Supplementary Information I

Programmable autonomous synthesis of single-stranded DNA

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1 Kinetic characterization of one-step PER

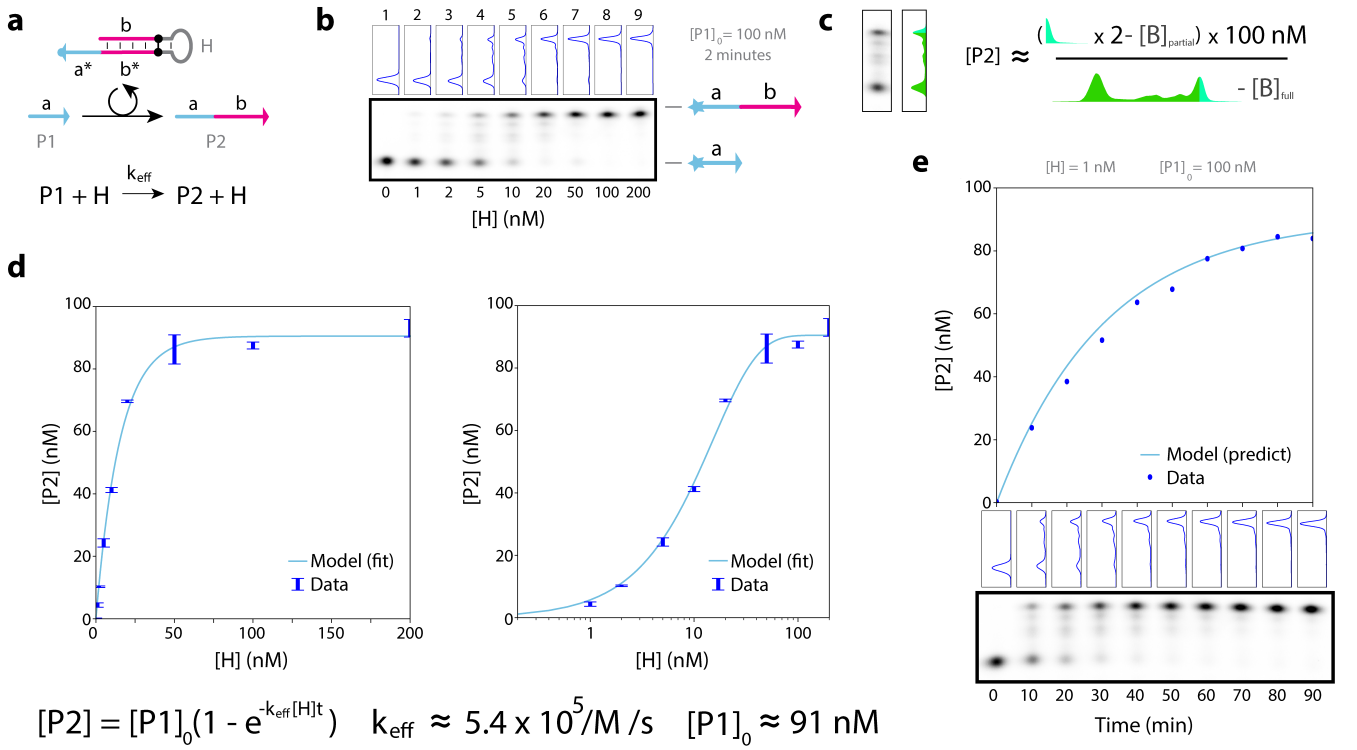

Figure 1: **Kinetics of one-step PER. (a)** In a single primer exchange reaction, a hairpin (H) acts catalytically to append a new domain **b** onto an initial primer sequence **a** (P_1) to create a transcript of the form **a-b** (P_2). **(b)** A PAGE denaturing gel shows extension products for different hairpin concentrations with primer fixed at 100 nM. Reactions were run for two minutes and halted with a mixture of formamide and EDTA before heat inactivation of the enzyme. Gel lane plots are depicted above the gel. See Supplementary Section 10 for more details. **(c)** The amount of extension product (P_2) is estimated by doubling the area under the pixel intensity curve from the peak corresponding to the extended product to the edge of the curve, subtracting background calculated ($[B]_{partial}$) from an empty area of gel of the same size, and then dividing by the total area under the curve minus the background calculated from an empty area of gel of the same size ($[B]_{full}$). **(d)** Extended product amounts were estimated for each hairpin concentration in each of three replicates of the experiment described in Fig. 1b. Blue error bars corresponding to the standard deviation are shown projected around the mean value of the data at each hairpin concentration. A fitted curve corresponding to the equation and constants below the plot is shown in cyan. Both plots show the same data, with the hairpin axis on a log scale in the plot to the right. **(e)** The model with fitted parameters was then used to predict the time-dependent behavior of the time series reaction shown originally in Fig. 2d. The model and quantification data are shown in cyan and blue, respectively. Gel data is shown with lane plots depicted directly above.

We found that for our typical reaction condition, a single primer exchange reaction is well approximated by a simple bimolecular reaction model (Fig. 1a):

$$P_1 + H \xrightarrow{k_{eff}} P_2 + H$$

Where P_1 represents the initial, unextended primer species, P_2 represents the extended primer species, and H represents the catalytic hairpin species. We devised a framework for fitting the effective rate parameter k_{eff} by varying the hairpin concentration of a 2 minute PER reaction at 37 °C with the primer concentration fixed at 100 nM (Fig. 1b). Because the hairpin acts catalytically, the rate of reaction completion (converting unextended primer P_1 to its extended form P_2) is expected to increase with hairpin concentration. In the case of the simple model presented above, where

the hairpin concentration remains unvaried throughout the experiment, that relationship is expected to be linear:

$$d[P_1]/dt = -k_{eff}[H][P_1]$$

Solving this differential equation for the concentration of P_1 gives an exponential decay:

$$[P_1] = [P_1]_0 e^{-k_{eff}[H]t}$$

Assuming each primer is in one of the two states (P_1 or P_2), the equation for the concentration of P_2 would be:

$$[P_2] = [P_1]_0(1 - e^{-k_{eff}[H]t})$$

The quantity of primers in state P_2 can be estimated by dividing the integrated pixel densities of Cy5-labeled strands in the extended band by the total integrated pixel intensities in the lane. Our quantification was done by assuming the peaks of the pixel intensity values along the vertical axis of a rectangular region surrounding each lane were symmetric, so that the amount of primer in the extended primer band could be estimated by multiplying the area to the right of that band's peak location by two (minus estimated background), dividing by the total area under the pixel intensity curve (minus estimated background), and then normalizing to 100 nM total primer concentration (Fig. 1c). Raw lane data and schematics for how background was estimated can be seen in Fig. 27

Three replicates of the experiment were performed on three separate days with all dilutions prepared fresh from stocks. The average values of the data from the quantification of these experiments with error bars computed as the standard deviation are depicted in blue in Fig. 1d. It appears that some percentage of the primers never complete extension to reach the final extension band as computed by our quantification strategy. By eye, this is not very obvious, but turning up the contrast significantly on the gel indicates that primers are spread along the lengths between the 10nt (unextended) and 20nt (extended) bands. As such, two variables from the expression for the concentration of P_2 presented above were fit by the non-linear least squares curve_fit function in the Python SciPy optimize package: k_{eff} and an effective $[P_1]_0$. The function fit the values 5.4×10^5 $\mathrm{M}^{-1}\mathrm{s}^{-1}$ and $91nM$ for k_{eff} and $[P_1]_0$, respectively.

The fitted model was then used to predict the data for extension products over the 90 minute time series originally presented in Fig. 2d (Fig. 1.). The model prediction is shown in cyan, and the actual data are shown in blue.

This model has several limitations. For one, it ignores the possibility of the effective hairpin concentration being reduced if the hairpins' primer binding sites are saturated by primers in solution. Instead, it assumes that all hairpins are accessible at any given time (high k_{off} rates), which relies on both the transient nature of the primer-hairpin interactions and a low overall primer concentration. A more extended model of the form below could better account for the thermodynamics of the primer binding:

$$P_1 + H \underset{k_{off,1}}{\overset{k_{on}}{\rightleftharpoons}} P_1 \cdot H \xrightarrow{k_{pol}} P_2 \cdot H \underset{k_{on}}{\overset{k_{off,2}}{\rightleftharpoons}} P_2 + H$$

In this extended model, it is clear that an excessive primer concentration or low k_{off} would affect the amount of exposed hairpin species (H). A further complication to even this extended model is the primer concentration-dependent effect of the polymerase. We estimate that the 800 units / ml of Bst Large Fragment Polymerase corresponds to a concentration of around 46 nM,[1,2] which is not that different than our typical primer concentration of 100 nM. Therefore, changes in primer concentrations and particularly increases would be expected to affect the k_{pol} term, especially as the polymerases become saturated. It is also clear that all primers are not all in the P_1 and P_2 states, but rather that it may take several extension and dissociation events for a primer to reach the final extended state. This is likely due to the limited processivity of the polymerase, which doesn't necessarily copy the complete extension domain in one go. This is particularly evident early on in the time series, where primers are spread along different extended states and only resolve into the final extended band as time progresses. A more comprehensive polymerase processivity model would be necessary to model this behavior. Accordingly, the rates for the reactions would be expected to change outside of our specific primer, polymerase, dNTP, and buffer conditions (100 nM primer, 800 units / ml Bst Large Fragment Polymerase, 10µM each of dATP, dCTP, and dTTP, 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 12 mM $MgSO_4$, and 0.1% Triton®X-100) under the range of hairpin concentrations (≈1 nM - 200 nM) we presented.

2 PER hairpin modifications: stop sequences and 3' protection

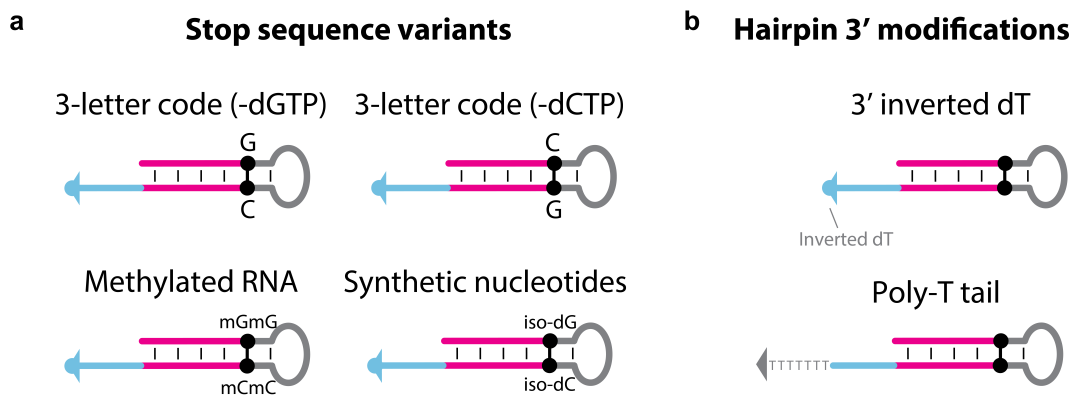

Figure 2: **PER hairpin modifications.** Two features are required for PER hairpins to operate - a stop sequence and a 3' modification that prevents extension by a polymerase. **(a)** Three stopper strategies were used in the work - 3-letter codes (-dGTP and -dCTP), methylated RNA bases, and iso-dG/iso-dC pairs. **(b)** Two strategies for inhibiting hairpin extension were used - an inverted dT modification on the 3' end and a poly-T tail consisting of 7 T's on the 3' end.

PER hairpins require two modifications to facilitate the catalytic elongation of ssDNA oligos: a stop sequence that prevents the polymerase from copying past the appended domain and a modification on the 3' end that prevents their extension.

Several strategies can be used to implement the requisite halting behavior of the polymerase. The work we present relies on three different strategies: a three-letter code, a three-letter code with methylated RNA stoppers, and a four-letter code with an iso-dG-iso-dC base pair (Fig. 2a). Some previous works have also encoded stopping points for polymerases, such as for PCR primers to create single-stranded extensions on the 5' ends of amplicons[3] and Scorpions primers that create sequence-specific fluorescence of PCR-amplified products.[4] Other enzyme-driven systems,[5,6] including our lab's previous work,[7] have typically used three-letter codes, unnatural bases, or polyethylene glycol spacers (such as Sp9 and Sp18) to stop polymerases.

To prevent extension of the PER hairpins, two strategies were utilized - an inverted dT or a polyT tail on the 3' end of the hairpin sequence (Fig. 2b). The inverted dT is particularly effective at blocking spurious extension of the hairpin strands, but the polyT tail was a more cost-effective option for the PER hairpins used to synthesize the origami staple strands.

3 Sequence dependence of first copied base

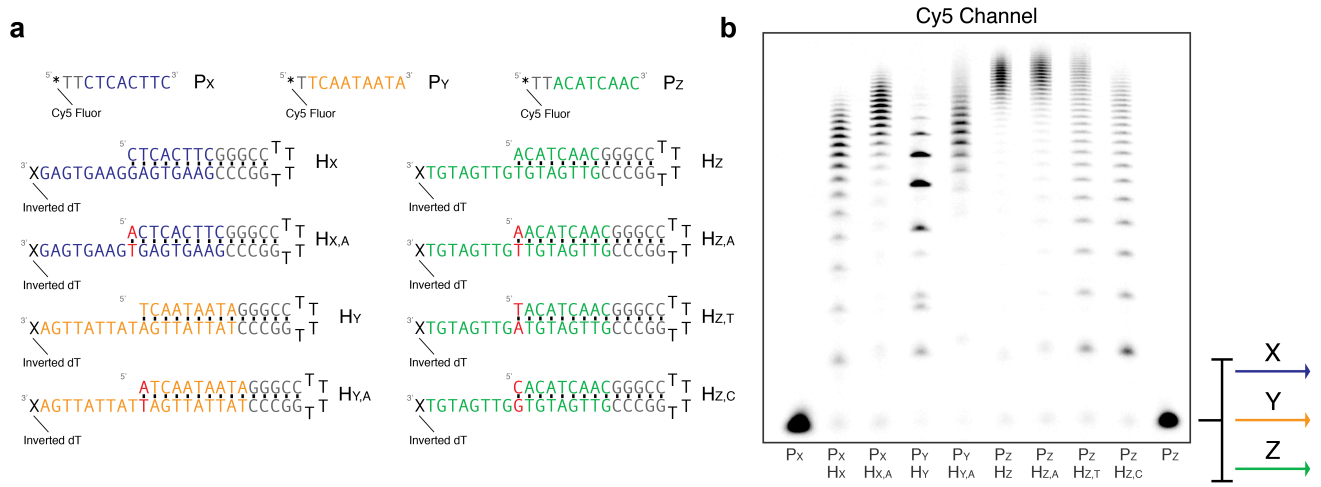

Figure 3: **A copy strategy.** After initial success with several sequences, we developed the design heuristic of always having an A base as the first base copied on a hairpin. **(a)** Three primers and several variations of hairpins were tested, and laddering efficiency was considered with respect to the first base copied on the hairpin stem. **(b)** Reactions were incubated for 2 hours with 10 nM hairpin and 100 µM dATP, dTTP, and dCTP. Pre-incubation strategy and all other methods remain the same as those for Fig. 5. For primers X and Y, which started with C and T respectively, adding an additional A base between the copied primer sequences improved the laddering. Primer Z, which originally started with an A base, continues to perform well when an additional A nucleotide is added between primer regions. However, adding a T or C base between Z domains results in a wider distribution of lengths.

While testing primer and hairpin sequences, we found that many of the telomerase systems were more efficient and ran with tighter band distributions when the first base copied on the hairpin stem was an A (Fig. 3). We adopted this principle for all other experiments and suspect it is due to the misincorporation of an A nucleotide at the stop sequence junction for the three-letter code systems.

4 DNA origami design

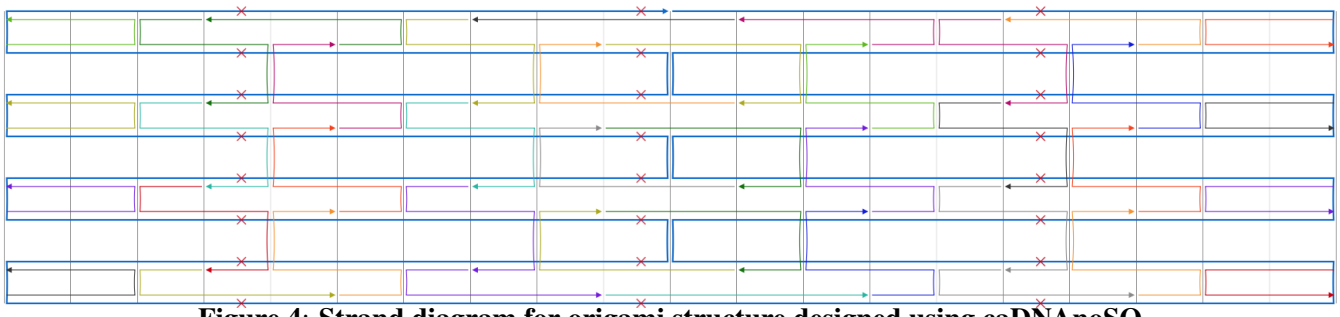

Figure 4: **Strand diagram for origami structure designed using caDNAnoSQ.**

A 1394 base pair scaffold sequence comprised of only A's, T's, and G's was used for the origami structures shown in Fig. 3d. The three letter code is helpful in both reducing undesired secondary structure folding of the scaffold and staple strands and allowing G-C pairs to serve as stop sequences for three-letter PER synthesis reactions. However, a three-letter code scaffold also has the disadvantage of reducing the diversity of sequence space compared to a 4-letter code. So, we designed it to avoid long sequence repeats - no subsequence of size 8 or longer is repeated in the scaffold. The scaffold sequence is listed in Supplementary Section 9.

The DNA origami structure was designed using the caDNAnoSQ software[8] (http://cadnano.org/legacy.html) (Fig. 4). The first 1256 bases of the scaffold is routed through a rectangular structure, expected to be approximately 23nm by 52nm, with a seam through the middle of the structure.

40 staples were added to hold the structure together (Table 2). A two step PER reaction was designed to synthesize each staple from a short primer, and the sequences for the oligos used can be found in Table 3. The general strategy used to split the staple strand into three segments was as follows:

1. The first 10 bases of the staple sequence were chosen as the primer.
2. An A base close to the middle of the remaining staple sequence was located, and the first hairpin was designed to append the region between the end of the primer sequence and this A base onto the cognate primer. The binding site was chosen to be a length that was appropriate for transient binding.
3. The second hairpin was designed to concatenate the region starting at the discovered A base and continuing to the end of the staple sequence onto the sequence copied by the first hairpin for that staple. As before, a binding site was chosen to be an appropriate length for PER binding and dissociation.

Full experimental details, larger field AFM images of the aggregated origami structures, and gels can be found in Supplementary Sections 10 and 11. Although we believe the structures without edge staples form based on the agarose gel results, the individual origami structures (which do not aggregate) proved to be extremely difficult to image successfully on the AFM. Therefore, we show only images of the aggregated structures (with edge staples).

