

## PCR: NAT1 deletion cassette (ClonNAT)

### Mix

Stock for 6 reactions à 100 µl (for 1 deletion):

|                          |  |
|--------------------------|--|
| 209 µl                   | H <sub>2</sub> O                         |
| 120 µl                   | 5x PCR Buffer (Roche: GC rich kit)       |
| 60 µl                    | Resolution solution (Roche: GC rich kit) |
| 60 µl                    | dNTPs (Stock: 2 mM)                      |
| 25 µl                    | MgCl <sub>2</sub>                        |
| 6 µl (more if low conc.) | pUC19NATPS template (100 – 300 ng)       |
| 30 µl                    | Primer NS1 (Stock: 10 µM = 10 pmol/µl)   |
| 30 µl                    | Primer NS2                               |

Aliquot 90 µl to 6 PCR tubes, heat to 95 °C and add 10 µl polymerase mix  
Polymerase Mix: **3.5 µl Poly** (Roche: GC rich kit) + **66.5 µl H<sub>2</sub>O**

### Cycle Parameters

|    |              |                          |
|----|--------------|--------------------------|
| 1. | 95 °C        | 5' 00"                   |
|    |              | Add 10 µl polymerase mix |
| 2. | <b>95 °C</b> | <b>0' 30"</b>            |
| 3. | <b>53 °C</b> | <b>0' 45"</b>            |
| 4. | <b>72 °C</b> | <b>2' 35"</b>            |
|    | GOTO 2.      | REP 30 x                 |
| 5. | 72 °C        | 30' 00"                  |
| 6. | 4 °C         | ∞                        |

Collect the 6 reactions, precipitate, redissolve in 75 µl H<sub>2</sub>O and transform 200 µl mycelium.

Complications: The buffers of the GC rich kit lead to high salt in the DNA pellet, which would reduce efficiency of electroporation.

⇒ use 50 % EtOH for the washing steps of DNA precipitation.

⇒ redissolve in 75 µl instead of 50 µl H<sub>2</sub>O