PCR: NAT1 deletion cassette (ClonNAT)

Mix

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

209 µl	H_2O
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	$MgCl_2$
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_2O**

Cycle Parameters

1.	95 °C	5’ 00”
		Add 10 µl polymerase mix
2.	**95 °C**	**0’ 30”**
3.	**53 °C**	**0’ 45”**
4.	**72 °C**	**2’ 35”**
	GOTO 2.	REP 30 x
5.	72 °C	30’ 00”
6.	4 °C	∞

Collect the 6 reactions, precipitate, redissolve in 75 µl H_2O 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_2O