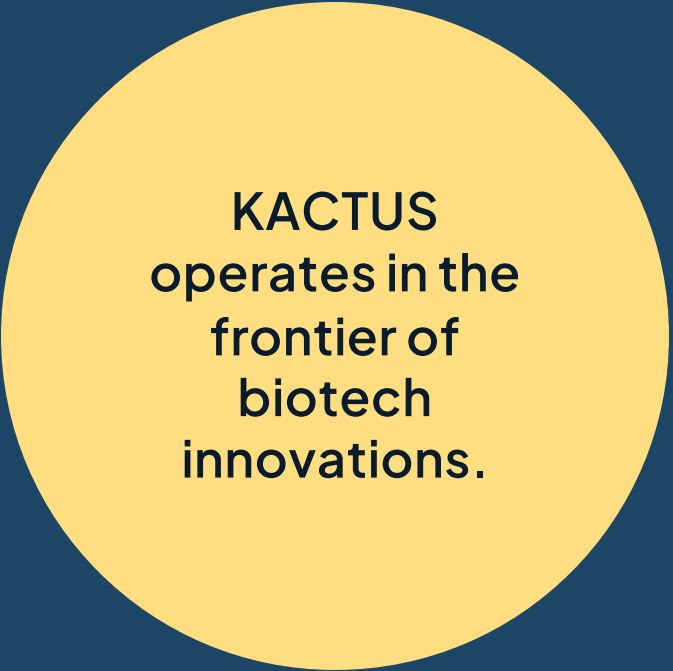


Engineered T7 RNAP for decreased production of dsRNA

May 18 2023

ManHee Suh, Ph.D

Chief Technology Officer



**KACTUS
operates in the
frontier of
biotech
innovations.**

**We focus on the unmet
market needs and provide
first-to-market protein and
enzyme products to
accelerate biopharma and
diagnostic breakthroughs.**

2018

Founded

3

Global Sites

200+

Employees

2400+

Catalog
Products

300
+

Pharma &
Biotech Clients

ISO
13485

Accredited

GMP
Standards

Certified & Audited

Waltham, MA

- Global Operations
- Order Fulfillment
- Customer Service

Shanghai, China

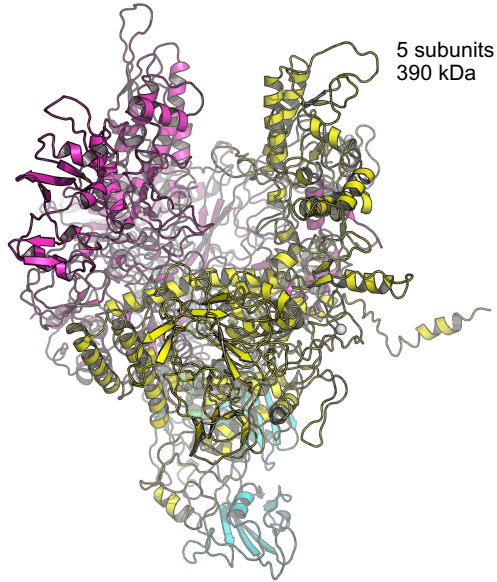
- Research & Development
- GMP for Cell & Gene Therapy
- Quality Control & Assurance
- Order Fulfillment
- Customer Service



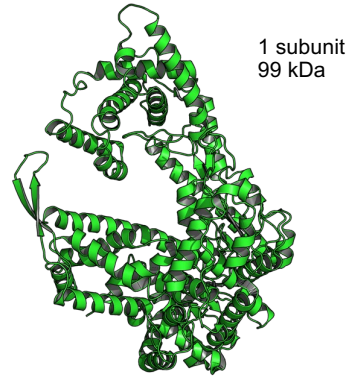
Lin-Gang, Shanghai

- GMP for Enzymes
- Large Scale
- Production
- Regulatory Filing
- Supply Chain Auditing

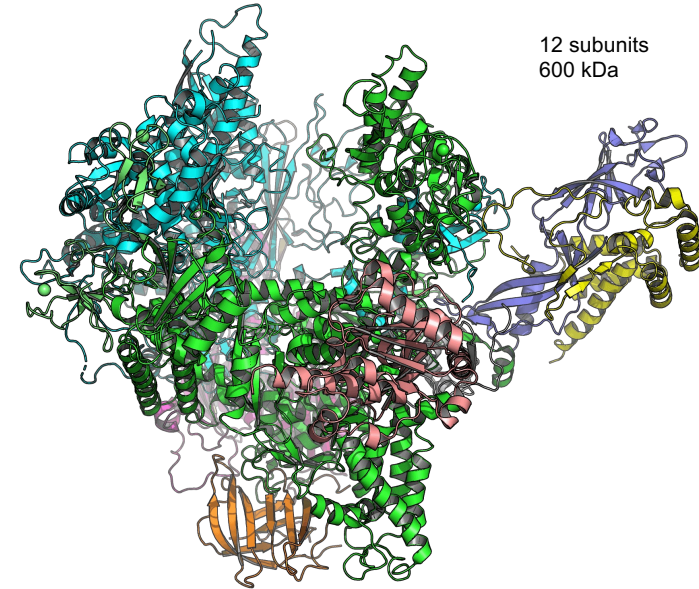
Bacterial RNAP



T7 RNAP



Eukaryotic RNAP II



- SGC (synthetic gene circuits)
 - Split versions of T7RNAP
 - Transcriptional AND gates
 - 3-input AND gate
- RNA editing
- Industrial biotechnology
- RNA Interference

Two Concerning RNA Contaminants

- Abortive initiation

Biochemistry **27**, 3966-3974 (1988).
Martin, C.T., Muller, D.K. & Coleman, J.E.

