Whiplash PCR

History:

- Invented by Hagiya et all 1997]
- Improved by Erik Winfree 1998
- Made Isothermal by John Reif and Urmi Majumder 2008
Polymerization Reaction

Primer Extension via Polymerase

extension of primer strand bound to the template by DNA polymerase
Polymerase Chain Reaction (PCR) is a protocol used to amplify a template strand.

It uses repeated stages of thermal cycling between two temperatures $t_1 < t_2$

**At temperature $t_1$:**
- a primer hybridizes to a segment of the template sequence and
- polymerase enzyme extends the primer sequence to form a complementary copy of the template sequence

**At temperature $t_2$:**
- the copied sequence melts off so both the original template sequence and the complementary copy can be used for further PCR cycles.

http://en.wikipedia.org/wiki/Polymerase_chain_reaction
Whiplash PCR (WPCR)

**Whiplash PCR** is a protocol used to compute using a single strand $s$ of single stranded DNA consisting of $n$ pairs of a primer sequence and an extension sequence, followed by a stop sequence (that stops the polymerization on each stage).

**Note:** multiple identical primer sequences may be paired with distinct extension sequences to allow for nondeterministic operation.

It uses repeated stages of thermal cycling between two temperatures $t_1 < t_2$

**At temperature $t_1$:**
- The 3’ end of $s$ hybridizes to a primer segment of $s$ and
- polymerase enzyme extends the 3’ end of $s$ to form a complementary copy of the corresponding extension sequence.

**At temperature $t_2$:**
- the copied sequence melts off the 3’ end of $s$ so a further stage of Whiplash PCR can be performed.
In IR-WPCR with non-reusable rules, computation comprises of the following steps after the end of the WPCR strand $W$ binds to current state in rule $R_i$: (a) as with the original WPCR protocol, copying the next state at the end of the WPCR strand $W$, (b) dislodging a secondary primer sequence $P_i$, which is specific to the transition rule $R_i$ from its initial position triggered by the primer extension on $W$, (c) subsequent hybridization of $P_i$ to its final position in rule $R_i$ and (d) dislodging of the $3'$ end of $W$ by primer extension of $P_i$, allowing the $3'$ end of $W$ to bind to the new transition rule. Observe that (b) and (d) act like a logical toggle switch allowing for an isothermal, autocatalytic reaction.

In this version of WPCR, each rule is encoded as a 7-tuple $x_i; y_i; z_i; a_i; b_i; w_i; y_i$ where $a_i$ still represents the current state and $b_i w_i y_i$ represents the next state where the $b_i$ in IR-WPCR is not the same as $b_i$ in the original WPCR strand. Rather, the original $b_i$ is now divided into 3 subsequences $b_i, w_i$ and $y_i$. The other regions in this tuple are required for destabilizing the $3'$ end of the strand once the next state is copied at the end of it. In this machine, the transition table with $n$ rules is encoded on a single stranded DNA as

Fig. 1 Schematic of the protocol for the original Whiplash PCR machine: $S1$: initial state of the WPCR strand $W$ with current state being $a_i^*$. $S2$: polymerase binds to the $3'$ end of $W$ (bearing the current state). $S3$: next state $b_i^*$ is copied at the head of $W$ by primer extension. $S4$: the mixture is heated so that $W$ loses its hairpin structure. $S5$: the solution is cooled so that the head of $W$ can bind to the new current state $b_i^* = a_j^*$ encoded at the $3'$ end of the strand and the whole state transition repeats again beginning with State $S2$.
Finite State Machine

![Finite State Machine Diagram]
Original Whiplash PCR Machine

Original Whiplash PCR Machine (Contd)

State $S_2$

Next state copied

State $S_3$
Original Whiplash PCR Machine (Contd)

Heat

Cool

State $S_4$
Original Whiplash PCR Machine (Contd)

Transition from State $S_i$ to State $S_j$

State $S_2$

State $S_5$
**Importance**

- Allows sequential molecular computations
- Also allows parallel execution of distinct programs

