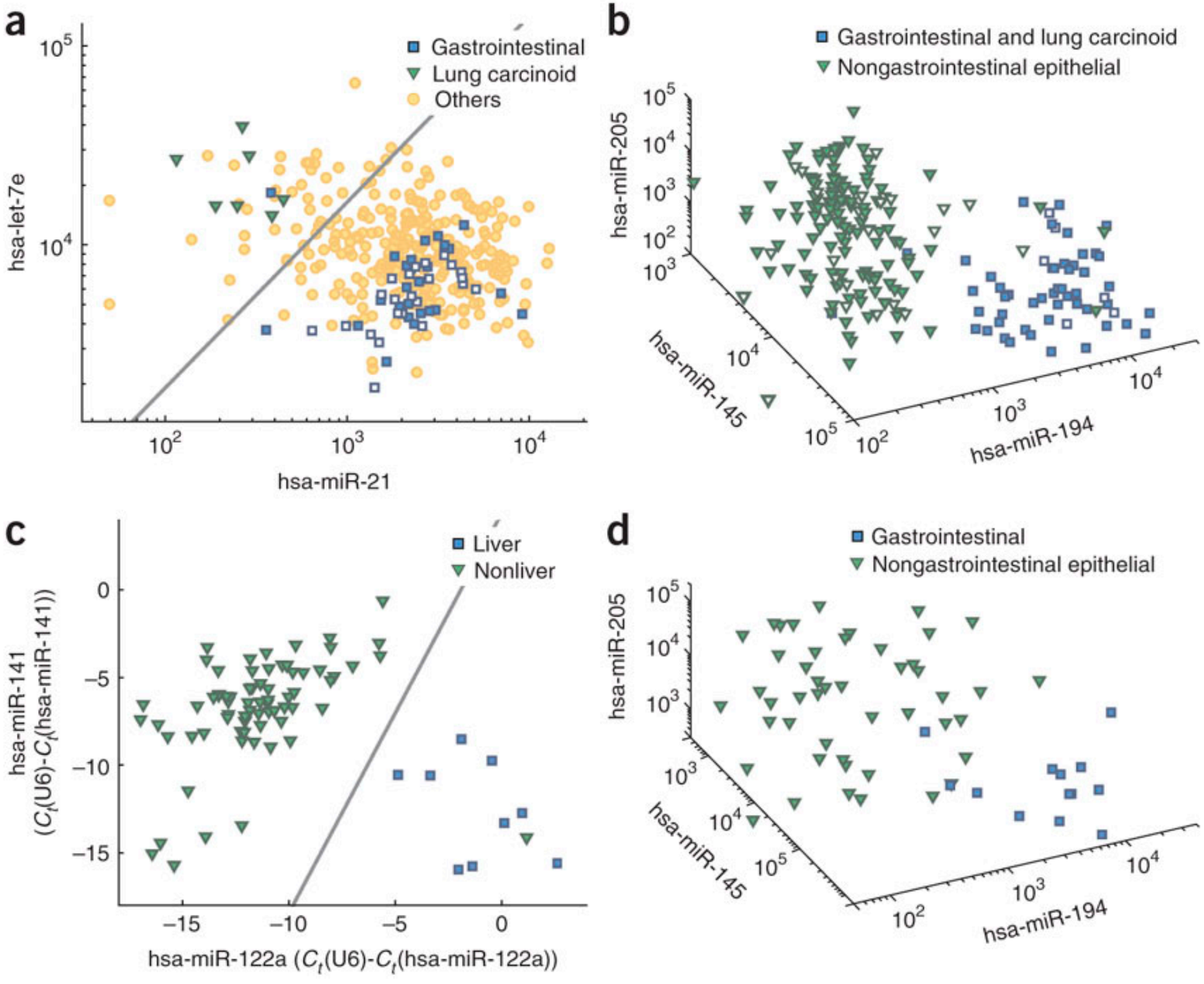
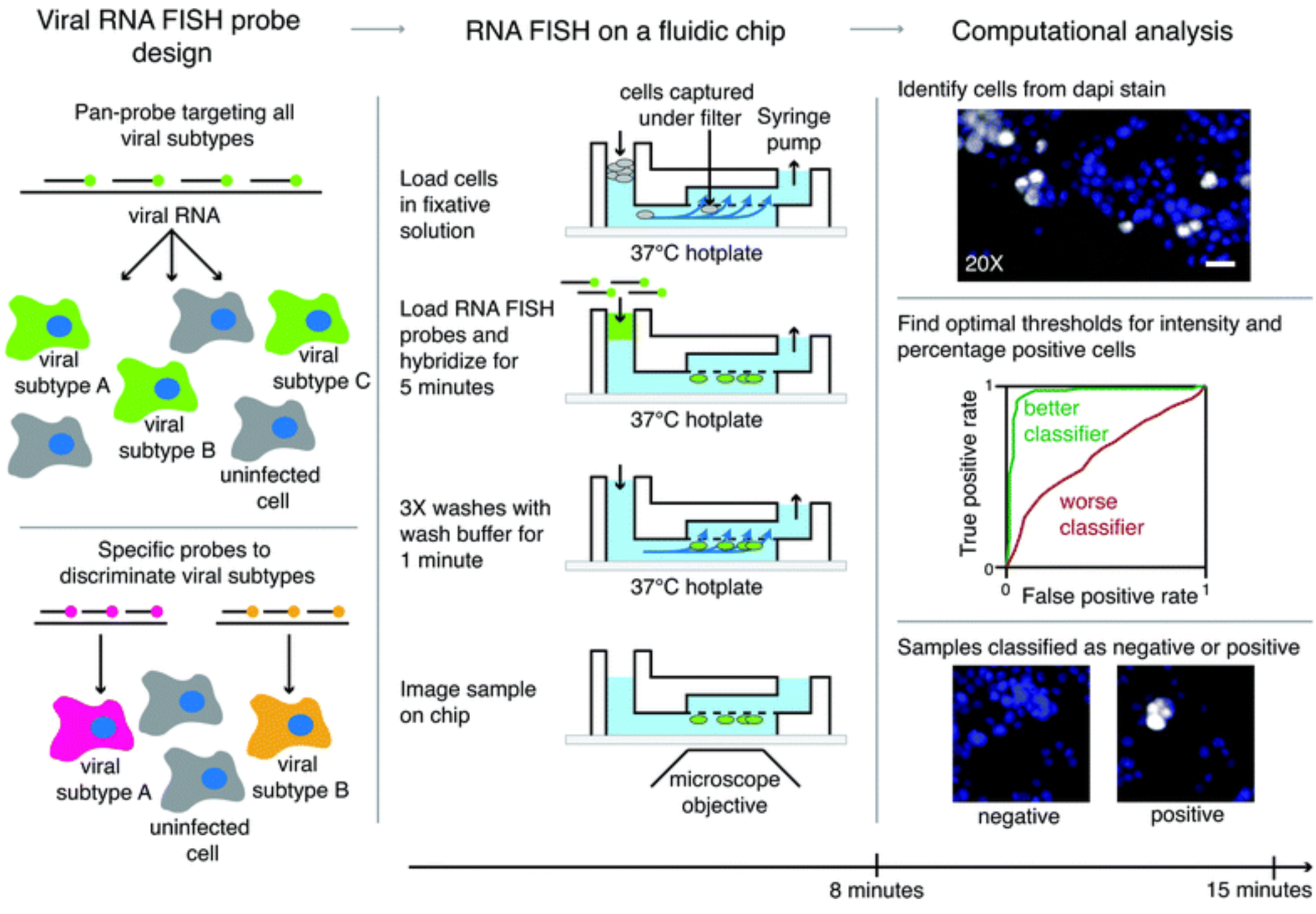
MiRNA-based cancer classifier



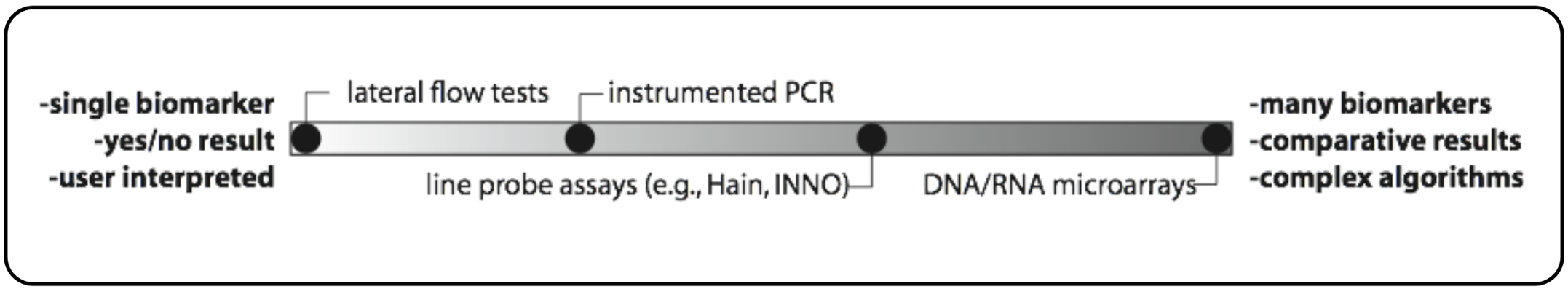
MicroRNAs accurately identify cancer tissue origin
N Rosenfeld et al. Nature biotechnology 26 (4), 462-469

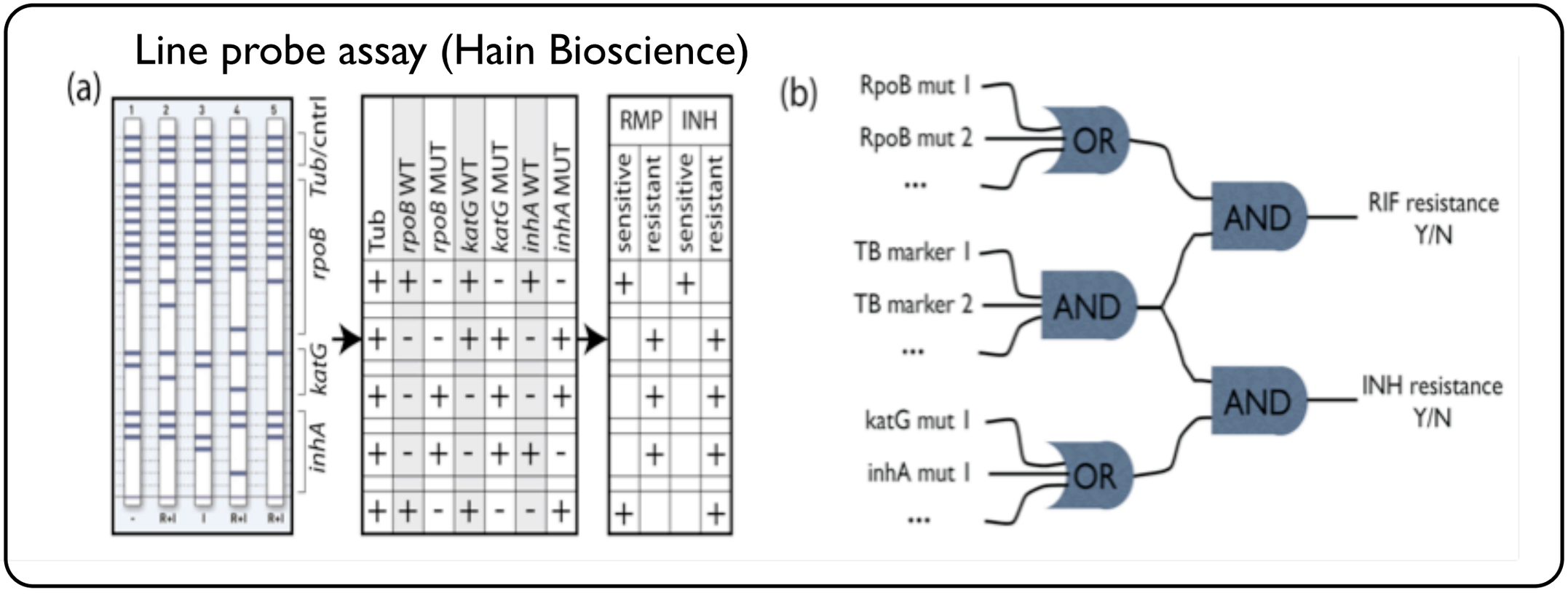
Influence diagnostic based on FISH



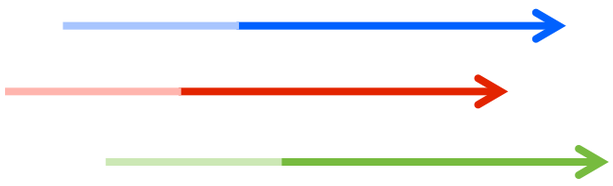
MDCK cells infected with different influenza strains. Arjun Raj lab.

There is a need for multiplexed detection and analysis



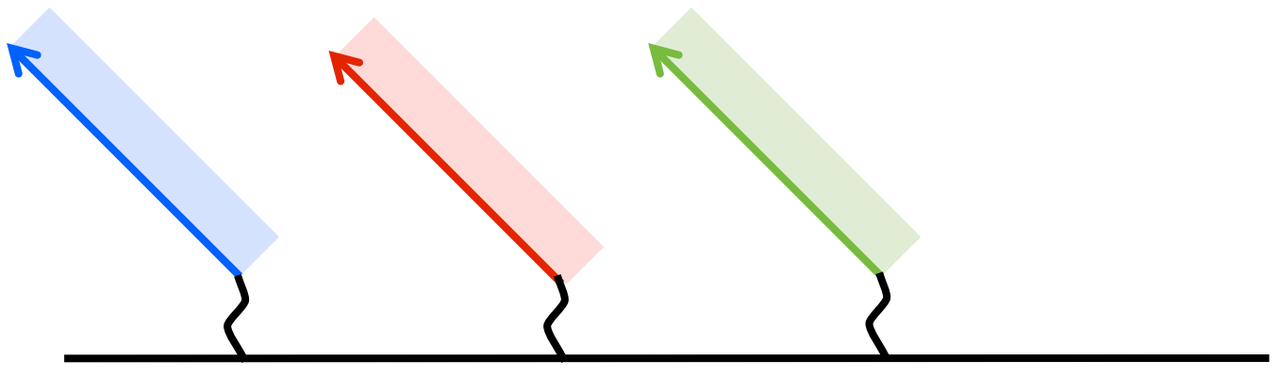


Nucleic acid detection uses Watson-Crick base pairing



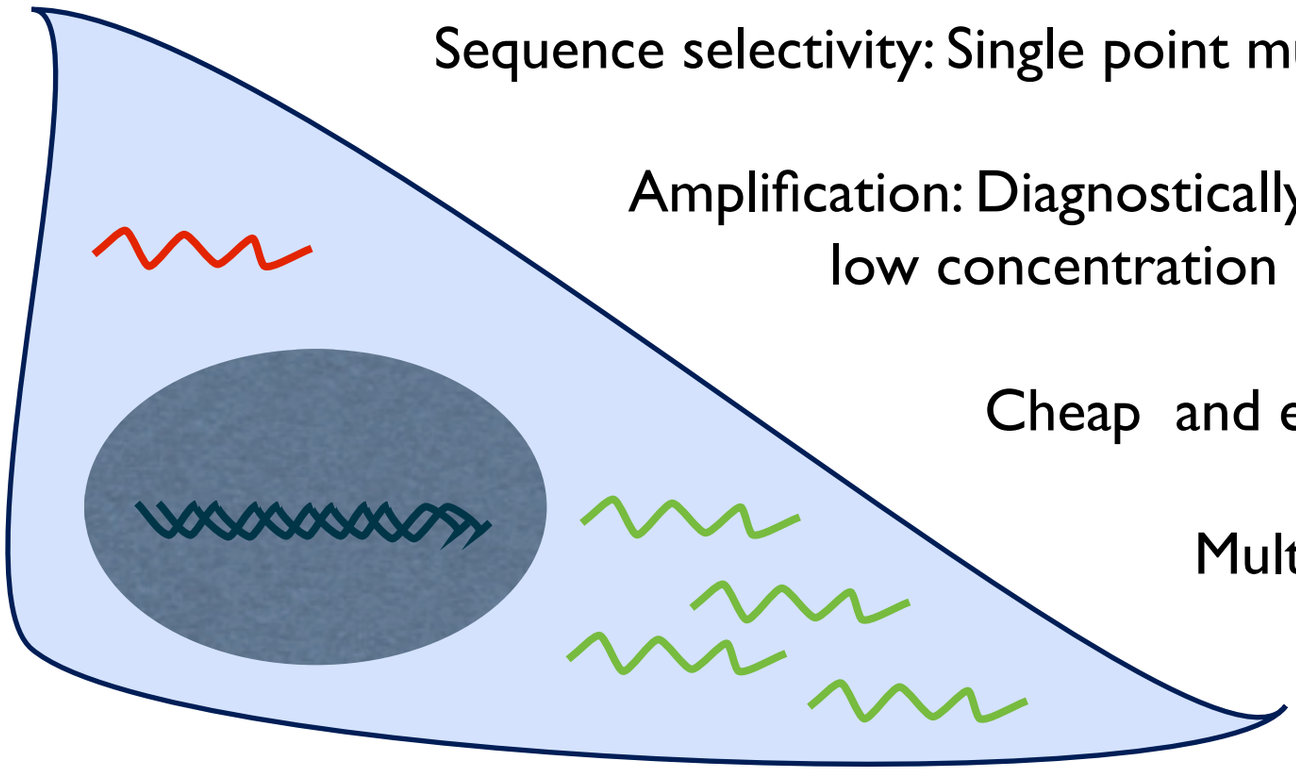
RNA sequence:
5’-AAUUCAGAUCCACCCAAAGAG-3’

Probe sequence:
3’-UUAAGUCUAGGUG-5’



Microarrays, qPCR and similar technologies typically require expensive instruments and complex sample preparation.

