Building Synthetic Circuits.

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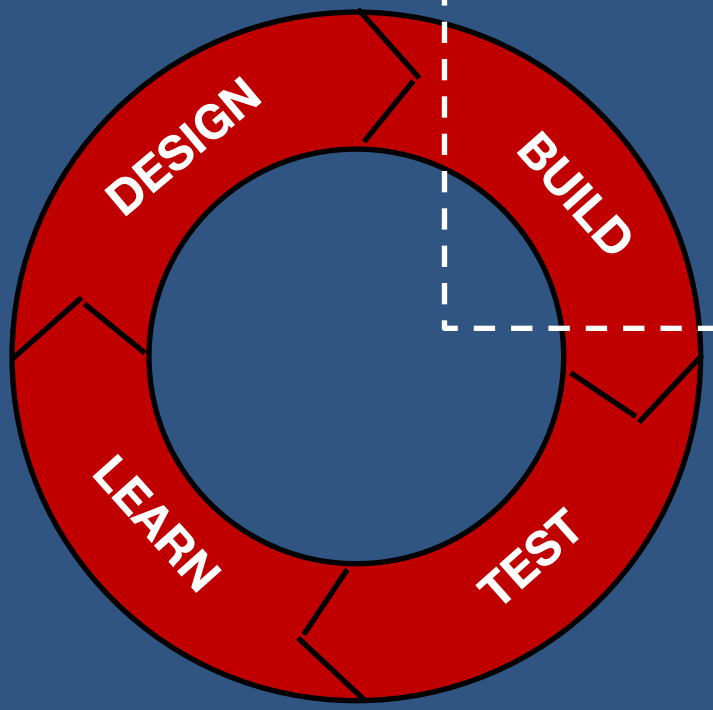
Learning Goals

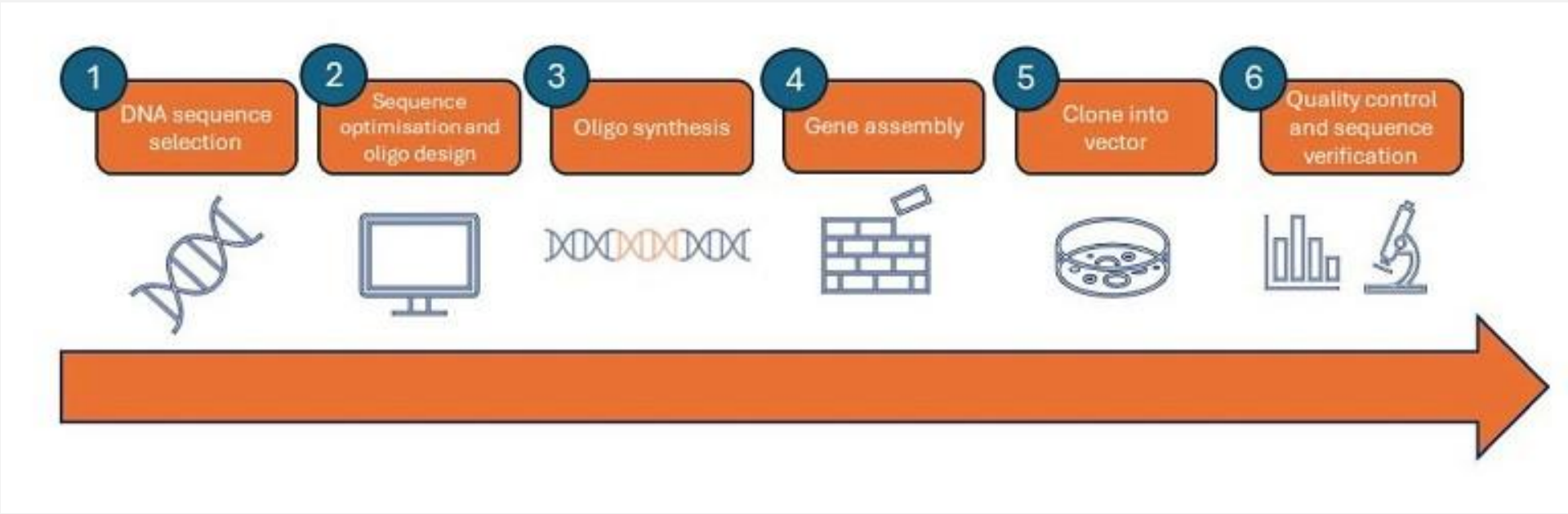
To understand:

- How to decide when and what to synthesise
- What are standardised DNA parts
- How to assemble multiple DNA parts in a single reaction
- How standardisation aids construct design and assembly

