Protocol: IV-HSL Emitter Module

Printable Version

Overview

This protocol reconstitutes the BjaI/BjaR quorum sensing components from *Bradyrhizobium japonicum* to establish IV-HSL-producing synthetic cells (emitters) and IV-HSL-responsive *Escherichia coli* cells (receivers), implementing the IV-HSL Emitter Cell.

BjaI is expressed inside Emitter Cells containing PURExpress to produce the enzyme BjaI from the template `pT7-bjaI`. BjaI will catalyze a reaction between the membrane impermeable IV-CoA and SAM substrates to yield membrane *permeable* IV-HSL.

E. coli cells expressing BjaR act as receiver cells, providing an easy means to detect IV-HSL production. When BjaR binds IV-HSL, expression of a fluorescent reporter gene controlled by a BjaR-regulated promoter is triggered.

Successfully built IV-HSL Emitter Cells will release IV-HSL and induce GFP expression in XL10-Gold cell with increasing green fluorescence over time.

There are five key stages to making the IV-HSL Emitter Cell:

Step	Process	Hands-on Time	Total Time	Notes
1	**Pre-culture BjaR receiver cells**	30 mins	3.5 hr	
2	**Prepare lipids-in-oil solution, outer solution, and substrate stock solutions**	1 hr	4 h	Buffers and lipids may be prepared in advance and used for experiments on subsequent days.
3	**Assemble PURE reactions**	30 mins	30 mins	
4	**Encapsulate liposomes**	30 mins	30 mins	
5	**Measure and image**	30 mins	6–12 h	Total time depends on the exact experiment and incubation conditions. GFP expression should be seen over the first 6 hours at 37C.

Materials and Equipment

Name	Product	Manufacturer	Part #	Price	Link
Buffers					
Glucose	D-(+)-Glucose, 99%	Thermo Scientific	A16828-36	**$**41.65	[link]
Sucrose	Sucrose, 99%	Thermo Scientific	A15583-36	$41.65	[link]
Lipids					
Egg PC	25mg/mL	Avanti Lipids	840051C-200mg	$186	[link]
Liss-Rhod-PE	18:0 Liss Rhod PE 1 mg/mL	Avanti Lipids	810179P-1mg	$273.47	[link]
Mineral Oil	Mineral oil, mixed weight	Thermo Scientific	AC415080010	$53.40	[link]
Glass Syringe 250 uL		Hamilton	14-815-238	$150.15	[link]
PURE					
PURE	PURExpress	NEB	E6800S	$295.00	[link]
RNase Inhibitor	RNase Inhibitor, Murine	NEB	M0314S	$81.00	[link]
DNA	`pT7-bjaI`	b. next			[link]
	`bjaR-GFP-native`	b.next			[link]
OptiPrep	OptiPrep - Density Gradient Media (Iodixanol)	COSMO BIO USA	AXS-1114542	$172	[link]
SAM	S-adenosylmethionine (SAM)	NEB	B9003S	$45	[link]
IV-CoA	Isovaleryl coenzyme A lithium salt hydrate	Millipore Sigma	I9381-10MG	$348	[link]
IV-HSL	3-Methyl-N-[(3S)-tetrahydro-2-oxo-3-furanyl]butanamide	LGC	TRC-M282980-50MG	$171	[link]

Name	Product	Manufacturer	Part #	Price	Link
DMSO	Dimethyl sulfoxide	Thermo Scientific	042780.M1	$342	[link]
Cell culture					
XL10-Gold Cells	XL10-Gold Ultracompetent Cells	Agilent	200314	$223	[link]
M9 Media	M9, Minimal Salts, 5X, powder, minimal microbial growth medium	Sigma-Aldrich	M6030-1KG	$260	[link]