Requirements for a good nucleic acid detection technology



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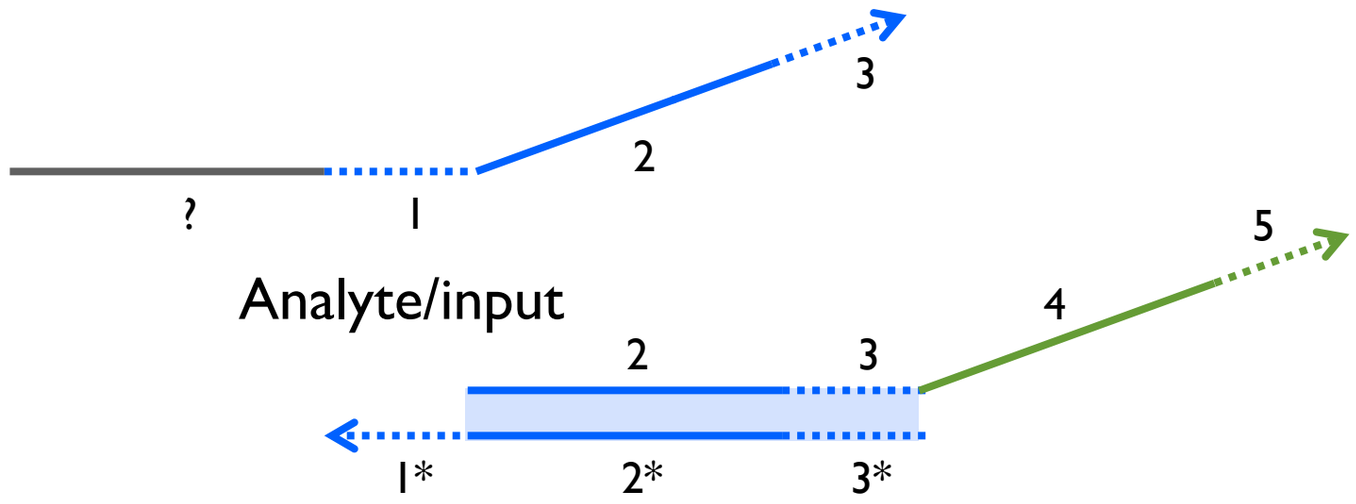
Strand displacement provides a mechanism for RNA sensing and analysis



Typical reaction conditions:
1-100 nM Gates, inputs, etc
20C-40C, TAE Mg^{++} or similar salt buffer

RNA sequence:
5'-AAUUCAGAUCCACCCAAAGAG-3'

1 2

For a review see D.Y. Zhang and G. Seelig, Nature Chemistry (2011)

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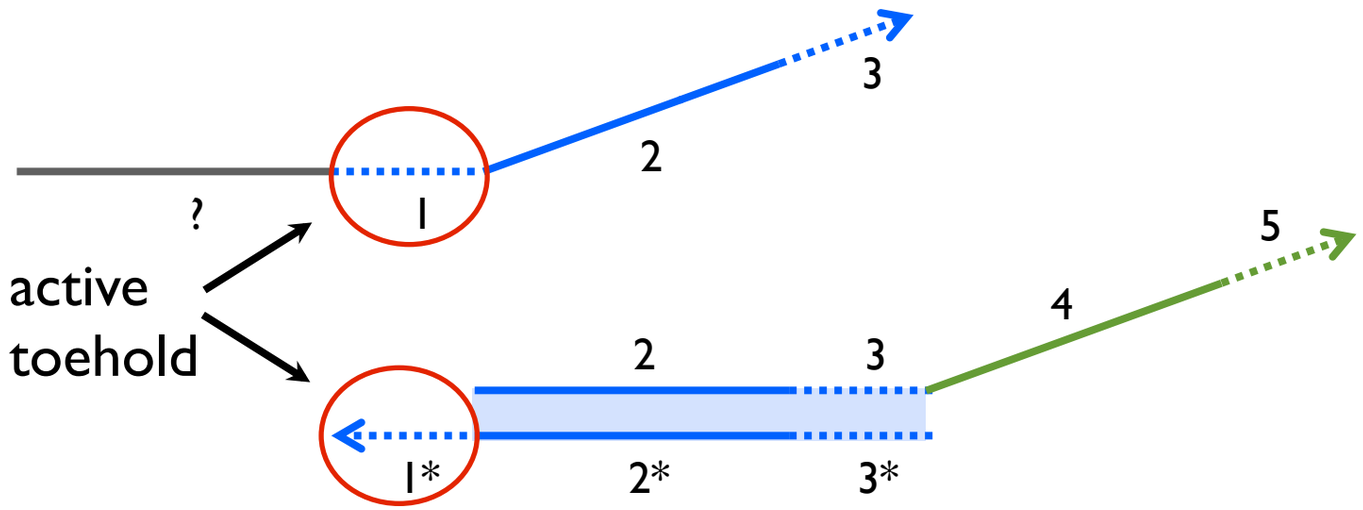

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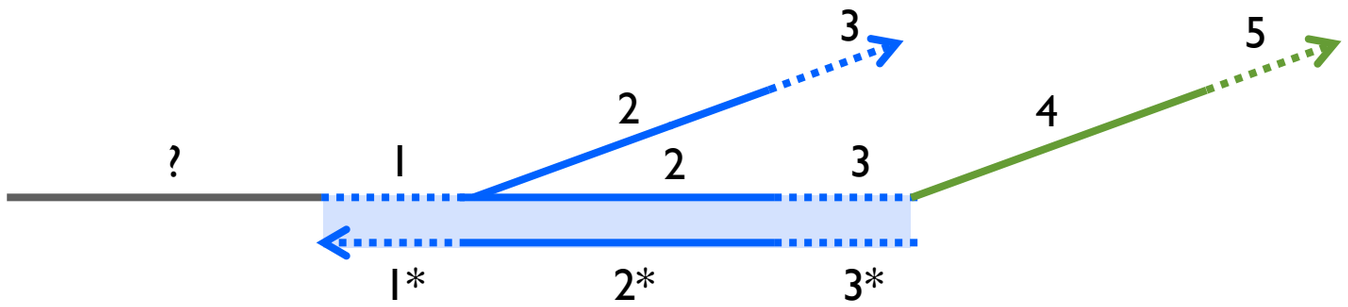
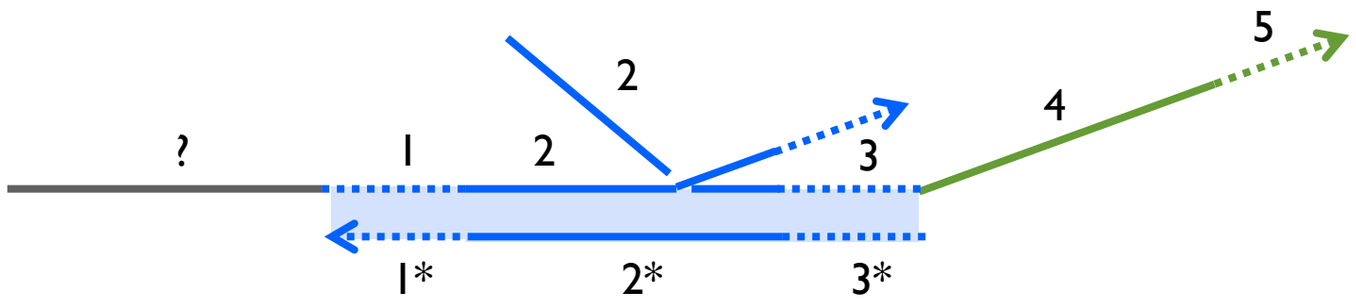


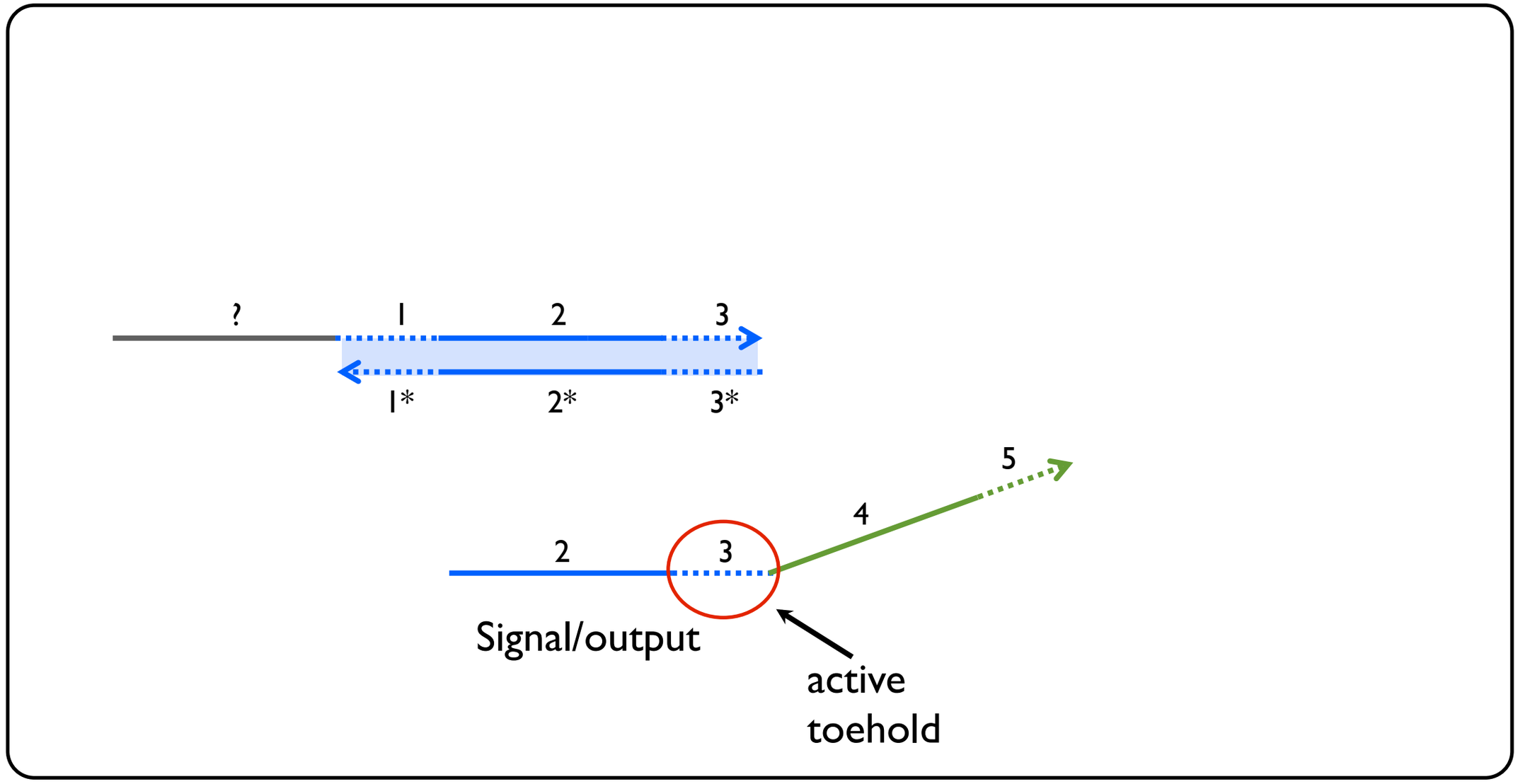
Strand displacement is initiated at the single-stranded toeholds. Toehold binding is a reversible process.

Strand displacement provides a mechanism for RNA sensing and analysis



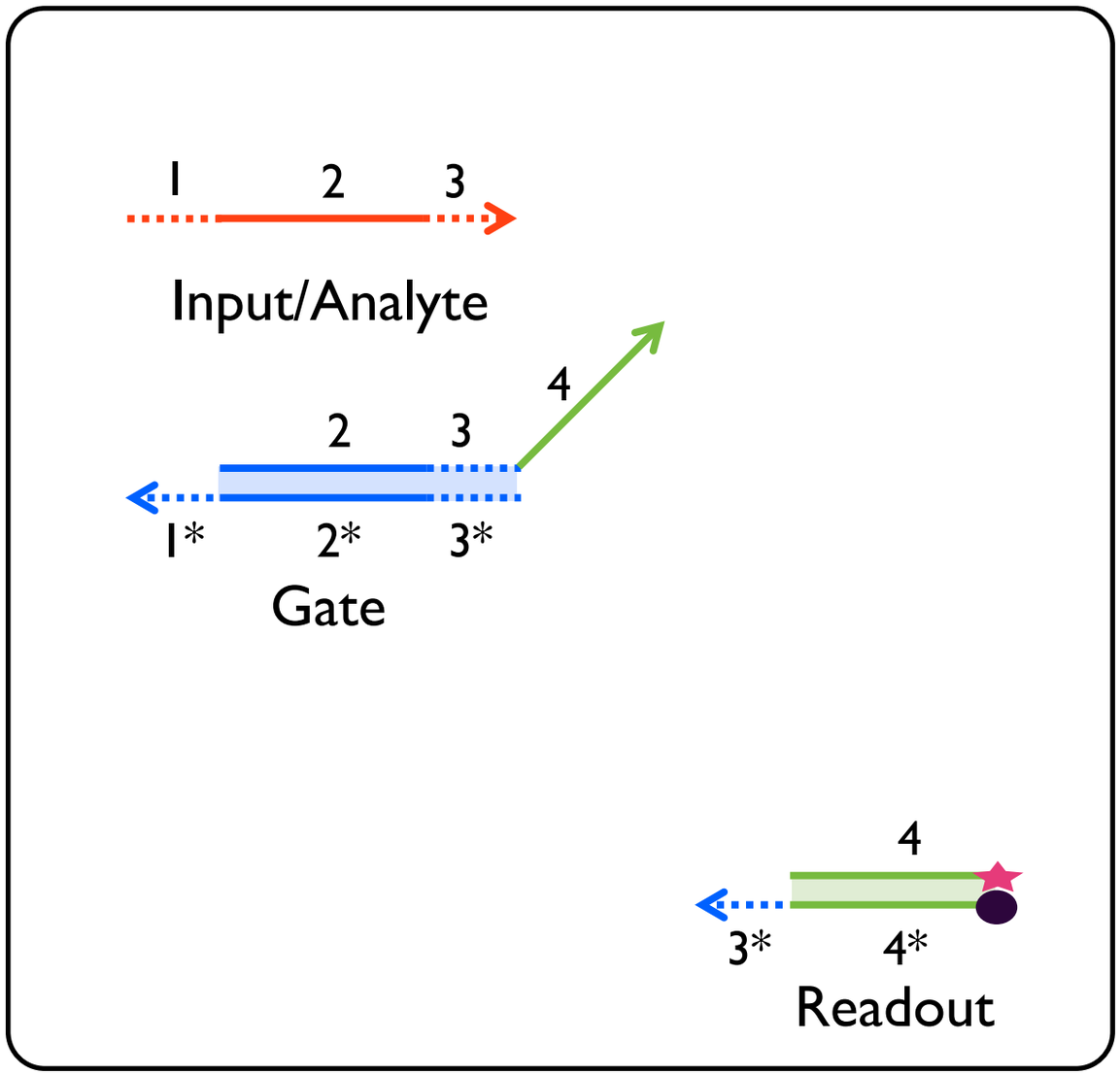
Strand displacement proceeds through a branch migration. Branch migration is a random walk.

Strand displacement provides a mechanism for RNA sensing and analysis



Release of the output strand is (almost) irreversible in the absence of a toehold for the reverse reaction.

Signals can propagate through multiple layers



The sequences of inputs and outputs can be completely independent.

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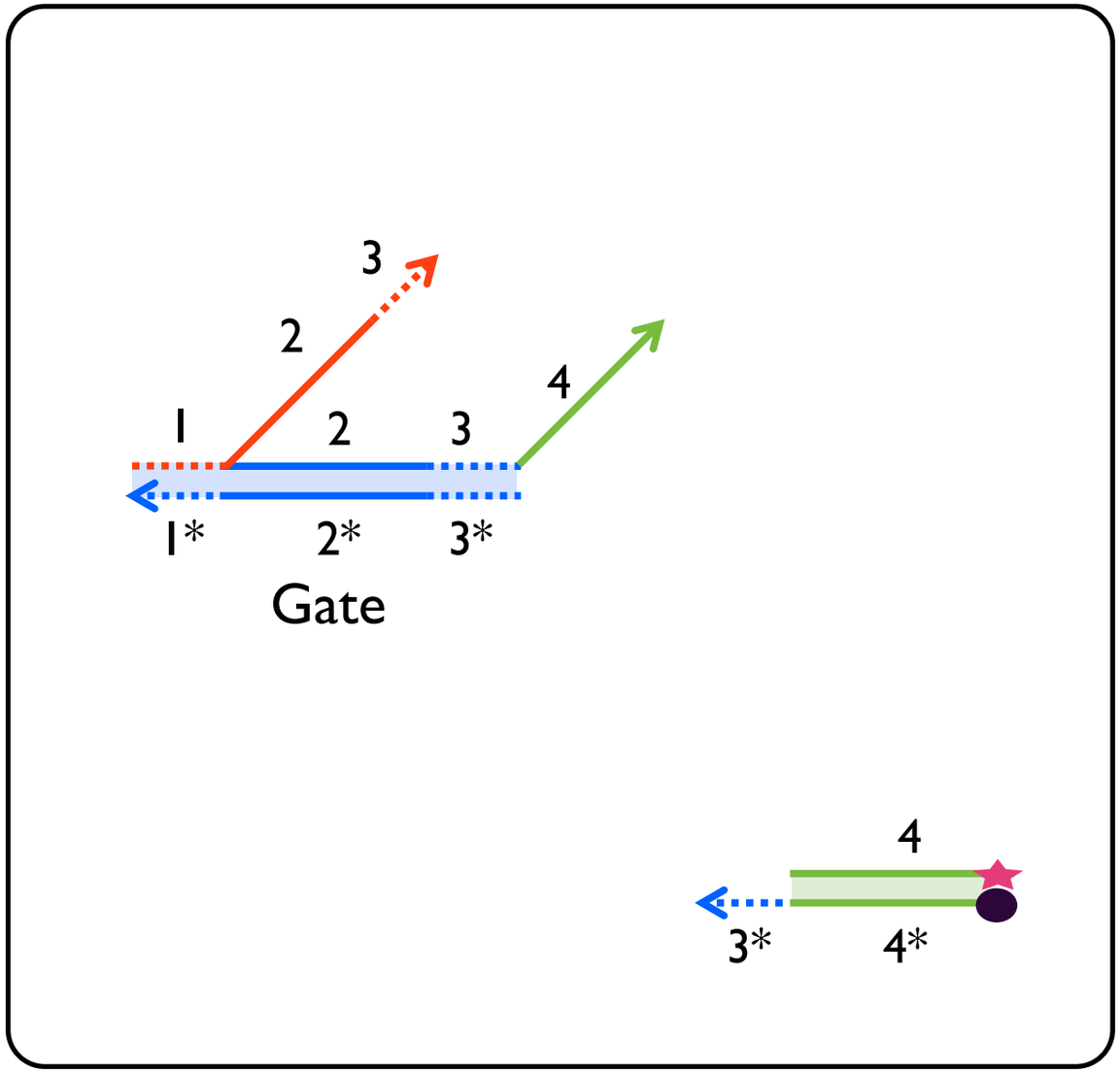

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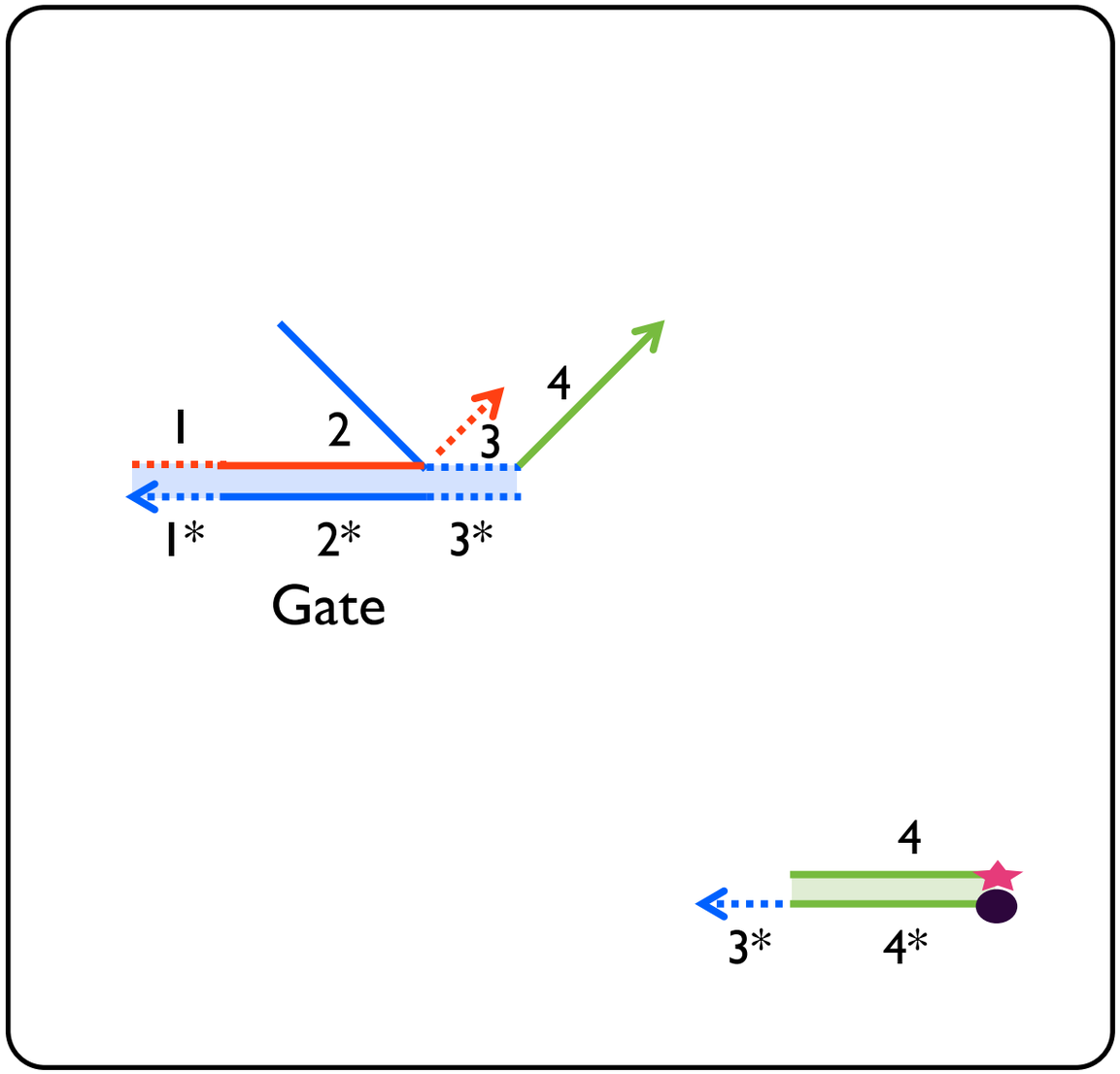

