Expanded View Figures

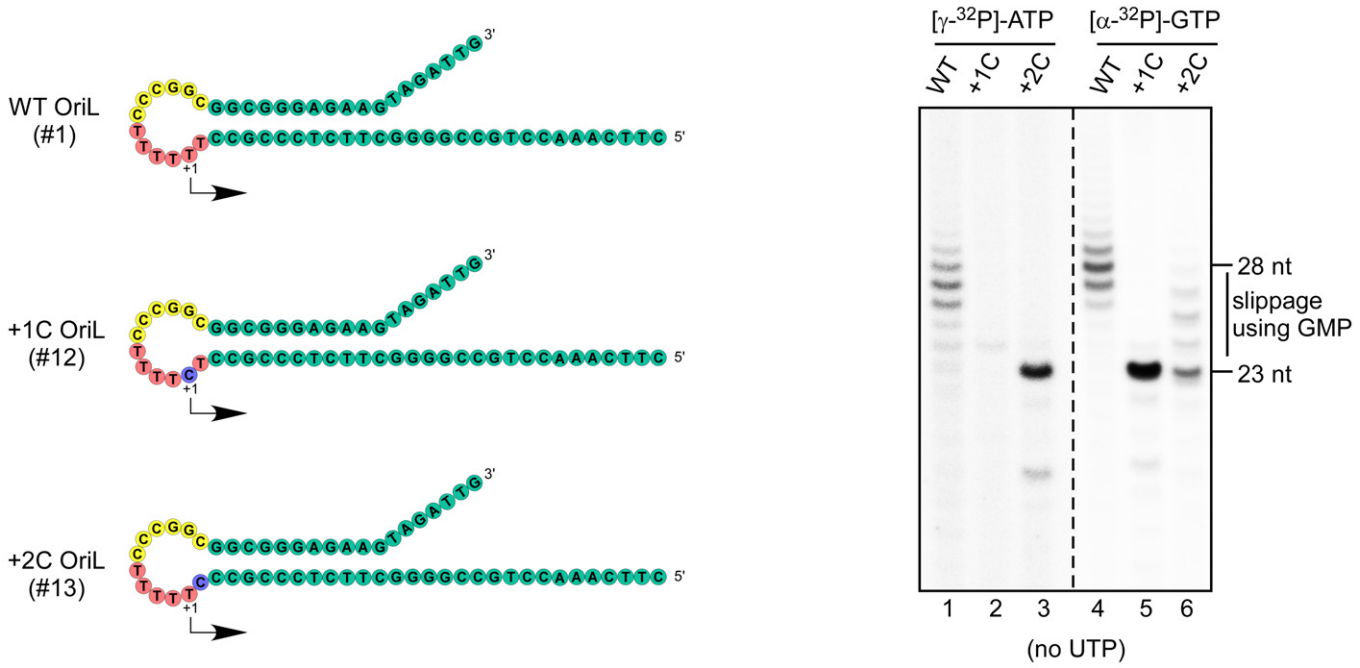

Figure EV1. Transcript slippage during initiation at OriL.

Left Panel—schematic representation of the mutated OriL templates used in the experiment. Right panel—the products of transcription by mtRNAP in the presence of a limiting set of substrates (ATP, CTP, and GTP) are shown. The 5' ends of the transcripts in lanes 1–3 were labeled by incorporation of [γ-^{32}P]-ATP, while transcripts in the reactions shown in lanes 4-6—internally, by incorporation of [α-^{32}P]-GTP. Disruption of the T-stretch in OriL prevents transcript slippage using AMP (lanes 2,3). Transcript slippage using GMP is observed at +2C OriL due to generation of the C-stretch in this OriL variant (lane 6).

