Whiplash PCR

Whiplash PCR: A DNA Computation method for do Finite State Computation using DNA Hybridization & Polymerase Enzyme

History:

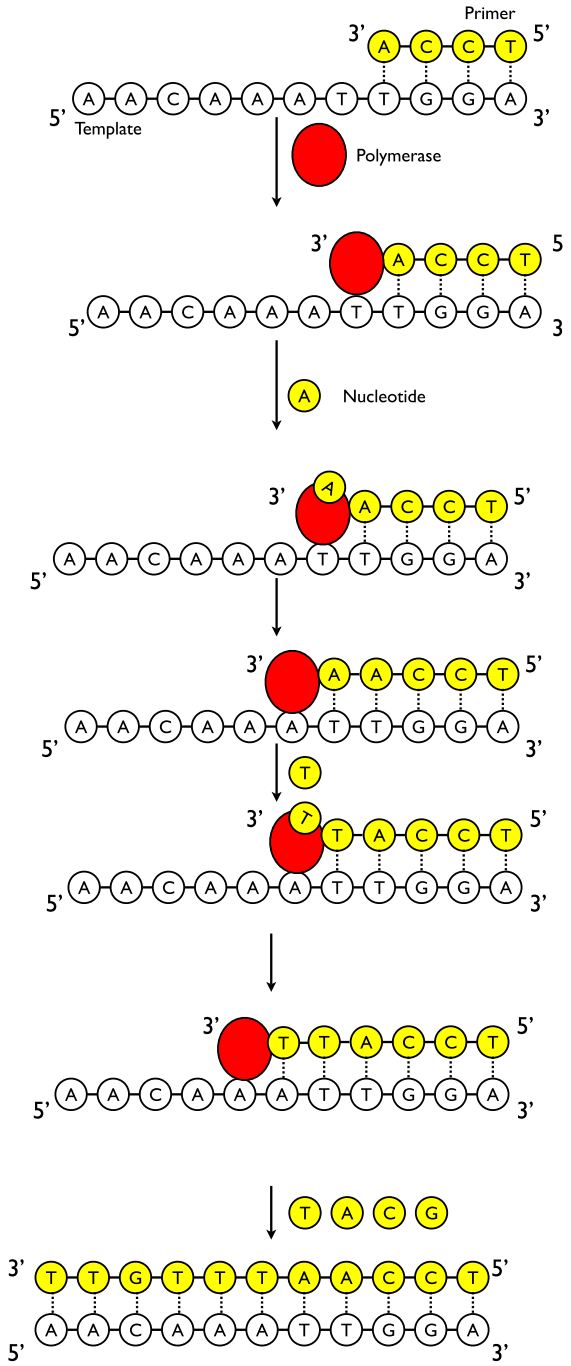
- Invented by Japanese researchers: Hagiya et all [1997]

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

- Made Isothermal by John Reif and Urmi Majumder [2008]

Review of Polymerization Reaction

Primer Extension via Polymerase



extension of primer strand bound to the template by DNA polymerase

Review of PCR

Polymerase Chain Reaction (PCR): a protocol used to amplify a template strand.

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

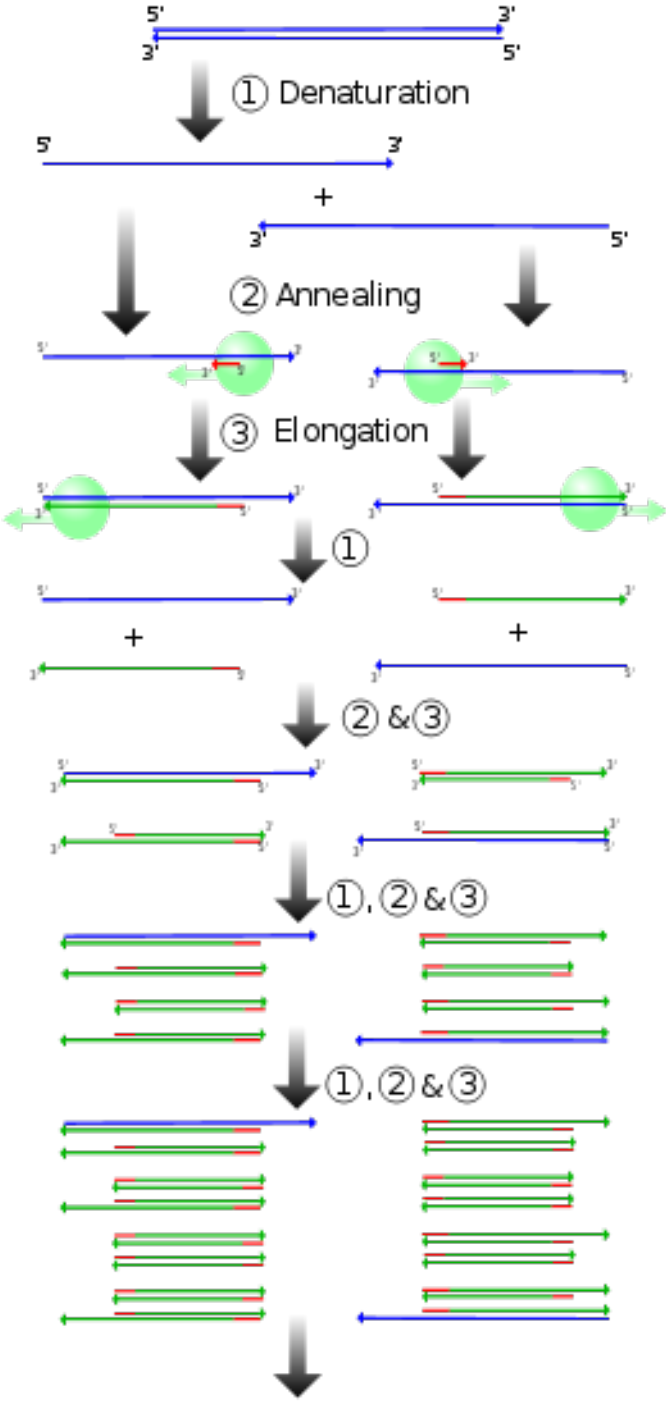
At temperature t_1:

- 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

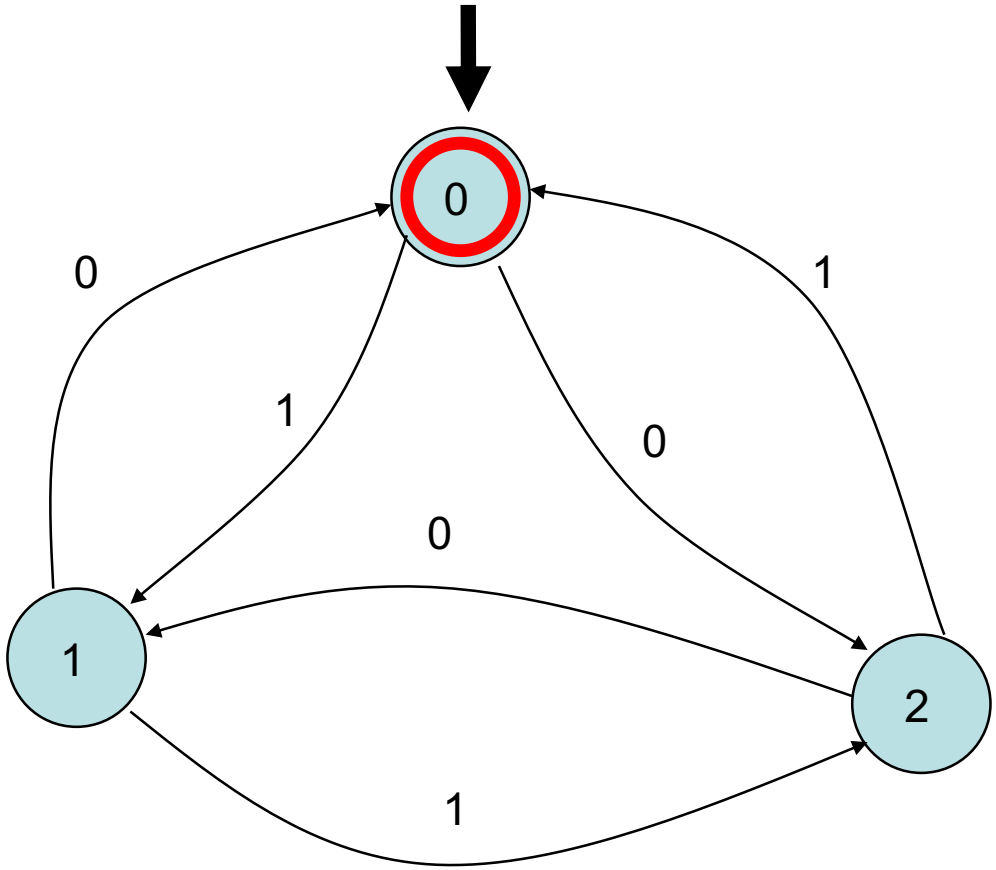
At temperature t_2:

- 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

Example of a Finite State Machine



Whiplash PCR (WPCR)

Whiplash PCR is a protocol used do Finite State Computation using a single strand S of single stranded DNA consisting of n pairs of a primer sequence and a 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 so a further stage of Whiplash PCR can be performed.

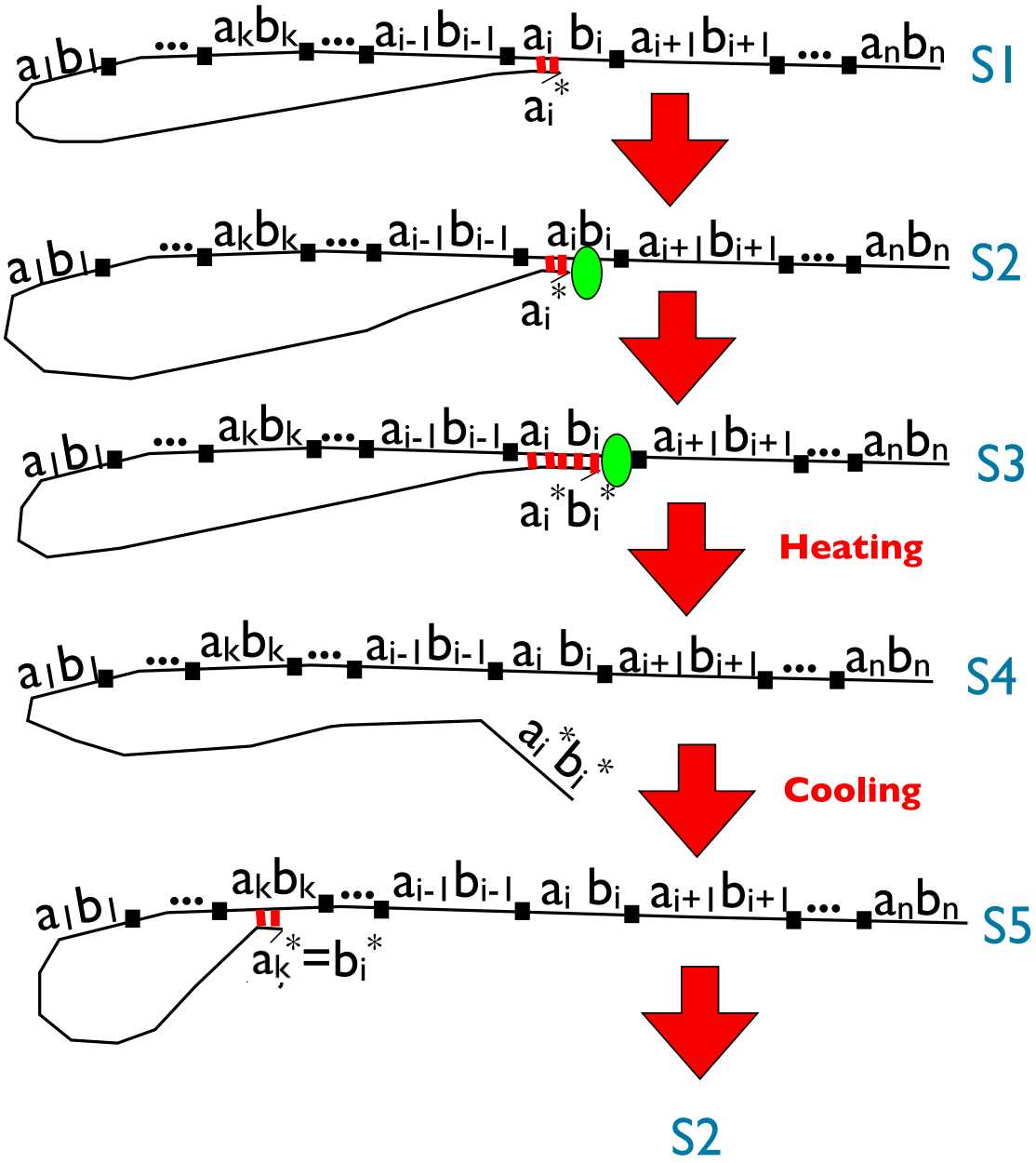
To limit polymerization:

- Use a stopper sequence ■ that stops polymerase extension

(eg, ■ is a G-Complex consisting of a series of consecutive G bases).