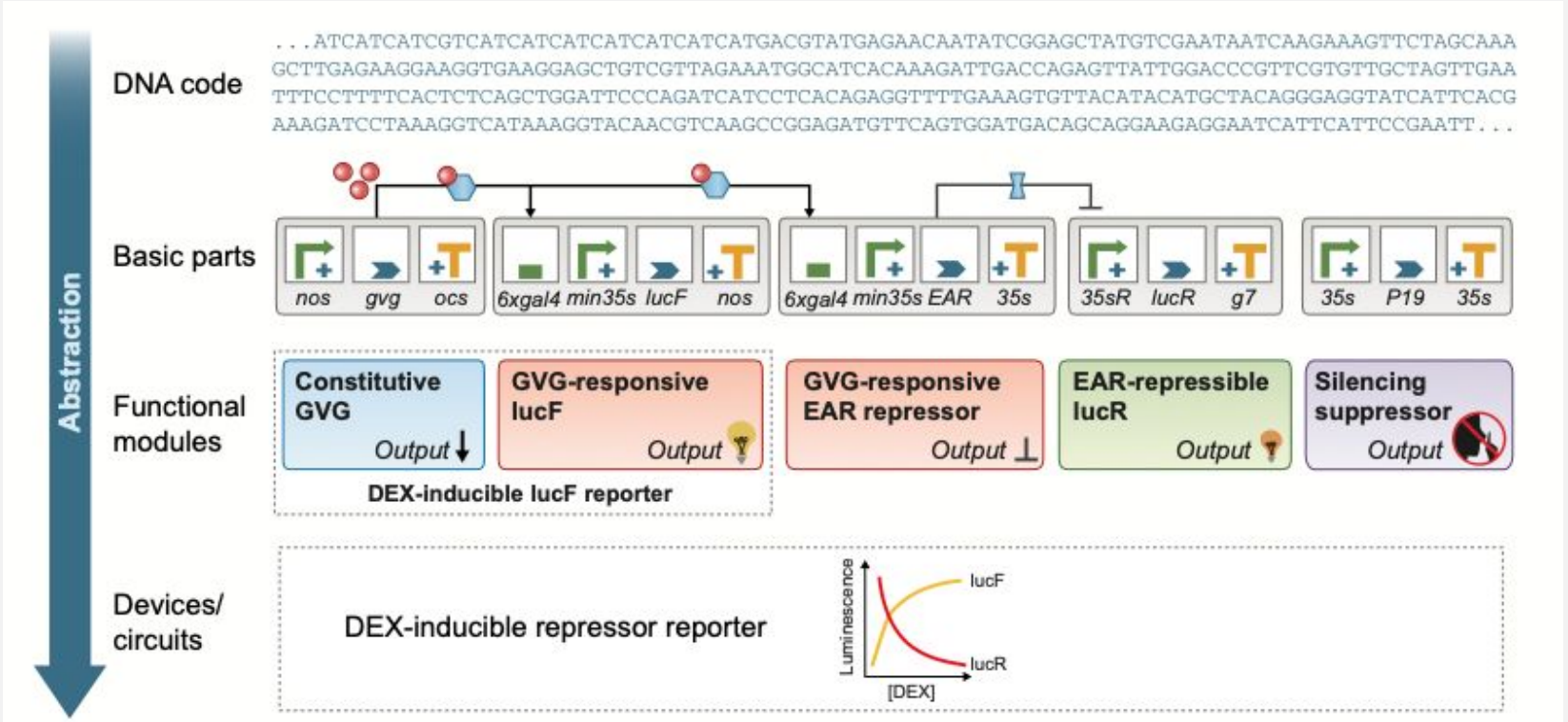


Synthesis vs. Assembly

Synthesis – oligos, blocks & genes



Abstraction

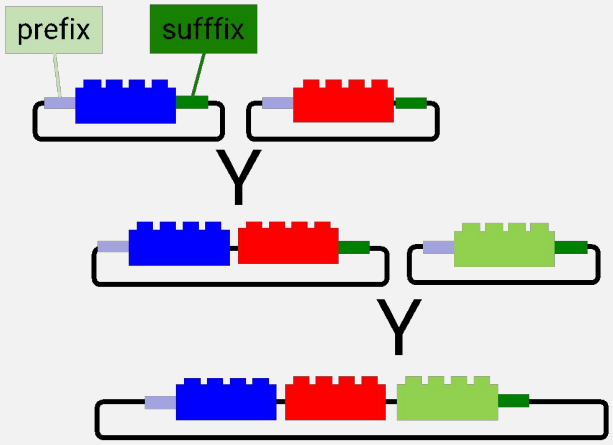


Figures: Patron (2020) New Phytologist

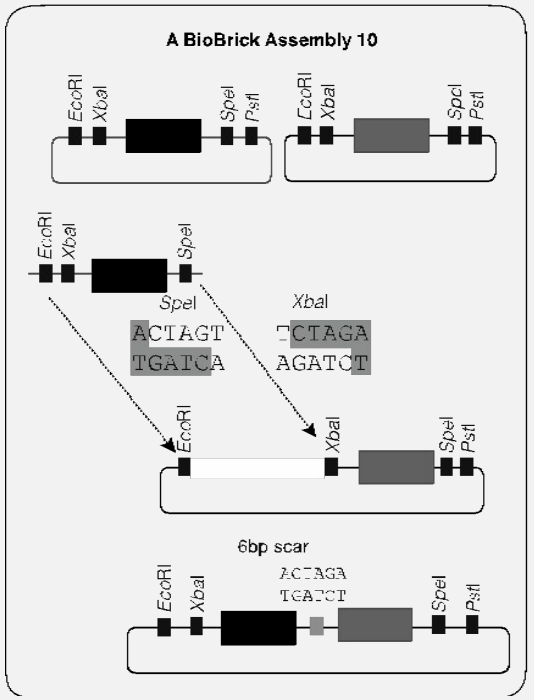
Standardisation

Biological Standards:
The BioBrick (BBF RFC10)

