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)
pClon Red = J119137

pSB1A2 = plasmid

receiving plasmid
Promoter made of Self-Assembled Oligos

boil & cool

left sticky end

right sticky end
Golden Gate Assembly

+ Bsa I and ligase

New promoter annealed oligos
Promoter Ready for Transformation
Promoter Ready for Transformation