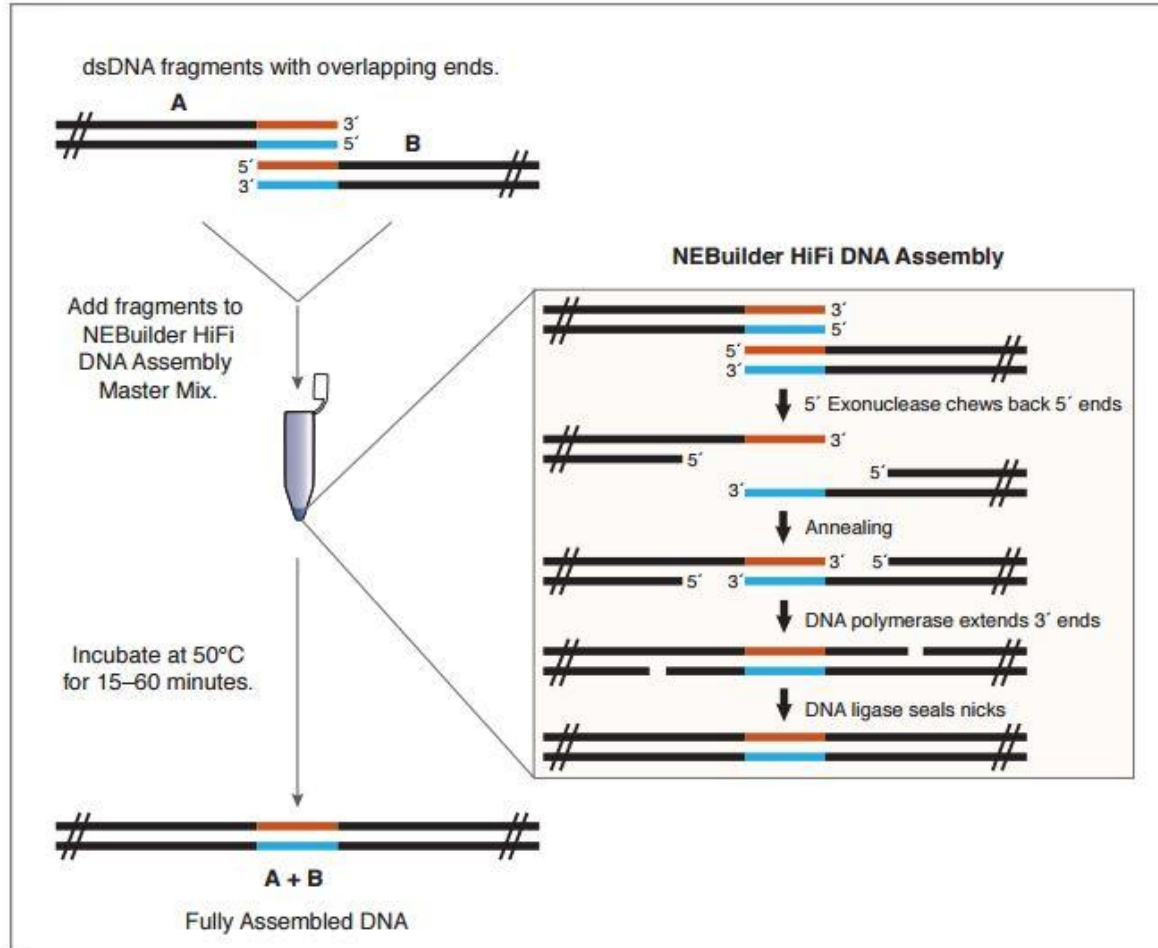


Gibson assemblies

Team IISc_Bangalore 2016

Principle of Gibson/Hifi assembly