Whiplash PCR (WPCR)

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



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

Whiplash PCR (WPCR)

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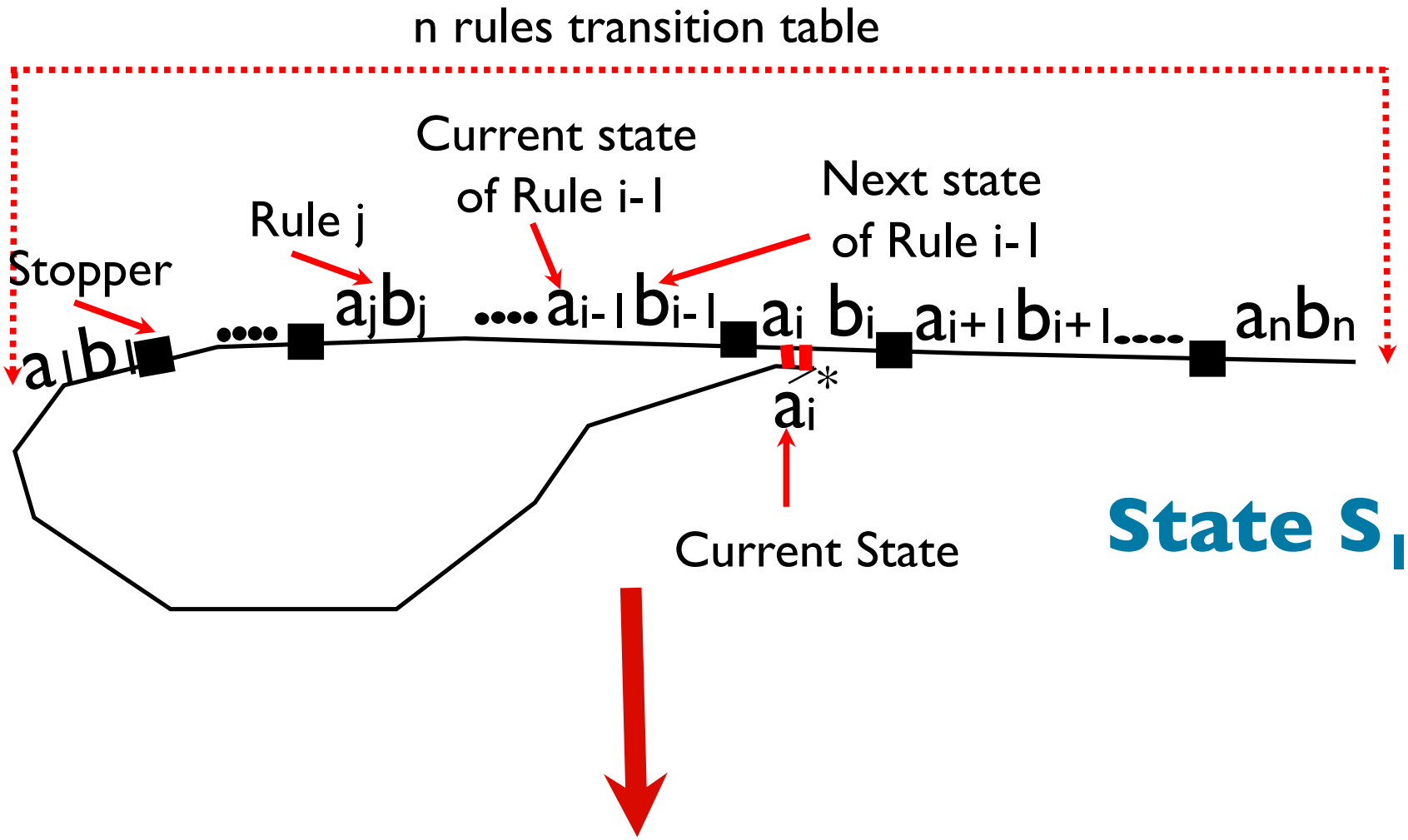
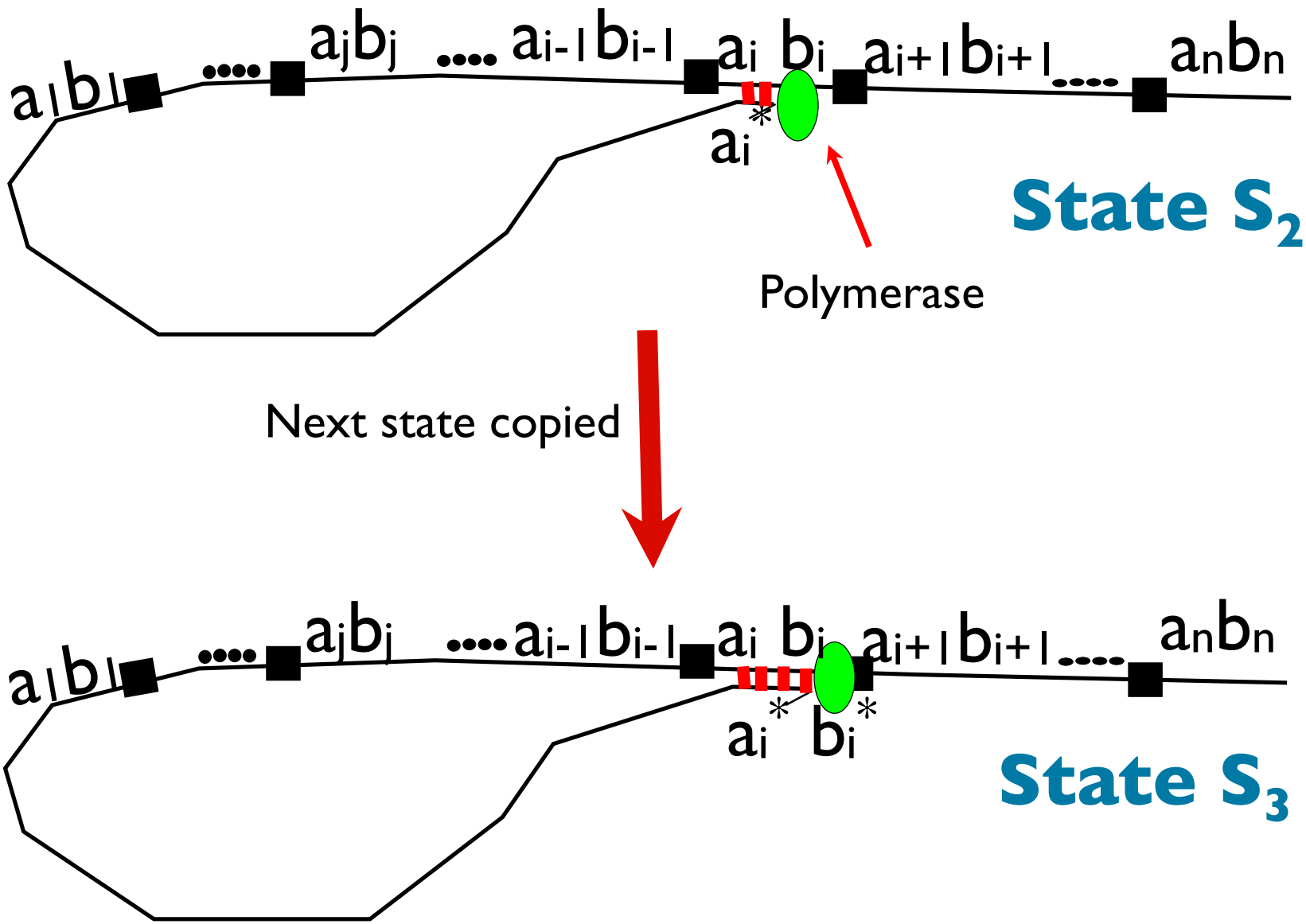
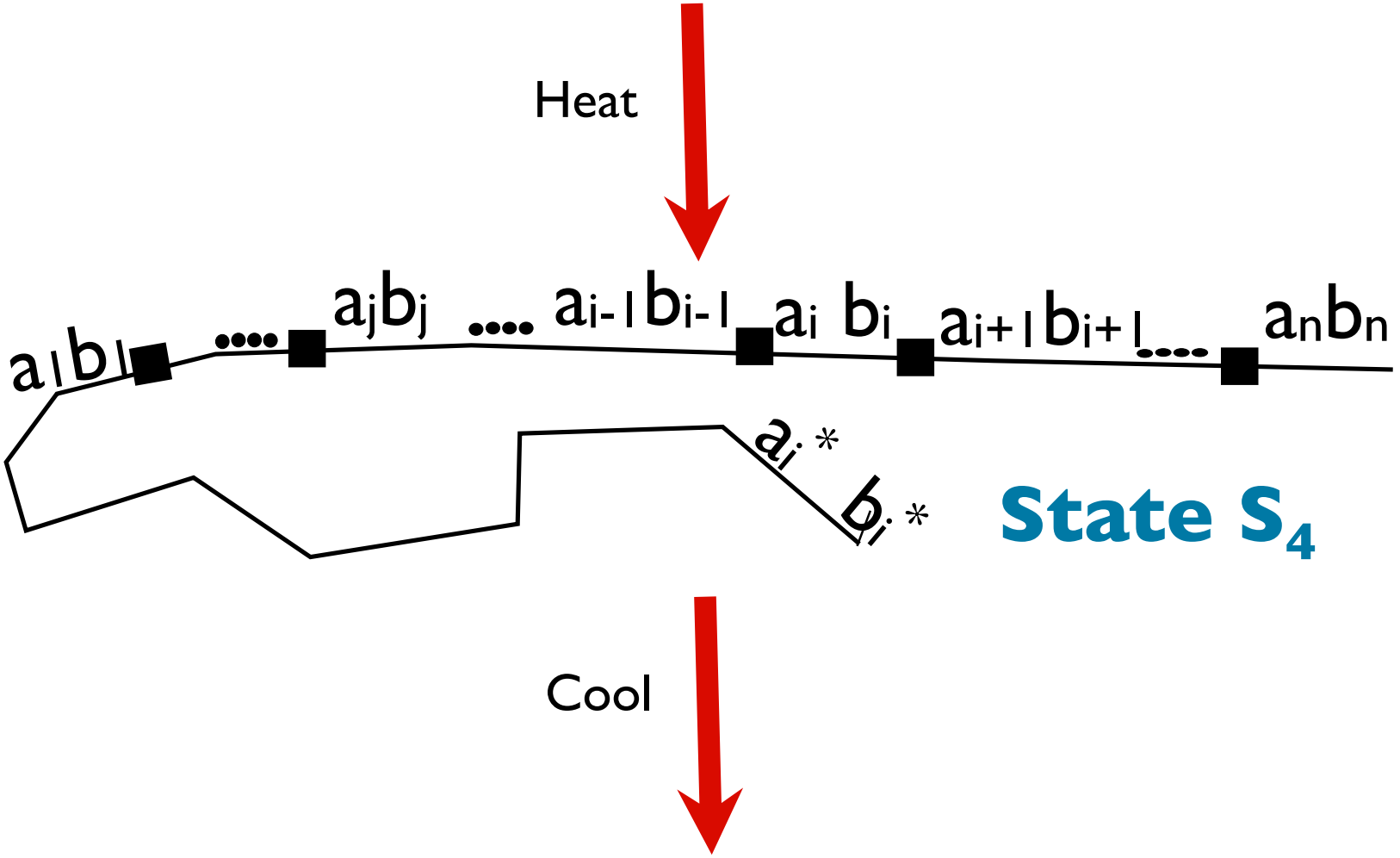
To limit polymerization:
Uses stopper sequence ■ that stops polymerase extension



