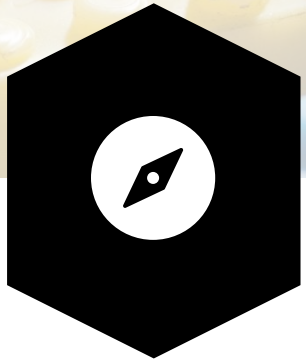
YNTEX

Golden Gate Webinar

OVERVIEW



WHY Goldengate?

Applications

Previous Work

Modular principle

101 GOLDEN GATE

Type IIS enzymes

Different Level

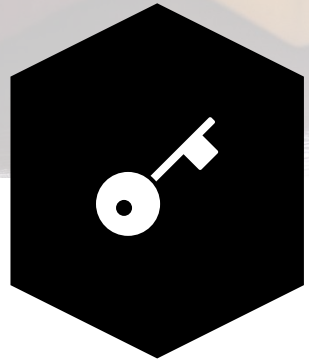
Modular cloning

METHOD IN YOUR LAB

One pot reaction

Protocols

Enzymes



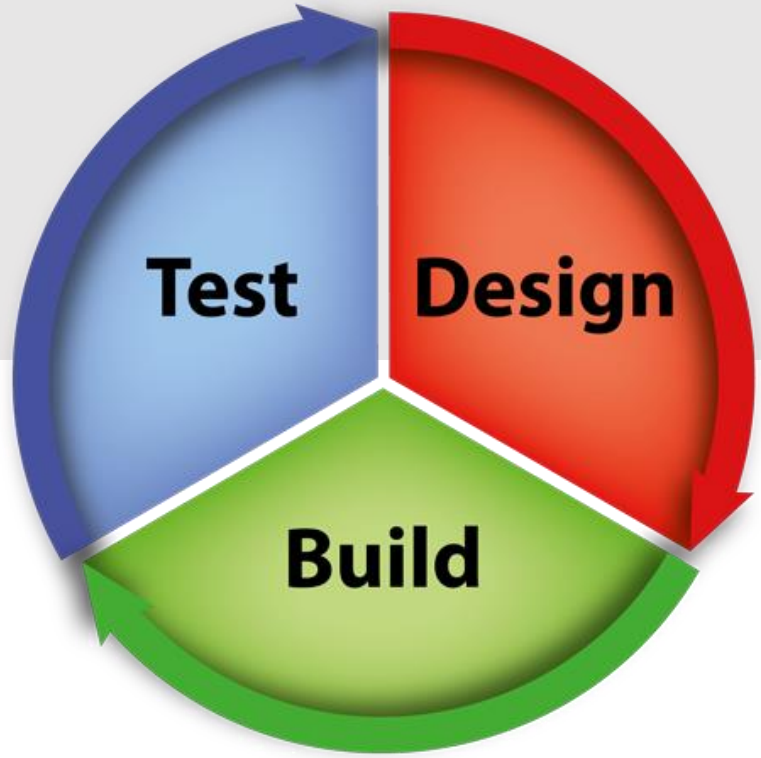
CREATE YOUR OWN TOOLBOX!

Toolbox architecture

Software tools

Practical part

Why should you use Golden Gate/MoClo?



Applications

- Modular designs with exchangeable parts
- Gene cluster refactoring
- Metabolic engineering/Synthetic Metabolism
- Genetic circuits
- Multi gene constructs

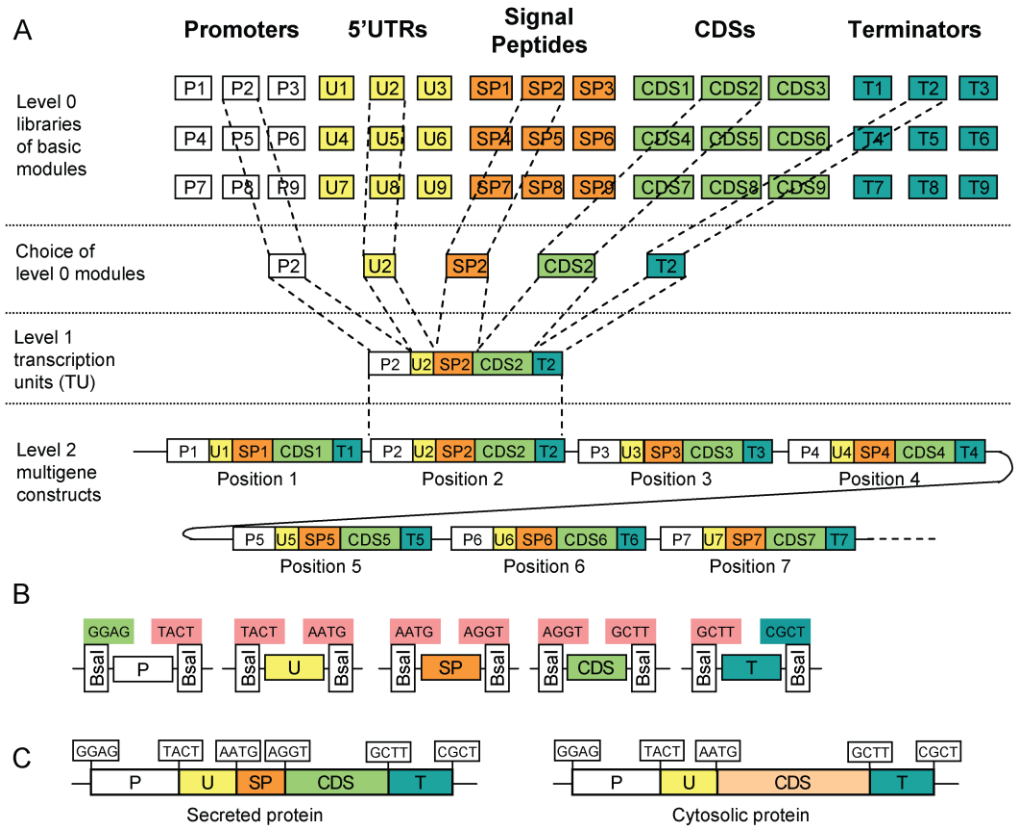
Advantages

- Up to 24 part cloning in one pot reaction
- Sequencing not necessary (if parts are sequenced)
- No time consuming primer ordering for new designs
- High throughput very easy achievable
- Can be automated

What has been done before?

Origin of Modular cloning (Moclo)

- A Modular Cloning System for Standardized assembly of multigene constructs (Weber, Marillonnet *et al* , 2011)
- Based on many vectors as a toolkit



What has been done before?