- Template strand switching

J Biol Chem **273**, 10253-10260 (1998).
Rong, M., Durbin, R.K. & McAllister, W.T.

- Runoff mRNA

Nucleic Acids Res **13**, 6223-6236 (1985).
Schenborn, E.T. & Mierendorf, R.C., Jr.

- slippage

J Mol Biol **232**, 1030-1047 (1993).
Macdonald, L.E., Zhou, Y. & McAllister, W.T.

- 3'-extensions**

Gene **72**, 75-89 (1988).
Krupp, G.

- Duplex RNA**

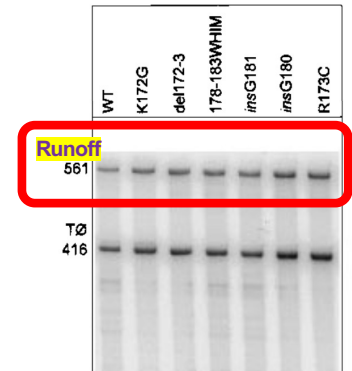
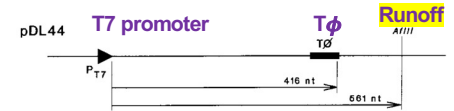
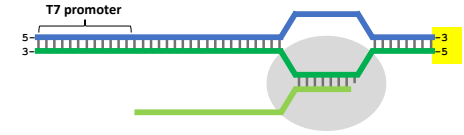
Nucleic Acids Res **46**, 5239-5249 (2018).
Mu, X., Greenwald, E., Ahmad, S. & Hur, S.

3'-extensions (self-primed; looping back)



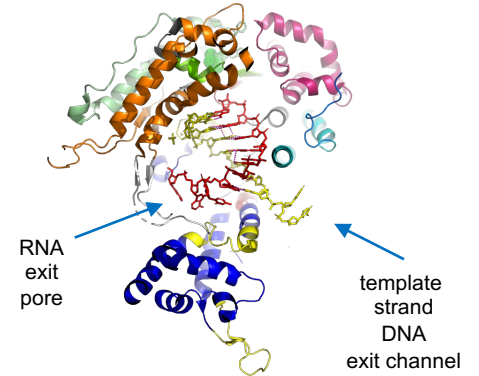
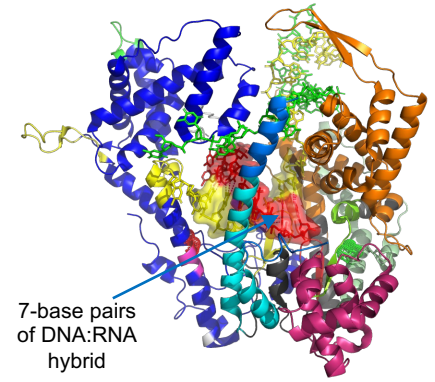
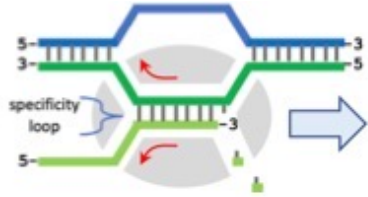
Duplex RNA

(Long, complete complementary sense/antisense RNA)

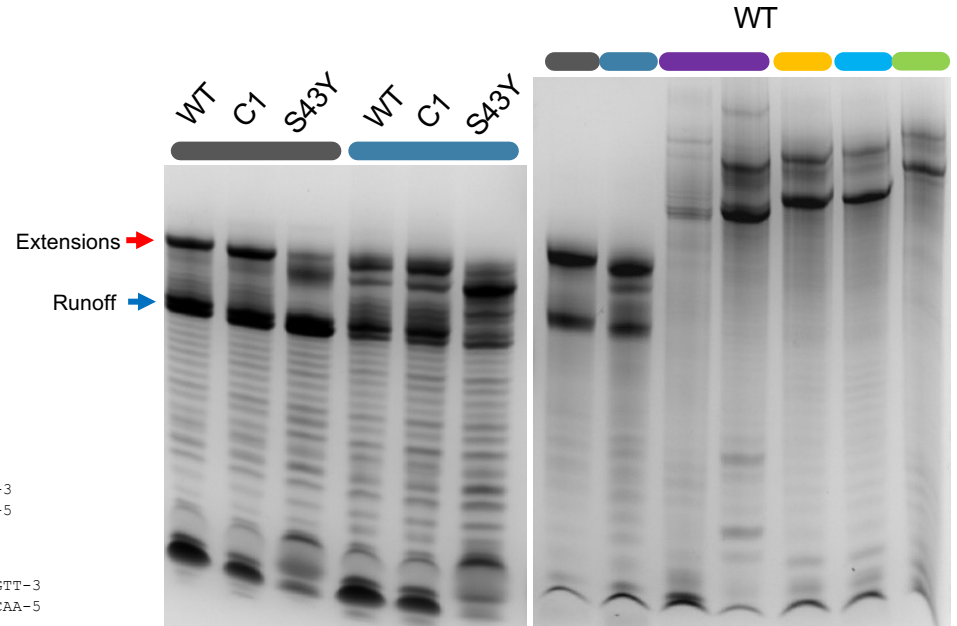


J Mol Biol **269**, 28-40 (1997).
Lyakhov, D.L. et al.