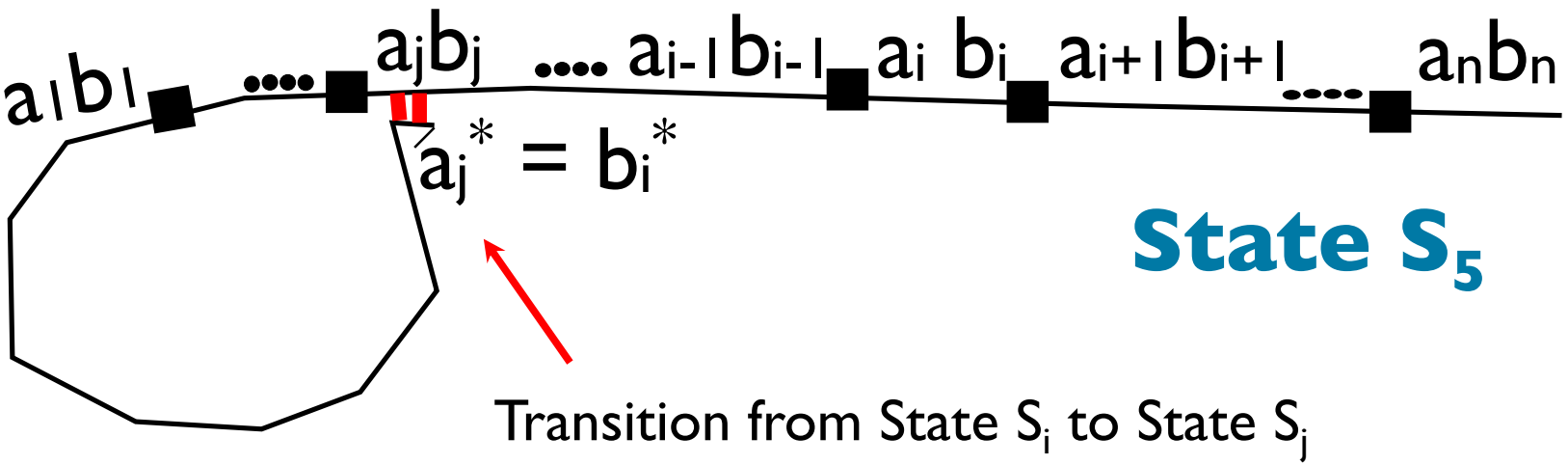
Whiplash PCR (WPCR), Continued



Whiplash PCR (WPCR), Continued



Whiplash PCR (WPCR), Continued



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):

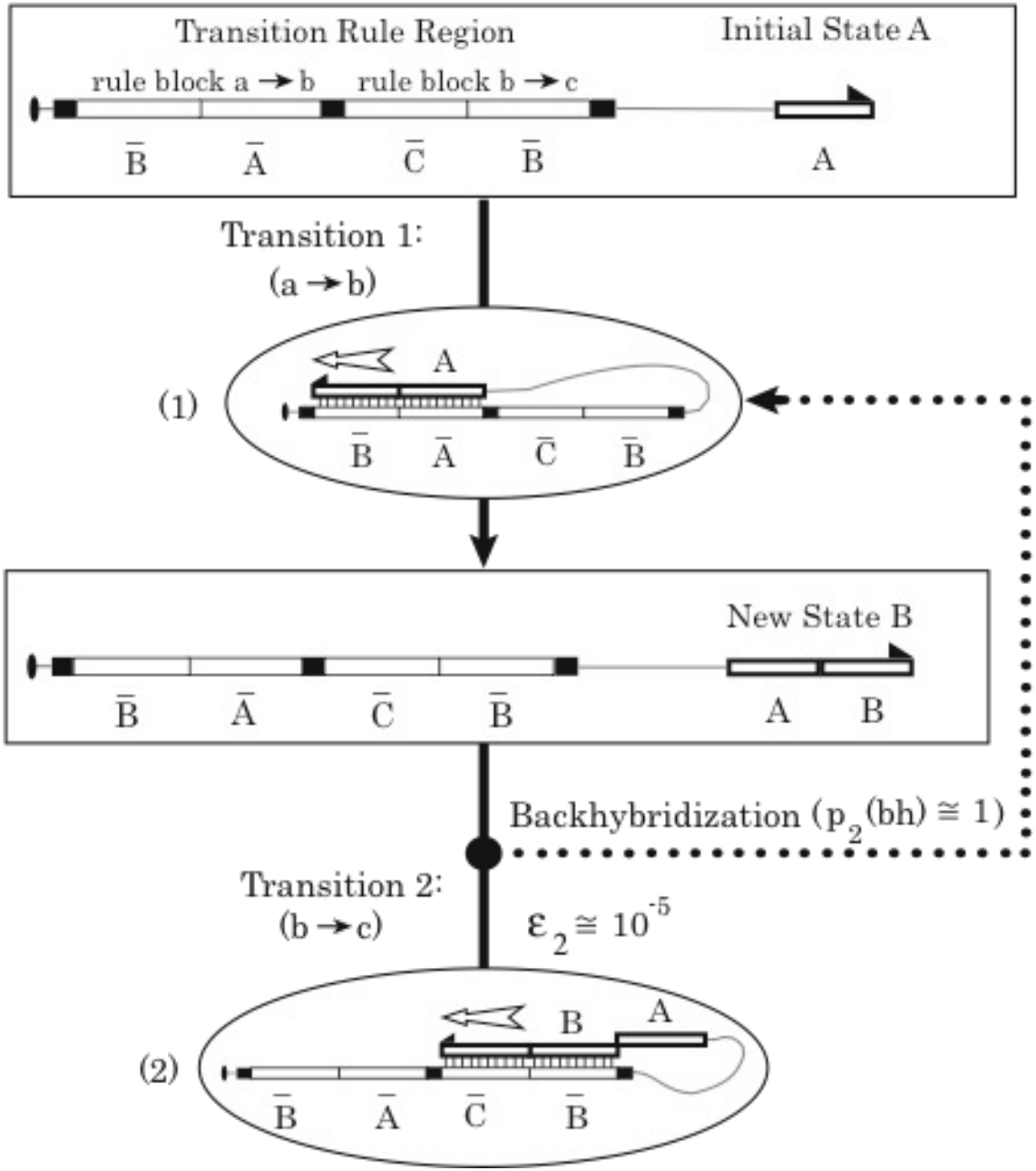
- *Each WPCR machine holds its own program*
- *Operation on local rules rather than global rules*

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

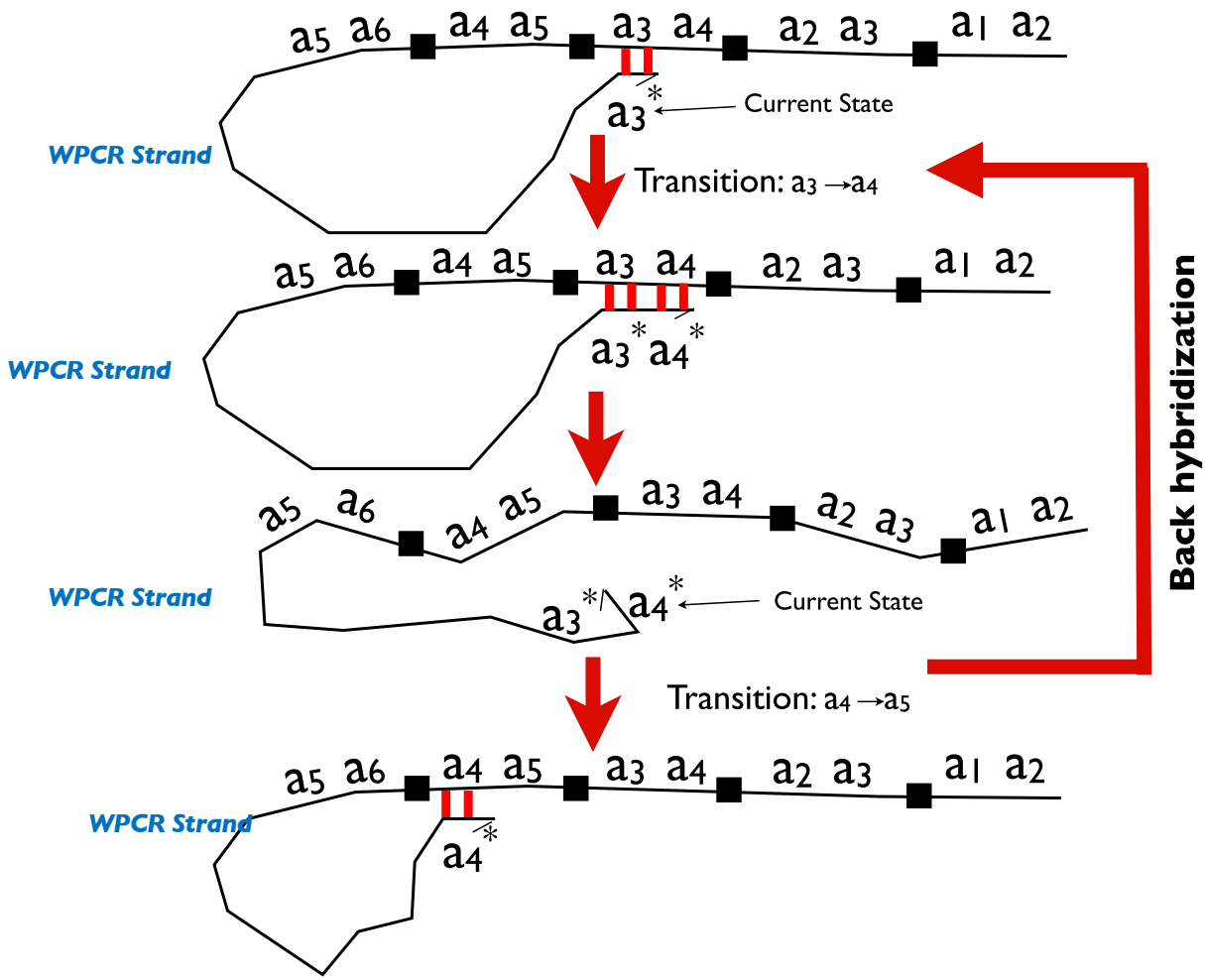
- Requires thermal cycling and hence its computing is **not isothermal**
- *Need a controlled laboratory environment*
- *No flexibility of application*
- **Back-hybridization problem**
- Program execution is limited to only a few steps

Back-hybridization in WPCR



Back-hybridization in WPCR 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.