EcoFlex: A Multifunctional MoClo Kit for *E. coli* Synthetic Biology

Origin of MoClo

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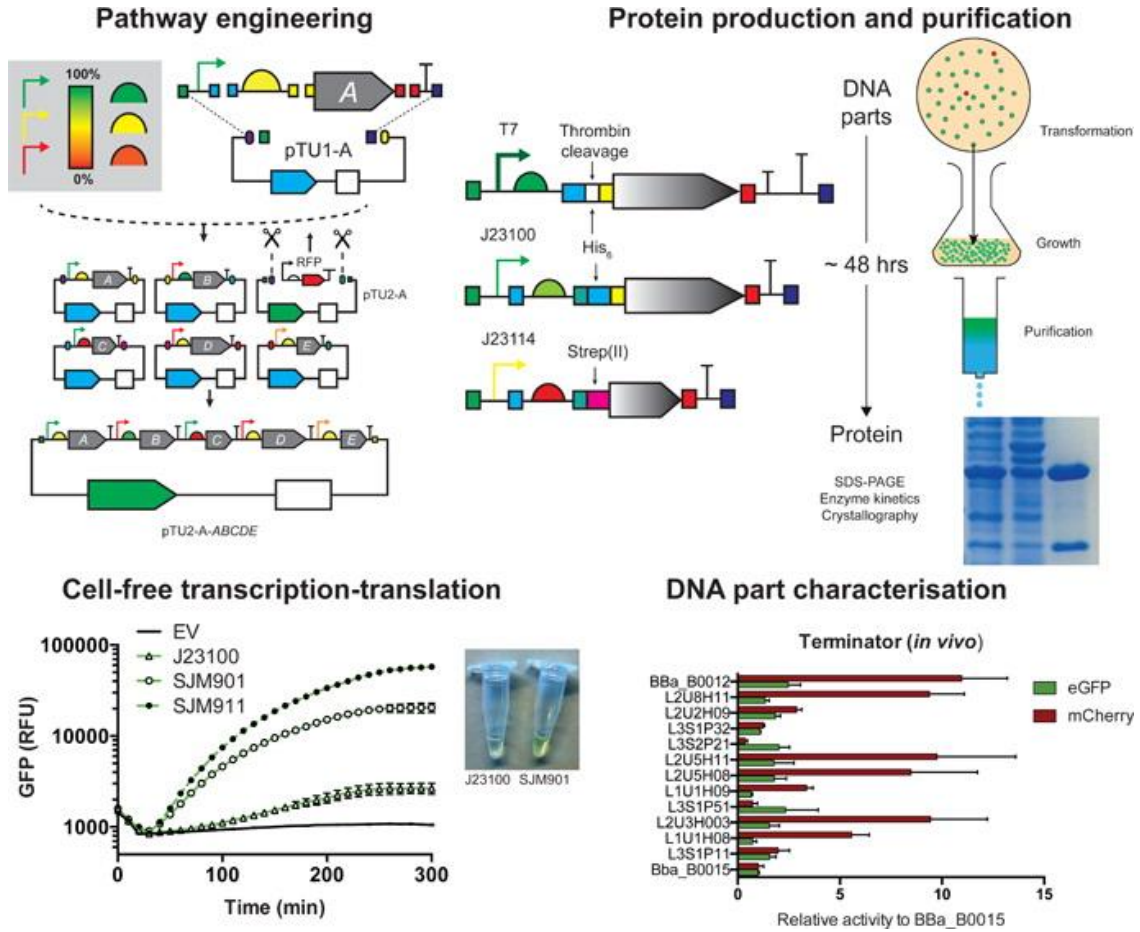
- Based on many vectors as a toolkit

Different Variants/ Chassis Organisms

- common standard for plant/phototrophic chassis
- Bacterial (*E.Coli*/*Vibrio*), bakers yeast, *pichia*, *yarrowia*, mammalien cells, cyanobacteria
- Iterative cloning / hierarchical cloning



What has been done before?

OpenPlant

sharing tools for a sustainable future

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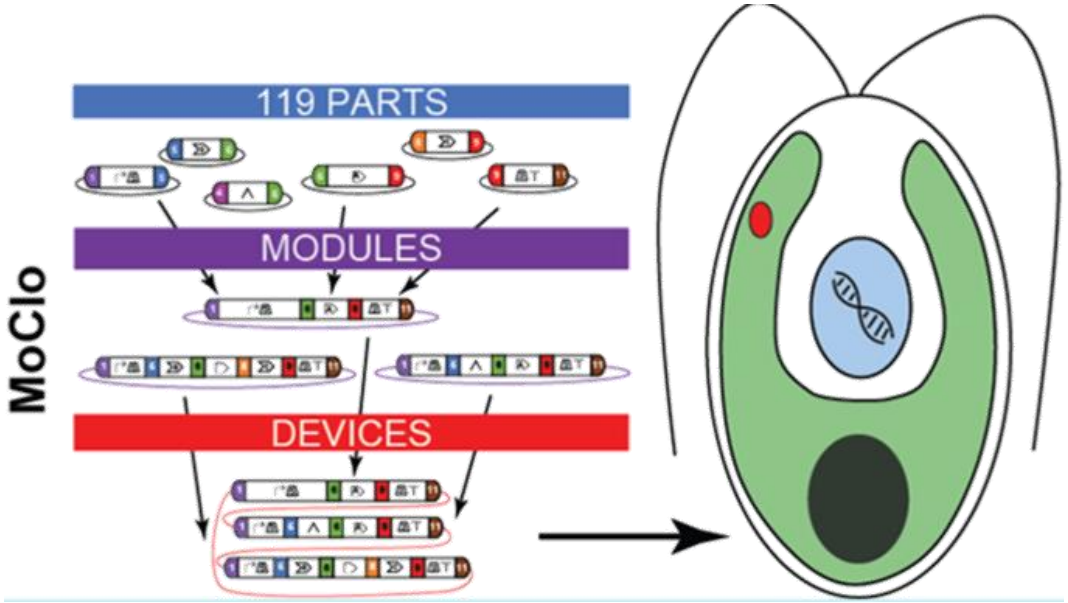

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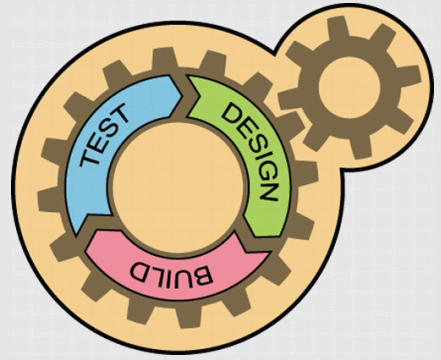

