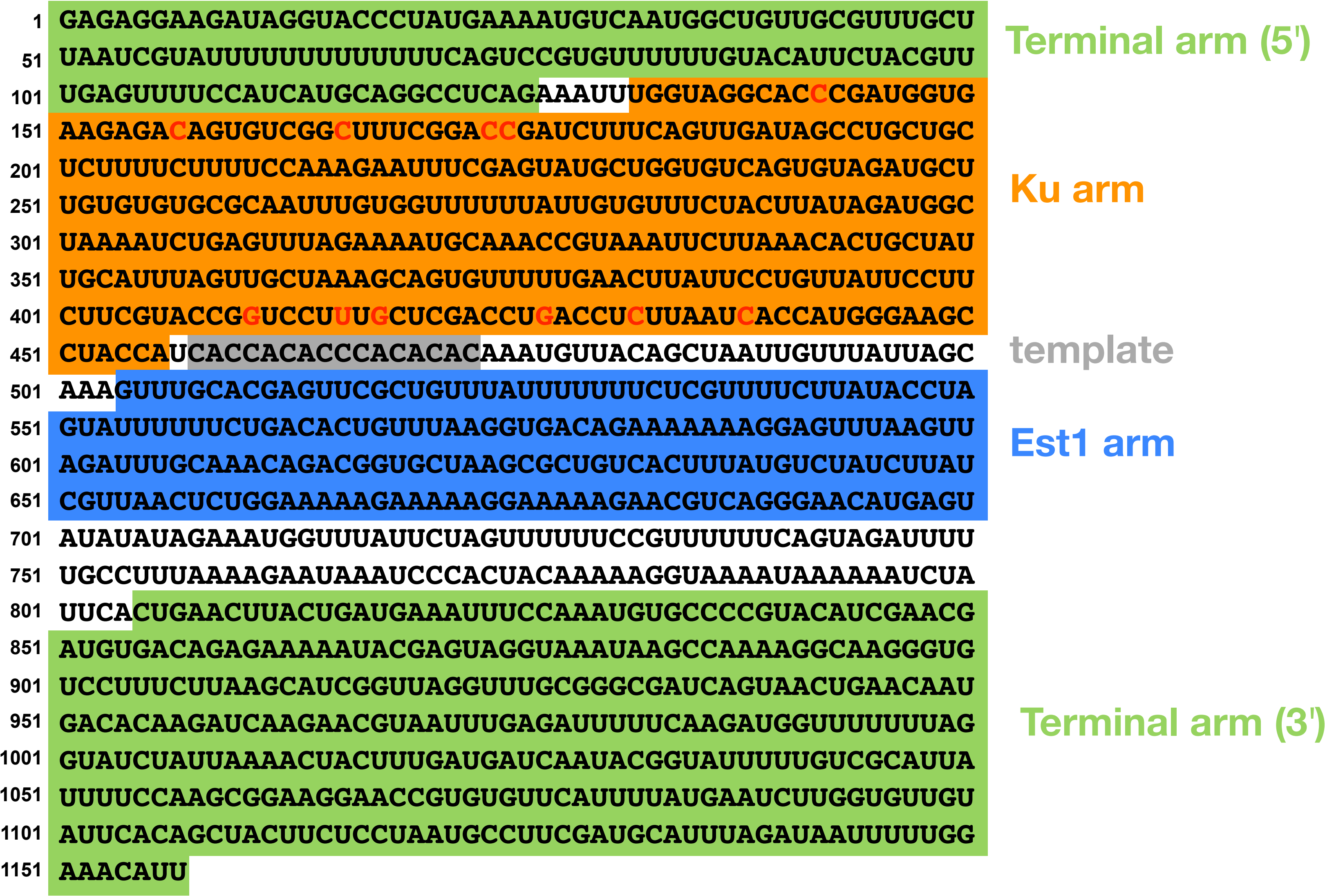
Supp. Fig. S1B: DA-TLC1



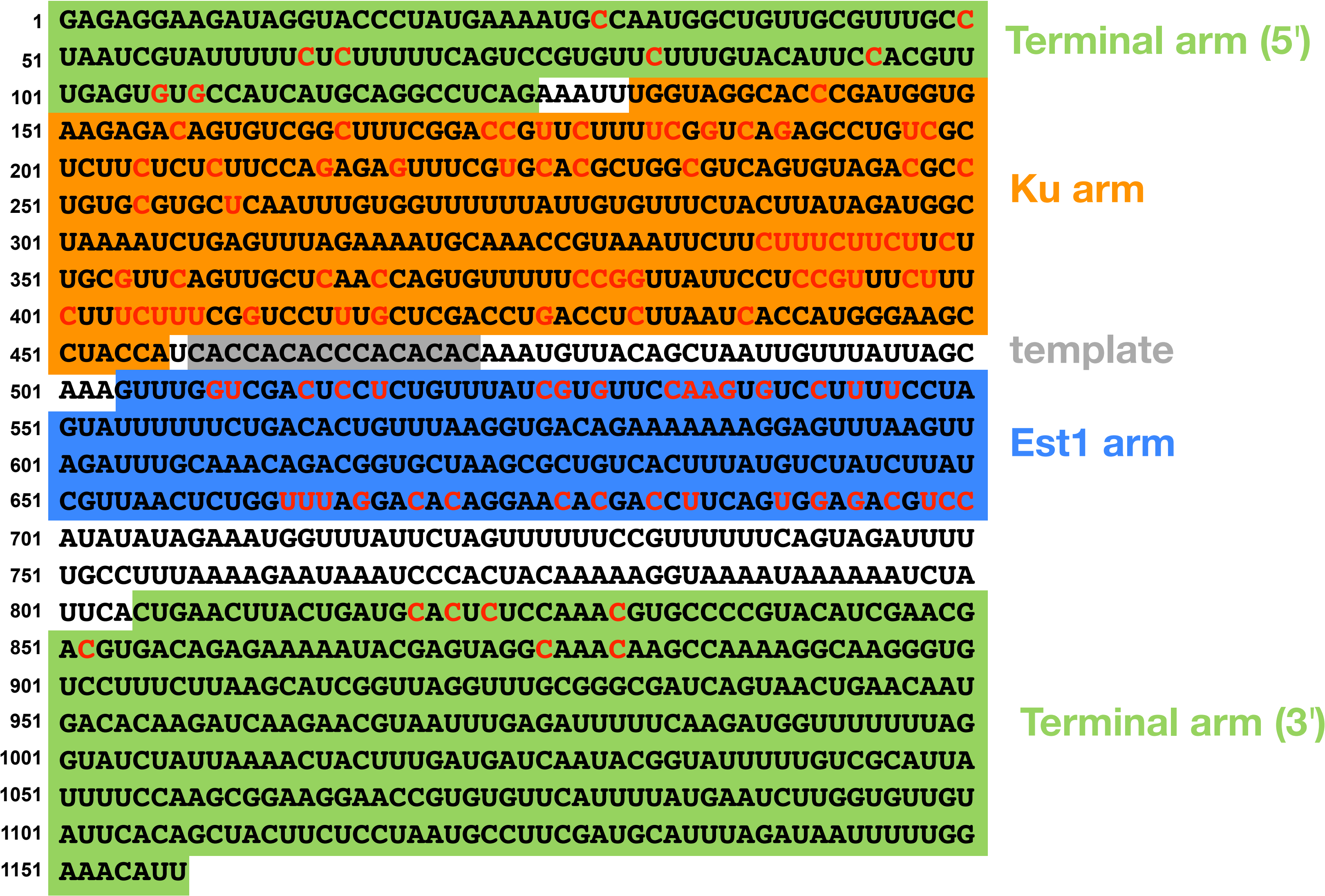
Supp. Fig. S1C: DET-TLC1



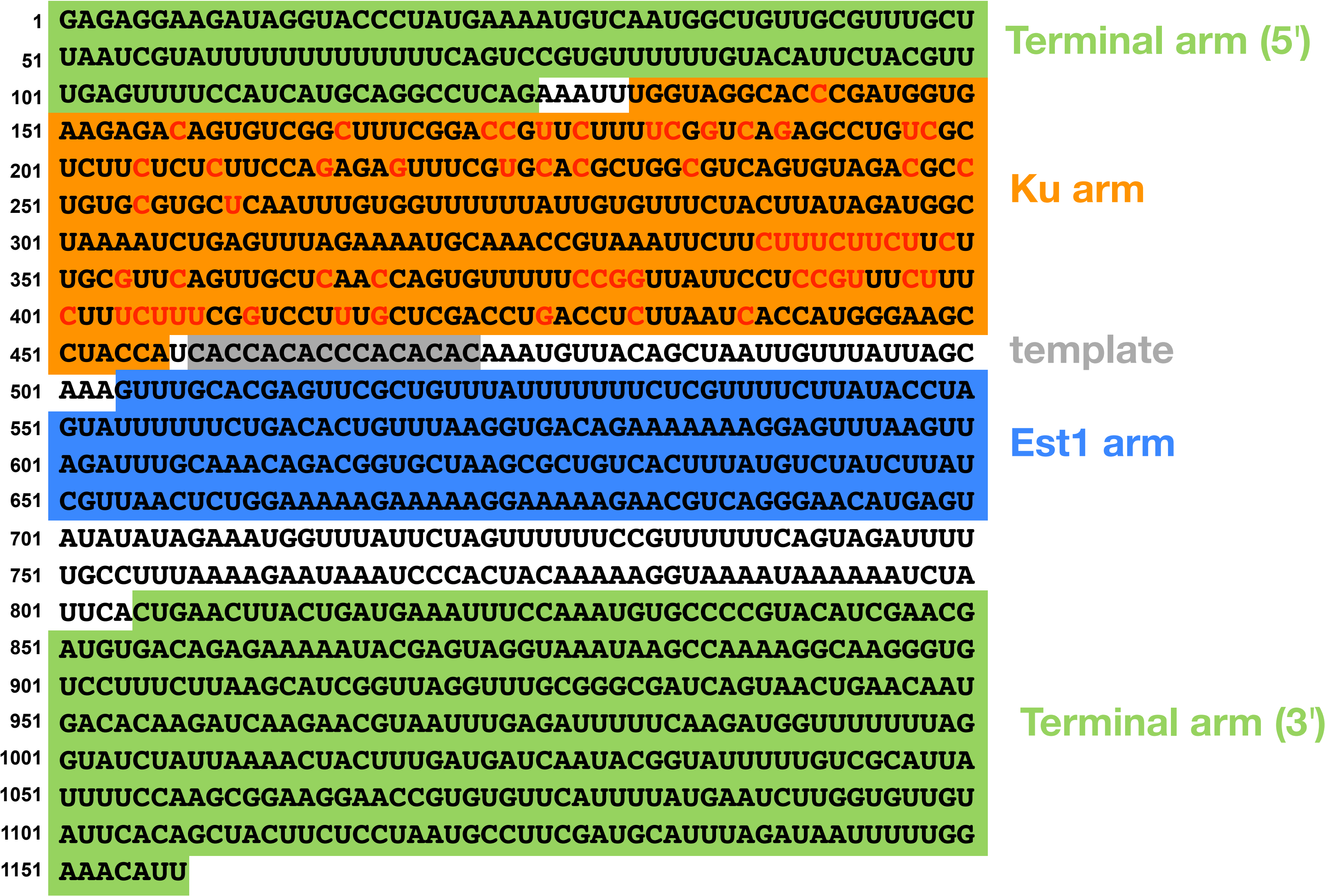
Supp. Fig. S1D: DK-TLC1



Supp. Fig. S1E: DPhyK-DA-TLC1



Supp. Fig. S1F: DPhyK-TLC1



Supplemental Figure S2

***Mfold* secondary structure predictions of DA-TLC1 alleles**

Mfold secondary structure predictions without constraints, with *P-num* output.

