Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2014
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Insert + vector ligation

Technische Universiteit
Eindhoven
University of Technology

Table of contents

1 Insert + vector ligation

- First determine the amount of vector you will need.
  - Aim for a 100 ng of vector:
    $$\frac{100\ ng}{[Vector]\frac{ng}{\mu L}} = x\ \mu L\ \text{vector needed}$$
- Now determine the amount of insert you will need:
  - We will need molar equality for the ligation to work.
  - Find the average number of bases in the insert (432bp for the repeat samples): N_a
  - Find the average number of bases in the vector: N_b
  - Molar equality is found by:
    $$\frac{100\ ng}{N_b} \times N_a = X\ ng\ of\ insert\ needed$$
    N_A = (6,022 141 29 ± 0,000 000 27) × 10^{23} mol^{-1}
- Instead of aiming for molar equality (to increase success rate) use 5 times X ng of the insert.
  $$\frac{5 * X\ ng}{[Insert]\frac{ng}{\mu L}} = Y\ \mu L\ \text{of insert needed.}$$

Component	**Quantity/mass/final concentration**	**Volume (uL)**
H_2O		Fill up to 20 µL
T4 Ligase Buffer 10x		2 µL
Insert DNA	X ng (stock … ng/µL)	Y µL
Vector DNA	100 ng (stock … ng/µL)	X µL
T4 DNA ligase	0.01 µL (400 U/µL stock)	1 µL
Total		20 µL

Important: add the ligase at the very end!!

- Gently mix the solution by pipetting up and down
- Incubate at 25°C for 2 hours in a PCR machine
- Heat inactivate for 10 min at 65°C