2004



Idempotent



BioBricks FOUNDATION

iGEM

Registry of Standard Biological Parts

Standardisation

cannot , not do understand
I what I
create

Richard Feynman

Standardisation

Biological Standards: Common Genetic Syntax (BBF RFC106)

2015

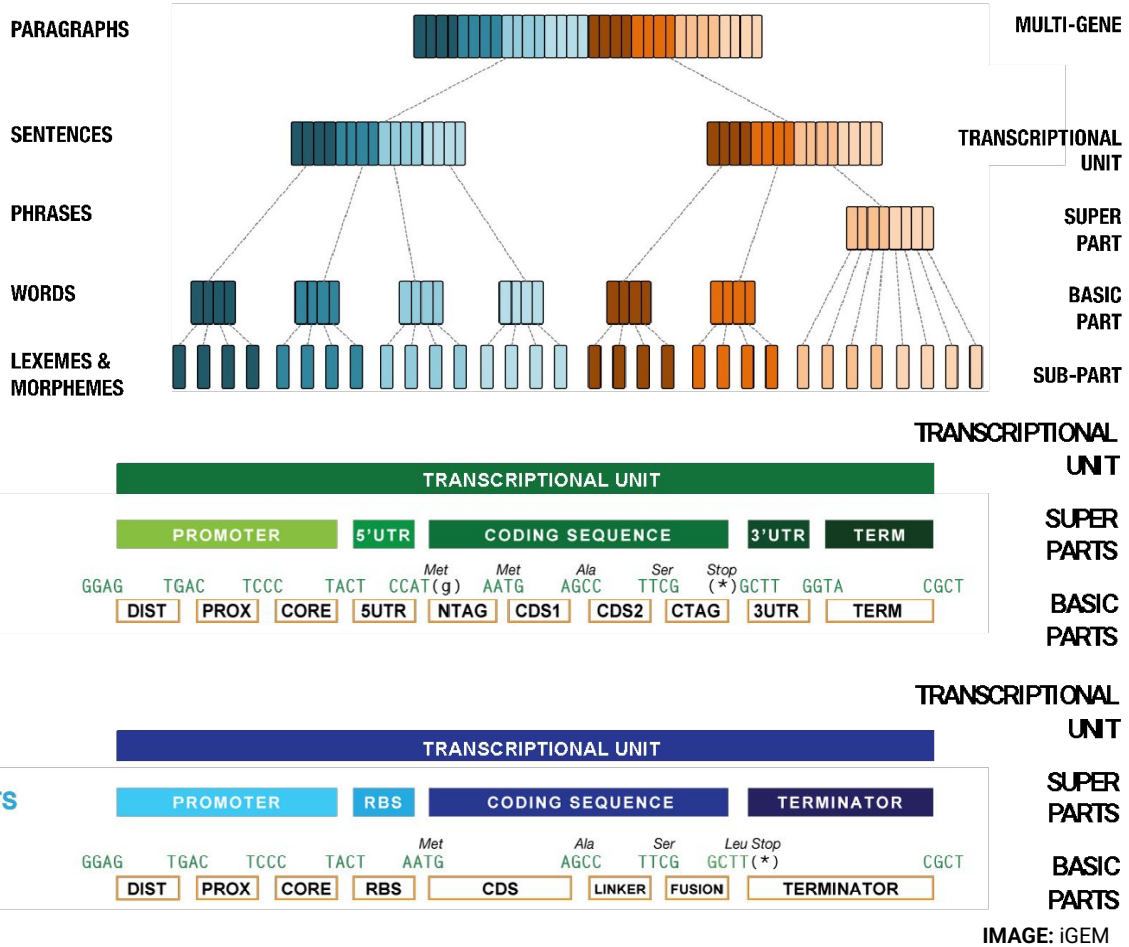
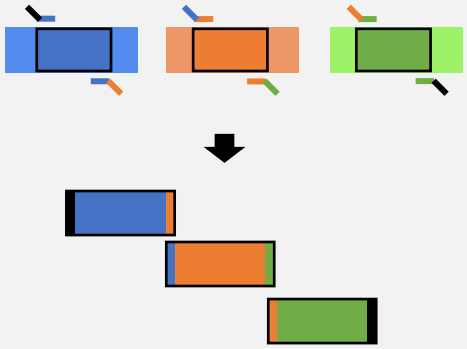