5 Examples of molecular primitives

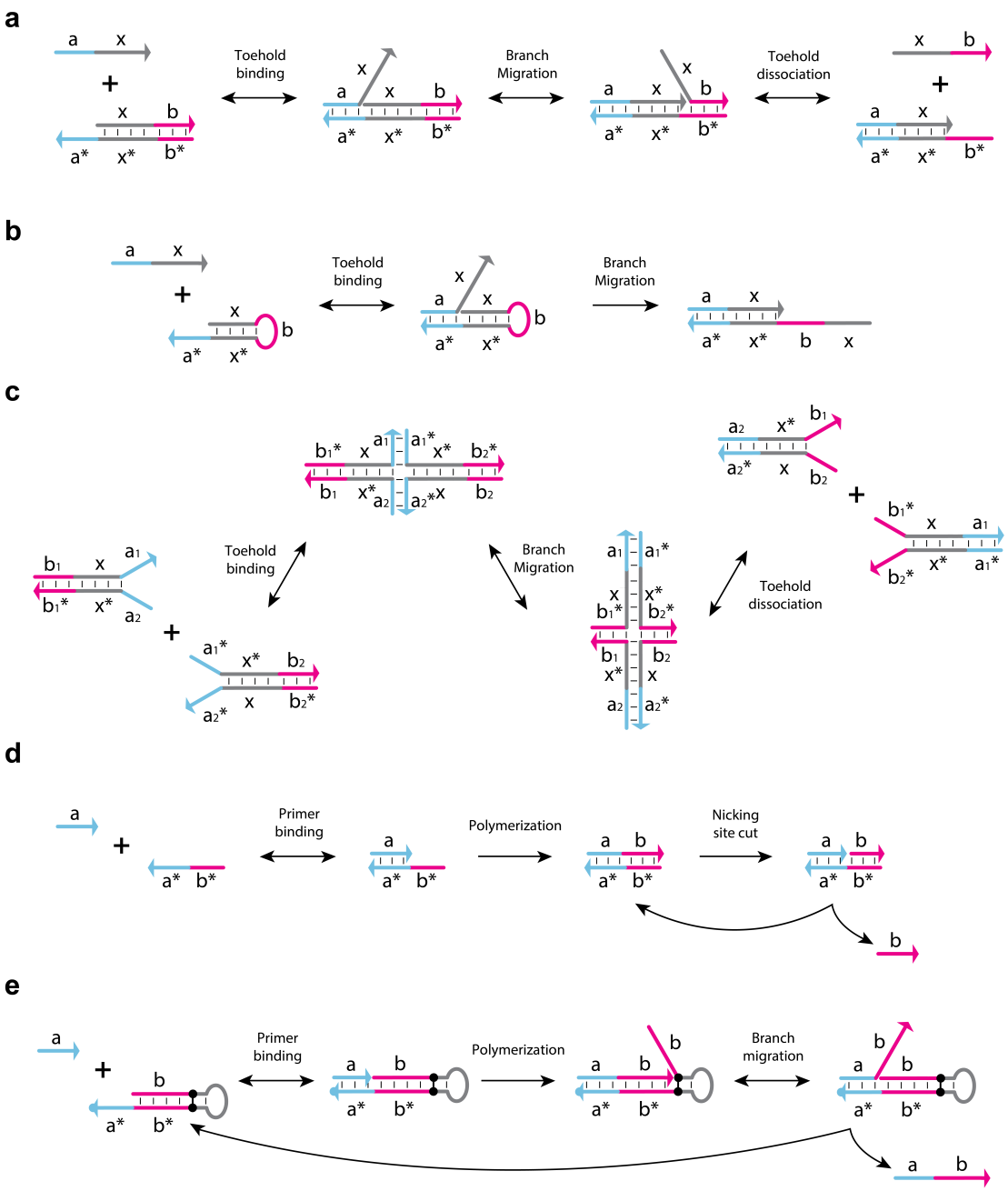

Figure 5: **Examples of molecular primitives.** **(a)** Toehold exchange.[9] **(b)** Hybridization chain reaction (HCR).[10] **(c)** Strand exchange (double-stranded toehold exchange).[11–13] **(d)** Polymerase/exonuclease/nickase (PEN) DNA toolbox reactions.[14] **(e)** Primer exchange.

Several examples of molecular primitives, which use specific reaction motifs that can be cascaded together to implement molecular behaviors, are shown in Fig. 5.

6 dGTP cleanup strategy

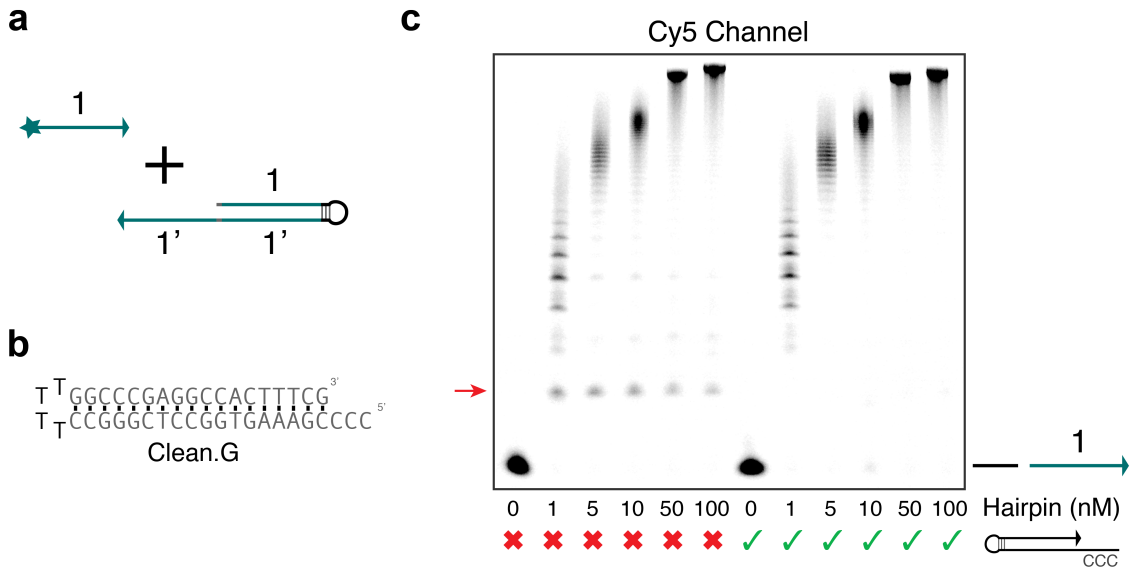

Figure 6: **dGTP cleanup strategy.** A special hairpin oligo was pre-incubated with the synthetic "telomerase" experiment (Fig. 5b) before primer introduction to improve conversion efficiencies. **(a)** The telomerase system consists of a primer and a hairpin. **(b)** The cleanup hairpin, Clean.G, has a template of C's upon which its 3' end can extend. **(c)** Results using pre-incubation with the Clean.G hairpin show fewer primers left behind than experiments without the hairpin.

A special hairpin was used to clean up extra dGTPs in solution before the telomerization primer was introduced for the experiment in Fig. 5b. This pre-incubation took place for 15 minutes at 37 °C in the presence of all reagents except the primer. Pre-incubation volume was 18 µL, and 2 µL 1 µM primer was introduced for the 4 hour incubation of 20 µL reaction. The gel shown is a wider view of that shown in Fig. 5b.

As the gel above shows, without the pre-incubation cleanup, a noticeable amount of the primer appears to get stuck after the first extension band. We hypothesized that this might be due to impurity in the dNTP solutions, specifically a small amount of dGTP contamination in the dATP, dTTP, and dCTP solutions. Because we use a G-C pair as the stop sequence within each hairpin, having dGTP in solution would allow the polymerase to pair a base with the C on a template, extending the primer sequences past where they are meant to go. Having an extra G base on the end of the strand would render the primers very unlikely to undergo subsequent telomerization steps. Introducing the Clean.G hairpin appears to eliminate this band and promote further telomerization.

7 Magnesium dependence of telomerization

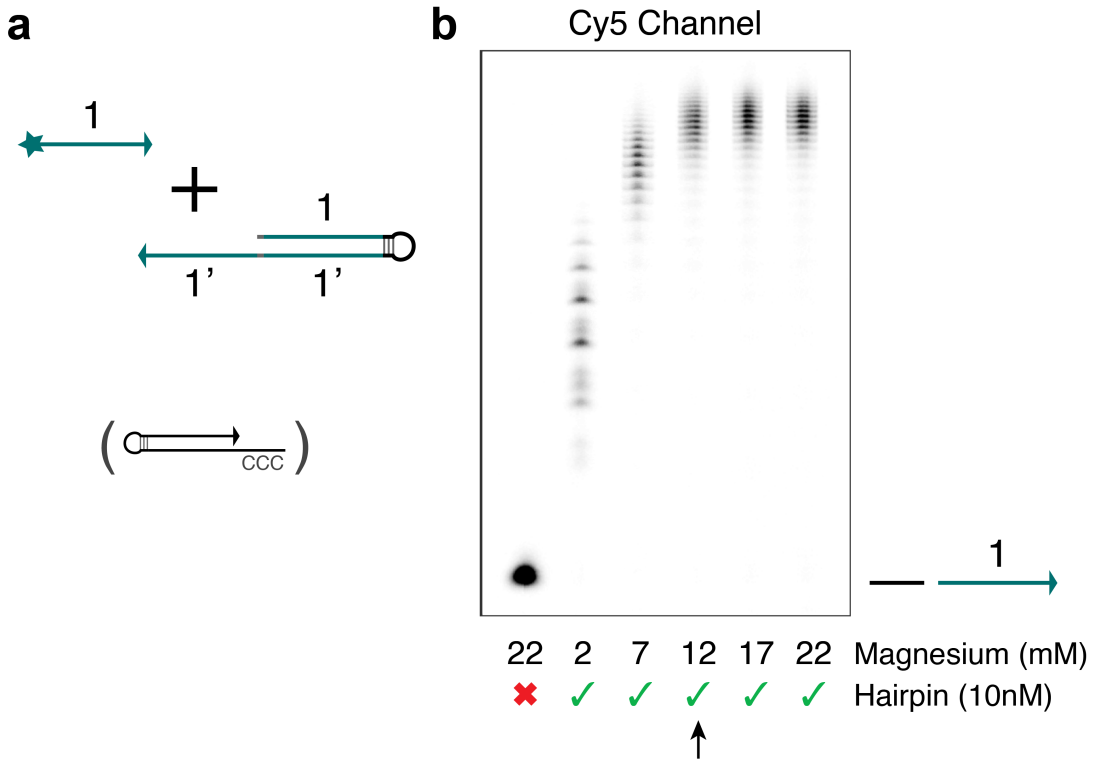

Figure 7: **Magnesium dependence of telomerization.** Supplementing magnesium dramatically increased the rate of telomerization. **(a)** The telomerase system consists of a primer, a catalytic hairpin, and the dGTP cleanup species described previously (see Fig. 6). **(b)** The default polymerase buffer contains 2 mM magnesium cations. Supplementing with increments of 5 mM shows increasing telomerization rates. For all other experiments, 12 mM of magnesium was included in the system. Reactions were incubated for 2 hours with 10 nM hairpin and 100 µM dATP, dTTP, and dCTP. Pre-incubation strategy and all other methods remain the same as those for Fig. 5b.

We found that supplementing magnesium increased the rate of telomerization (Fig. 7). Although the PER reaction operates at physiological salt conditions, increasing the magnesium cations can dramatically increase the reaction rate.

8 Hairpin protection for signal detection

Figure 8: **Hairpin protection strategy.** PER hairpins are incubated with an excess of protector strands to block their primer binding sites. **(a)** A toehold exchange reaction[9] is used to conditionally expose the primer binding site of a PER hairpin when a target RNA is present. **(b)** The protector strand sequence typically extends one base past the primer domain, and this 'clamp' sequence is typically a 'C' or 'A'.

For many of the PER hairpins, a protector strand was designed to block the primer binding site of a PER hairpin in the absence of a cognate RNA signal. When present, the RNA target can bind an exposed single-stranded portion on the protector strand, and a toehold exchange reaction[9] exposes the primer binding site (Fig. 8a). All protector strands terminated in an inverted dT on the 3' end to prevent their extension, and we designed them and the hairpins' primer binding sites to contain a one base overhang past the region where the primer binds. We refer to this extra region as a 'clamp' sequence, typically a 'C' or 'A' base, and its purpose was to reduce leakage in the system.

9 Sequences of oligos

9.1 Sequences for all figures (minus origami).

	Species	Figure(s)	Type	Sequence	Purification
1	Primer	2d, 3c, 5b, 1bde, 6c, 7b	DNA	/5Cy5/TTCTCTTATT	HPLC
2	Hairpin	2d, 1bde	DNA	ACTAAATTCAGGGCCTTTTGGCCCTGAATTTAGTAATAAGAGA/3InvdT/	STD
3	Hairpin A	3c	DNA	ACTAAATTCAGGGCCTTTTGGCCCTGAATTTAGTAATAAGAGA/3InvdT/	MinElute
4	Hairpin B	3c	DNA	ATATCCCATAGGGCCTTTTGGCCCTATGGGATATTGAATTTAG/3InvdT/	MinElute
5	Hairpin C	3c	DNA	ATTACACTACGGGCCTTTTGGCCCGTAGTGTAATTATGGGATA/3InvdT/	MinElute
6	Hairpin D	3c	DNA	ATATTAAACCGGGCCTTTTGGCCCGGTTTAATATGTAGTGTAA/3InvdT/	MinElute
7	Hairpin E	3c	DNA	ATCATTTTTCGGGCCTTTTGGCCCGAAAAATGATGGTTTAATA/3InvdT/	MinElute
8	Hairpin	5b, 6c, 7b	DNA	ATCTCTTATTGGGCCTTTTGGCCCAATAAGAGATAATAAGAGA/3InvdT/	HPLC
9	Primer 2	5b*, 6c*, 7b*	DNA	/5Cy5/TTTACACTAC	HPLC
10	Cleaner	5b, 6c, 7b, 3b	DNA	CCCCGAAAGTGGCCTCGGGCCTTTTGGCCCGAGGCCACTTTCG	STD
11	Primer	5e, **??**cd	DNA	TTTGGAGATG	STD
12	Hairpin A	5e, **??**cd	DNA	TTAGGGCCCGGTTTTCCGGGCCCTAACCATCTCCTGTGCAAATC/3InvdT/	STD
13	Protector A	5e, **??**cd	DNA	TCAGTTTTGCATAGATTTGCACAGGAGATGG/3InvdT/	STD
14	Hairpin B	5e, **??**cd	DNA	TTAGGGCCCGGTTTTCCGGGCCCTAACCCTAACC/3InvdT/	HPLC
15	miR-19a	5e, 4e, **??**cd	RNA	/5Cy5/UGUGCAAAUCUAUGCAAAACUGA	RNASE
16	TWT	5e*, 4e	RNA	/56-FAM/UCCAUGUCCGCGUCCCACUAGCAG	RNASE
17	Primer	7c, 6bce	DNA	/5Cy5/TTTTACACTA	HPLC
18	Hairpin A (miR-19a)	7c, 6bc	DNA	ATTATTAAACmGmGGCCTTTTGGCmCmCGTTTAATAATGTAGTGTAATGTGCAAATC/3InvdT/	HPLC
19	Hairpin B (TWT)	7c	DNA	ATCTCTTATTmGmGGCCTTTTGGCmCmCAATAAGAGATGGTTTAATATCCATGTCCGC/3InvdT/	HPLC
20	Hairpin C (TWT)	7c	DNA	ACTTATTCATTTTTmGmGGCCTTTTGGCmCmCAAAAATGAATAAGTGTAGTGTAATCCATGTCCGC/3InvdT/	HPLC
21	Hairpin D (miR-19a)	7c	DNA	ACTTACTAAATTCAmGmGGCCTTTTGGCmCmCTGAATTTAGTAAGTGAAAAATGATGTGCAAATC/3InvdT/	HPLC
22	Protector A (miR-19a)	7c, 6bc	DNA	TCAGTTTTGCATAGATTTGCACATTACACTAC/3InvdT/	STD
23	Protector B (TWT)	7c	DNA	CTGCTAGTGGGTCGCGGACATGGATATTAAACC/3InvdT/	STD
24	Protector C (TWT)	7c	DNA	CTGCTAGTGGGTCGCGGACATGGATTACACTAC/3InvdT/	STD
25	Protector D (mir-19a)	7c	DNA	TCAGTTTTGCATAGATTTGCACATCATTTTTC/3InvdT/	STD
26	miR-19a	7c, 6bce	RNA	UGUGCAAAUCUAUGCAAAACUGA	STD
27	TWT	7c, 6bce	RNA	UCCAUGUCCGCGUCCCACUAGCAG	STD
28	Hairpin (TWT)	6be	DNA	ATTATTAAACmGmGGCCTTTTGGCmCmCGTTTAATAATTTAGTGTAATCCATGTCCGC/3InvdT/	HPLC
29	Protector (TWT)	6be	DNA	CTGCTAGTGGGTCGCGGACATGGATTACACTAA/3InvdT/	STD
30	Hairpin (TWT)	6c	DNA	ATCTCTTATTmGmGGCCTTTTGGCmCmCAATAAGAGATTGTTTAATATCCATGTCCGC/3InvdT/	HPLC
31	Protector (TWT)	6c	DNA	CTGCTAGTGGGTCGCGGACATGGATATTAAACA/3InvdT/	STD
32	Primer	6d	DNA	/5Cy5/TTTATTAAAC	HPLC
33	Hairpin (miR-21)	6de	DNA	ATCTCTTATTmGmGGCCTTTTGGCmCmCAATAAGAGATTGTTTAATATAGCTTATC/3InvdT/	HPLC
34	Protector (miR-21)	6de	DNA	TCAACATCAGTCTGATAAGCTATATTAAACA/3InvdT/	STD
35	Hairpin	6de	DNA	ACTTATTCATTTTTmGmGGCCTTTTGGCmCmCAAAAATGAATAAGTGTTTAATA/3InvdT/	HPLC
36	miR-21	6de	RNA	UAGCUUAUCAGACUGAUGUUGA	STD
37	Hairpin (miR-19a)	6e	DNA	ATTATTAAACmGmGGCCTTTTGGCmCmCGTTTAATAATTTAGTGTAATGTGCAAATC/3InvdT/	HPLC
38	Protector (miR-19a)	6e	DNA	TCAGTTTTGCATAGATTTGCACATTACACTAA/3InvdT/	STD
39	Hairpin A	4e	DNA	ATAGTGGGAGGCT/iisodG/CCGGGTTTTCCCGG/iMe-isodC/AGCCTCCCACTATTCAGTTTTG/3InvdT/	HPLC
40	Hairpin B	4e	DNA	AGCTACAACG/iisodG/CCGGGTTTTCCCGG/iMe-isodC/CGTTGTAGCTAGCCTCCC/3InvdT/	HPLC
41	Hairpin C	4e	DNA	AGCGGACATTT/iisodG/CCGGGTTTTCCCGG/iMe-isodC/AAATGTCCGCTCGTTGTAGC/3InvdT/	HPLC
42	DzFull	4e*	DNA	ATAGTGGGAGGCTAGCTACAACGAGCGGACAT	STD
43	P_X	3b	DNA	/5Cy5/TTCTCACTTC	HPLC
44	P_Y	3b	DNA	/5Cy5/TTCAATAATA	HPLC
45	P_Z	3b	DNA	/5Cy5/TTACATCAAC	HPLC
46	H_X	3b	DNA	CTCACTTCGGGCCTTTTGGCCCGAAGTGAGGAAGTGAG/3InvdT/	STD
47	H_X,A	3b	DNA	ACTCACTTCGGGCCTTTTGGCCCGAAGTGAGTGAAGTGAG/3InvdT/	STD
48	H_Y	3b	DNA	TCAATAATAGGGCCTTTTGGCCCTATTATTGATATTATTGA/3InvdT/	STD
49	H_Y,A	3b	DNA	ATCAATAATAGGGCCTTTTGGCCCTATTATTGATATTATTGA/3InvdT/	STD
50	H_Z	3b	DNA	ACATCAACGGGCCTTTTGGCCCGTTGATGTGTTGATGT/3InvdT/	STD
51	H_Z,A	3b	DNA	AACATCAACGGGCCTTTTGGCCCGTTGATGTTGTTGATGT/3InvdT/	STD
52	H_Z,T	3b	DNA	TACATCAACGGGCCTTTTGGCCCGTTGATGTAGTTGATGT/3InvdT/	STD
53	H_Z,C	3b	DNA	CACATCAACGGGCCTTTTGGCCCGTTGATGTGGTTGATGT/3InvdT/	STD
54	Primer 2	1bd	DNA	/5Cy5/TCTAAATTCA	HPLC

Table 1: Oligos for all experiments except those for the DNA origami structure, ordered from IDT. An asterisk (*) indicates an oligo that was present in a control for the figure experiment but not shown in the cropped gels. For experiments with multiple gated hairpins, the target that activates that hairpin is indicated in parentheses.

Oligos for all experiments except those for the DNA origami structure are listed in Table 1. All oligos were ordered from IDT, and they were unpurified (STD), HPLC-purified (HPLC), or RNase-free HPLC-purified (RNASE).Oligos listed as MinElute purified were purified in-house by running 100 µL of 10 µM unpurified oligo through a Qiagen MinElute PCR purification column and washed per kit instructions. Column-bound oligos were eluted to 15 µL and concentrations were measured using a Nanodrop and their extinction coefficients.