Back-hybridization in WPCR



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 in WPCR

- 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

Isothermal Reactivating Whiplash PCR (IR-WPCR) for Locally Programmable Molecular Computation

- John Reif and Urmi Majumder
- Department of Computer Science
- Duke University

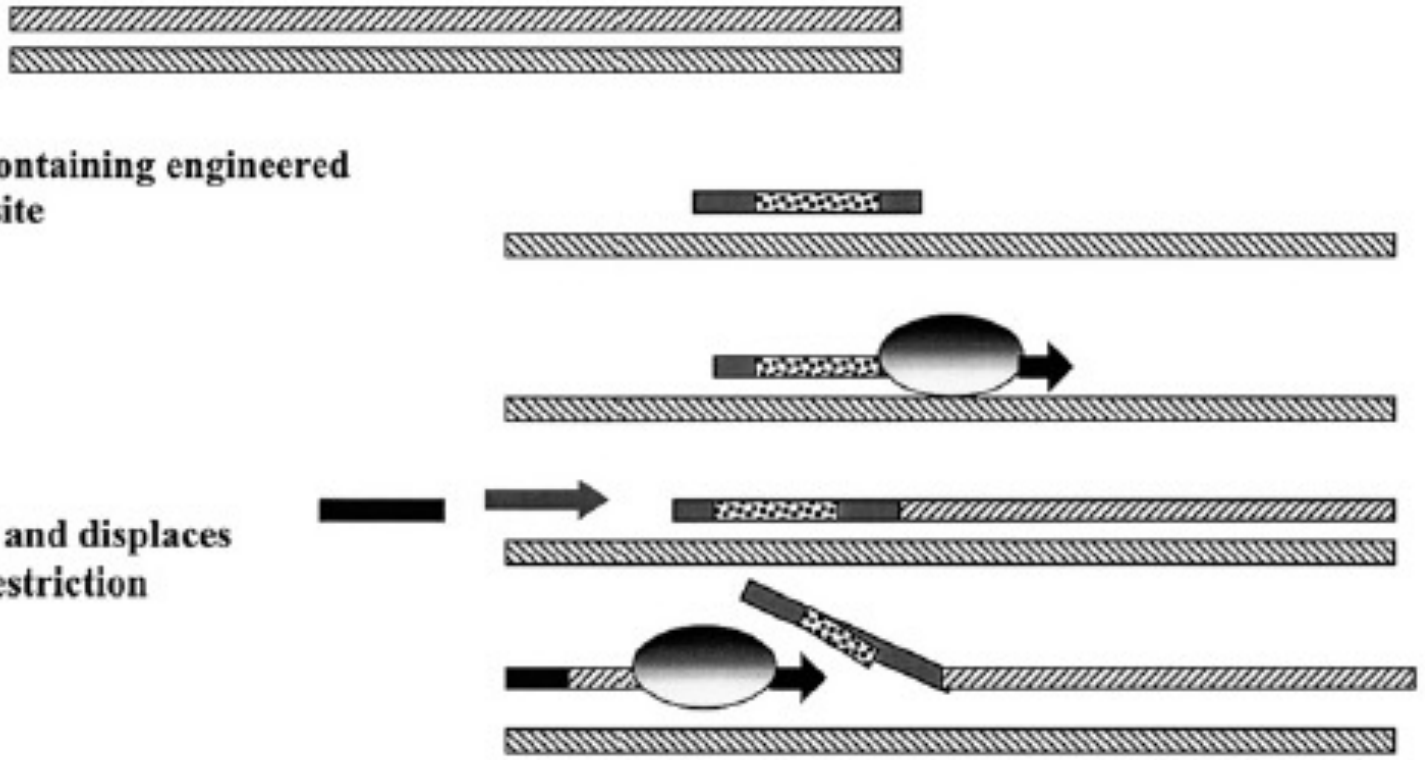
John H. Reif and Urmi Majumder, Isothermal Reactivating Whiplash PCR for Locally Programmable Molecular Computation, Natural Computing, Vol. 9, Number 1, pp. 183-206, (March 2010). Springer Netherlands, ISSN: 1567-7818 DOI: 10.1007/s11047-009-9148-6

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**

Key technique
to get system
Isothermal:
Use Strand Displacing Polymerase

Review of Strand Displacing Polymerase Polymerase



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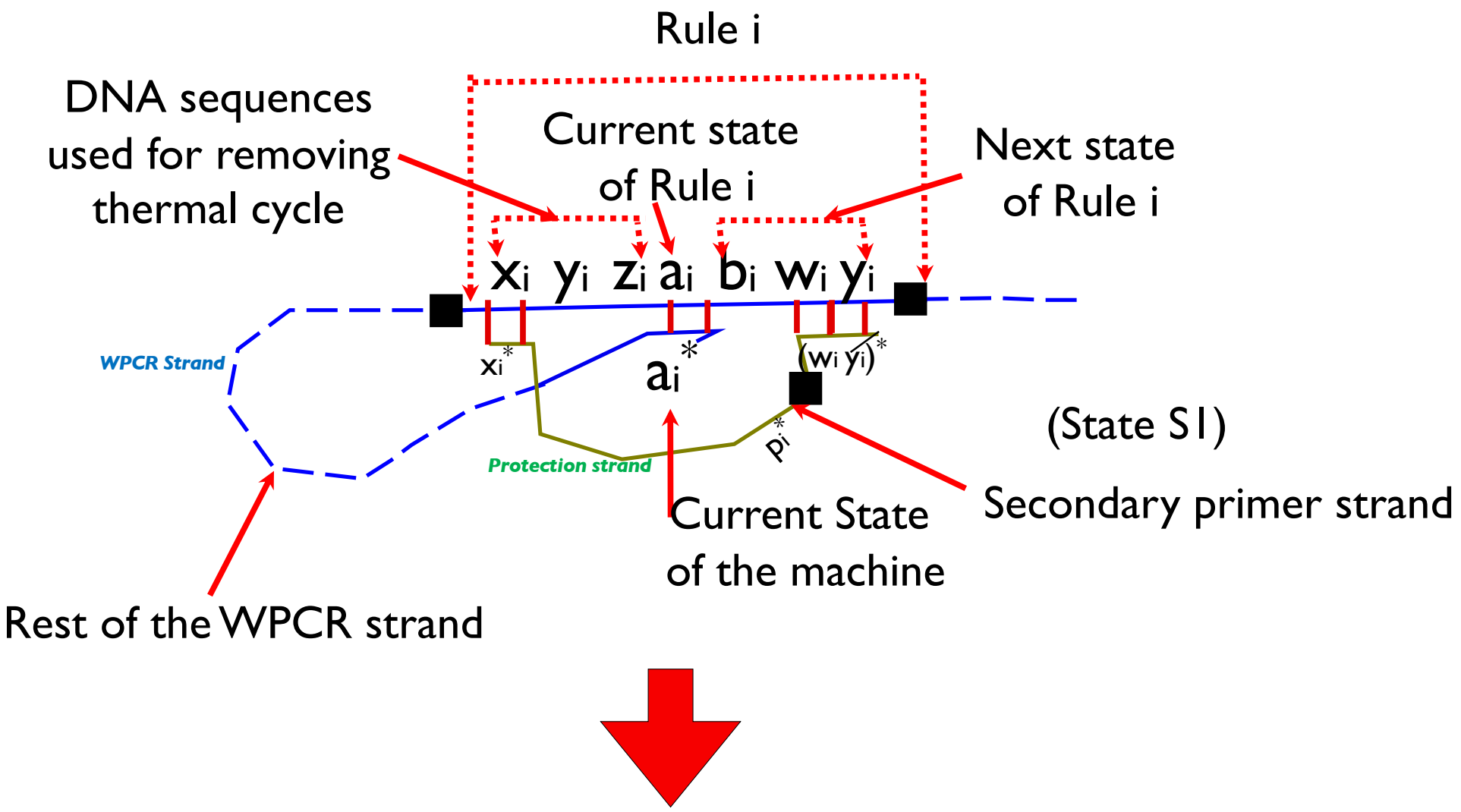
Isothermal Reactivating WPCR (IR-WPCR)

- uses extension of a secondary primer by a DNA polymerase with good strand displacement capability to trigger state transition
- First describe IR-WPCR with non-reusable states
  - prevents back-hybridization
- Next describe IR-WPCR with reusable states
  - similar to original WPCR machine but **isothermal**
- Proof of correctness of IR-WPCR machine

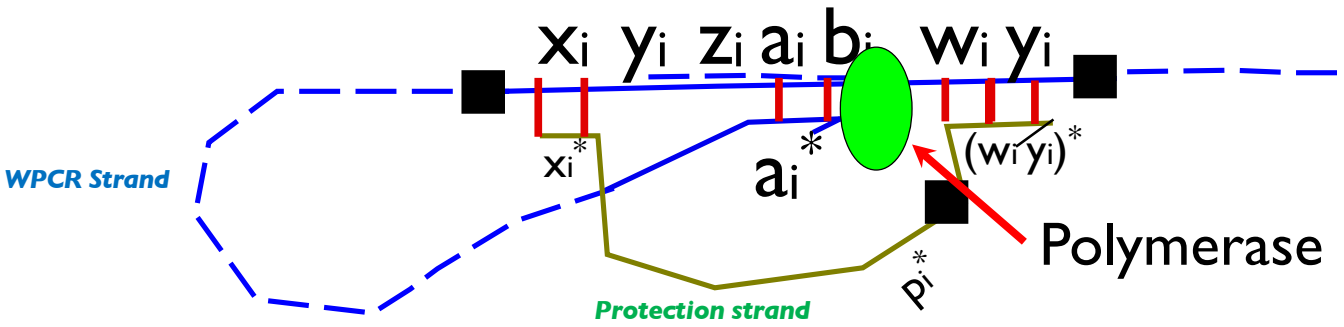
Isothermal Reactivating WPCR (IR-WPCR)

- Addresses all the cons of a WPCR
- **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

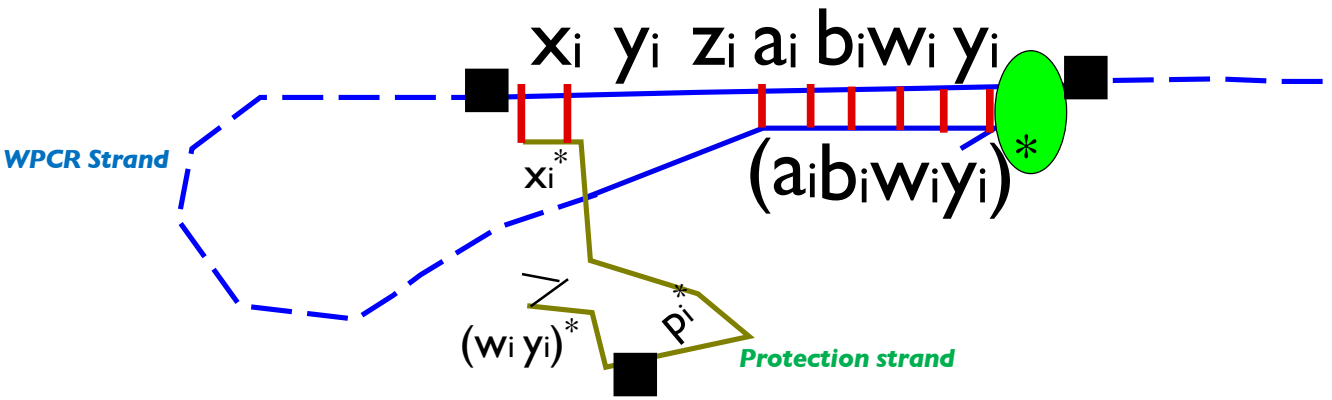


IR-WPCR machine with non-reusable states



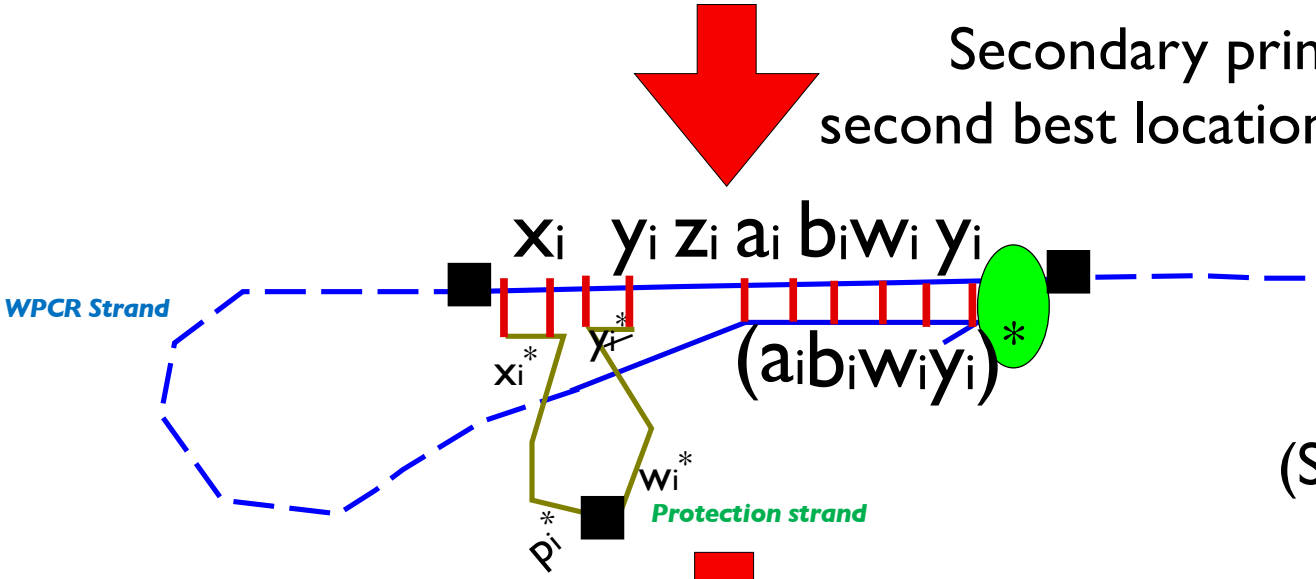
(State S2)

