Turn-on RNA Mango Beacons for Trans-Acting Fluorogenic Nucleic Acid Detection

Amir Abdolahzadeh[1], Quiana R. Ang[1], Jana R. Caine[2], Shanker Shyam S. Panchapakesan[3], Shinta Thio[1], Razvan Cojocaru[1], and Peter J. Unrau[1]*

[1]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

[2]Department of Chemistry, The University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z1

[3]School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamilnadu, India, 613401

*To whom correspondence should be addressed:

Phone 778-782-3448; punrau@sfu.ca

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Supplementary Tables

Table S1. DNA suppressor sequences for **Figure S1** with contrast and relative F_{max}.

Identity	**Sequence**	**Contrast**	**F_{max}**
Extended Beacon Reporter	GGA UCA CAU ACG GAC AAU AGC AGG AGA GGA GAG GAA GAG GAG AAC GUA GCC UGG CUA AUG CCC	n/a	n/a
Complementary Trigger	GGG CAU UAG CCA GGC UAC GUG CUA UUG UCC GUA UGU GAU CC	10	98
D6	CTC TCC TGC TAT	113 ± 4	89 ± 3
DNA A	CTC TCC TGC TAT TGT CCG	126 ± 2	79 ± 2
DNA B	AGG CTA CGT TCT CCT CTT	62 ± 1	72 ± 1
DNA C	CCT CTC CTG CTA TTG TCC G	176 ± 10	64 ± 4
DNA D	CCT CTC CTG CTA TTG TCC	38 ± 1	79 ± 3
DNA E	CCT GCT ATT GTC CGT ATG	142 ± 10	51 ± 4

Sequences start with 5′ end.

Supplementary Figure Legends

Figure S1. The effect of DNA suppressor length and position on contrast and fluorescence maxima. Relative fluorescence maximum and contrast reported, standard deviation shown (error bars). Sequences available in **Table S1**. Sample X is the indicated beacon sequence in the absence of any DNA oligonucleotide.

Figure S2. Initial screening for adapter nucleotides flanking Mango cores and trigger gaps. Six Mango I type Beacons (top to bottom) with various flanking nucleotides were tested with triggers containing either 0, 1, or 2 nucleotides of separation between hybridization sites (left to right). 5' and 3' flanking adenosines on the Mango core with a trigger containing 0 nucleotides of separation gave the highest contrast, and were therefore used in the future construct designs. Experiments were performed in singlet as an initial screen on a SpectramaxM5 Plate Reader. Final fluorescence values are given in RFU and contrast (C) calculated as described in the main text.

Figure S3. Mango Beacons are sensitive to single point mutations (SPMs). SPMs are colored in red (**Table 3**, bolded) while wildtype sequences are in green and black. D: addition of TO1-Biotin (100 nM final), S: Addition of Mango Beacon (50 nM final), T: Addition of Trigger (100 nM final).

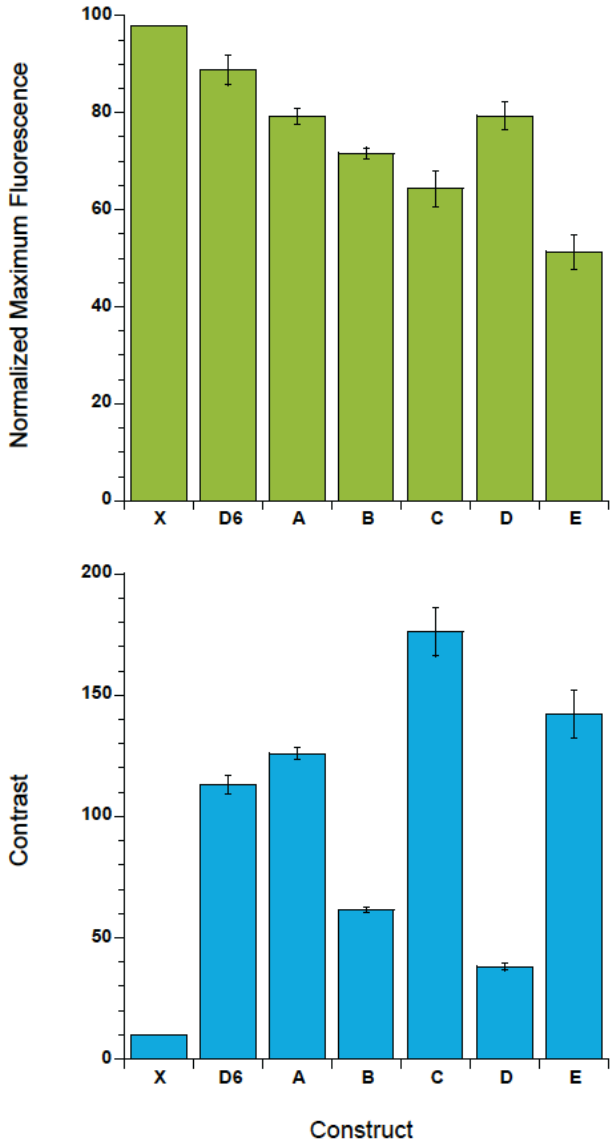
Figure S4. Kinetics of Mango Beacon folding for on-rate determination. Kinetics of linear fluorescence emergence upon target RNA addition for: **A)** A self-hybridizing Mango Beacon (MIIB + 4i) or **B)** A DNA oligo inhibited reporter (MIIB and D5). **C)** Calculated molar rate