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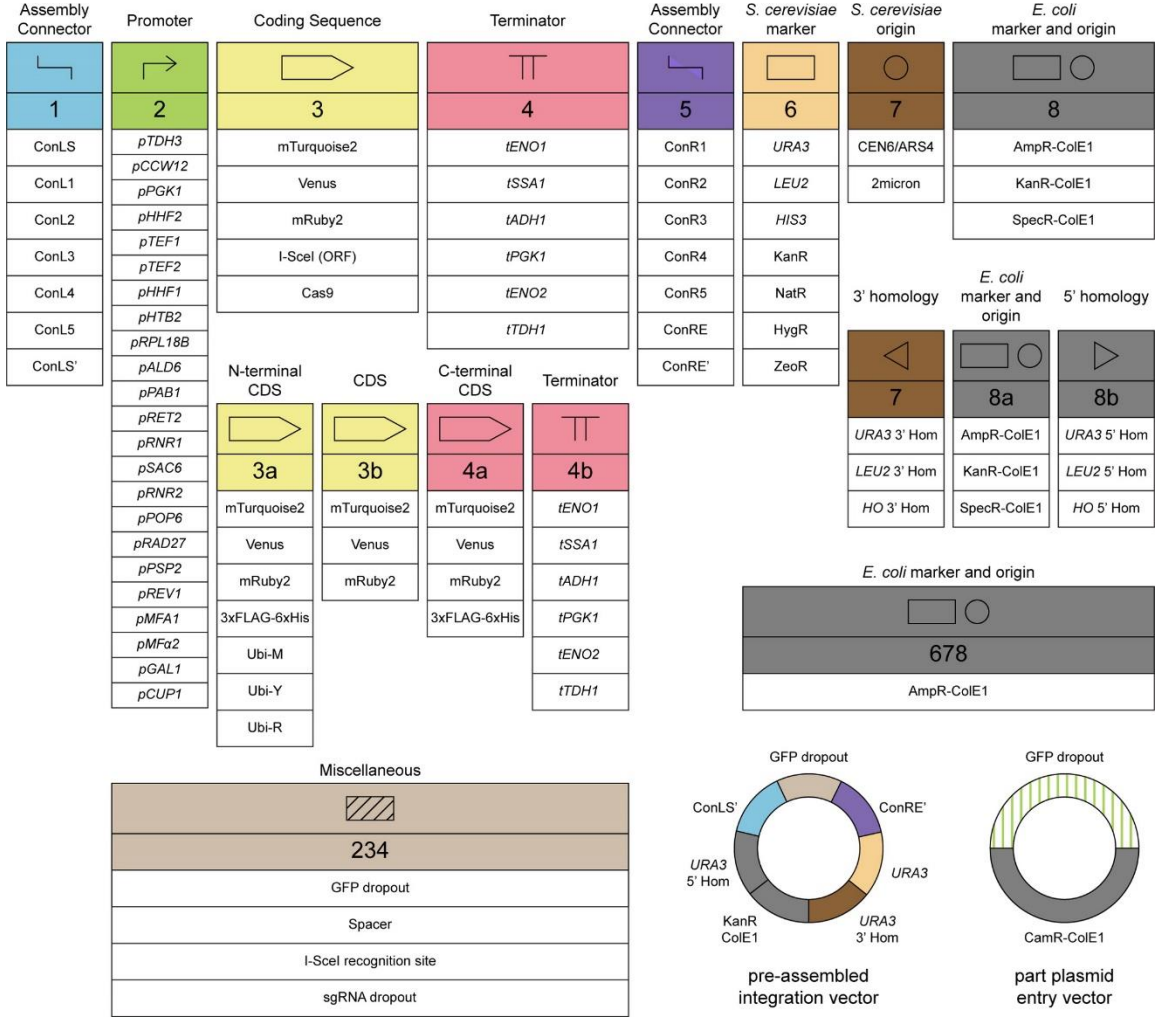