(A) TLC1

(B) DA-TLC1

(C) DET-TLC1

(D) DK-TLC1

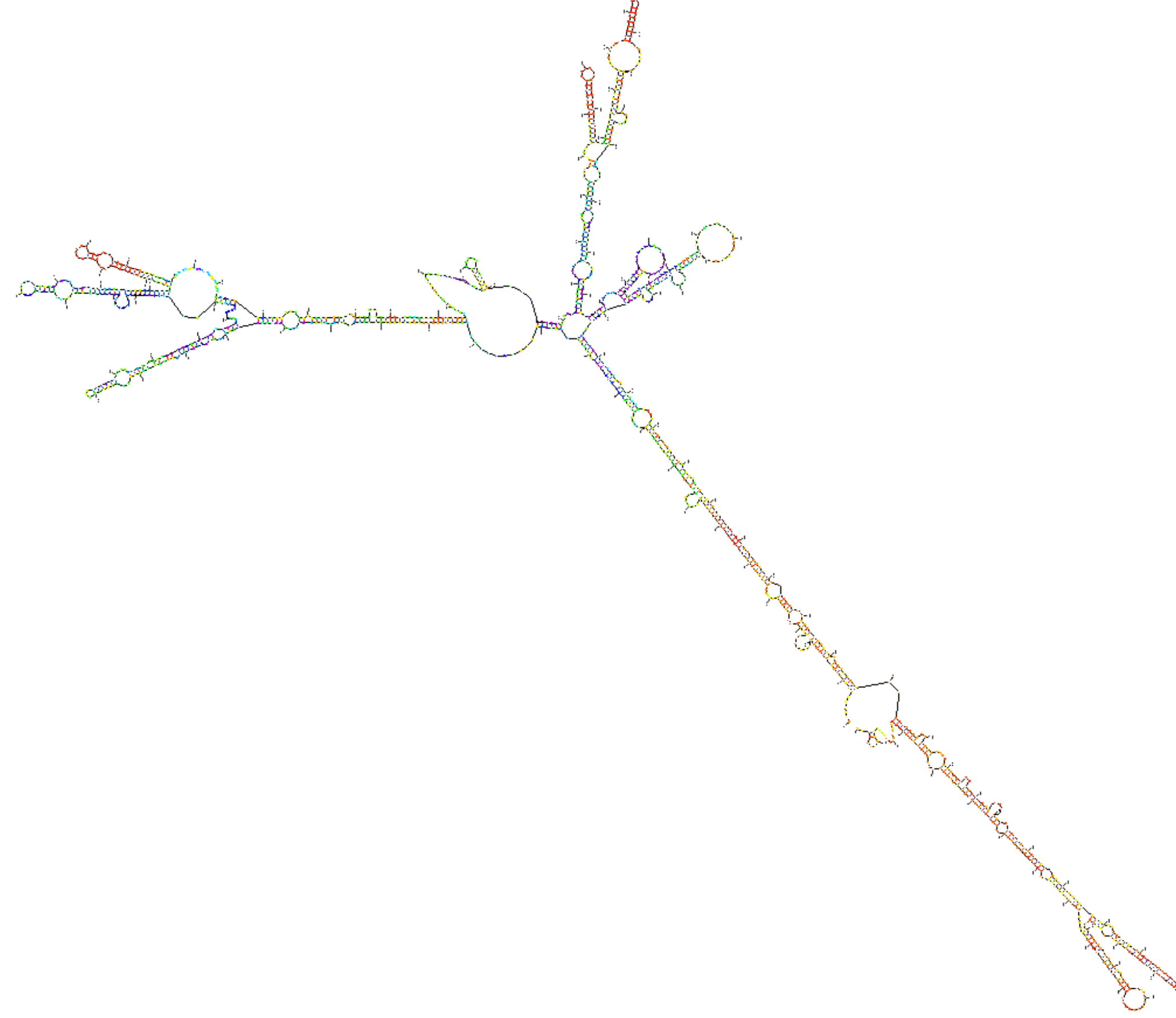
(E) DPhyK-DA-TLC1

(F) DPhyK-TLC1

Supp. Fig. S2A: TLC1

Created Sun Feb 5 04:51:33 2023

Output of sv_graph (8)
mfold_v11.4.7