**unlike other forms of molecular computation (e.g. tiling assembly):**

- Each WPCR machine holds its own program
- Operation on local rules rather than global rules

Note: Tiling assembly can be made to do multiple programs in parallel if we start with a universal cellular automata tile set with different seed rows. However, it is not very practical to generate such a large til
Whiplash PCR

- Applied to solve NP search Problems by Erik Winfree 1998
Figure 1: (a) A branching program for computing the \( \mu \)-formula \( (x_1 \lor \overline{x_3}) \land (\overline{x_2} \lor x_4) \). A possible input would be \( x_1 = 1, x_2 = 1, x_3 = 0, x_4 = 1 \), which leads to output \( + \). The computation follows a path through the diagram, and thus can only access variables in the order prescribed. (b) A branching program which does not correspond to a \( \mu \)-formula.

Winfree 1998
Figure 2: Probable secondary structures during the computation of the $\mu$-formula $(x_1 \lor \bar{x}_3) \land (\bar{x}_2 \lor x_4)$ on the input 1101. “Probable” is in the mind of the artist. Note that the tick marks denote the stop sequence; because the 3′ head sequence will never contain the complement to the stop sequence, this will be the site of a small bulge in regions that are shown as double-stranded.

Whiplash PCR

Winfree 1998
Assembly Graph
derived from Branching Programs
for Evaluating Boolean Formula

Figure 3: An assembly graph for generating input to the formula \((x_1 \lor \overline{x_2}) \land (\overline{x_1} \lor x_3)\). Up to \(2n + 1\) oligos are required, and additional symbols \(P_i\) are used. For convenience, the node \(P_0\) is written twice. Since there will be a restriction site in \(P_0\), this results effectively in paths from the leftmost node to the rightmost.
Deriving GOTO program to Evaluate Boolean Formula

Figure 4: Reducing BP-SAT to GG-SAT: the \( n = 3, \hat{n} = 5 \) example. (a) The direct construction, combining the assembly graph from Figure 3 and the \( \mu \)-formula program for \( (x_{11} \lor \overline{x}_2) \land (\overline{x}_{12} \lor x_3) \). (b) The optimized construction obtained by following GOTO statements in the fixed region of (a). All GOTO programs are of length 5.
Whiplash PCR

Figure 7: (a) The polymerization stop step on a standard frame, where a single symbol is copied, and its representation as an edge in a BP. (b) The polymerization stop step on an enhanced frame, where two hidden frames are made active, and its representation as an edge in a WOBP.
Figure 5: A GOTO graph for solving the Independent Set Problem. Inputs are generated in which exactly $k = 3$ out of $n = 8$ variables have value 1. The edge labels “0” and “1” in column $i$ are shorthand for GOTO statements setting the value of variable $x_i$; as in FSAT, variables which are referenced more than once in the formula must be duplicated, and the corresponding edges in the graph will be labelled with more than one GOTO statement. Note that concentration ratios of the oligos could be adjusted to make all paths equally likely (for ligation-based assembly, at least; it is not so clear for assembly PCR).
GOTO program for Hamiltonian Path Problem

Figure 6: Solving the Hamiltonian Path Problem: A graph $G$ (a) and its corresponding GOTO graph $GG$ (b). This is Adleman’s example with 2 additional edges added to prevent pruning from simplifying the GOTO graph to triviality. For convenience the nodes show only the vertex index $i$, and not the full symbol $P_{ik}$. 