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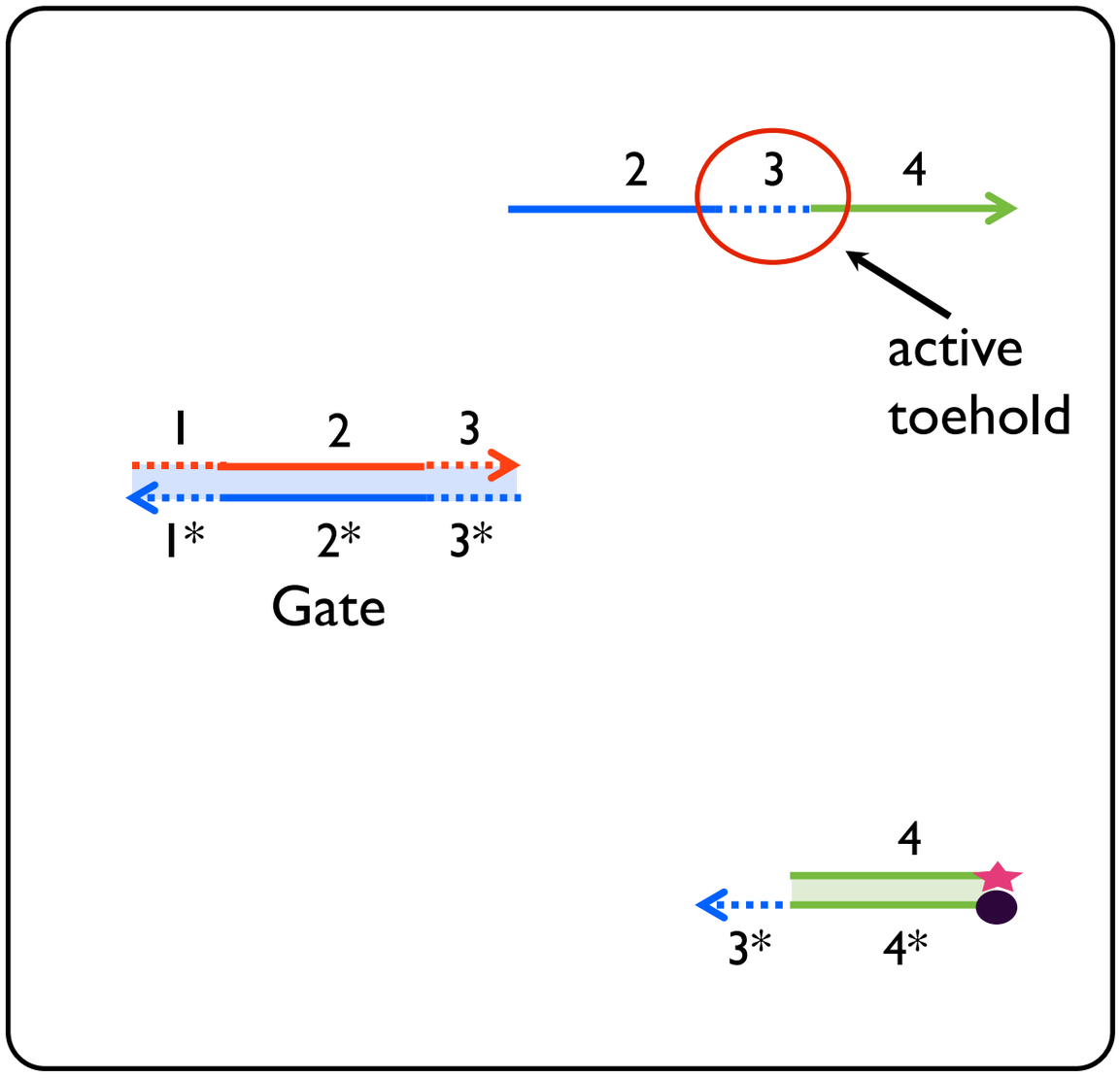

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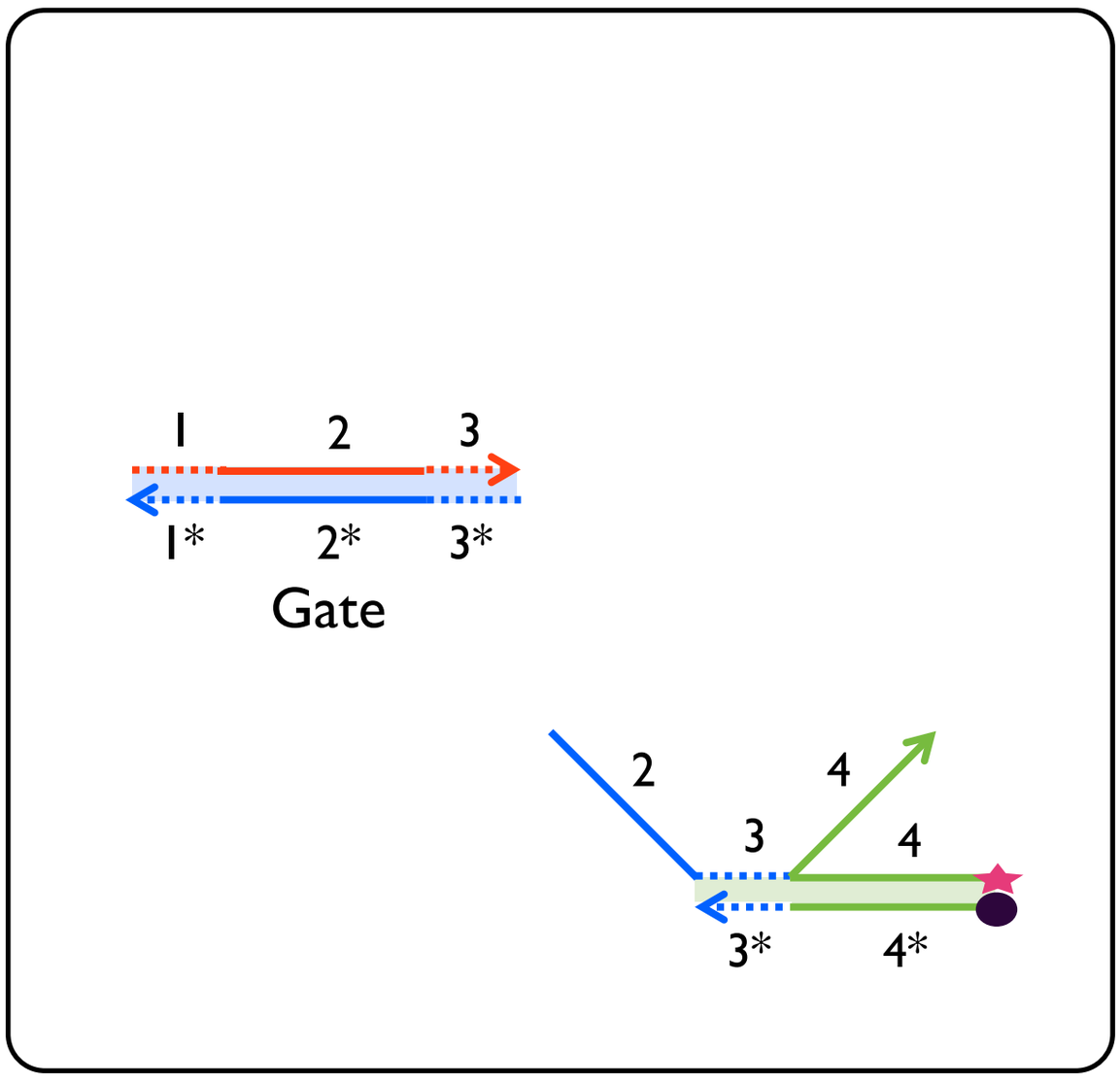

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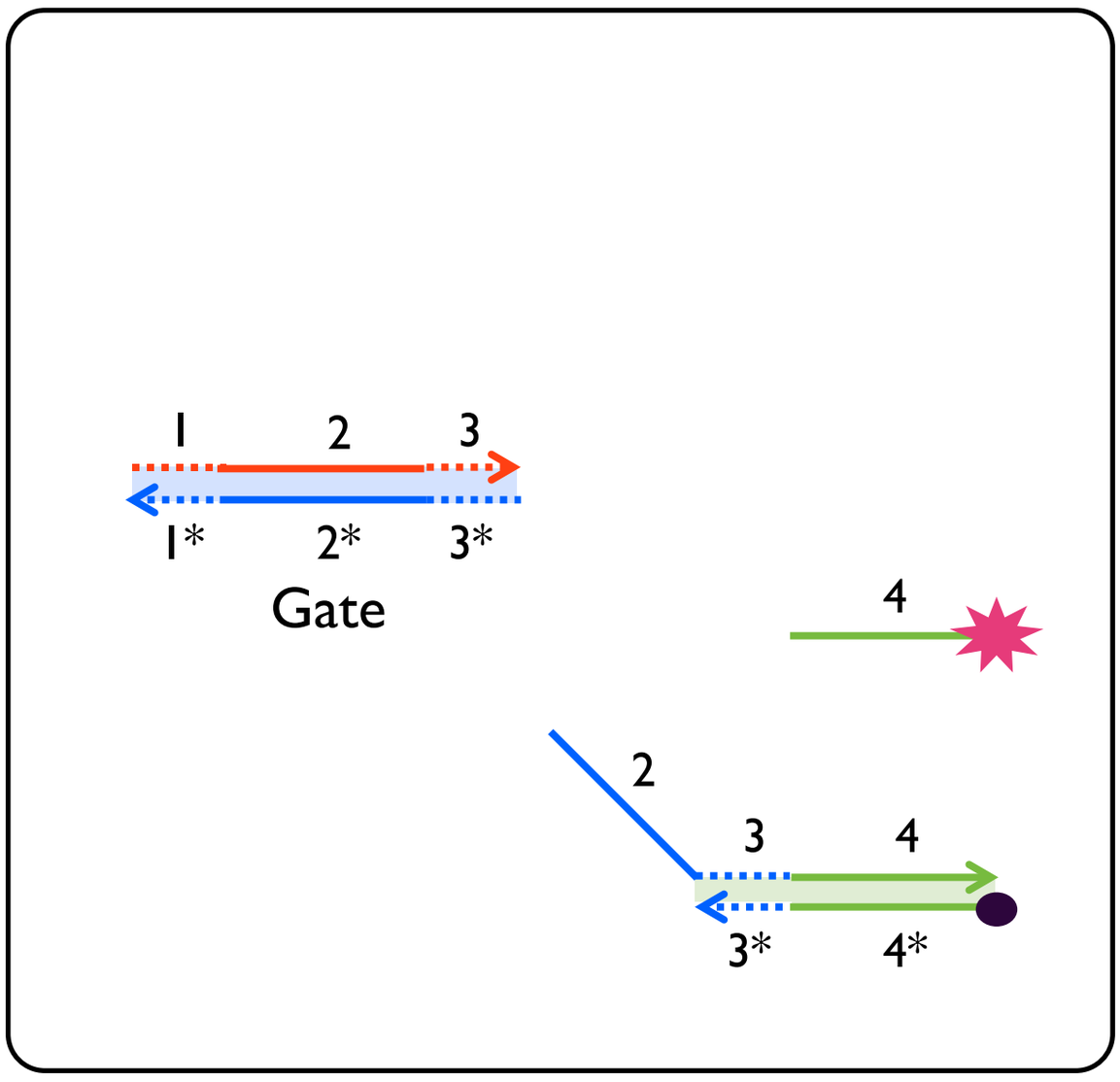

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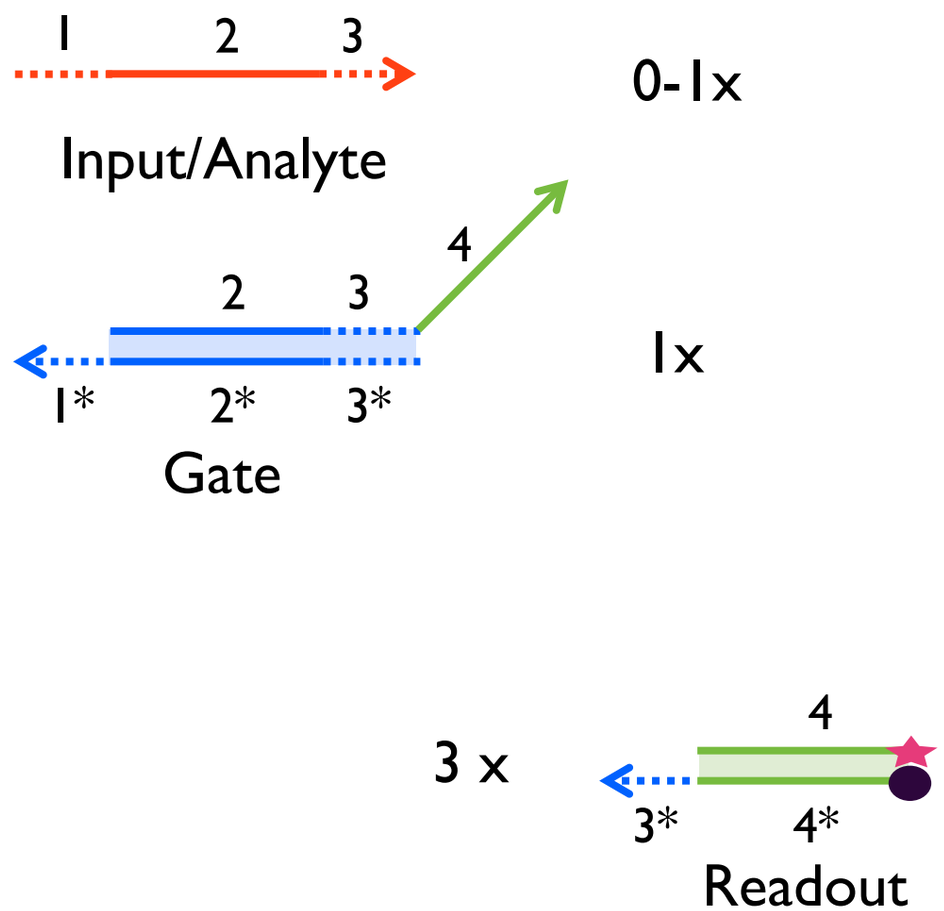
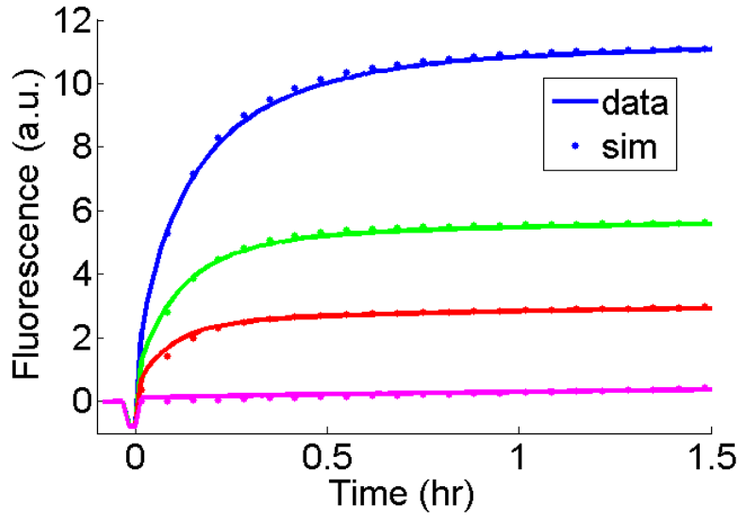
Reaction kinetics are approximately bimolecular