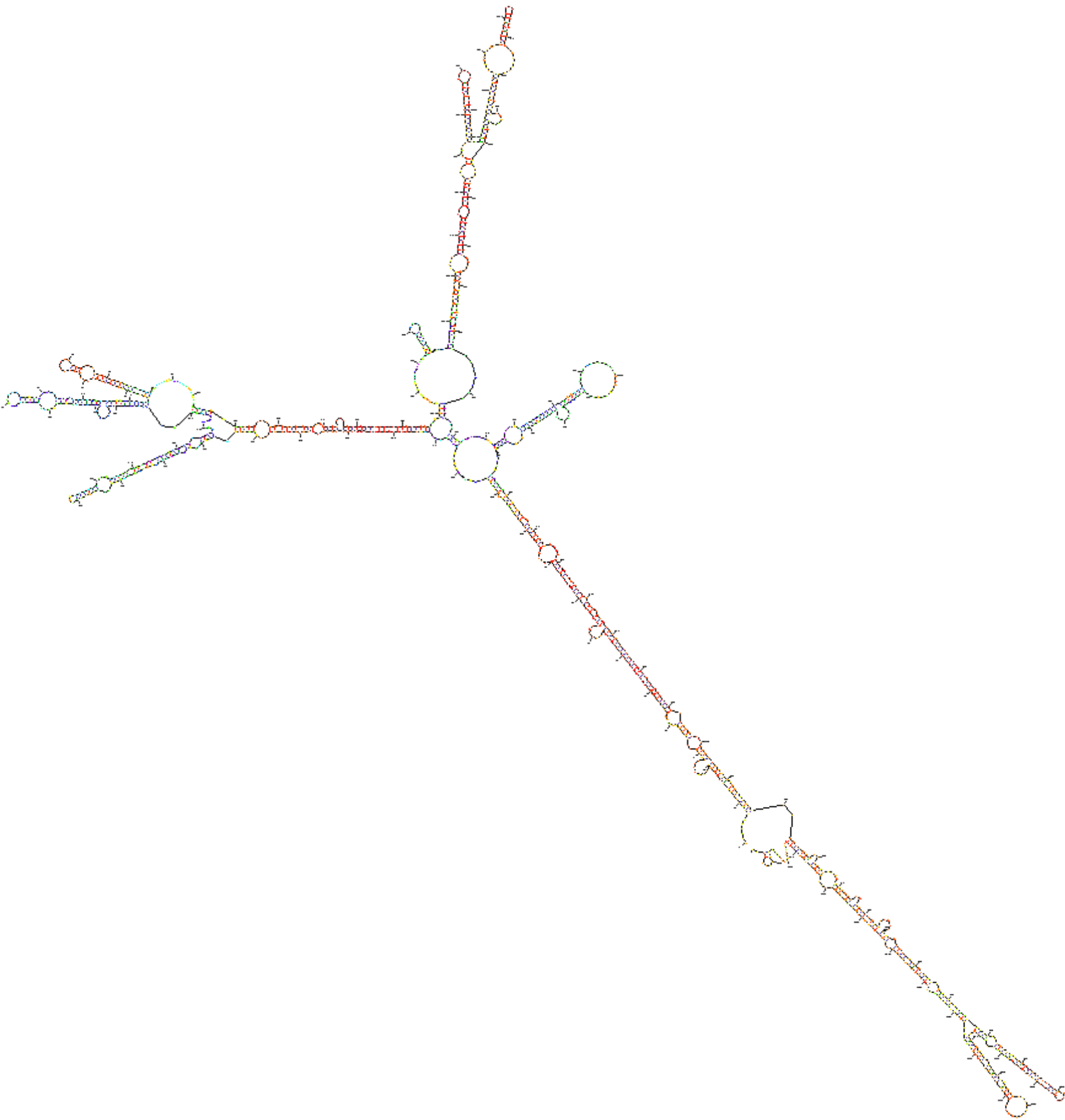


$\Delta G = -304.05$ [Initially -321.00] A

Supp. Fig. S2B: DA-TLC1

Output of sr_graph (6)
mfold_v4.7

Created Sun Feb 5 04:52:17 2023

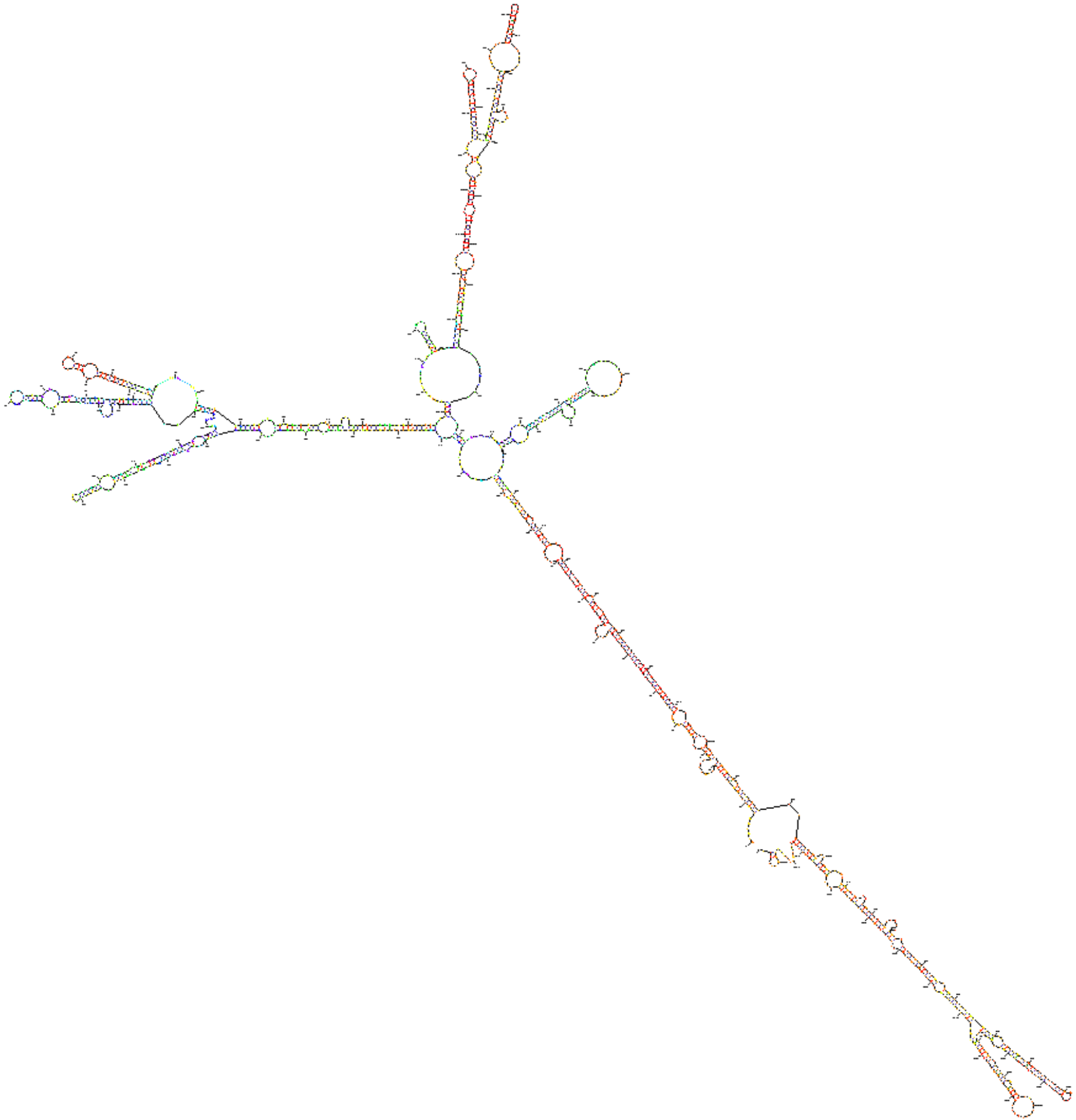


dG = -359.52 [Initially -382.20] B