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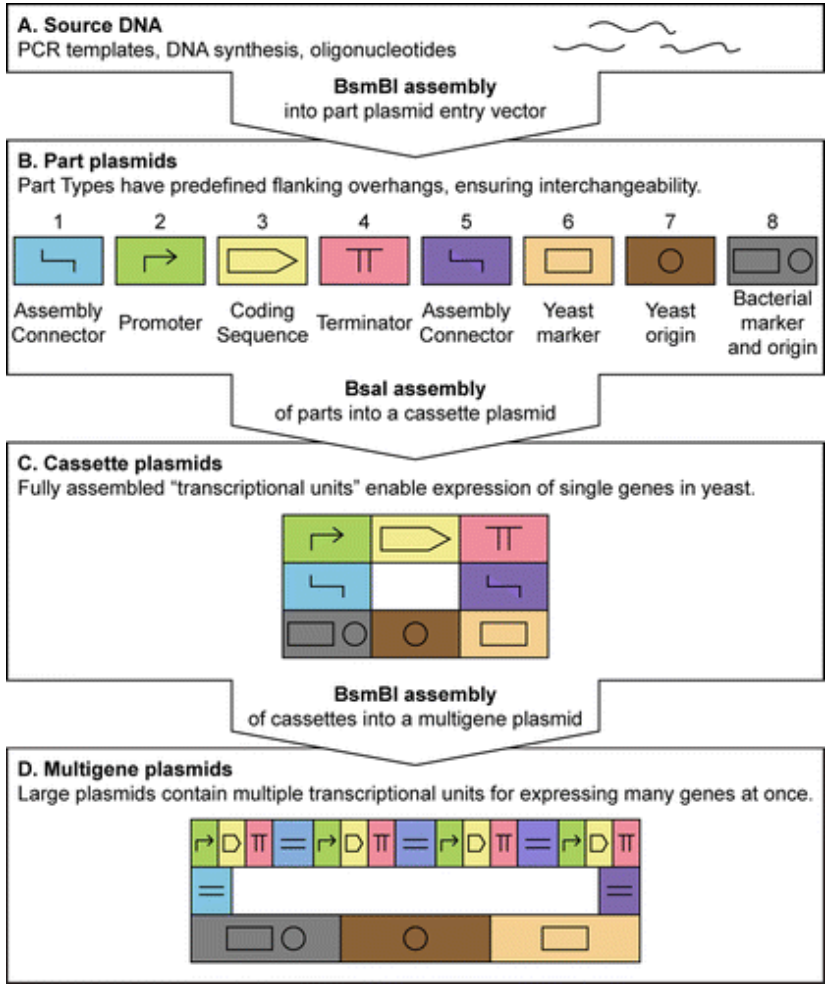
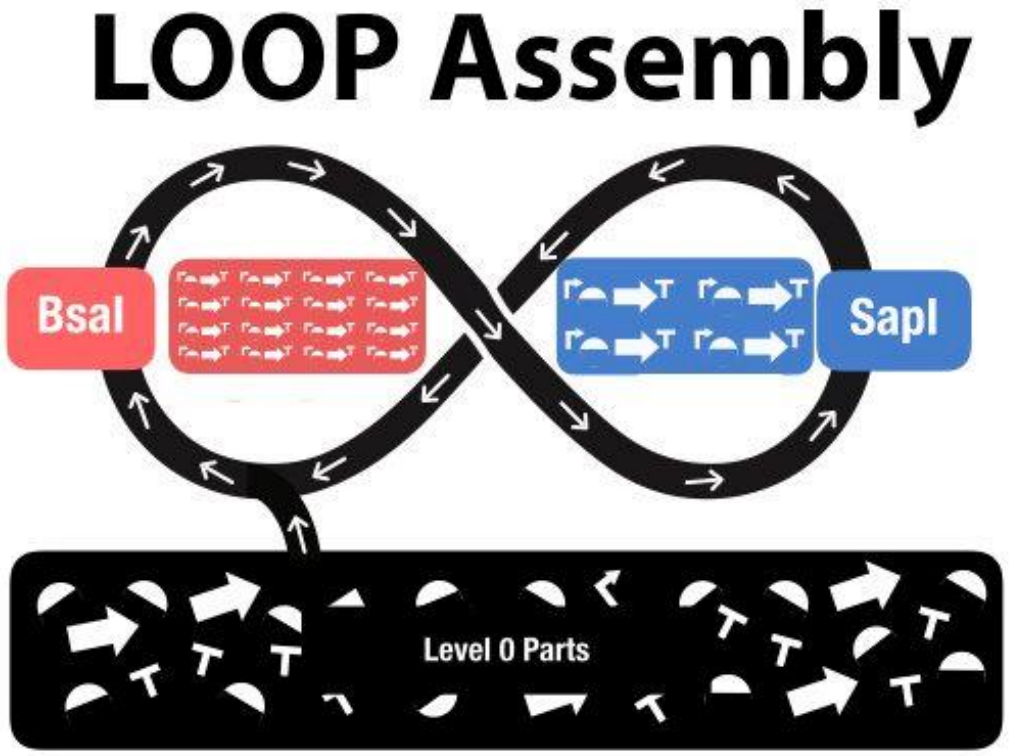
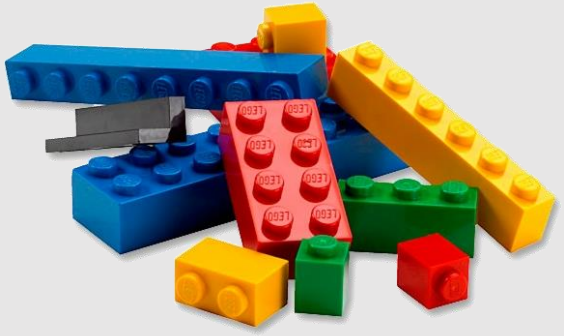


VS.



The Modular cloning principle



BsaI

BsmBI

Level 0

Single genetic part.

Level 1

Transcriptional unit (TU)

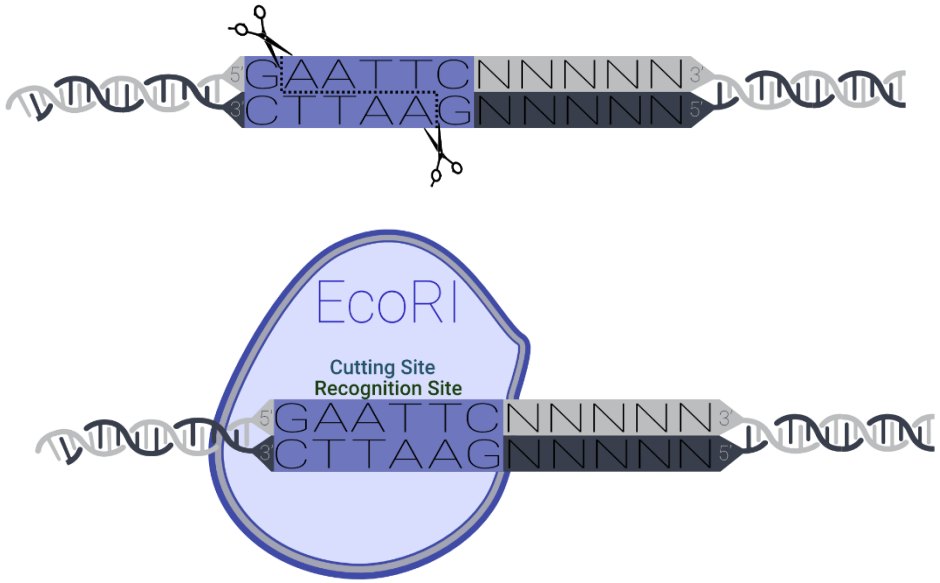
built out of level 0 parts.

Level 2

Multigene casette

built out of level 1 plasmids.