Winfree 1998
Limitations of WPCR

- Requires thermal cycling and hence its computing is not isothermal
- Need a controlled laboratory environment
- No flexibility of application
- Back-hybridization
- Program execution is limited to only a few steps
Previous techniques to address back-hybridization

- Protocol with successive transitions in one step (Sakamoto et al., 1999):
  - did not significantly increase number of steps of program execution
- PNA Mediated WPCR (Rose et al., 2001):
  - not autocatalytic
- Displacement Whiplash PCR (Rose et al., 2006):
  - not autocatalytic
Need for isothermal & autocatalytic WPCR machine

- Elimination of thermal cycles will allow more flexibility of applications
- Improve the yield of the system by minimizing back-hybridization
Isothermal Reactivating Whiplash PCR for Locally Programmable Molecular Computation

• John Reif and Urmila Majumder

• Department of Computer Science

• Duke University
Key technique to get system Isothermal: Strand Displacement

The 3' end of the single strand still encodes the current state as in original WPCR. We also tether the transition table portion of W to another stable nanostructure to prevent formation of any undesired secondary structure (Figs. 2, 3). The latter is mostly a double stranded DNA intercepted by sections of DNA that is bound to each transition rule. Since the rigidity of a double-stranded DNA is well known we use this particular nanostructure as a support in our designs.

3.1 Computing with a non-reusable rule IR-WPCR strand

Suppose we have the single strand in the form shown in Fig. 3 prior to the addition of polymerase. In Sect. 2, we will discuss how we can obtain this particular secondary structure. W.l.o.g we will assume that the 3' end of the single strand encodes for the complement of the current state \( a_i \) in rule \( R_i \). For clarity, we will refer to a figure that focuses only on the events at \( R_i \) (Figs. 4, 5).

Once \( a_i/C_3 \) binds to \( a_i \) in \( R_i \) (Fig. 5: State S1) in presence of polymerase (Fig. 5: State S2), the next state \( b_i/c_i \) is copied at the 3' end of \( W \), thus dehybridizing the \( \{w_i/y_i\}/C_3 \) portion of the protection strand \( P_i \) encoded as \( \{x_i/p_i/w_i/y_i\}/C_3 \) (Fig. 5: State S3). The \( y_i/C_3 \) portion of \( P_i \) is now free to hybridize with the \( y_i \) portion on the rule \( R_i \) that is closer to \( x_i \) (Fig. 5: State S4).

The 3' end of \( P_i \), in presence of polymerase (Fig. 5: State S5), then extends up to the stopper sequence \( S \) (shown in black filled squares in the figure), thus displacing the 3' end of Fig. 2. Left: branch migration; Right: extension of primer strand bound to the template by DNA polymerase.
Outline

✦ Original Whiplash PCR (WPCR) Machine
✦ Pros and Cons of the original WPCR Machine
✦ Our Contribution: Isothermal and Reactivating WPCR (IR-WPCR) machine
  ✦ IR-WPCR machine with non-reusable rules
  ✦ IR-WPCR machine with reusable rules
✦ Preparation Stage
✦ Proof of correctness of the system
✦ Experimental Verification Plan
✦ Conclusion
Original Whiplash PCR Machine

Reference: M Hagiya, M Arita, D Kiga, K Sakamoto and S Yokomaya,
DNA Based Computers III, pp:55-72, American Mathematical Society, 1999

$n$ rules transition table

Current state of Rule $i-1$

Next state of Rule $i-1$

Reference: M Hagiya, M Arita, D Kiga, K Sakamoto and S Yokomaya,
DNA Based Computers III, pp:55-72, American Mathematical Society, 1999

Stopper

Current State

State S1
Original Whiplash PCR Machine (Contd)

State S2

Next state copied

State S3
Original Whiplash PCR Machine (Contd)

Heat

State S4

Cool
Original Whiplash PCR Machine (Contd)

State S5

Transition from State i to State j

State S2

$a_j^* = b_i^*$
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Importance

- Allows autonomous molecular computation
- Allows parallel execution of distinct programs unlike other forms of molecular computation (e.g. tiling assembly)
  - Each WPCR machine holds its own program
  - Operation on local rules rather than global rules

Note: Tiling assembly can be made to do multiple programs in parallel if we start with a universal cellular automata tile set with different seed rows. However, it is not very practical to generate such a large tile set.
Limitations

- Requires thermal cycling and hence its computing is **not isothermal**
- *Need a controlled laboratory environment*
- *No flexibility of application*
- **Back-hybridization**
  - Program execution is limited to only a few steps
Back-hybridization is a phenomenon where a hairpin with a longer double stranded (ds) DNA region is preferentially formed over one with a shorter ds-DNA region.