Supp. Fig. S2C: DET-TLC1

Output of arc_graph [8]
infold_vtl 4.7

Created Sun Feb 5 04:54:27 2023

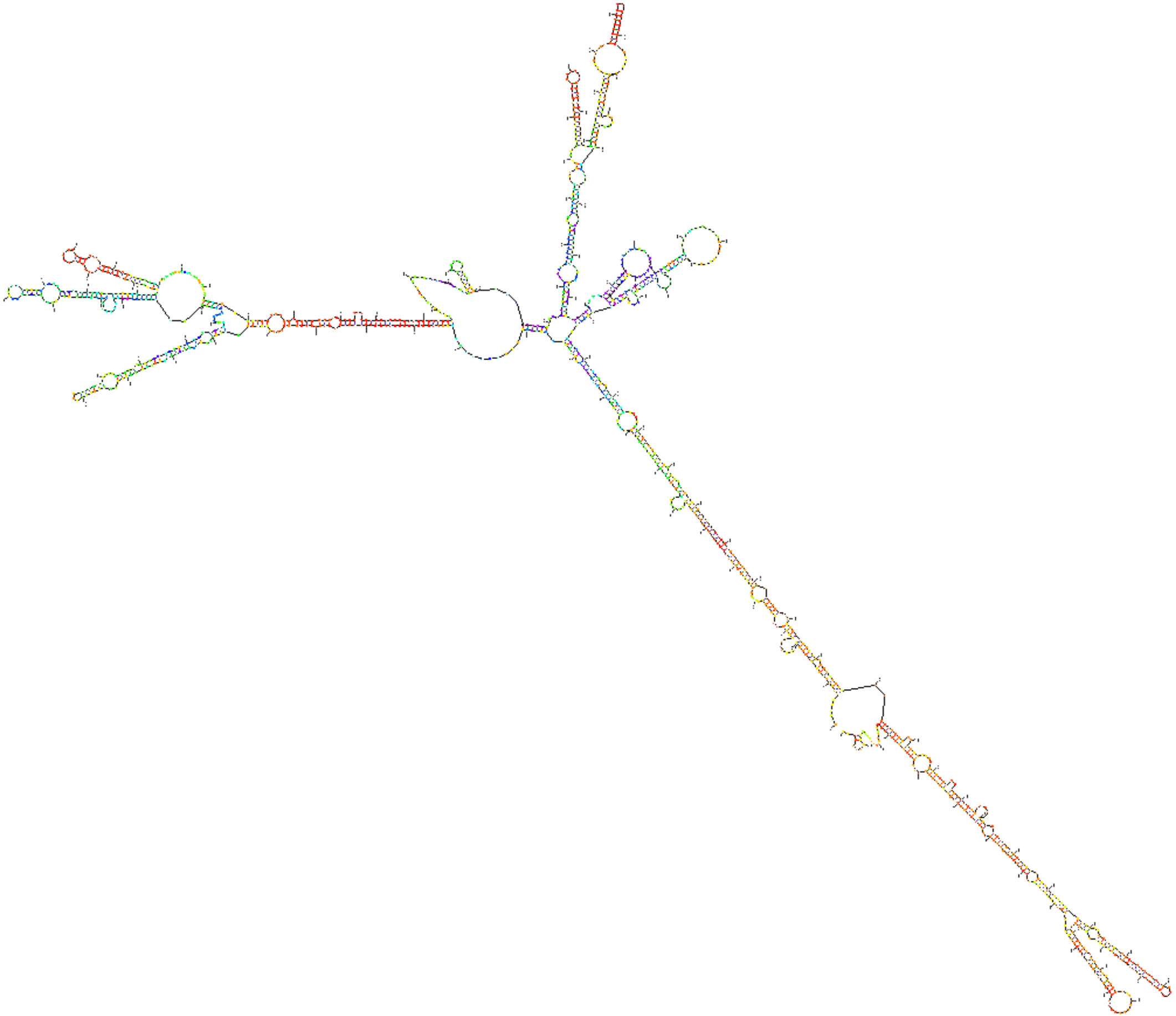


dG = -346.96 [Initially -367.30] C

Supp. Fig. S2D: DK-TLC1

Created Sun Feb 5 04:54:59 2023