Next state copied while displacing the secondary primer

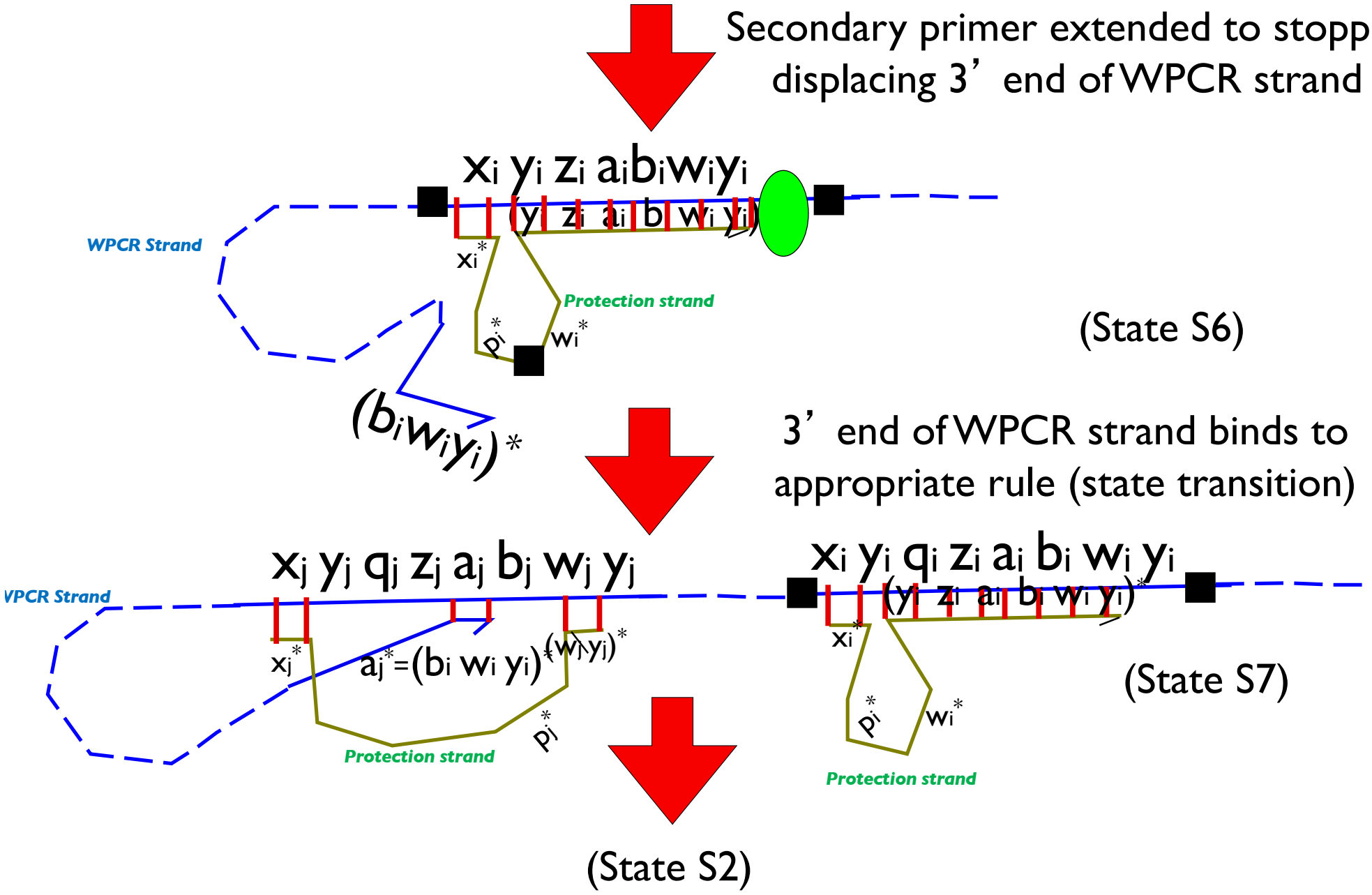


(State S3)

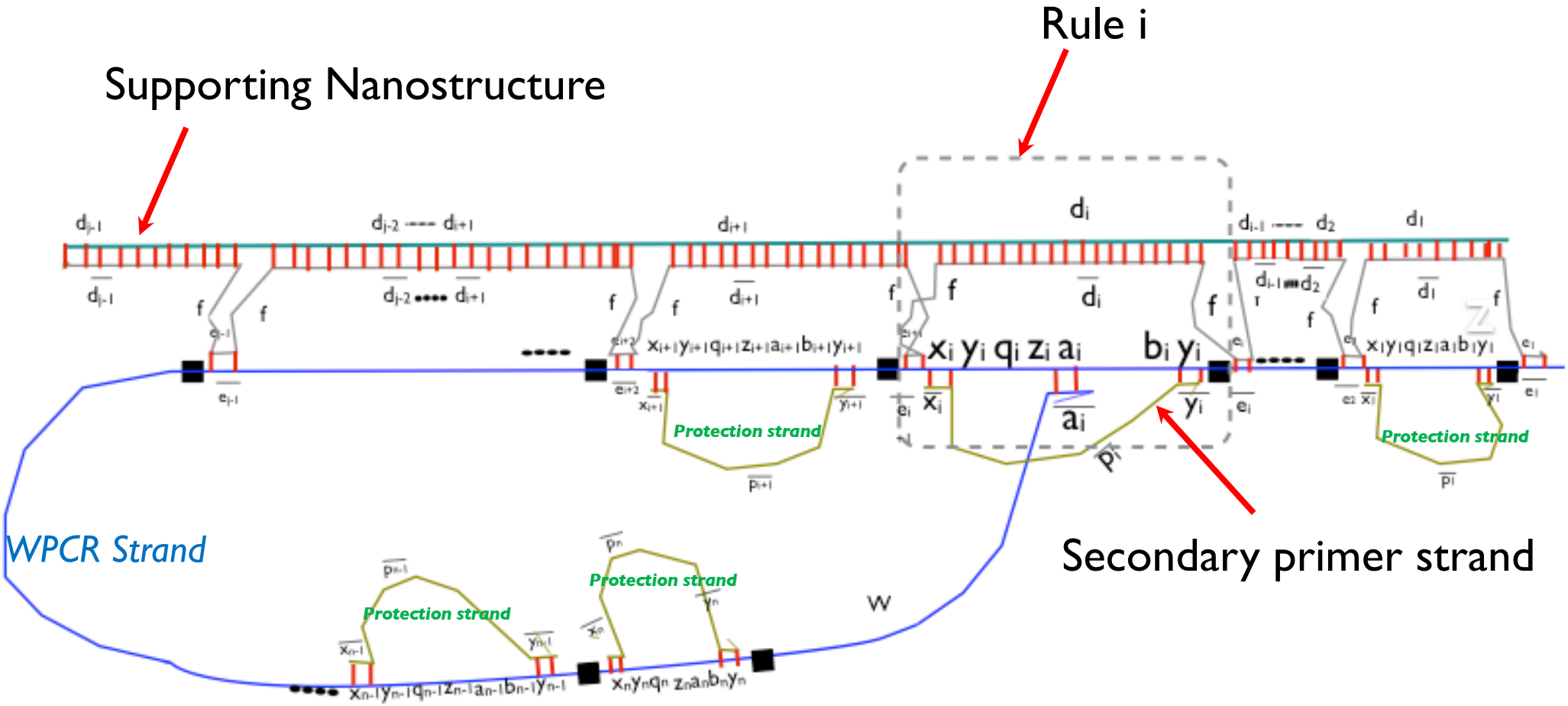
IR-WPCR machine with non-reusable states