Figure from Displacement Whiplash PCR: Optimized Architecture and Experimental Validation, DNA 10, LNCS 4287, pgs: 393-403, 2006
Previous techniques to address back-hybridization

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- Improve the yield of the system by minimizing back-hybridization
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**Isothermal Reactivating WPCR Machine**

- Addresses all the cons of a WPCR machine
- **Key concept:** use extension of a secondary primer by a DNA polymerase with good strand displacement capability to trigger state transition
- A non-isothermal preparation stage precedes the computation stage
- Two types:
  - IR-WPCR machine with non-reusable states
    - Prevents *back-hybridization*
  - IR-WPCR machine with reusable states
    - Original WPCR machine but isothermal
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The 30 end of the single strand still encodes the current state as in original WPCR. We also tether the transition table portion of W to another stable nanostructure to prevent formation of any undesired secondary structure (Figs. 2, 3). The latter is mostly a double stranded DNA intercepted by sections of DNA that is bound to each transition rule. Since the rigidity of a double-stranded DNA is well known we use this particular nanostructure as a support in our designs.

3.1 Computing with a non-reusable rule IR-WPCR strand

Suppose we have the single strand in the form shown in Fig. prior to the addition of polymerase. In Sect. 2, we will discuss how we can obtain this particular secondary structure. W.l.o.g we will assume that the 30 end of the single strand encodes for the complement of the current state $a_i$ in rule $R_i$. For clarity, we will refer to a figure that focuses only on the events at $R_i$ (Figs. 4, 5).

Once $a_i$ binds to $a_i$ in $R_i$ (Fig. 5: State S1) in presence of polymerase (Fig. 5: State S2), the next state $b_i$ is copied at the 30 end of W, thus dehybridizing the $w_i$ portion of the protection strand $P_i$ encoded as $x_i$ (Fig. 5: State S3). The $y_i$ portion of $P_i$ is now free to hybridize with the $y_i$ portion on the rule $R_i$ that is closer to $x_i$ (Fig. 5: State S4).

The 30 end of $P_i$, in presence of polymerase (Fig. 5: State S5), then extends up to the stopper sequence $S$ (shown in black filled squares in the figure), thus displacing the 30 end Fig. 2 Left: branch migration; Right: extension of primer strand bound to the template by DNA polymerase.
IR-WPCR Strand after preparation stage

Supporting Nanostructure

Rule i

Secondary primer strand
**Details of WPCR Strand for Isothermal execution**

- **Fig. 3** Complete WPCR Strand for isothermal and autocatalytic program execution (Rule $R_i$ on focus). Although details are provided in this figure, the emphasis is on the layout of the overall strand. In particular, note that most of the strand representing the transition rules is stabilized using a supporting DNA nanostructure and only the current state of the machine is allowed to freely bind to an appropriate rewrite rule using a lag region $W$. 

- **Details of WPCR Strand for Isothermal execution** 
  - This rule site is now completely unavailable for further hybridization and hence this protocol is called non-reusable rules IR-WPCR. 
  - The new current state $a_j/C3$ at the 30 end of $W$ then binds to $a_j$ which is the current state for transition rule $R_j$ (Fig. 5: State S7). At this stage, the next state transition begins with the polymerase binding to the head (30 end) of $W$, encoding the current state $a_j/C3$ (Fig. 5: State S2). Hence the state machine operates without thermal cycles and uses only polymerase to facilitate denaturation of the 30 of $W$ from the old rule. Moreover, each rule allows copying. 
  - Back-hybridization: transition from state $a_3$ to state $a_4$ happens as usual but for the next transition $a_4$ to $a_5$, the 30 end of the machine preferentially binds with the old transition rule. This is because $a_j/C3_3$ along with $a_j/C3_4$ at the 30 end of the machine has a longer hybridization region when bound with rewrite rule $a_3$!$a_4$ compared to when only $a_j/C3_4$ binds with the current state of the rewrite rule $a_4$!$a_5$:

Consequently, the machine is stuck in state $a_4$. 

