# **Benchling**

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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

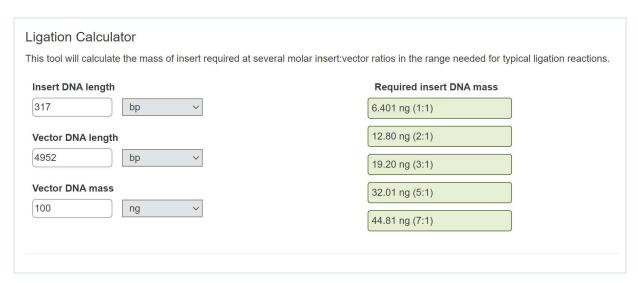
## **Procedure**

### 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.



- 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:  $(12.8ng)/(68.5ng/\mu L) = 0.19\mu L. \text{ This is too small a volume to pipette directly. If this happens, you need to dilute it (for example, <math>1.9\mu L$  of the insert in  $8.1\mu L$  of MQ) This would yield a solution with MFa concentration of  $12.8ng/\mu L$  so you could just add  $1\mu L$  of this dilution to your reaction to add 12.8ng. Use the formula c1xV1 = c2xV2 to dilute your inserts.

V<sub>1</sub>=amount of stock you add to dilution

C<sub>1</sub>= conc. of stock (NANODROPED)

 $V_2$ = final volume= added stock plus MQ to dilute (10 $\mu$ L)

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

 $V_1$  uL + (  $10\mu$ L- $V_1$  uL) uLMQ= dilution (total volume of  $10\mu$ L)

#### LI construct DIGLIG

	А	В	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 μΙ	1 μΙ
5	T4 DNA Ligase (5 units/µl)	0.25 μΙ	0.25 μΙ
6	Enzyme (5 units/µI)	0.25 μΙ	0.25 μΙ
7	ATP (10 mM)	1 μΙ	1 µI

### LII constrsucts DIGLIG

	А	В
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.

