Lab tasks Schedule

Task 1: Lab work. September 19th 2017

Tasks:

1- Reconstitution of the fragments
2- Reconstitution of the primers
3- PCR amplification for circ RNA circuit and CRISPR circuit

1- Reconstitution of the fragments :
Following the instructions written in the specification sheet of the IDT of each fragment. We made reconstitution of the lyophilized fragments by deionized RNAse free water.

N.B : the volume of added RNase free water was calculated by : http://www.molbiol.edu.ru/eng/scripts/01_07.html

when performing the calculation we found that 105 uL of the deionized water was added to each fragment to reach the desired concentration 10ng / uL

2- Reconstitution of primers :
Following the instructions written in the specification sheet of the IDT of each fragment. We made reconstitution of the lyophilized fragments by deionized RNAase free water

3- Preparation of cas9 from distribution kit according to IGEM's instructions (fragment 3 in CRISPR circuit).

4- Preparation for PCR according to protocol and amplification
PCR products
1-5 fragments of regular circuit
1-4 fragments of CRISP circuit

PCR reaction mix : according to kit taq PCT master mix kit / Cat No. 201443 (QIAGEN)

Serial	Reagent	Volume
1	Taq Master Mix	25 uL
2	RNAase free water	19 uL
3	Primer Mix (25 pmol)	5 uL
4	gDNA (1 ug / Reaction)	1 uL
	Total volume	50 uL

PCR protocol:
Was done according to the instruction manual
Annealing temperature: 55 C
No. of cycles: 12 cycles (as recommended by IDT instructions) as most of our gDNA is less than 1 KB

Task 2: Lab work 21st September

Steps:

Real Time {PCR amplification for all fragments from IdT:

Purpose: To ensure that all fragments used in Gibson assembly is pure DNA with satisfactory amplification.

1) Run PCR for fragments for fragments to see curves instead of gel electrophoresis (ensure quantification).
2) Reconstitution of primes to reach a 100ql concentration.
3) Dilution before PCR by Rnase free water to reach a concentration of 20 ql.
4) Preparation of the reaction mix for PCR

Serial	Reagent	Volume
1	QuantiTect Master Mix	10 uL
2	RNAase free water	3 uL
3	Primer Mix (25 pmol)	2 uL
4	gDNA (1 ug / Reaction)	5 uL
	Total volume	20 uL

Working on 2 parallel circuits

-determine cyclic condition of PCR

-Red rotter in ram (volume 20 ql)

-Protocol of kit in Q-PCR

Technique optimized from after the non-specific 1st 10-15 cycles initial, log exponential, plateau.

Output: 9 ependorph 0.2

Labled from 1-9

1-5 circ-RNA

6-9 CRISPR

Gibson Assembly Protocol E5510

Gibson for target + positive control

Target: single band on gel electrophoresis

Equation: weight in ng * 1000 / (bp * 650 dalton)

By Gibson online calculator (NE, bio calculator) we calculated the total amount of fragments

Fragment no.	Mass in pmol	Volume in ųl
Circ-RNA		
Fragment 1	1.378	1.5
Fragment 2	1.46	1.5
Fragment 3	1.63	1.5
Fragment 4	1.45	1.5
Fragment 5	2.2	2.0
CRISPR		
Fragment 6	2.26	3.0
Fragment 7	1.33	2.0
Fragment 8 (cas9)	0.00096	2.0
Fragment 9	1.62	2.0

-insert into thermal cycles to assemble the fragments into gibson master mix to do function

Output 3 ependorph 0.2ųl

1= Gibson assembly for circ-RNA circuit (fragment 1,2,3,4,5)

2= Gibson assembly for CRISPR circuit (fragment 6,7,8,9)

3= positive control for Gibson assembly (provided by the kit)

Task 3: IGEM 2017 Technical Work – 25th September, 2017

IGEM Participants:

Tasks:

- Download and analyze pictures of Gel Electrophoresis of assembled fragments
- Download and analyze pictures of PCR curves
- Transformation of assembled fragment into competent cells

Methods:

1) Preparation of a semisolid media- LB agar plates via application of (Protocol 6)
   Main steps: preparation of 0.75 gm agar powder in 50 ml of LB media
   Incubate in 37 degrees Celsius
   Solvate ampicillin Na salt in 20 ml distilled H2O
   Pour LB agar media into 3 plates to culture a transformed Gibson PC, Crisp, and Circ

http://2011.igem.org/LB_Broth
P.S. to each 1ml of broth, add 1ul of 50 mg/mol ampicillin; therefore, for a 50 ml of broth add 250ul of 10 mg/mol ampicillin

2) Transformation into competent cell (Bioengland e.coli)
   Application of Protocol 5
   Gibson Assembly® Chemical Transformation Protocol (E5510)
   https://www.neb.com/protocols/2012/12/11/gibson-assembly-transformation-protocol-e5510

Task 4: IGEM 2017 Technical Work – 27th September, 2017

Tasks:

1- We checked the culture media and took pictures and the results were as follows:

Culture	Results
+ve control culture plate	No bacterial Colonies
Circular RNA culture plate	Bacterial colonies Present
CRISPR culture plate	Bacterial colonies Present and of more density

2- We have followed protocol 7 and for DNA extraction “Monarch® Plasmid Miniprep Kit” with inoculation of cells with phosphate buffer saline.

3- We worked on step 3 of protocol 2 to run a new gel of Cas9 for improving characterization

4- We documented output samples and associated them to previous output samples.