GGS Shuffling Protocol

Once entry constructs and the recipient vector are made and sequenced, performing DNA shuffling only requires pipetting all components into a reaction mix, incubating the mix in a thermocycler,and transforming it into competent cells. An important factor is to add an equimolar amount of DNA for each of the module sets and the destination vector. Since a module set usually contains several modules, the amount of DNA for each individual module of a set containing *x* different modules (*x* alternative homologous sequences) should contain only 1/*x* the amount of DNA compared to the vector; for example, each module from a set containing three modules should have a third of the amount of DNA compared with the recipient vector (Golden\_Gate\_Mehtods).

Restriction/Ligation Cocktail

1. Figure out how much DNA to use
2. Then construct a table similar to the one below:

* XμL DNA (100ng if Vector and DNA are similar bps)
* 2μL 10X Ligation Buffer
* 1μL BsaI
* 1μL Ligase for assembly of 2–4 module sets or (1μL) of HC ligase for assembly of more than four module sets
* 16μL – X Water
* 20μL Total Volume

1. Incubate for 60-120min at 37°C (Depending on how many modules: If more than 6 module sets are ligated together,the incubation time is increased to 6 h, or cycling is used as following: 2 min for 37°C followed by 3 min for 16°C, both repeated 50 times)
2. Incubate for 5 min at 50°C
3. Incubate for 5min at 80°C

The final incubation step at 80°C is very important and should not be omitted. Its purpose is to inactivate the ligase at the end of the restriction–ligation. Omitting this step would lead to religation of some of the insert and plasmid backbone fragments still present in the mix, when it is taken out of the thermocycler before transformation.

1. Proceed to transformation or to PCR screening for size