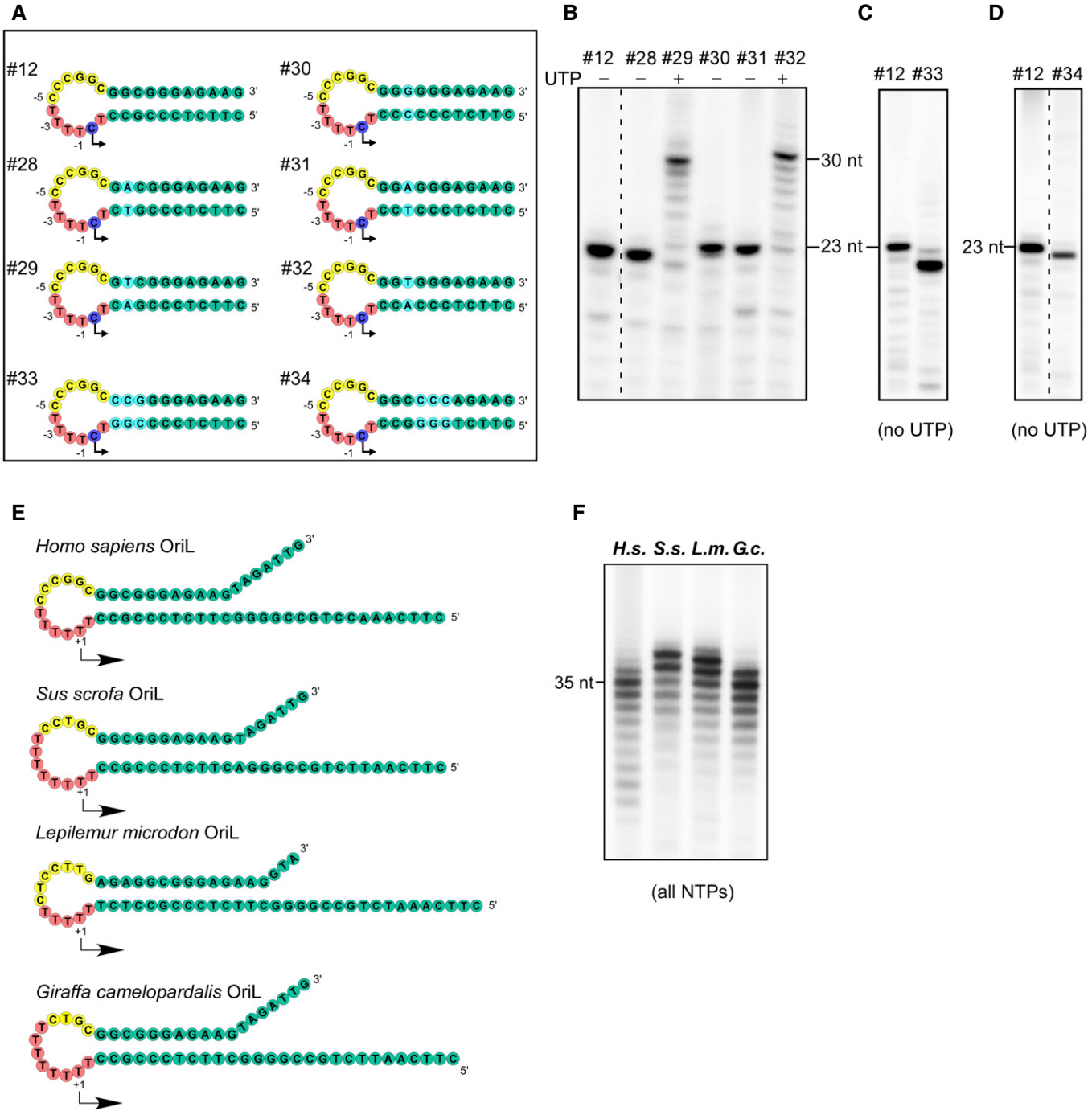

Figure EV2. Transcription initiation at OriL is sequence-independent.

A Schematic representation of the mutated OriL templates used in panels B through D. The templates are grouped by experiments shown below. The color scheme is as indicated in Fig 1A. The purple circles indicate the transcription start site in the templates having +1T to C substitutions and thus initiating with GTP. Other substitutions in the WT sequence are shown in cyan. Partial sequence of the transcribed region is shown. The templates used in the experiments shown in panels B–F are identified atop of each lane. Substitutions in the OriL stem have been chosen to match the ones used in the previous study (Wanrooij, 2012b).