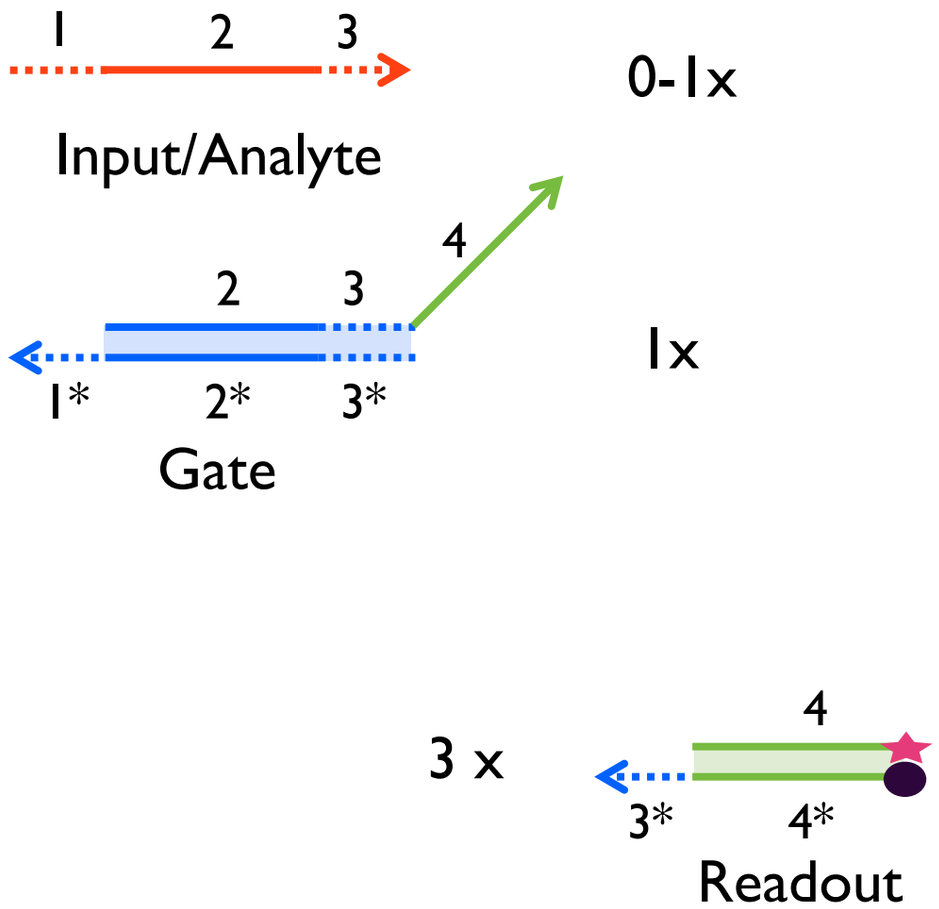


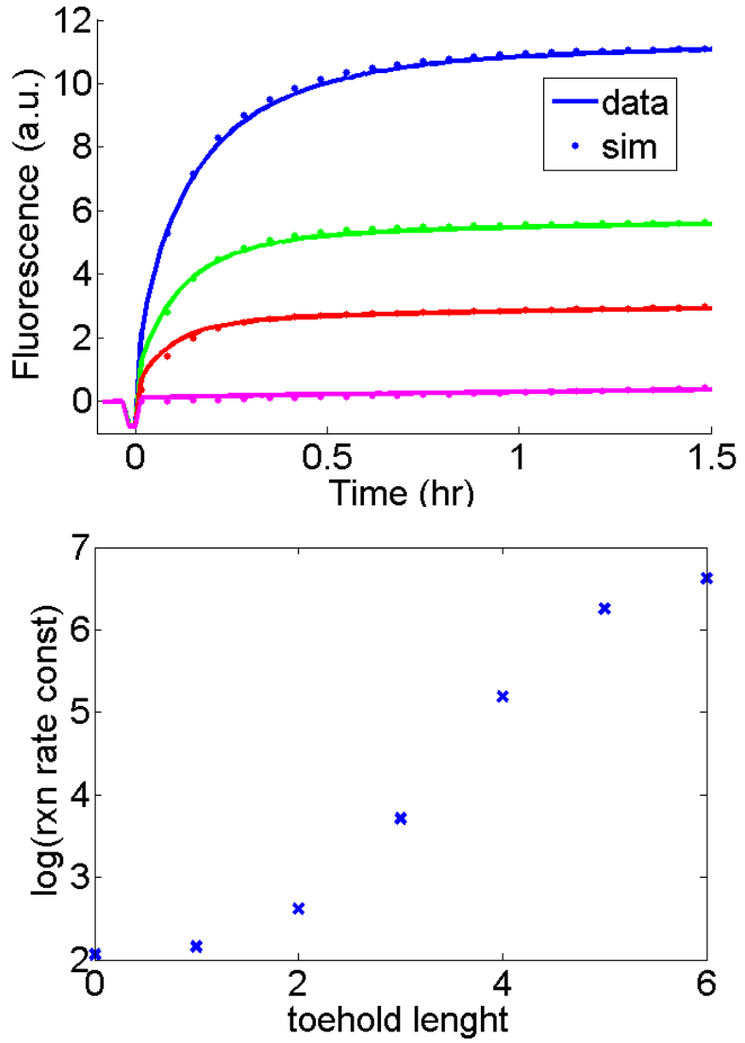


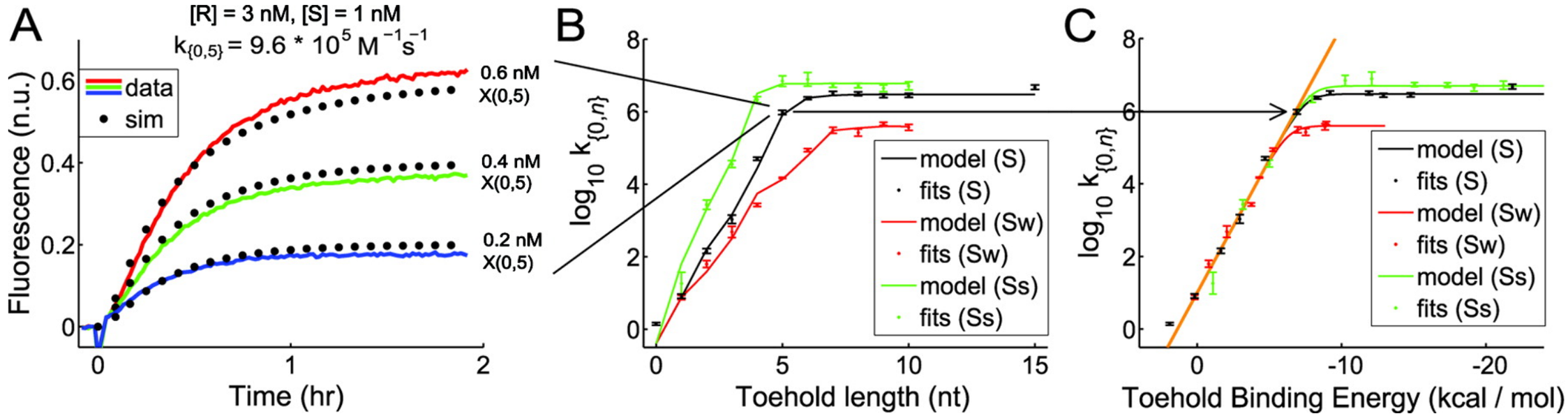
$$In + Gate \xrightarrow{k} Out + Waste$$

(10 nM gates, 30nM readout, TAE 12.5 mM Mg^{++})

Toehold length determines reaction rate



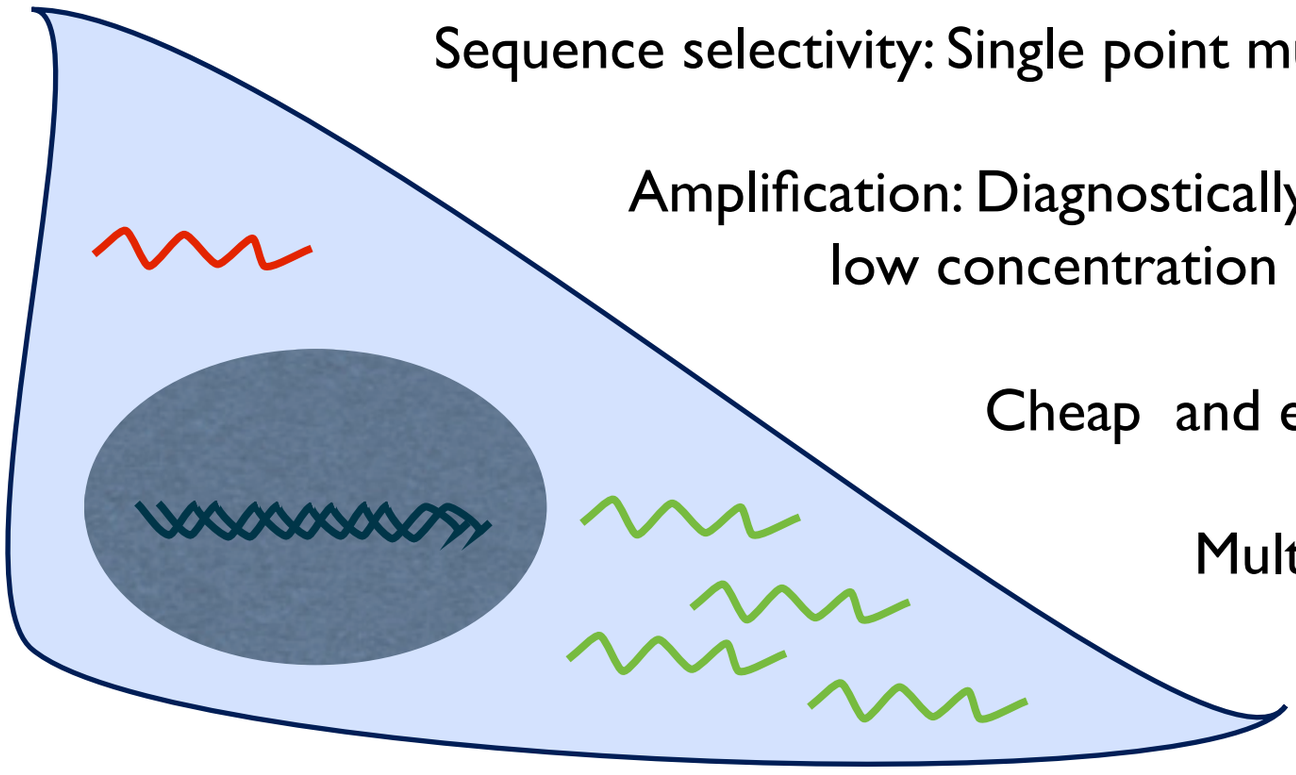




Toehold-mediated strand displacement experiments (*m* = 0). Results presented in this figure also use domains γs and γw in place of domain γ where specified. (A) Sample trajectories for *n* = 5. S and R were initially in solution at the displayed concentrations, and X(0, 5) was added to solution at *t* ≈ 0 to achieve the final concentration displayed. The black dotted lines labeled “fit” denote simulations of a bimolecular reaction with with the experimental best-fit rate constant *k*{0, 5} = 1.0 × 106 M−1 s−1, and the reporter reaction with rate constant *k*rep = 1.3 × 106 M−1 s−1. The black lines spanning A, B, and C indicate that the three traces shown in A are represented by a single data point in B and C. (B) Summary of strand displacement rate constants plotted against the invading toehold length *n*. The *y*-axis shows the base-10 logarithm of the experimental best-fit values (“fits”) and three-step model predicted values (“model”) of *k*s{0, *n*}, *k*{0, *n*}, and *k*w{0, *n*}, expressed in M−1 s−1. (C) Summary of strand displacement rate constants plotted against the calculated binding energy of the toehold. The orange line shows the asymptotic behavior predicted by the three-step model as *k*f approaches ∞.

Published in: David Yu Zhang; Erik Winfree; *J. Am. Chem. Soc.* **2009,** 131, 17303-17314.
DOI: 10.1021/ja906987s

Requirements for a good nucleic acid detection technology



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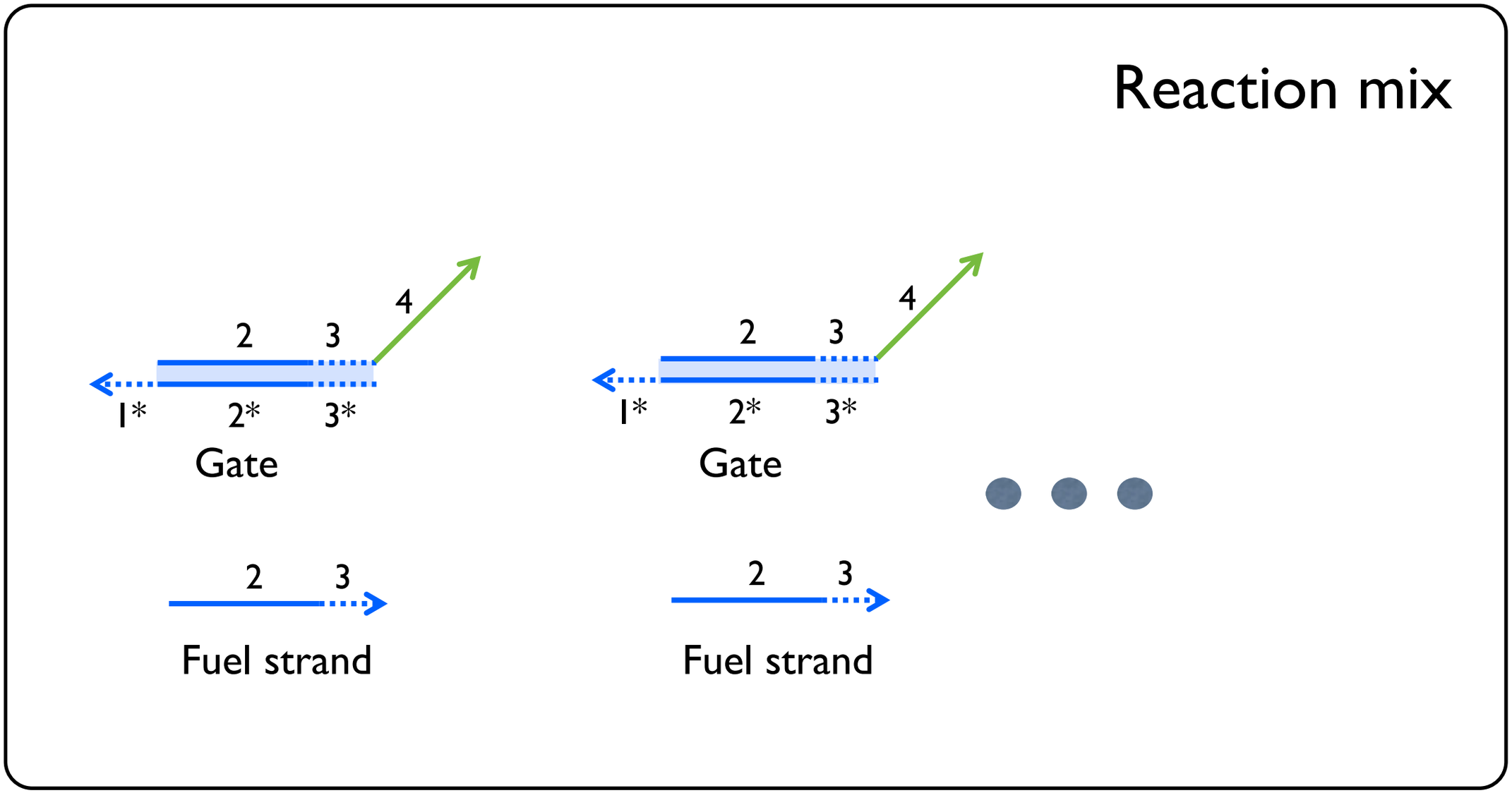
Strand displacement-based signal amplification: An input can release more than one output



Qian and Winfree, Science (2011)
(see also Zhang et al. Science (2007), Seelig et al. JACS (2006), Turberfield et al. PRL (2004))

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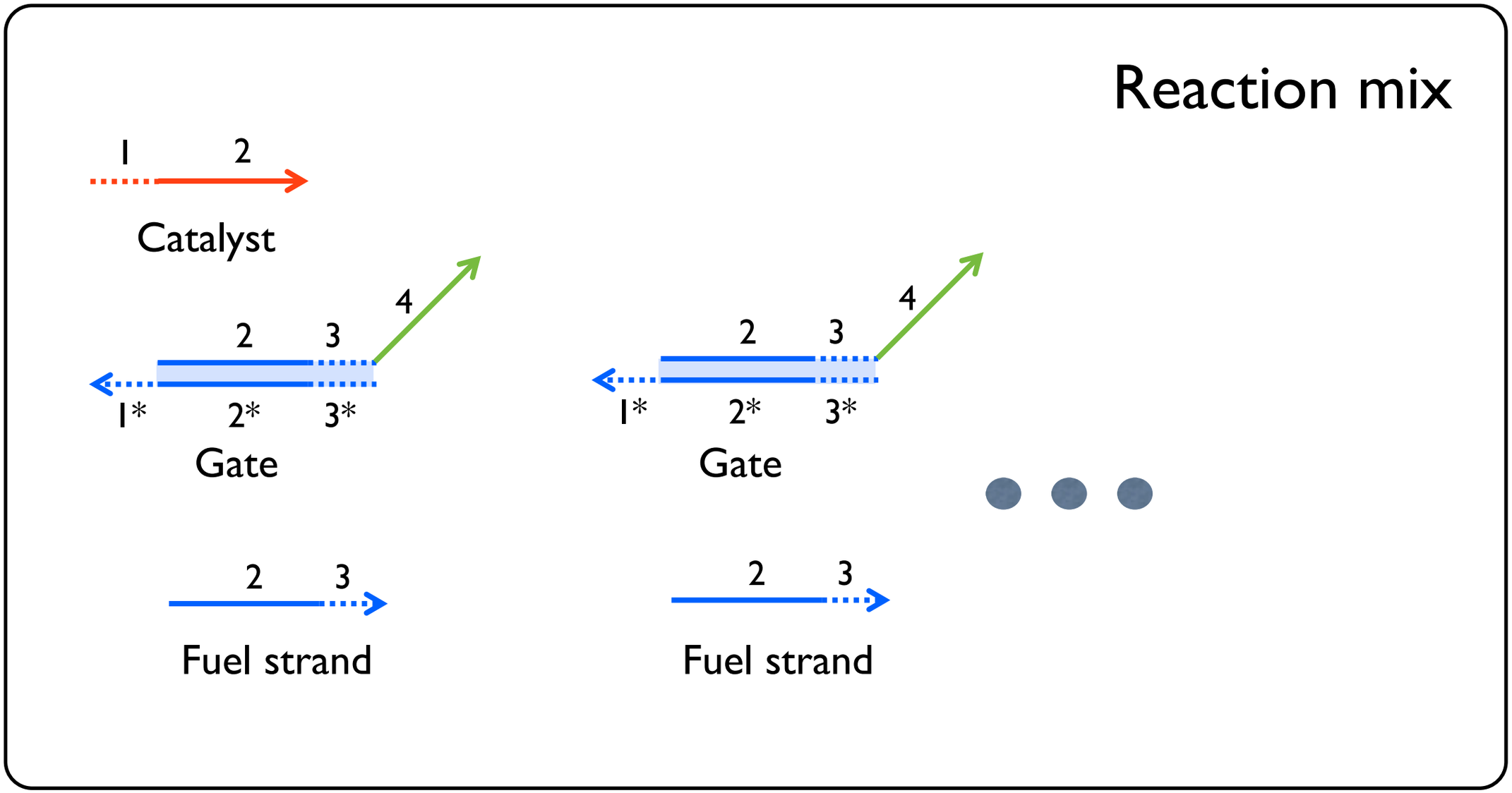

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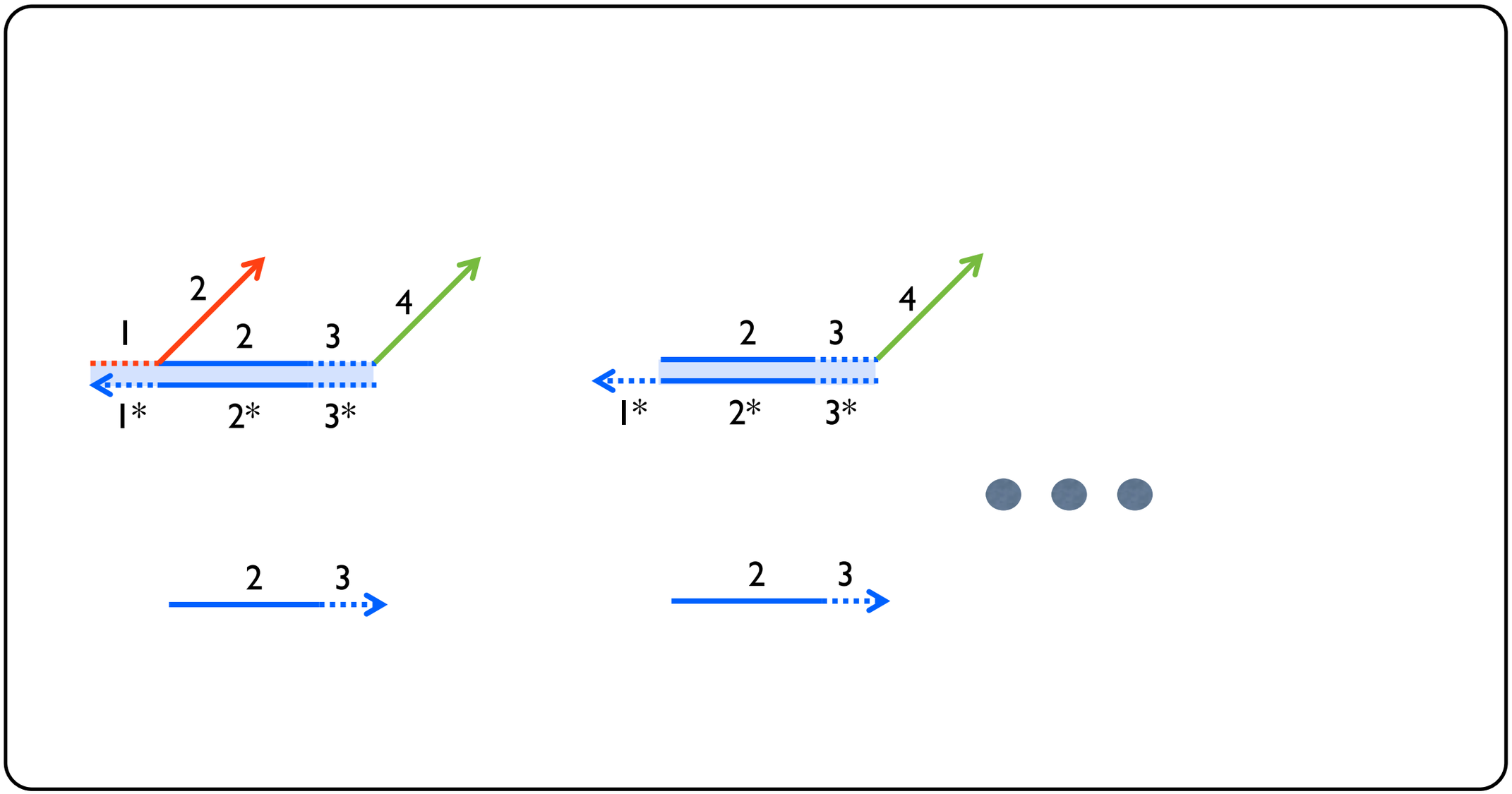