Step 1: Pre-culture BjaR receiver cells

- [ ] Prepare glycerol stock of BjaR receiver cells
  - [ ] Transform XL-10 Gold competent *E. coli* with `bjaR-GFP-native`:
    - [ ] Add 1–5 µl containing 1 pg–100 ng of plasmid DNA `bjaR-GFP-native` to 50 µl of XL10-Gold cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. **Do not vortex.**
    - [ ] Place the mixture on ice for 15 minutes. Do not mix.
    - [ ] Heat shock at exactly 42°C for 40 seconds. Do not mix.
    - [ ] Place on ice for 5 minutes. Do not mix.
    - [ ] Pipette 950 µl of room temperature SOC into the cell mixture.
    - [ ] Shake the cell mixture vigorously (250 rpm) at 37°C for 60 minutes.
    - [ ] Warm Ampicilin LB agarose plates at 37°C for 10 mins.
    - [ ] Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in LB.
    - [ ] Spread 50–100 µl of each dilution onto a Ampicilin agarose plate and incubate overnight for ~15 hrs at 37°C.
  - [ ] [if we're including making a glycerol stock, need overnight culture and glycerol stock preparation here]
- [ ] Prepare a streak plate from the glycerol stock (reference)
  - [ ] Streak a Ampicillin LB plate from the glycerol stock and incubate overnight at 37C.
- [ ] Prepare M9 Media containing 1× M9 salts, 0.34 mg/ml−1 thiamine hydrochloride, 0.2% casamino acids, 2 mM $MgSO_4$, 100 µM $CaCl_2$ and 0.4% (wt/vol) glucose.

☐ Pick a colony from the *E. coli* streak plate, and inoculate a 5 mL culture tube containing the M9 media with 100 ug/mL carbenicillin.

☐ Incubate the cells at 37 °C, 225 rpm, for 3 h. *Prepare Emitter liposomes while the cells incubate.*

☐ Dilute the culture media with the pre-warmed M9 media until OD600 = ~0.1.

☐ Balance osmolarity of the culture media with PURE (inner solution in liposomes) by adding glucose to the M9 media:

	Volume to mix (uL)
M9 media	1000
3M Glucose	293.81

Step 2: Prepare lipids-in-oil solution, outer solution, and substrate stock solutions

Prepare lipids-in-oil (mineral oil) solution

☐ Clean glass syringes.

- ☐ Pour a small amount of 95% ethanol into a glass container (e.g. a 10 mL beaker).
- ☐ Assemble the glass syringe and prime it by drawing ethanol into the glass syringe, then empty into a waste bottle.

☐ Use glass syringes to add lipids, as shown in the table below, into the 10 ml glass vial containing 1 ml of mineral oil (final lipid concentration is 5 mg/ml).

Lipids	Stock Concentration (mg/mL)	Volume to add (uL)	Target percentage
Egg PC	25	160	66.68
Cholesterol	50	20	33.32
18:0 Liss Rhod PE	1	5	0.01

☐ Heat the lipids-in-oil mixture on a hotplate at 55 C for 3 hrs.

☐ Vortex the lipids-in-oil mixture for 1 min.

- The lipids-in-oil mixture can be stored at 4 C for up to 3 days.

Prepare outer solution

Final concentration of sugar stock solution is 900 mM

Buffer	Volume to add (uL)
3M Glucose Stock	700
H2O	300

Prepare substrate stock solutions

Substrate	Concentration (uM)	MW (g/mol)	Weight (g)	Final Volume (mL)
SAM	5000	398.44	1.99	1
IV-CoA	5000	851.65	4.26	1
IV-HSL	10	183.21	1.83	1