B–D Changes in the OriL sequence do not affect transcription initiation. Transcription assays were performed using WT OriL (left lanes in B–D) and the OriL templates with substitutions in the stem region as indicated in panel A. Partial NTP mixture (no UTP) has been used in all assays except for the templates #29 and #32, which required all NTPs to make a run-off product. Note that the RNA products obtained on templates #33 and #34 appear to be smaller than the RNA made on WT OriL due to difference in their G-C content.

E Schematic representation of the OriL templates used in panel F.

F Human mtRNAP efficiently transcribes OriL from other mammalian species.

Figure EV3. PolG and mtRNAP assemble into a ternary complex on WT and −1A OriL.

A, B EMSA using OriL and mtRNAP (A) and PolG (B).
C Relative affinity of mtRNAP and PolG to OriL as observed in A and B.
D Relative affinity of mtRNAP to WT and −1A OriL assayed using EMSA.
E The primosome forms on both WT and −1A OriL. EMSA was performed using WT (left) or −1A OriL (right). Note the change of mobility of the labeled species in the presence of both mtRNAP and PolG ("super shift").
F Elution profile of PolG-OriL-mtRNAP, PolG and mtRNAP during size-exclusion chromatography on Superdex 200 column.
G Coomassie stained SDS–PAGE showing the composition of fractions obtained in size-exclusion experiment with the primosome complex. Molecular weight markers Mark12 (Invitrogen) are show in lane M.

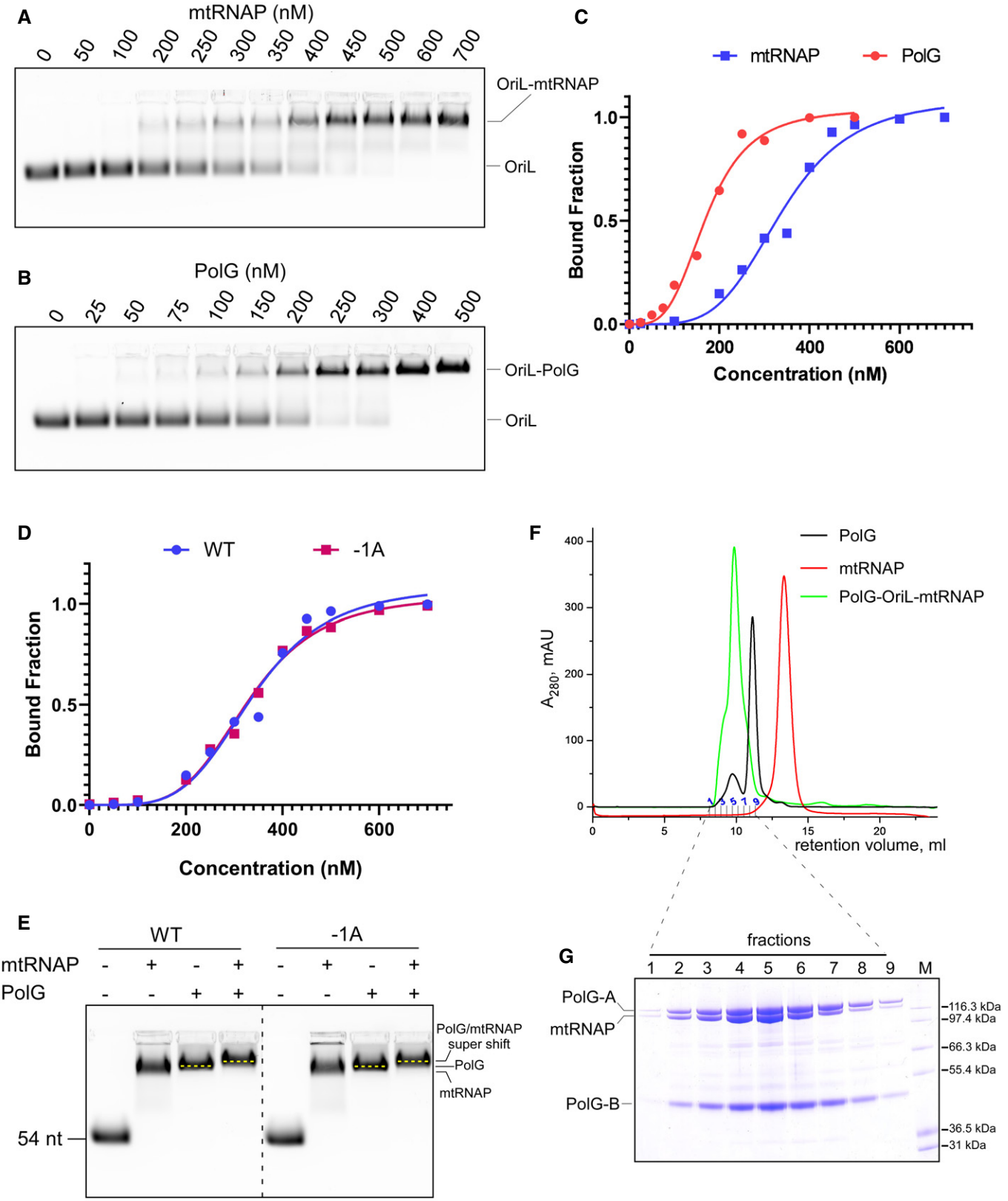
A
mtRNAP (nM)
0 50 100 200 250 300 350 400 450 500 600 700
OriL-mtRNAP
OriL
B
PolG (nM)
0 25 50 75 100 150 200 250 300 400 500
OriL-PolG
OriL
C
mtRNAP
PolG
Bound Fraction
1.0
0.5
0.0
0 200 400 600
Concentration (nM)
D
WT
-1A
Bound Fraction
1.0
0.5
0.0
0 200 400 600
Concentration (nM)
E
WT
-1A
mtRNAP - + - + - + - +
PolG - - + + - - + +
PolG/mtRNAP super shift
PolG
mtRNAP
54 nt
F
PolG
mtRNAP
PolG-OriL-mtRNAP
A280, mAU
400
300
200
100
0
0 5 10 15 20
retention volume, ml
1 5 6 7 8
G
fractions
1 2 3 4 5 6 7 8 9 M
PolG-A
mtRNAP
PolG-B
116.3 kDa
97.4 kDa
66.3 kDa
55.4 kDa
36.5 kDa
31 kDa