(M/s) vs. trigger concentration (nM) was used to estimate on-rates. RNA beacon was held at 50 nM throughout.

Figure S5. Mango Beacons of MIIB + 4i type can misfold. A) The dominant band induced by Trigger A (^{32}P label: left panel) displays Mango TO1-B fluorescence (TO1-B stained gel, right panel), whereas Trigger B and a DNA trigger induce minimal in-gel fluorescence. **B)** Self-inhibiting Mango Beacons can misfold creating multiple bands as visualized by native gel analysis. Three trigger strands (A, B and C) were added to ^{32}P radiolabeled MIIB + 4i and analyzed in a native gel.

Figure S6. DMS probing of Mango II and a MIIB + 5i. A) Mango II aptamer sequence (left) with stem (gray and dark gray) and a GAA^A tetraloop (violet). **B)** MIIB + 5i shown hybridized to its trigger strand with colored hybridization arms (light hybridized to dark shades respectively), adapter A residues (violet) flanking the quadruplex core, and self-inhibitory sequence is shown in orange. Guanines participating in the G-quadruplex are bolded. Regions of quadruplex indicated by solid black bars. * indicates location of ^{32}P radiolabel used in DMS probing. T1: T1 RNase ladder, OH: alkaline hydrolysis ladder.

Figure S1.