Figure 1: Overview of the NEBuilder HiFi DNA Assembly Method

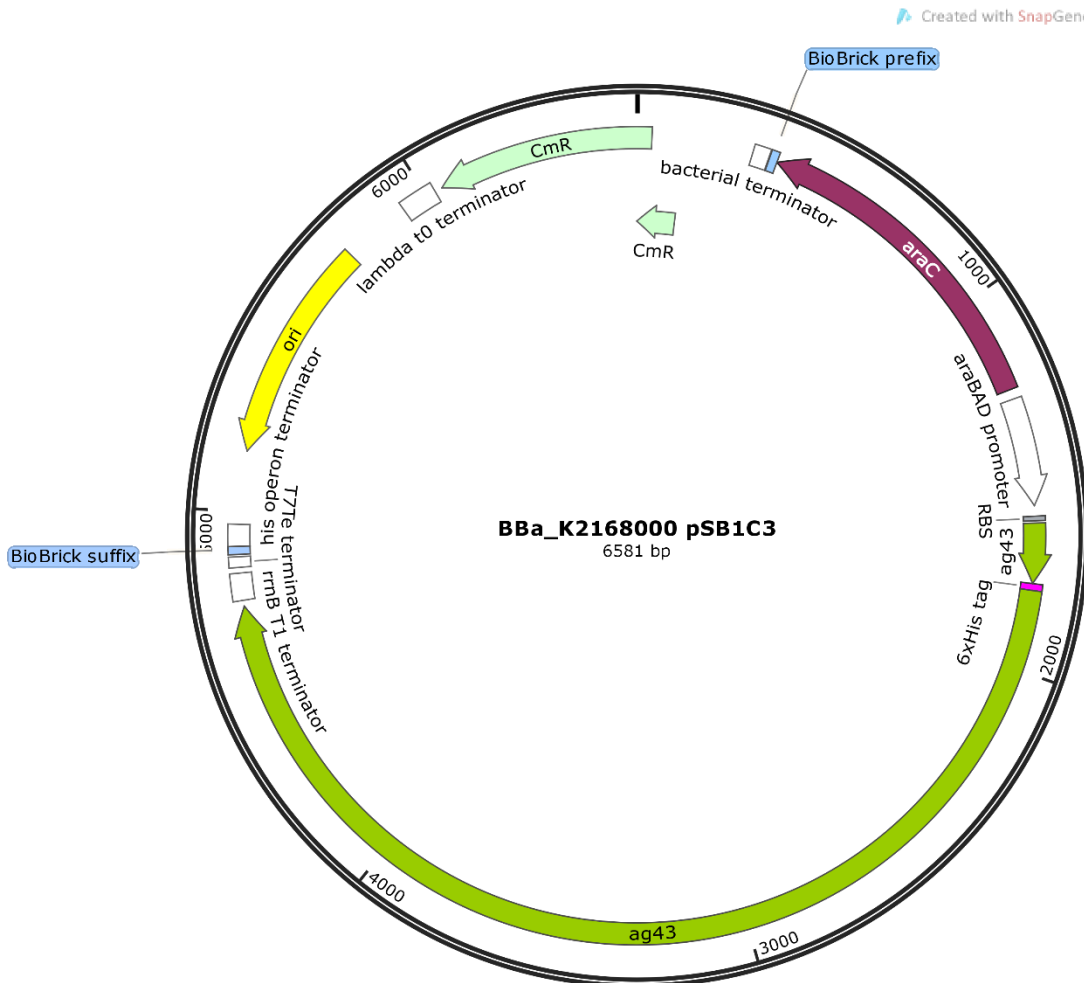


(Ref: NEB Hifi DNA Assembly MasterMix instruction manual)