3'-extensions have a limited range



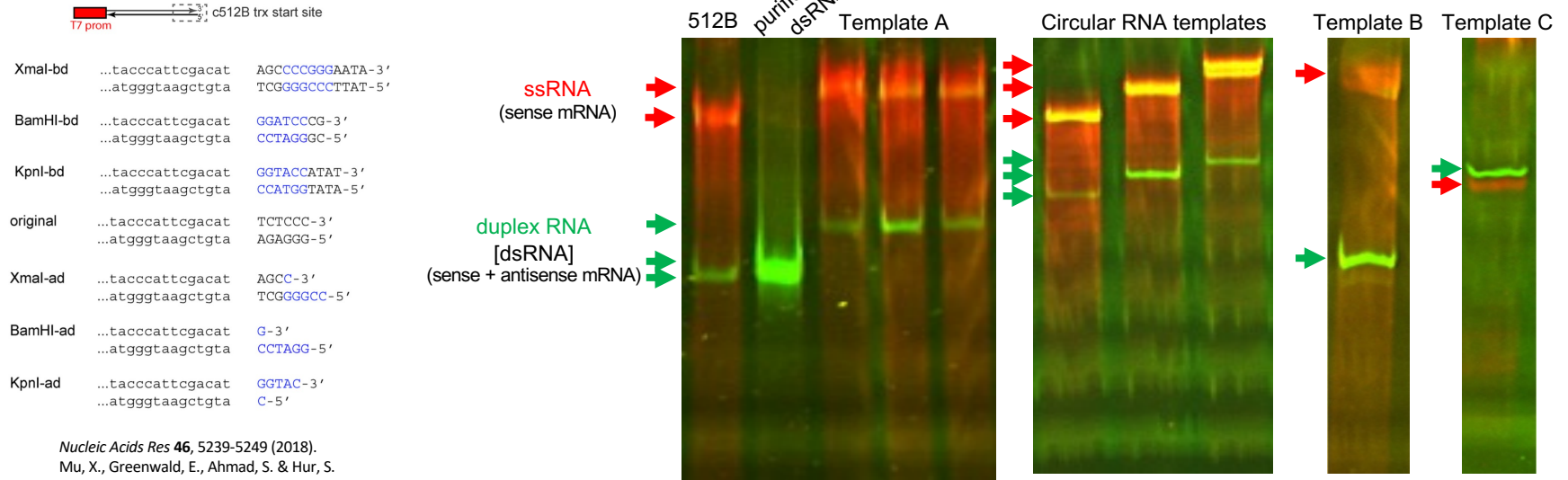
3'-extensions are ubiquitous



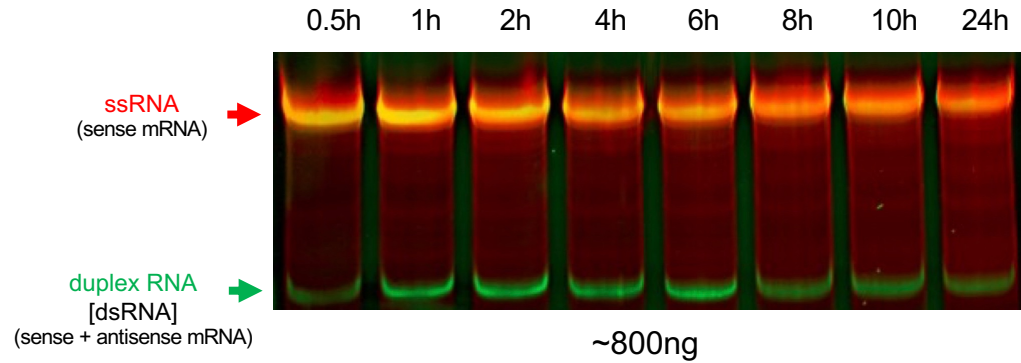
Duplex RNA contaminants are ubiquitous

Long, sense-antisense duplex RNA (dsRNA)

[hybridization of the intended mRNA transcript with a fully complementary antisense RNA]

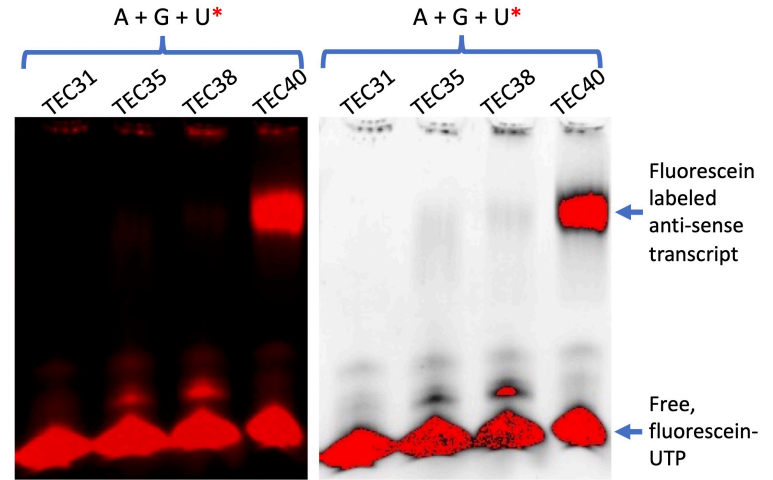
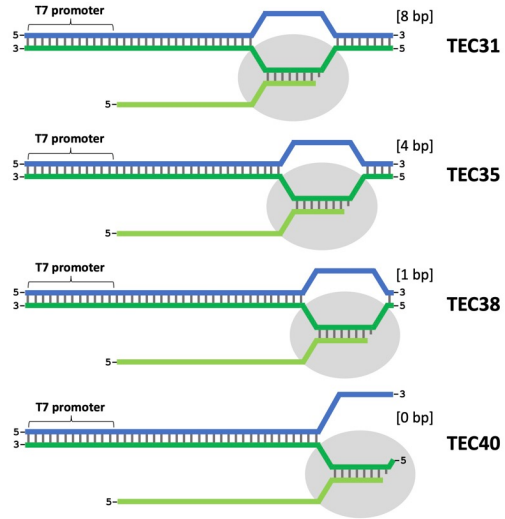


