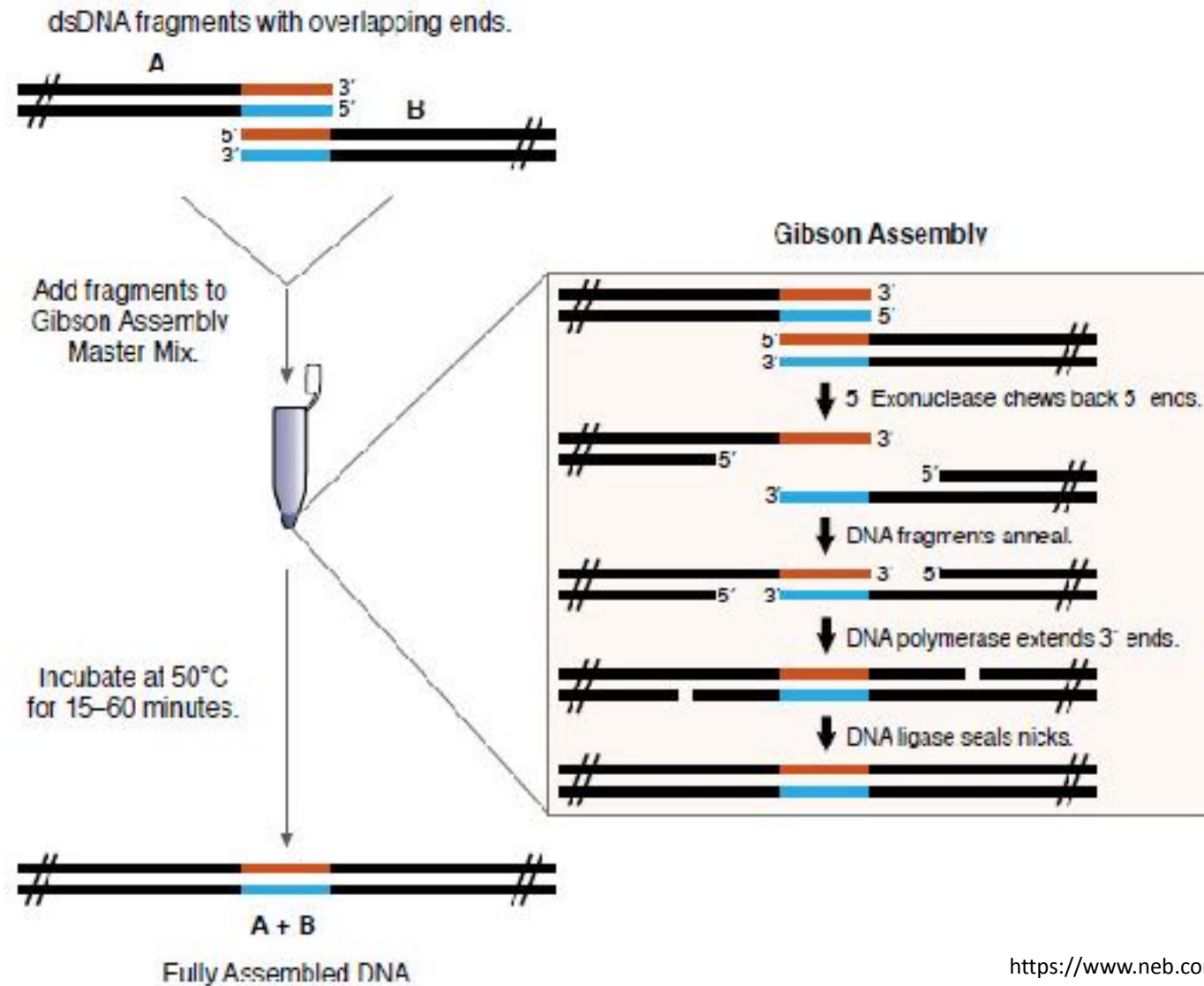
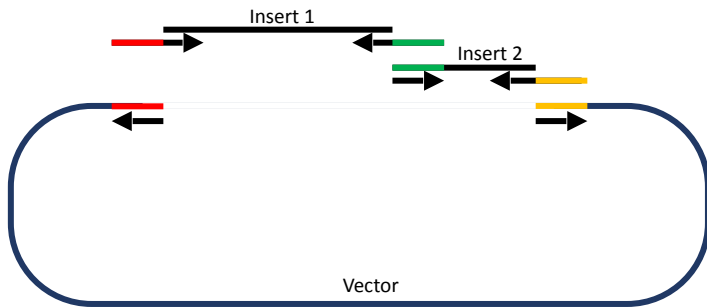


Gibson assembly



Circular polymerase extension cloning (CPEC)