Oligos were ordered pre-suspended in 1 x TE buffer at 100 µM, and these concentrations were assumed for all dilutions. DNA oligos were diluted in 1 x TE to working concentrations of 10uM, with stock and working solutions stored at -20 °C. The RNA oligos were suspended in lab in 1 x TE buffer and stored at -80 °C.

Schematics depicting oligo sequences 1 through 42 aligned according to their designed secondary structures and colored based on the figure domain designs can be seen in Fig. 9 through Fig. 11.

9.2 Sequence schematics (oligos 1-14)

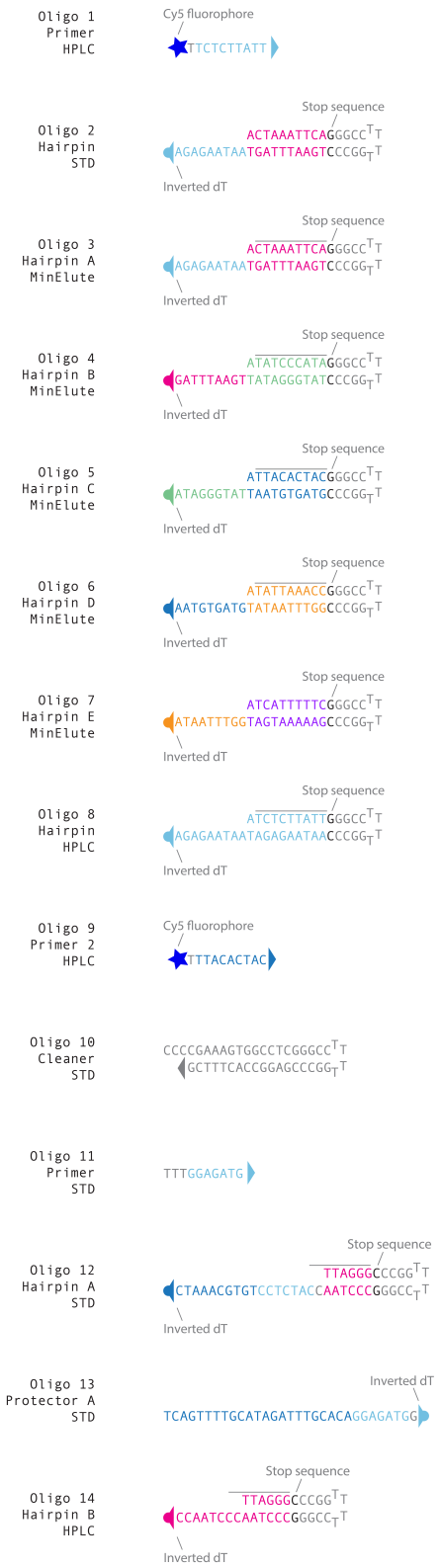

Figure 9: **Sequence schematics for oligos 1 through 14.** Primer sequences within the hairpin copy regions that cascade into subsequent primer exchange reactions are annotated with a bar above the sequence.

9.3 Sequence schematics (oligos 15-28)

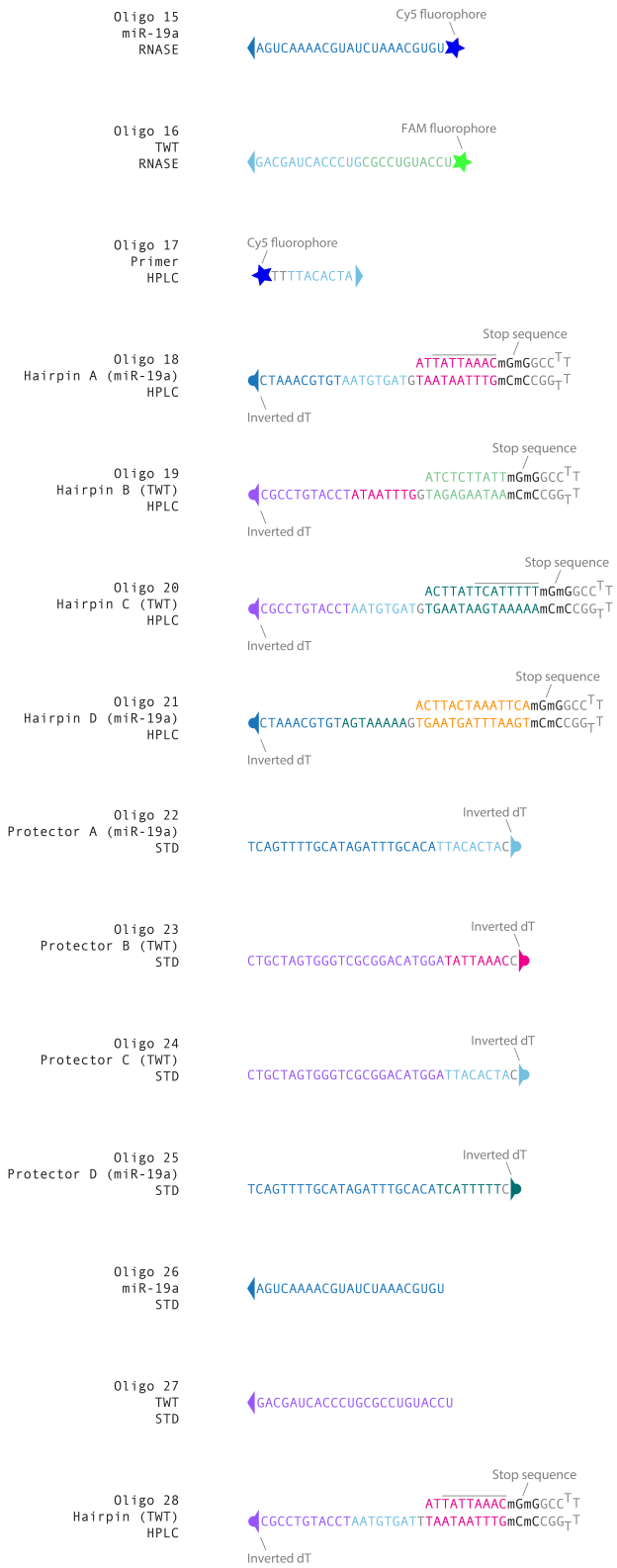

Figure 10: **Sequence schematics for oligos 15 through 28.** Primer sequences within the hairpin copy regions that cascade into subsequent primer exchange reactions are annotated with a bar above the sequence.

9.4 Sequence schematics (oligos 29-42)

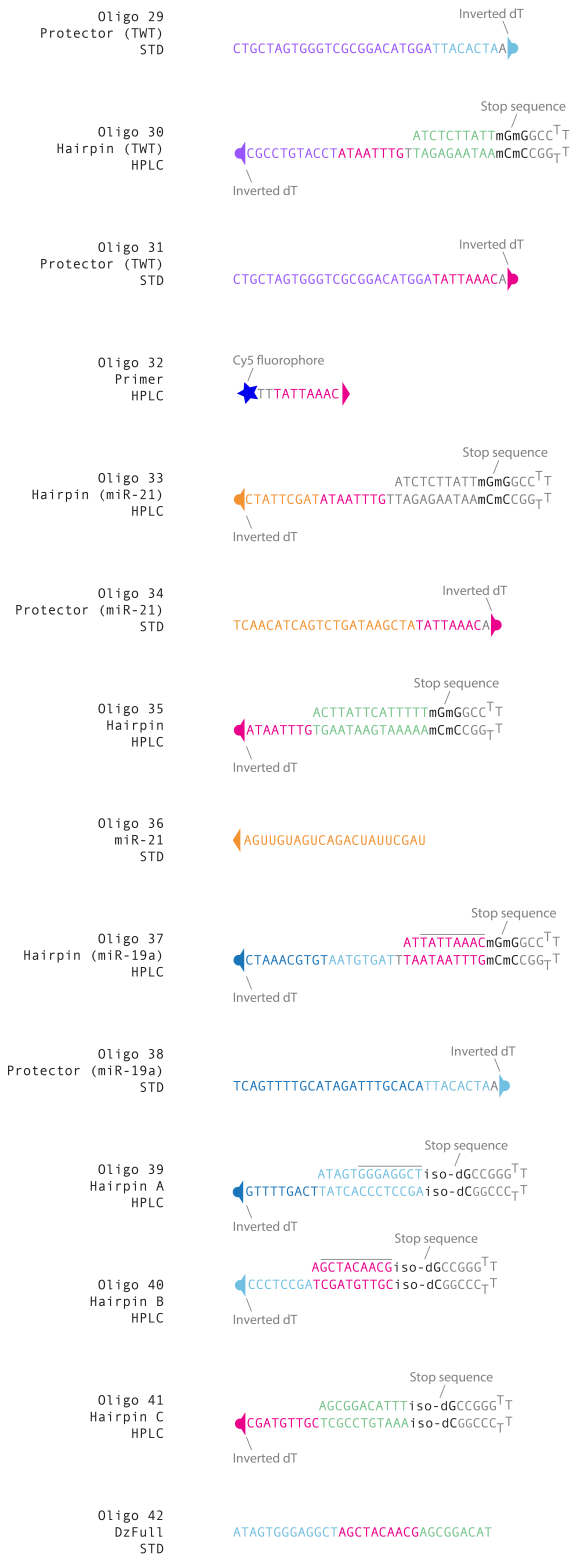

Figure 11: **Sequence schematics for oligos 29 through 42.** Primer sequences within the hairpin copy regions that cascade into subsequent primer exchange reactions are annotated with a bar above the sequence.

9.5 Sequences for origami structure.

The scaffold sequence used for the origami is listed below. It was ordered as a gBlock from IDT and PCR amplified. The primer for the complementary strand was phosphorylated, so it could be digested post-PCR by lambda exonuclease to leave behind the single-stranded scaffold.

TGTTAGTAGTTGGATGTGTTTGGTGATAGGTATTAAAGTAAGGTATGGAGGAGATTTTGGTGGAATAAGTAGTAA
GAAATGATTAGGTTTGAATGAGAGAATTGAAGAGGATGTTATAATGATAGTGAGGGATAAAATGTAATGTGAATG
GGTAGAAGATATGTATGTGGTATTGTAAAGGATTATGGAGTGGAATTAGATATGGTGTTAGAGTTTGTAATTTAGT
AAAGGTGTTTAATTGAGGATGGTGATGAGGTAAATAGAGGGATGATAGGATATATAGTGAAGAGATTTATAGTGG
AGTTGAAAAGTTTTGGAAAATTAGAGTGGTGGATAGATTAGATTAAGAGGAGGTTGGATAATGTAGGATAGGTGT
TATATGGATAGTAAATTTGATAGAGTGTGGAATGAGGTTGTTTAGTTAGTTAAAAAGATGTTAATGAGTAATGTTG
GTTGTATATAAGGAAGAAGGTTGAATGGATGAAAAGGAGGAATAGTGATGTTTATTTGATGTTGGGTATTTATGA
TTAAGTTGAAGGAGTTTAATAGAATAGGAGTTAGTGGATGGGTATGTAGTGTGGTAAGAATATTGGAGGTGAGGA
AGTTTGATATTTAGTTGATATGGAGAGTTTAGTATTAATGTGGAGTGATAGAAAATAGTTAATAGGGAGGGAGTT
AAGTAAAGATGAGTTGAGATTTGATTGTTGTGTTTATGGTAGGTGATAAGTTATGGAAATAGATTGAGAATGGAA
AGAAGTTATTAAGATAGTTATGAGGAATGGAGTTTTTGAATTGGTGTAGTGATATTAGGTAGGTATGTGTGAAGA
ATGATTTGAAGTGTTAGTGAGTAAGAGTATGGATGGTTGTGAGAGTATTTAAGTTTGGATTGTAGAAGGTGATGTG
TAATGGTAATGTATGGTGAAAGAAATTAGGGATGTTGATGATTATGAGTGAATGAAGGTGGAAATTGGAGAAGAT
GTATTGGATAGGAGAGATTAGGATGATTGAGTAGGTTATTAGATGGTTATTGTTTATAAGAAGGAGAAAATGTTA
TTTTGGATGGATTAGTAATAGGAAGATAGGTTTTTATGGGTAAGAGAAATGGATTGGTTATAGGGAATTTGTAAA
AAGTTGTAGGAGGAGTTGTTGAAGAAGAGAATGAATAATTGTAAGTGGTTATGTTATGAATATATGGGTTTGTGG
ATTAAGGGATGGTATGGTATAGAGAAAGATATAGTTTATGTTGGAATTGTGAGTATTAGGAGGTGTAGAGATTGG
GAGTAATTGAAAATGGTTGATAAAAAGGTTGTAAATAAGATAATGAAGATGGGTGAGAAGGTATTGAAATGAAT
GATGTGAAAATATGTTAGGGTGGTTGTTATAGAGTTATAAGG

	Staple
1	CACAAACCTCATTCTCTTCTTCATAACATT
2	ATCTTTCTCTTTTTACAAATTCCCAACCTATC
3	TACTAACACTCACAATTCCAACATAAACTAT
4	TACTTTAAACTATCATTATAACACCATATC
5	TTTCTTACTACTTATTCATTCAAACCTAATCA
6	AACCACTTACAATTATCATATATTCATAACAT
7	TCCTACAACTATACCATACCATCCCTTAATC
8	ATCCATTCTTCTACCCATTCACAAATACCAC
9	TATCCCTCTACCTATCACCAAACACATCCAAC
10	AATTCTCTCCACCAAAATCTCCTCCATACCT
11	TTCTCCTTAATCATCCTAATCTCACCTTCT
12	TTCCTATTTTCTCCAATTTCCACCATTTCTTT
13	ATACATATTCTCTTACCCATAAATATAACCA
14	TAATTCCAACTATATATCCTATCATTATCC
15	TAAACACCTTTACTAACATCACCATCCTCAAT
16	ATCTAATAACCTACTCCTTATAAACAATAACC
17	AATACATCACTAATCCATCCAAAAACAACTCC
18	CTCATAACAAAACTTTTCAACTCCACCACTC
19	ATCTCTTCCTCCATAATCCTTTACTTACATTT
20	ATTTACCTATTACAAACTCTAACATCCTCTTC
21	ACAATCCATACTCACTAACACTTATCTATT
22	CACCATACCATACCTACCTAATATATAACTAT
23	TAATTTTCTCATCAACATCCCTATTCATTCA
24	AACCTCCTCATTAACATCTTTTTATACCCA
25	CAAATTTACTATCCATTCATTCCACACTCTAT
26	CAACCATCCATACTCTAACTTAAATACTCTCA
27	TCTTCACAATTACCATTACACATCTCCTATCC
28	CAATTCAAACCTTCTTCCTTATATCCTTTTC
29	ACATTACTCTTAATCTAATCTATCCACTATAA
30	AAACAACCATAACACCTATCCTACATCCCTCT
31	TCCATAACTCAACTCATCTTTACTTAACTCC
32	CTTAATAATAACTATTTTCTATCACTCCACAT
33	ATCCATTCAAAACTCCATTCCTCCACTACAC
34	ACATCAAACTCACCTCCAATATTCTTACCAC
35	TATTCTATTAAACTCCCCCATCCACTAACTCC
36	ACACAACAATCAAATCTTATCACCTACCATAA
37	CTCCCTATCTTCTTTCCATTCTCACAAATCAT
38	TAATACTAAACTCTCCATATCAACTAAATAT
39	CAAACTTCTAAACATCACTATTCCTACAACCA
40	ACTACATATTCAACTTAATCATAAAACTAACT

Table 2: Synthetic staples for positive control DNA origami structures, ordered from IDT. Edge staples correspond to staples 5, 6, 15, 16, 25, 26, 35, and 36.