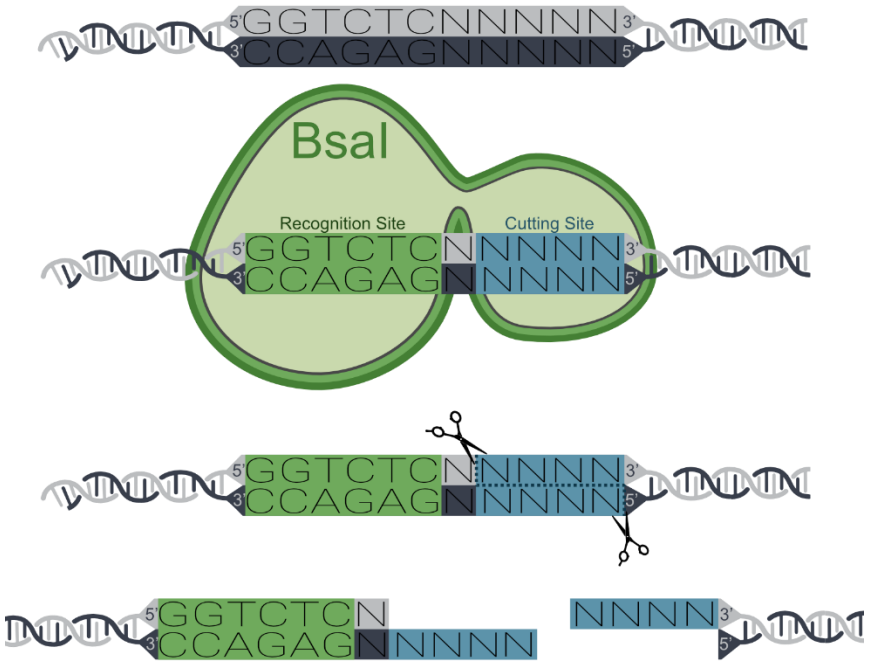
101 Golden Gate



TYPE II ENZYMES

Type II Enzymes cut inside of there recognition sequence
Can be scarless

101 Golden Gate



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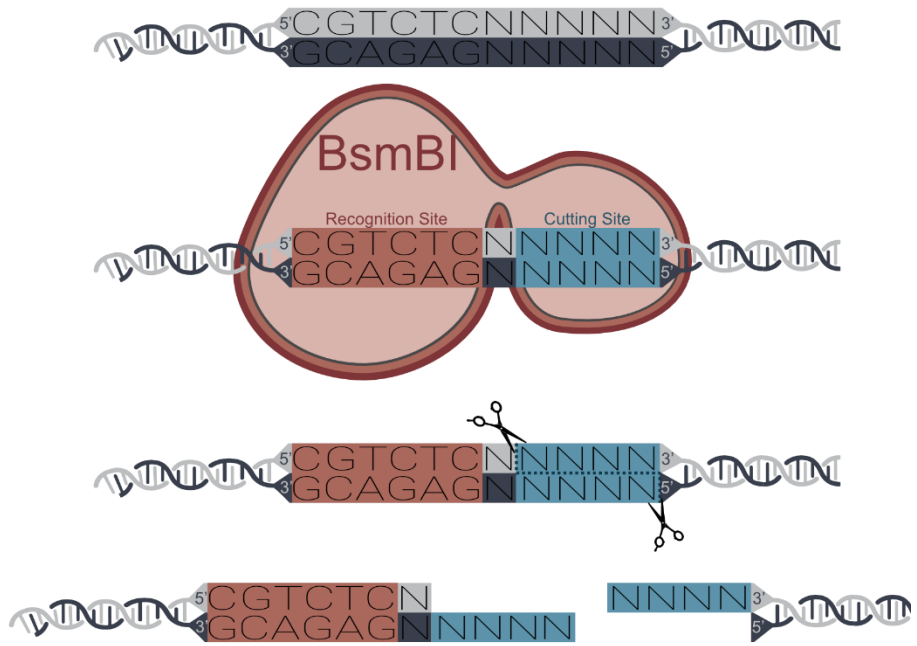

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101 Golden Gate

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Part

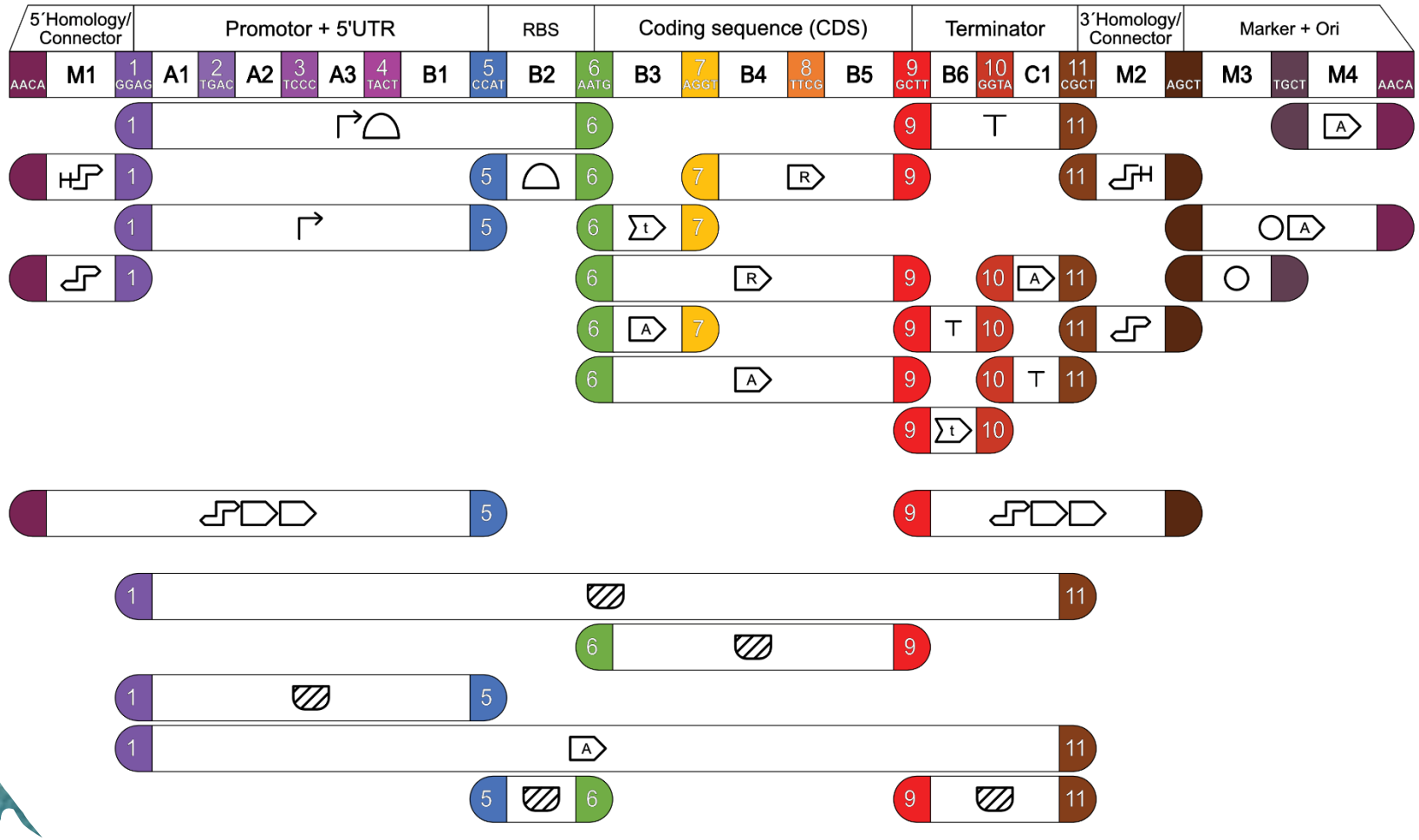
101 Golden Gate

Modular Cloning

Fusion sites are standardized
Allows exchangeability of parts (level 0, e.g promoter) with other labs/groups

