Template and primer DNA sequences

Autosticky PCR was used to generate the single-stranded overhangs for labeling the DNA transcription templates. Following primers were used:

5'-autosticky PCR primer:
5'-TCACG AAAGC TGAGT AGTCA CGAGT CTTCT /idSp/ ctggcagttttagg ctgatttgg-3'

3'-autosticky PCR primer:
5'-CCttaatcatactcaccaaattaccatcCC /idSp/ ATAAACGCAGAAAGGCCCAC -3'

idSp denotes an abasic site.

To label the 3'-ends of the DNA transcription templates, following DNA oligonucleotide was hybridized to the single-stranded overhang of the DNA transcription templates:

5'-Cy3.5-GGG ATG GTA ATT TGG T /iCy3.5/ GA GTA TGA TTA AGG -3'

Following sequences for the DNA transcription templates were ordered from IDT as geneBlocks (these are the templates used during autosticky PCR with the primers mentioned above):

All sequences have following common backbone:
RNA polymerase promoter (green), hybridization sequence for immobilization of stalled complex through 5'-end of nascent RNA (red), potential binding site for DNA oligo binding to 5'-end of nascent RNA (blue), sequence of interest (see further below), binding site for labeled DNA oligo that is binding to 3'-end of nascent RNA (orange) and triple transcription terminator (black).

This is the DNA sequence:
ctggcagttt taggctgatt tggttgaatg ttgcgcggtc agaaaattat tttaaatttc ctcttgtcag gccggaataa ctccctataa tgcgccacc
ACT ACCAC CACCC AACCA ACACA CC AAC CAC TCC AAT TAC ATA CAC C
(Sequence of interest)
CCCTA TCCCT TATCT TAAC
GGCTCCTTTTGGAGCCTTTTTTTTTGGAGATTTTCTAAAACGAAAGGCTCAGTCGAAA GACTGGGCCTTTCGTTTTATCT TAATC ACACT GGCTC ACCTT CGGGT GGGCC TTTCT GCGTT TAT

These are the inserted sequences of interest:

3'domain:
tgacgggggc ccgcacaagc ggtggagcat gtggtttaat tcgatgcaac gcgaagaacc
ttacctggtc ttgacatcca cggaagtttt cagagatgag aatgtgcctt cgggaaccgt
gagacaggtg ctgcatggct gtcgtcagct cgtgttgtga aatgttgggt taagtcccgc
aacgagcgca acccttatcc tttgttgcca gcggtccggc cgggaactca aaggagactg
ccagtgataa actggaggaa ggtggggatg acgtcaagtc atcatggccc ttacgaccag

ggctacacac gtgctacaat ggcgcataca aagagaagcg acctcgcgag agcaagcgga
cctcataaag tgcgtcgtag tccggattgg agtctgcaac tcgactccat gaagtcggaa
tcgctagtaa tcgtggatca gaatgccacg gtgaatacgt tcccgggcct tgtaca

Δ147 construct:
tgacgggggc ccgcacaagc ggtggagcat gtggtttaat tcgatgcaac gcgaagaacc
ttacctggtc ttgacatcca cggaagtttt cagagatgag aatgtgcctt cgggaaccgt
gagacaggtg c tgTCACca gccc ttacgaccag
ggctacacac gtgctacaat ggcgcataca aagagaagcg acctcgcgag agcaagcgga
cctcataaag tgcgtcgtag tccggattgg agtctgcaac tcgactccat gaagtcggaa
tcgctagtaa tcgtggatca gaatgccacg gtgaatacgt tcccgggcct tgtaca

Δ270 construct:
tgacgggggc ccgcacaagc ggtggagcat gtg TCAC cac gtgctacaat ggcgcataca aagagaagcg acctcgcgag
agcaagcgga cctcataaag tgcgtcgtag tccggattgg agtctgcaac tcgactccat gaagtcggaa
tcgctagtaa tcgtggatca gaatgccacg gtgaatacgt tcccgggcct tgtaca

Δ318 construct:
tgacgggggc ccgcacaagc ggtggagcat gtg TCAC cac gtgctacaat ggcgca cTCACg
tgcgtcgtag tccggat tgg cTTCGg ccat gaagtcggaa tcgctagtaa tcgtggatca gaatgccacg
gtgaatacgt tcccgggcct tgtaca

The stalled transcription elongation complex was immobilized through the nascent RNA with a biotinylated double-stranded DNA with single-stranded overhang. The double-stranded sequence was:
cgggcc tcttcgctat tacgccagct ggcgaaaggg ggatgtgctg caaggcgatt aagttgggta acgccagggt
tttcccagtc acgacgttgt aaaacgacgg ccagtgaatt cgagctcggt acccggggat cctctagagt cgacctgc

Biotinylation and single-stranded overhang were obtained by amplifying above sequence (from pUC19 vector) with autosticky PCR and following two primers:
5'-primer: 5'-BiotinTEG/CG GGC CTC TTC GCT ATT AC-3'
3'-primer: 5'-GGT GTG TTG GTT GGG TGG TGG TAG T
/idSp/ GCAGGTCGACTCTAGAGGAT -3'

These are the sequences of the labeled DNA oligonucleotides for co-transcriptional hybridization to the 5'- or 3'-ends of the nascent RNA:

5'-end:
p66_Cy3_FQ: 5'- /5Cy3/GG TGT ATG TAA TTG GAG TGG TT/3IABkFQ/ -3'
p106_Cy3: 5'- GG TGT ATG TA /iCy3/ A TTG GAG TGG TT -3'

3'-end:
p28_Cy3_BHQ2: 5'- /5BHQ-2/GTT AAG ATA AGG GAT AGG G/3Cy3Sp/ -3'

p73_Cy3: 5'- GTT AAG ATA AGG GAT AGG G/3Cy3Sp/ -3'
p107_Cy3.5: 5'- GTT AAG ATA AGG GAT AGG G /3Cy3.5Sp/ -3'

If not otherwise stated, p28 or p73 were used for all the experiments. p66 was used to detect real co-transcriptional binding of Cy5-S7 by FRET (Figure S1A). p106 and p107 were used for the helix H28 formation experiments.