Output of a_graph [6]
mod_all 4,7



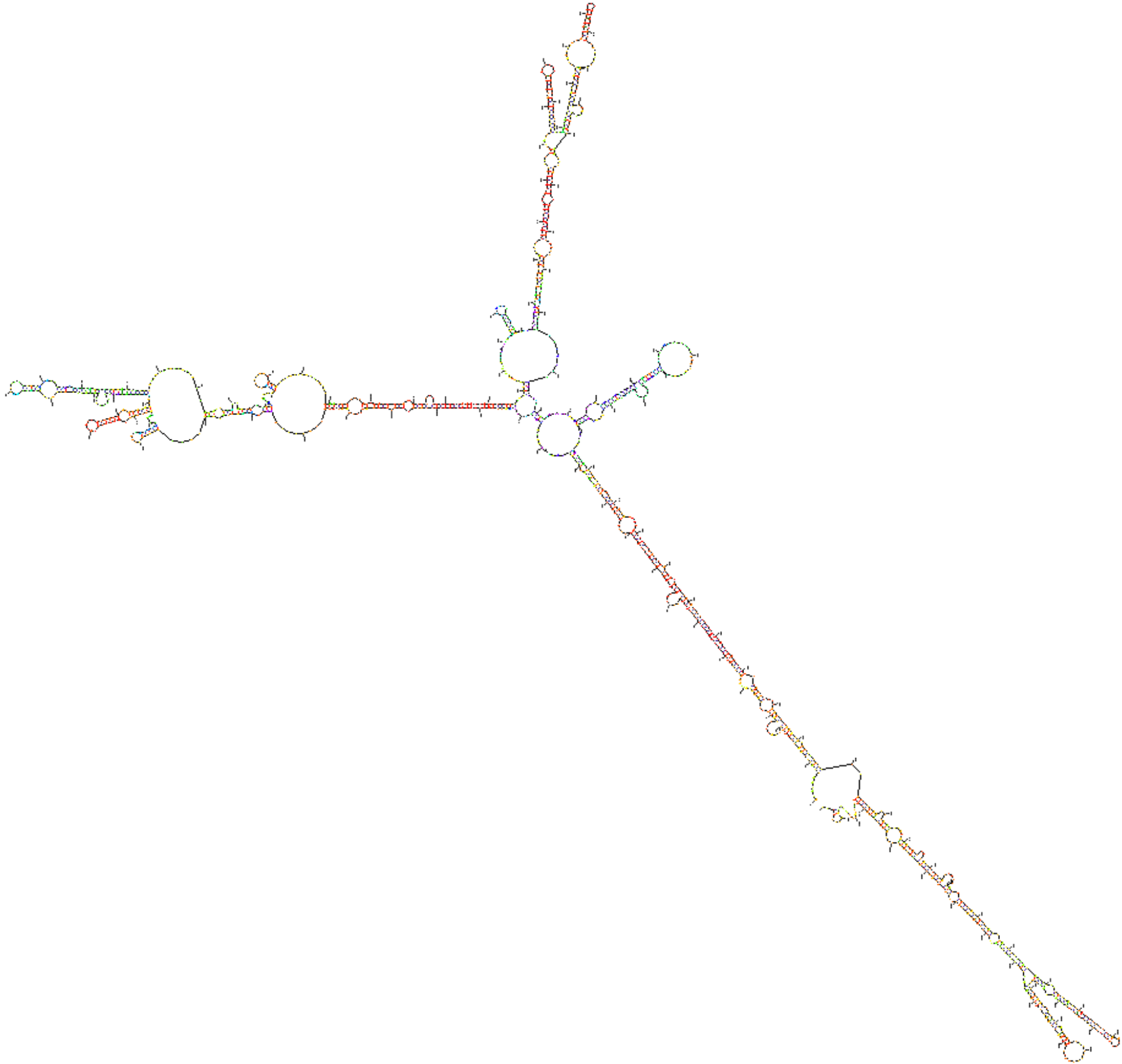
dG = -316.61 [Initially -335.90] D

Supp. Fig. S2E: DPhyK-DA-TLC1

Created Sun Feb 5 04:56:11 2023

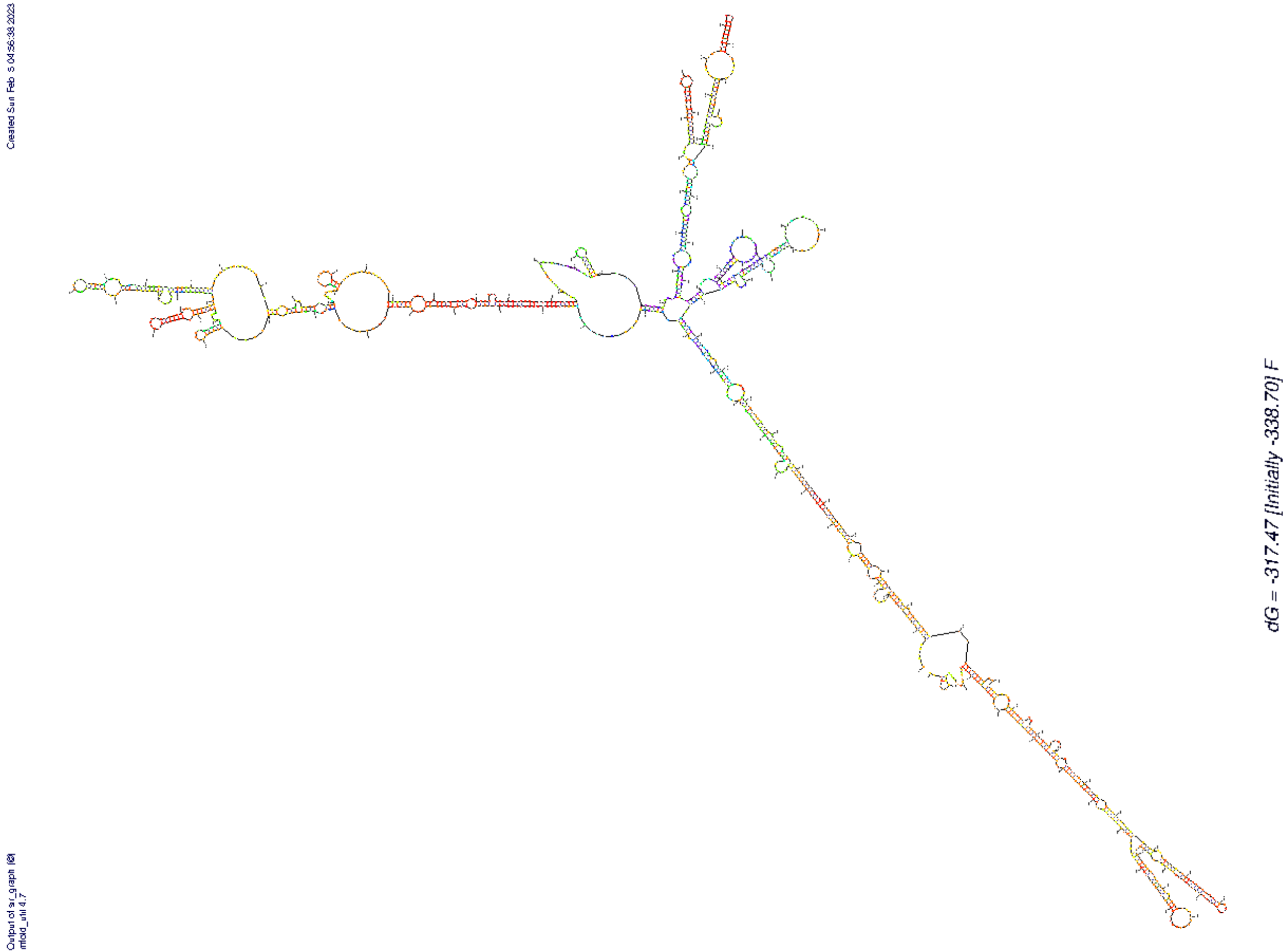
Output of s_graph (0)

