MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	340
MMRRC Strain Name	B6.Cg-*Tcrd*^{tm1Mom} Tg(UBC-GFP)30Scha/Mmnc
Gene Name(s)	T-cell receptor delta chain(Tcrd), Ubiquitin C (UBC Human), GFP
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	9/3/13

Genotyping for this strain includes Neo and EGFP PCR reactions

Neo PCR for strain #340

	1X
ddH_2O	13
5X Buffer	5.0
25 mM $MgCl_2$	2
10 mM dNTPs	0.5
10 µM Primer Forward	1.0
10 µM Primer Reverse	1.0
Taq	0.5
DNA	2

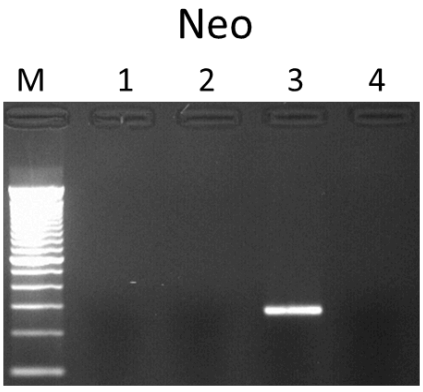
Thermal Cycler:
Step 1: 94^{0}C for 5 min
Step 2: 94^{0}C for 1 min
Step 3: 61^{0}C for 1 min
Step 4: 72^{0}C for 1 min,
Step 5: Step 2 to 4; Cycles: 35
Step 6: 72^{o}C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 µM with respect to each primer.
Neo1: CTT TTT GTC AAG ACC GAC CTG TCC G
Neo2: CTC GAT GCG ATG TTT CGC TTG GTG

Bands expected: Neo$^+$ 290bp

Run on 1.0% agarose gel in TAE.



Lane 1, 2: WT; Lane 3: Neo+; Lane 4: H_2O;
M: 100 bp DNA ladder (Invitrogen)

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www.med.unc.edu/mmrrc www.mmrrc.org

EGFP PCR for the strain # 340

	1X
ddH_2O	13.8
5X Buffer	5.0
25mM $MgCl_2$	2
10mM dNTPs	0.5
50 µM each EGFP F/R	0.4
50 µM each Actin F/R	0.8
Taq	0.5
DNA	2

Thermal Cycler:
Step 1: 95°C for 5 min
Step 2: 95°C for 45 sec
Step 3: 60°C for 45 sec
Step 4: 72°C for 1 min
Step 5: 30x from step 2 to step 4
Step 6: 72°C for 10 min

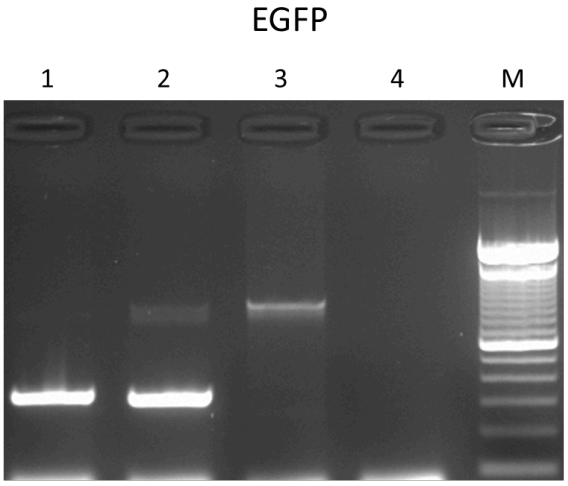
Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 50 µM with respect to each primer
EGFP F: CCT ACG GCG TGC AGT GCT TCA GC
EGFP R: CGG CGA GCT GCA CGC TGC GTC CTC
Actin PCR control/forward primer: GAT GAC GAT ATC GCT GCG CTG GTC G
Actin PCR control/reverse primer: GCC TGT GGT ACG ACC AGA GGC ATA CAG

Bands expected: Actin ~1kb (DNA control)
EGFP ~300bp

Run on 1% agarose gel.

Note: 1 kb band of Actin was not shown in some reactions.



Lane 1, 2: EGFP+; Lane 3: WT; Lane 4: H_2O; M: 100 bp DNA ladder (Invitrogen)

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