dsRNA formation occurs continuously throughout the IVT



Acridine Orange stain

How does the antisense RNA occur?



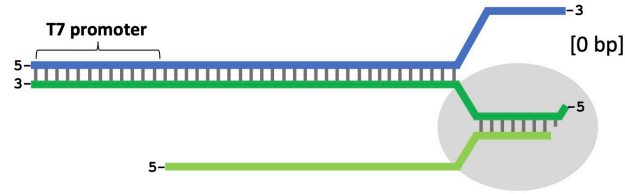
20% Urea-PAGE

Duplex RNA

(Long, complete complementary sense/antisense RNA)



T7 RNAP does not release the template DNA & mRNA



Promoter-driven IVT

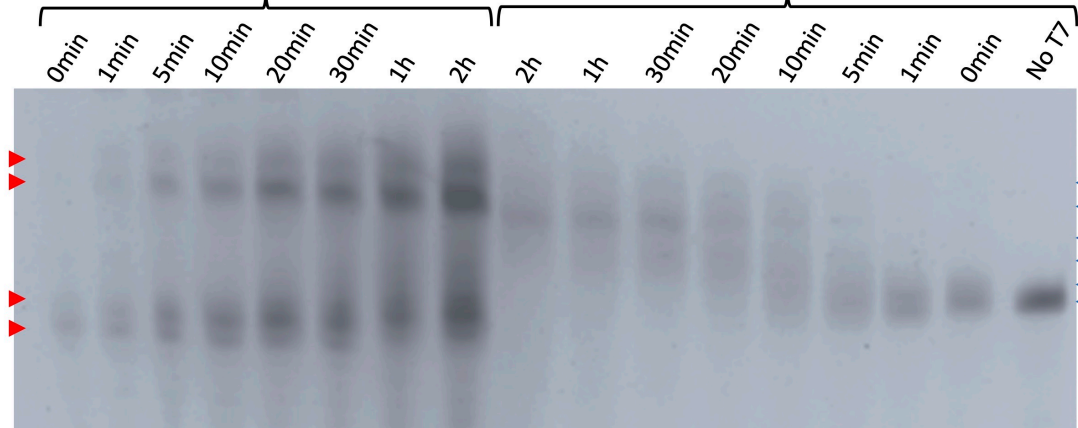
5-AATTAATACGACTCACTATAAGGAATAAGTAGAGGTGAAGATTTA-3
3-TTAATTATGCTGAGTGATATCCTTATTCATCTCCACTTCTAAAT-5



5-GGAAUAAGUAGAGGUGAAGAUUUA-3

Promoter-independent IVT (synthetic RNA)

5-GGAAUAAGUAGAGGUGAAGAUUUA-3

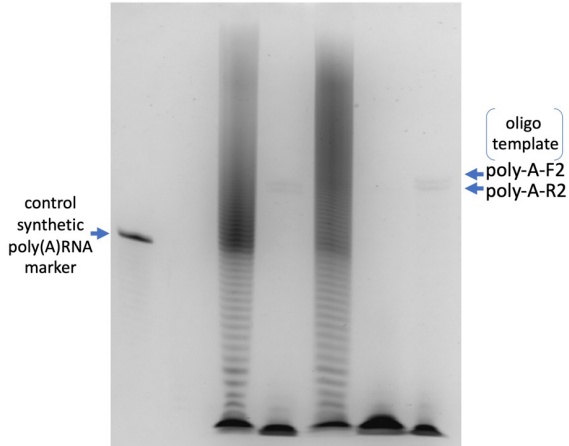


Urea-PAGE

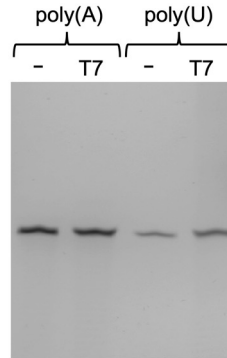
T7 RNAP does not release the template DNA & mRNA

>poly-A-F2
 5-AAATTAAATACGACTCACTATAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3
 3-TTAAATTATGCTGAGTGATACTCTTTTTTTTTTTTTTTTTTTTTTTTTTT-5
 >poly-A-R2
 ↻ >poly(A)RNA
 5-GGAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3

