Autor: Johanna W
Eintrag 1/4: mit YFP (D,E)
In Projekt: Schönas lvl1 GG
Keine Tags verwendet
12.09.2019

lvl 1 Golden Gate:

1. Parts on ice, buffer on ice
2. Prepared Mastermixes in PCR-tube (out of parts)
3. Added 1 µL T4 Lig buffer
4. Added 1 µL lvl 1 Ori part (UTEX bb) (c = 12.6 ng/µL)
6. Enzymes in ice
7. Added 1 µL of T4 ligase
8. Added 1 µL of Bsa1
9. Pipet-mixing
10. Maurice’s Golden Gate program in Mastercycler (Eppendorf) (GG Standard)

Mastermix D

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Mastermix E

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13.09.2019
**Transformation:**

1. 5 µL of the GG lvl1 (D, E) in top10
2. incubation on ice for 30 minutes
3. heatshock (43°C, 90 sec)
4. incubation in ice for 5 minutes
5. 500 µL of LB medium added
6. incubation (37°C, 2h)
7. centrifugation
8. remove supernatant
9. plate on agar plate with Spec
10. in incubator 37°C

**14.09.2019**

1. see plate pictures below
2. picked 3 white colonies from plate D and 2 green colonies from plate E for overnight cultures
3. in incubator 37°C, 190rpm

**15.09.2019**

DNA plasmid purification (Macherey-Nagel kit) of **D1, D2, D3, E1, E2**:

see protocol; warmed Elution buffer to 45°C before use

**16.09.2019**

Retransformation of **D1, D2, D3, E1, E2**:

1. 2 µL (50 ng/µL) in DH5
2. incubation on ice for 30 minutes
3. heatshock (43°C, 90 sec)
4. incubation in ice for 5 minutes
5. 500 µL of LB medium added
6. incubation (37°C, 2h)
7. plate 100 µL on agar plate with Spec
8. in incubator 37°C

18.09.2019
1. see plate pictures below
2. picked 1 white colony of each plate (D1, D2, D3, E1, E2) for overnight culture
3. in incubator 37°C, 190rpm

19.09.2019
Plasmid DNA purification (Macherey-Nagel kit) of D1, D2, D3, E1, E2:
see protocol; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

BsmbI digest of D1, D2, D3, E1, E2:
1. mixed components: 1.00 µL BsmbI + 5.00 µL NEBuffer + 38 µL water dest. = 44 µL in each tube
2. 1µg of DNA:
   6.00 µL of D1 added in one tube
   2.00 µL of D2 added in other tube
   4.00 µL of D3 added in other tube
   2.00 µL of E1 added in other tube
   6.00 µL of E2 added in last tube
3. 2h, 37°C in Mastercycler
4. Agarosegel with EtBr (120 V, 60 min), see picture below

24.09.2019

repetition of GG E --> E sub 2

lvl 1 Golden Gate:
see above

Mastermix E sub 2

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Transformation of E sub 2:
see above

26.09.2019

picked 3 green colonies from the plate for overnight cultures (see plate Transformation E-2)
27.09.2019

nothing grew in overnight cultures, so 3 white colonies (E₂4, E₂5, E₂6) were picked from the plate for overnight cultures

28.09.2019

Plasmid DNA purification (Macherey-Nagel kit) of E₂4, E₂5, E₂6:

see protocol; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

BsmBI digest of E₂4, E₂5, E₂6:

1. 2.70 µL of E₂4 (380 ng/µL) + 41.3 µL water added in one tube

2. 2.50 µL of E₂5 (404 ng/µL) + 41.5 µL water added in one tube

3. 2.40 µL of E₂4 (430 ng/µL) + 41.6 µL water added in one tube

2. added 5.00 µL of NEBuffer and 1.00 µL of Esp3I in each tube

3. 2h, 37°C in Mastercycler

4. Agarose gel with EtBr (70 V, 45 min), see picture below (test digest)
Projekt: Schönas lvl1 GG

Retrafo_E.jpg

Gel_GGiVL1_A,B,C,D,E.jpg
Transformation_E-2.jpg
test_digest.jpg
13.09.2019

lvl 1 Golden Gate:

1. Parts on ice, buffer on ice
2. Prepared Mastermixes in PCR-tube (out of parts)
3. Added 1 μLT4 Lig buffer
4. Added 1 μL lvl 1 Ori part (UTEX bb) (c = 12.6 ng/μL)
5. Enzymes in ice
6. Added 1 μL of T4 ligase
7. Added 1 μL of Bsa1
8. Pipet-mixing
9. Golden Gate program in Mastercycler (Eppendorf) (GG Standard)