- **Supporting Nanostructure** 

- **Fig. 4** Back-hybridization: transition from state $a_3$ to state $a_4$ happens as usual but for the next transition $a_4$ to $a_5$, the 30 end of the machine preferentially binds with the old transition rule. This is because $a_j/C3_3$ along with $a_j/C3_4$ at the 30 end of the machine has a longer hybridization region when bound with rewrite rule $a_3$!$a_4$ compared to when only $a_j/C3_4$ binds with the current state of the rewrite rule $a_4$!$a_5$:
Evaluation Stage for Non-Reusable Rules

Fig. 5 Evaluation stage for non-reusable rules IR-WPCR protocol with the focus being only on the transition rule $R_i$ to which the current state is hybridized: S1 WPCR strand $W$ with protection strand $P_i$ encoded as $(x_i p_i y_i)^*$ partially hybridized with rule $R_i$. Also the 3' end of $W$, bearing the current state $a_i^*$ is hybridized to $a_i$ of $R_i$. S2: polymerase binds to the 3' end of $W$. S3: polymerase extends $a_i^*$ to copy $b_i w_i y_i$, thus displacing $w_i^* y_i^*$ of $P_i$ from $w_i y_i$ of rule $R_i$ located further away from $x_i$ in $R_i$. S4: $y_i^*$ of $P_i$ binds to $y_i$ located next to $x_i$ in $R_i$. S5: polymerase binds with the 3' end of $P_i$. S6: 3' end of $P_i$ is extended by the polymerase to copy $z_i a_i b_i w_i y_i$, thus displacing 3' end of $W$ which has the new current state $a_j = b_i w_i y_i$. S7: 3' end of $W$ bearing $a_j^*$ binds to the $a_j$ in rule $R_j$ and the process repeats starting with the polymerase binding to the 3' end of $W$ as shown in State S2.
IR-WPCR machine with non-reusable states

DNA sequences used for removing thermal cycle

Current state of Rule i

Next state of Rule i

Current State of the machine

Secondary primer strand

(State S1)

Rest of the WPCR strand
**IR-WPCR machine with non-reusable states**

**State S2**

Next state copied while displacing the secondary primer

**State S3**
Secondary primer binds to the second best location in the neighborhood

(State S4)

(Polymerase binds to the 3’ end of bound secondary primer)

(State S5)
IR-WPCR machine with non-reusable states

Secondary primer extended to stopper displacing 3’ end of WPCR strand

(State S6)

3’ end of WPCR strand binds to appropriate rule (state transition)

(State S7)

(State S2)
IR-WPCR machine with non-reusable states

Pros & Cons

✦ **Pros of IR-WPCR with non-reusable states:**
  ✦ Prevents Back-hybridization since rule once used is not available any more
  ✦ Isothermal

✦ **Cons of IR-WPCR with non-reusable states:**
  ✦ Rule cannot be reused
  ✦ Needs redundant encodings of a rule for complex finite state machine

✦ IR-WPCR Machine with reusable states has all the power of the original WPCR machine and yet operates isothermally
Back-Hybridization

Fig. 4  Back-hybridization: transition from state $a_3$ to state $a_4$ happens as usual but for the next transition $a_4$ to $a_5$, the 3’ end of the machine preferentially binds with the old transition rule. This is because $a_3^*$ along with $a_4^*$ at the 3’ end of the machine has a longer hybridization region when bound with rewrite rule $a_3 \rightarrow a_4$ compared to when only $a_4^*$ binds with the current state of the rewrite rule $a_4 \rightarrow a_5$. Consequently, the machine is stuck in state $a_4$.
Protocol for Folding
Whiplash PCR to avoid back-hybridization

