

Highly Efficient Single-Pot Scarless Golden Gate Assembly

Background/objective

Golden Gate assembly is one of the most widely used DNA assembly methods and an important tool for synthetic biology and metabolic engineering. However, challenges preventing more widespread use include lack of *Bsal*-free parts, introduction of scars between junctions, and the need for a comprehensive understanding of linker choice on assembly outcome. Here, researchers address all three of these key challenges.

Approach

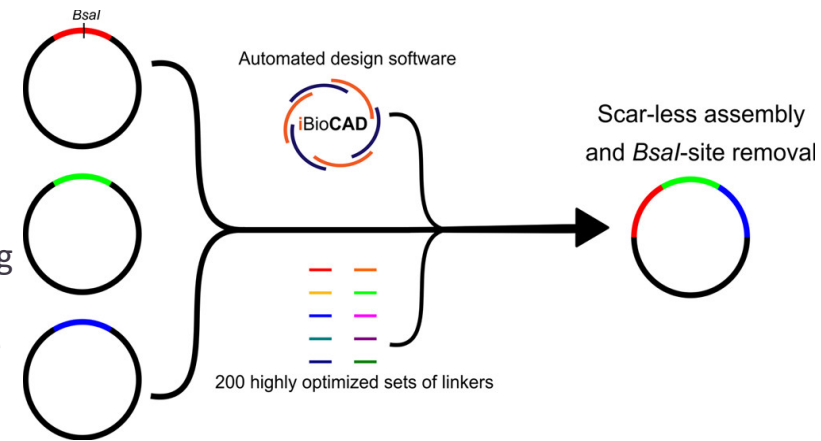
- ❖ Developed a plasmid-based Golden Gate assembly system to test ligation efficiency of 96 different linkers — and evaluated results via assembly of the six-part zeaxanthin pathway.
- ❖ Used experimental data to design 200 sets of optimized linkers in conjunction with iBioCAD GGA, a web-based application for the design of scarless and regular Golden Gate assemblies using the optimized linker sets.
- ❖ Tested iBioCAD GGA output by cloning the ampicillin resistance gene in a pET26b vector.

Results

- ❖ Results from testing the 96 linkers tended to validate general Golden Gate guidelines, with some key exceptions.
- ❖ A three-piece scarless assembly (3kb) and *Bsal* removal were performed on the ampicillin gene with 100% efficiency, validating the accuracy of iBioCAD GGA outputs.

Significance

- ❖ These tools eliminate the trade-off between scarless and efficient Golden Gate assemblies and the need to remove *Bsal* recognition sites before starting the assembly.
- ❖ Study results improve the efficiency of Golden Gate assembly, a fundamental tool for synthetic biology and genetic engineering.



iBioCAD GGA recommends linkers from a set of 200 optimized linker sets based on fragment sequences and allows for *Bsal*-site removal and scarless ligation via one-pot Golden Gate assembly.