	Primer	First hairpin	Second hairpin
1	CACAAACCTC	ATTCTCTTCTTCGGGCCTTTTGGCCCGAAGAAGAGAATGAGGTTTGTTTTTTT	ATAACATTGGGCCTTTTGGCCCAATGTTATGAAGAAGATTTTTTT
2	ATCTTTCTCT	TTTTACAAGGGCCTTTTGGCCCTTGTAAAAAGAGAAAGTTTTTTT	ATTCCCAACCTATCGGGCCTTTTGGCCCGATAGGTTGGGAATTTGTAAAAAGTTTTTTT
3	TACTAACACT	CACAATTCCAGGGCCTTTTGGCCCTGGAATTGTGAGTGTTAGTTTTTTT	ACATAAACTATGGGCCTTTTGGCCCATAGTTTATGTTGGAATTGTTTTTTT
4	TACTTTAAAC	TATCATTATAGGGCCTTTTGGCCCTATAATGATAGTTTAAAGTTTTTTTT	ACACCATATCGGGCCTTTTGGCCCGATATGGTGTTATAATGATATTTTTTT
5	TTTCTTACTA	CTTATTCATTCGGGCCTTTTGGCCCGAATGAATAAGTAGTAAGATTTTTTT	AAACCTAATCAGGGCCTTTTGGCCCTGATTAGGTTTGAATGAATATTTTTTT
6	AACCACTTAC	AATTATCATATGGGCCTTTTGGCCCATATGATAATTGTAAGTGGTTTTTTT	ATTCATAACATGGGCCTTTTGGCCCATGTTATGAATATATGATAATTTTTTTTT
7	TCCTACAACT	ATACCATACCGGGCCTTTTGGCCCGGTATGGTATAGTTGTAGTTTTTTT	ATCCCTTAATCGGGCCTTTTGGCCCGATTAAGGGATGGTATGGTTTTTTTT
8	ATCCATTCTT	CTACCCATTCGGGCCTTTTGGCCCGAATGGGTAGAAGAATGGTTTTTTT	ACAAATACCACGGGCCTTTTGGCCCGTGGTATTTGTGAATGGGTTTTTTTT
9	TATCCCTCTA	CCTATCACCAAGGGCCTTTTGGCCCTTGGTGATAGGTAGAGGGATTTTTTT	ACACATCCAACGGGCCTTTTGGCCCGTTGGATGTGTTTGGTGATTTTTTTT
10	AATTCTCTCC	ACCAAAGGGCCTTTTGGCCCTTTGGTGGAGAGAATTTTTTT	ATCTCCTCCATACCTGGGCCTTTTGGCCCAGGTATGGAGGAGATTTTGGTGGTTTTTTT
11	TTCTCCTTAA	TCATCCTAGGGCCTTTTGGCCCTAGGATGATTAAGGAGATTTTTTT	ATCTCACCTTCTGGGCCTTTTGGCCCAGAAGGTGAGATTAGGATGATTTTTTT
12	TTCCTATTTT	CTCCAATTTCCGGGCCTTTTGGCCCGGAAATTGGAGAAAATAGGATTTTTTT	ACCATTTCTTTGGGCCTTTTGGCCCAAAGAAATGGTGGAAATTGTTTTTTT
13	ATACATATTC	TCTTACCCATGGGCCTTTTGGCCCATGGGTAAGAGAATATGTATTTTTTT	AAATATAACCAGGGCCTTTTGGCCCTGGTTATATTTATGGGTAATTTTTTT
14	TAATTCCAAC	TATATATCCTGGGCCTTTTGGCCCAGGATATATAGTTGGAATTTTTTTT	ATCATTATCCGGGCCTTTTGGCCCGGATAATGATAGGATATATTTTTTTT
15	TAAACACCTT	TACTAACATCGGGCCTTTTGGCCCGATGTTAGTAAAGGTGTTTTTTTTT	ACCATCCTCAATGGGCCTTTTGGCCCATTGAGGATGGTGATGTTAGTTTTTTT
16	ATCTAATAAC	CTACTCCTTATGGGCCTTTTGGCCCATAAGGAGTAGGTTATTAGATTTTTTT	AAACAATAACCGGGCCTTTTGGCCCGGTTATTGTTTATAAGGAGTTTTTTTT
17	AATACATCAC	TAATCCATCCAGGGCCTTTTGGCCCTGGATGGATTAGTGATGTATTTTTTT	AAAACAACTCCGGGCCTTTTGGCCCGGAGTTGTTTTTGGATGGTTTTTTT
18	CTCATAACAA	AACTTTTCAGGGCCTTTTGGCCCTGAAAAGTTTTGTTATGATTTTTTT	ACTCCACCACTCGGGCCTTTTGGCCCGAGTGGTGGAGTTGAAAAGTTTTTTTT
19	ATCTCTTCCT	CCATAATCCTTTGGGCCTTTTGGCCCAAAGGATTATGGAGGAAGAGTTTTTT	ACTTACATTTGGGCCTTTTGGCCCAAATGTAAGTAAAGGATTATTTTTTT
20	ATTTACCTAT	TACAAACTCTAGGGCCTTTTGGCCCTAGAGTTTGTAATAGGTAAATTTTTTT	ACATCCTCTTCGGGCCTTTTGGCCCGAAGAGGATGTTAGAGTTTGTTTTTTT
21	ACAATCCATA	CTCACTAACGGGCCTTTTGGCCCGTTAGTGAGTATGGATTGTTTTTTT	ACTTATCTATTGGGCCTTTTGGCCCAATAGATAAGTGTTAGTGATTTTTTT
22	CACCATACCA	TACCTACCTAGGGCCTTTTGGCCCTAGGTAGGTATGGTATGGTTTTTTT	ATATATAACTATGGGCCTTTTGGCCCATAGTTATATATTAGGTAGGTTTTTTT
23	TAATTTTCTC	ATCAACATCCCTGGGCCTTTTGGCCCAGGGATGTTGATGAGAAAATTTTTTTT	ATTCATTCAGGGCCTTTTGGCCCTGAATGAATAGGGATGTTTTTTTT
24	AACCTCCTCA	TTAACATCTTTTTGGGCCTTTTGGCCCAAAAAGATGTTAATGAGGAGTTTTTTT	ATACCCAGGGCCTTTTGGCCCTGGGTATAAAAAGATGTTTTTTT
25	CAAATTTACT	ATCCATTCATTCCGGGCCTTTTGGCCCGGAATGAATGGATAGTAAATTTTTTTTTT	ACACTCTATGGGCCTTTTGGCCCATAGAGTGTGGAATGAATTTTTTT
26	CAACCATCCA	TACTCTAACTTGGGCCTTTTGGCCCAAGTTAGAGTATGGATGGTTTTTTT	AAATACTCTCAGGGCCTTTTGGCCCTGAGAGTATTTAAGTTAGAGTTTTTTT
27	TCTTCACAAT	TACCATTACACGGGCCTTTTGGCCCGTGTAATGGTAATTGTGAATTTTTTT	ATCTCCTATCCGGGCCTTTTGGCCCGGATAGGAGATGTGTAATGTTTTTTT
28	CAATTCAAAC	CTTCTTCCTTGGGCCTTTTGGCCCAAGGAAGAAGGTTTGAATTTTTTTT	ATATCCTTTTCGGGCCTTTTGGCCCGAAAAGGATATAAGGAAGATTTTTTT
29	ACATTACTCT	TAATCTAATCTGGGCCTTTTGGCCCAGATTAGATTAAGAGTAATGTTTTTTT	ATCCACTATAAGGGCCTTTTGGCCCTTATAGTGGATAGATTAGATTTTTTTT
30	AAACAACCAT	AACACCTATCCTGGGCCTTTTGGCCCAGGATAGGTGTTATGGTTGTTTTTTTT	ACATCCCTCTGGGCCTTTTGGCCCAGAGGGATGTAGGATAGGTTTTTTT
31	TCCATAACTC	AACTCATCTTTGGGCCTTTTGGCCCAAAGATGAGTTGAGTTATGGTTTTTTT	ACTTAACTCCGGGCCTTTTGGCCCGGAGTTAAGTAAAGATGATTTTTTT
32	CTTAATAATA	ACTATTTTCTGGGCCTTTTGGCCCAGAAAATAGTTATTATTAAGTTTTTTT	ATCACTCCACATGGGCCTTTTGGCCCATGTGGAGTGATAGAAAATAGTTTTTTT
33	ATCCATTCAA	AACTCCGGGCCTTTTGGCCCGGAGTTTTGAATGGTTTTTTT	ATTCCTCCACTACACGGGCCTTTTGGCCCGTGTAGTGGAGGAATGGAGTTTTTTTTTT
34	ACATCAAACT	CACCTCCAATGGGCCTTTTGGCCCATTGGAGGTGAGTTTGATTTTTTTT	ATTCTTACCACGGGCCTTTTGGCCCGTGGTAAGAATATTGGAGGTTTTTTT
35	TATTCTATTA	AACTCCCCCGGGCCTTTTGGCCCGGGGGAGTTTAATAGAATTTTTTTT	ATCCACTAACTCCGGGCCTTTTGGCCCGGAGTTAGTGGATGGGGGAGTTTTTTT
36	ACACAACAAT	CAAATCTTATCGGGCCTTTTGGCCCGATAAGATTTGATTGTTGTTTTTTTT	ACCTACCATAAGGGCCTTTTGGCCCTTATGGTAGGTGATAAGATTTTTTTTT
37	CTCCCTATCT	TCTTTCCATTCTCGGGCCTTTTGGCCCGAGAATGGAAAGAAGATAGGGTTTTTTT	ACAAATCATGGGCCTTTTGGCCCATGATTTGTGAGAATGGTTTTTTT
38	TAATACTAAA	CTCTCCATATCGGGCCTTTTGGCCCGATATGGAGAGTTTAGTATTATTTTTTT	AACTAAATATGGGCCTTTTGGCCCATATTTAGTTGATATGGATTTTTTT
39	CAAACTTCTA	AACATCACTGGGCCTTTTGGCCCAGTGATGTTTAGAAGTTTTTTTTTT	ATTCCTACAACCAGGGCCTTTTGGCCCTGGTTGTAGGAATAGTGATGTTTTTTTT
40	ACTACATATT	CAACTTAATCGGGCCTTTTGGCCCGATTAAGTTGAATATGTAGTTTTTTT	ATAAAACTAACTGGGCCTTTTGGCCCAGTTAGTTTTATGATTAAGTTTTTTTTT

Table 3: Oligos for the DNA origami structure staple strands, ordered from IDT. Edge staples correspond to staples 5, 6, 15, 16, 25, 26, 35, and 36.

Oligos used for the staples in the origami structures are listed in Tables 2 and 3. Each staple strand is associated with a synthetic staple sequence, a primer sequence, and two hairpin sequences. All oligos were ordered unpurified and unmodified in 96 well plates from IDT, pre-suspended in 1 x TE buffer at 100 µM. As before these concentrations were assumed for all dilutions, and stocks of 1 µM of synthetic staples, primers, first hairpins, and second hairpins were mixed and stored at -20 °C with the plate.

10 Full experimental details

Experimental and gel setups are described below, based on the oligo identifying numbers in Table 1 (except for the origami structure experiments). Gel lanes are listed as 1 through 15, left to right on each scan.

10.1 Time series for single primer exchange reaction (Fig. 2d)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL 10 nM hairpin if present, and water to 20 µL. Reactions were set up at 4 °C, incubated at 37 °C for the listed amount of time, and quenched through mixing with 10 µL 100% formamide and 2 µL 0.5 M EDTA and incubation at 80 °C for at least 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea), with 5 µL of the quenched reactions loaded into the lanes. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. 10bp ladder
3. Oligo 1 (primer) for 90 minutes.
4. Oligos 1 (primer) and 2 (hairpin) for 0 minutes.
5. Oligos 1 (primer) and 2 (hairpin) for 10 minutes.
6. Oligos 1 (primer) and 2 (hairpin) for 20 minutes.
7. Oligos 1 (primer) and 2 (hairpin) for 30 minutes.
8. Oligos 1 (primer) and 2 (hairpin) for 40 minutes.
9. Oligos 1 (primer) and 2 (hairpin) for 50 minutes.
10. Oligos 1 (primer) and 2 (hairpin) for 60 minutes.
11. Oligos 1 (primer) and 2 (hairpin) for 70 minutes.
12. Oligos 1 (primer) and 2 (hairpin) for 80 minutes.
13. Oligos 1 (primer) and 2 (hairpin) for 90 minutes.
14. Oligo 2 (hairpin) for 90 minutes.
15. (empty)

10.2 5-step elongation (Fig. 3c)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of pre-mixed combinations of 100 nM of each hairpin present, and water to 20 µL. Reactions were set up at 4 °C, incubated at 37 °C for 4 hours, and heat inactivated by incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. 10bp ladder
4. (empty)
5. Oligo 1 (primer).
6. Oligos 1 (primer) and 4-7 (hairpins B-E).
7. Oligos 1 (primer) and 3 (hairpin A).
8. Oligos 1 (primer) and 3-4 (hairpins A-B).
9. Oligos 1 (primer) and 3-5 (hairpins A-C).
10. Oligos 1 (primer) and 3-6 (hairpins A-D).
11. Oligos 1 (primer) and 3-7 (hairpins A-E).
12. Oligos 3-7 (hairpins A-E).
13. (empty)
14. (empty)
15. (empty)

10.3 PER staple synthesis for origami (Fig. 3d)

The scaffold was prepared through PCR and exonuclease treatment. It was ordered as a gBlock hybridized pair from IDT, and amplified symmetrically by PCR (20 cycles, NEB M0257 Vent exo -, 95 °C x 30 s denaturing, 64 °C x 30 s anneal, and 72 °C x 1:40 min extension) with 0.2uM primers (CCTTATAACTCTATAACAACCACCCTAACATATTTTC and TGTTAGTAGTTG-GATGTGTTTGGTGATAG). The product was then washed (Qiagen QiaQuick 28104, eluted in half of the original volume) and treated with Lambda exonuclease (NEB M0262, 37 °C x 30 min, followed by 75 °C x 15 min heat inactivation) per manufacturer instructions, and used in origami construction.

PER reactions were prepared by mixing 4 µL 10 x ThermoPol reaction buffer, 4 µL 8000 units/ml Bst Large Fragment polymerase, 4 µL 100 mM $MgSO_4$, 4 µL 1 mM dATP/dTTP/dCTP solution, 4 µL 1 µM pre-mixed primer solution if present, 4 µL of 100 nM pre-mixed first hairpin solution if present, 4 µL of 100 nM pre-mixed second hairpin solution if present, and water to 40 µL. Reactions were set up at 4 °C, incubated at 37 °C for 1 hour, and heat inactivated by incubation at 80 °C for 20 minutes.

After PER synthesis, five annealing reactions corresponding to lanes 2-6 of the agarose gel below were set up at room temperature. All reactions consisted of 1.5 µL of about 89 nM scaffold (see Supplementary Section 9 for scaffold synthesis details) mixed with 30 µL solution containing 1 x ThermoPol reaction buffer and an additional 10 mM magnesium ($MgSO_4$) and one of: PER synthesized staples, synthetic staples, or no staples. For the PER synthesized staple reactions (lanes 2 and 5), 30 µL of the 40 µL reactions above was directly mixed with the appropriate amount of scaffold. For the other reactions, 30 µL of solution was prepared which contained mixed staple solutions at a final concentration of 100 nM before mixing with the scaffold. All annealing reactions were then annealed from 80 °C to 20 °C over 1 hour, with one degree change per minute. Reactions were then held at room temperature during AFM visualization and run on an agarose gel as described below.

While solutions annealed, reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 5 µL of reactions were mixed with 5 µL 100% formamide with the bromophenol blue dye, heated to 95 °C for 2 minutes, transferred directly to a cold plate, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

While annealed structures were visualized on the AFM, they were also visualized on a 50g 0.8% agarose gel (1 x TAE + 10 mM Mg++) which was pre-stained with 0.8x Sybr Gold dye. 15 µL of annealing reactions was mixed with 3 µL of 6x loading dye (50% glycerol, 1 x TE, 10 mM Mg++, bromophenol blue), and 17 µL was loaded into each lane. The gel was run in 1 x TAE + 10 mM Mg++ buffer at 80 volts for 90 minutes, while the gel box was sitting in a room temperature water bath. The gel was then imaged under the Sybr Gold channel.

AFM images were taken on a Nanoscope V machine. 2 µL of sample was deposited onto freshly cleaved mica, let sit for 2 minutes, then washed twice with 20 µL (with 1 x TE + Mg++) before visualization with tapping mode in fluid.

On a later date, a control agarose gel was run to demonstrate the effect of the PER buffer conditions (including polymerase, dNTPs, and hairpins) on the aggregation and band intensities of the agarose gel. Heat inactivated polymerase solutions were prepared by mixing 4 µL 10 x ThermoPol reaction buffer, 4 µL 8000 units/ml Bst Large Fragment polymerase, 4 µL 100 mM $MgSO_4$, 4 µL 1 mM dATP/dTTP/dCTP solution, 4 µL of 100 nM pre-mixed first hairpin solution, 4 µL of 100 nM pre-mixed second hairpin solution, and water to 36 µL. Reactions were set up at 4 °C and directly heat inactivated by incubation at 80 °C for 20 minutes.

As before, five annealing reactions corresponding to lanes 2-6 of the control agarose gel below were set up at room temperature. All reactions consisted of 1.5 µL of about 89 nM scaffold (see Supplementary Section 9 for scaffold synthesis details) mixed with 30 µL solution containing 1 x ThermoPol reaction buffer and an additional 10 mM magnesium ($MgSO_4$) and either synthetic staples or no staples. For the lanes containing heat-inactivated polymerase (lanes 2 and 5), 3 µL of 1 µM synthetic staple solution was added to 27 µL of the appropriate deactivated polymerase solution. For the other reactions, 30 µL of solution was prepared which contained mixed staple solutions (if present) at a final concentration of 100 nM before mixing with the scaffold.

Annealed reactions were again visualized on a 50g 0.8% agarose gel (1 x TAE + 10 mM Mg++) which was pre-stained with 0.8x Sybr Gold dye. 20 µL of annealing reactions was mixed with 4 µL of 6x loading dye (50% glycerol, 1 x TE, 10 mM Mg++, bromophenol blue), and 17 µL was loaded into each lane. The gel was run in 1 x TAE + 10 mM Mg++ buffer at 80 volts for 90 minutes, while the gel box was sitting in a room temperature water bath. The gel was then imaged under the Sybr Gold channel.

PAGE gel lanes:

1. (empty)
2. 10bp ladder.
3. Primers (Table 3).
4. Primers and second hairpins (Table 3).
5. Primers and first hairpins (Table 3).
6. Primers, first hairpins, and second hairpins (Table 3).
7. First and second hairpins (Table 3).
8. (empty)
9. 10bp ladder
10. Primers minus edge primers (Table 3, minus rows indicated in caption).

11. Primers minus edge primers and second hairpins minus edge hairpins (Table 3, minus rows indicated in caption).
12. Primers minus edge primers and first hairpins minus edge hairpins (Table 3, minus rows indicated in caption).
13. Primers minus edge primers and first and second hairpins minus edge hairpins (Table 3, minus rows indicated in caption).
14. First and second hairpins minus edge hairpins (Table 3, minus rows indicated in caption).
15. (empty)

Agarose gel lanes (annealed structures):

1. Invitrogen 1kB Plus Ladder
2. Scaffold mixed with full PER reaction (lane 6 from PAGE gel above). (Visualized on AFM, images in Fig. 3d, Supplementary Section 11).
3. Scaffold mixed with synthetic staples (Table 2). (Visualized as control on AFM, image in Supplementary Section 11).
4. Just scaffold.
5. Scaffold mixed with full PER reaction minus edge staples (lane 13 from PAGE gel above).
6. Scaffold mixed with synthetic staples minus edge staples (Table 2 minus rows indicated in caption).

Agarose control gel lanes (annealed structures):

1. Invitrogen 1kB Plus Ladder
2. Scaffold mixed with synthetic staples (Table 2) and heat-inactivated PER buffer solution (hairpins, dNTPs, polymerase).
3. Scaffold mixed with synthetic staples (Table 2).
4. Just scaffold.
5. Scaffold mixed with synthetic staples minus edge staples (Table 2 minus rows indicated in caption) and heat-inactivated PER buffer solution (hairpins, dNTPs, polymerase).
6. Scaffold mixed with synthetic staples minus edge staples (Table 2 minus rows indicated in caption).

10.4 Synthetic "telomerase" (Fig. 5b)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 1 mM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of hairpins with varied concentrations, 2 µL of 1 µM cleaner species if present, and water to 20 µL. Reactions were set up with all components except the primer at 4 °C and pre-incubated at 37 °C for 15 minutes before the primers were introduced. Reactions were then incubated for 4 hours and heat inactivated by incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. 10bp ladder
2. Oligo 1 (primer).
3. Oligos 1 (primer) and 8 (hairpin). Hairpin at final concentration of 1 nM.
4. Oligos 1 (primer) and 8 (hairpin). Hairpin at final concentration of 5 nM.
5. Oligos 1 (primer) and 8 (hairpin). Hairpin at final concentration of 10 nM.
6. Oligos 1 (primer) and 8 (hairpin). Hairpin at final concentration of 50 nM.
7. Oligos 1 (primer) and 8 (hairpin). Hairpin at final concentration of 100 nM.
8. Oligo 1 (primer) and 10 (cleaner).
9. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). Hairpin at final concentration of 1 nM.
10. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). Hairpin at final concentration of 5 nM.
11. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). Hairpin at final concentration of 10 nM.
12. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). Hairpin at final concentration of 50 nM.
13. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). Hairpin at final concentration of 100 nM.
14. Oligo 8 (hairpin). Hairpin at final concentration of 100 nM.
15. Oligos 9 (orthogonal primer) and 8 (hairpin). Hairpin at final concentration of 100 nM.

10.5 In situ signal amplifier (Fig. 5e)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 1 mM dATP/dTTP/dGTP solution, 2 µL 10 mM ThT solution, 2 µL 1 µM primer if present, 2 µL of 10 µM telomerase hairpin if present, 2 µL of 100 nM detector hairpin if present, 2 µL of 150 nM protector if present, 2 µL 100 nM RNA target if present, and water to 20 µL. Reactions were set up with all components except the primer at 4 °C and pre-incubated at 37 °C for 15 minutes before the primers were introduced. Reactions were then incubated for 4 hours and immediately removed for results visualization. Reactions were visualized on a 4-20% Novex native PAGE gel (1 x TBE). 10 µL of each reaction was mixed with 1.43 µL 80% glycerol, and 5 µL of this mix was loaded into each well. The gel was run with 1 x TBE buffer at 90V and room temperature for 75 minutes. The gel was then stained for several minutes with 101 µM ThT in 1 x TBE and scanned under the Cy5 and FAM channels. The gel was then stained for several minutes with 1 x Sybr Gold solution and imaged with Sybr Gold. While the gel was running, the remaining 10 µL of the reactions were visualized first under a a Safe Imager 2.0 Transilluminator, through the amber filter unit, and a picture was taken with the camera on a Samsung Galaxy S6 mobile phone (vis). Then, the same reactions were scanned under the FAM channel (FAM).