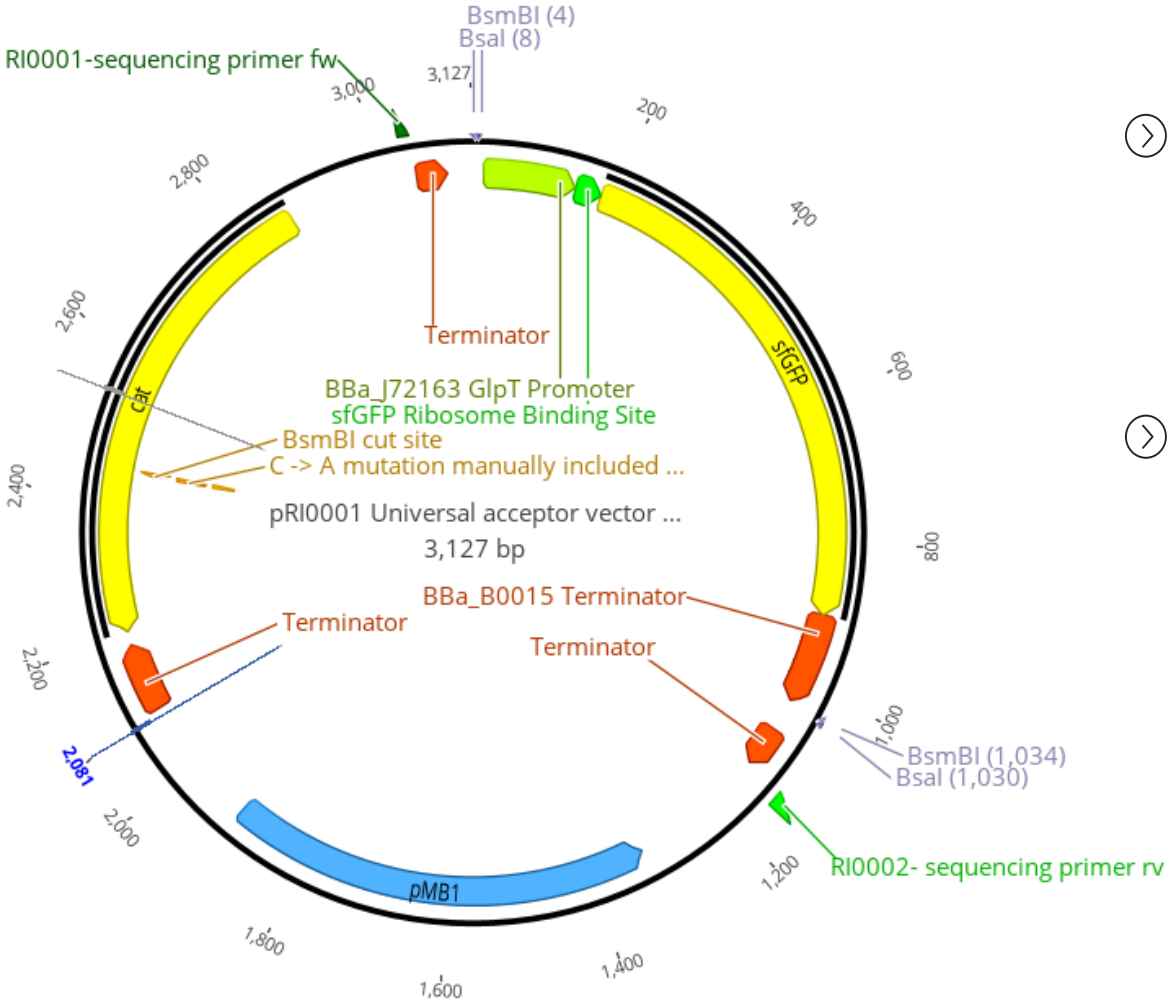
M1,M2, M3 and M4 were introduced by the iGEM Team Marburg 2018 and rationally designed

101 Golden Gate



Level 0

Basis Parts



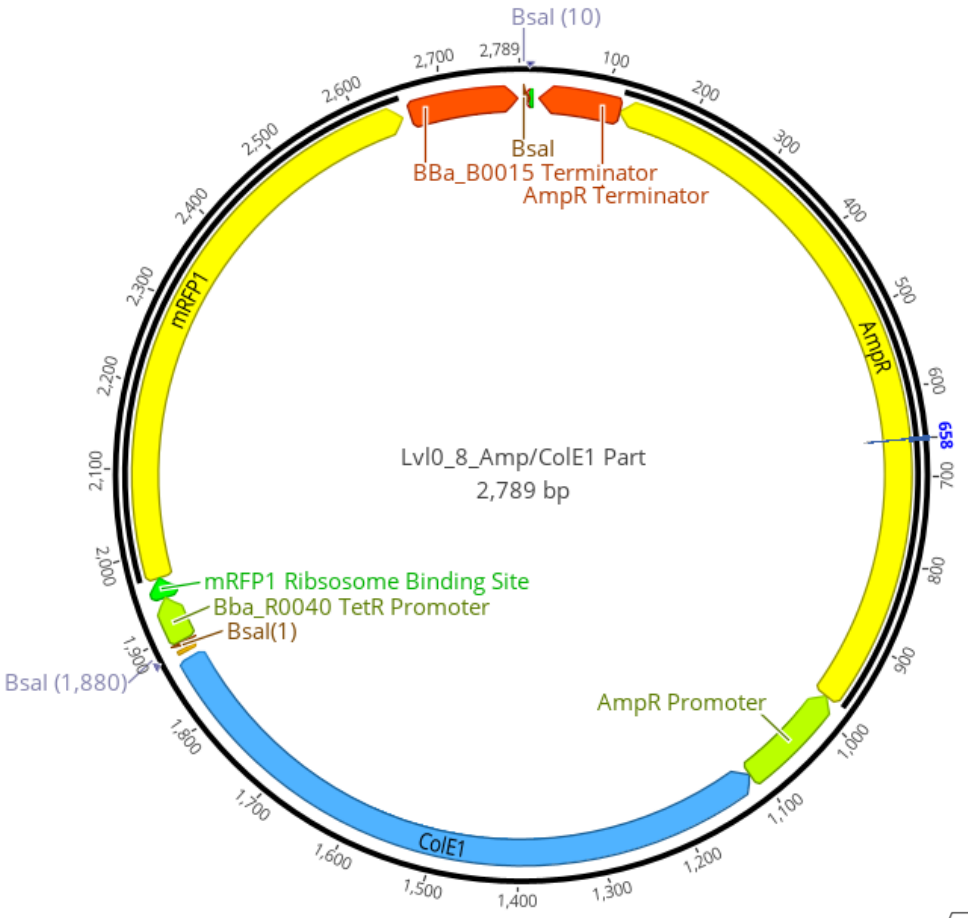
Universal Acceptor Vector

E.Coli GFP cassette for selecting the colonies
Chloramphenicol resistance

Level 0 Part creation

Parts can be created by PCR or DNA Synthesis
Must be free of BsaI and BsmBI recognition sites
Primer w/ specific overhangs to create fusion site
Level 0 golden gate reaction