modu_001 4.7



$dG = -360.38$ [Initially -385.00] E

Supp. Fig. S2F: DPhyK-TLC1



Supplemental Figure S3

Comparison of *Mfold* secondary structure predictions for wild-type, inverse-designed, and phylogenetic models of TLC1 arms. Regions of TLC1 arm secondary structures are shown, comparing the *Mfold* secondary structure prediction and phylogenetically determined (Zappulla and Cech, *PNAS* 2004) structures of wild-type TLC1 to the *Mfold*-predicted structures of the inverse-designed “determined” arms. Nucleotide changes in inverse-designed alleles are circled in red.

(A) Ku-binding arm

(B) Est1-binding arm

(C) Terminal arm

Supp. Figure S3A

(See Fig. 1A for distal portion of Ku arm Mfold models)

Ku arm

boxes indicate similarly folded portions

WT TLC1
(Mfold)

DA-TLC1

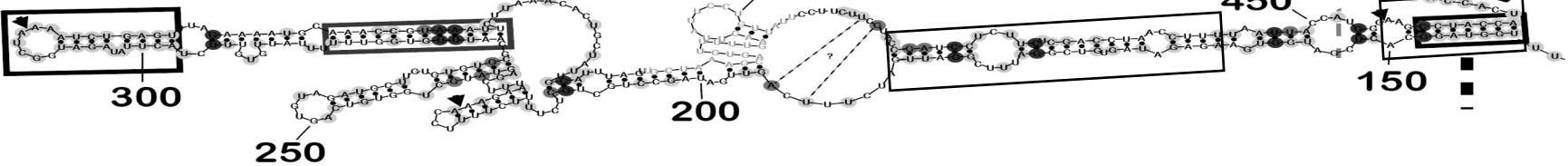
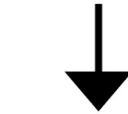
DPhyK-TLC1

11 nts changed (O)

62 nts changed (O)

C = WT

Ku



WT TLC1
(Mfold +
phylogenetics)
(Zappulla and Cech,
PNAS, 2004)

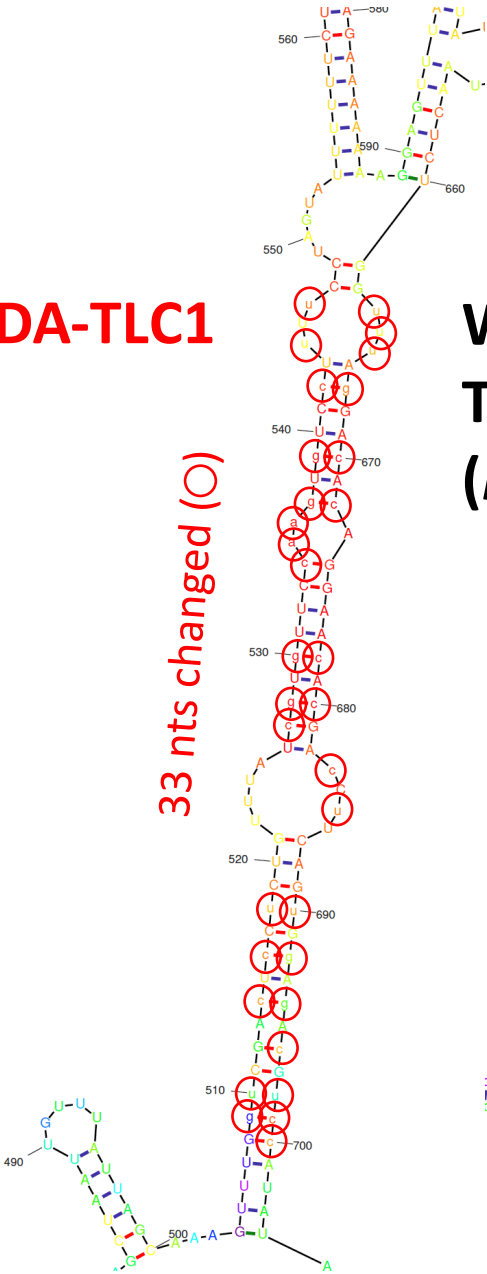
Supp. Figure S3B

(See Fig. 1A for distal portion of Est1 arm Mfold models)