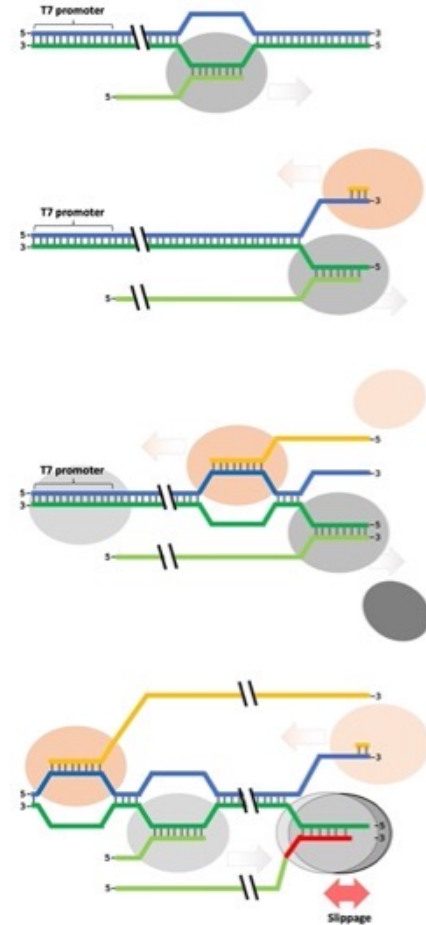
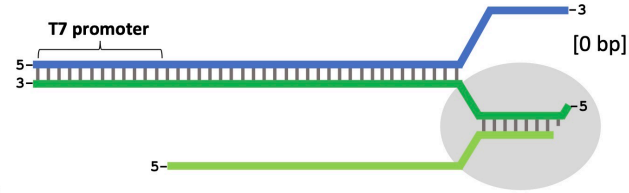
1	2	3	4	5	6	7	lanes
-	-	+	+	+	-	+	G
-	-	+	-	+	-	-	A
-	-	+	-	-	+	-	C
-	-	+	-	-	-	+	U



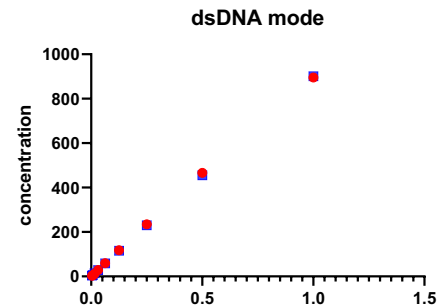
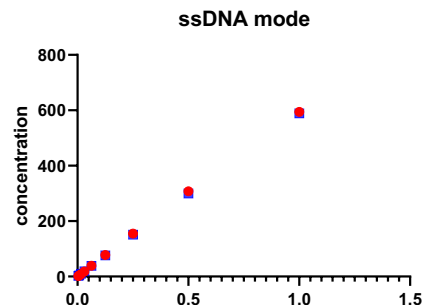
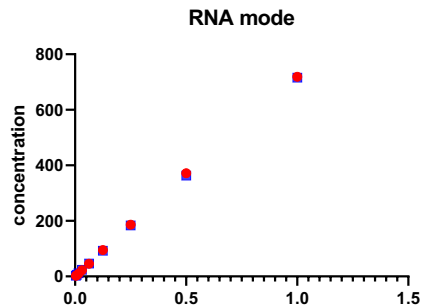
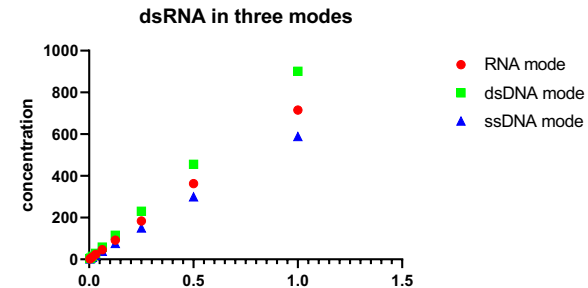
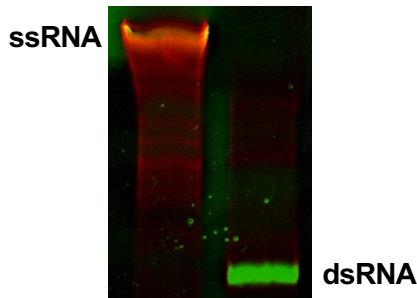
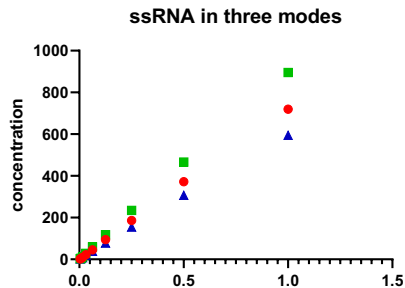
oligo template
 poly-A-F2
 poly-A-R2



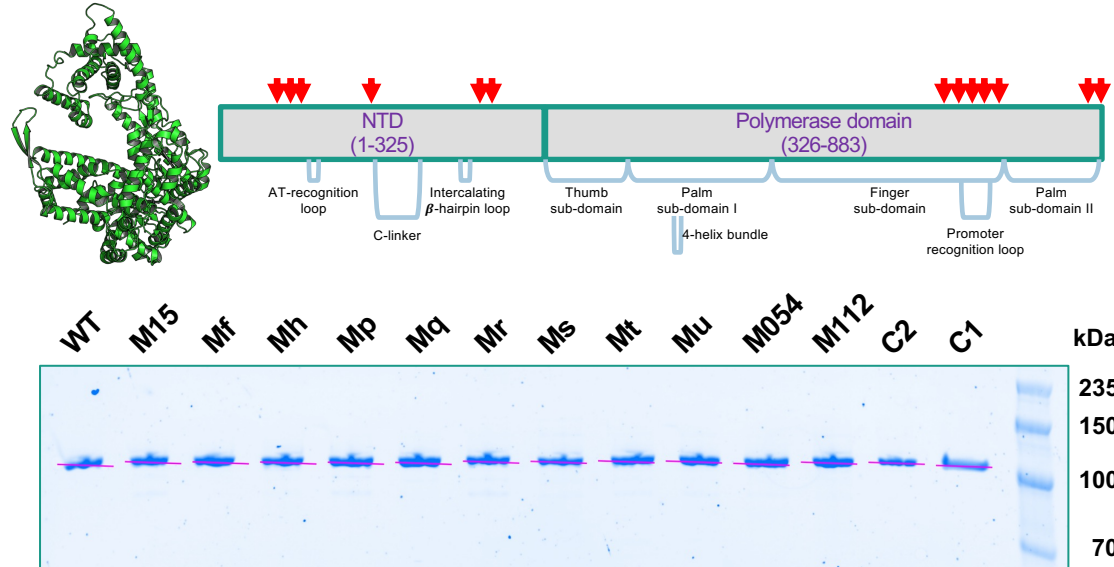
>poly(A)RNA
 5-GGAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3
 >poly(U)RNA
 5-GGUUUUUUUUUUUUUUUUUUUUUUUUUUU-3