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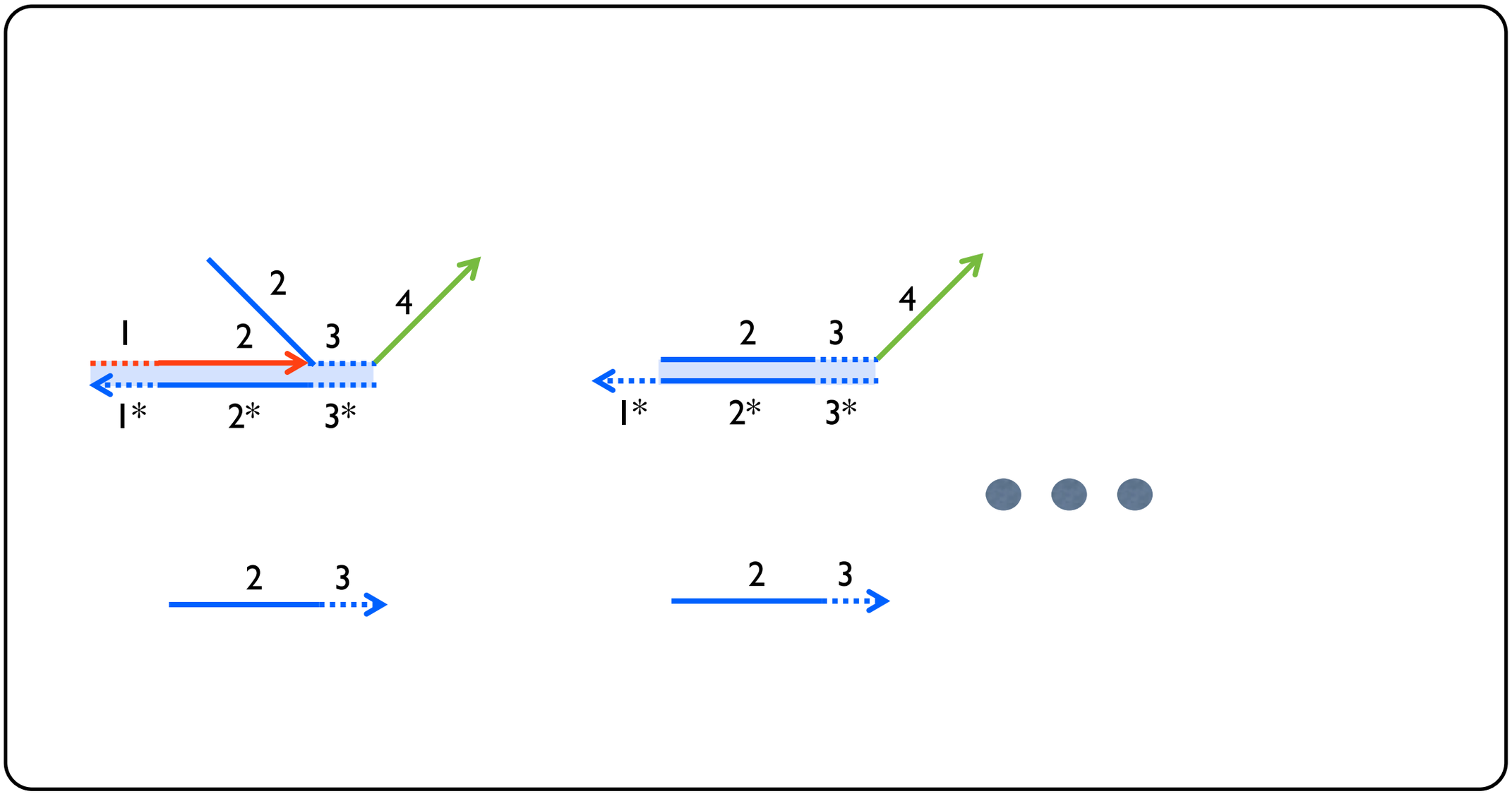

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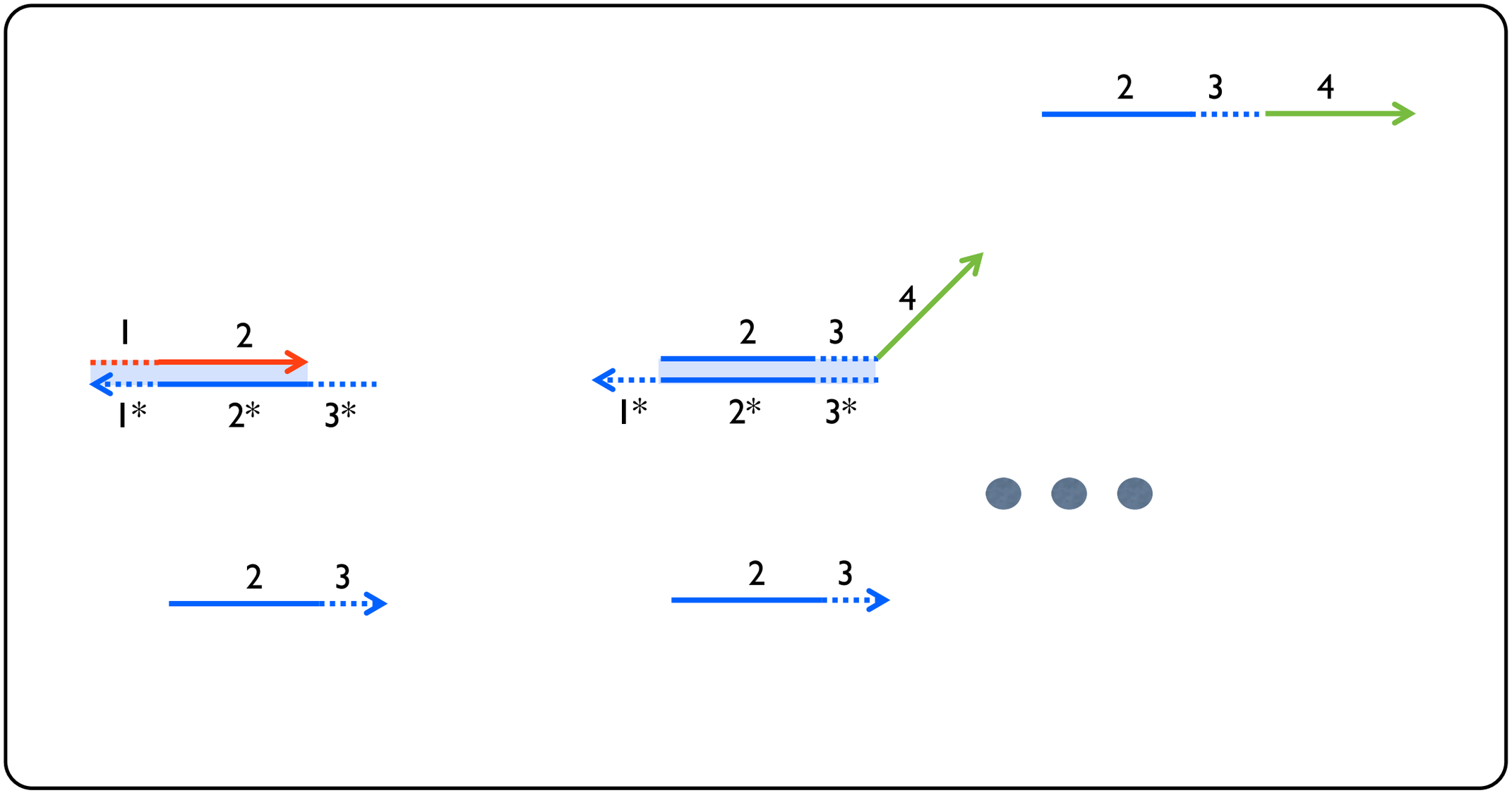

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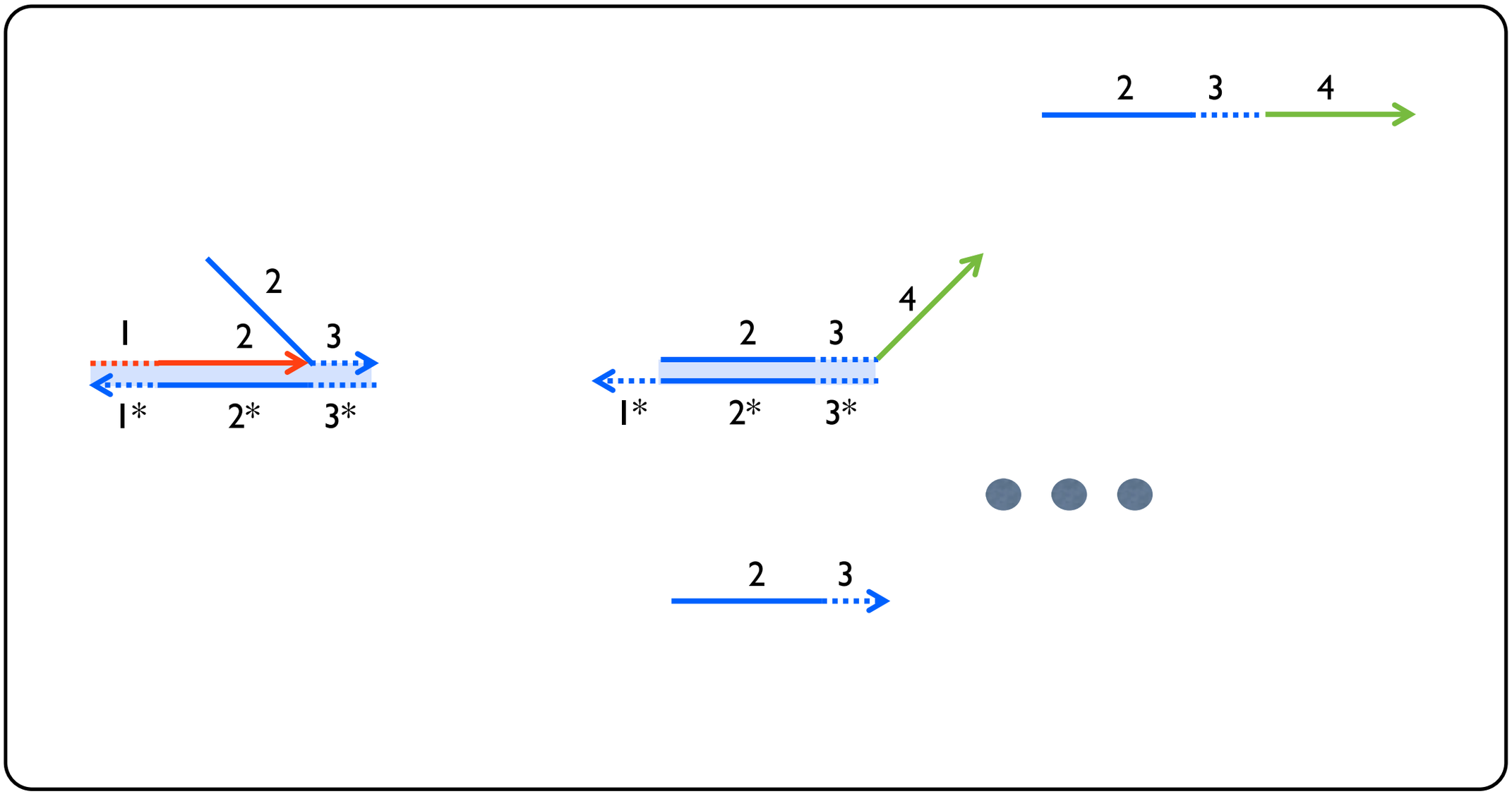

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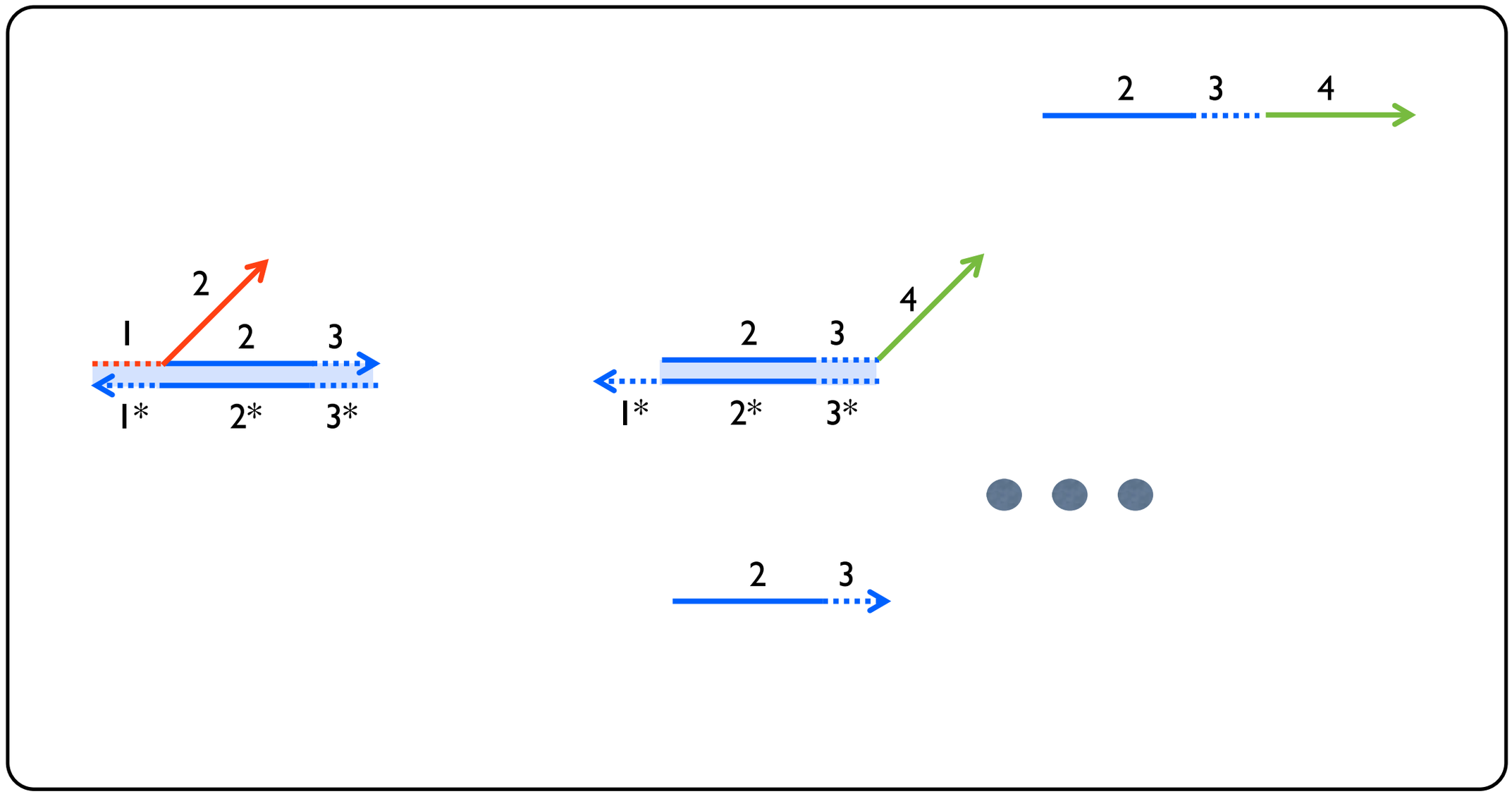

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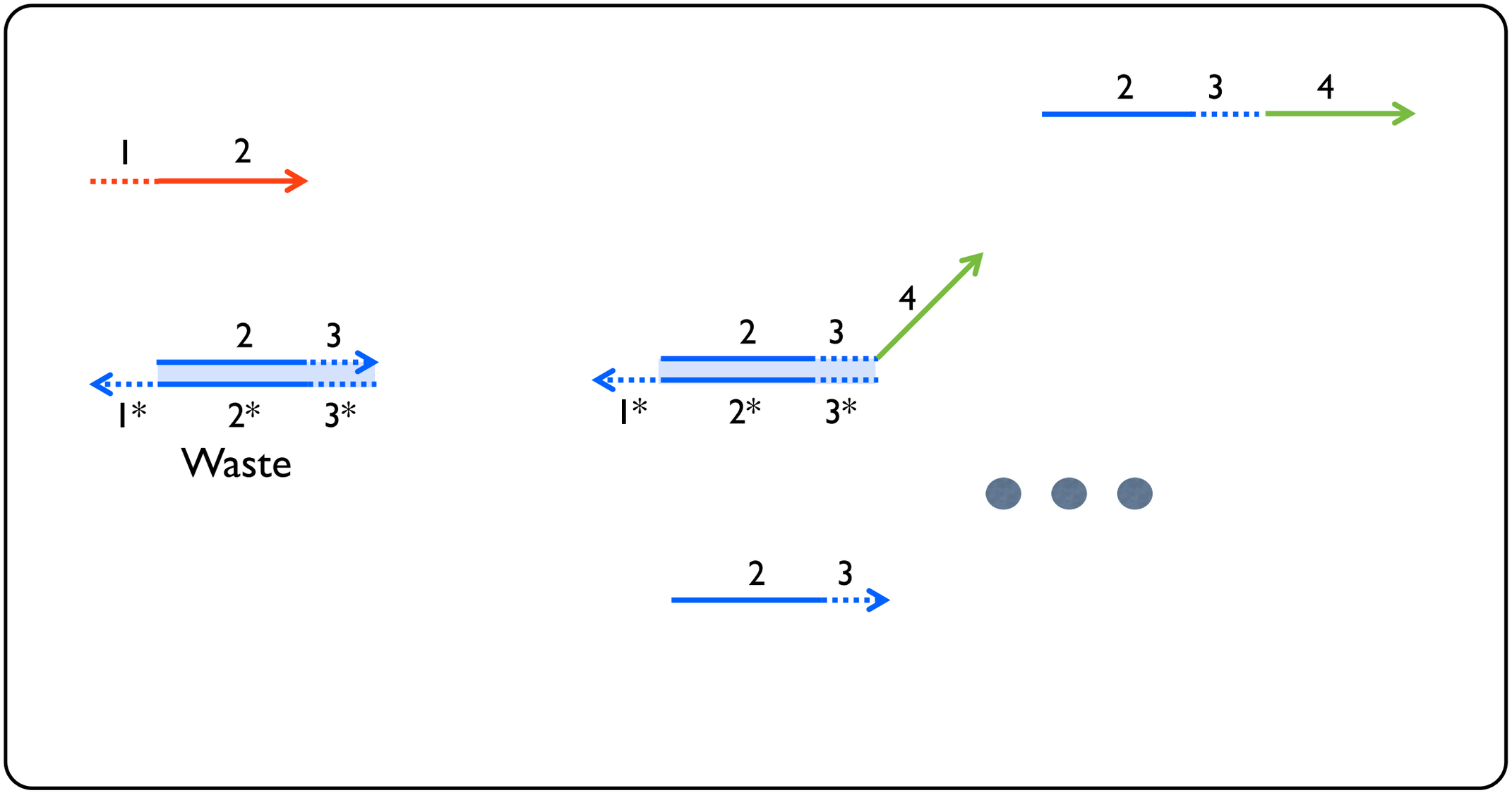