Level 1
Transcription Units



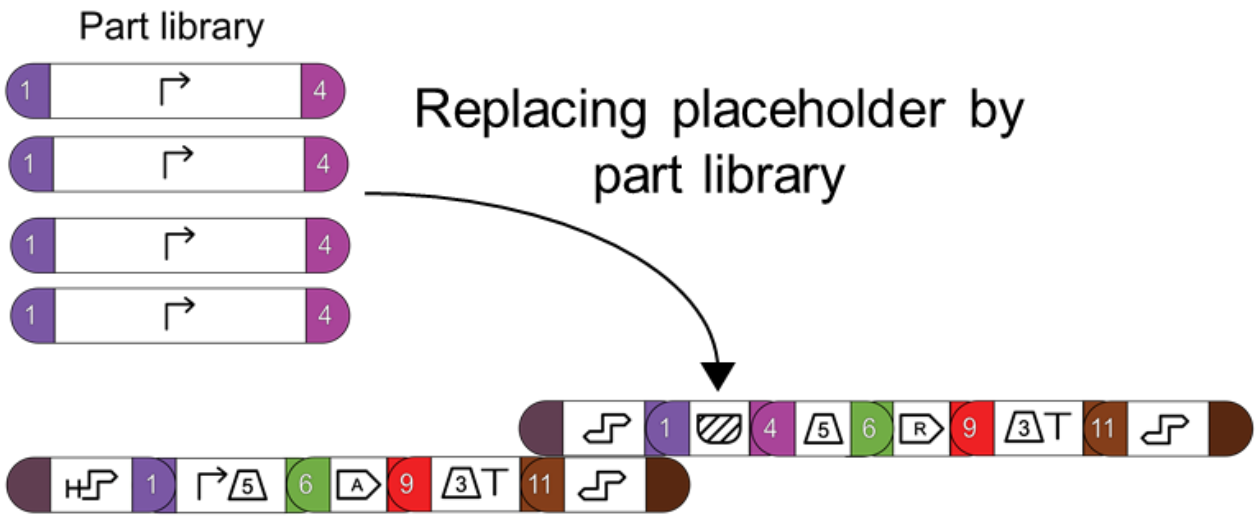
Transcription unit assembly

Parts from different Level 0 types are assembled in one pot

Highly modular, libraries for fast testing

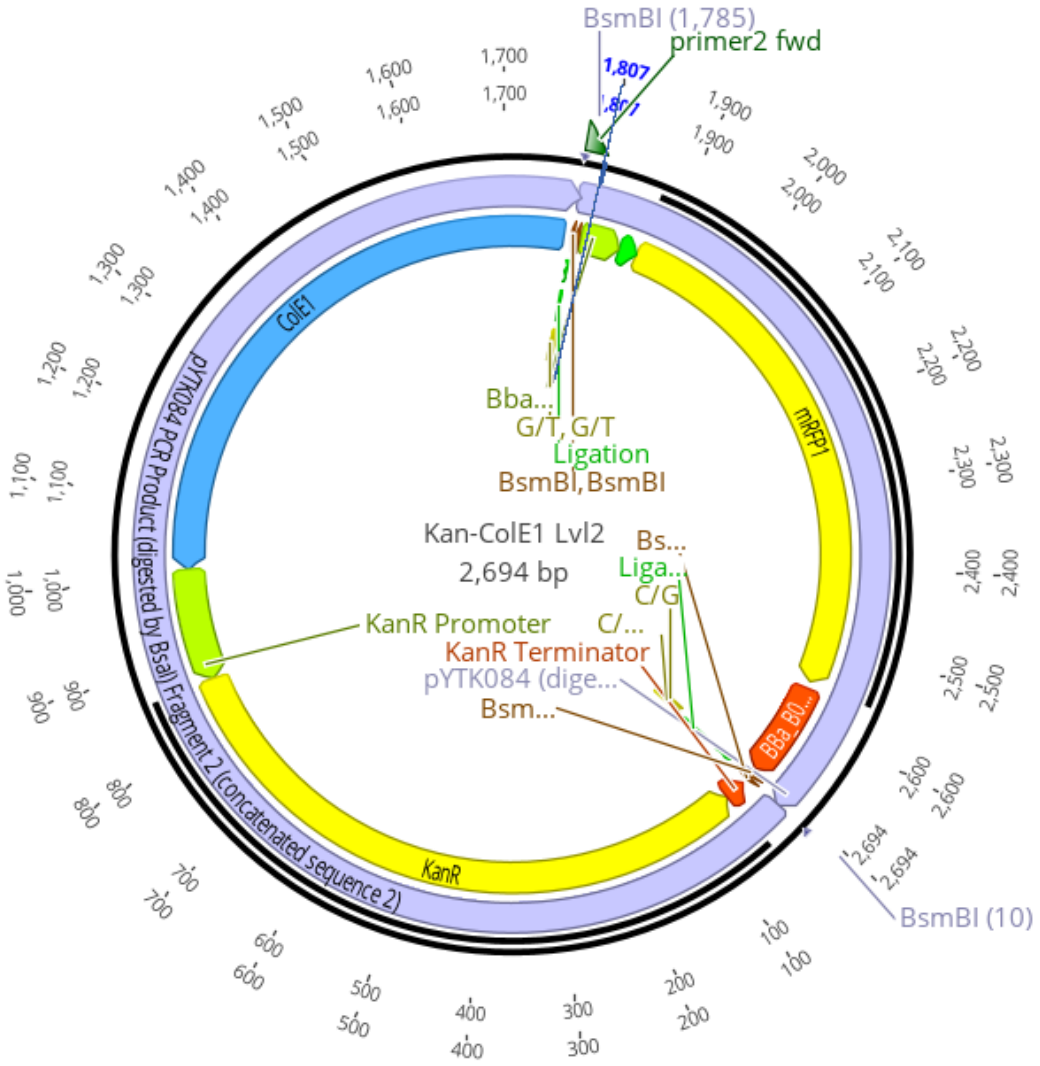
Usually standardized antibiotic resistance for level 1 (Amp)

