08-10-16

PCR

- Amplification of 110 matrix with :
    1 and 10 primers
    11 and 12 primers
    13 and 3 primers
    20 and 6 primers
    + Control

Hybridation temperature used : 69°C
Elongation time used : 1 min 30 sec

- Gel verification of amplifications
    Agarose gel : 1%
    100 mV, 30 min

=> Successful amplification of all pcr products

08-11-16

PCR

- Amplification of 113 matrix with :
    7 and 14 primers
    15 and 16 primers
    17 and 8 primers
    + Control

Hybridation temperature used : 69°C
Elongation time used : 20 sec

- Gel verification of amplifications
    Agarose gel : 1%
    100 mV, 30 min

=> Successful amplification of :
7 + 14
8 + 17

08-12-16

PCR

- Amplification of Dnmt matrix with :
    23 and 9 primers
    21 and 4 primers
    5 and 2 primers
    22 and 4 primers
    + Control

Hybridation temperature used : 68°C
Elongation time used : 50 sec

- Gel verification of amplifications
    Agarose gel : 1%
    100 mV, 30 min

=> Successful amplification of all pcr products

GIBSON ASSEMBLY (dCas9)

psB1C3 : 0,5 µL
1 + 10 : 1 µL
11 + 12 : 1µL
13 + 3 : 1µL
Mix : 10µL
H_2O : 6,5µL

(Control done with circular plasmid)

- Put on the thermocycler at 50°C during 60 min

- Put at -20°C until the transformation step

08-15-16

TRANSFORMATION

- Transformation in DH5α of :
  psB1C3 containing 3 fragments of 110 matrix (LB + Choramphenicol)

- Put plates at 37°C

08-16-16

PCR

- Amplification of 2 matrix with:
    15 and 16 primers (113 matrix)
    18 and 19 primers (gDNA matrix)
    + Control

Hybridation temperature used : 69°C
Elongation time used : 15sec

- Gel verification of amplifications
    Agarose gel : 1%
    100 mV, 30 min

=> No amplification observed

- No colony on the plate containing the cloning of 110 matrix (08-15-16)

08-17-16

CLONING

- Digest of
    psB1C3 with EcoRI and PstI
    10X GCN4 with EcoRI and PstI
    Dnmt3a with EcoRI and PstI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10 min

- Ligation
    10X GCN4 in psB1C3
    Dnmt3a in psB1C3
    Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing 10X GCN4 (LB + Choramphenicol)
    psB1C3 containing Dnmt3a

- Put on the incubator at 37°C

08-18-16

PCR

- Amplification of 2 matrix with:
    15 and 16 primers (113 matrix)
    18 and 19 primers (gDNA matrix)
    + Control

Hybridation temperature used : 74°C
Elongation time used : 15sec

- Gel verification of amplifications
    Agarose gel : 1%
    100 mV, 30 min

=> No amplification observed

GIBSON ASSEMBLY

psB1C3 with :
    1 + 10 fragment
    11 + 12 fragment
    13 + 3 fragment
    Control

- Put on the thermocycler at 50°C during 60 min

TRANSFORMATION

- Transformation in DH5α of :
    psB1C3 containing 3 fragments of 110 matrix (LB + Choramphenicol)

- Put plates at 37°C

08-19-16

NEW TRY OF TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing 10X GCN4 (LB + Choramphenicol)
    psB1C3 containing Dnmt3a

- Put on the incubator at 37°C

- No colony on plates containing 10X GCN4 cloning and Dnmt3a cloning (08-17-16)

08-22-16

- No colony on the plate containing the cloning of 110 matrix (08-18-16)

SET UP MINICULTURES

- Pick selected colony of agar plates and put it on 6mL of LB + Chloramphenicol
    psB1C3 containing 10X GNC4 (X2)
    psB1C3 containing Dnmat3a (X2)

- Put on the shaking incubator at 37°C

GIBSON ASSEMBLY (Dnmt3a3l)

psB1C3 with:
    21 + 4 fragment
    5 + 2 fragment
    Control

- Put on the thermocycler at 50°C during 60 min

GIBSON ASSEMBLY (Dnmt3l)

psB1C3 with:
    22 + 4 fragment
    5 + 2 fragment
    Control

- Put on the thermocycler at 50°C during 60 min

TRANSFORMATION

- Transformation in DH5α of :
    psB1C3 containing Dnmt3a3l (LB + Choramphenicol)
    psB1C3 containing Dnmt3l

- Put plates at 37°C

08-23-16

MINIPREP

- Miniprep of:
    psB1C3 10X GCN4 1
    psB1C3 10X GCN4 2
    psB1C3 Dnmt3a 1
    psB1C3 Dnmt3a 2

GEL MIGRATION

- Gel verification of miniprep tubes
    Fisrt, the plasmid is digested 1h at 37°C
    Agarose gel: 1%
    100 mV, 30 min

=> Nothing

08-24-16

- No colony on the plate containing the cloning of Dnmt3a3l (08-22-16)
- No colony on the plate containing the cloning of Dnmt3l (08-22-16)