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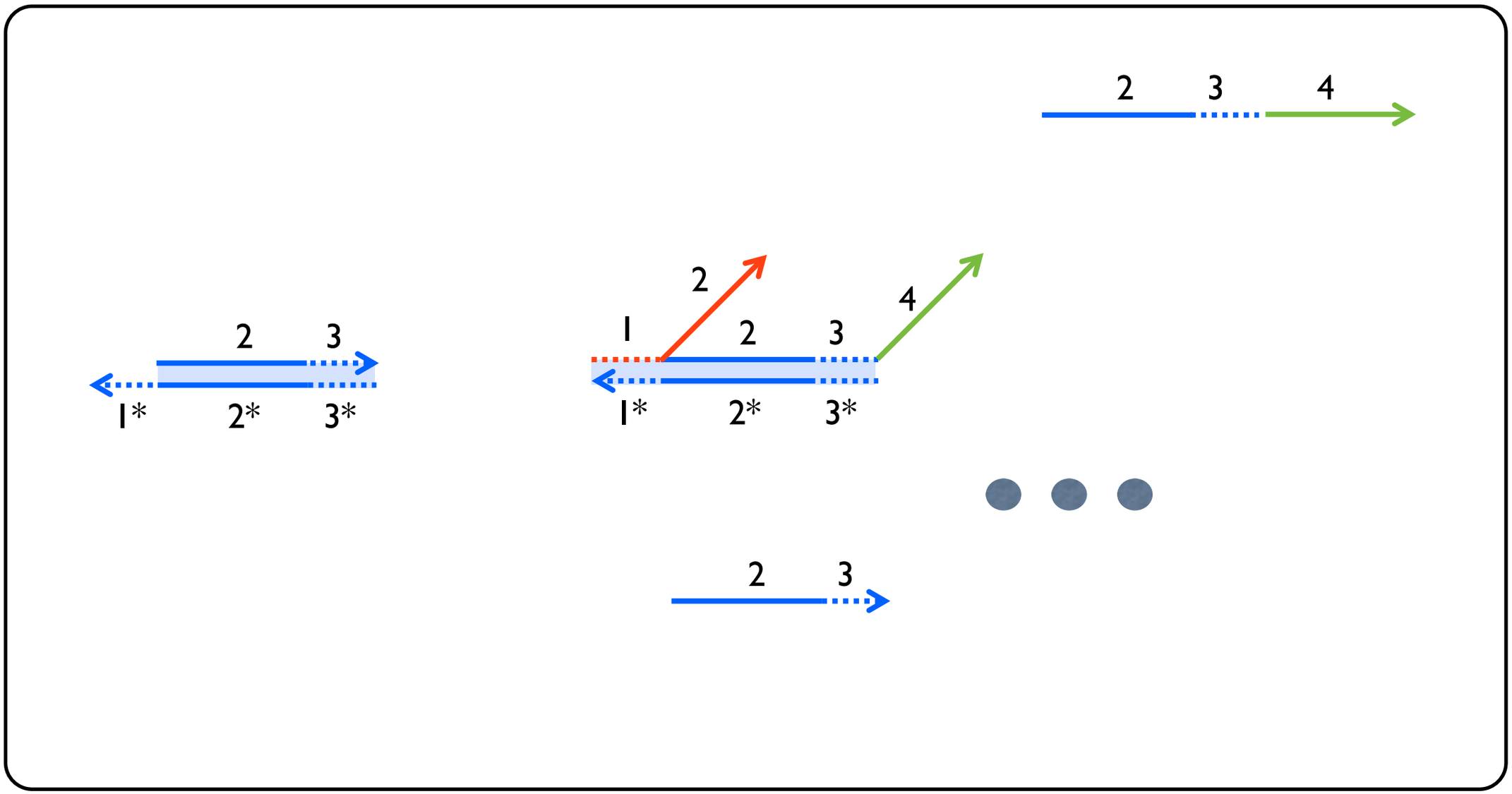

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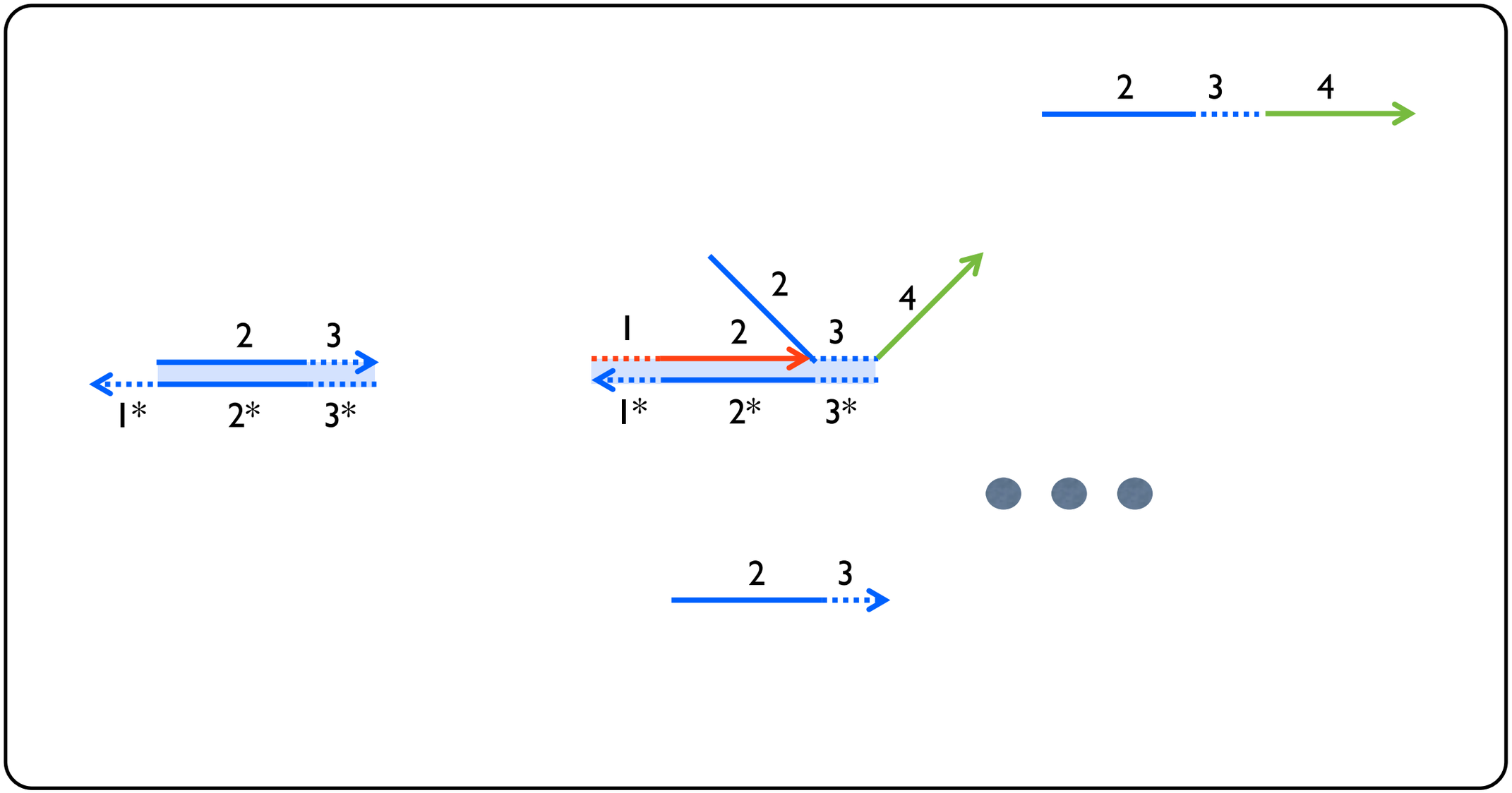

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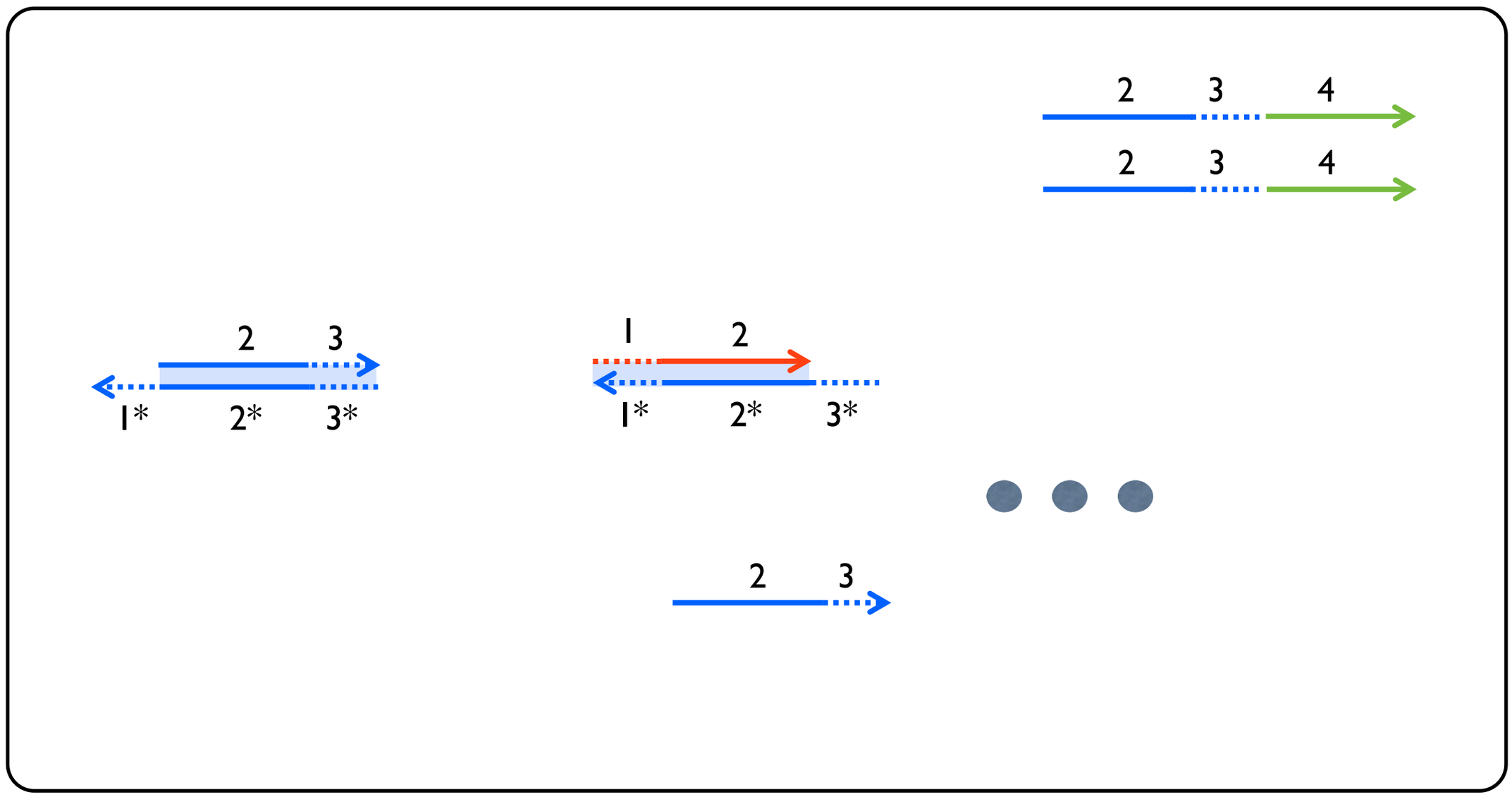

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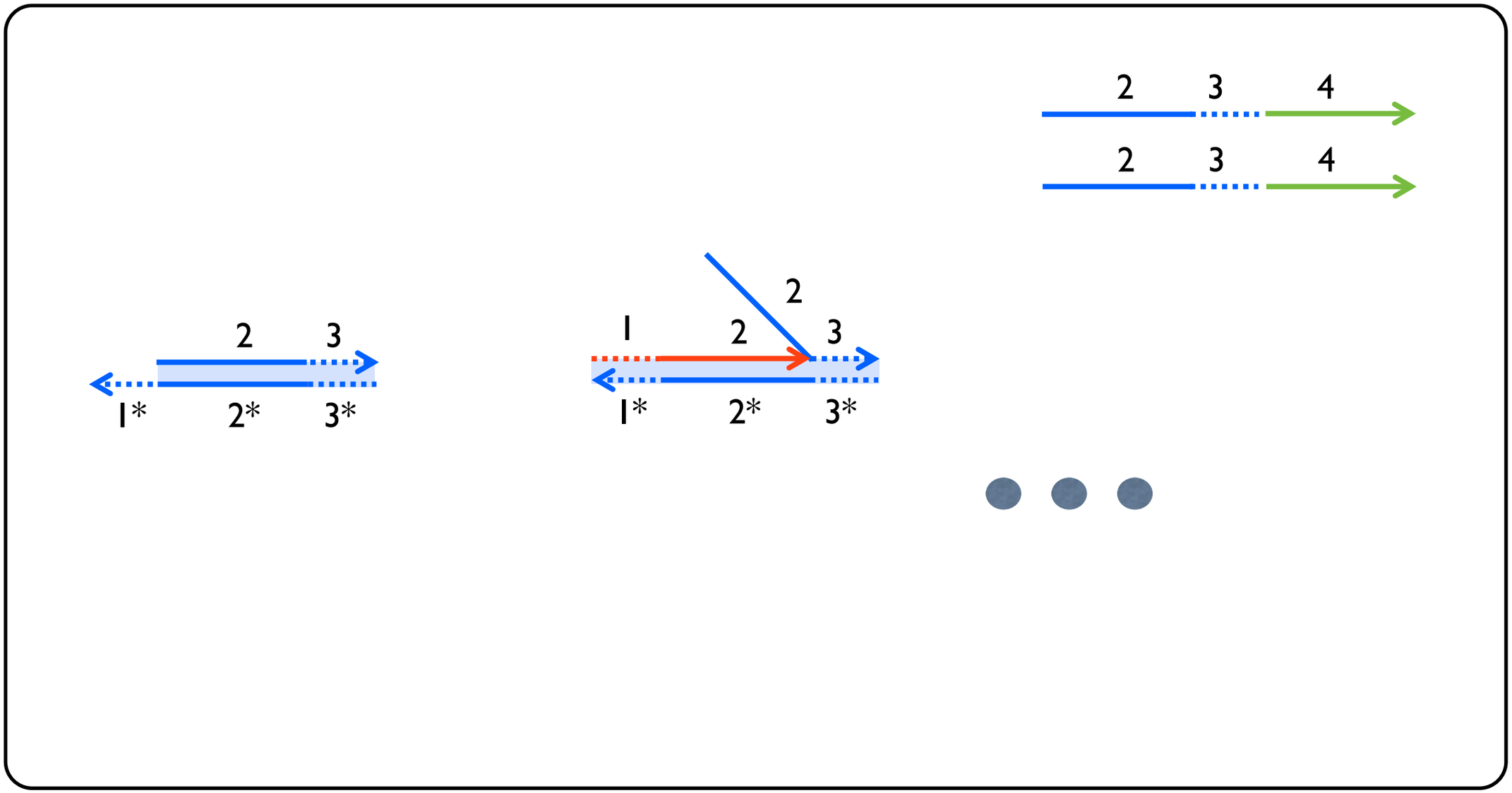

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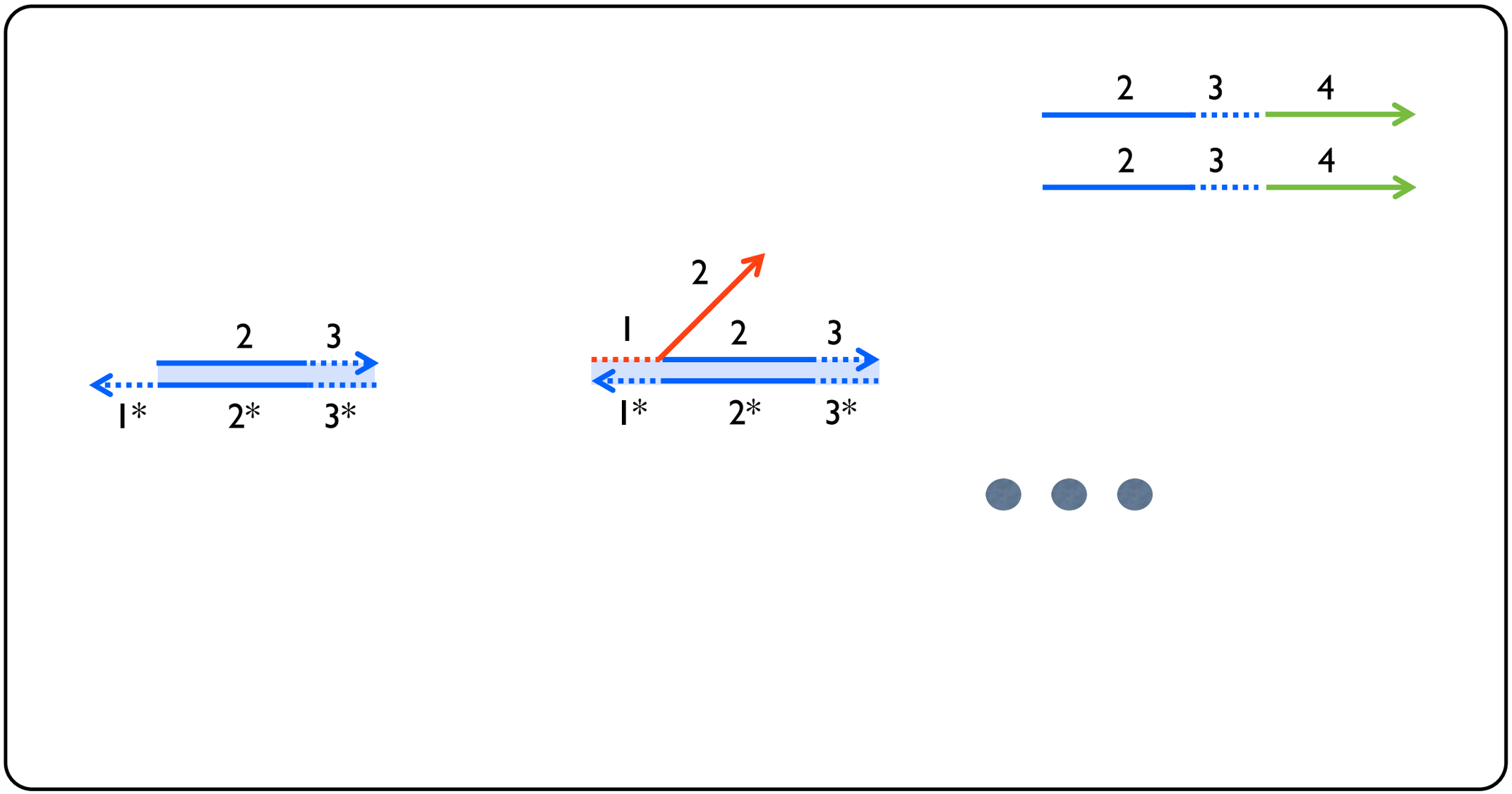

