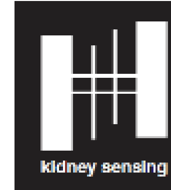


PCR Product Cloning

We have performed DNA ligations with different plasmids due the availability of vectors in the different labs. All the vectors cited below have blue/white selection.

Insert calculation:

$$ng\ of\ insert = \frac{ng\ of\ vector\ \times kb\ size\ of\ insert}{kb\ size\ of\ vector} \times insert\!:\!vector\ molar\ ratio$$

Blunt-end Cloning – PUC19/ *Sma*I

The PCR product amplified by the High Fidelity Enzyme Mix doesn´t have adenine overhang, so we used the PUC19 vector digested with the restriction enzyme *Sma*I.

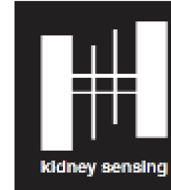
Materials

- Blunt-ended DNA
- PUC19 previously digested with *Sma*I
- 10X T4 DNA Ligase Buffer
- 50% PEG 4000 Solution
- T4 DNA Ligase (5U/µL) (Thermo Scientific)
- Water, nuclease free
- 0.5 mL microtube

Method

1. After thawing and vortexing the solutions (except the enzyme solution), prepare the following mixture in a 0.5 sterile microtube. Keep the microtube on ice.

Blunt-ended DNA (vector:insert)	1:3 to 6:1
PUC19 previously digested with *Sma*I	50 ng
10X T4 DNA Ligase Buffer	2 µL
50% PEG 4000 Solution	2 µL
T4 DNA Ligase (5U/µL)	1 µL (5U)
Water, nuclease free	to 20 µL



2. Spin the mixture.
3. Incubate overnight at 4°C.
4. Transform the ligation in *E. coli* DH5α. (**see the Transformation in *E. coli* Protocol**) [http://2014.igem.org/wiki/images/a/a5/Transformation_in_Escherichia_coli_DH5.pdf]
5. Plate out the suspension on a LB agar plate with ampicillin (100 µg/mL), X-Gal (80 µg/mL) and IPTG (0.5 mM) and incubate at 37° for 12 hours.
6. Select the white colonies.

TOPO® TA Cloning® Kit - Invitrogen

Materials

- PCR product (A overhang)
- Salt Solution
- TOPO® vector
- Water, nuclease free
- 0.5 mL microtube

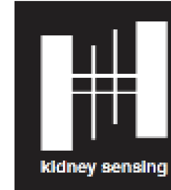
Method

1. After thawing and vortexing the solutions (except the enzyme solution), prepare the following mixture in a 0.5 sterile microtube. Keep the microtube on ice.

PCR product (A overhang)	0.5–4 µL
Salt Solution	1 µL
TOPO® vector	1 µL
Water, nuclease free	to 6 µL

2. Spin the mixture.
3. Incubate at room temperature for 5-30 minutes.
4. Transform the ligation in *E. coli* DH5α. (**see the Transformation in E. coli Protocol**) [http://2014.igem.org/wiki/images/a/a5/Transformation_in_Escherichia_coli_DH5.pdf]
5. Plate out the suspension on a LB agar plate with ampicillin (100 µg/mL), X-Gal (80 µg/mL) and IPTG (0.5 mM) and incubate at 37° for 12 hours.
6. Select the white colonies.

For more information, consult the kit specifications available on the website:
[http://tools.lifetechnologies.com/content/sfs/manuals/topota_man.pdf]



pGEM-T-Easy Cloning - Promega

Materials

- PCR product (A overhang)
- 2X Rapid Ligation Buffer
- pGEM-T Easy vector (50 ng/µL)
- T4 DNA ligase (3U/µL) (Promega)
- Water, nuclease free
- 0.5 mL microtube

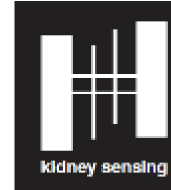
Method

1. After thawing and vortexing the solutions (except the enzyme solution), prepare the following mixture in a 0.5 sterile microtube. Keep the microtube on ice.

2X Rapid Ligation Buffer	5 µL
PGEM-T Easy (50 ng)	1 µL
PCR product (A overhang)	x µL
T4 DNA Ligase	1 µL
Water, nuclease free	to 10 µL

2. Spin the mixture.
3. Incubate overnight at 4°C.
4. Transform the ligation in *E. coli* DH5α. (**see the Transformation in E. coli Protocol**) [http://2014.igem.org/wiki/images/a/a5/Transformation_in_Escherichia_coli_DH5.pdf]
5. Plate out the suspension on a LB agar plate with ampicillin (100 µg/mL), X-Gal (80 µg/mL) and IPTG (0.5 mM) and incubate at 37° for 12 hours.
6. Select the white colonies.

For more information, consult the kit specifications available on the website: [http://www.promega.com.br/~/media/files/resources/protcards/pgem%20t%20and%20pgem%20t%20easy%20vector%20systems%20quick%20protocol.pdf]



InsTAclone PCR Cloning Kit/pTZ57R – Thermo Scientific

Materials

- 5X Ligation Buffer
- Vector pTZ57R/T (55 ng/µL)
- PCR product
- T4 DNA ligase (5U/µL)
- Water, nuclease free
- 0.5 mL microtube

Method

1. After thawing and vortexing the solutions (except the enzyme solution), prepare the following mixture in a 0.5 sterile microtube. Keep the microtube on ice.

5X Ligation Buffer	6 µL
Vector pTZ57R/T (165 ng)	3 µL
PCR product (A overhang)	x µL
T4 DNA Ligase	1 µL
Water, nuclease free	to 30 µL

2. Spin the mixture.
3. Incubate overnight at 4°C.
4. Use 2.5 of the ligation mixture to transform in *E. coli* DH5α. (**see the Transformation in E. coli Protocol**) [http://2014.igem.org/wiki/images/a/a5/Transformation_in_Escherichia_coli_DH5.pdf]
5. Plate out the suspension on a LB agar plate with ampicillin (100 µg/mL), X-Gal (80 µg/mL) and IPTG (0.5 mM) and incubate at 37° for 12 hours.
6. Select the white colonies.

For more information, consult the kit specifications available on the website: [http://www.thermoscientificbio.com/uploadedfiles/resources/k121-product-information.pdf]