IMAGE: iGEM

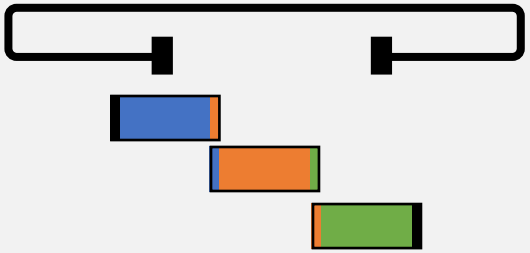
Overlap-dependent Parallel Assembly

Isothermal (aka Gibson) Assembly

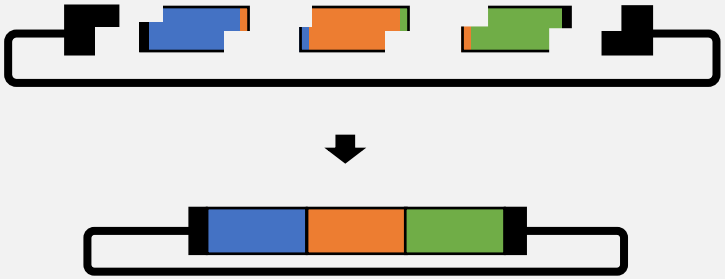
Production of overlapping double-stranded DNA fragments



Overlapping linear fragments are combined with a three-enzyme cocktail:

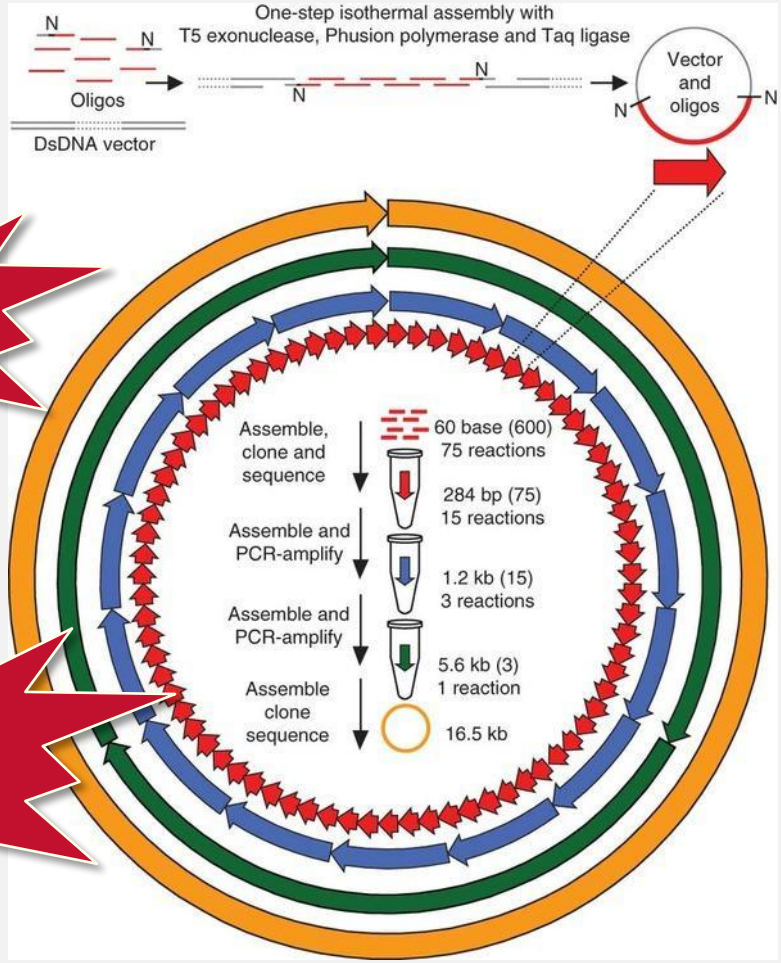


T5 exonuclease performs a 5’-3’ resection of all components allowing complementary overhangs to self-anneal.
A proof-reading **DNA polymerase** & **Taq-ligase** fill in and join gaps



Overlap-dependent Parallel Assembly

16,520 bp mouse mitochondrial genome from 600 fragments



44 parts assembled in 3 steps

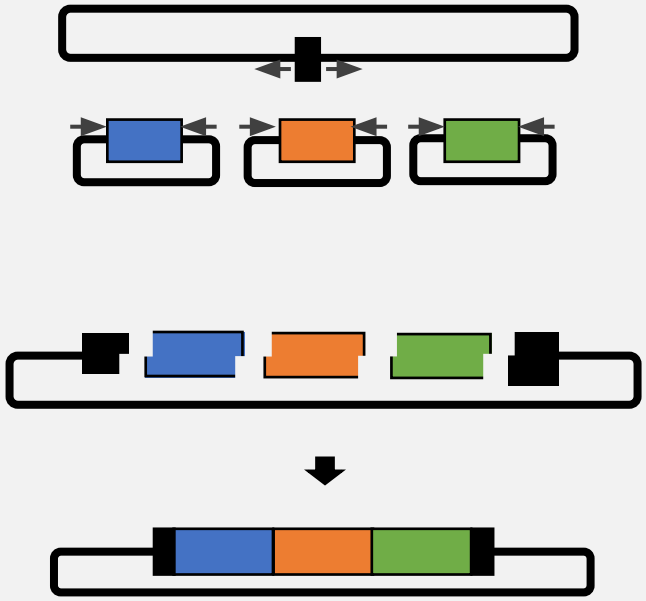
Entirely synthetic. Assembled 600 oligos in 5 steps