Lanes:

1. (empty)
2. (empty)
3. (empty)
4. 10bp ladder
5. Oligo 15 (RNA target).
6. Oligos 11 (primer), 14 (telomerase hairpin), and 15 (RNA target).
7. Oligos 11 (primer), 12 (detector hairpin), 13 (protector strand), and 15 (RNA target).
8. Oligos 11 (primer), 12 (detector hairpin), 13 (protector strand), 14 (telomerase hairpin), and 15 (RNA target).
9. Oligos 11 (primer), 12 (detector hairpin), 13 (protector strand), and 14 (telomerase hairpin).
10. Oligos 11 (primer), 12 (detector hairpin), 13 (protector strand), 14 (telomerase hairpin), and 16 (orthogonal RNA target).
11. Oligos 12 (detector hairpin), 13 (protector strand), and 14 (telomerase hairpin).
12. (empty)
13. (empty)
14. (empty)
15. (empty)

10.6 Event recorder (Fig. 7c)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of the graph solution if present, and water to 18 µL. Graph solution consisted of Hairpins A and C at 250 nM, protectors A and C at 500 nM, hairpins B and D at 750 nM, and protectors B and D at 1 µM. Reactions were set at 4 °C and pre-incubated at 37 °C for 15 minutes before the primer was introduced. The reactions were then incubated for 5 hours, with 1 µL of 5 µM miR-19a RNA target, 1 µL of 5 µM TWT RNA target, or 1 µL of water added at 1 hour and 3 hours into this incubation (see list below). Reactions were heat inactivated by incubation at 80 °C for 20 minutes and visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. 10bp ladder
4. Oligo 17 (primer). Water at 1 hour. Water at 3 hours.
5. Oligos 17 (primer) and 18-25 (graph). Water at 1 hour. Water at 3 hours.
6. Oligos 17 (primer) and 18-25 (graph). Oligo 26 (miR-19a) at 1 hour. Water at 3 hours.
7. Oligos 17 (primer) and 18-25 (graph). Water at 1 hour. Oligo 26 (miR-19a) at 3 hours.
8. Oligos 17 (primer) and 18-25 (graph). Oligo 27 (TWT) at 1 hour. Water at 3 hours.
9. Oligos 17 (primer) and 18-25 (graph). Water at 1 hour. Oligo 27 (TWT) at 3 hours.
10. Oligos 17 (primer) and 18-25 (graph). Oligo 26 (miR-19a) at 1 hour. Oligo 27 (TWT) at 3 hours.
11. Oligos 17 (primer) and 18-25 (graph). Oligo 27 (TWT) at 1 hour. Oligo 26 (miR-19a) at 3 hours.
12. Oligos 18-25 (graph). Water at 1 hour. Water at 3 hours.
13. (empty)
14. (empty)
15. (empty)

10.7 AND/OR logic (Fig. 6bc)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of the circuit solution if present, 2 µL of 2.5 µM miR-19a RNA target if present, 2 µL of 2.5 µM TWT RNA target if present, and water to 20 µL. OR circuit solution consisted of oligos 18 and 28 at 1 µM and oligos 22 and 29 at 2.5 µM. AND solution consisted of oligo 18 at 1 µM, oligo 30 at 2 µM, and oligos 22 and 31 at 2.5 µM. Reactions were set at 4 °C and pre-incubated at 37 °C for 15 minutes before the primer was introduced. The reactions were then incubated for 2 hours followed by heat inactivation via incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. 10bp ladder
3. Oligo 17 (primer).
4. Oligo 17 (primer) and AND solution.
5. Oligo 17 (primer), AND solution, and oligo 27 (TWT).
6. Oligo 17 (primer), AND solution, and oligo 26 (miR-19a).
7. Oligo 17 (primer), AND solution, oligo 26 (miR-19a), and oligo 27 (TWT).
8. AND solution.
9. Oligo 17 (primer) and OR solution.
10. Oligo 17 (primer), OR solution, and oligo 27 (TWT).
11. Oligo 17 (primer), OR solution, and oligo 26 (miR-19a).
12. Oligo 17 (primer), OR solution, oligo 26 (miR-19a), and oligo 27 (TWT).
13. OR solution.
14. (empty)
15. (empty)

10.8 NOT logic (Fig. 6d)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of the circuit solution if present, 2 µL of 2.5 µM miR-21 RNA target if present, and water to 20 µL. NOT circuit solution consisted of oligo 35 at 300 nM, oligo 33 at 1.5 µM, and oligo 34 at 2.5 µM. Reactions were set at 4 °C and pre-incubated at 37 °C for 15 minutes before the primer was introduced. The reactions were then incubated for 3 hours followed by heat inactivation via incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. 10bp ladder
4. (different experiment)
5. (different experiment)
6. (different experiment)
7. (different experiment)
8. (different experiment)
9. (different experiment)
10. Oligo 32 (primer).
11. Oligo 32 (primer) and NOT solution.
12. Oligo 32 (primer), NOT solution, and oligo 36 (miR-21).
13. NOT solution.
14. (empty)
15. (empty)

10.9 (X OR Y) AND NOT Z logic (Fig. 6e)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of the circuit solution if present, 2 µL of 2.5 µM miR-19a RNA target if present, 2 µL of 2.5 µM TWT RNA target if present, 2 µL of 2.5 µM miR-21 target if present, and water to 20 µL. Circuit solution consisted of oligo 35 at 400 nM, oligos 37 and 28 at 1 µM, oligo 33 at 2 µM, and oligos 38, 29, and 34 at 2.5 µM. Reactions were set at 4 °C and pre-incubated at 37 °C for 15 minutes before the primer was introduced. The reactions were then incubated for 5 hours followed by heat inactivation via incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. 10bp ladder
4. Oligo 17 (primer).
5. Oligo 17 (primer) and circuit solution.
6. Oligo 17 (primer), circuit solution, and oligo 36 (miR-21).
7. Oligo 17 (primer), circuit solution, and oligo 27 (TWT).
8. Oligo 17 (primer), circuit solution, and oligo 26 (miR-19a).
9. Oligo 17 (primer), circuit solution, oligo 27 (TWT), and oligo 36 (miR-21).
10. Oligo 17 (primer), circuit solution, oligo 26 (miR-19a), and oligo 36 (miR-21).
11. Oligo 17 (primer), circuit solution, oligo 26 (miR-19a), and oligo 27 (TWT).
12. Oligo 17 (primer), circuit solution, oligo 26 (miR-19a), oligo 27 (TWT), and oligo 36 (miR-21).
13. Circuit solution.
14. (empty)
15. (empty)

10.10 DNAzyme nanodevice (Fig. 4e)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dNTPs solution, 2 µL 100 nM miR-19a target if present, 2 µL of 200 nM Hairpin A if present, 100 nM of hairpin B if present, 2 µL of 100 nM Hairpin C if present, 2 µL of 200 nM TWT RNA target if present, 2 µL pre-synthesized DNAzyme sequence if present, and water to 20 µL. Reactions were set up at 4 °C, incubated at 37 °C for 4 hours, and quenched through mixing with 10 µL 100% formamide and 2 µL 0.5 M EDTA. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 5 µL of each quenched solution was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. (empty)
4. 10bp ladder
5. Oligo 16 (TWT).
6. Oligo 15 (miR-19a).
7. Oligos 15 (miR-19a), 40-41 (hairpins B-C), and 16 (TWT).
8. Oligos 15 (miR-19a), 39 (hairpin A), and 16 (TWT).
9. Oligos 15 (miR-19a), 39-40 (hairpins A-B), and 16 (TWT).
10. Oligos 15 (miR-19a), 39-41 (hairpins A-C), and 16 (TWT).
11. Oligos 39-41 (hairpins A-C) and 16 (TWT).
12. Oligos 42 (pre-synthesized DNAzyme) and 16 (TWT).
13. (empty)
14. (empty)
15. (empty)

10.11 Supplemental: Kinetics of one-step PER (Fig. 1bde)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 100 µM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL of varied concentrations of hairpin if present, and water to 20 µL. Reactions were set up at 4 °C, incubated at 37 °C for 2 minutes, quenched through mixing with 10 µL 100% formamide (with bromophenol blue dye) and 2 µL 0.5 M EDTA, and then incubated at 80 °C for at 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea), with 5 µL loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C (except replicate 2, which was run at room temperature) for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold. This experiment was repeated 3 times.

Note that these gels were scanned at a higher voltage under the Cy5 channel than most other gels in the paper, as it was discovered after those experiments that this was required to get the pixel values under a more linear regime in order to retrieve quantitative data from the gel bands. As such, the exact samples used for the original time series gel (Fig. 2d) were retrieved from freezer storage and re-run on a gel that was also scanned at a higher voltage in order to be quantified for Fig. 1e. The lower voltage scanning for other gels does not affect the interpretation of the paper's results, as no quantitative claims were made about those data.

Lanes:

1. (empty)
2. 10bp ladder
3. (empty)
4. Oligo 1 (primer).
5. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 1 nM.
6. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 2 nM.
7. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 5 nM.
8. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 10 nM.
9. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 20 nM.
10. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 50 nM.
11. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 100 nM.
12. Oligos 1 (primer) and 2 (hairpin). Hairpin at final concentration of 200 nM.
13. Oligos 54 (primer) and 2 (hairpin). Hairpin at final concentration of 200 nM.
14. Oligo 2 (hairpin). Hairpin at final concentration of 200 nM.
15. (empty)

10.12 Supplemental: A copy strategy (Fig. 3)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 2 µL 100 mM $MgSO_4$, 2 µL 1 mM dATP/dTTP/dCTP solution, 2 µL 1 µM primer, 2 µL 100 nM hairpin if present, 2 µL of 1 µM cleaner species, and water to 20 µL. Reactions were set up with all components except the primer at 4 °C and pre-incubated at 37 °C for 15 minutes before the primers were introduced. Reactions were then incubated for 2 hours and heat inactivated by incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. 10bp ladder.
4. Oligos 43 (P_X) and 10 (cleaner).
5. Oligos 43 (P_X), 10 (cleaner), and 46 (H_X).
6. Oligos 43 (P_X), 10 (cleaner), and 47 (H_X,A).
7. Oligos 44 (P_Y), 10 (cleaner), and 48 (H_Y).
8. Oligos 44 (P_Y), 10 (cleaner), and 49 (H_Y,A).
9. Oligos 45 (P_Z), 10 (cleaner), and 50 (H_Z).
10. Oligos 45 (P_Z), 10 (cleaner), and 51 (H_Z,A).
11. Oligos 45 (P_Z), 10 (cleaner), and 52 (H_Z,T).
12. Oligos 45 (P_Z), 10 (cleaner), and 53 (H_Z,C).
13. Oligos 45 (P_Z) and 10 (cleaner).
14. (empty)
15. (empty)

10.13 Supplemental: dGTP cleanup (Fig. 6)

This figure shows a wider view of the same gel for the experiment in Fig. 5b.

10.14 Supplemental: magnesium dependence of telomerization (Fig. 7)

Reactions were prepared by mixing 2 µL 10 x ThermoPol reaction buffer, 2 µL 8000 units/ml Bst Large Fragment polymerase, 0-4 µL 100 mM $MgSO_4$, 2 µL 1 mM dATP/dTTP/dCTP solution, 2 µL 1 µM primer if present, 2 µL 100 nM hairpin if present, 2 µL of 1 µM cleaner species, and water to 20 µL. Reactions were set up with all components except the primer at 4 °C and pre-incubated at 37 °C for 15 minutes before the primers were introduced. Reactions were then incubated for 2 hours and heat inactivated by incubation at 80 °C for 20 minutes. Reactions were visualized on a 15% Novex PAGE denaturing gel (1 x TBE and 7 M Urea). 20 µL reactions were mixed with 10 µL 100% formamide, and 5 µL was loaded into each lane. The gel was run with 1 x TBE buffer at 200V and 65 °C for 35 minutes, scanned under the Cy5 and FAM channels, stained for several minutes with 1 x Sybr Gold solution, and imaged with Sybr Gold.

Lanes:

1. (empty)
2. (empty)
3. (empty)
4. 10bp ladder
5. Oligos 1 (primer) and 10 (cleaner). 4 µL added magnesium.
6. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). No added magnesium.
7. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). 1 µL added magnesium.
8. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). 2 µL added magnesium.
9. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). 3 µL added magnesium.
10. Oligos 1 (primer), 10 (cleaner), and 8 (hairpin). 4 µL added magnesium.
11. Oligos 10 (cleaner) and 8 (hairpin). 4 µL added magnesium.
12. Oligos 9 (orthogonal primer), 10 (cleaner), and 8 (hairpin). 4 µL added magnesium.
13. (empty)
14. (empty)
15. (empty)

11 Full experimental gel and imaging results

For all experiments, gels were scanned on a Typhoon FLA 9000 with the Cy5 and FAM gels before being stained with Sybr Gold and scanned under that dye's channel. Full gels for all these experiments can be found in Fig. 12-29. Note that most of the experiments didn't have any oligos labeled with the FAM fluorophore, in which case that channel shows a blank gel. Because the Cy5 channel has such little autofluorescence, we found it helpful to include that scan in most of the experiments, so that the outline of the gel could be understood and band locations in specific lanes could be verified.

Gels were converted to 8 bit format and contrasted in the ImageJ software[15] by adjusting the pixel value maximum only. The overlay for Fig. 4 was done in the same software after contrasting by merging channels, converting to RGB, and inverting the image. Rotating and cropping for figure images was also done with ImageJ, and rotating and cropping for supplemental gel images was done in Adobe Illustrator using object rotation and clipping mask functionalities.

AFM images were taken on a Nanoscope V and flattened using the Nanoscope Analysis software. Scale bars and color scales (from -3nm to 3nm) were added using the Gwyddion software package.

11.1 Time series for single primer exchange reaction (Fig. 2d)

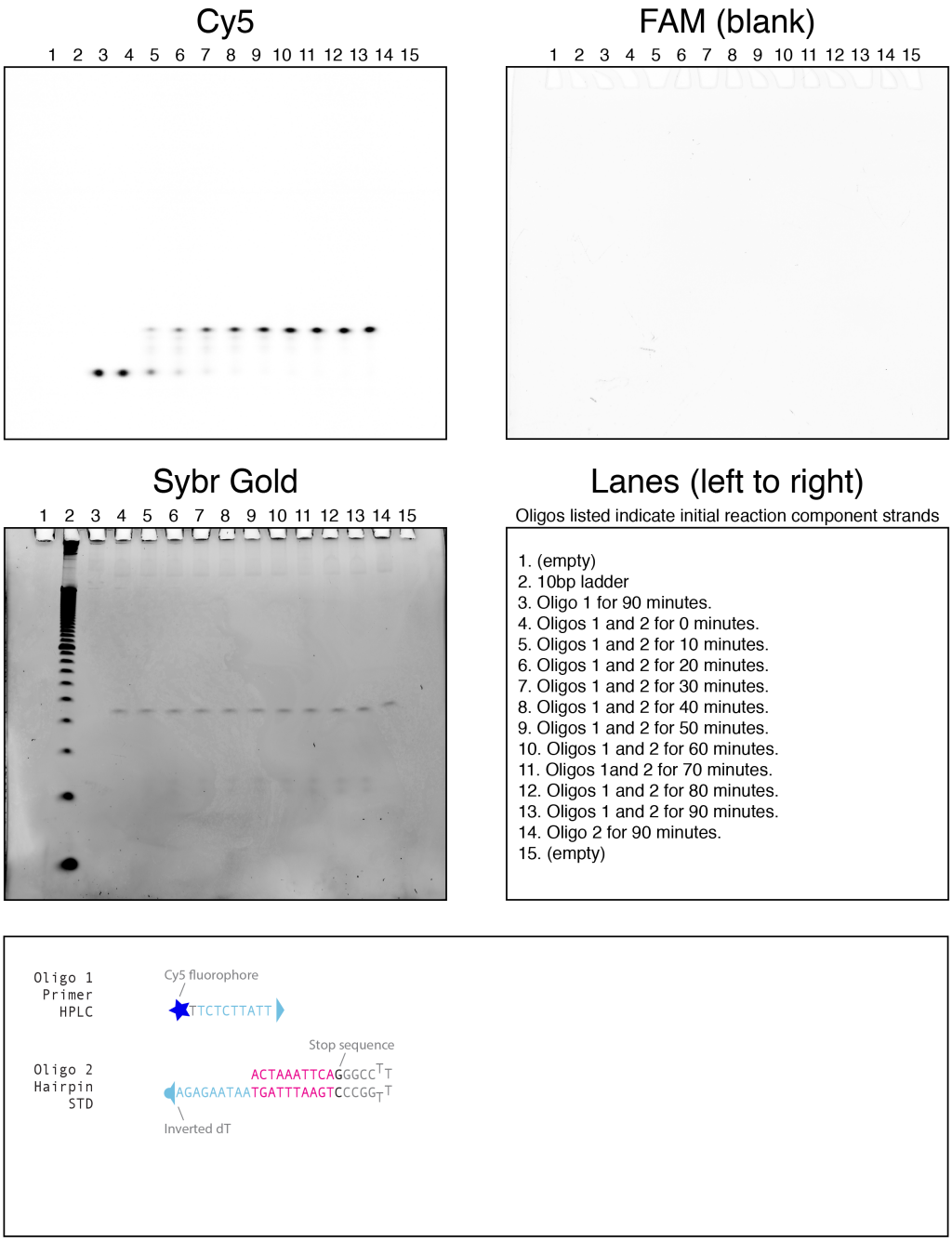

Figure 12: **Gels for Fig. 2d time series.** Gel scans for Fig. 2d time series results.

11.2 5-step elongation (Fig. 3c)

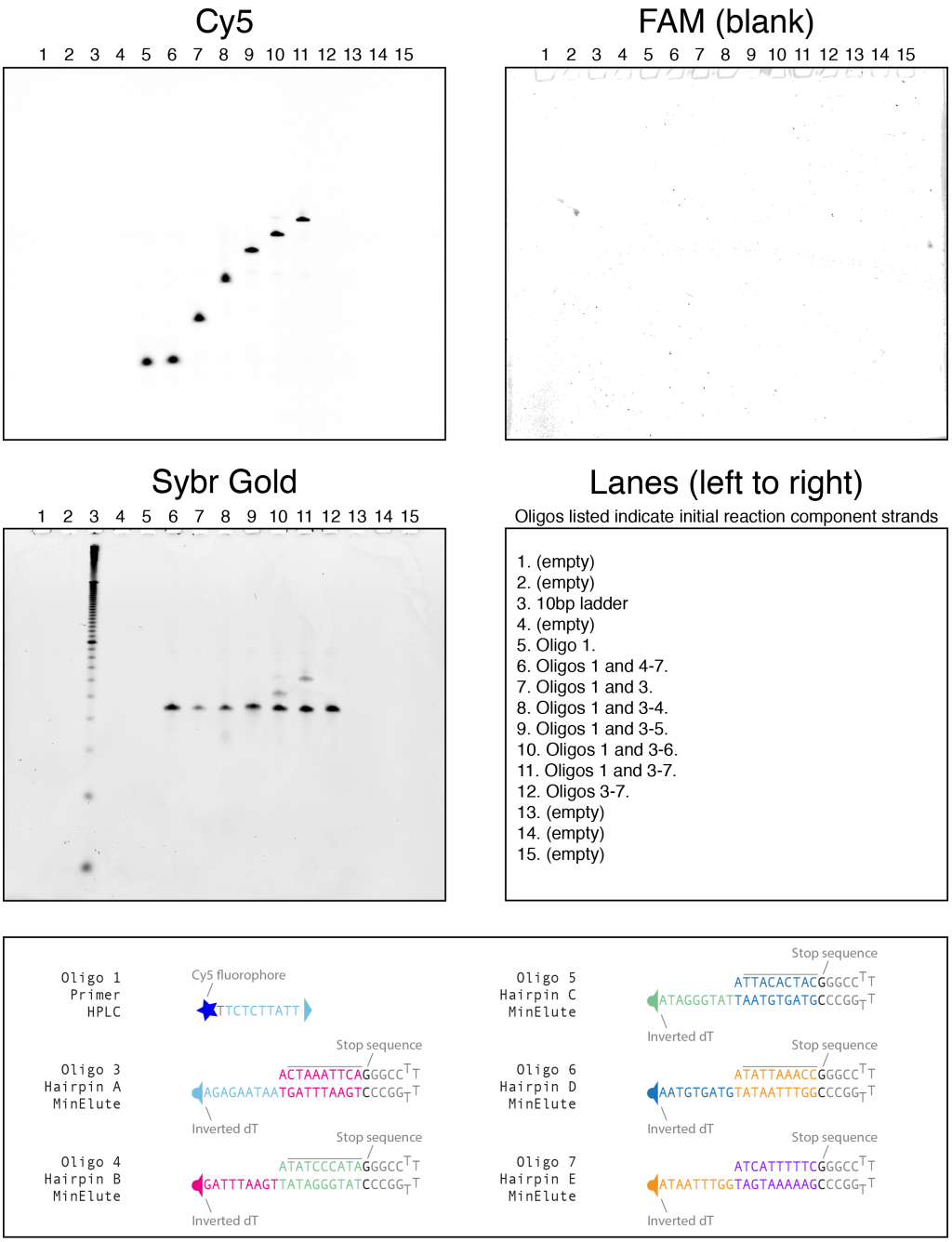

Figure 13: **Gels for Fig. 3c.** Gel scans for Fig. 3c results.

