

Golden Gate

Why Golden Gate?

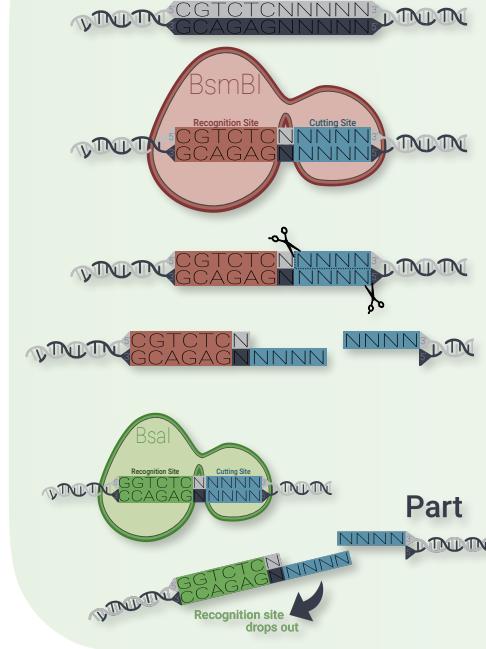
You can use it for many Applications:

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

And there are many Advantages:

- You can assemble up to 24 parts in a one pot reaction
- Sequencing of assembled parts is not necessary
- No time consuming primer ordering for new designs
- High throughput is very easy achievable
- Cloning can be automated

Type IIS Restriction Enzymes



Type IIS restriction enzymes cut **outside** of their recognition sequence. In opposition to type II restriction enzymes, which cut inside of their recognition sequence.

Cloning with type IIS restriction enzymes can be scarless, due to the fact, that the recognition side can drop out of the cloning constructs.

Two of the most used type IIS restriction enzymes for golden gate cloning are **BsaI** and **BsmBI**.

Using Type IIS Restriction Enzymes for Scarless Cloning:

Golden Gate Reaction:

Level 0 with PCR Product	Level 0 with annealed oligos	Level 1 & 2	Level 1 & 2 (optimised)
0,5 µl Entryvector (50-70 ng)	0,5 µl Entryvector (50-70 ng)	0,5 µL per Insert (37,5 ng)	20 fmol per Insert
1 µl PCR Product (50-70 ng)	2 µl annealed oligos		

- 1 µl T4 ligase buffer
- 1 µl T4 or T7 ligase
- 0,5 µl BsmBI / BsaI
- fill up to 10 µl with H₂O

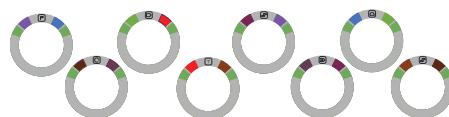
Cycler Conditions:

for the restriction enzyme

- 37 °C for 2 min
 - 16 °C for 5 min } 25-50x for the ligase
 - 50 °C for 10 min for a final digest (to remove original plasmid)
 - 80 °C for 10 min
 - 4 °C ∞ for enzyme inactivation
- Transform 5 µl of the golden gate mix into competent cells



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



At the first step the fragments are being cut by the restriction enzymes and stay behind with 4 bp overhangs at both sides.



In the next step the parts with complementary overhangs assemble and are being assembled via the ligase.



To increase the efficiency this process is repeated many times. In opposition to the correct assembled plasmid wrong assembled or uncut vectors still have the enzyme recognition site and get cut in the next cycling step.

Molecular Cloning

Standardised Golden Gate Cloning Parts

For Modular Cloning the 4 bp overhangs also called fusion sites are standardised. For example all RBS-parts (B2) have the standardised overhangs at the beginning and at the end. This allows to build exchangeable parts, which can also be shared with other labs and groups. These overhangs and the standardised position names are listed below. M1, M2 and M3 were introduced by the iGEM Team Marburg 2018.

5' Homology/Connector	Promotor			RBS		Coding sequence (CDS)					Terminator		3' Homology/Connector	Marker + Ori												
AACA	M1	1 GGAG	A1	2 TGAC	A2	3 TCCC	A3	4 TACT	B1	5 CCAT	B2	6 AATG	B3	7 AGGT	B4	8 TTGG	B5	9 GCCT	B6	10 GCTA	C1	11 CGCT	M2	AGCT	M3	AACA

Create your own “Single Genetic Part” (Level 0)

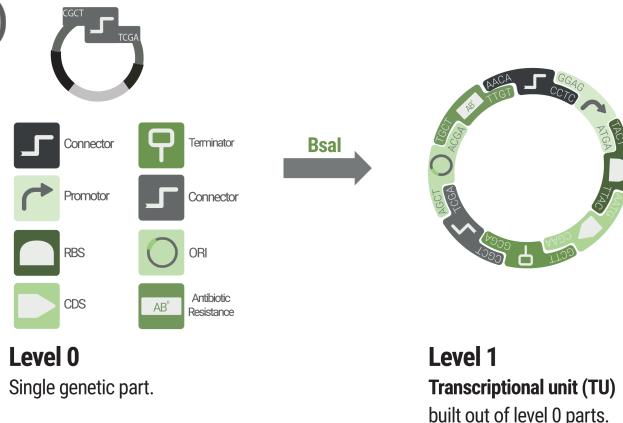
All single genetic parts are cloned via GoldenGate (**BsmBI**) into a “Universal Acceptor Vector”. The acceptor vector has a Chloramphenicol-resistance-cassette and a GFP cassette which will be replaced by the basic parts. This allows to select if the part entered the acceptor vector - if the colonies grow on Chloramphenicol and don't have GFP expression, then there is an extremely high chance of having cloned the correct vector - but the vector should still be sequenced. These standardised plasmids with single genetic parts are called level 0.

These Parts can be created by PCR, DNA-Synthesis or primer ligation. The sequence must be free of **BsaI** and **BsmBI** recognition sites. Fusion sites can be created via primers with specific overhangs.

Building Transcriptional-Units (Level 1)

Different level 0 parts can be assembled to Transcriptional-Units (level 1) in a one pot GoldenGate reaction with **BsaI**. This assembly is specific enough that sequencing should not be necessary.

Due to the high variety of different level 0 parts different level 1-constructs can be built fast and easy e.g. to find the best suiting promoter for the application. The usually standardised antibiotic resistance for level 1 is Ampicillin.



Building multiple Transcriptional-Units (level 2)



Level 1
Transcriptional unit (TU)
built out of level 0 parts.

Level 2
Multigene cassette
built out of level 1 plasmids.

Level 1 parts can be assembled to build plasmids with more than one Transcriptional-Units (level 2). For this application, level 1 vectors are equipped with “Connenctor-Parts”. These parts introduce new **BsmBI** restriction sites with new fusion sites.

So in an one pot GoldenGate reaction with **BsmBI** the Transcriptional-Units can be assembled. With the Marburg Collection it is possible to connect up to 5 level1-constructs. Transcriptional-Units can also be fused in inverted direction.