Spectroscopically, ssRNA and dsRNA are indistinguishable



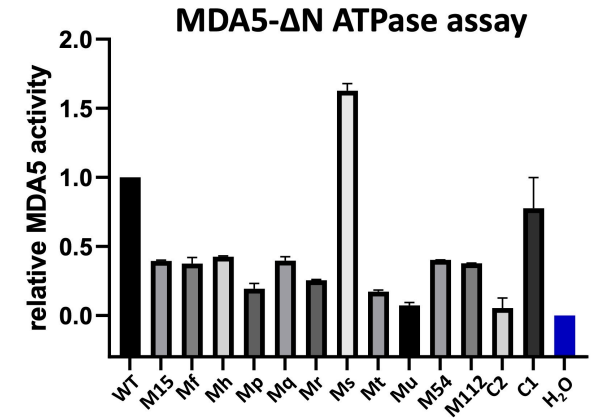
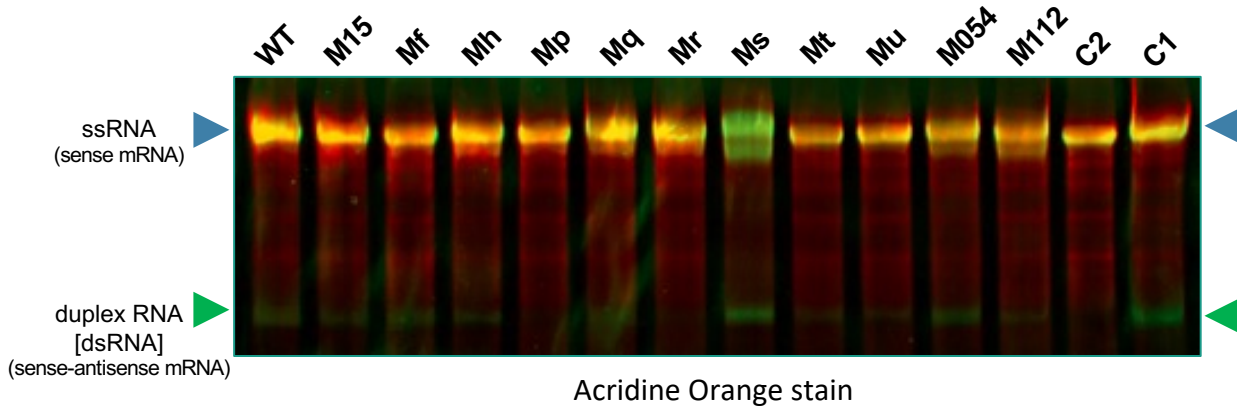
Rational designing of T7 RNA polymerase



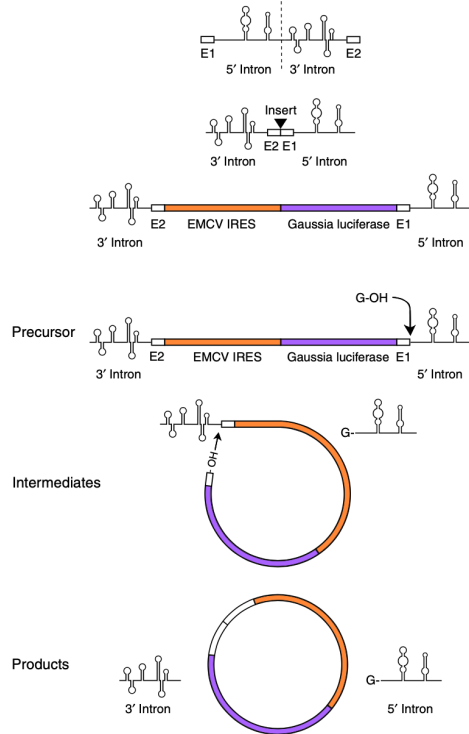
Engineered T7 RNAPs can reduce duplex RNA

Long, sense-antisense duplex RNA (aka, dsRNA)