Mastermix B

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Mastermix C
### 14.09.2019

**Transformation:**

1. 5 µL of the GG lvl1 (B, C) in top10
2. incubation on ice for 30 minutes
3. heatshock (42.5°C, 90 sec)
4. incubation in ice for 5 minutes
5. 500 µL of LB medium added
6. incubation (37°C, 2h)
7. centrifugation
8. remove supernatant
9. plate on agar plate with Spec
10. in incubator 37°C

### 15.09.2019

picked 3 white colonies of each plate for overnight cultures

### 16.09.2019
DNA plasmid purification (Macherey-Nagel kit) of B1, B2, B3, C1, C2, C3:

see protocol; warmed Elution buffer to 80°C before use and used 30µL

19.09.2019

BsmbI digest of B1, B2, B3, C1, C2, C3:

1. mixed components: 1.00 µL BsmbI + 5.00 µLNEBuffer + 38 µL water dest. = 44 µL in each tube

2. 1µg of DNA:
   2.00 µL of B1 added in one tube
   2.00 µL of B2 added in other tube
   2.00 µL of B3 added in other tube
   6.00 µL of C1 added in other tube
   3.00 µL of C2 added in other tube
   6.00 µL of C3 added in last tube

3. 2h, 37°C in Mastercycler

4. Agarosegel with EtBr (120 V, 60 min), see picture below

24.09.2019

repetition of C2

lvl 1 Golden Gate:

see above

Mastermix C2,a
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Mastermix $C_{2b}$

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<th>V (part) /µL</th>
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<tr>
<td>6_17</td>
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Transformation of $C_{2a}$ and $C_{2b}$:

see above

26.09.2019

picked 3 white colonies of each plate for overnight cultures (see plate Transformation C-2a, C-2b)

27.09.2019

DNA plasmid purification (Macherey-Nagel kit) of $C_{2a1}, C_{2a2}, C_{2a3}, C_{2b1}, C_{2b2}$:

see protocol; warmed Elution buffer to 80°C before use and used 30 µL
$C_{2a2}$ was only 17 ng/µL, so a retransformation was done with 2 µL in DH5, see transformation above just no centrifugation, 100 µL were plated

**BsmBl digest of $C_{2a1}$, $C_{2a3}$, $C_{2b1}$, $C_{2b2}$:**

1. **mixed components:**
   - $C_{2a1}$: 3.90 µL of C2a1 (258 ng/µL) + 40.1 µL of water added in one tube
   - $C_{2a3}$: 3.20 µL of C2a3 (321 ng/µL) + 40.8 µL of water added in other tube
   - $C_{2b1}$: 25.0 µL of C2b1 (37 ng/µL) + 19.0 µL of water added in other tube
   - $C_{2b2}$: 4.50 µL of C2b2 (227 ng/µL) + 39.5 µL of water added in other tube

2. added 5.00 µL of NEBuffer and 1.00 µL of BsmBl in each tube

3. 2h, 37°C in Mastercycler

4. Agarosegel with EtBr (70 V, 45 min), see picture below

   digest $C_{2a1}, C_{2a3}, C_{2b1}, C_{2b2}$ or test digest

--> $C_{2b1}$ is looking good, need sequencing

**28.09.2019**

Retransformation of $C_{2b1}$

2 µL of $C_{2b1}$ in DH5, see transformation above just no centrifugation, 100 µL were plated

**29.09.2019**

C2a2: three white colonies and two green ones were picked for overnight cultures

C2b1: four white colonies were picked for overnight cultures

**30.09.2019**
DNA plasmid purification (Macherey-Nagel kit) of $C_{a2-w1}$, $C_{a2-w2}$, $C_{a2-w3}$, $C_{a2-G1}$, $C_{a2-G2}$, $C_{b1-w1}$, $C_{b1-w2}$, $C_{b1-w3}$, $C_{b1-w4}$:

see protocol; warmed Elution buffer to 80°C before use and used 30 µL

sent $C_{b1-w1}$ to sequencing

**BsmbI digest of $C_{a2-w1}$, $C_{a2-w2}$, $C_{a2-w3}$, $C_{a2-G1}$, $C_{a2-G2}$:**

1. mixed components:
   
   $C_{a2-w1}$: 3.80 µL of $C_{a2-w1}$ (264 ng/µL) + 40.2 µL of water added in one tube
   
   $C_{a2-w2}$: 6.10 µL of $C_{a2-w2}$ (164 ng/µL) + 37.9 µL of water added in other tube
   
   $C_{a2-w3}$: 10.5 µL of $C_{a2-w3}$ (96 ng/µL) + 33.5 µL of water added in other tube
   
   $C_{a2-G1}$: 2.50 µL of $C_{a2-G1}$ (410 ng/µL) + 41.5 µL of water added in other tube
   
   $C_{a2-G2}$: 3.90 µL of $C_{a2-G2}$ (259 ng/µL) + 40.1 µL of water added in other tube

