NET MICE GENOTYPING PROTOCOL

NET mouse lines:

NET KO: genotyped in house, only breed HETs
NET Floxed: genotyped in house, only breed floxed, genotype every other generation
Phox-2B-CRE/NET Floxed (P2BC/NF): genotyped in house, breed CRE (-) females and CRE (+) males, genotype for NET Floxed every other generation

DNA EXTRACTION (applies for all lines):

*All buffer from Sigma REDExtract-N-Amp tissue PCR kit

50 uL of extraction solution per tail
12.5 uL of tissue prep per tail
Set for 10 mins at room temperature
Boil at 95C for 3 mins
Add 50 uL of neutralization buffer per tail

PHOX2B-CRE Master Mix:

6 uL PCR water (ultrapure water, aliquot in hood)
10 uL red extract (from kit above, green kappa also works well)
1 uL primer MKH 38R
1 uL primer MKH 39F
1 uL primer MKH 40R

19 uL of master mix + 1 uL of DNA

Thermocycler settings:

94 degrees: 3:00 min
94 degrees: 30s
54 degrees: 1 min
72 degrees: 1 min
72 degrees: 2 min
*Repeat steps 2-4 35x

CRE (+) = HET [300 and 600 bp]
CRE (-) = WT [300 bp]

NET Floxed Master Mix:

7 uL PCR water
10 uL red extract (or green kappa)
1 uL primer MKH 28F
1 uL primer MKH 29R

19 uL of master mix + 2 uL of DNA

Thermocycler settings:

99 degrees: 2:00 min

95 degrees: 30s
60 degrees: 1 min
72 degrees: 1 min

*Repeat steps 2-4 30x

NET Floxed= 503 bp

NET KO Master Mix:

7 uL PCR water
10 uL red extract (or green kappa)
1 uL primer NET 1
1 uL primer NET 2
1 uL primer NET 3

19 uL of master mix + 2 uL of DNA

Thermocycler settings:
95 degrees: 5 min
95 degrees: 30s
56 degrees: 1s
68 degrees: 2 min
72 degrees: 7 min
*Repeat steps 2-4 30x

400 bp= KO
800 bp= WT

GEL:

1.5% agarose gel
KB ladder

PRIMER STOCKS:

Kept at 200 uM and then diluted and aliquoted to 5 uM
Keep working stock (5 uM) at 4C, found the primers work better when reducing freeze/thaw cycles

OTHER TIPS:

PCR worked best when the DNA was extracted on the same day as tagging and tailing
For trouble shooting bad PCRs, try freshly made primers, and even stripping the tails