Gibson Cloning

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Basic idea

- Fragments are joined based on double-stranded 15 base pair overlaps at each end
- Generate overlap sequences using PCR
- Vector can be restriction digested or amplified by PCR

Timeline

- Several days before: order primers
- Day 1:
  - PCR your insert
  - PCR or restriction digest your vector
  - Gel purify both fragments (can skip this in some cases)
  - Mix vector and insert in Gibson enzyme/buffer mix, incubate 15 min
  - Transform
- Day 2: Pick colonies, grow overnight
- Day 3:
  - Check for correct insert
  - Send in sequencing
- Day 4: Results! Yay!

Advantages

- No need to find compatible restriction sites (or any sites at all)
- No ligation step
- If you get colonies, you can be pretty sure they’re correct - less time spent trying to find the correct clone!

Disadvantages

- Expensive
- Risk of introducing errors during PCR step
- Need to wait for primers to arrive

Mechanism

Exonuclease

Polymerase

Ligase

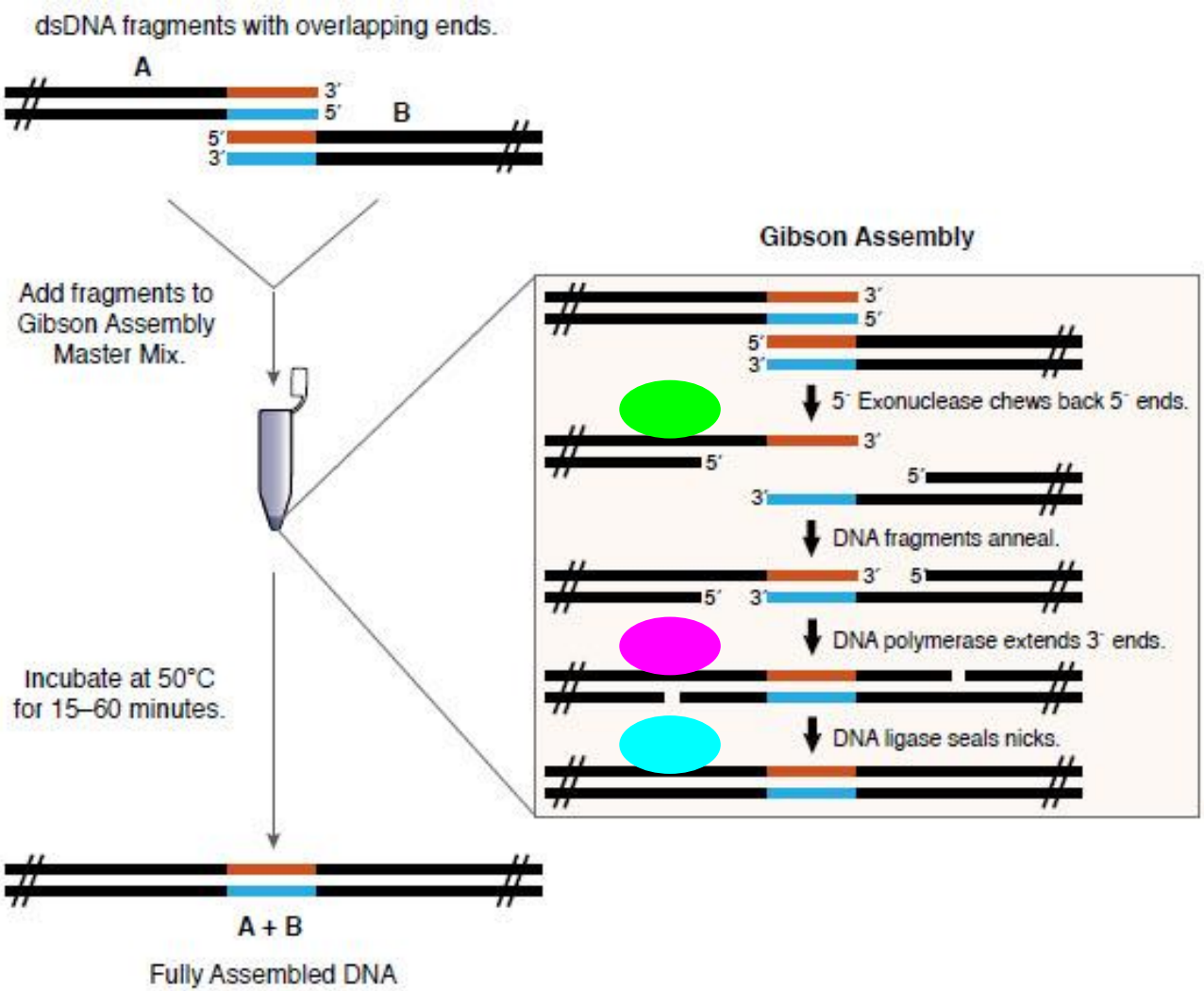
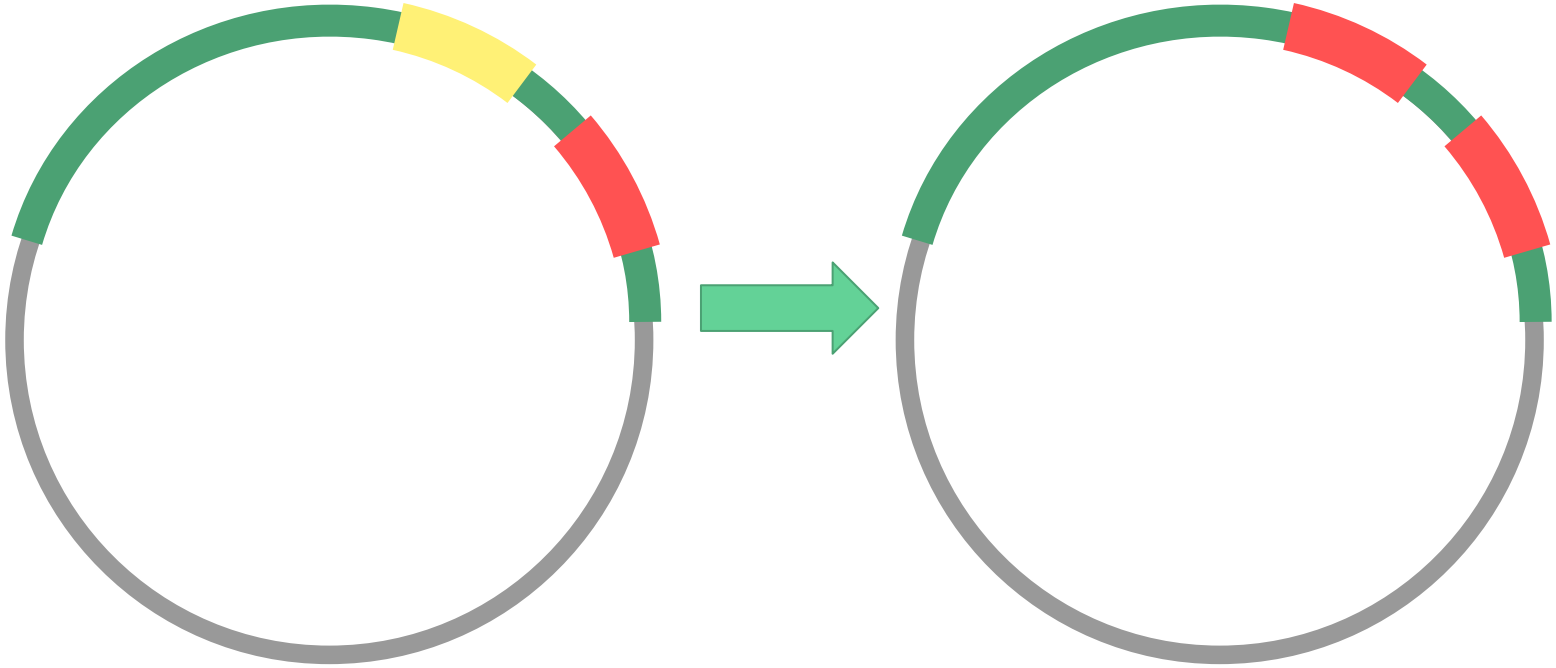
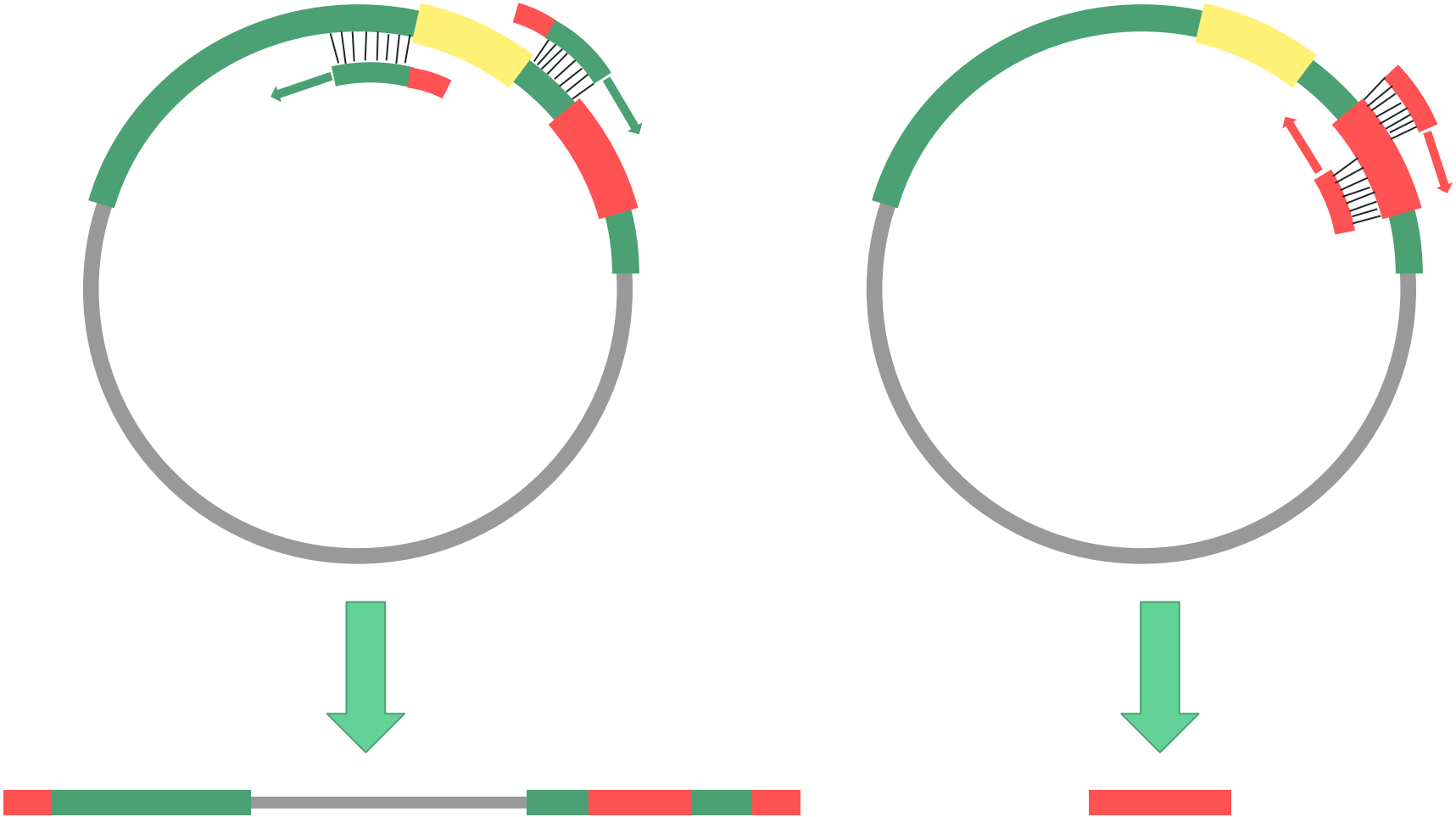
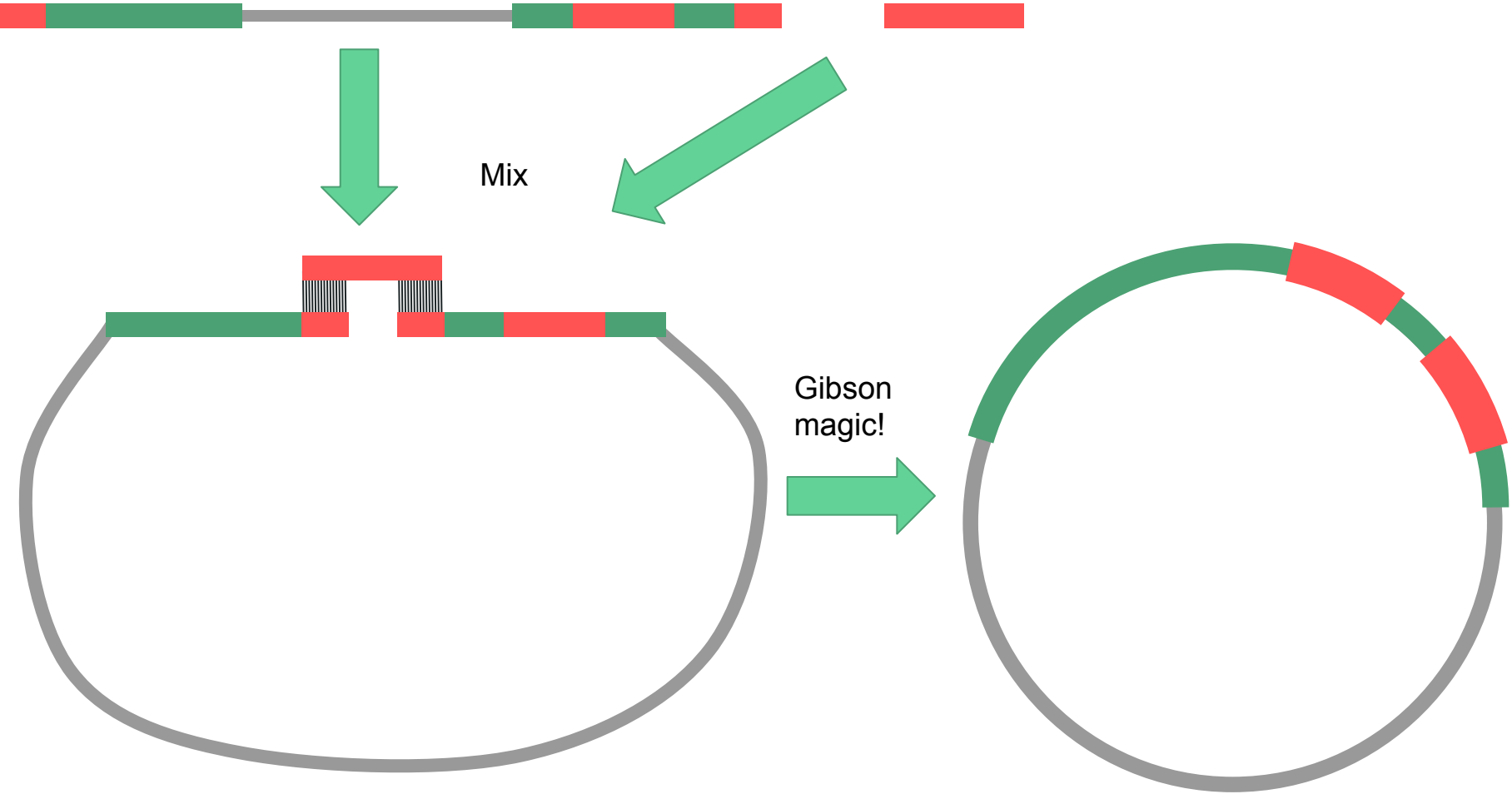