IR-WPCR machine with non-reusable states

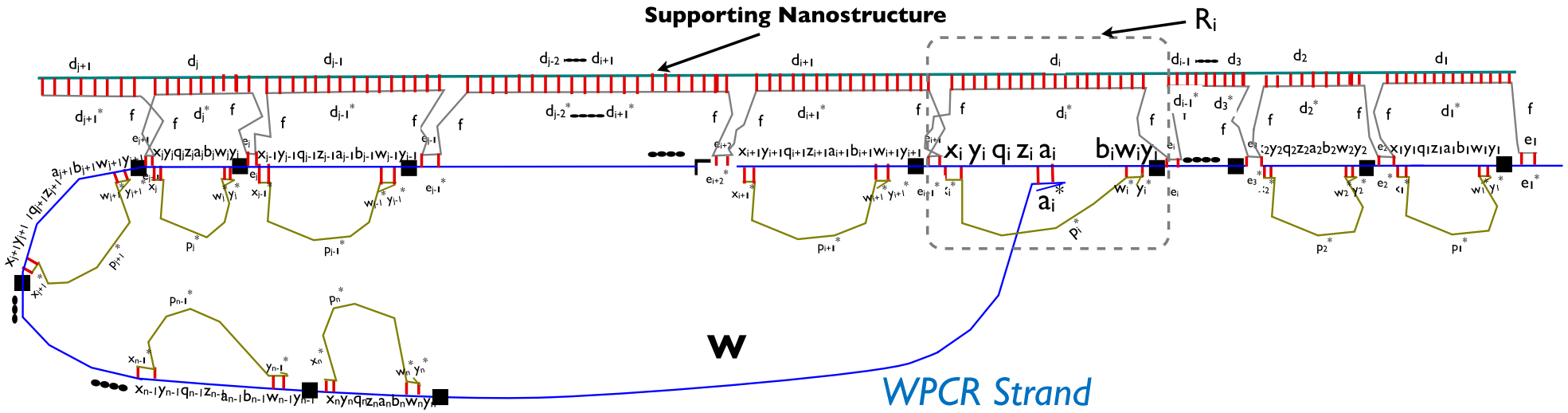


preparation stage for IR-WPCR Strand



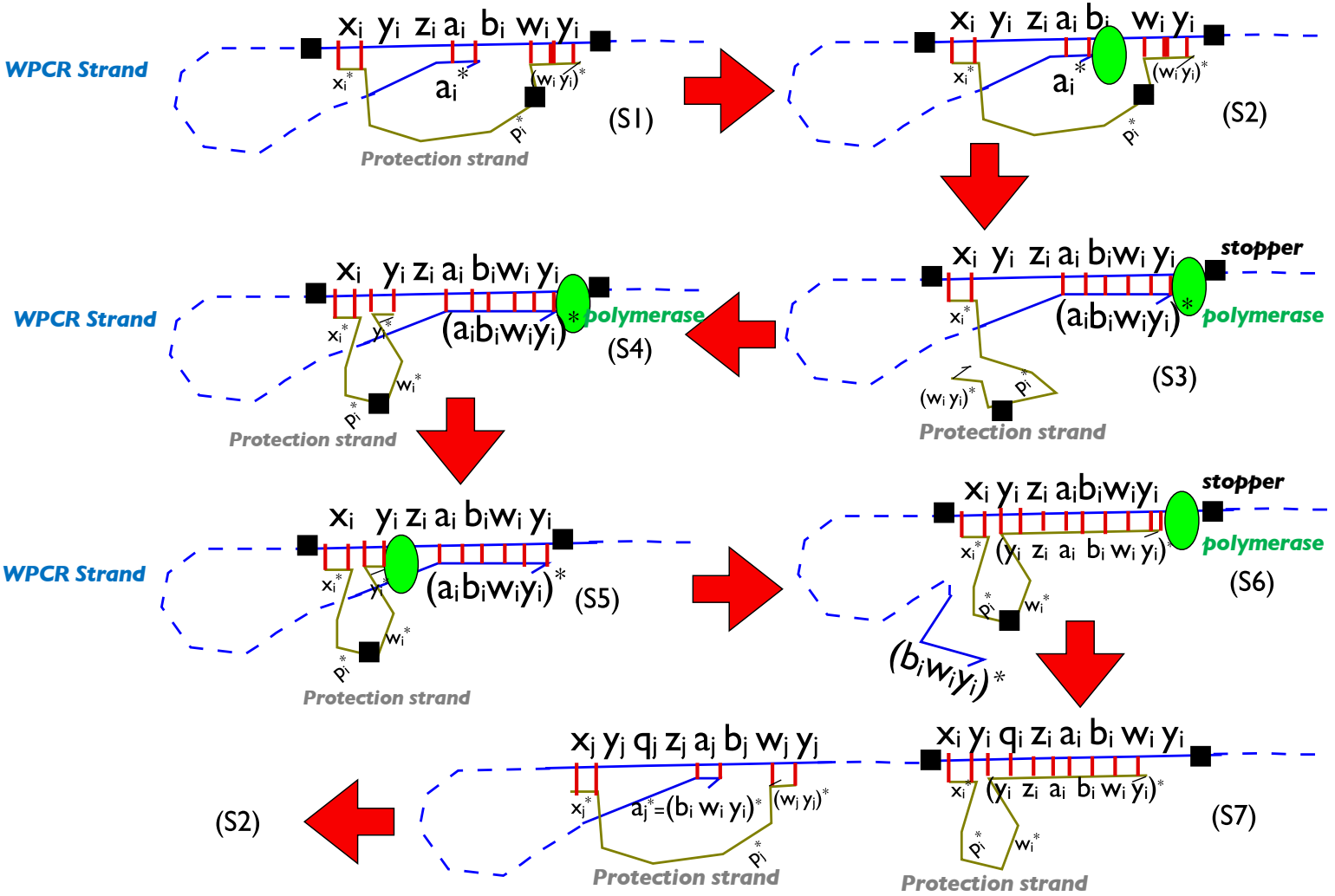
To limit polymerization by strand-displacing polymerase:
Uses stopper sequence ■ that stops polymerase extension
(can be a G-quadraplex that forms a very hard-to-displace nanostructure).

Details of WPCR Strand for Isothermal execution



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

Evaluation Stage for Non-Reusable Rules

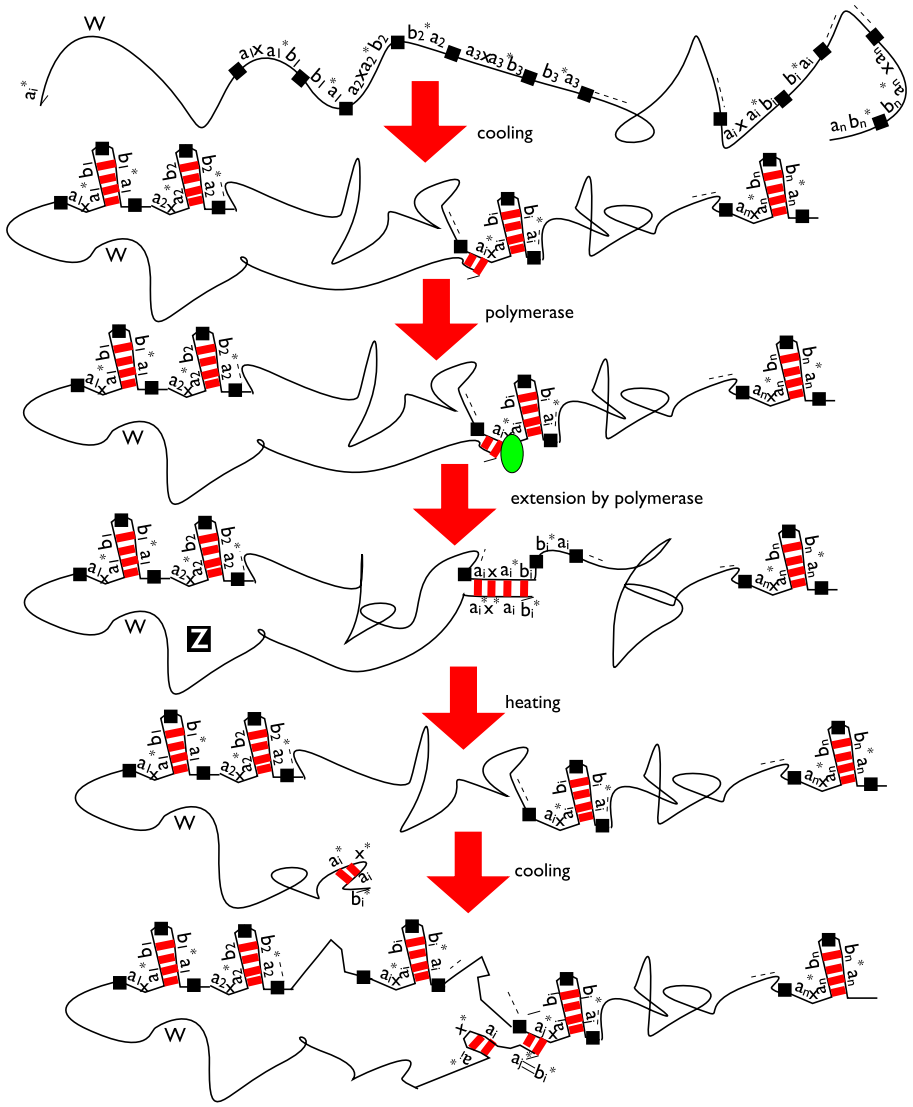


Evaluation stage for non-reusable rules IR-WPCR protocol with the focus being only on the transition rule R_i to which the current state is hybridized: *S1* WPCR strand W with protection strand P_i encoded as $(x_i p_i y_i)^*$ partially hybridized with rule R_i. Also the 3′ end of W, bearing the current 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 with non-reusable states Pros & Cons

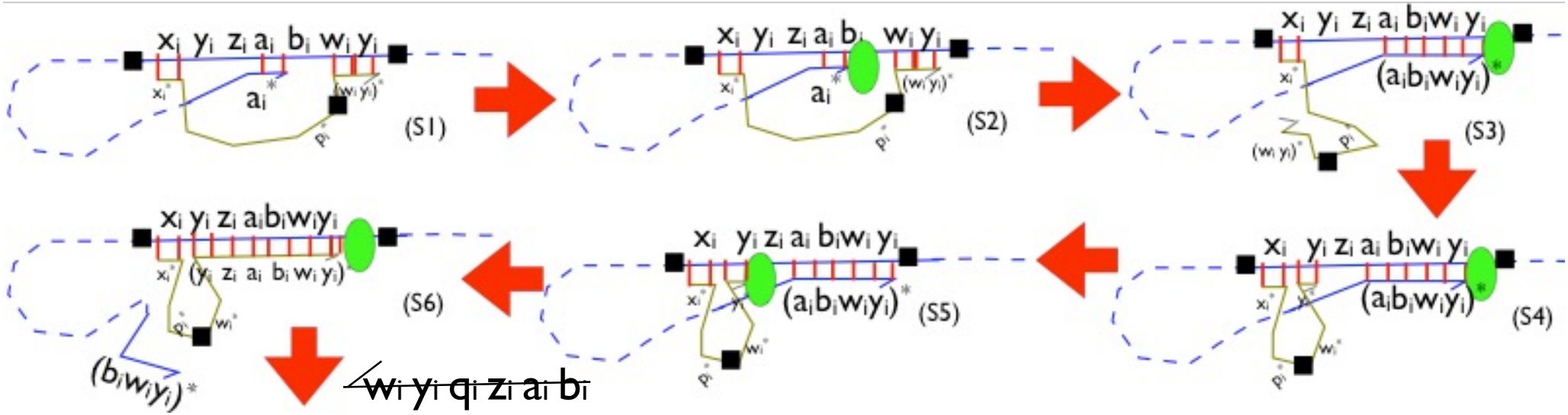
- **Pros of IR-WPCR with non-reusable states:**
  - Prevents Back-hybridization since rule once used is not available any more
  - Is Isothermal
- **Cons of IR-WPCR with non-reusable states:**
  - Rule cannot be reused
  - Needs redundant encodings of a rule for complex finite state machine
- IR-WPCR Machine with reusable states has all the power of the original WPCR machine and yet operates **isothermally**

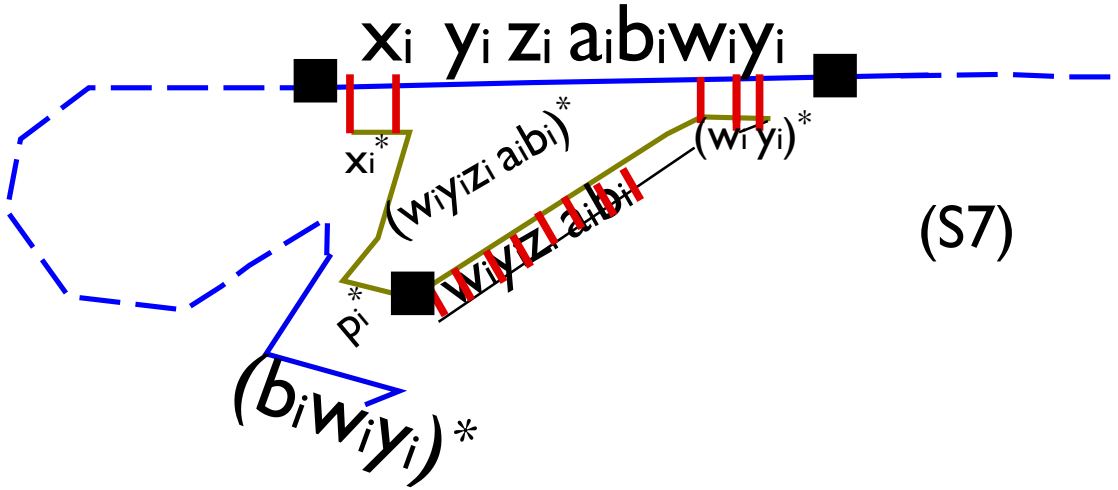
Protocol for Folding Whiplash PCR to avoid back-hybridization



