Whiplash PCR

History:

- Invented by Japanese researchers: Hagiya et al [1997]

- Improved (and renamed to Whiplash PCR) by Erik Winfree [1998]

- Made Isothermal by John Reif and Urmia Majumder [2008]
Polymerization Reaction

Primer Extension via Polymerase

extension of primer strand bound to the template by DNA polymerase
Polymerase Chain Reaction (PCR): 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 do computation 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.

**To limit polymerization:**
Uses stopper sequence that stops polymerase extension (in non-strand-displacing polymerase, can be a hybridized sequence).
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 3' 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 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 $\langle x_i; y_i; z_i; a_i; b_i; w_i; y_i \rangle$ 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

M Hagiya, M Arita, D Kiga, K Sakamoto and S Yokomaya, DNA Based Computers III, pp.55-72, American Mathematical Society, 1999
Finite State Machine
Original Whiplash PCR

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

To limit polymerization:
Uses stopper sequence that stops polymerase extension
(in non-strand-displacing polymerase, can be a hybridized sequence).

n rules transition table

Current state of Rule i-1

Next state of Rule i-1

Current State

State $S_1$
Original Whiplash PCR (Contd)

State $S_2$

Next state copied

State $S_3$
Original Whiplash PCR (Contd)

Heat

Cool

State $S_4$
Original Whiplash PCR (Contd)

$\text{State } S_5$

Transition from State $S_i$ to State $S_j$

$\text{State } S_2$
Importance of WPCR

- 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...
Limitations of WPCR

- Requires thermal cycling and hence its computing is **not isothermal**
  - *Need a controlled laboratory environment*
  - *No flexibility of application*

- **Back-hybridization problem**
  - 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
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 $a_4$. 
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 Urmi Majumder

• Department of Computer Science

• Duke University

Key technique to get system isothermal:
Use Strand Displacing Polymerase
Strand Displacing Polymerase Polymerase

Target Generated containing engineered restriction enzyme site

Bumper Primer binds and displaces strand generated by restriction engineered primer

Restriction Enzyme cleaves primer

Donna C. Sullivan, Division of Infectious Diseases, University of Mississippi
Outline

- 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
- Conclusion
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 WPCR Machine

- Addresses all the cons of a WPCR machine
- **Key concept:** use extension of a secondary primer for a DNA polymerase with good strand displacement capability to trigger state transitions
- 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
**IR-WPCR machine with non-reusable states**

- DNA sequences used for removing thermal cycle
- Secondary primer strand
- Rest of the WPCR strand
- (State S1)
- Current State of the machine
- Rule i
- Current state of Rule i
- Next state of Rule i
IR-WPCR machine with non-reusable states

(State S2)

Next state copied while displacing the secondary primer

(State S3)
IR-WPCR machine with non-reusable states

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 S2)
preparation stage for IR-WPCR Strand

To limit polymerization by strand-displacing polymerase:
Uses stopper sequence ■ that stops polymerase extension
(can be a G-quadruplex that forms a very hard-to-displace nanostructure).
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 $W$. 

$WPCR$ Strand

Supporting Nanostructure

$R_i$
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 $b_iw_iy_i$, thus displacing $w_i^*y_i^*$ of $P_i$ from $w_i^*$ 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 $a_j = b_iw_iy_i$. $S7$: 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

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.
IR-WPCR machine with reusable states
IR-WPCR machine with reusable states

Transition from state i to state j

(S2)

(S8)
Summary IR-WPCR machine with reusable states

- **WPCR** machine with reusable states forms a stem loop and the protection strand \( P_i \) hybridizes with \( x_i \) and \( w_i y_i \). W.l.o.g., assume that the current state is \( a_i \) and thus the 3' end of the WPCR strand binds to rule \( R_i \). When the polymerase binds to the 3' end of the WPCR strand, it opens up the stem loop hiding part of the next state encoding in this rule. The 3' end of the WPCR strand is extended as far as the first stopper sequence in rule \( R_i \). This event, in turn, displaces the 3' end of the protection strand \( P_i \). The latter now binds to its second best match in rule \( R_i \) namely \( y_i \). Polymerase now binds to the 3' end of \( P_i \) and extends it to displace the 3' end of the WPCR strand (this end has the new current state of the machine, \( b_i w_i y_i \) encoded in it). This event marks the completion of a state transition. An auxiliary strand already in solution, then resets rule \( R_i \) to its original configuration. However, the only difference between this protocol and the reusable rule IR-WPCR machine that cannot prevent back-hybridization is that the former uses folding.
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
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
**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: $(y_i z_i c_i)^*$ 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 $(y_i z_i c_i)^*$ 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 $(x_i p_i w_i y_i)^*$ is introduced and it hybridizes with the $x_i$ and free $w_i y_i$ of rule $R_i$. S5: the copy of $(y_i z_i c_i)^*$ 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

\[ x_i y_i z_i a_i b_i w_i y_i \]

\[ (\bar{y}_i \bar{z}_i \bar{c}_i)^* \]

\[ (S1) \]

\[ x_i y_i z_i a_i b_i w_i y_i \]

\[ (\bar{y}_i \bar{z}_i \bar{c}_i)^* \]

\[ (S2) \]

\[ x_i y_i z_i a_i b_i w_i y_i \]

\[ (\bar{y}_i \bar{z}_i \bar{c}_i)^* \]

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

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

Continuous Time Markov Chain for reusable rule $R_i$

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]
Experimental Verification Plan

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 $h(b\|w\|y)h^*$ 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