11.3 PER staple synthesis for origami (Fig. 3d)

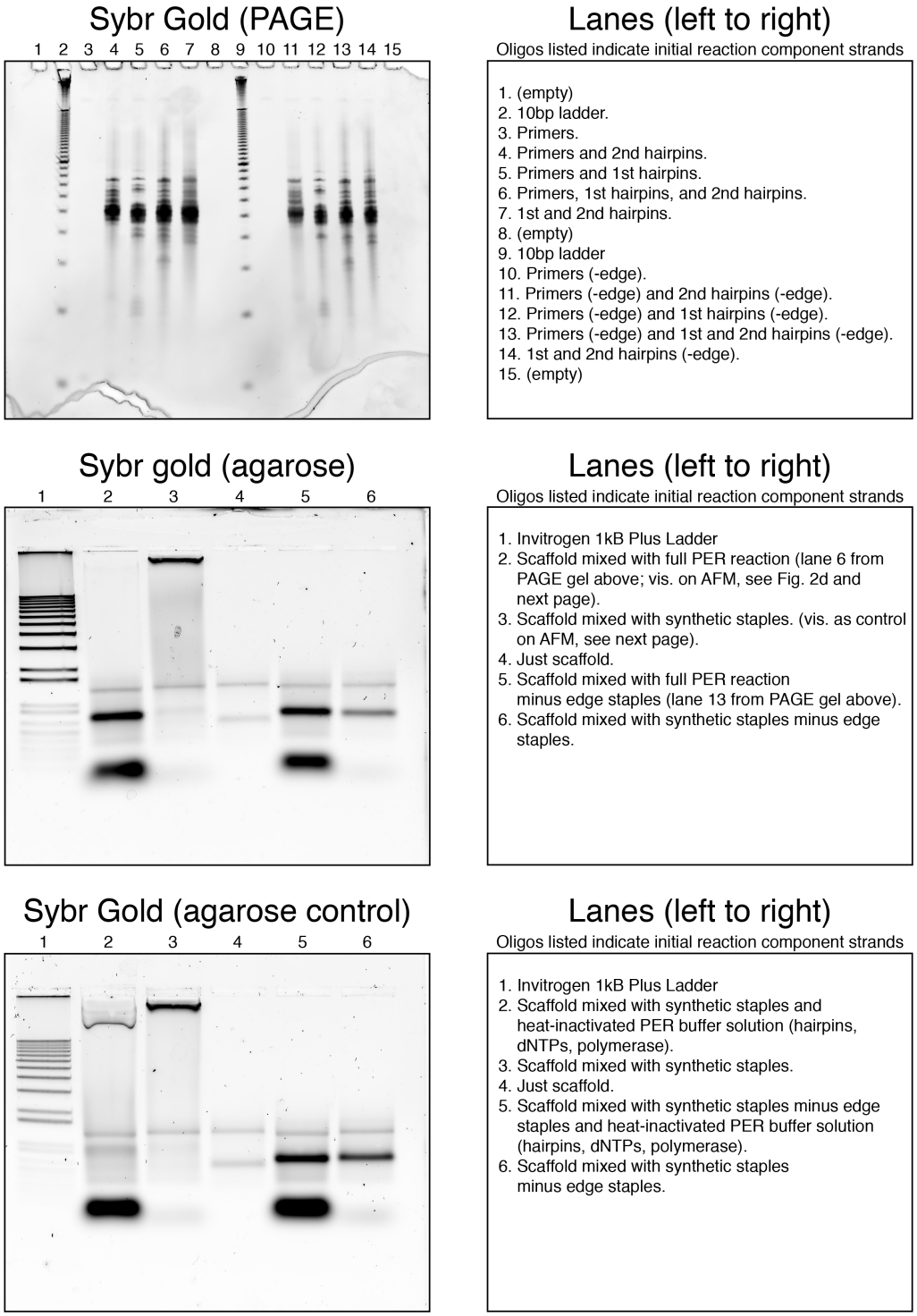

Figure 14: **Gels for Fig. 3d experiments.** Gel scans for origami results from Fig. 3d.

11.4 AFM images of individual origami structures (Fig. 3d)

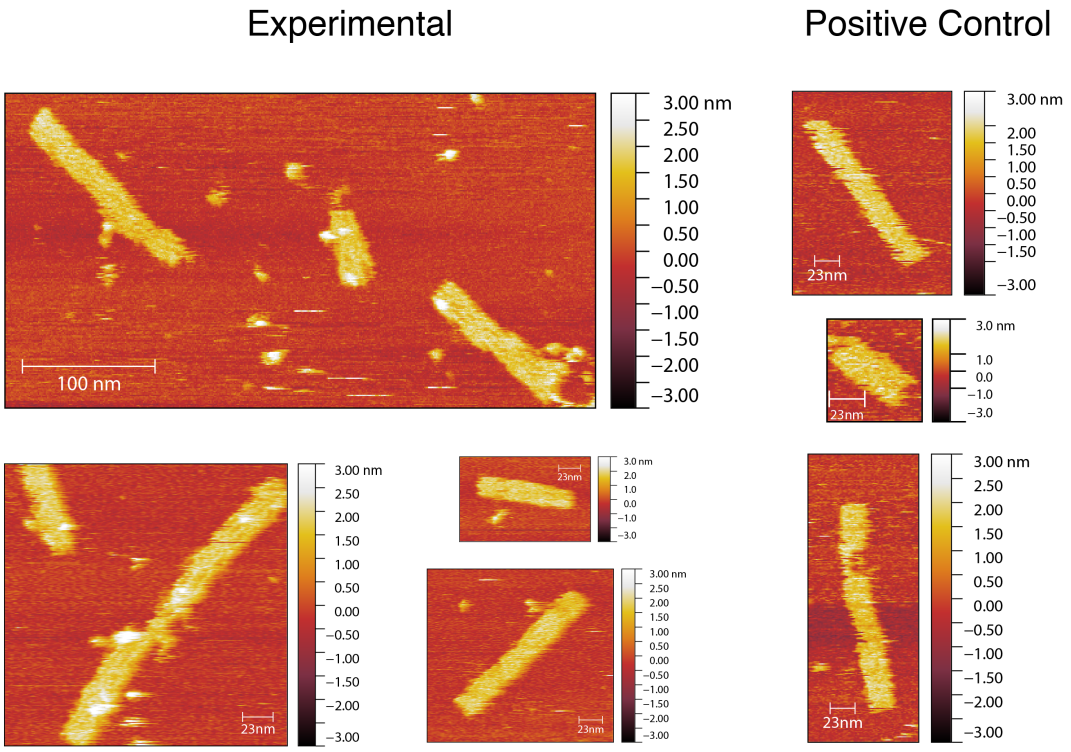

Figure 15: **Additional AFM images for Fig. 3d.** Experimental AFM images correspond to structures formed with PER synthesized staple strands. Scale bars are indicated on the images, and all use the same color scale as in Fig. 3d.

11.5 Synthetic "telomerase" (Fig. 5b)

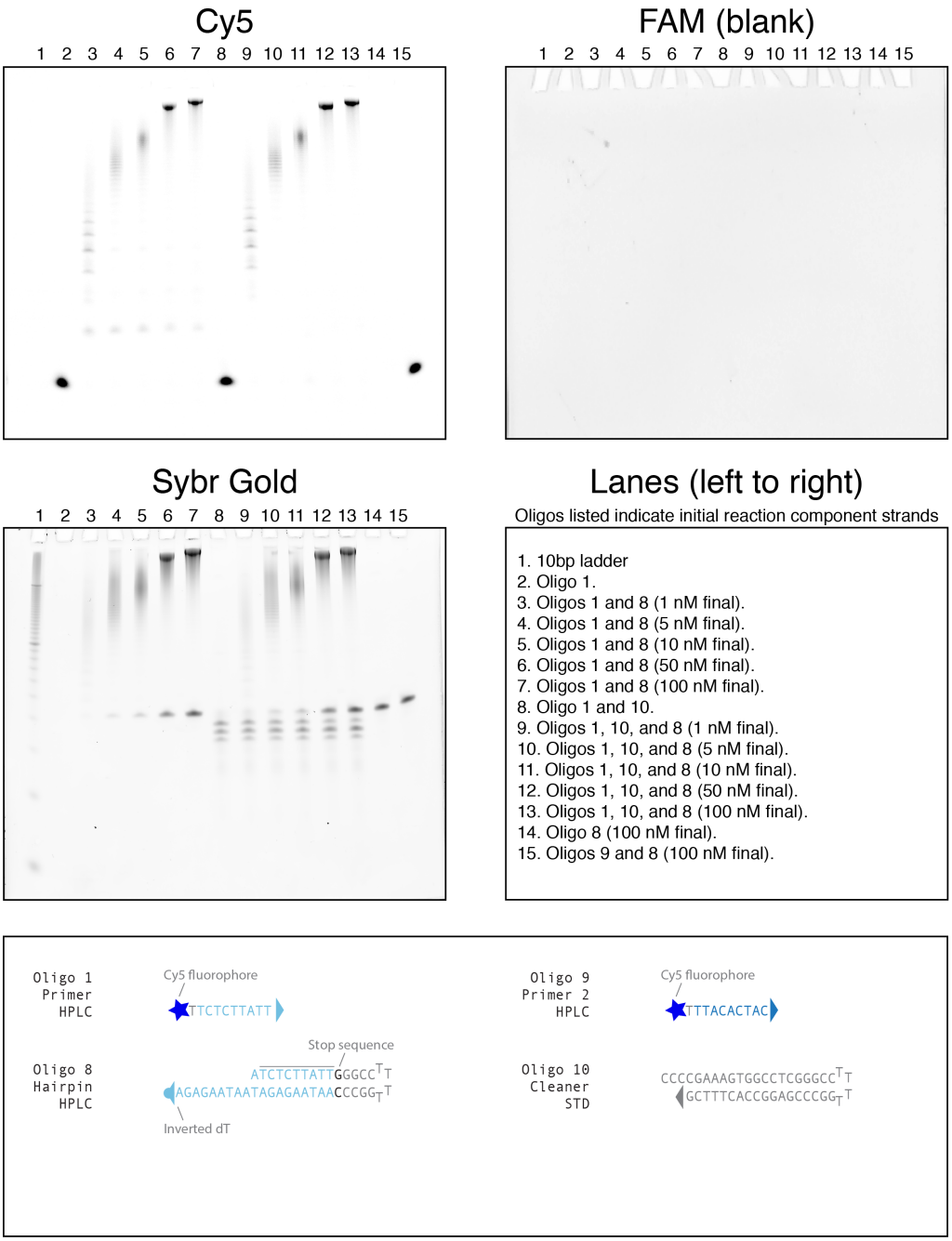

Figure 16: **Gels for Fig. 5b.** Gel scans for Fig. 5b results.

11.6 In situ signal amplifier (Fig. 5e)

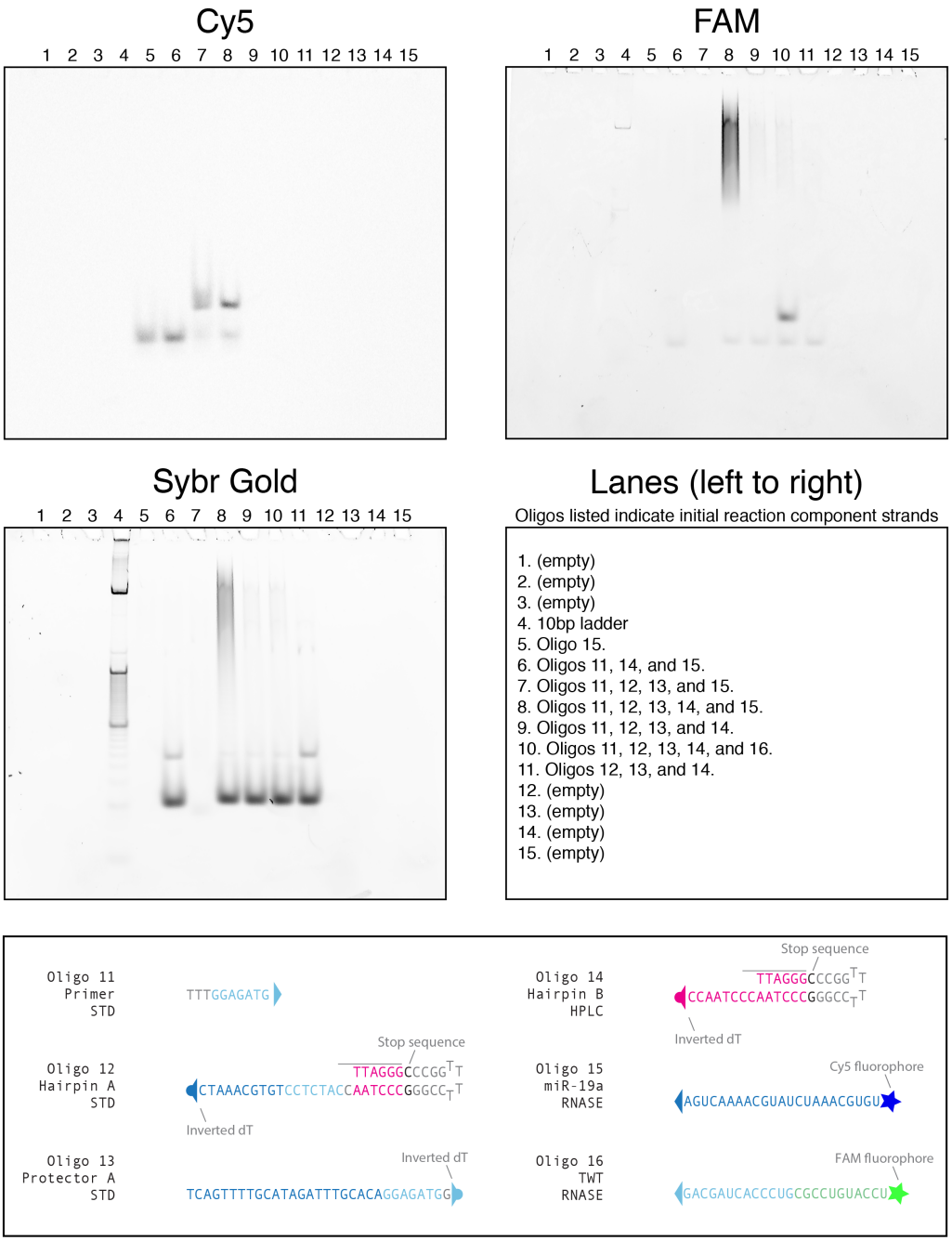

Figure 17: **Gels for Fig. 5e.** Gel scans for Fig. 5e results.

11.7 Event recorder (Fig. 7c)

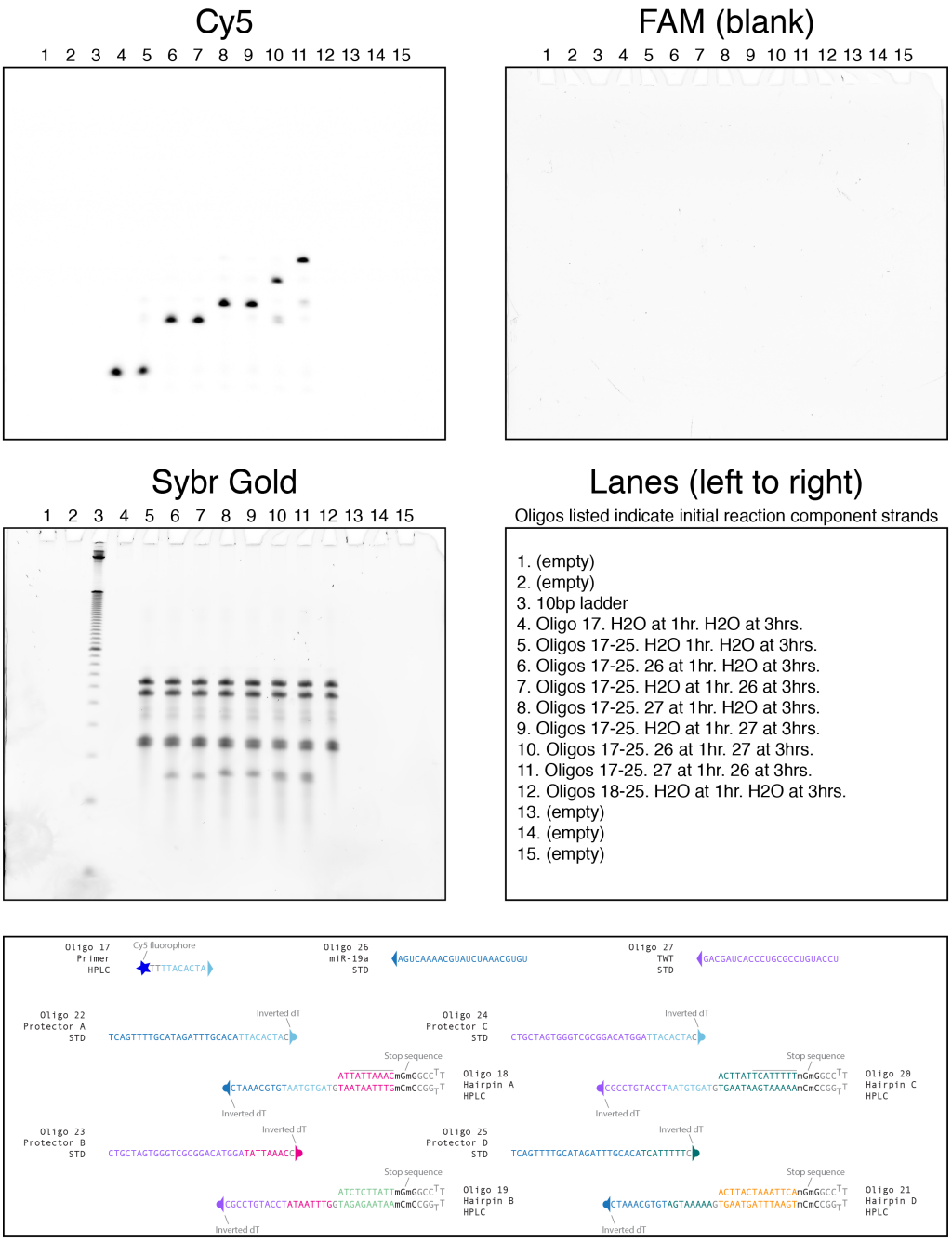

Figure 18: **Gels for Fig. 7c.** Gel scans for Fig. 7c results.

11.8 AND/OR logic (Fig. 6bc)

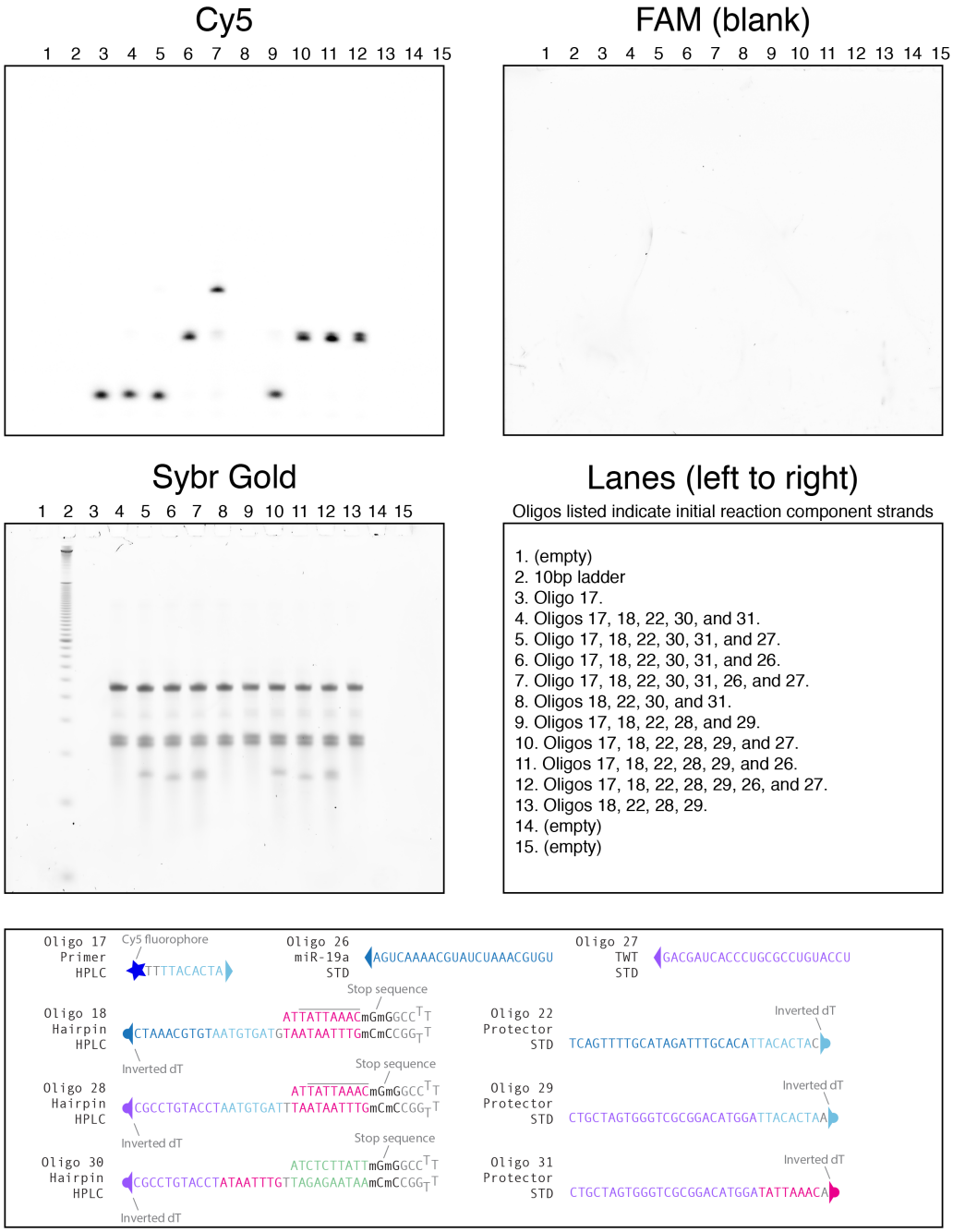

Figure 19: **Gels for Fig. 6bc.** Gel scans for Fig. 6bc results.