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     5'GGAUCACAUACGGACAAUAGCAGGAGAGGAGAGGAAGAGGAGAACGUAGCCUGGCUAAUGCCC
Suppressor 6            TATCGTCCTCTC'5
DNA A                GCCTGTTATCGTCCTCTC'5
DNA B                                          TTCTCCTCTTGCATCGGA'5
DNA C                GCCTGTTATCGTCCTCTCC'5
DNA D                 CCTGTTATCGTCCTCTCC'5
DNA E           GTATGCCTGTTATCGTCC'5
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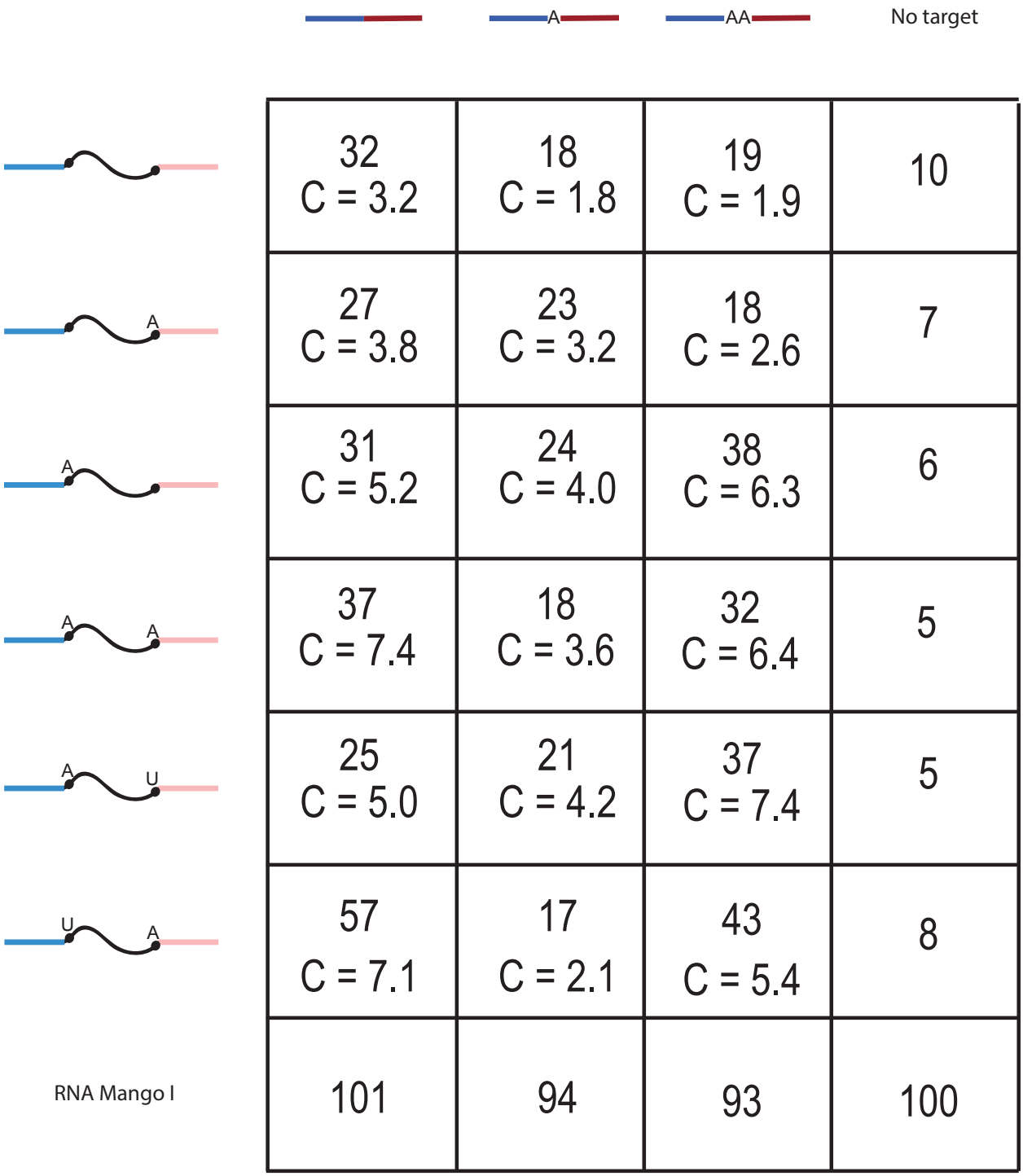
Figure S2.



32 C = 3.2	18 C = 1.8	19 C = 1.9	10
27 C = 3.8	23 C = 3.2	18 C = 2.6	7
31 C = 5.2	24 C = 4.0	38 C = 6.3	6
37 C = 7.4	18 C = 3.6	32 C = 6.4	5
25 C = 5.0	21 C = 4.2	37 C = 7.4	5