Intended BioBricks

Created with SnapGene®

Created with SnapGene®



BioBrick suffix



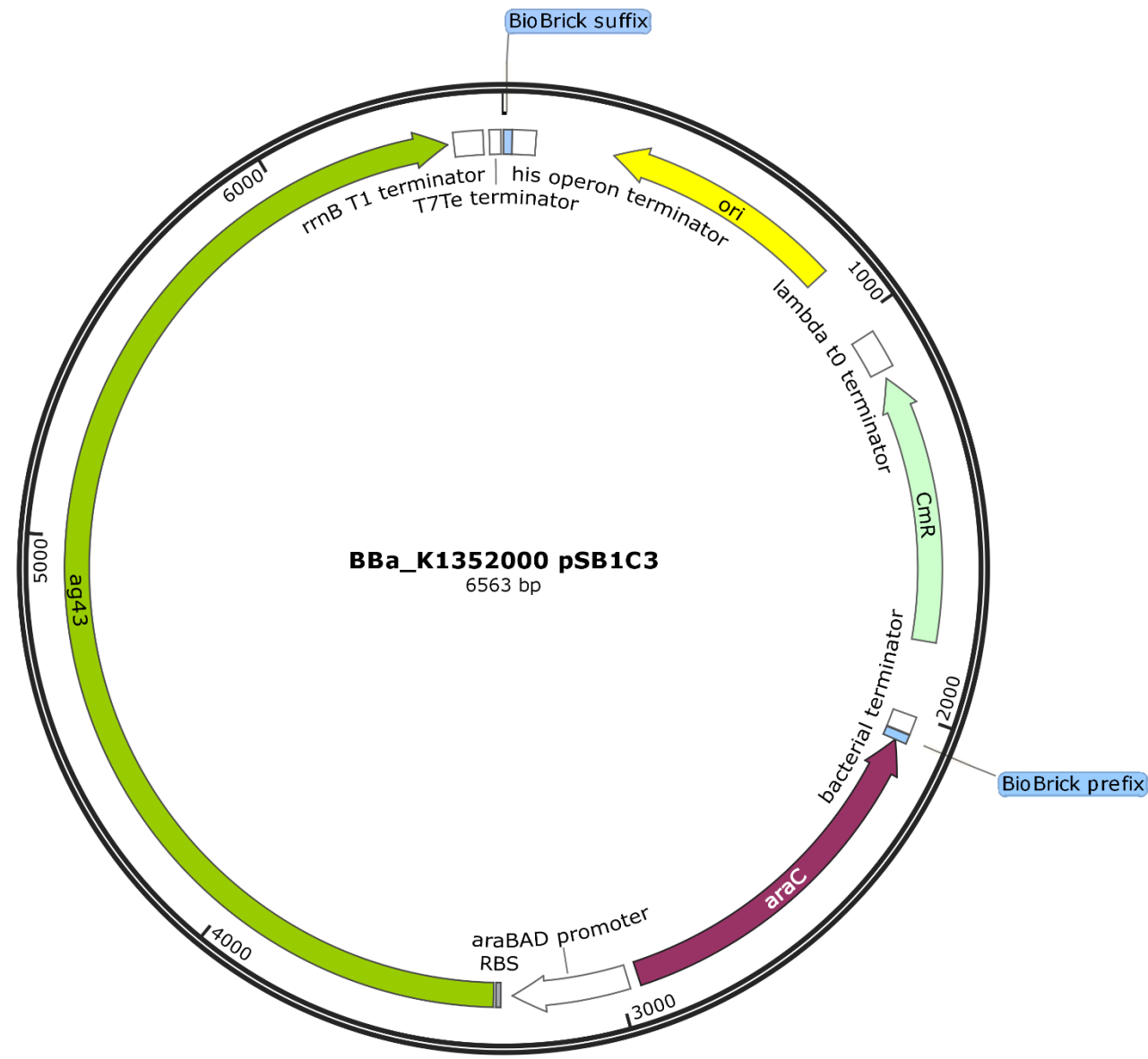
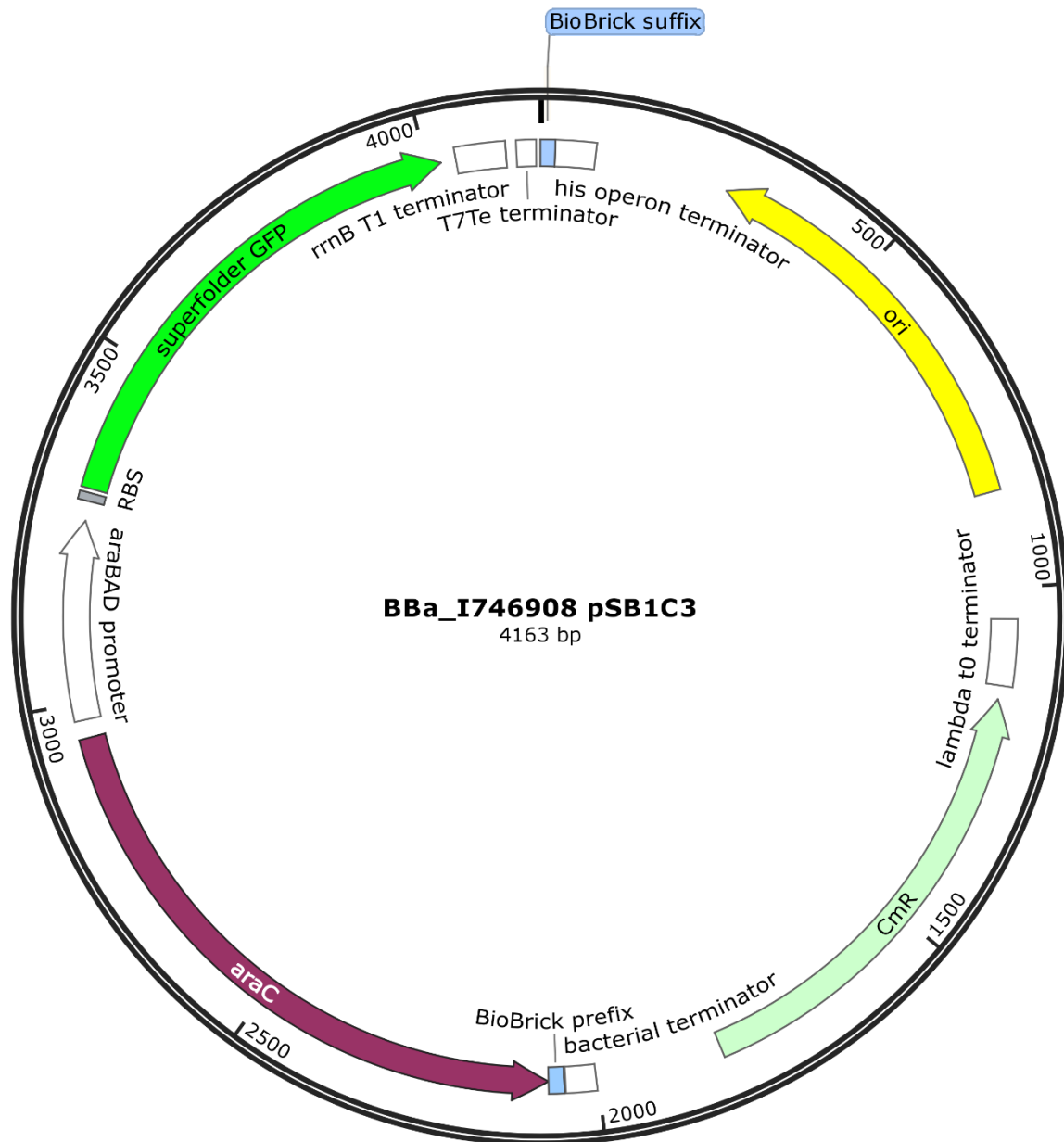
RBS

