

PCR: GEN3 deletion cassette (G418)

Mix

Stock for 6 reactions of 100 μ l (for 1 deletion):

357 μ l	H ₂ O
60 μ l	Thermo-Pol Buffer (Vent)
60 μ l	dNTPs (Stock: 2 mM)
3 μ l (6 μ l if low conc.)	pGEN3 template (100 – 300 ng)
30 μ l	Primer S1 (Stock: 10 μ M = 10 pmol/ μ l)
30 μ l	Primer S2

Aliquot 90 μ l to 6 PCR tubes, heat to 95 °C and add 10 μ l polymerase mix

Polymerase Mix: **1.9 μ l Vent** (NEB) + **3.7 μ l Taq** (Roche/Amersham) + **64.4 μ l H₂O**

Cycle Parameters

1.	95 °C	5' 00"
		Add 10 μ l polymerase mix
2.	95 °C	0' 45"
3.	50 °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 50 μ l H₂O and transform 150 μ l mycelium.