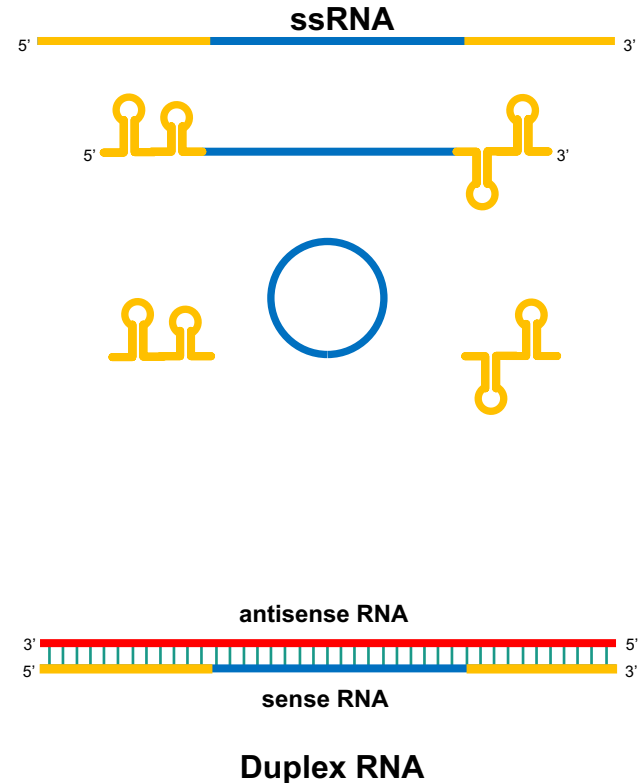
[hybridization of the intended mRNA transcript with a fully complementary antisense RNA]



The presence of dsRNA influences circRNA production

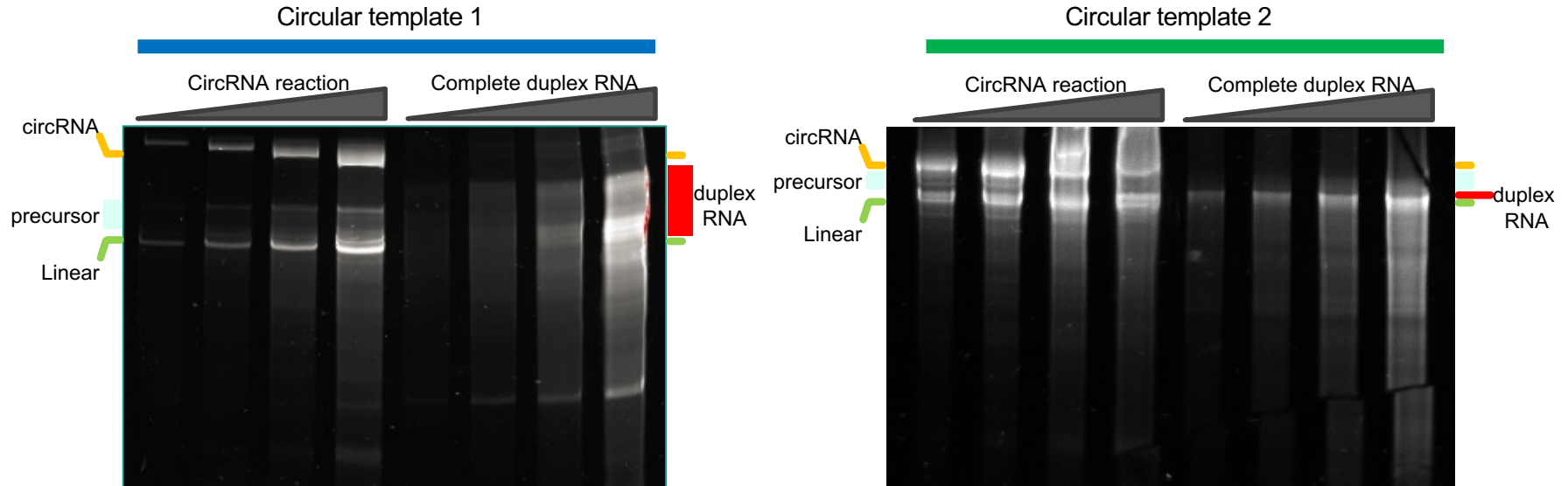


Nat Commun 9, 2629 (2018).
Wesselhoeft, R.A., Kowalski, P.S. & Anderson, D.G.