6xHis tag

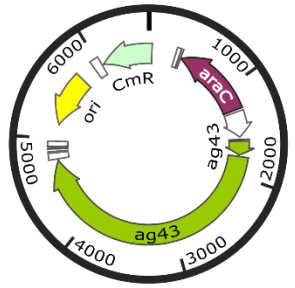
Template BioBricks

Created with SnapGene®

Created with SnapGene®

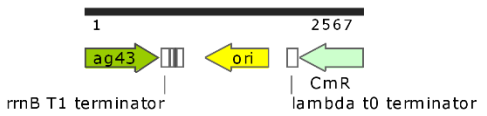
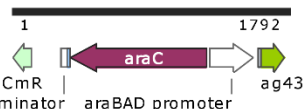
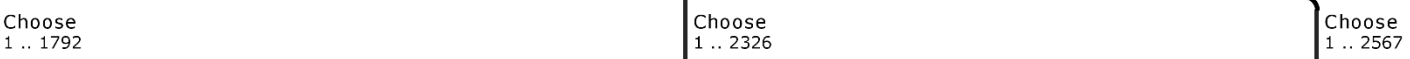


Hifi assembly of BBa_K2168000



BBa_K2168000 pSB1C3
6581 bp

GIBSON ASSEMBLY



Fragment 1
1792 bp

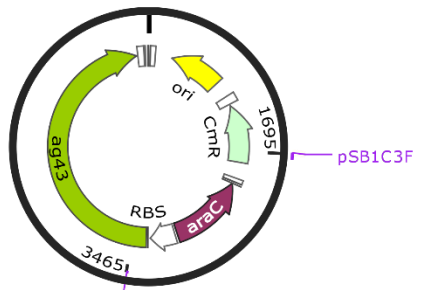
Fragment 2
2326 bp

Fragment 3
2567 bp

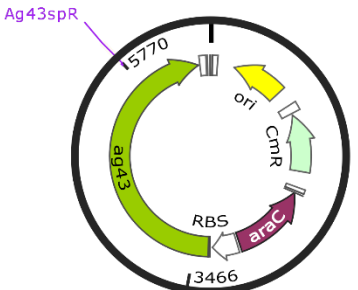
PCR
Amplify 1695 .. 3465 using:
pSB1C3F
Ag43HNR

PCR
Amplify 3466 .. 5770 using:
Ag43HNF
Ag43spR

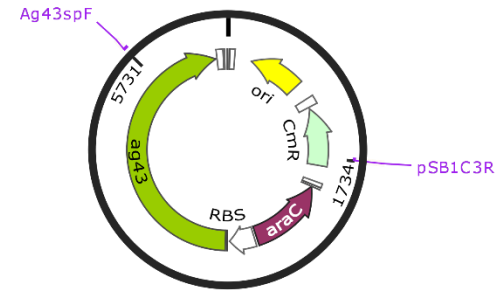
PCR
Amplify 5731 .. 1734 using:
Ag43spF
pSB1C3R