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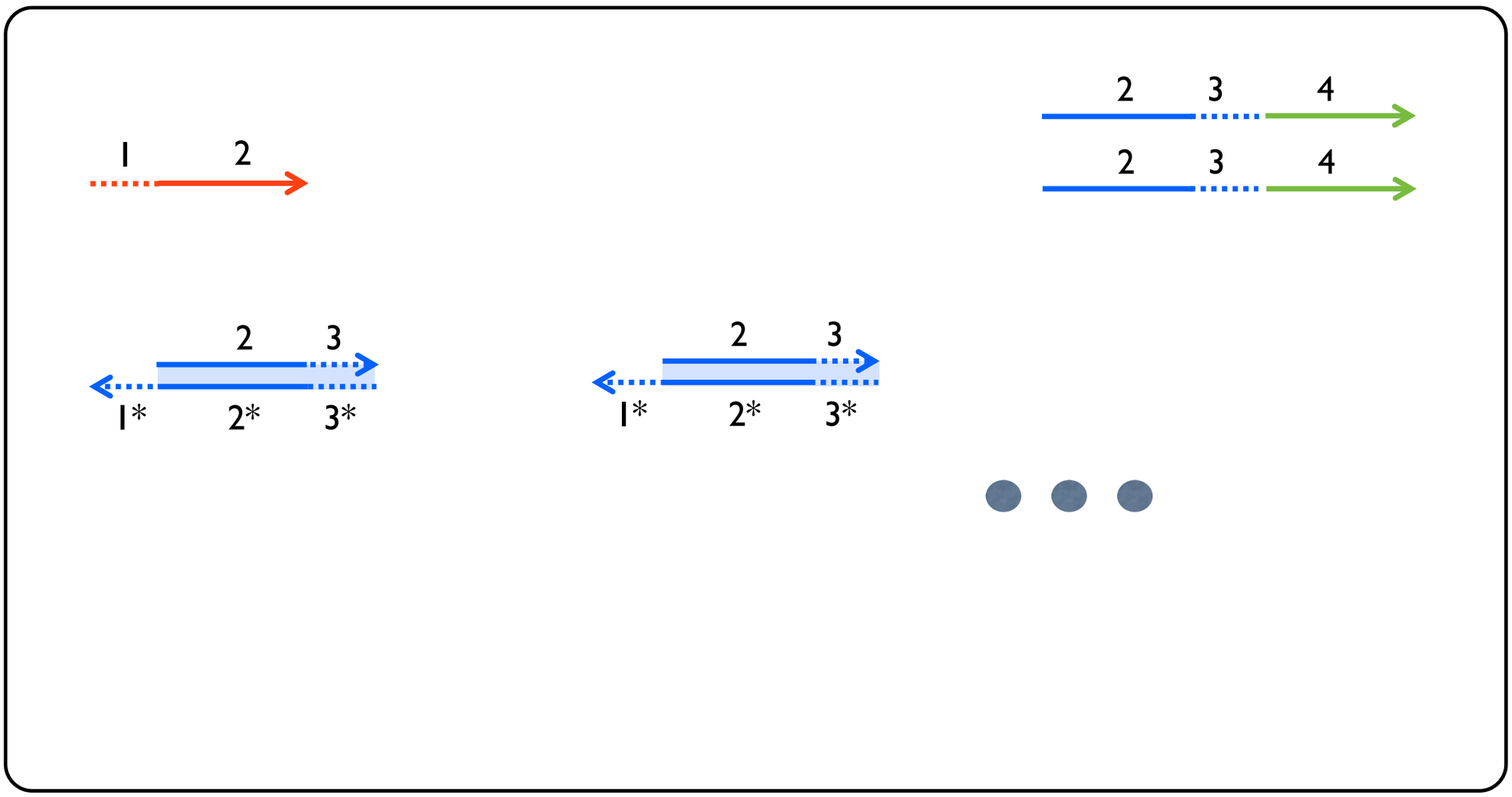

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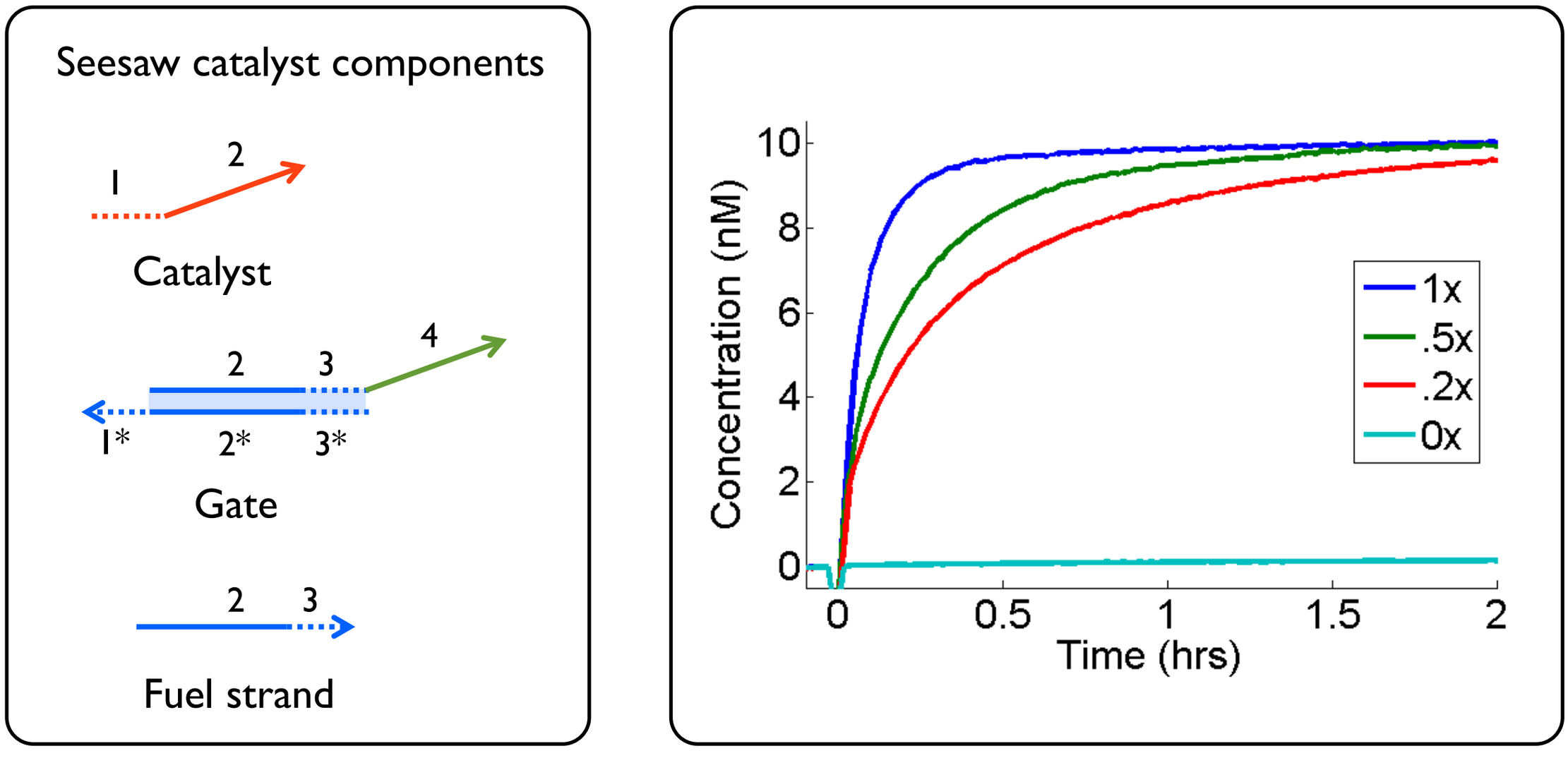

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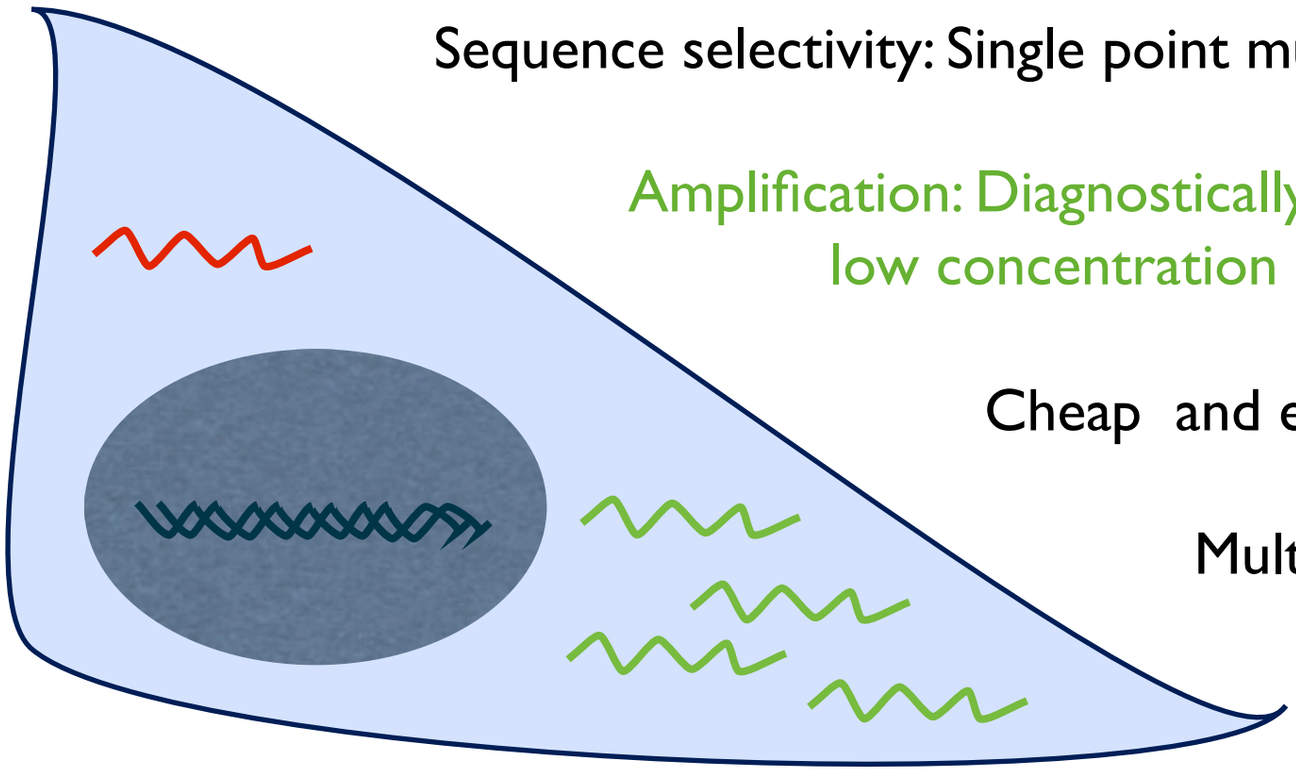
Requirements for a good nucleic acid detection technology



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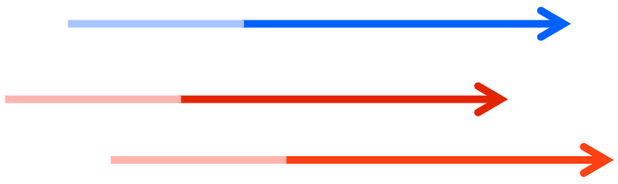
An ideal detection scheme can identify individual point mutations

```
let-7a: ugagguaguagguuguauaguu
let-7b: ugagguaguagguugugugguu
let-7c: ugagguaguagguuguaugguu
let-7d: agagguaguagguugcauaguu
let-7e: ugagguaggagguuguauaguu
let-7f: ugagguaguagauuguauaguu
let-7g: ugagguaguaguuuguacaguu
let-7i: ugagguaguaguuugugcuguu

let-7a*:aacuauacaaccuacuaccuca
```

Single mutations distinguish between multiple members of a miRNA family.

Individual mismatches can be hard to detect



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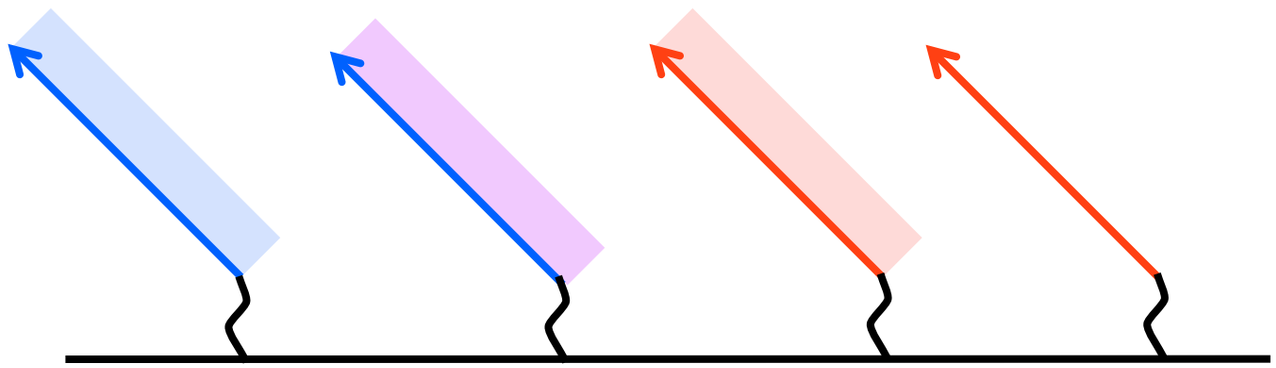
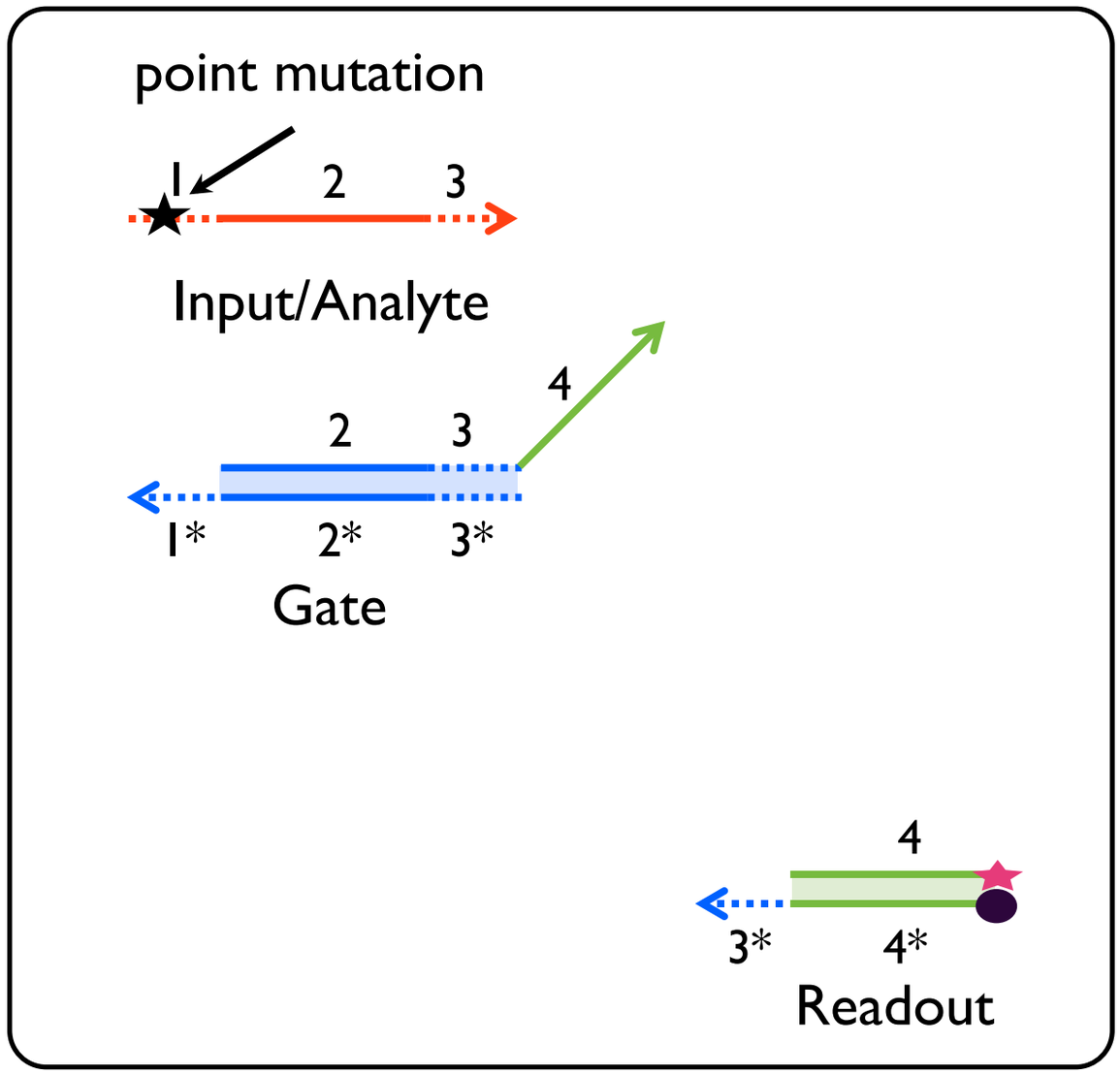
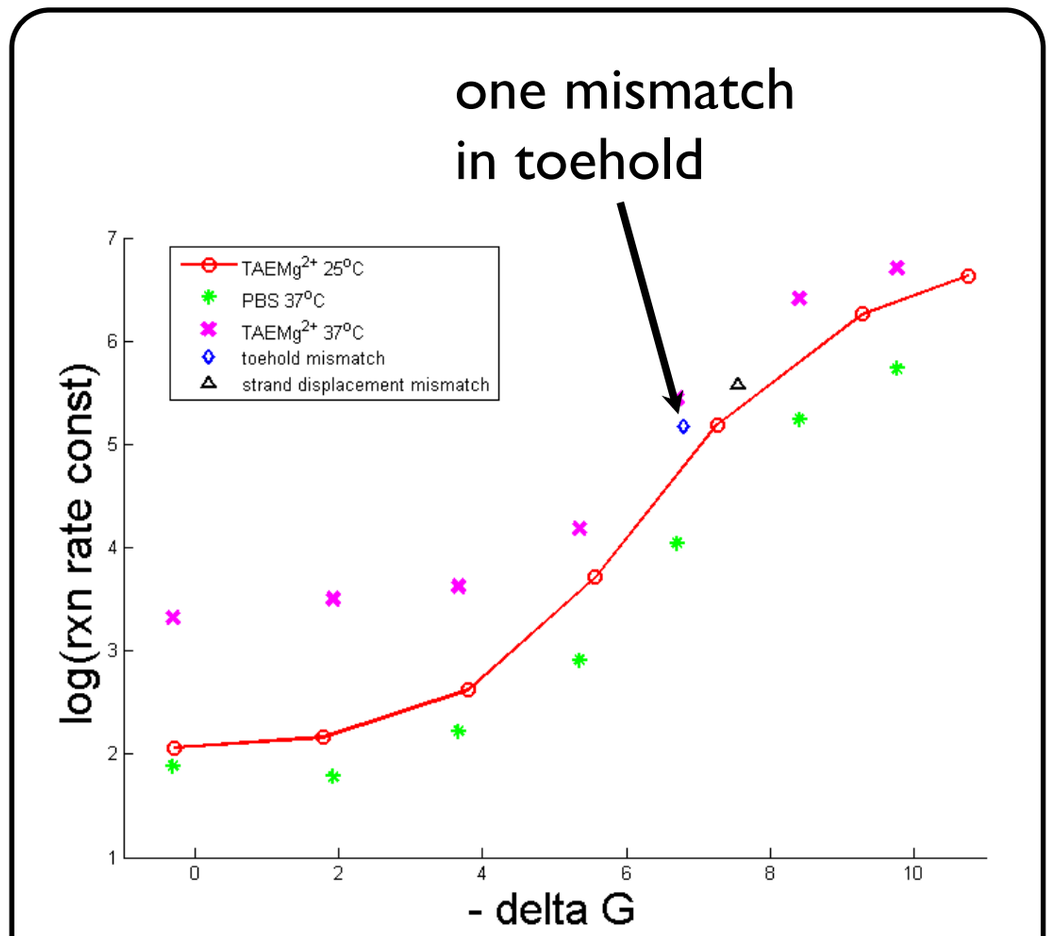


Mismatch discrimination works best near the melting temperature of the probe.

