

Bio113 Synthetic Biology

Promoter Discovery

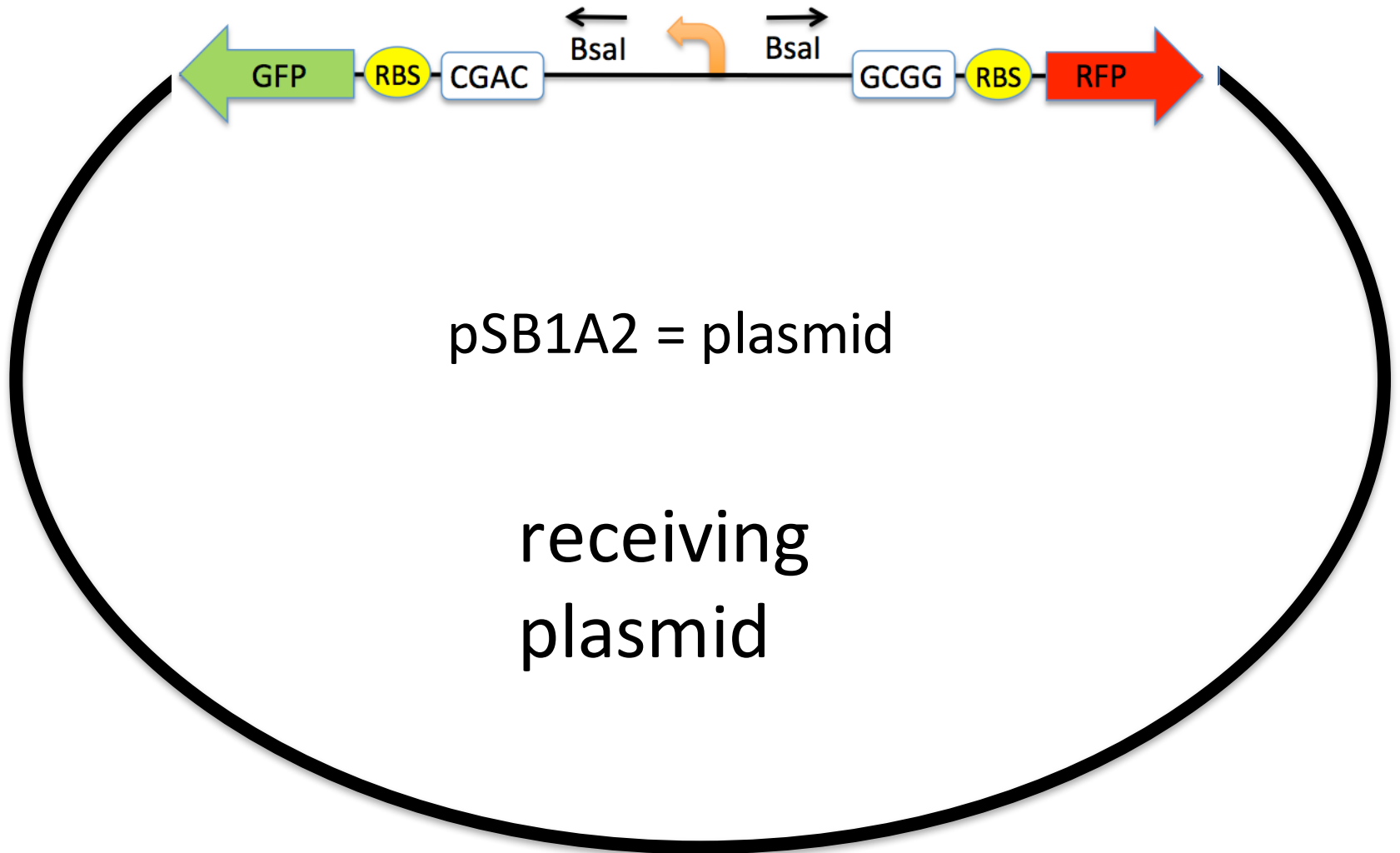
developed by Todd Eckdahl and A. Malcolm Campbell

Goal: Clone a new promoter and test its effectiveness.