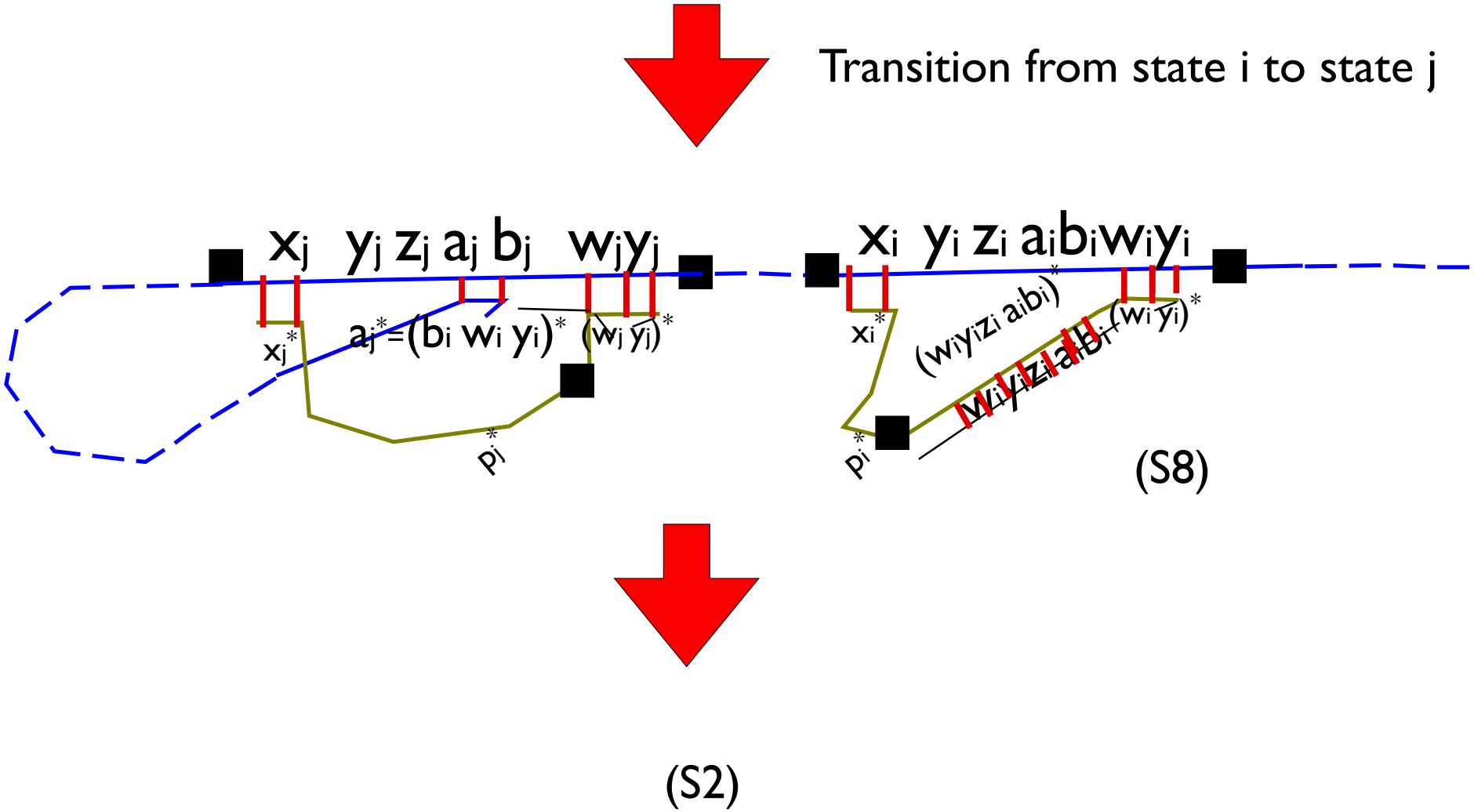
Schematic of the protocol for the folding Whiplash PCR machine: *S1*: initial state of the WPCR strand *W*. *S2*: the solution is heated such that the next state in each rule hidden in a hairpin loop with current state of the machine being a_i^*. *S3*: polymerase binds to the 3′ end of *W* (bearing the 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

