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Introduction

Digestion Ligation is a procedure in restriction cloning and it is the procedure with which we assemble the constructs in our iGEM project. ALWAYS: when we do DIGLIG, which needs 4h time then first check if one of the small blocks A or B is available! In this protocol, we use the insertion of MFa into the p02 Vector as an example

Materials

- MQ
- 10X Tango Buffer
- ATP
- T4 DNA Ligase
- Enzymes
- BSA
- T4 Buffer (as an alternative to 10X Tango Buffer and ATP)
- Plasmids
  - Backbone and insert

Procedure

How to Calclulate the Concentrations in the case of our MFalpha:

1. https://nebiocalculator.neb.com/#!/ligation

   Go to this website and type in the DNA concentrations and bp lengths that you have for insert and vector, choose 2:1 ratio. So in this example we need around 13ng of insert.

   Note: For LII assembly, You have to put in the bp length of the whole plasmid and not that of the insert it contains.

Ligation Calculator

This tool will calculate the mass of insert required at several molar insert:vector ratios in the range needed for typical ligation reactions.

Input	Value	Unit	Required insert DNA mass
Insert DNA length	317	bp	6.401 ng (1:1)
			12.80 ng (2:1)
Vector DNA length	4952	bp	19.20 ng (3:1)
			32.01 ng (5:1)
Vector DNA mass	100	ng	44.81 ng (7:1)

2. The length of our Mfa is 317bp (including the amplification overhangs) and we choose (2:1) ratio, so you need 12.8ng of insert Mfa.
3. Nanodrop your insert: we have 68.5ng/µL Mfa

   Calculate how much of the stock DNA solution needs to be added. in the case of MFa: (12.8ng)/(68.5ng/µL)=0.19µL. This is too small a volume to pipette directly. If this happens, you need to dilute it (for example, 1.9µL of the insert in 8.1µL of MQ) This would yield a solution with MFa concentration of 12.8ng/µL so you could just add 1µL of this dilution to your reaction to add 12.8ng. Use the formula c1xV1=c2xV2 to dilute your inserts.

   V_1=amount of stock you add to dilution

   C_1= conc. of stock (NANODROPED)

   V_2= final volume= added stock plus MQ to dilute (10µL)

   C_2 = conc. of dilution (=required insert DNA mass)

   V_1 uL + (10µL-V_1 uL) uLMQ= dilution (total volume of 10µL)

LI construct DIGLIG

	A	B	Example for MFa in p02
1	H20	fill up to 15 ul	10.5 µl
2	Vector (7.5 nM)	calculate as described above	dilute 1 ul in 3 ul MQ, then add 1 ul
3	Insert (15 nM)	calculate as described above	dilute 1.9 ul in 8.1 ul MQ, then add 1 ul
4	10X Tango Buffer	1 µl	1 µl
5	T4 DNA Ligase (5 units/µl)	0.25 µl	0.25 µl
6	Enzyme (5 units/µl)	0.25 µl	0.25 µl
7	ATP (10 mM)	1 µl	1 µl

LII constrsucts DIGLIG

	A	B
1	H20	fill up to 15 ul
2	Vector (7.5 nM) p(10) for all!	calculate as described above
3	Insert A-B Promoter (15 nM)	calculate as described above
4	Insert B-C MFa or dummy BB6 for B+ (15 nM)	calculate as described above
5	Insert C-D Receptor (15 nM)	calculate as described above
6	Insert D-E Fluorescent protein(15 nM) 125.9 ng (2:1)	calculate as described above
7	Insert E-F Terminator (15 nM)	calculate as described above
8	Insert F-G BB9 dummy (15 nM)	calculate as described above
9	10X Tango Buffer	1 .5 µl

10	T4 DNA Ligase (5 units/µl) our tube has 400000 u per ml-> 400u/uL	0.25uL
11	Enzyme (5 units/µl)	0.25 µl dilution: (1uL EZ in 3uL MQ =to volume 4uL) -> then add 1uL
12	ATP (10 mM)	1 µl

4. The program on the thermocycler is **diglig long,** which can also be used for short insert as well as for the LII constructs.