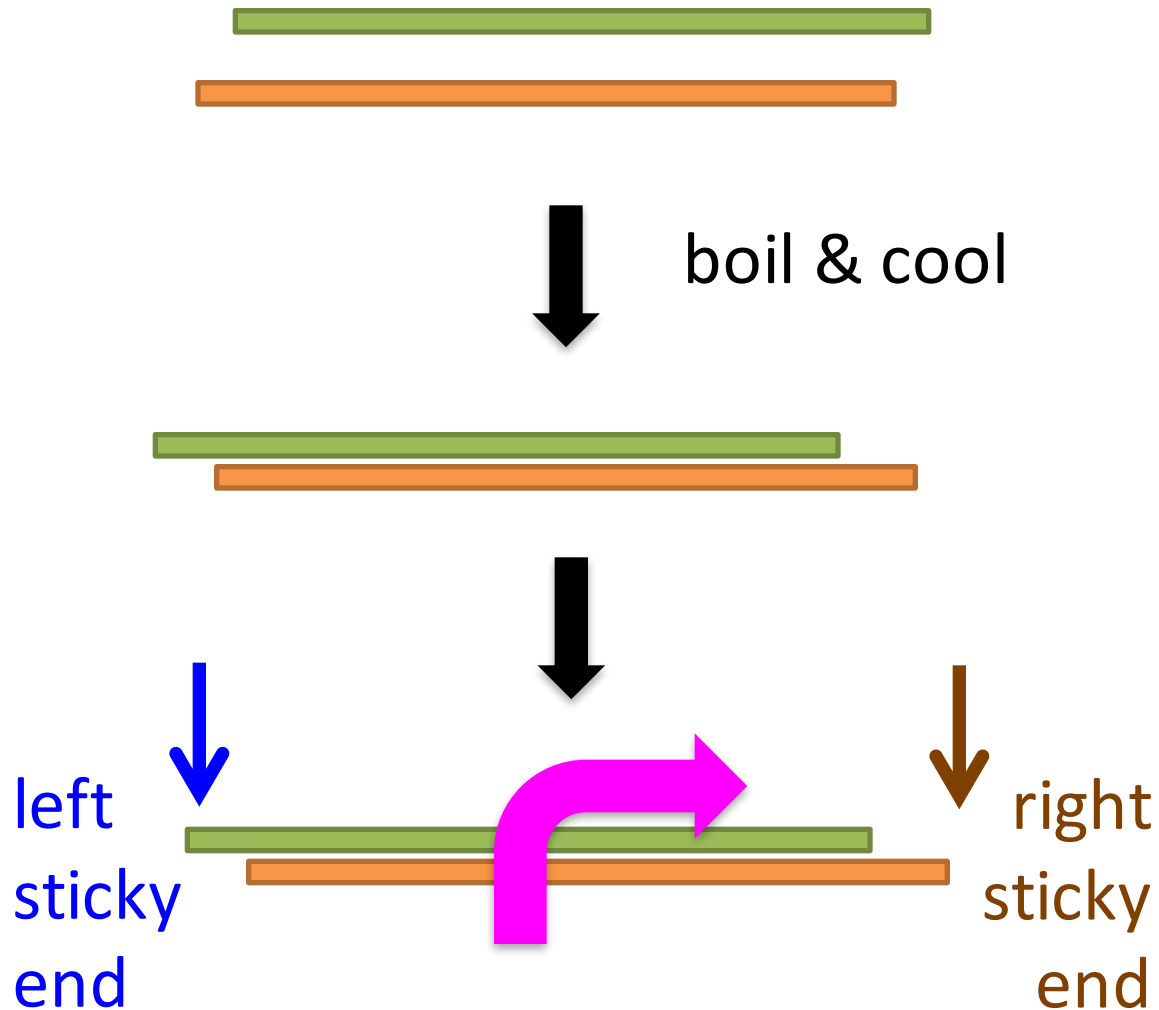
Golden Gate Assembly Method

- DNA: promoter + receiving plasmid
- Ligation Protocol
 - Bsa I + ligase simultaneously
 - 37° C for 1 minute (optimal for Bsa I)
 - 16° C for 1 minute (optimal for ligase)
 - Total of 5, 10, 20, or 30 cycles
 - 37° C for 15 minutes (optimal for Bsa I)