57 C = 7.1	17 C = 2.1	43 C = 5.4	8
101	94	93	100

Figure S3.

Wild Type Mango Beacon

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUAA

3′CCUGUUAUCG ---------- UGCAUCGGAC ---- CGAUUGGG

C20A

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUAA

3′CCUGUUAUAG ---------- UGCAUCGGAC ---- CGAUUGGG

C20U

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUAA

3′CCUGUUAUUG ---------- UGCAUCGGAC ---- CGAUUGGG

U21G

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUAA

3′CCUGUUAGCG ---------- UGCAUCGGAC ---- CGAUUGGG

U23G

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUAA

3′CCUGUGAUCG ---------- UGCAUCGGAC ---- CGAUUGGG

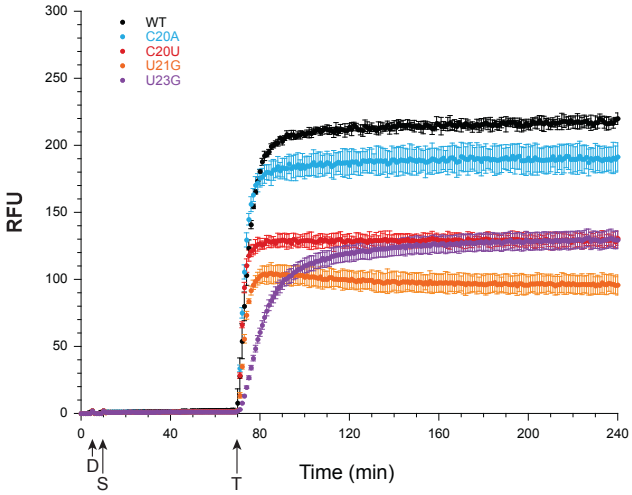


Figure S4.

