pJET PCR cloning

Purpose:

To clone the synthesis sequence made from IDT into plasmids for further experiments.

Materials:

- IDT synthesis DNA oligoneucleotide
- Thermofisher cloneJET kit

Procedures:

1. Premix solution as following:

name	DNA	ddH_2O	pJET1.2	T4 ligase	2x reaction buf.
	(ul)				
LF pre	0.9	7.1	1	1	10
Gal Lip suf	1.0	7.0			
Glu Xyn pre	1.0	7.0			
xyn	0.9	7.1	1	1	10
glu	0.5	7.5			
lip	0.5	7.5			
ste12p	1.0	7.0			
pste12	0.6	7.4			

2. DNA should be well resuspended, the molecular ends of each fragment is calculated through the following chart:

name	length (bp)	pmol end	mass (ng)	conc. (ng/ul)	add (ul)
LF pre	1827	0.15	84.68	100	0.85
Gal Lip suf	2030	0.15	94.09	100	0.94
Glu Xyn pre	2080	0.15	96.40	100	0.96
xyn	1886	0.15	87.41	100	0.87
glu	1067	0.15	49.45	100	0.49
lip	1010	0.15	46.81	100	0.47
ste12p	2126	0.15	98.54	100	0.99
pste12	561	0.15	26.00	50	0.52

3. After well mixing the reaction solution on ice, the mixture should be centrifuge rapidly and lay at room temperature for 10 minute reaction.

4. The ligation product could then go through transformation and be checked with PCR.