pClone Red = J119137



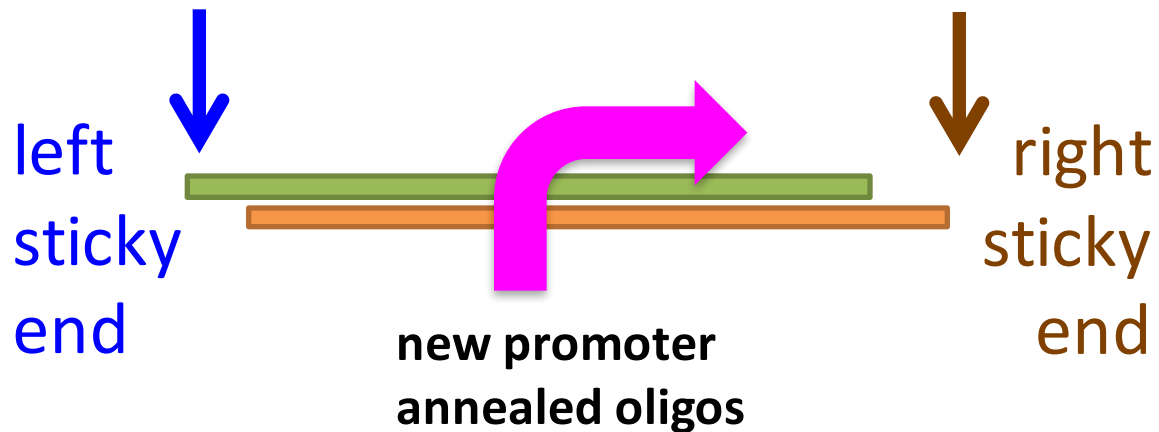
Promoter made of Self-Assembled Oligos



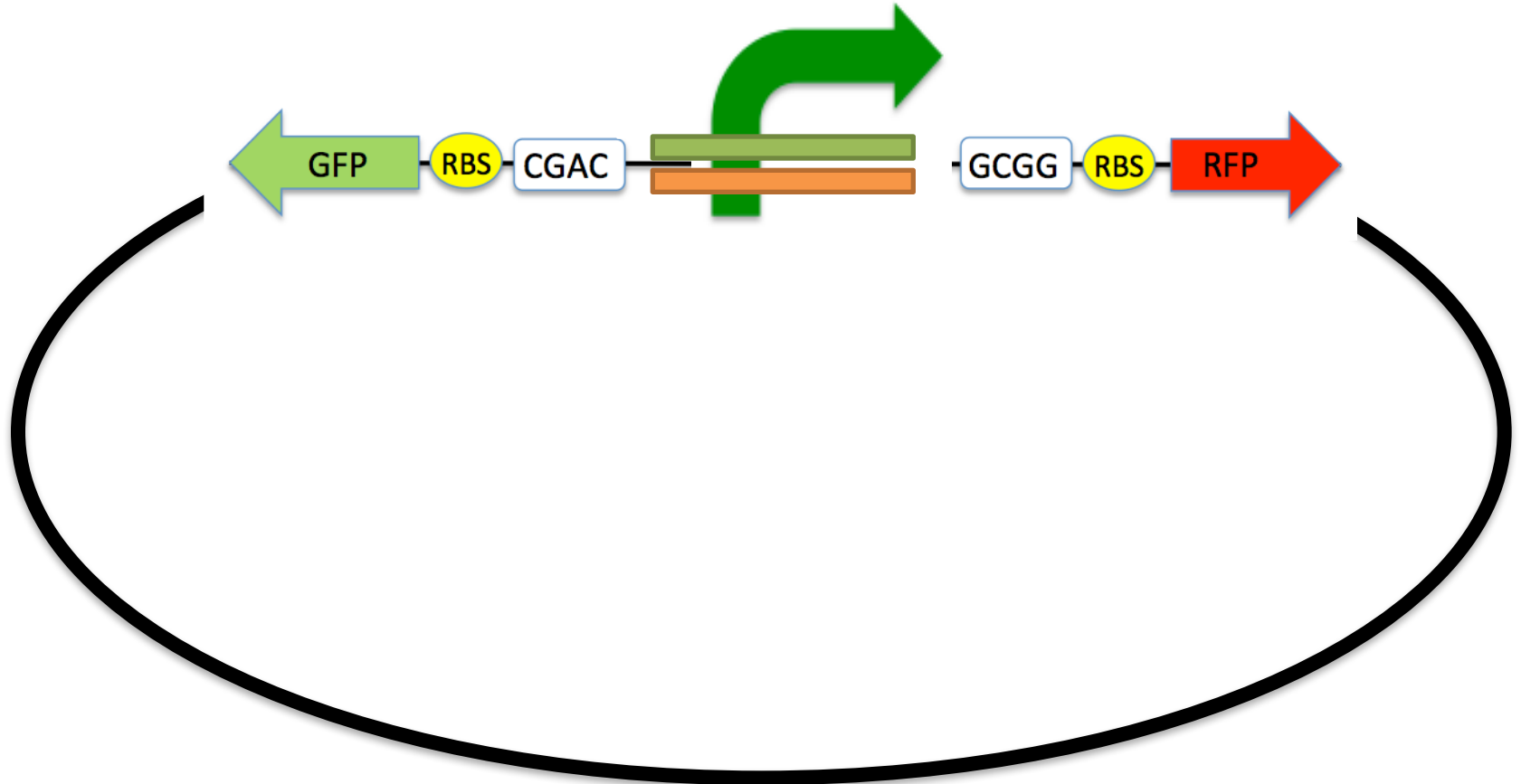
Golden Gate Assembly



+ Bsa I and ligase



Promoter Ready for Transformation



Promoter Ready for Transformation