Figure EV3.

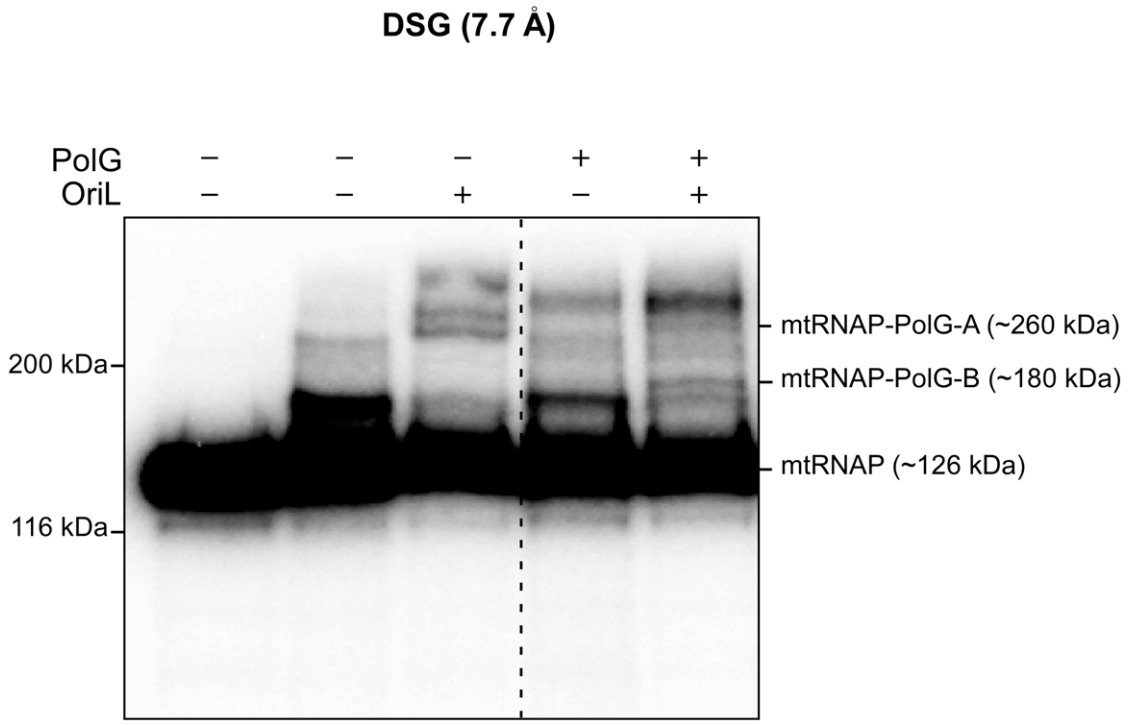

Figure EV4. Proximity of mtRNAP and PolG in the primosome revealed by DSG cross-linking.

Cross-linking was performed using ^{32}P-labeld mtRNAP in the presence or absence of PolG and OriL, as indicated. The leftmost lane represents a control reaction, in which the DSG reagent has not been added. The products of the reaction were resolved using 8% Tris-glycine SDS–PAGE. The positions of the protein markers are indicated to the left of the gel. The positions of the adducts representing cross-link between mtRNAP and PolG are indicated to the right.

A

Pos. in mtDNA	stem, bp	loop, nt	-ΔG, kcal
●1334-1368	9	13	10.3
1570-1600	9	4	8.2
2096-2125	10	6	11
2684-2705	6	8	7.6
2808-2842	7	17	6.7
3418-3450	11	4	9.4
3655-3700	12	18	6
3890-3920	8	7	10.7
4109-4137	6	10	5.4
4357-4377	6	5	5.4
5208-5229	6	6	6.1
●5723-5760	11	12	14.8
●5970-6000	6	12	6.1
6228-6280	10	21	7.7
7602-7625	9	5	5.9
8000-8040	8	6	5.5
8180-8200	6	6	7.3
8521-8560	10	5	5.9
8841-8890	9	21	5
9291-9350	10	29	6
11171-11200	5	4	5.5
11361-11394	7	4	5.6

B

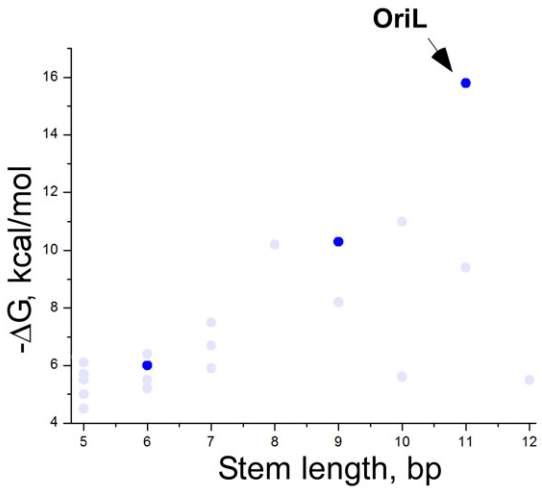

Figure EV5. OriL is the only stem loop that can drive efficient primer generation by mtRNAP.

A Putative stem loops found in the light strand of human mtDNA. The size of the stem and the loop of the hairpins, and the stability of the hairpins (ΔG) are indicated. Dark blue dots indicate stem loops that satisfy the topological requirements to serve as a potential template for transcription.

B Stability of the stem loops found in human mtDNA as a function of the stem length. Stability of stem loops (−ΔG) found in human mtDNA as a function of the stem length. Light blue dots—stem loops that have a "disallowed" number of nucleotides in the loop (< 10 or more than 16). Note that neither of these stem loops, except OriL, encodes a stretch of the dTMP residues required for efficient transcription and primer generation.