2. added 5.00 µL of NEBuffer and 1.00 µL of BsmbI in each tube

3. 2h, 37°C in Mastercycler

4. Agarosegel with EtBr (110 V, 30 min; then 100 V, 15 min), see picture below ($C_{2a2-W1_W2_W3_G1_G2}$)

sent $C_{a2-w1}$ to sequencing
Transformation_C-2a__C-2b.jpg
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<th>Autor: Maurice Mager</th>
<th>erstellt: 17.09.2019 11:58</th>
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12.10.2019

Ivl 1 Golden Gate:

1. Parts on ice, buffer on ice
2. Prepared Mastermixes in PCR-tube (out of parts)
3. Added 1 µL lvl 1 Ori part (amp col E 1) (c = 10.4 ng/µL)
4. Added 1 µL T4 Lig buffer
5. Enzymes in ice
6. Added 1 µL of T4 ligase
7. Added 1 µL of Bsa1
8. Pipet-mixing
9. Golden Gate program in Mastercycler (Eppendorf) (GG Standard)

### Mastermix F

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### Mastermix G
### Projekt: Schönas lvl1 GG

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**Mastermix H**

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**13.10.2019**

**Transformation:**

1. 5 µL of the GG lvl1 in top10
2. incubation on ice for 30 minutes
3. heatshock (42°C, 90 sec)
4. incubation in ice for 5 minutes
5. 500 µL of LB medium added
6. incubation (37°C, 1h)
7. centrifugation
8. remove supernatant
9. plate on agar plate with Amp
10. in incubator 37°C

lvl 1 Golden Gate:

1. Parts on ice, buffer on ice
2. Prepared Mastermixes in PCR-tube (out of parts)
3. Added 1 µL lvl 1 Ori part (amp col E 1) (c = 10.4 ng/µL)
4. Added 1 µL T4 Lig buffer
6. Enzymes in ice
7. Added 1 µL of T4 ligase
8. Added 1 µL of Bsa1
9. Pipet-mixing
10. Golden Gate program in Mastercycler (Eppendorf) (GG Standard)

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14.10.2019

1. picked 3 colonies from each plate for overnight cultures

$F_{\text{new}}$: 2 white colonies
G: G1 green
    G2 white
    G3 white

H: 3 white colonies

2. in incubator 37°C, 210rpm

15.10.2019

Plasmid DNA purification (Macherey-Nagel kit):
see protocol; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

Retrafo of H1, H3, F_{new}³ (see Transformation above, just used 3 µL in DH5)

BsmbI digest of H2, G1, G2, G3, F_{new}¹, F_{new}²:

1. mixed components:
   - H2: 15.0 µL of H2: (67 ng/µL) + 29.0 µL of water added in one tube
   - G1: 6.90 µL of G1 (146 ng/µL) + 37.1 µL of water added in other tube
   - G2: 4.80 µL of G2 (208 ng/µL) + 39.1 µL of water added in other tube
   - G3: 3.00 µL of G3 (340 ng/µL) + 41.0 µL of water added in other tube
   - F_{new}¹: 7.00 µL of F_{new}¹ (142 ng/µL) + 37.0 µL of water added in other tube
   - F_{new}²: 21.7 µL of F_{new}² (46 ng/µL) + 22.3 µL of water added in other tube

2. added 5.00 µL of NEBuffer and 1.00 µL of BsmbI in each tube

3. 2h, 37°C in Mastercycler

4. Agarosegel with EtBr (100 V, 45 min, then 110 V, 15 min), see picture below
16.10.2019

1. picked colonies from retrafo plates H2, G1, G2, G3, Fnew1, Fnew2 for overnight cultures

2. in incubator 37°C, 200 rpm

17.10.2019

Plasmid DNA purification (Macherey-Nagel kit):

see protocoll; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

-> not good concentrations

18.10.2019

Transformation:

1. 5 µL of the 2nd half of the GG lvl1 in DH5α

2. incubation on ice for 30 minutes

3. heatshock (42°C, 90 sec)

4. incubation in ice for 5 minutes

5. 500 µL of LB medium added

6. incubation (37°C, 1h)

7. centrifugation

8. remove supernatant

9. plate on agar plate with Amp

10. in incubator 37°C

19.10.2019

1. picked 3 colonies of each plate for overnight cultures
2. in incubator 37°C, 200 rpm

20.10.2019

Plasmid DNA purification (Macherey-Nagel kit):

see protocol; warmed Elution buffer to 80°C and just added 30 µL Elution buffer

BsmBI digest of 2. H2:

1. mixed components:
   2. H2 5.80 µL of 2. H2 + 38.2 µL of water added in one tube

2. added 5.00 µL of CutSmartBuffer and 1.00 µL of BsmBI in each tube

3. 2h, 37°C in Mastercycler

4. Agarose gel with EtBr (70 V, 45 min)

--> looks like an uncomplete digest