University of Delaware iGEM team
Laboratory Instructions for Gibson Assembly and Transformation
Monday July 31, 2017

Overview:
The objective of these procedures is to assemble plasmid constructs, each containing a single bacteriocin or accessory gene and use these constructs to transform competent E.Coli. The double stranded DNA provided to us by IDT includes the following 13 components:

CvaA/B bp 1 - 2140	ABC Transporter	1000ng**	CvaAB
CvaA/B bp 2116 - 4257	ABC Transporter	1000ng**	CvaAB
Curvacin A	Bacteriocin	1000ng**	CurA
Enterocin A	Bacteriocin	1000ng**	EntA
Pediocin PA-1	Bacteriocin	1000ng**	PedPA1
Sakacin P	Bacteriocin	1000ng**	SakP
pSB1C3 linearized vector	Chloramphenicol resistant plasmid	1000ng**	pSB1C3
pSB1K3 linearized vector	Kanamycin resistant plasmid	1000ng**	pSB1K3
Col_5_Acidocin_Ad	Bacteriocin	1000ng**	AcdAD
Col_5_Lactocin_705 beta	Bacteriocin*	1000ng**	LasBeta
Col_5_Lactocin_705 alpha	Bacteriocin*	1000ng**	LasAlpha
Col_5_Sakacin_QC	Bacteriocin	1000ng**	SakQC
PelB_PediocinPA1	Bacteriocin	1000ng**	PBPedPA1
*Individual components of a 2 part system **Nominal mass			

Except for the two Lactocins, all of the above bacteriocin and transport genes will be assembled into constructs. Bacteriocins will be ligated into a chloramphenicol resistant backbone. The ABC transporter will be ligated into a Kanamycin resistant backbone. Following assembly, the reaction products will be used to transform competent e.coli. Transformed cells will be plated on selective media and incubated for 48 hours. The final product of this day's work will be a set of master plates for a total of 8 transformed cell lines. Each plate will be labeled with the date, gene symbol, plasmid, dilution level and operator's initials. The lactocins may be used at a later date.

Materials and Equipment for G-Block re-suspension

- TE Buffer 10 mM Tris, 1mM EDTA, pH 8
- Microcentrifuge, Vortexer
- Water bath heated to 50 degrees C

Materials and Equipment for Gibson Assembly:

- Microfuge tubes
- Gibson HiFI Kit reagents (Master Mix)
- Wet Ice
- Water Bath at 50 degrees C (and floaty tube holders)

Materials and Equipment for Transformations

- Microfuge tubes -- labeled appropriately
- Competent Cells -- thawed on ice within 20 minutes of use
- Water Bath at 42 degrees C (and floaty tube holders)
- Shaking Incubator
- Dilution tubes, prepared in advance with 450 uL of SOC

General Procedure

5:30 pm -- 6:30 pm

1. Gather all required materials, turn on water baths and fetch ice
2. If Necessary, prepare TE Buffer.
3. Calculate molarities for gibson reactions, double checked by a second operator
4. Label 8 microfuge tubes for Gibson Reactions:
    a. Prefix RX
    b. Symbol, eg SakP, PedPA1 etc. . .
5. Label 8 microfuge tubes for transformation, and set aside.
6. Re-suspend GBlocks following procedure on package insert.
    a. Add 100 uL TE buffer, vortex, incubate 50 C for 20 minutes
    b. Vortex again and do a quick spin to collect all liquid at the bottom of the vial
7. Check DNA concentration on the spectrophotometer
    a. Dilute 10 uL of each resuspended G-Block in 90 uL TE, measure absorbance at 260nm and 320 nm

6:30 pm -- 8:00 pm

8. Set up Gibson reactions on ice, Taking care not to contaminate microfuge tubes
    a. For Bacteriocins:
        i. 5 uL Bacteriocin + 5 uL pSB1C3 Vector + 10 uL master mix
    b. For CvaAB
        i. 6 uL CvaAB-1 + 6uL CvaAB-2 + 3 uL pSB1K3 Vector + 10 uL master mix
9. Incubate Reactions at 50 C for 15 to 60 minutes
    a. Start time: 7:03 pm End Time: 7:23

8 :00 pm -- 10:00 pm

9. 10 minutes prior to the end of Gibson Assembly, fetch competent cells from -80 (on ice)
10. Transfer Competent cells ~ 50uL to sterile microfuge tubes
11. Follow Incubation procedure as described in NEB Protocol
    a. 30 minutes on ice. Start time: End Time:
    b. 30 second heat shock at 42 C
    c. Add 950 uL SOC
    d. Shake and incubate for 60 minutes at 37 C, 250 rpm
12. At the end of incubation, prepare a 10x dilution of each transformation mixture
    a. 50uL transformation mixture in 450 uL SOC
13. Prepare the following master plates for each transformation
    a. 100 uL of transformation mixture, spread on plate
    b. 100 uL of the 10 x dilution spread on selective plate
    c. Streak a loop full of the transformation mixture
14. Incubate plates inverted at 37 C for ~ 48 hours.

Work sheet for Gibson Reactions.

pSB1C3
OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

pSB1K3
OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Curvacin A Reaction
OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	5 uL	50ng	0.04pmol
Bacteriocin dsDNA	5 uL	50ng	0.075pmol

Enterocin A
OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	5 uL	50ng	0.04pmol
Bactericon dsDNA	5 uL	50ng	0.074pmol

Pedicocin PA1
OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	5 uL	50ng	0.04pmol
Bacteriocin dsDNA	5 uL	50ng	0.071pmol

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Sakacin P
OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	5 uL	50ng	0.04pmol
Bacteriocin dsDNA	5 uL	50ng	0.0745pmol

Acidocin AD

OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	4 uL	40ng	0.032pmol
Bacteriocin dsDNA	5 uL	50ng	0.0685pmol

Add 1 uL water

Sakacin QC

OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	5 uL	50ng	
Bacteriocin dsDNA	5 uL	50ng	0.07pmol

PelB-Pediocin PA1

OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1C3 dsDNA	5 uL	50ng	0.04pmol
Bacteriocin dsDNA	5 uL	50ng	0.073pmol

CvaAB

OD260: **OD320:** **Actual Mass:** **Fragment fm/ng:**

Fragment	Volume Added	Actual Mass	Actual Moles
pSB1K3 dsDNA	3 uL	30 ng	0.0225 pmol
CvaAB-1	6 uL	60 ng	0.0456pmol
CvaAB-2	6 uL	60 ng	0.0456pmol

15 uL of Master mix