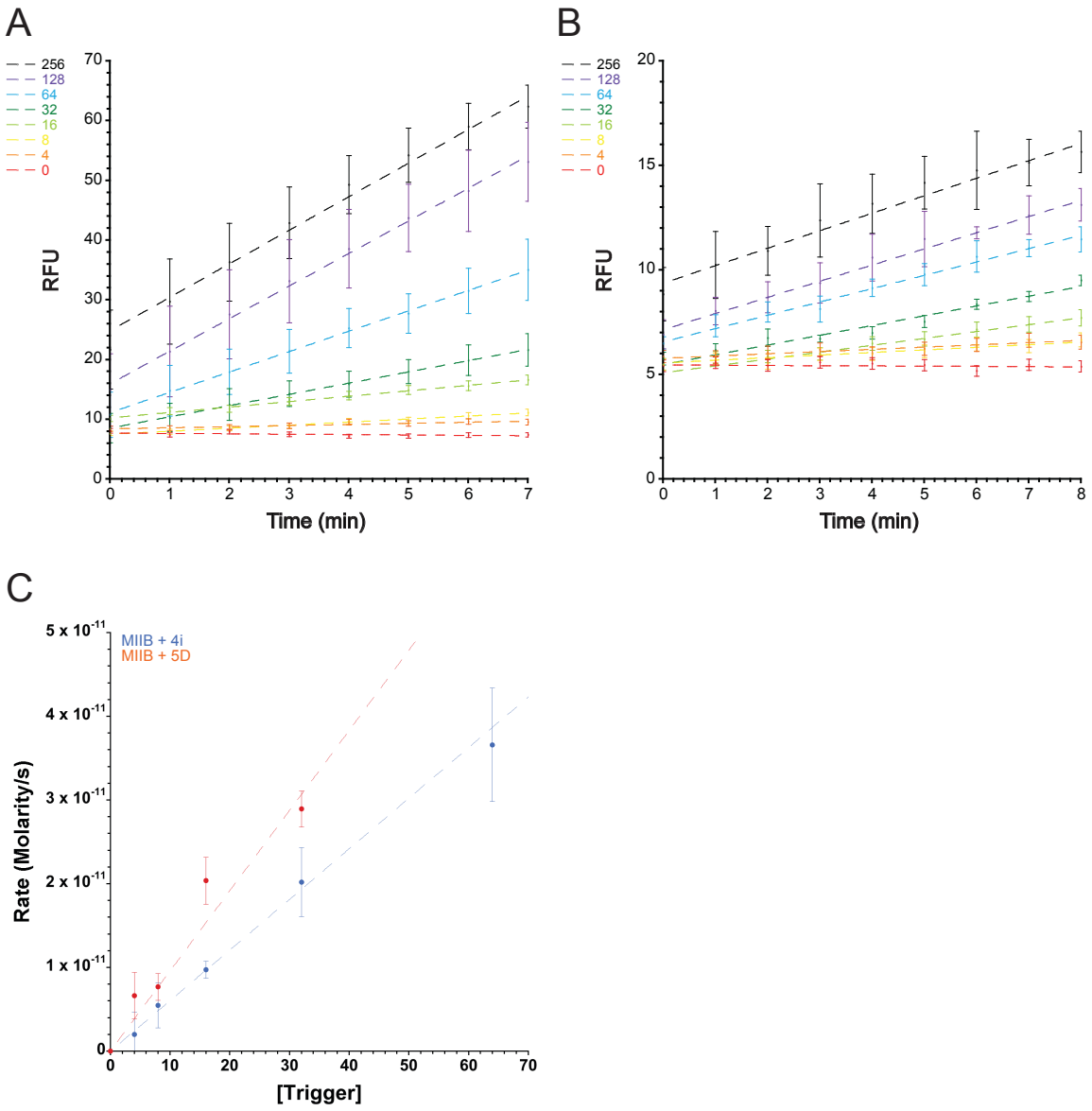
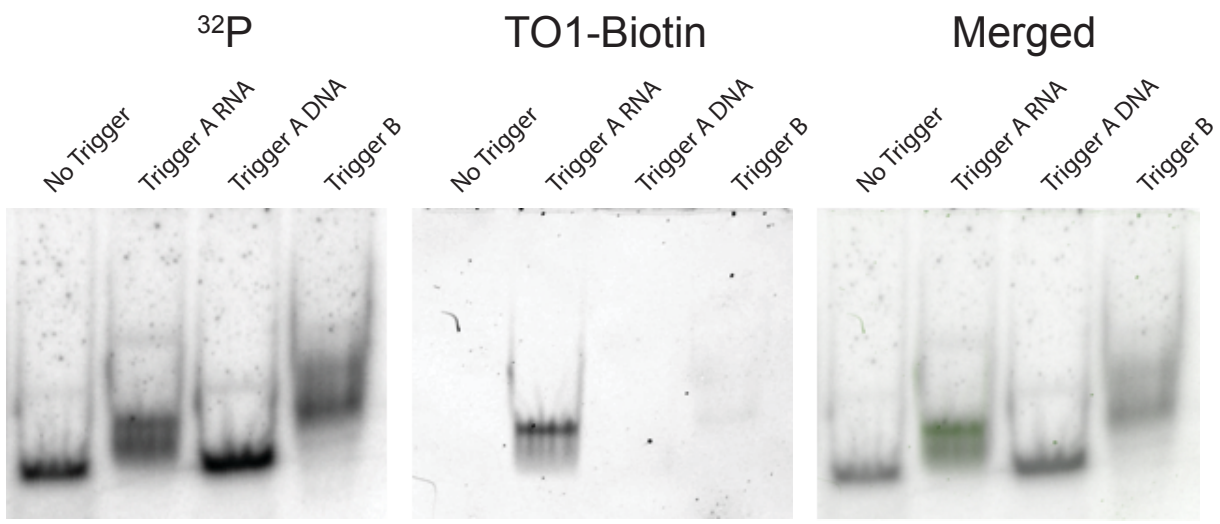

