Supporting Information for 'A clocked finite state machine built from DNA'

1. DNA sequences

Transition Rules	
$T_{(1\text{-}2)}{}^{a}$	CATTTTAGTTACGAAGATAGCGGTGGAATGTGGCTATTATCCCACACACCACCGCTATCT
$T_{(2\text{-}1)}{}^{b}$	CGCTATCTCCACACACCACCTAAAATGGTGGAATGTGGAGATAGCGAGTCTTGATAATAG
$T_{(3\text{-}4)}{}^{a}$	TATTGATATTACGAAGATAGCGGTGGAATGTGGTTATTCCTCCACACACCACCGCTATCT
$T_{(3\text{-}2)}{}^{c}$	TATTGATATTAGCAAGATAGCGGTGGAATGTGGCTATTATCCCACACACCACCGCTATCT
$T_{(4\text{-}3)}{}^{b}$	CGCTATCTCCACACACCACTATCAATAGTGGAATGTGGAGATAGCGAGTCTTAGGAATAA
Inputs	
I_a	CCACATTCCACCGCTATCTTCGTGTGGTGTGTGGTTTGACTCTTGGTA
I_b	ATGGTTCTCAGTTTGTGGTGTGTGGGACTCGCTATCTCCACATTCCAC
I_c	CCACATTCCACCGCTATCTTGCTGTGGTGTGTGGTTTGACTCTTGGTA
Clocks	
C_0	TACCAAGAGTCAAGATAGCG
C_1	AGATAGCGACTGAGAACCAT
Reporters	
R_1	Cy5-TATGATTTTAGGTGGCATA-Iowa Black RQ
R_4	Iowa Black RQ-AGCAGTGGATAAGGATGCT-Cy3
Initiators	
S_1	CGCTATCTCCACACACCACCTAAAATG
S_3	CGCTATCTCCACACACCACTATCAATA
OH_3	TTATTCCTAAGACTCGCTATCTCCACATTCCAC
OH_4	CCACATTCCACCGCTATCTTCGTAATATCAATA

Underlined sequences indicate the position of the mismatches in the stem of the transition and input strands that are repaired when incorporated into the polymer.

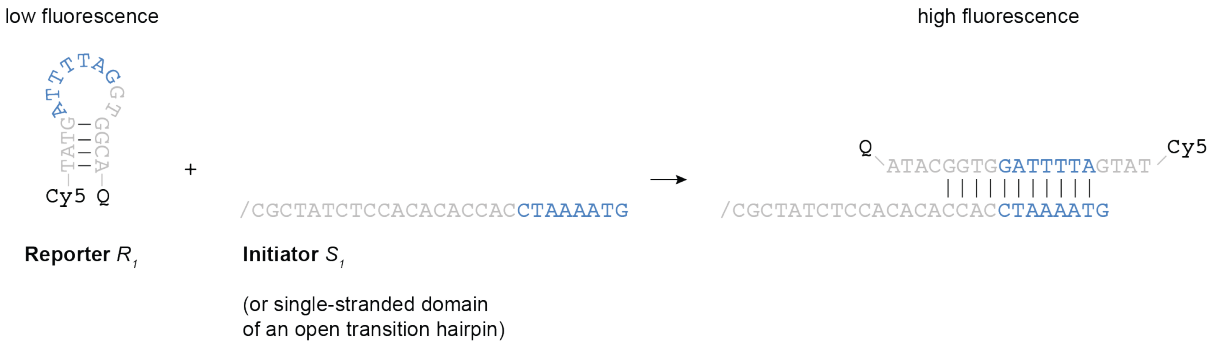

Figure S1. The molecular beacon R_1 is used to report State 1. It is a stem-loop structure labeled at opposite ends with a fluorophore and a quencher (Q). The loop and part of the neck of the R_1 are complementary to the single-stranded sequence that is displayed at the end of the growing polymer when the state machine is in State 1. An increase in fluorescence is seen when the reporter hybridizes to its target sequence because the separation between fluorophore and quencher increases. When the state changes, the molecular beacon is released and the fluorescence signal is quenched as the stem-loop structure refolds. The reporter R_4 is designed to report State 4 in a similar way.