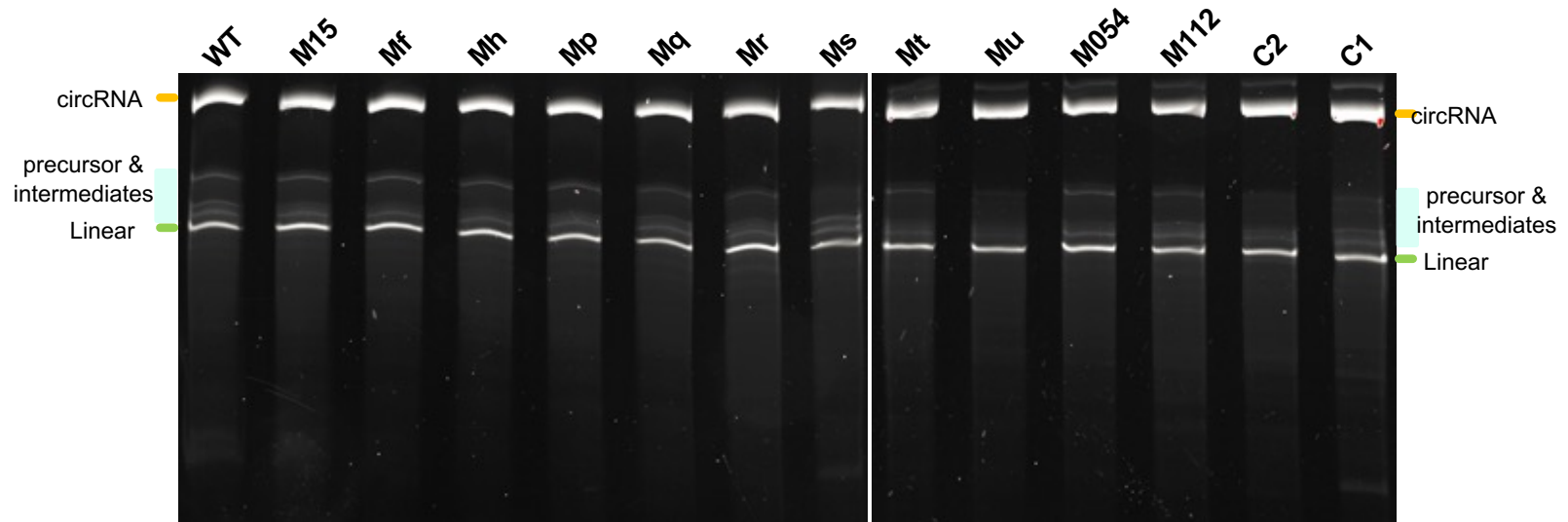


Why should we care about dsRNA in circRNA?

Long duplex RNA (dsRNA) cannot become circRNA

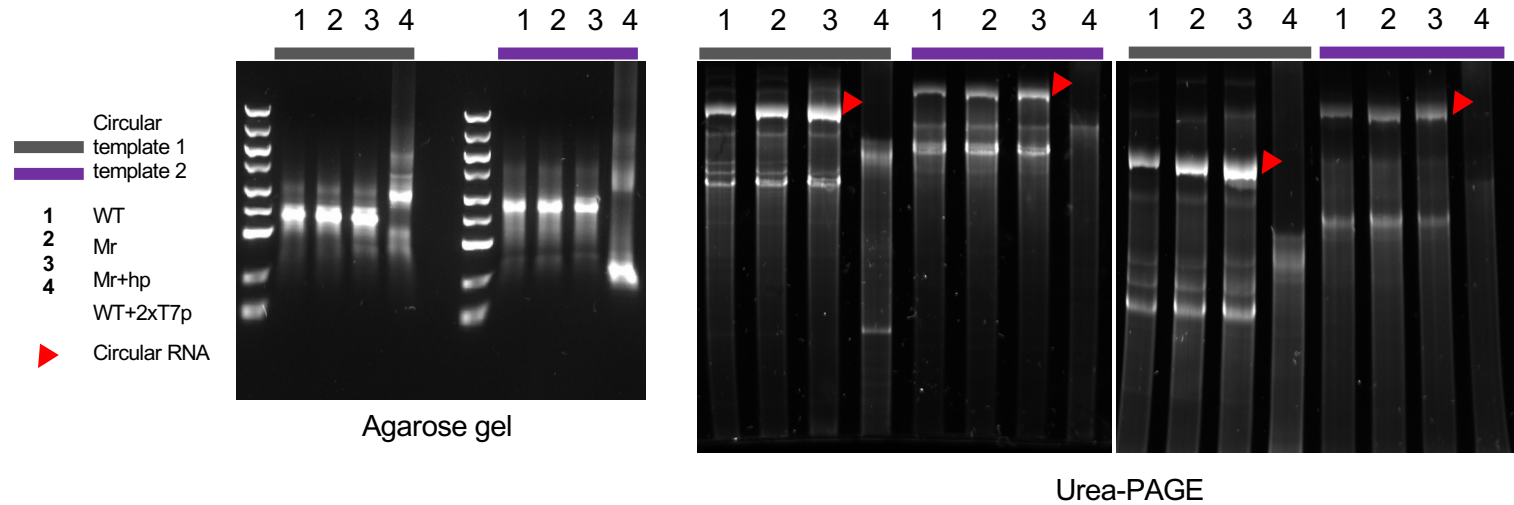


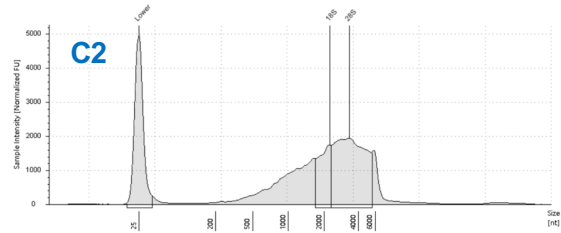
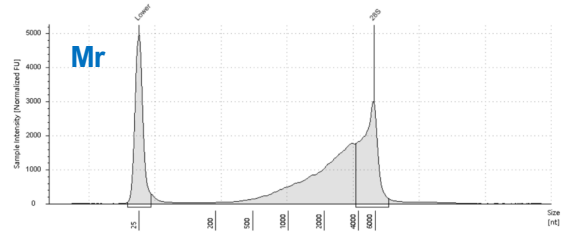
T7 RNAP circular RNA formation abilities cont.



~80ng

Our T7 mutant produces more circular RNA





CE analyses on the 10k base-long mRNA
by Capillary electrophoresis instrument
(4150 TapeStation, Agilent)

- 3'-extensions do not occur continuously
- Template DNA and RNA are not released promptly from T7 RNAP
- dsRNAs are immunostimulatory molecules
- dsRNAs in the IVT reactions cannot become circRNA
- Suppressing the production of dsRNA byproducts increases the target mRNA
- Certain T7 mutant RNA polymerases can reduce the dsRNA byproducts
- Use of versatile T7 RNAP simplify the mRNA production

Thank you!

KACTUS