11.9 NOT logic (Fig. 6d)

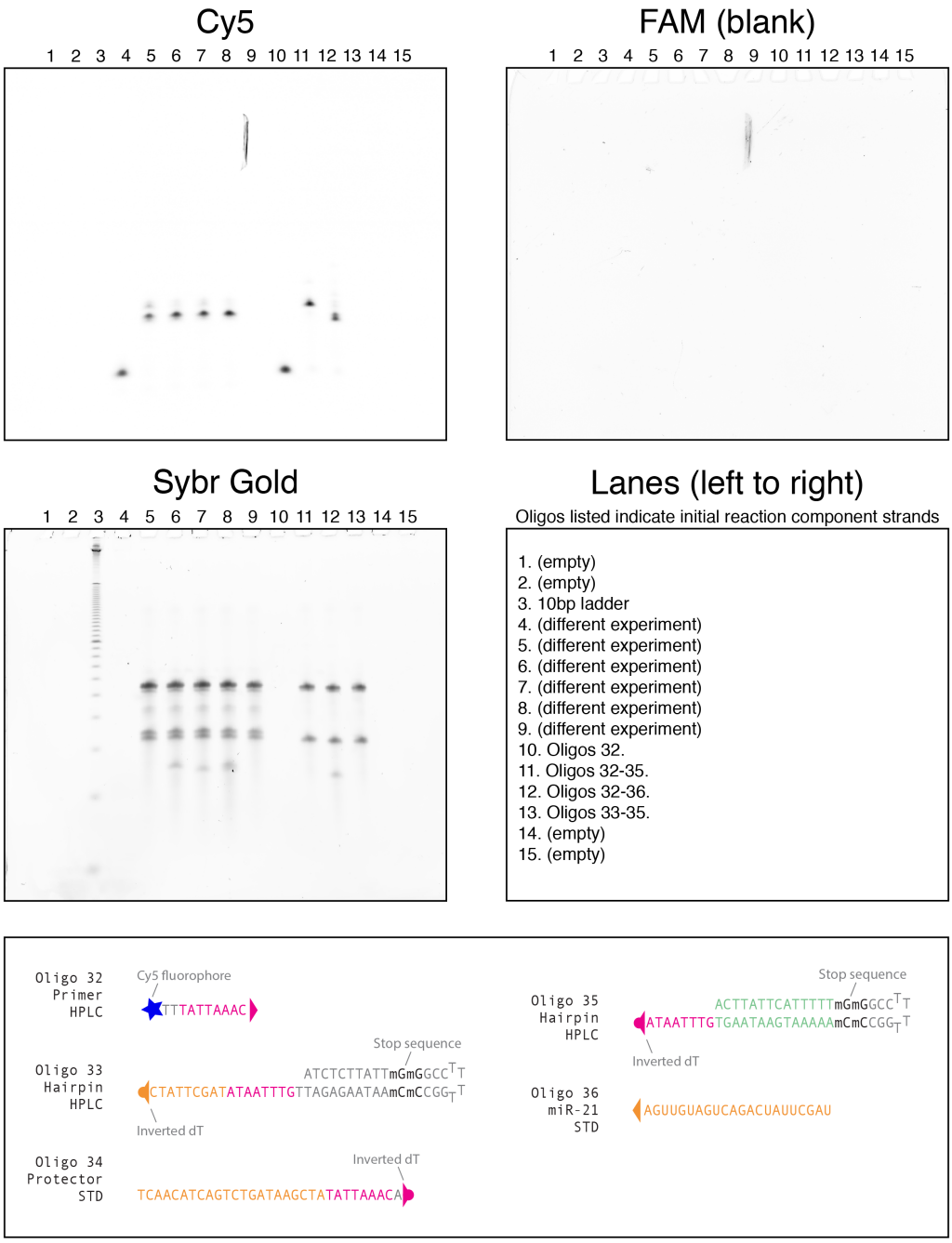

Figure 20: **Gels for Fig. 6d.** Gel scans for Fig. 6d results.

11.10 (X OR Y) AND NOT Z logic (Fig. 6e)

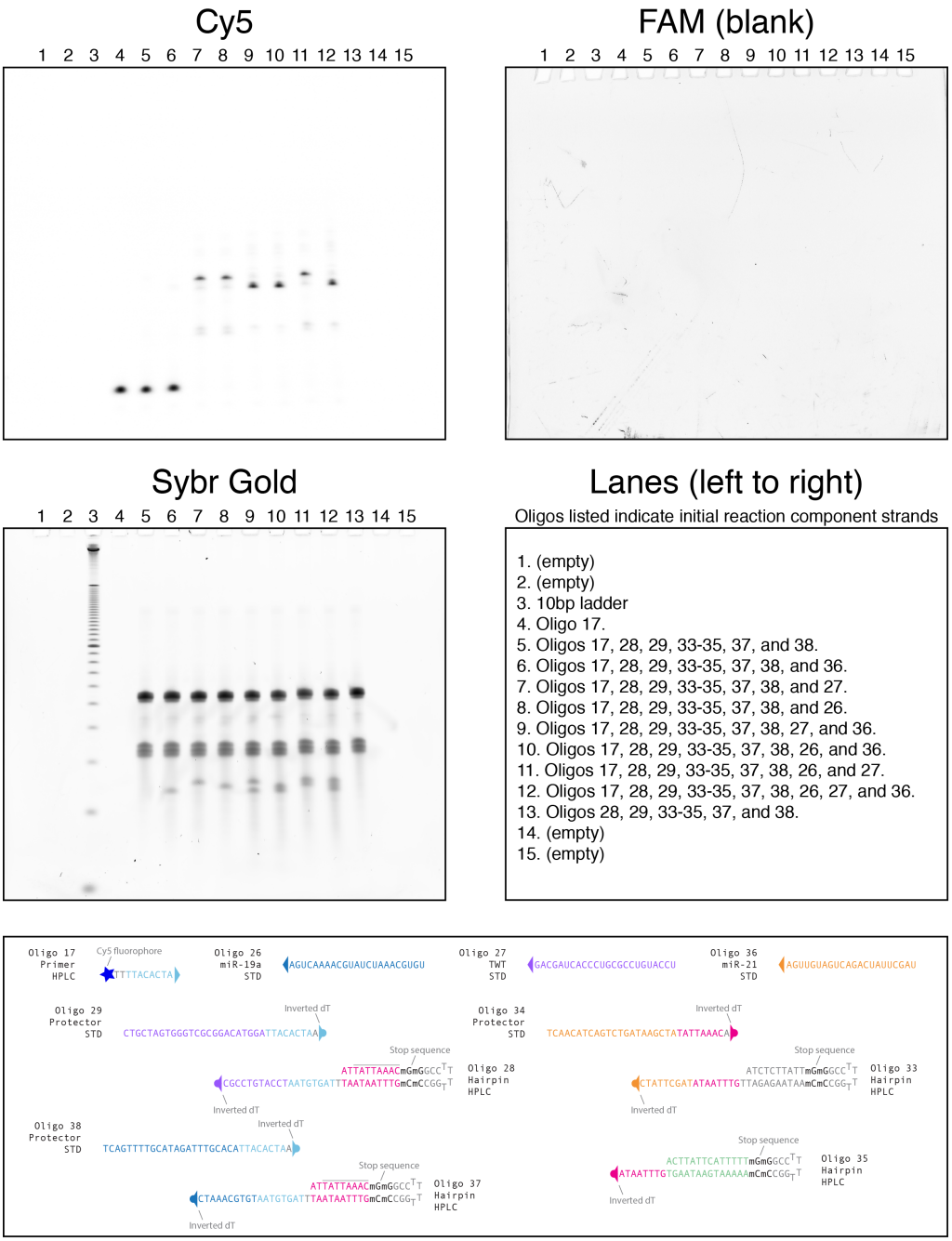

Figure 21: **Gels for Fig. 6e.** Gel scans for Fig. 6e results.

11.11 DNAzyme nanodevice (Fig. 4e)

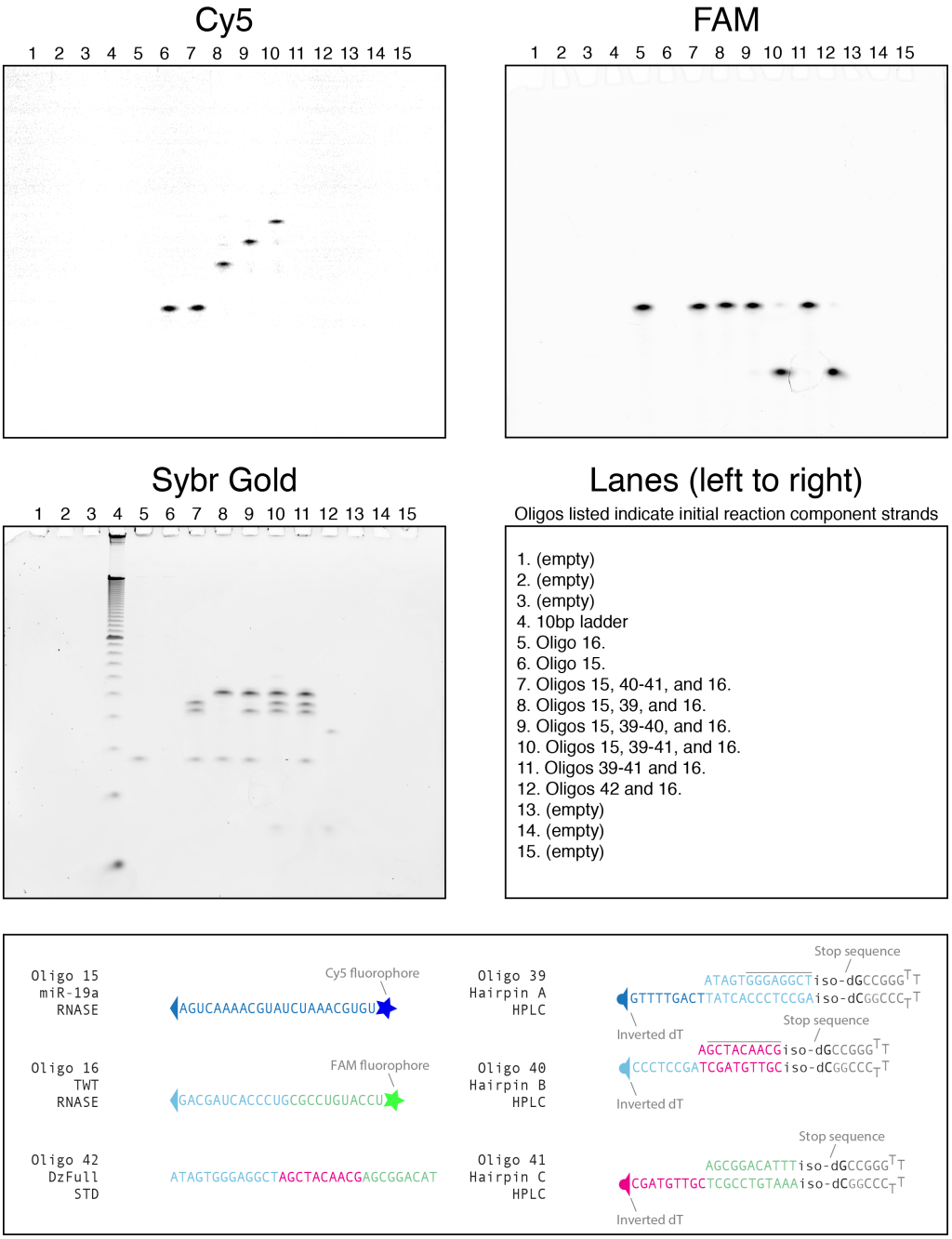

Figure 22: **Gels for Fig. 4e.** Gel scans for Fig. 4e results.

11.12 Supplemental: Kinetics of one-step PER (Fig. 1bde)

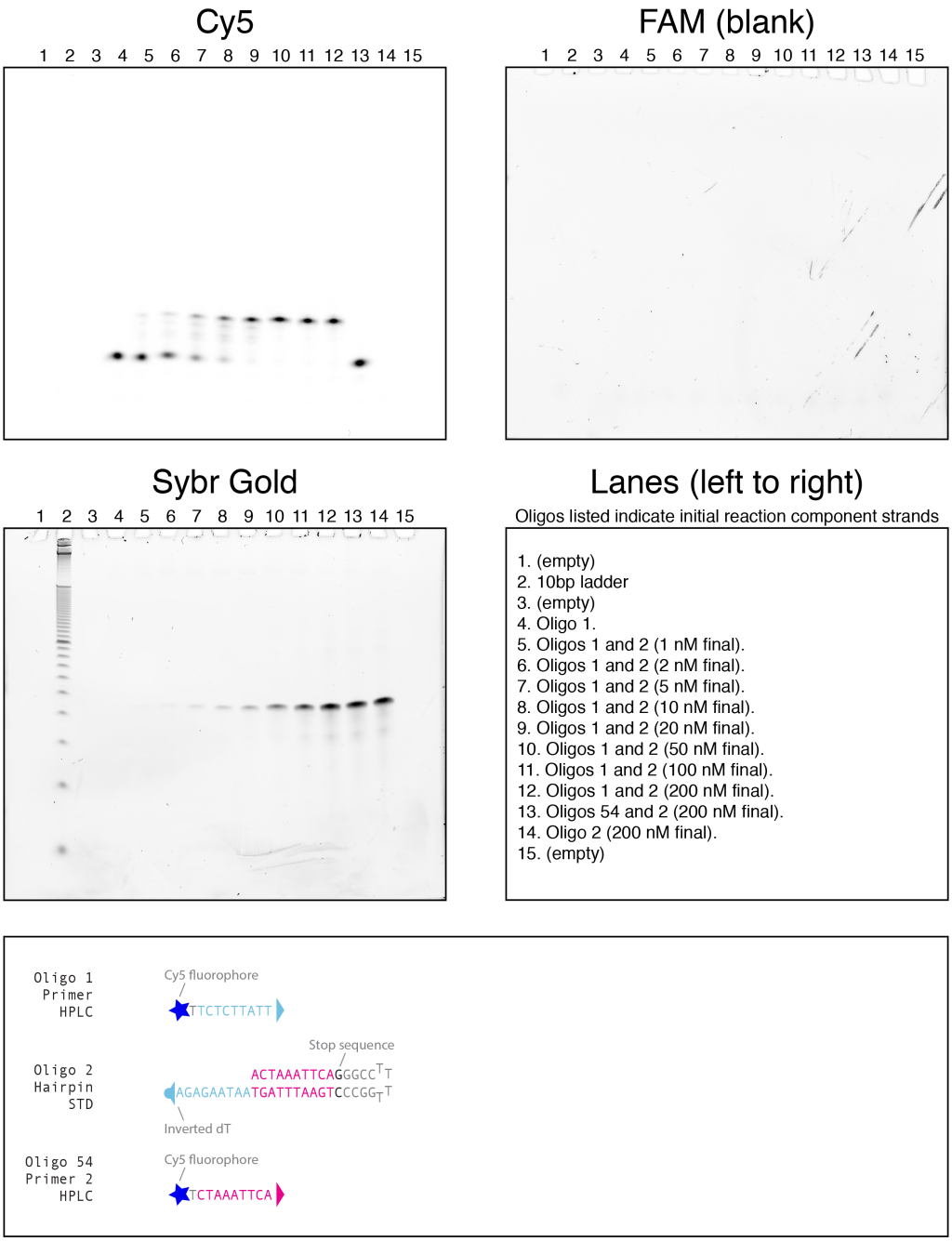

Figure 23: **Gels for Fig. 1d, replicate 1.** Gel scans for Fig. 1d results, replicate 1.

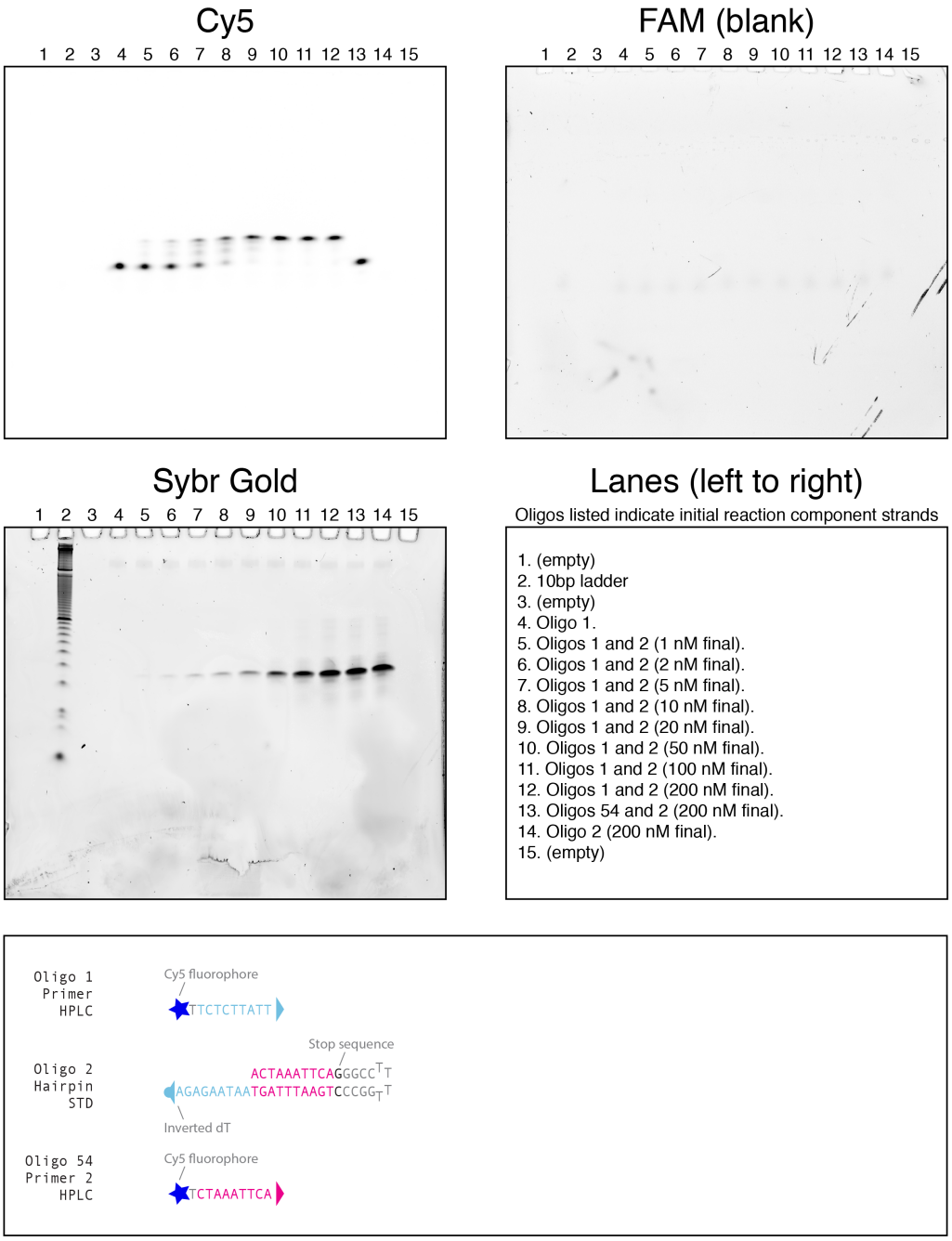

Figure 24: **Gels for Fig. 1d, replicate 2.** Gel scans for Fig. 1d results, replicate 2.