Strand displacement kinetics is sensitive to mismatches





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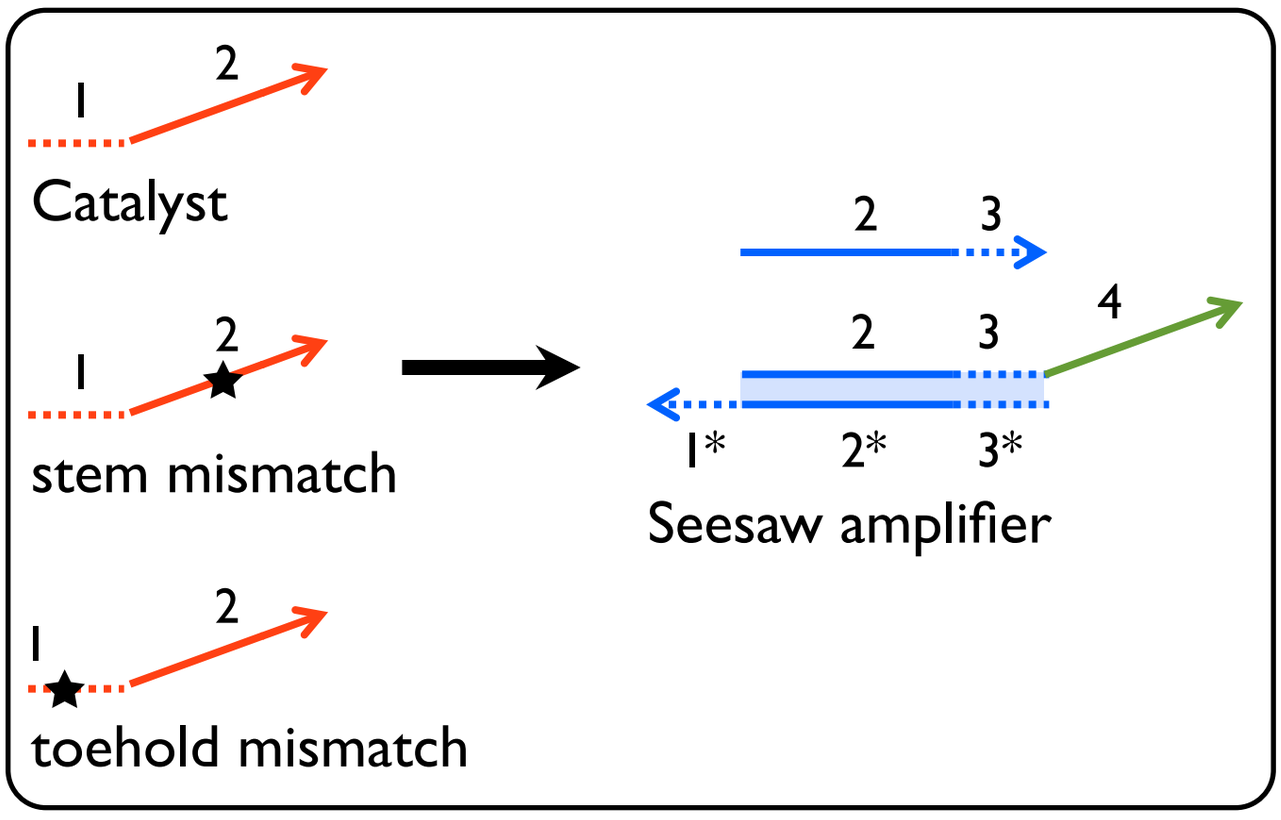
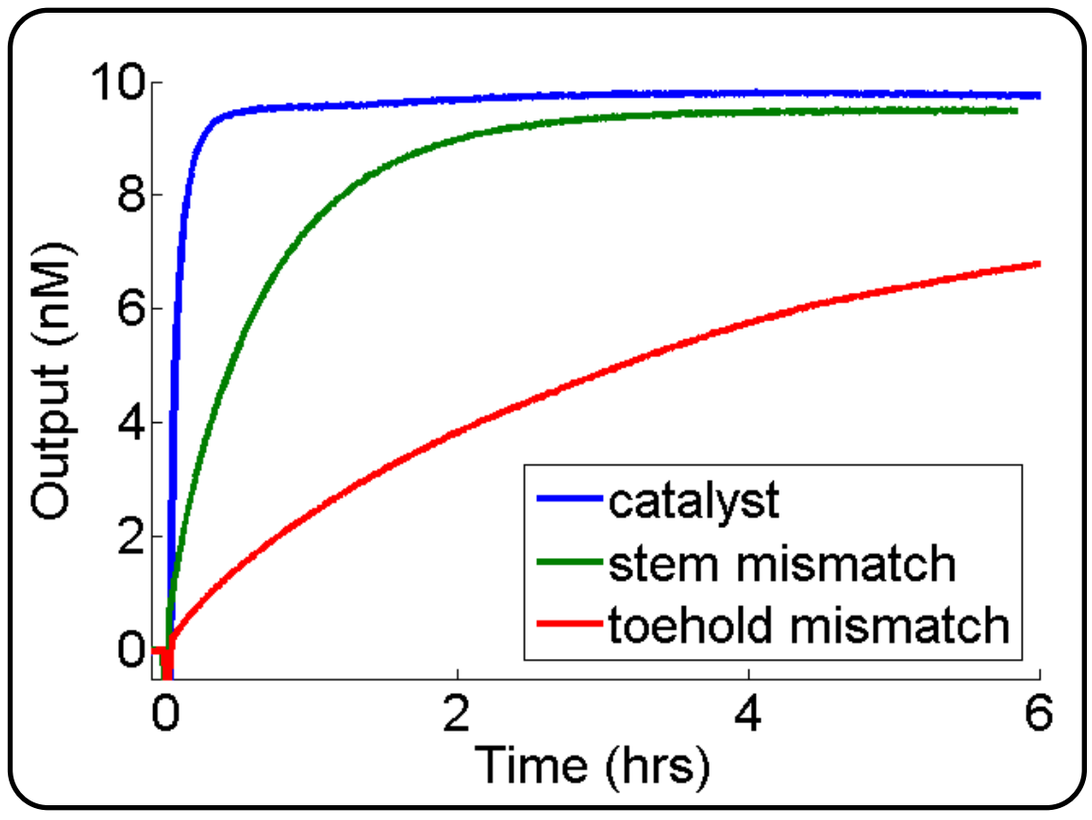
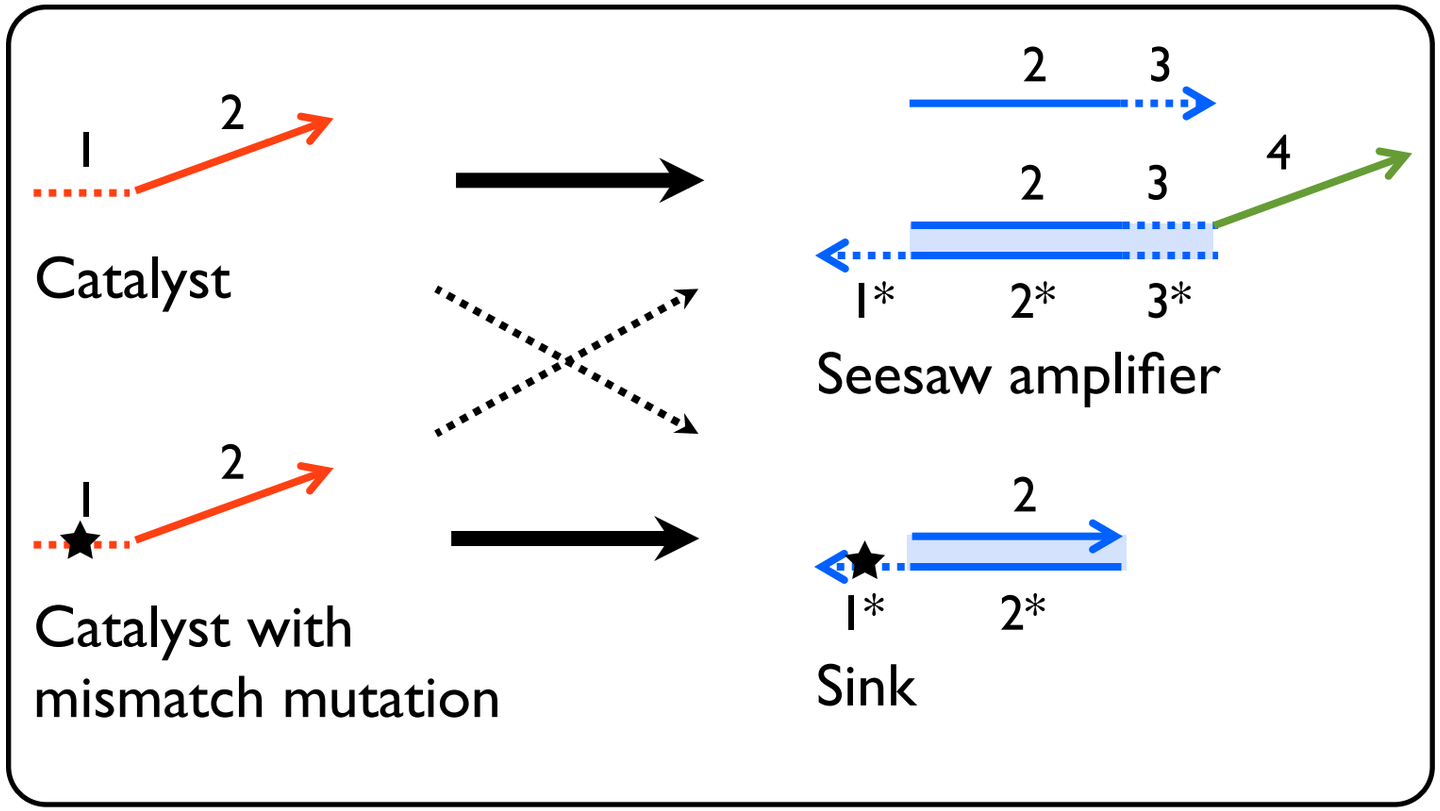
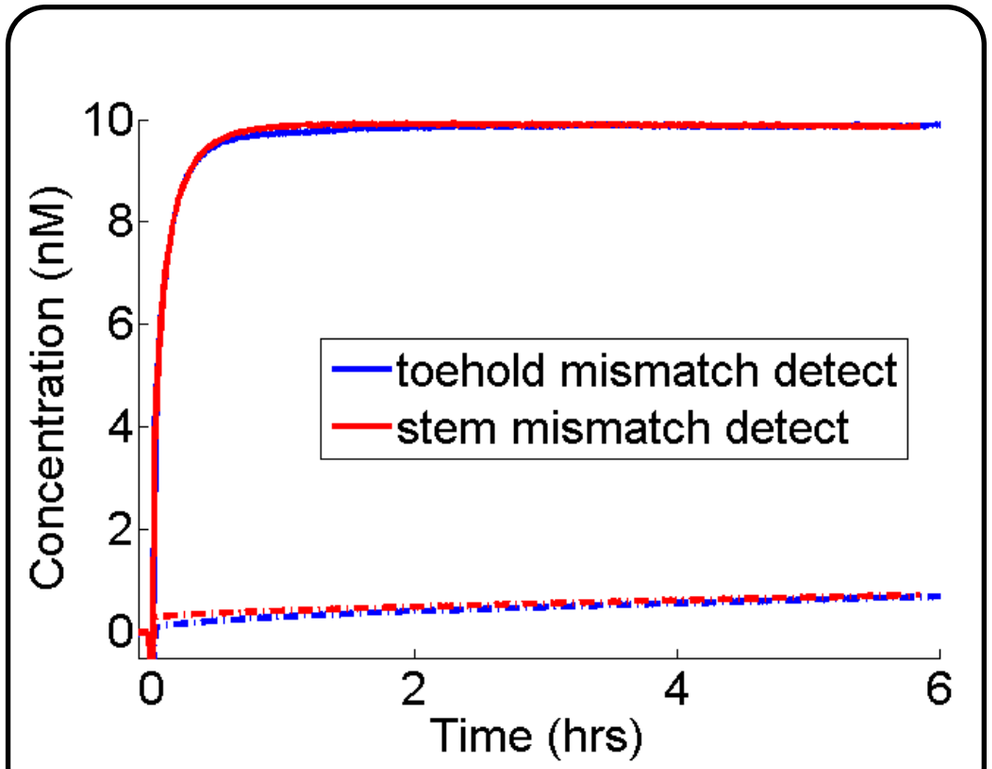




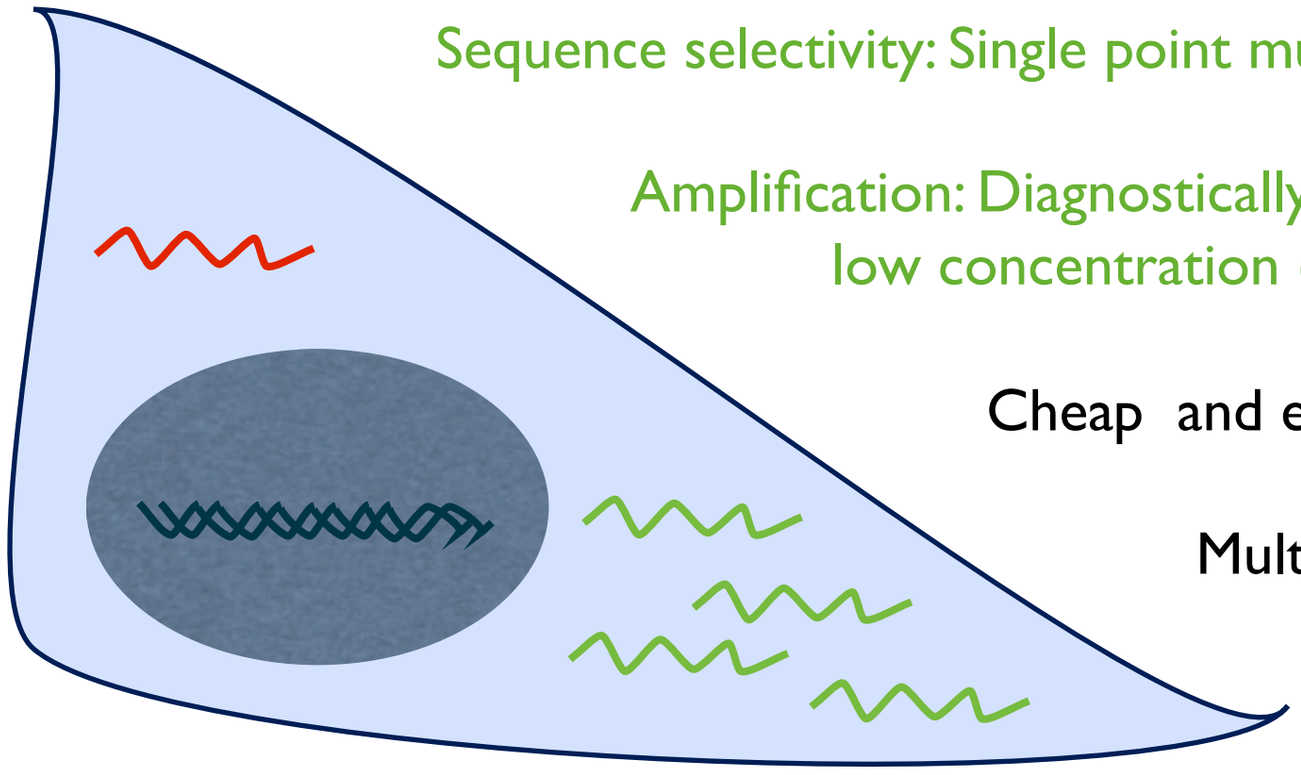
End point discrimination is not possible and kinetics depend on concentrations.

Strand displacement provides a mechanism for mismatch discrimination at the end-point





Requirements for a good nucleic acid detection technology



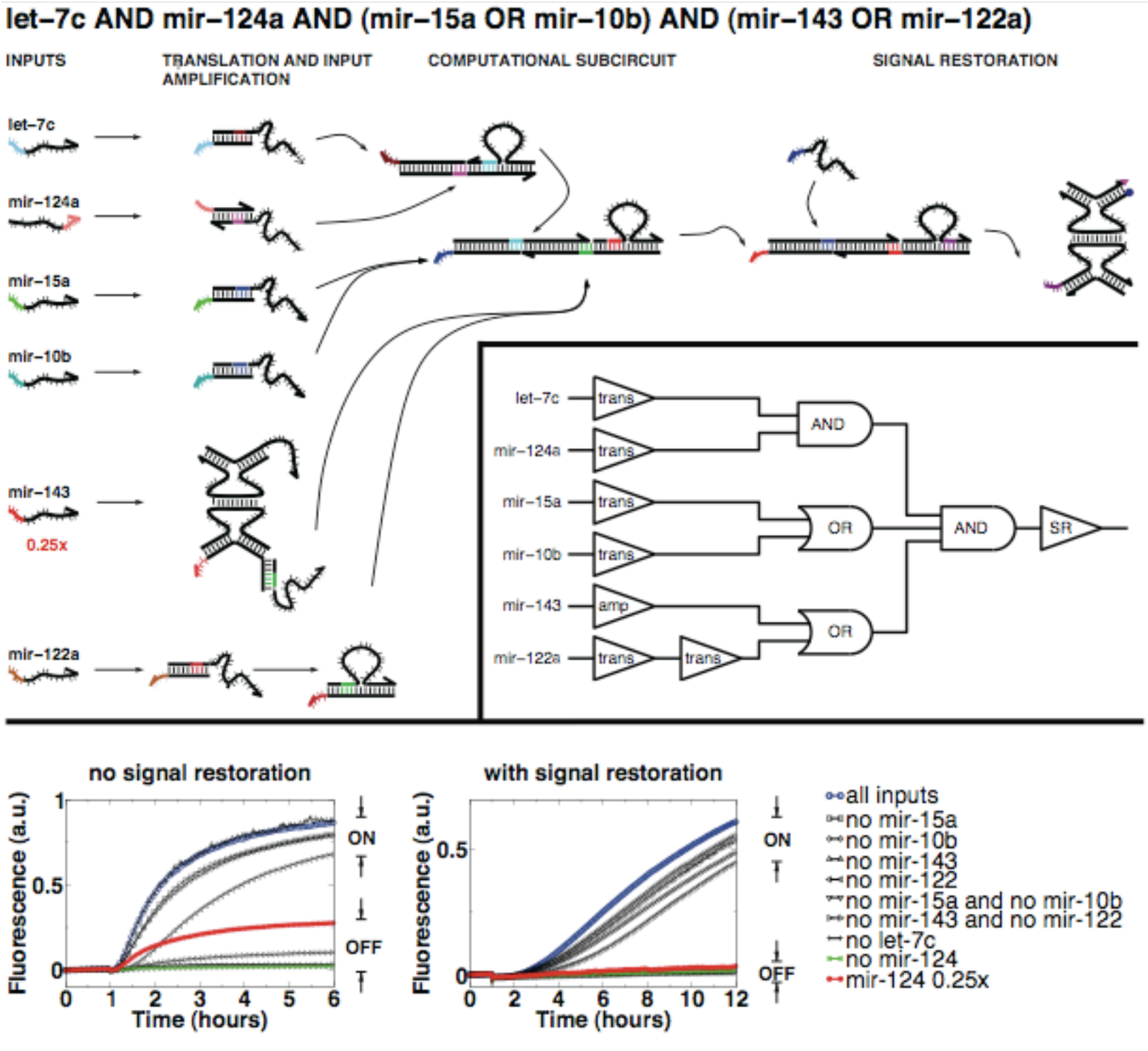
Sequence selectivity: Single point mutations can be diagnostic of a disease

Amplification: Diagnostically relevant nucleic acids can be at very low concentration (mir-141: 100 ‘000 copies/ul in serum)

Cheap and easy to use (for low resource settings)

Multiplexed detection of multiple markers

Strand displacement can be used for multiplexed detection and analysis



Seelig, Soloveichik, Zhang, Winfree Science (2006)

Seelig, Yurke, Winfree JACS (2006)

Soloveichik, Seelig, Winfree, PNAS (2011)

see also Qian, Winfree, Science (2011)