BBa_K1352000 pSB1C3
6563 bp

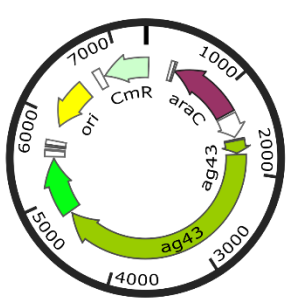


BBa_K1352000 pSB1C3
6563 bp



BBa_K1352000 pSB1C3
6563 bp

Hifi assembly of BBa_K2168001



BBa_K2168001 pSB1C3
7328 bp

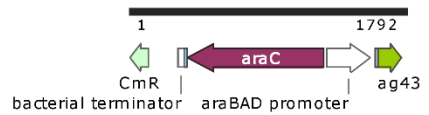
GIBSON ASSEMBLY

Choose 1 .. 1792

Choose 1 .. 2326

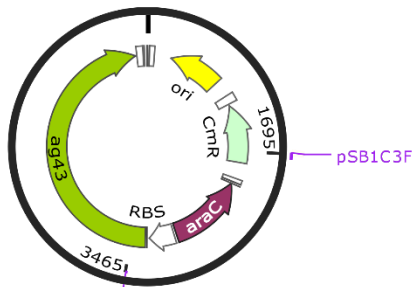
Choose 1 .. 715

Choose 1 .. 2624

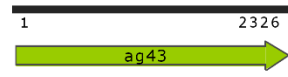


Fragment 1
1792 bp

PCR Amplify 1695 .. 3465 using:
pSB1C3F
Ag43HNR

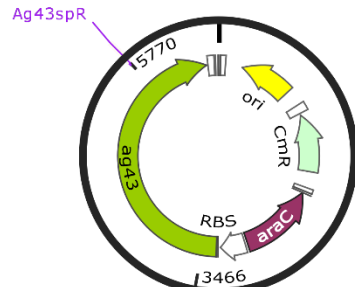


BBa_K1352000 pSB1C3
6563 bp

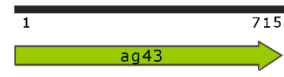


Fragment 2
2326 bp

PCR Amplify 3466 .. 5770 using:
Ag43HNF
Ag43spR

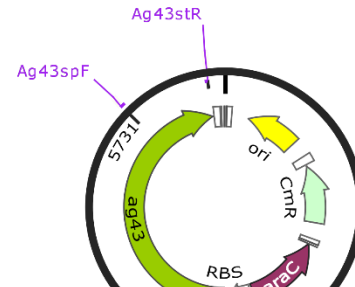


BBa_K1352000 pSB1C3
6563 bp

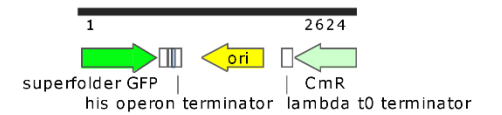


Fragment 3
715 bp

PCR Amplify 5731 .. 6423 using:
Ag43spF
Ag43stR

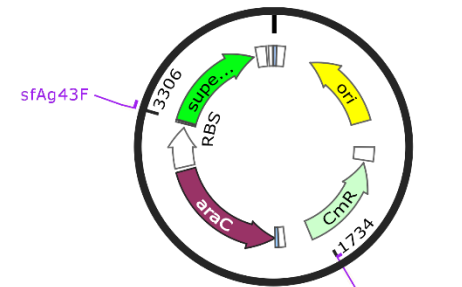


BBa_K1352000 pSB1C3
6563 bp



Fragment 4
2624 bp

PCR Amplify 3306 .. 1734 using:
sfAg43F
pSB1C3R



BBa_I746908 pSB1C3
4163 bp

Primers

Primer name	Sequence(5'-3')	Remarks
Ag43stR	CACCACCTGAACCTCCACCACCgaaggtcacattcagtgtg gcctgac	Reverse primer to eliminate stop codon for Ag43-sfGFP chimera and add linker sequence between Ag43 and sfGFP.
Ag43spF	gatgatgacggctcccgtgc	Splits BBa_K1352000 into smaller, manageable PCRs.
Ag43spR	catcatcccggaccgtgcc	Splits BBa_K1352000 into smaller, manageable PCRs.
pSB1C3F	gtaatatccagctgaacggctctggttatag	Splits pSB1C3 CAT gene from template.
pSB1C3R	ctcaatgtacctataaccagaccggttcag	Splits pSB1C3 CAT gene from template.
sfAg43F	TTCGGTGGTGGAGGTTTCAGGTGGTGGTGGCTCGATG CGTAAAGGCGAAGAGCTGttcac	Forward primer to isolate sfGFP from BBa_I746908, adds linker sequence between Ag43 and sfGFP.
Ag43HNF	gctCATCATCACCATCACCACgacatcgttgtgcaccgggag	Forward primer to add 6xHis tag to Ag43.
Ag43HNR	gtcGTGGTGATGGTGATGATGagcagccagcaccgggagtg	Reverse primer to add 6xHis tag to Ag43.

Primers pairs with complementary overhangs

- pSB1C3F-pSB1C3R
- Ag43HNF-Ag43HNR
- Ag43spF-Ag43spR
- Ag43stR-sfAg43F

The 5' ends of the following primer-pairs give rise to 3' complementary overhang ends of PCR fragments during the Gibson/Hifi assembly, whereas the 3' ends of the primers anneal the PCR templates.