Fig. 8 Schematic of the protocol for the folding Whiplash PCR machine: S1: initial state of the WPCR strand W. S2: the solution is heated such that the next state in each rule hidden in a hairpin loop with current state of the machine being $a_i^*$. S3: polymerase binds to the 3′ end of W (bearing the current state). S4: next state $b_i^*$ is copied at the head of W by primer extension and hairpin loop is opened. S5: the mixture is heated so that W loses its hairpin structure (It may even open up the individual hairpin loops in each rule, not shown here). S6: the solution is cooled so that the head of W can bind to the new current state $b_i^* = a_j^*$ encoded at the 3′ end of the strand and the whole state transition repeats again beginning with State S2. Note that the next state in each rule is hidden in a stem loop as is the old current state encoded at the 3′ end of the WPCR strand. These two stem loop formations are key to preventing back-hybridization in this protocol.
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IR-WPCR machine with reusable states
IR-WPCR machine with reusable states

Transition from state $i$ to state $j$

$\pi^*$

$(S8)$
Summary IR-WPCR machine with reusable states

**Main Protocol for Preventing Backhybridization**

- **S1**: WPCR strand \( W \) with protection strand \( P \) encoded as \( x_i a_i z_i b_i w_i y_i \) \( \ast \) partially hybridized with rule \( R_i \). Also the 3’ end of \( W \), \( a_i^* \) is hybridized to \( a_i \) of \( R_i \).

- **S2**: The temperature of the solution is such that the 3’ part of the next state \( b_i w_i y_i \) forms part of a stem loop.

- **S3**: Polymerase binds to the 3’ end of \( W \).

- **S4**: Polymerase extends \( a_i^* \) to copy \( b_i w_i y_i \), thus displacing \( w_i / C_3 \) of \( P \) from \( w_i y_i \) of rule \( R_i \) located further away from \( x_i \) in \( R_i \). Furthermore, it opens the stem loop in which part of the next state was hidden.

- **S5**: \( y_i / C_3 \) of \( P \) binds to \( y_i \) located next to \( x_i \) in \( R_i \).

- **S6**: 3’ end of \( P \) is extended by polymerase to copy \( z_i a_i x_i \) \( \ast \) \( \ast \) \( \ast \), thus displacing 3’ end of \( W \) which has the new current state \( a_j = b_i w_i y_i \).

- **S7**: A \( a_i \) encoded \( \ast \) \( \ast \) present in the solution displaces \( \ast \) region of the extended protection strand \( P \) so that the configuration of the latter can be reset. Furthermore, at the 3’ end of the WPCR strand the old state \( a_i \) forms part of a hairpin loop \( \ast \) because of the solution temperature.

- **S8**: The next state of \( R_i \) is reset to its stem loop configuration as well. Additionally, 3’ end of \( W \) bearing \( a_i / C_3 \) binds to the \( a_j \) in rule \( R_j \). At this stage, the process repeats starting with the polymerase binding to the 3’ end of \( W \) as shown in State S2.
IR-WPCR machine with reusable states

Pros & Cons

- **Pros of IR-WPCR with non-reusable states:**
  - Isothermal
  - States reusable allowing us to build complex finite state machines

- **Cons of IR-WPCR with non-reusable states:**
  - Back-hybridization
Handling of inputs in IR-WPCR machine

- Each input can be encoded between current and next state
- Symbols in input encoded uniquely to maintain sequentiality
- External input ligated at the 3’ end of WPCR strand at the start of the corresponding state transition
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Preparation Protocol

✦ Simple Preparation Protocol
  ✦ Secondary primer hybridizes as desired since wy since longer than just y on the rule encoding

✦ Complex Preparation Protocol
  ✦ Elaborate protocol to increase the probability of desired secondary structures of WPCR strand before computation starts
DNA Complex Preparation Protocol

