Quickchange site directed mutagenesis protocol

revised for ITR mutagenesis: 10.06.2010

Used vector: **P34.3** **(**pAAV_MCS_clone3) conc: 408 ng/µl we did a 1:20 delution, and used 0.5µl (so 10 ng)

PCR reaction:

		recommended
2.5 µL	10x Pfu Ultra II buffer	
0.5 µL	template (~10 ng) → 1:20 dilution!	2.5-25 ng
0.56 µL 0.61 µL	forward primer leftITR 1:10 forward primer rightITR 1:10	62.5 ng
0.56 µL 0.61 µL	reverse primer leftITR 1:10 reverse primer rightITR 1:10	62.5 ng
0.5 µL	DMSO	optional, 2-4 % for primers forming strong 2nd ry structures
0.5 µL	dNTP	250 µM each dNTP (1 mM total)
19.38 µL 19.28 µL	dH2O for leftITR dH2O for rightITR	ad 24.5 µL
0.5 µL	PfuUltra II fusion (1.25 U)	

Different amounts due to primer length (leftITR=34 bp, rightITR=31 bp)

If DMSO is not added, add equivalent extra amount of dH_2O

PCR program:

1	95 °C	2' (HotStart polymerase) (→ in PCR box!)
20	95 °C	30 s
	66 °C	1'
	68 °C	5' (4.645 kb) normal: 72 °C, leads to strand displacement

Increased to avoid priming in other ITR

- incubate the PCR sample on ice for 2'
- add 0.5 µL DpnI (10 U) and mix it gently by stirring with pipette tip
- incubate mix at 37 °C for 1 h
- place on ice and continue with transformation, use XL1 blue cells (standard for cloning)