Step 3: Assemble PURE Reactions

PURE reaction setup

	Sample	Negative control	Positive control	
Component	**Volume (uL)**	**Volume (uL)**	**Volume (uL)**	**Notes**
PURE Solution A	12	12	0	PURE energy solution: small molecules
PURE Solution B	9	9	0	PURE proteins and ribosomes
RNAse Inhibitor	1.5	1.5	0	Prevents RNAse activity
EM01-pOpen-pT7-BjaI (~200 ng/uL)	1.5	0	0	DNA encoding green fluorescent protein
SAM (5mM)	1.8	1.8	0	Substrate for IV-HSL production.
IV-CoA (5mM)	0.48	0.48	0	Substrate for IV-HSL production.
OptiPrep	1.5	1.5	1.5	Adds density for phase-transfer
IV-HSL (10 uM)	0	0	0.3	Commercial IV-HSL for positive control.
3M Glucose	0	0	8.46	
ddH2O	2.22	3.72	19.74	
Total	30	30	30	

☐ Thaw reagents on ice and then keep on ice.

☐ Prepare a PCR strip in a strip holder on ice for assembly of the three reactions (Sample, Negative, Positive).

Step 4: Encapsulate PURE reactions into Liposomes

Some tips and tricks can be found in "Hello, world" PURE Liposomes.

- [ ] Set up a microfuge tube rack, with three 1.5 mL microfuge tubes per liposome encapsulation:
  - [ ] Number the tubes per the number of reactions assembled in Step 3.
  - [ ] For each reaction, label the two tubes:
    - [ ] **I** — Oil emulsion
    - [ ] **O** — Outer solution
- [ ] Add 30 ul of PURE reactions prepared in **Step 3** to tubes labelled **I**.
- [ ] Add 180 uL of the lipids-in-oil mixture on top of the PURE reactions in tubes labelled **I** and pipette vigorously until the emulsion becomes cloudy.
- [ ] Add 300 uL of outer solution to each of the tubes labelled **O**.
- [ ] Add 210 uL of the milky solution carefully on top of the outer solution in the tubes labelled **O.**
- [ ] Centrifuge at 9000 rpm at 4c for 10 mins.
- [ ] Remove the top oil and resuspend the pellet in 100 ul of outer solution.
- [ ] Collect the liposomes.

Step 5: Measure and Image Liposomes and Cells

Imaging using confocal microscopy (Operetta CLS):

While microscopy setups may vary, our performance data was collected using the following configuration.

- [ ] Add BjaR receiver cells prepared in Step 1 into 384 Well Glass Bottom Microplates.
- [ ] Add 10 uL of liposomes made in Step 3 on top of the receiver cells in 384 Well Glass Bottom Microplates.
- [ ] Imaging conditions using Operetta:
  - Temperature: 37 C degree
  - Green fluorescence channel (200 us expsoure 95%) - excitation: 460-490 nm; emission: 500-550 nm.
  - Red fluorescence channel (50 us exposure 95%) - excitation: 530-560 nm; emission: 570-650 nm.
  - Brightfield (20 us 95%)
  - We capture a 6 h time lapse with 10 min intervals.
  - We also acquired z-stack images spanning from 0 µm to 80 µm of the focal plane.

Measuring usinng plate reader (BioTek Cytation 5):

☐ Add BjaR receiver cells prepared in Step 1 into 96 Well Glass Bottom Microplates.

☐ Add 10 uL of liposomes made in Step 3 on top of the receiver cells in 96 Well Glass Bottom Microplates.

☐ Procedures:

- Temperature: 37 C degree
- Read the fluorescence intensity from the bottom
- Excitation wavelength: 485 nm ; Emission wavelength: 528 nm
- We capture a 6 h time lapse with 5 min intervals

Background Protocols

☐ Prepare lipids for use in encapsulation: 〓 Lipid Preparation

☐ Prepare inner and outer buffers: 💧 PURE inner and outer solution

Resources and References

- **Other Protocols**
  - Transformation protocol: 🧫 Transformation v1.0.
- **Papers**
  - Smith, J. M., Hartmann, D. & Booth, M. J. Engineering cellular communication between light-activated synthetic cells and bacteria. *Nature Chemical Biology* **19,** 1138–1146 (2023). [https://www.nature.com/articles/s41589-023-01374-7]

Credits

- b.next