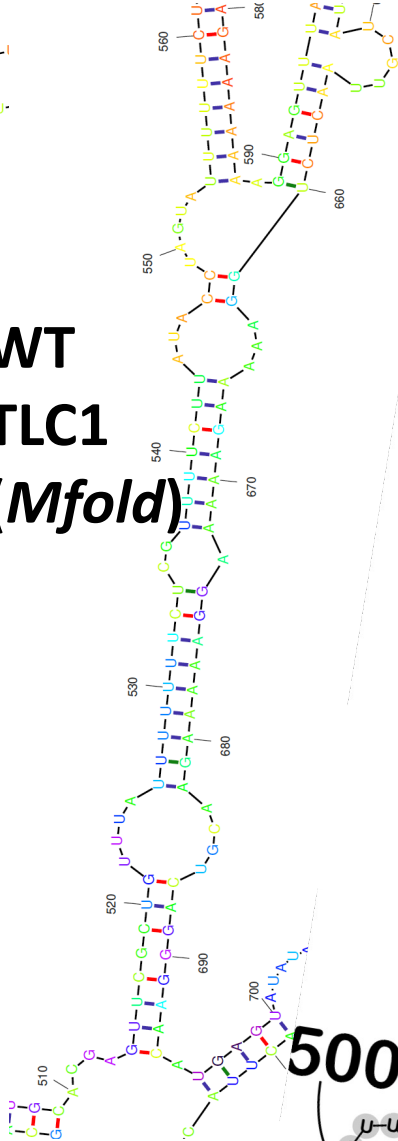
Est1 arm

DA-TLC1

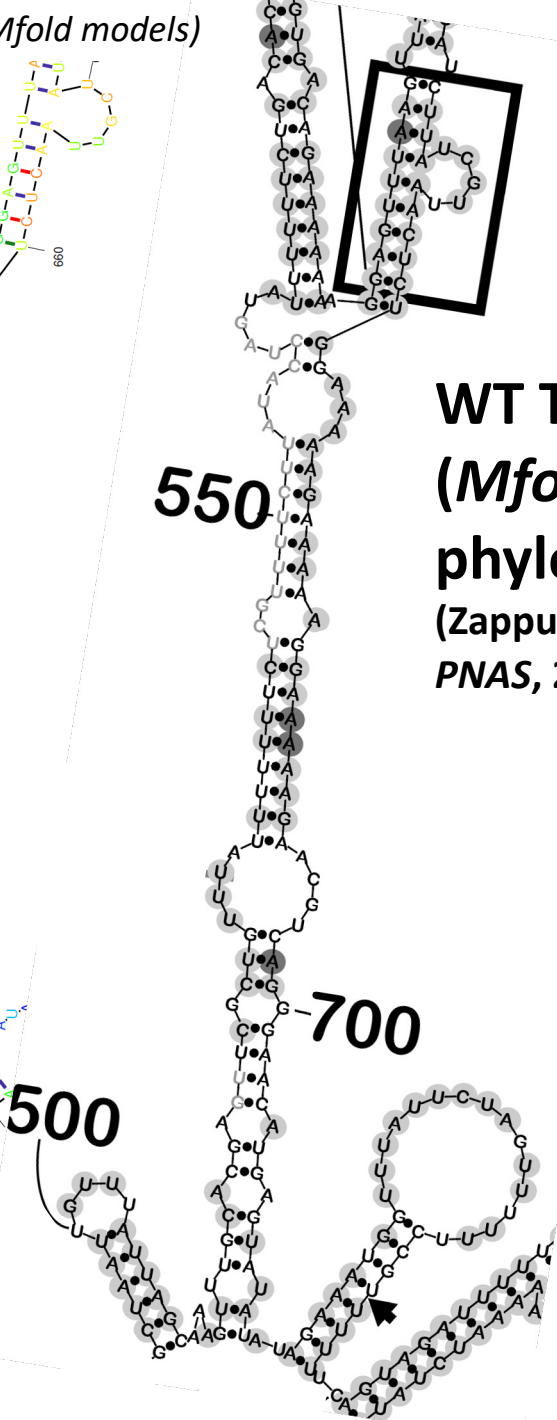
33 nts changed (○)



WT
TLC1
(Mfold)



WT TLC1
(Mfold +
phylogenetics)
(Zappulla and Cech,
PNAS, 2004)

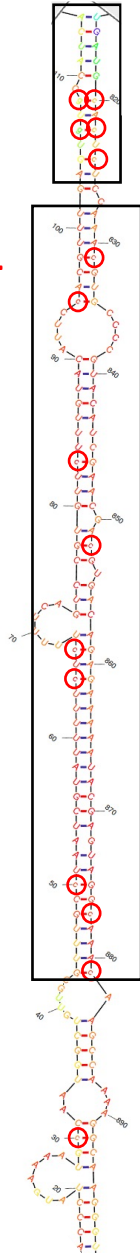


Supp. Figure S3C

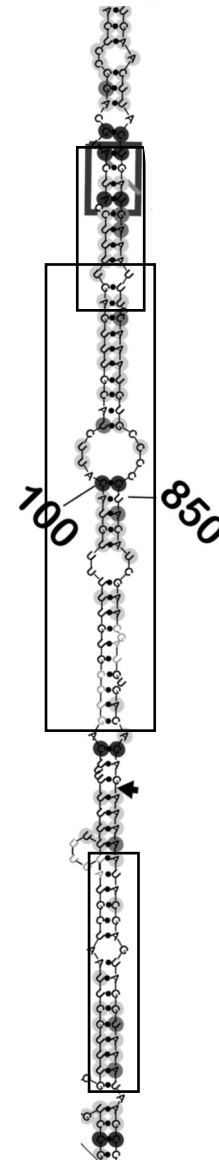
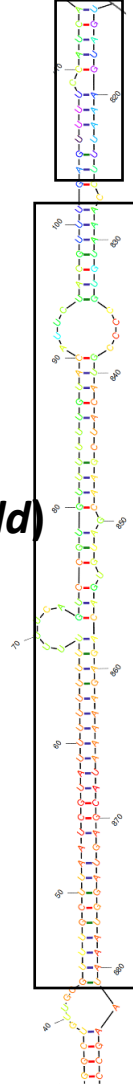
DA-TLC1

Terminal arm

15 nts changed (○)



WT
TLC1
(*Mfold*)



WT TLC1 (*Mfold*
+ phylogenetics)
(Zappulla and Cech,
PNAS, 2004)

(See Fig. 1A for distal portion
of terminal arm *Mfold* models)