2. Methods

DNA components were resuspended to a concentration of 100 µM in TE buffer (10 mM Tris•HCl, 0.5 mM EDTA pH 8.0). Fluorescence experiments were performed at 25°C in TE buffer supplemented with 0.5 M NaCl. Reactions were prepared by adding 1.5 µL of each component to 150 µM of buffer to give a final concentration of ~ 1µM. The clock strands C_0 and C_1 were added alternately at 20-minute intervals in stoichiometric quantity.

The initial components present in each experiment presented in the manuscript are listed below:

Fig. 2A Initiator S_1, Input I_a (2x) and I_b (2x), Transition Rules $T_{(1-2)}^{a}$ (2x) and $T_{(2-1)}^{b}$ (2x), Reporter R_1

Fig. 2B Initiator S_3, Input I_a (2x) and I_b (2x), Transition Rules $T_{(3-4)}^{a}$ (2x) and $T_{(4-3)}^{b}$ (2x), Reporter R_4

Fig. 2C Initiator S_1 and S_3, Input I_a (4x) and I_b (4x), Transition Rules $T_{(1-2)}^{a}$ (2x), $T_{(2-1)}^{b}$ (2x), $T_{(3-4)}^{a}$ (2x), $T_{(4-3)}^{b}$ (2x), Reporter R_1 and R_4

Fig. 3A Initiator S_3, Transition Rules $T_{(3-2)}^{c}$, $T_{(2-1)}^{b}$, $T_{(3-4)}^{a}$, $T_{(4-3)}^{b}$, Input I_a and I_b, Reporter R_1 and R_4

Fig. 3B Initiator S_3, Transition Rules $T_{(3-2)}^{c}$, $T_{(2-1)}^{b}$, $T_{(3-4)}^{a}$, $T_{(4-3)}^{b}$, Input I_b and I_c, Reporter R_1 and R_4

3. Clocked reaction kinetics compared to the un-clocked reaction kinetics

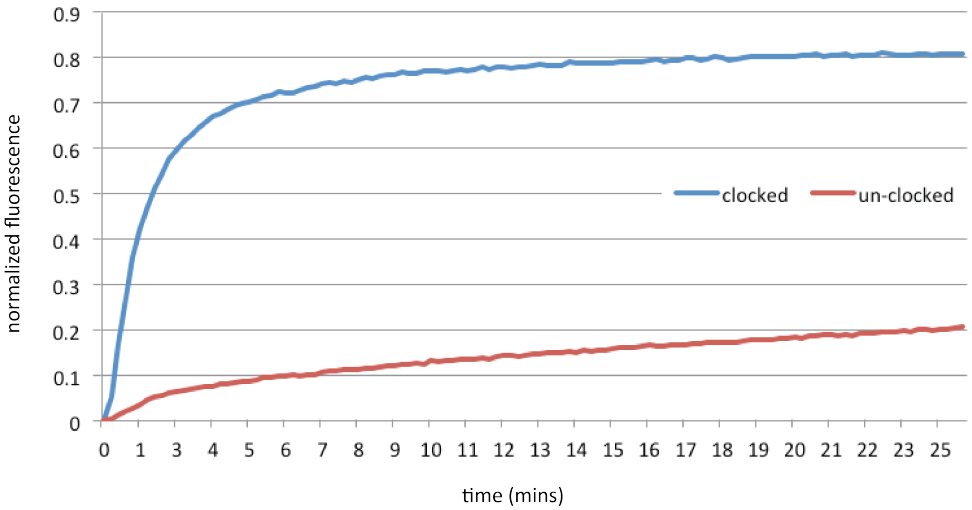

Figure S2. Clocked versus un-clocked state transition. Clocked and un-clocked reactions were prepared with the following components: transition hairpins $T_{(3-4)}^{a}$, $T_{(4-3)}^{b}$, reporter R_4 and OH_3 (a strand that is designed to open the transition hairpin $T_{(4-3)}^{b}$). At t=0 minutes, input I_a and clock C_0 were added to the clocked reaction, whereas only input I_a was added to the un-clocked reaction. The fluorescence signal was normalized by setting the initial fluorescence to 0 and the signal for fully opened R_4 to 1 (the signal for fully opened R_4 was measured using OH_4 to open $T_{(3-4)}^{a}$). After 20 minutes the clocked reaction (blue line) reached ~80% completion and the un-clocked reaction reached ~20% completion (red line).