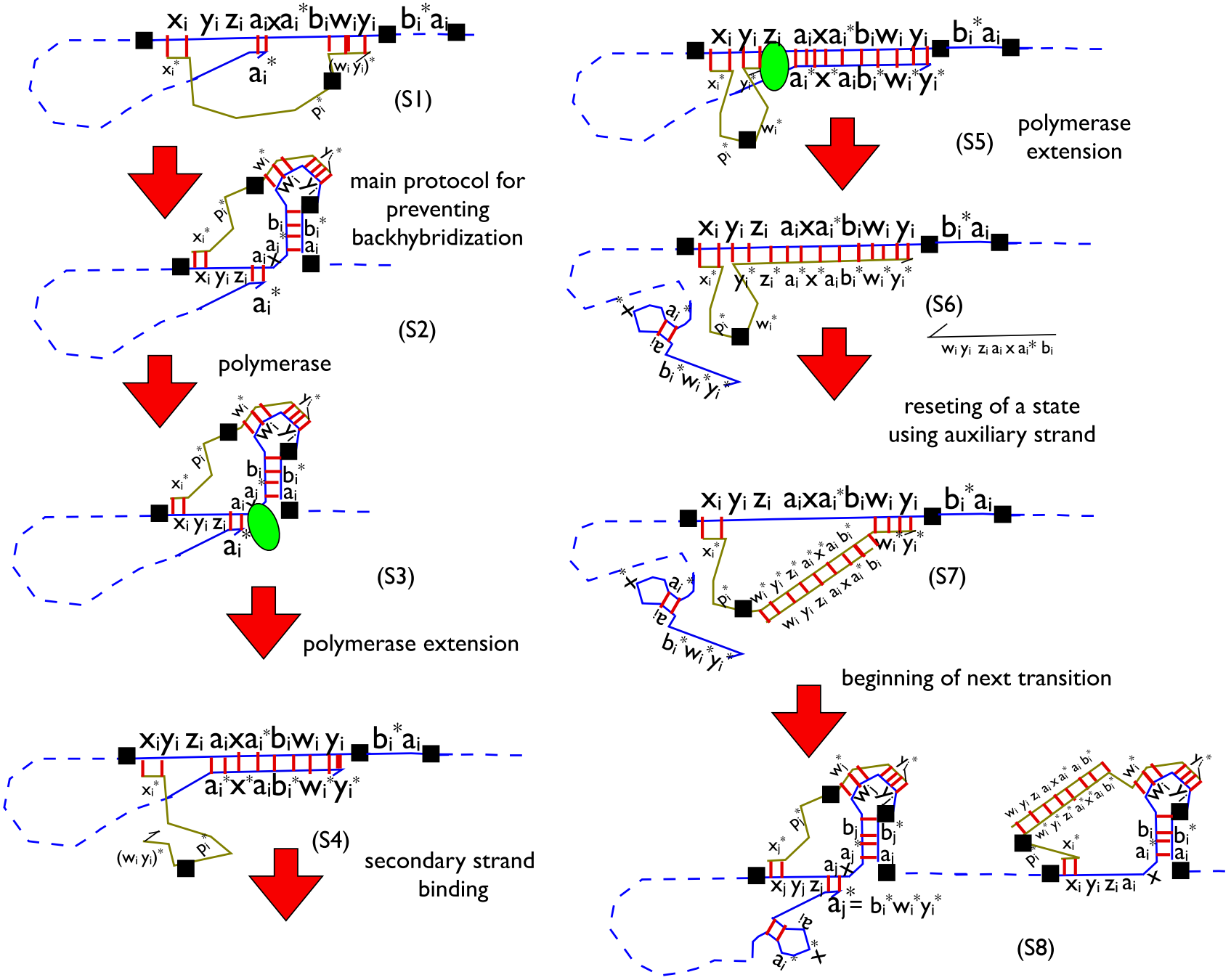




IR-WPCR machine with reusable states



Summary IR-WPCR machine with reusable states



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

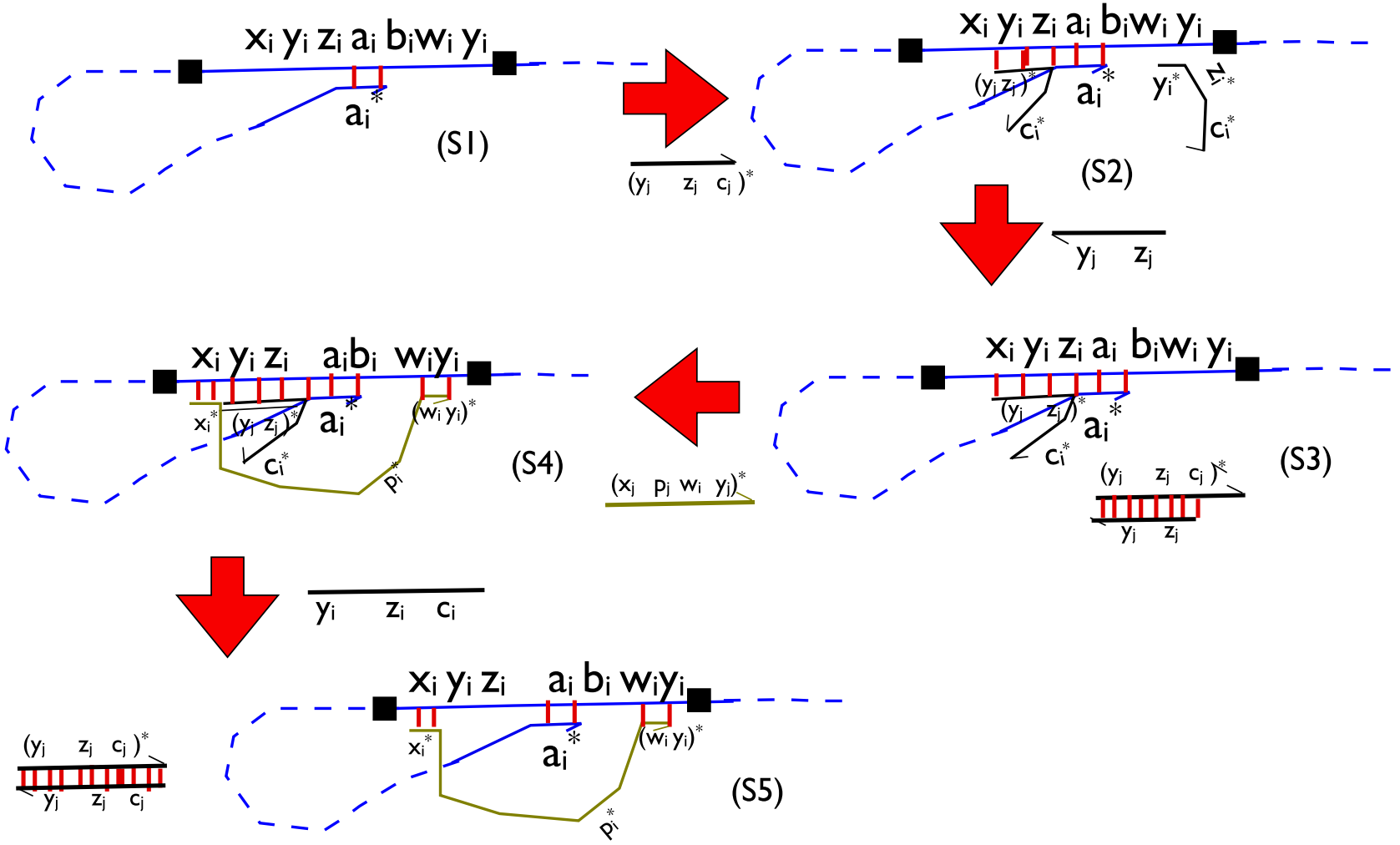
Outline

- 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

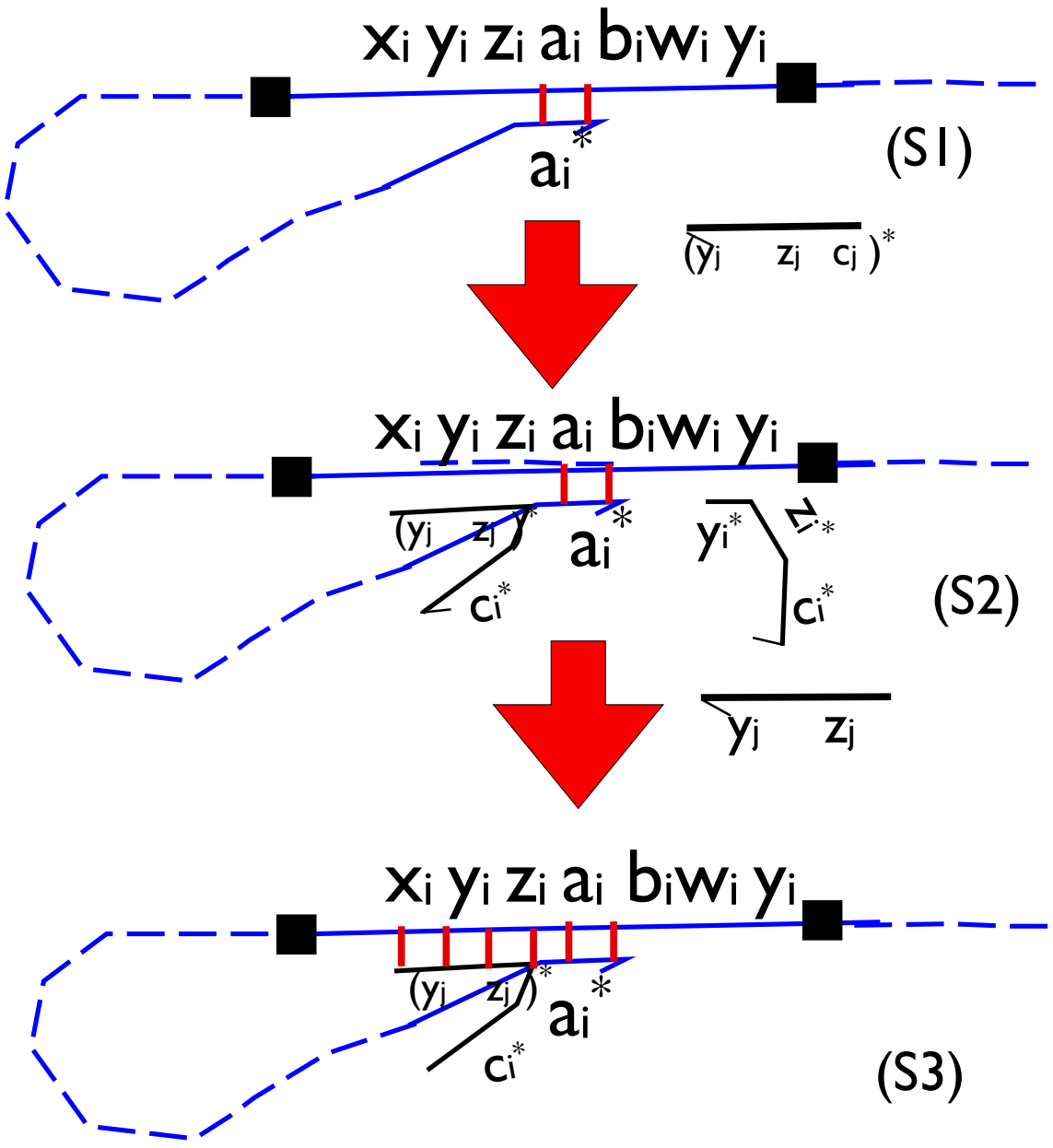
- Simple Preparation Protocol
  - Secondary primer hybridizes as desired since wy since longer than just y on the rule encoding
- Complex Preparation Protocol
  - Elaborate protocol to increase the probability of desired secondary structures of WPCR strand before computation starts

DNA Complex Preparation Protocol

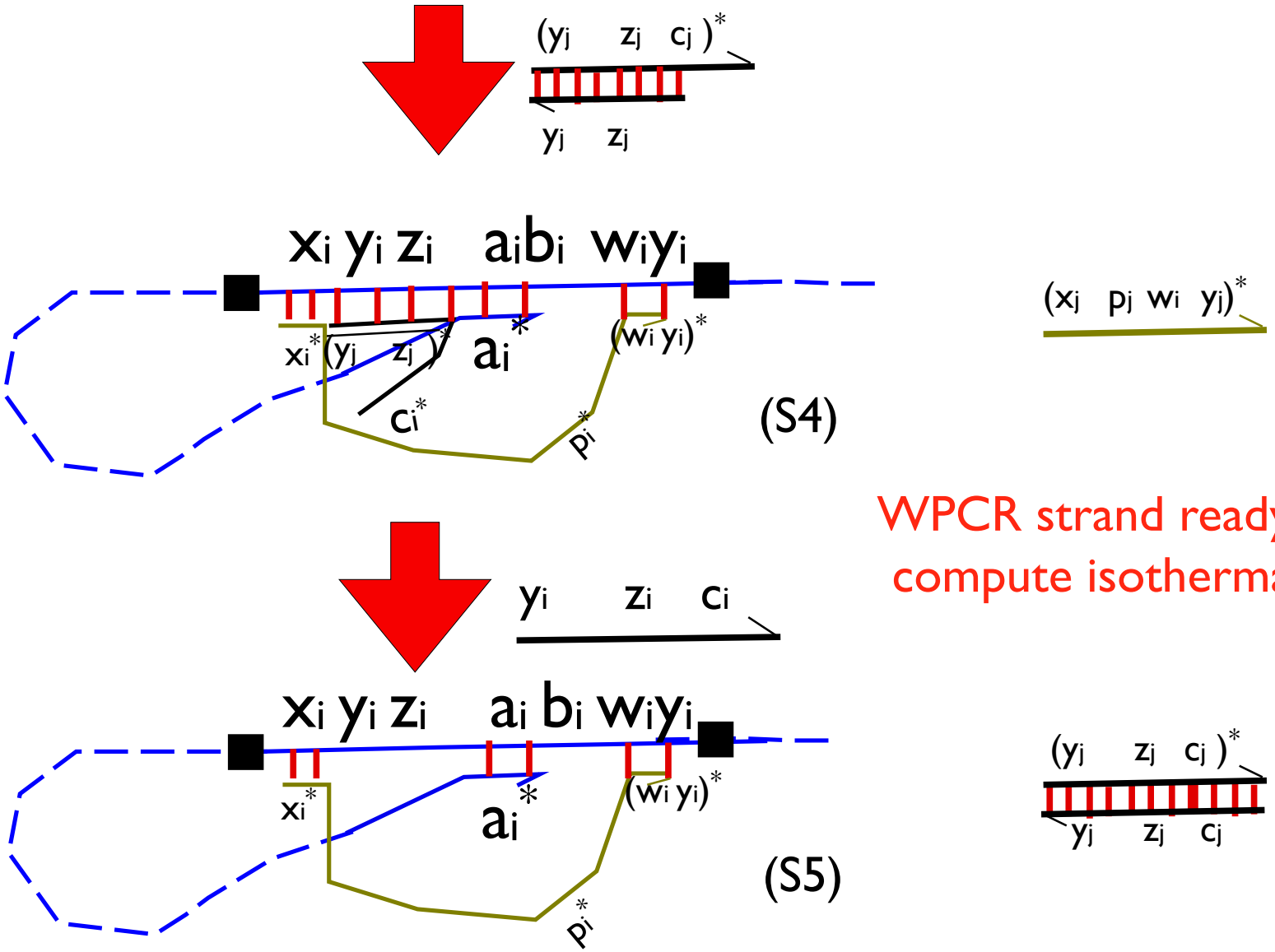


Complex preparation protocol with respect to only rule R_i: *S1* WPCR strand W tethered to support (not shown in the Figure). *S2*: $(y_i z_i c_i)^*$ is added to the solution. One copy binds to the y_i near x_i and another binds to y_i further away from it. *S3*: the copy of $(y_i z_i c_i)^*$ that binds to the y_i in R_i further away from x_i is removed by the addition of $y_i z_i$. 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

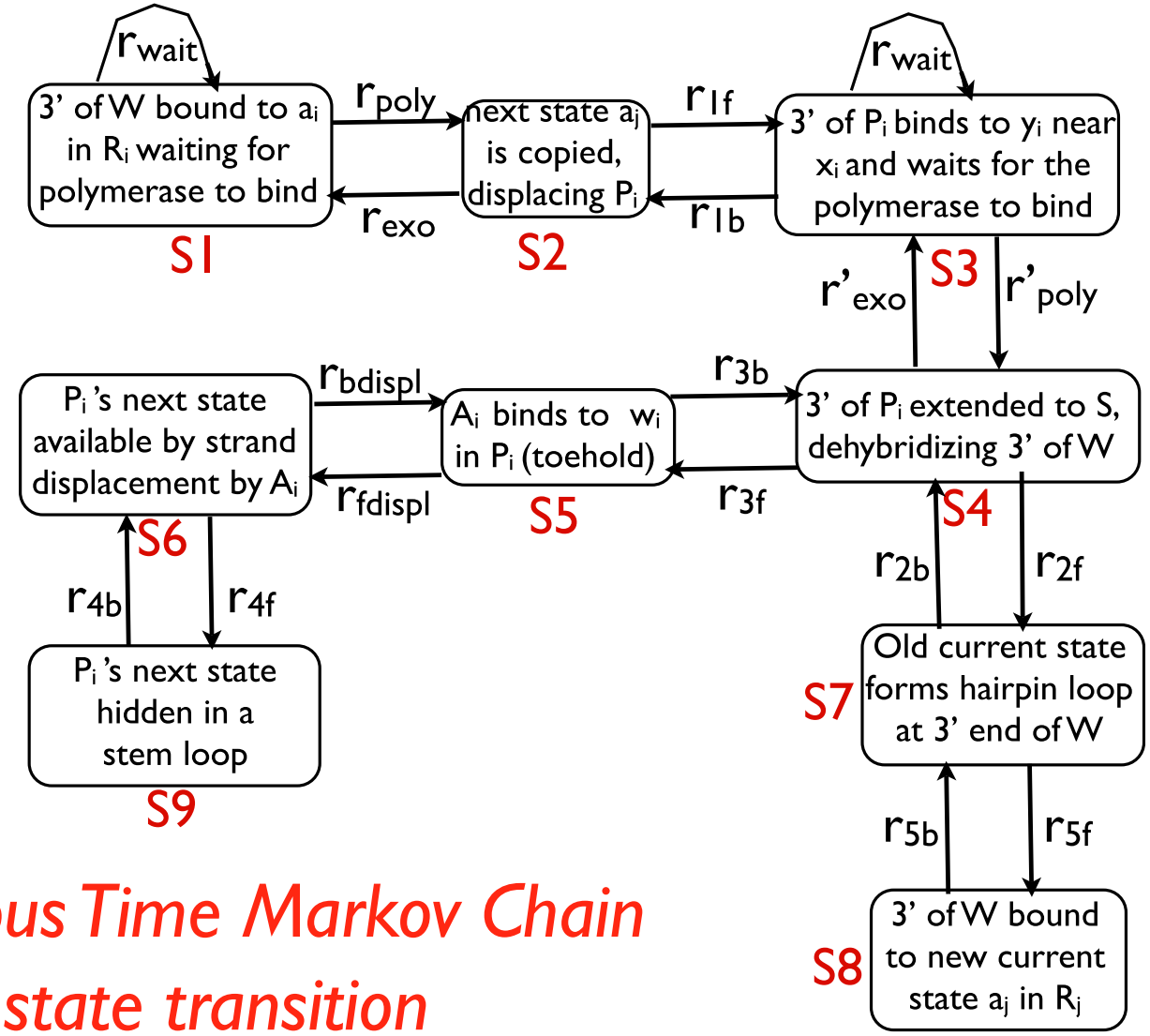


Complex Preparation Protocol (Contd)



Proof of Correctness of IR-WPCR Machine

Continuous Time Markov Chain for reusable rule R_i



A Continuous Time Markov Chain for state transition

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]