A
256
128
64
32
16
8
4
0
RFU
Time (min)
B
256
128
64
32
16
8
4
0
RFU
Time (min)
C
MIIB + 4i
MIIB + 5D
Rate (Molarity/s)
[Trigger]

Figure S5.

A



C:	165	ND	3.5
F:	217	ND	6.7

B

Beacon S3B with Trigger A

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUCC

3′CCUGU**UA**U**C**G ---------- UGCAUCGGAC ---- CGAUUGGG

Trigger B

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUCC

3′CCUGU**UA**U**C**G ---------- UGCAUCGGAC -CC- CGAUUGGG

Trigger C

5′GGACAAUAGC A **GGAG A GGAG A GGAAGA GGAG** A ACGUAGCCUG CUCC GCUCC

3′CCUGU**UA**U**C**G ---------- UGCAUCGGAC --C- CGAUUGGG

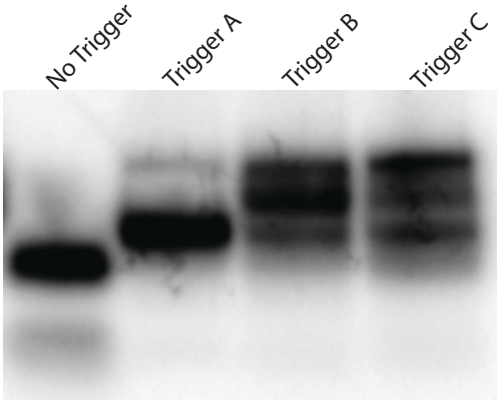
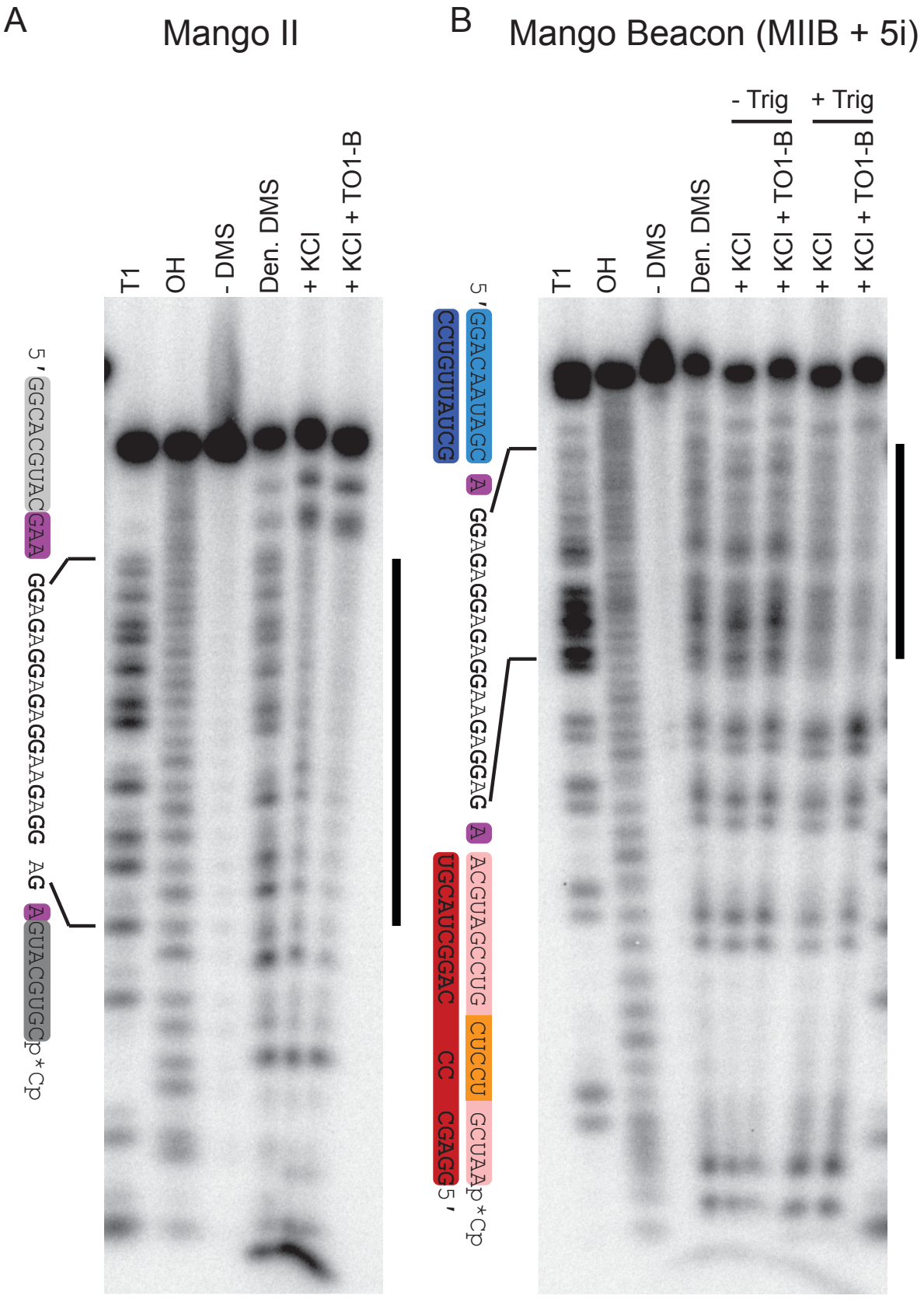


Figure S6.


A
Mango II
T1
OH
- DMS
Den. DMS
+ KCl
+ KCl + TO1-B
5′ GGCACGUAC GAA GGAGAGGAGAGGAAGAGG AG A GUACGUGC p*Cp
B
Mango Beacon (MIIB + 5i)
- Trig
+ Trig
T1
OH
- DMS
Den. DMS
+ KCl
+ KCl + TO1-B
+ KCl
+ KCl + TO1-B
5′ GGACAAUAGC A GGAGAGGAGAGGAAGAGGAG A ACGUAGCCUG CUCCU GCUAA p*Cp 5′
CCUGUUAUCG
UGCAUCGGAC CC CGAGG