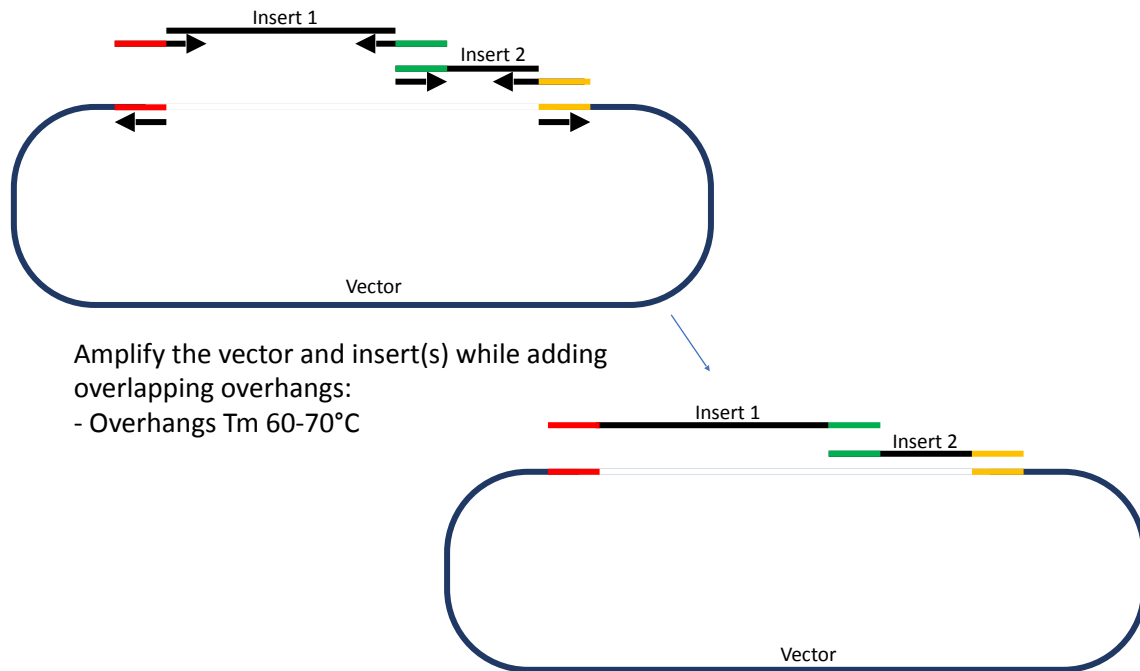
- Directional, ligase-free cloning method
- Allows assembly of multiple overlapping DNA fragments into a circular plasmid



Amplify the vector and insert(s) while adding overlapping overhangs:
- Overhangs T_m 60-70°C

Circular polymerase extension cloning (CPEC)

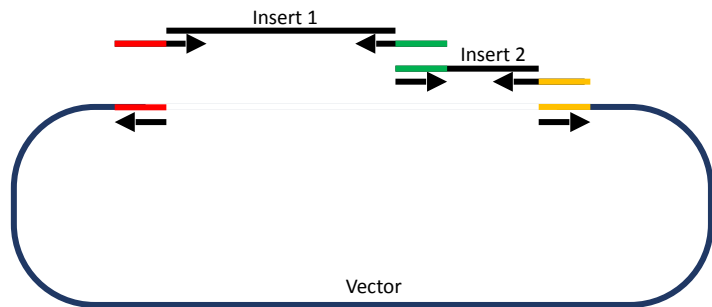
- Directional, ligase-free cloning method
- Allows assembly of multiple overlapping DNA fragments into a circular plasmid



- Combine purified DNA pieces at 1:1 molar ratio
- Low DNA concentration favors circularisation and prevents concatenation

Circular polymerase extension cloning (CPEC)

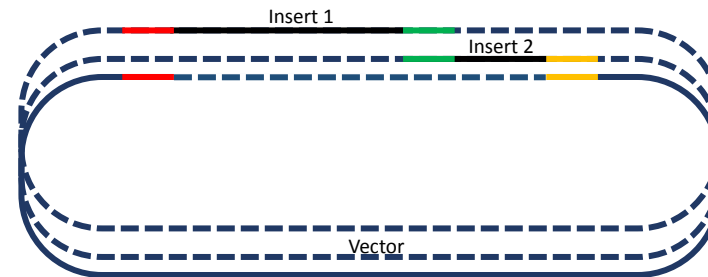
- Directional, ligase-free cloning method
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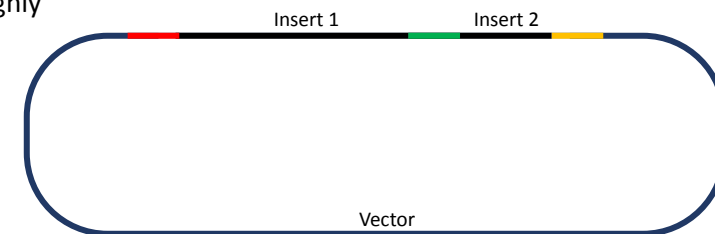
Amplify the vector and insert(s) while adding overlapping overhangs:
- Overhangs T_m 60-70°C



Combine purified DNA pieces at 1:1 molar ratio
- Low DNA concentration favors circularisation and prevents concatenation



Run PCR with high fidelity polymerase:
- single strands anneal with their overlapping ends and extend using each other as a template.
- Stringent annealing T (slow ramp 55-70°C) - highly accurate multiway assembly



All pieces assembled into ds circular plasmid, ready for transformation.