Image: NEB.com

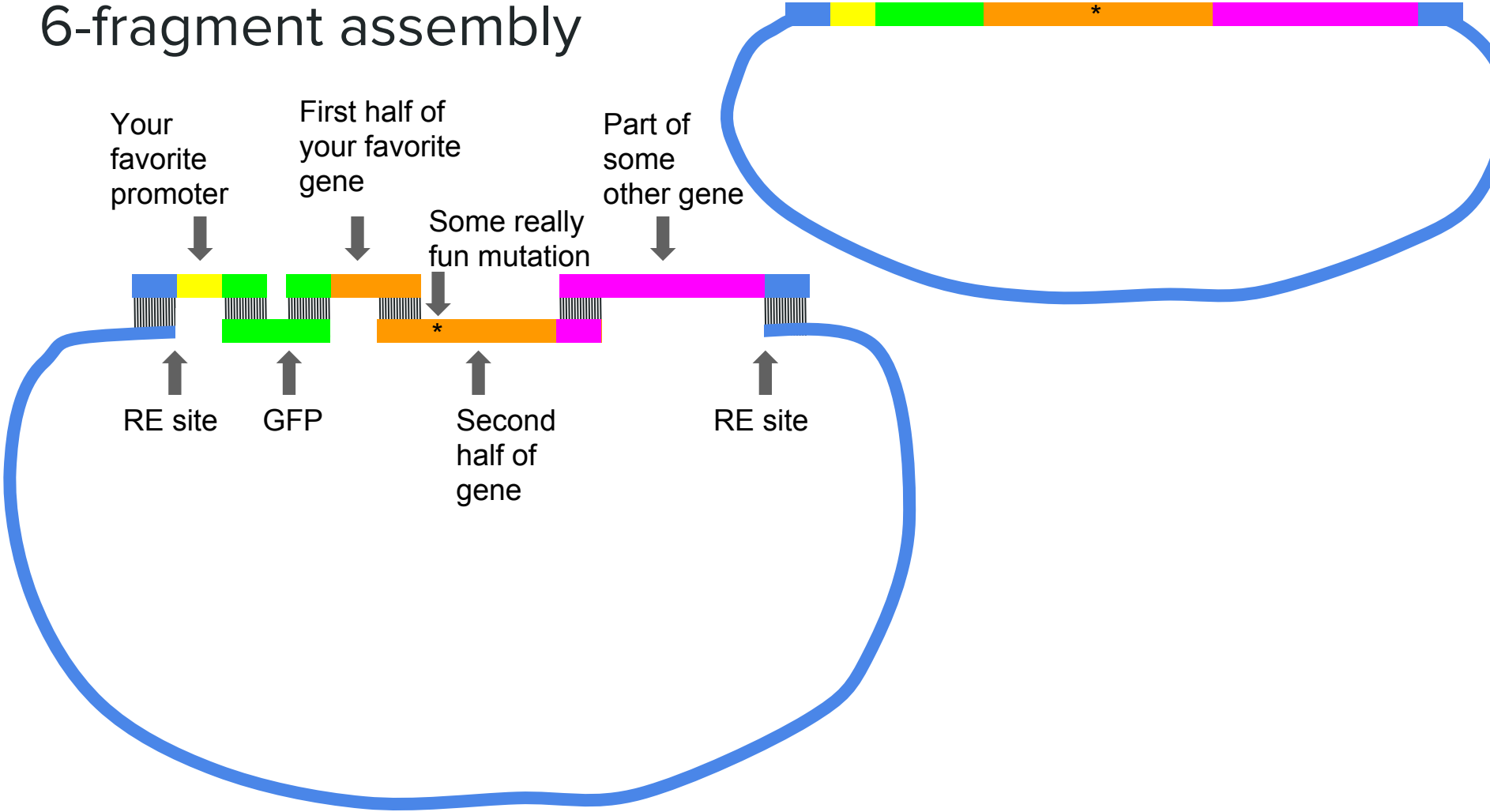
Example cloning project



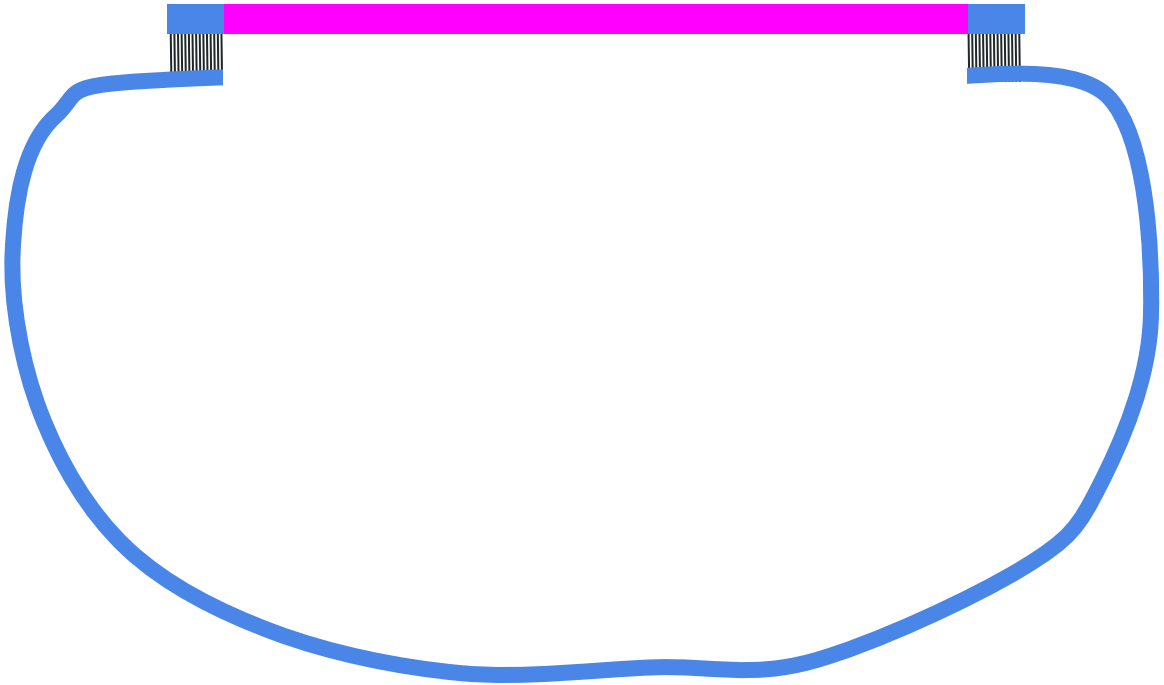



Mix
Gibson
magic!

6-fragment assembly



Primer Design



How do I get it?

- NEB Gibson Assembly / NEBuilder HiFi
  - $16/rxn for 10 rxns
  - $12.60/rxn for 50 rxns ($3.15 for 5uL rxn)
- Clontech In-Fusion (includes competent cells and other stuff)
  - $23.10/rxn for 10 rxns
  - $17.84/rxn for 50 rxns
  - $15.14/rxn for 100 rxns ($3.79 for 5uL rxn)
- Make your own: https://openwetware.org/wiki/Gibson_Assembly might be a good place to start?

For comparison:

- CloneAmp: $0.96/rxn
- XbaI: $0.23 per 10 units
- Sequencing: $5/rxn, or $35 to sequence my main gene of interest