Fig. 6  Complex preparation protocol with respect to only rule $R_i$: $S1$ WPCR strand $W$ tethered to support (not shown in the Figure). $S2$: $(y_iz_i c_i)^*$ is added to the solution. One copy binds to the $y_i$ near $x_i$ and another binds to $y_i$ further away from it. $S3$: the copy of $(y_iz_i c_i)^*$ that binds to the $y_i$ in $R_i$ further away from $x_i$ is removed by the addition of $y_i$. The duplex thus formed is then removed from the solution using magnetic beads (not shown here). $S4$: Protection strand $P_i$ encoded as $(x_ip_i w_i y_i)^*$ is introduced and it hybridizes with the $x_i$ and free $w_i y_i$ of rule $R_i$. $S5$: the copy of $(y_i c_i)^*$ that is bound to the $y_i$ in $R_i$ nearer to $x_i$ is removed by the addition of $y_i q_i z_i$. Here too, the duplex is later removed using magnetic beads.
Complex Preparation Protocol

\[ x_i y_i z_i a_i b_i w_i y_i \]

\[(S1)\]

\[ a_i^* \]

\[ (y_i z_i c_i)^* \]

\[ x_i y_i z_i a_i b_i w_i y_i \]

\[(S2)\]

\[ a_i^* \]

\[ y_i^* \]

\[ (y_i z_i)^* \]

\[ c_i^* \]

\[ y_i^* \]

\[ c_i^* \]

\[ c_i^* \]

\[ (y_i z_i)^* \]

\[ (S3)\]
Complex Preparation Protocol (Contd)

WPCR strand ready to compute isothermally!
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Proof of Correctness of IR-WPCR Machine

Continuous Time Markov Chain for reusable rule $R_i$

A Continuous Time Markov Chain for state transition

Fig. 10 Continuous time Markov Chain for rule $R_i$ in the reusable rules IR-WPCR protocol that prevents back-hybridization using folding WPCR.
Proof of Correctness of IR-WPCR Machine

- Assume proof of correctness of the original WPCR machine
- Stochastic system: Likelihood and rate of a state transition
  - Rate of Polymerization
    - Rate formulation [Rose et al, 2001]
    - Φ-29 Rates [Saturno et al, 1995]
  - Rate of hybridization [Winfree, 1998]
  - Rate of dehybridization [Winfree, 1998]
  - Rate of strand displacement
    - 1D random walk
  - Mean time for single base migration [Thompson 1976]
Outline

✦ Original Whiplash PCR (WPCR) Machine
✦ Pros and Cons of the original WPCR Machine
✦ Our Contribution: Isothermal and Reactivating WPCR (IR-WPCR) machine
  ✦ IR-WPCR machine with non-reusable rules
  ✦ IR-WPCR machine with reusable rules
✦ Preparation Stage
✦ Proof of correctness of the system
✦ Experimental Verification Plan
✦ Conclusion
Encode a 3 state machine in an IR-WPCR strand

\[ x_1 - y_1 - z_1 - a_1 - b_1 - w_1 - y_1 - S - x_2 - y_2 - z_2 - a_2 - b_2 - w_2 - y_2 - S - S' - a_1^* \]

**Two experiments**: to verify both transitions happen using FRET (molecular beacon technique)
Validate first transition

- Encode only first rule in the WPCR strand
- Encode a molecular beacon as $h(b_1w_1y_1)h^*$ with a fluorophore and quencher at the two ends (hybridized to WPCR strand and emitting signal)
- When next state is copied molecular beacon is released and forms a hairpin, thus quenching the fluorescence
- Other transition can be validated similarly
Summary

- Isothermal Reactivating WPCR machine
  - uses extension of a secondary primer by a DNA polymerase with good strand displacement capability to trigger state transition
- IR-WPCR machine with non-reusable states
  - prevents back-hybridization
- IR-WPCR with reusable states
  - similar to original WPCR machine but isothermal
- Proof of correctness of IR-WPCR machine
- Experimental verification plan using molecular beacons and polymerase Φ-29