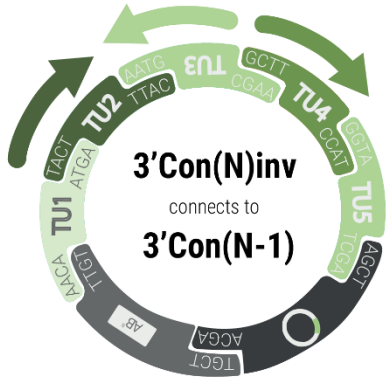
Library Cloning

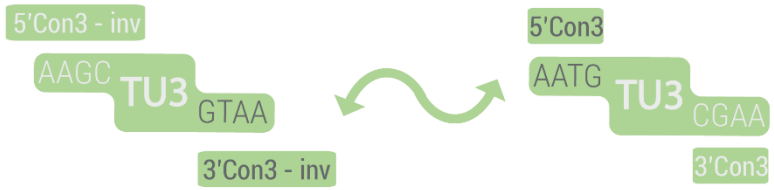


Level 2

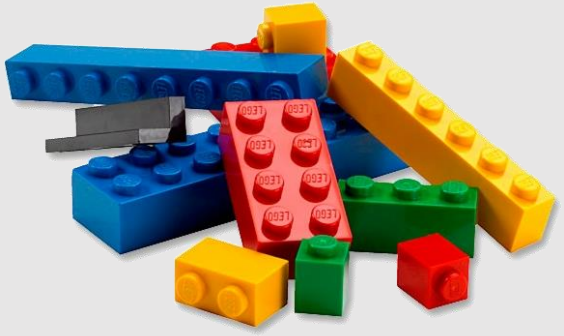
Multi TU assembly







The Cloning Workflow



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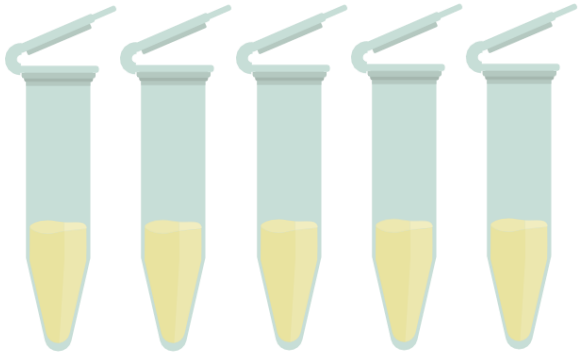
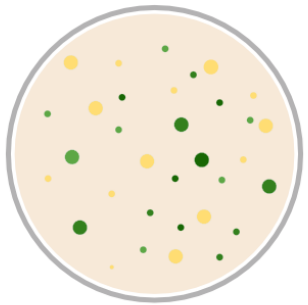




Golden Gate in your Lab

Pipette all your parts together

Golden Gate assembly allows for cloning in a one pot reaction. Parts, enzyme and ligase are pipetted together and put into a thermocycler.

Cyclic/thermocycler reaction

Step 1	37°C	2 min	x 50
Step 2	16°C	5 min	
Cycle steps 1 & 2 x 50			
Step 3	50°C	10 min	
Step 4	80°C	10 min	
Total Time ~6h (370min)			

37°C for the restriction enzyme
16°C for the ligase
50°C as a final digest (to remove original plasmid)
80°C for enzyme inactivation

Transformation of Golden Gate reactions

Competent cells

Dh5α or Top10 should be used. For level 0 the efficiency is not critical. For Level 1 (many fragments) the transformation efficiency is crucial.

Transformation

2-5 µL of each assembly reaction
50 µL competent cells
recovery for 1h (amp) to 2h (Kan, Chloramphenicol).

Picking the colonies

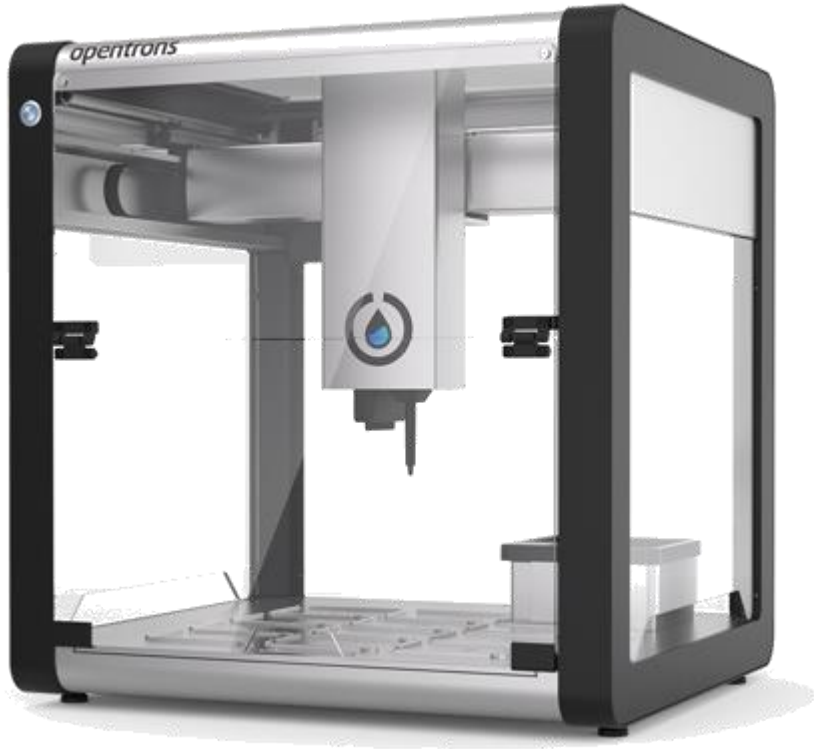
For level 0 white colonies should be picked, GFP cassette allows selection (green colonies are wrong).

For level 1 and 2 white colonies should be picked, RFP cassette allows selection (red colonies are wrong).

Automation for MoClo/ Golden Gate

Echo 525

The Echo is able to transfer nanoliter droplets in multi-well plates and enables golden gate reaction in 1-2 µl Volume



Opentrons OT-2

The Opentron is an affordable alternative to bring automation into your lab. The last years Marburg Team developed an easy to use software for Golden Gate library cloning.

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