Figure: Gibson et al (2010) Nature Methods 7, 901–903

Ligase-dependent Parallel Assembly

Type IIS (aka Golden Gate) Assembly

Parts flanked by Type IIS restriction endonuclease recognition sites

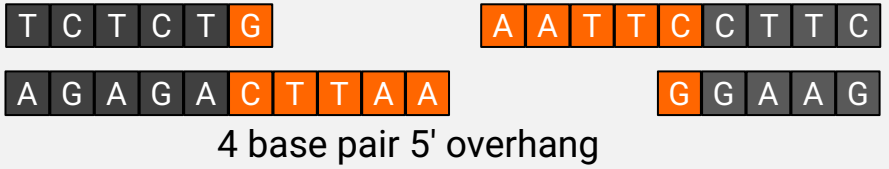


Ligase-dependent Parallel Assembly

Type II Restriction Endonucleases

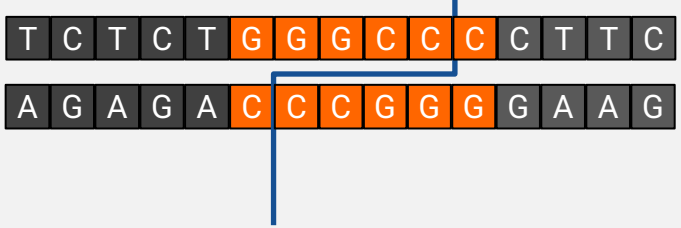
Palindromic, cleave inside of their recognition sequence:

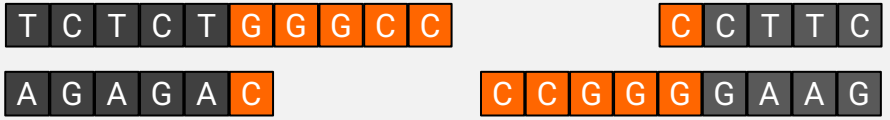
EcoRI G□AATTC



4 base pair 5' overhang

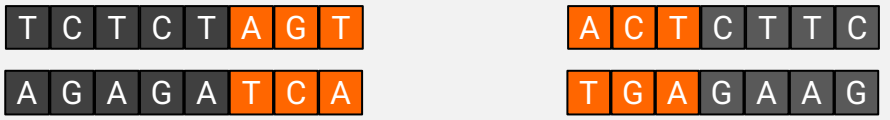
ApaI GGGCC□C





4 base pair 3' overhang

ScaI AGT□ACT

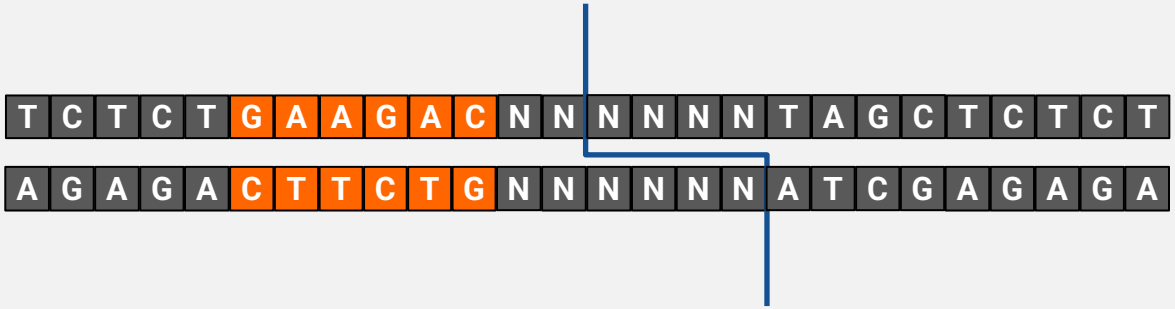


Ligase-dependent Parallel Assembly

Type IIS Restriction Endonucleases

Non-palindromic, cleave outside of their recognition sequence:

BpiI GAAGACNN□NNNN

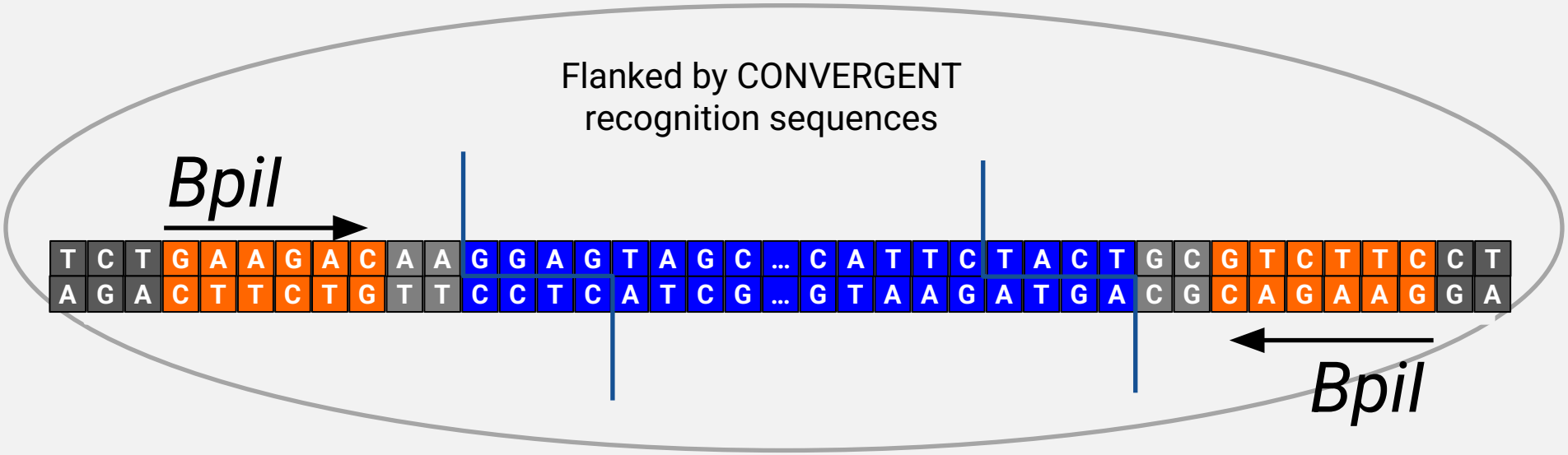


4 base pair 5' overhang

Ligase-dependent Parallel Assembly

Type IIS Restriction Endonucleases

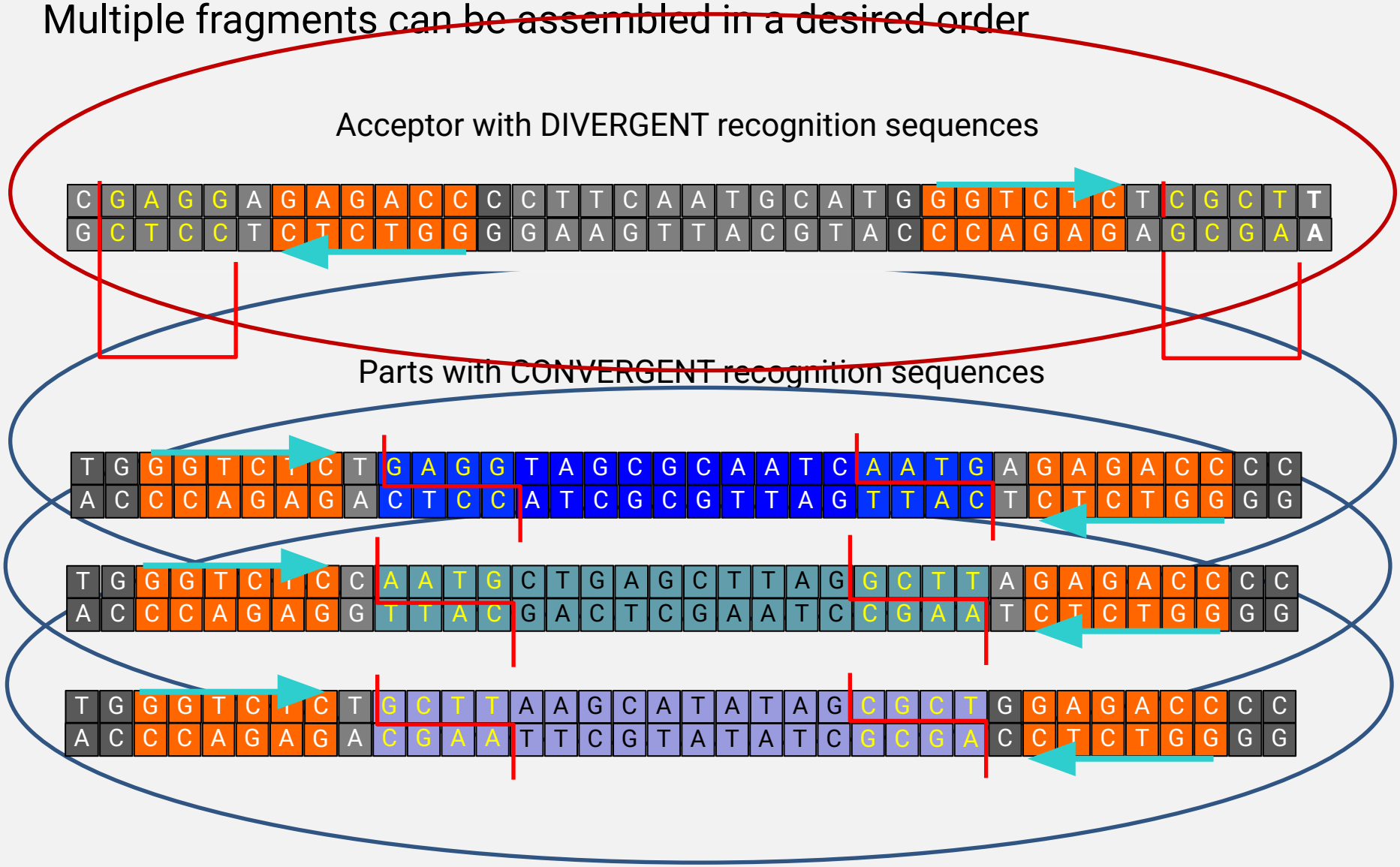
Non-palindromic, cleave **outside** of their recognition sequence:



No recognition-sequence
in released fragment

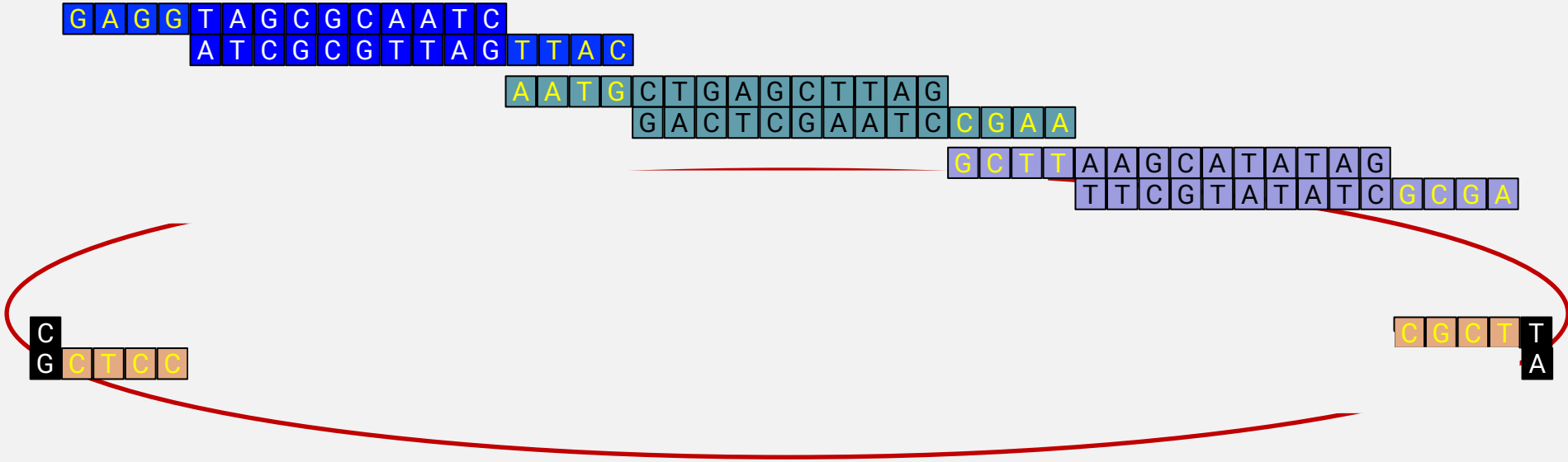
Ligase-dependent Parallel Assembly

Multiple fragments can be assembled in a desired order



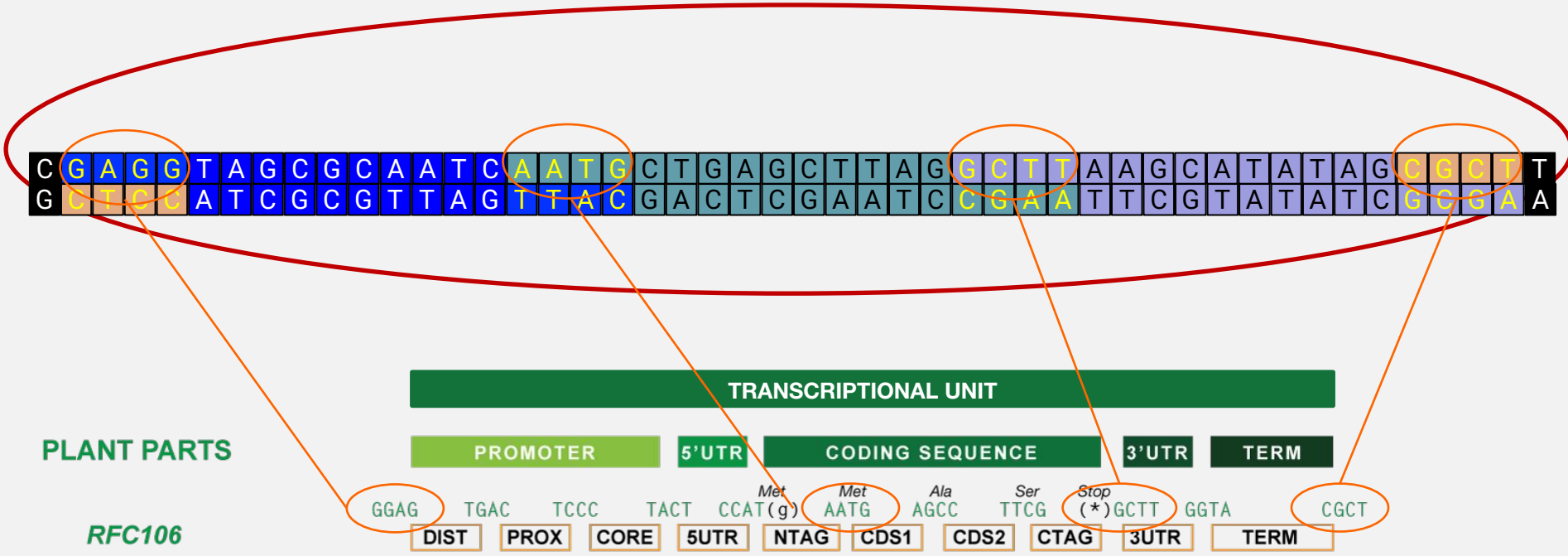
4 (ii) Ligase-dependent Parallel Assembly

Multiple fragments can be assembled in a desired order



Ligase-dependent Parallel Assembly

Multiple fragments can be assembled in a desired order
Recognition sequence is not present in the ligated product

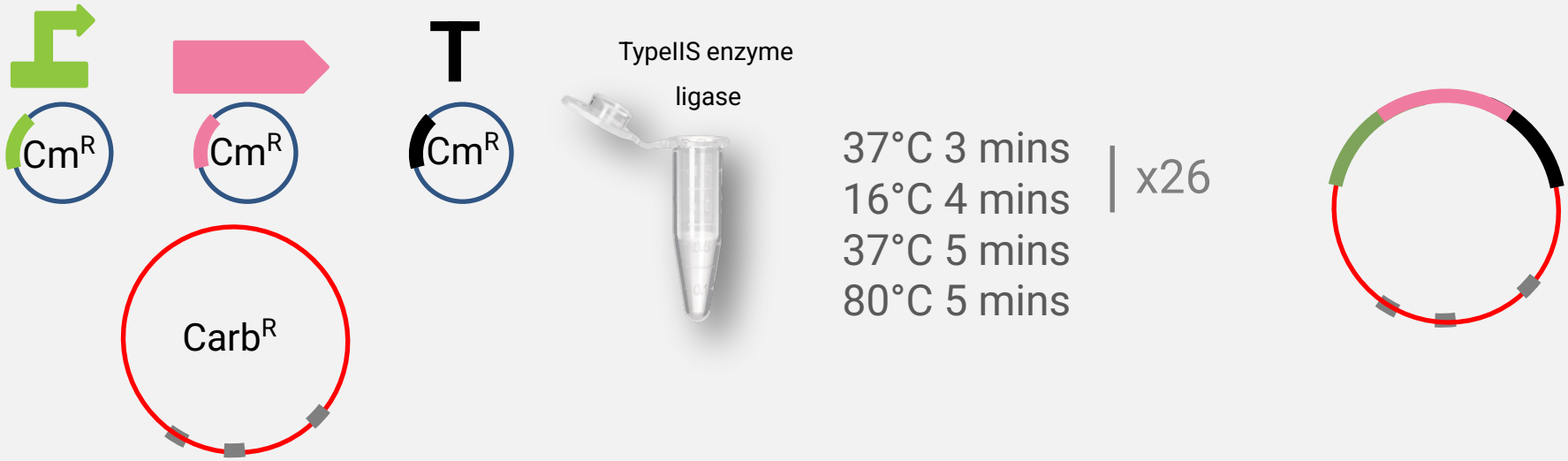


Ligase-dependent Parallel Assembly

One-step Type IIS Digestion-Ligation (Dig-Lig)

Since the recognition sequence is never present in the ligated product:

- The digestion and ligation are performed simultaneously
- The reaction can be repetitively cycled for maximum efficiency



Select on Carb

Standardisation + Abstraction + Automation = Scalability

BIO FOUNDRY

- Increases throughput - many experiments performed simultaneously
- Reduces reaction volumes
- Increases reproducibility by eliminating many human-errors

END OF PART

Building Synthetic Circuits

- How to decide when and what to synthesise
- What are standardised DNA parts
- How to assemble multiple DNA parts in a single reaction
- How standardisation aids construct design and assembly