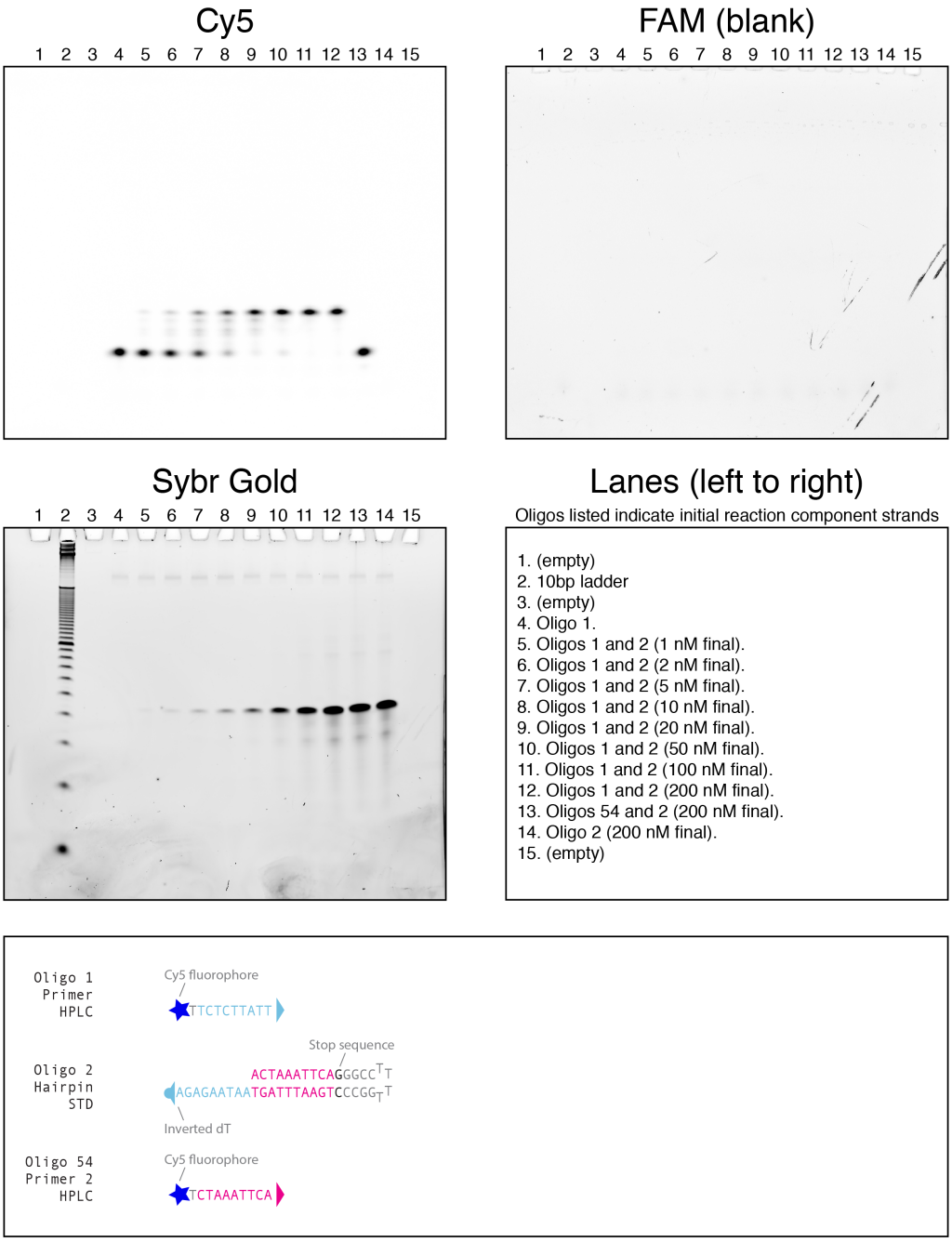

Figure 25: **Gels for Fig. 1bd, replicate 3.** Gel scans for Fig. 1bd results, replicate 3.

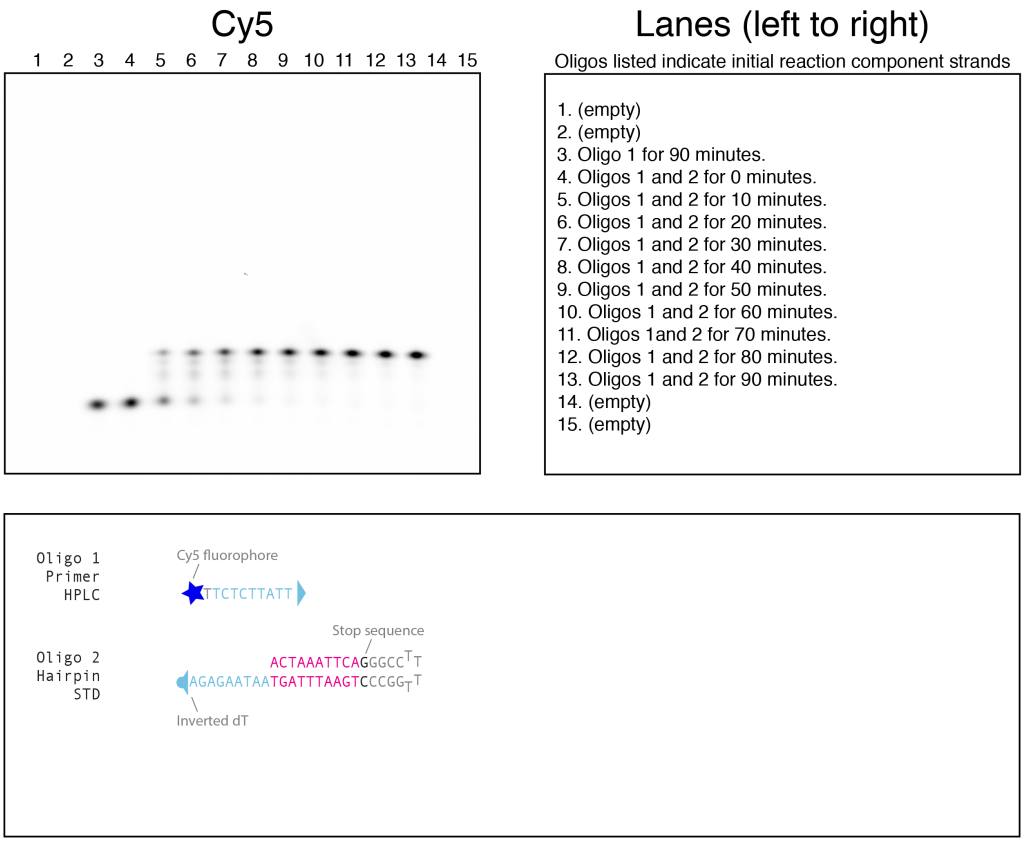

Figure 26: **Gels for Fig. 1e.** Gel scans for Fig. 1e results. Note that the samples for Fig. 2d were retrieved from freezer storage and re-run on a new gel to be scanned at a higher voltage for better quantification.

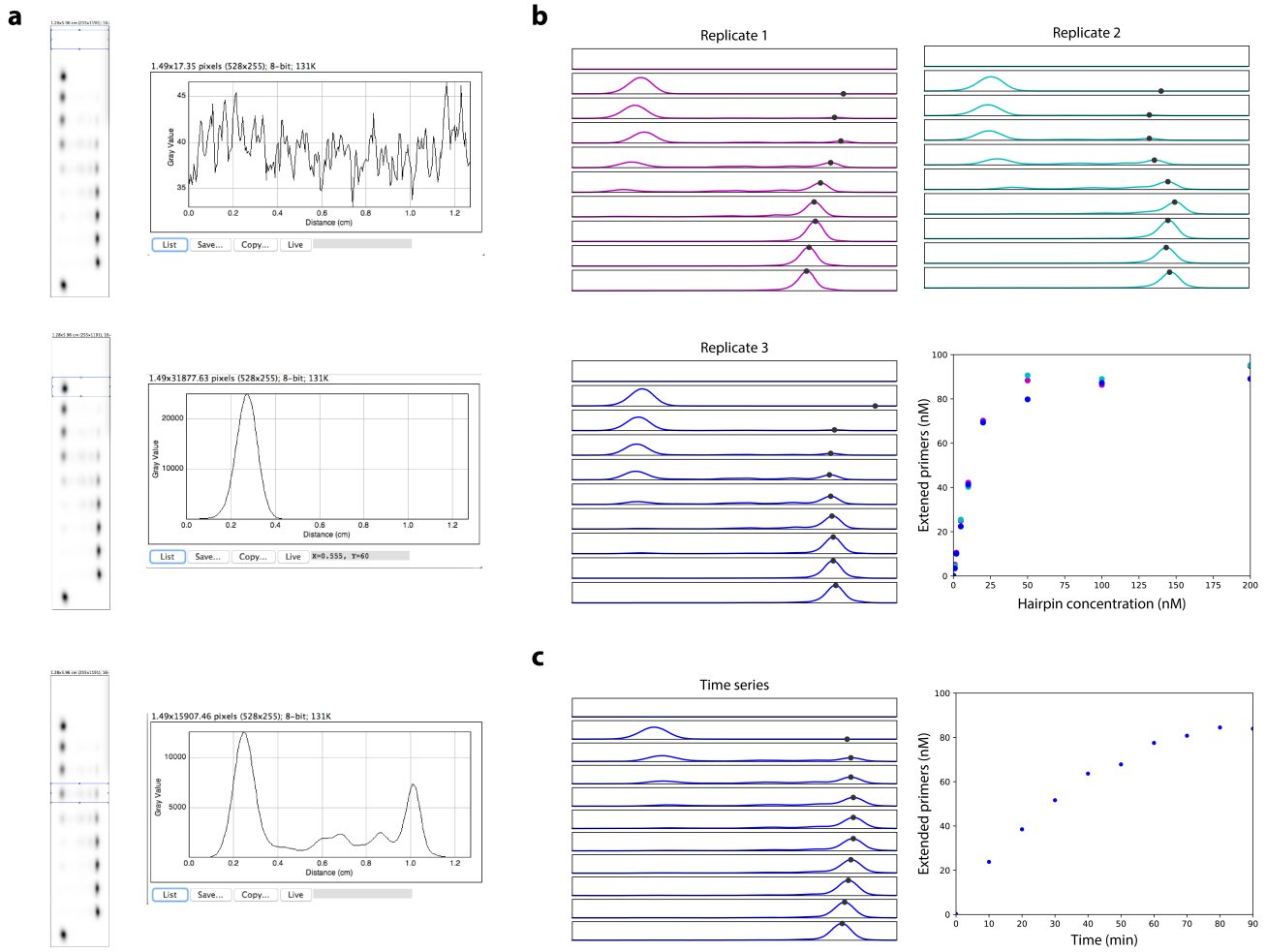

Figure 27: **Gel quantification data for Fig. 1bde.** Gel quantification data for Fig. 1bde results. **(a)** Screenshots that show how background (top) and lanes were plotted using the ImageJ software. Data were saved in text files and processed with a Python script. **(b)** Gel lane plots of the three replicates of the kinetics experiments used in Fig. 1bd (see Fig. 23- 25 for gel images) and a plot of the estimated amount of extended primer according to hairpin concentration. The top plot for all cases is the background plot, and points on the curves indicate where the maximum of the extended primer band was determined to be. **(c)** Gel lane plots of the time course data shown in Fig. 1e (see Fig. 26 for gel image) and plot of estimated amount of extended primer over time.

11.13 Supplemental: A copy strategy (Fig. 3)

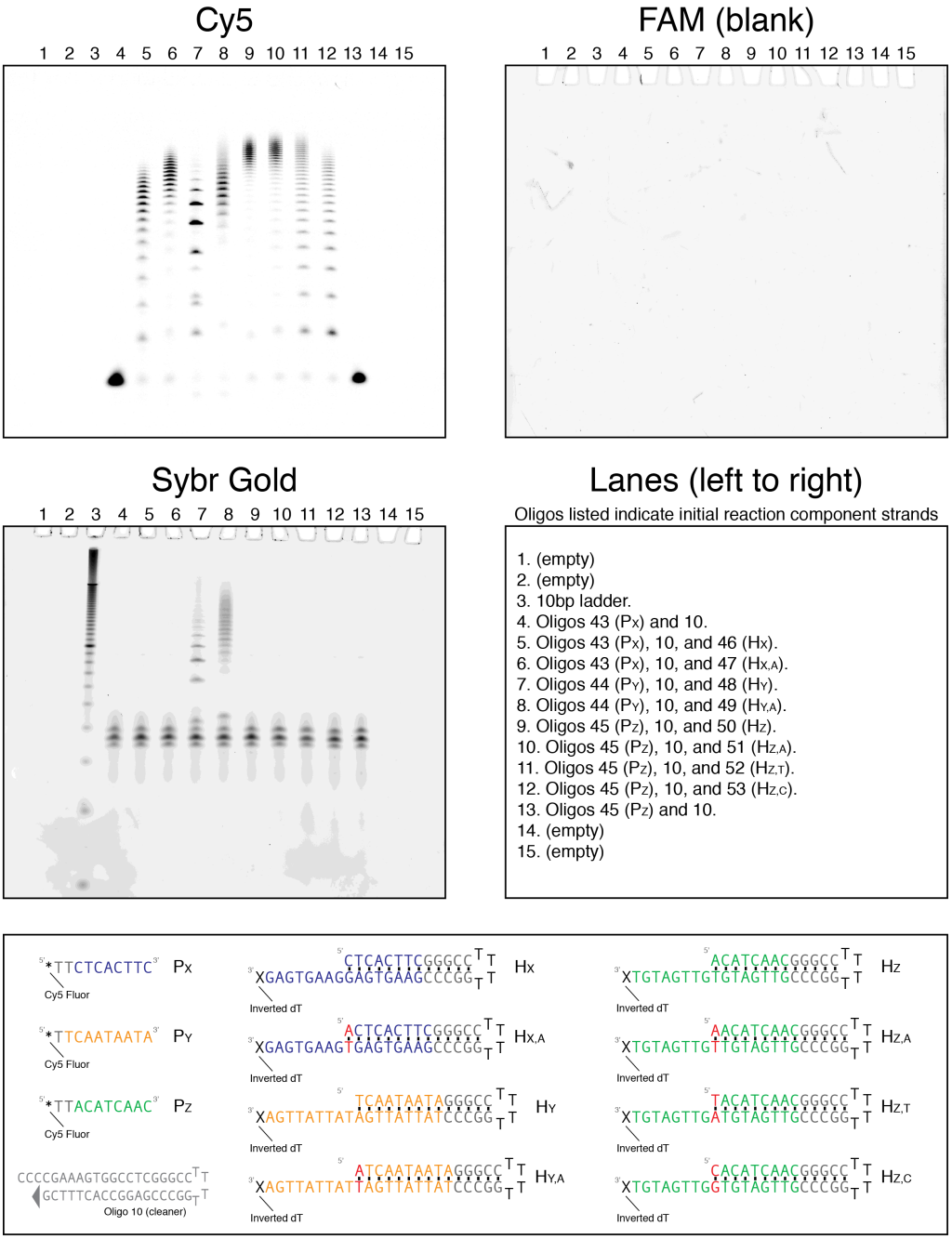

Figure 28: **Gels for Fig. 3.** Gel scans for Fig. 3 results.

11.14 Supplemental: dGTP cleanup (Fig. 6)

See gels for the synthetic “telomerase” (Fig. 5b).

11.15 Supplemental: magnesium dependence of telomerization (Fig. 7)

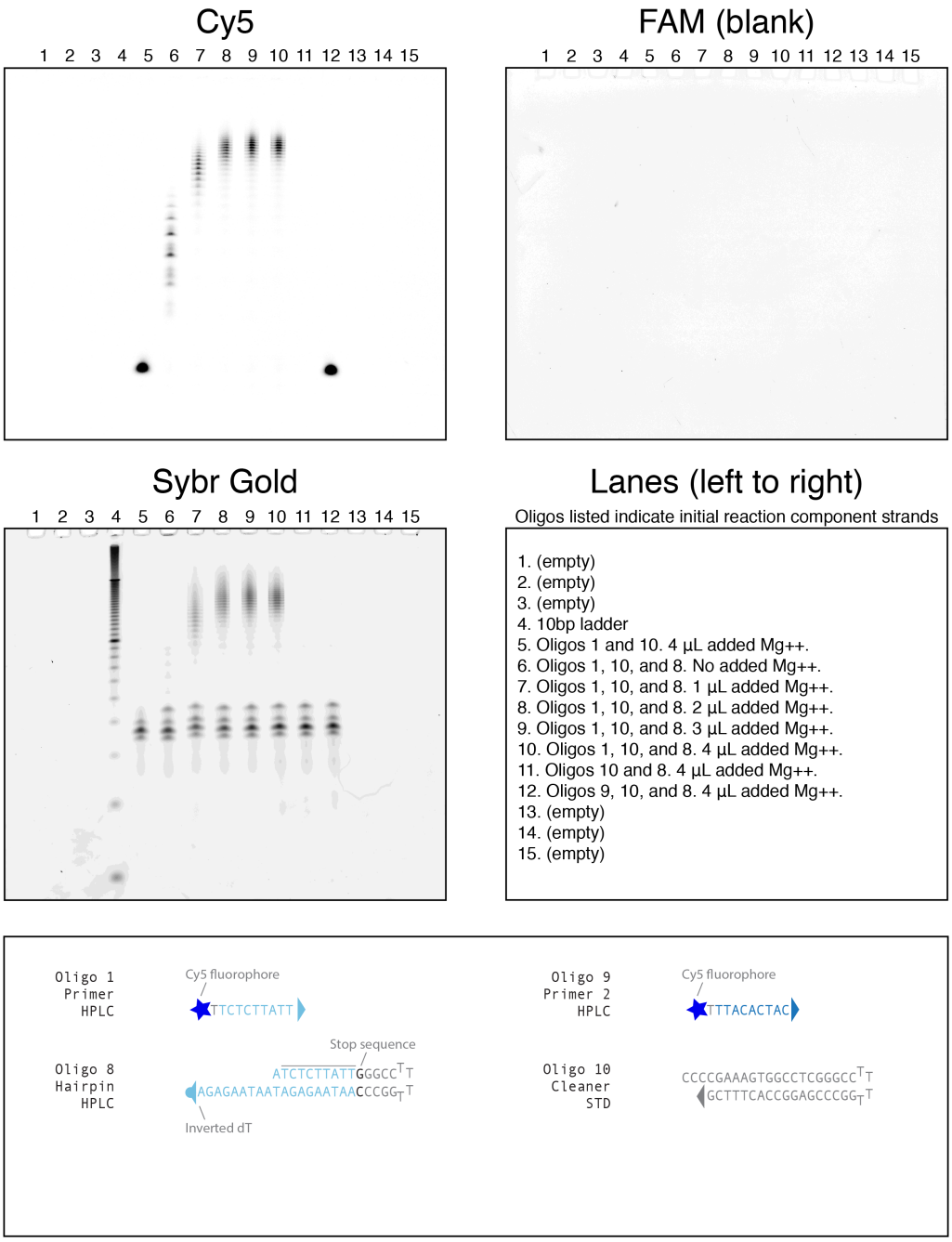

Figure 29: **Gels for Fig. 7.** Gel scans for Fig. 7 results.

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