

Ninja 2-band genotyping PCR:

WT sequence:

CCCTCTTCCCTCGTGATCTGCAACTCCAGTCTTTCTAGAAGATGGGCGGGAGTCTTCTG  
GGCAGGCTTAAAGGCTAACCTGGTGTGTGGGCGTTGTCCTGCAGGGGAATTGAACAGGT  
GTAAAATTGGAGGGACAAGACTTCCCACAGATTTTCGGTTTTGTCGGGAAGTTTTTTAA  
TAGGGGCAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGGTTTTATGCAGCAAAA  
CTACAGGTATTATTGCTTGTGATCCGCCTCGGAGTATTTTCCATCGAGGTAGATTAAA  
GACATGCTCACCCGAGTTTTATACTCTCCTGCTTGAGATCCTTACTACAGTATGAAATT  
ACAGTGTCGCGAGTTAGACTATGT

WT band: 378bp

Ninja band: 280 bp

Ninja band after Cre: 694 bp

New Rosa26 WT F	CCCTCTTCCCTCGTGATCTG
New Rosa26 WT R	ACATAGTCTAACTCGCGACTG
New Rosa26 Ninja R	GTCGAGGTGCCCGAAGGAC
New NinjaAftrCre R	GAATCTGCAGGGAGAGGAGTT

Ninja sequence (unmodified)

CCCTCTTCCCTCGTGATCTGCAACTCCAGTCTTTCTCCCGGTGCTAGCTACTCTCGAGG  
TCGACGGTATCGATAAGCTTGATATCGAATTCCCTGCAGCCCGGATAACTTCGTATAGT  
ACACATTATACGAAGTTATGGCTAAGCTTTGTGCCATGCCATTGATGCTAGGAACAAAC  
GACCCAACACCCGTGCGTTTTATTCTGTCTTTTTATTGCCACGCGTTCAGGCACCGGGC  
TTGCGGGTCATGCACCAGGTGCGCGGTCCTTCGGGCACCTCGACGT

Ninja sequence (after Cre)

CCCTCTTCCCTCGTGATCTGCAACTCCAGTCTTTCTCCCGGTGCTAGCTACTCTCGAGG  
TCGACGGTATCGATAAGCTTGATATCGAATTCCCTGCAGCCCGGATAACTTCGTATAGT  
ACACATTATACGAAGTTATGTTTACTACTACATGTTACTGGCGCGCCGAACAAACGACC  
CAACACCCGTGCGTTTTATTCTGTCTTTTTATTGCCACGCGTTCAAGCTGTGGCAGGGA  
AACCCTCTGCCTCCCCGTGATGTAATACTTTTGCAAGGAATGCGATGAAGTAGAGCC  
GCAGTGGCCAAGTGGCTTTGGTCCGTCTCCTCCACGGATGCCCTCCACGGCTAGTGGG  
CGCATGTAGGCGGTGGGCGTCCGCCGCTCCAGCAGCAGGTCATAGAGGGGCACCACGT  
TCTTGCACTTCATGCTGTACAGATGCTCCATGCCTTTGTTACTCATGTGCCTGATGTGG  
GAGAGGATGAGGAGGAGCTGGGCCAGCCGCTGGTGCTGCTGCTGCAGGGTCAGGCCTGC  
CTTGCCATCAGGTGGATCAAAGTGTCTGTGATCTTGTCCAGGACTCGGTGGATATGGT  
CCTTCTCTTCCAGAGACTTCAGGGTGCTGGACAGAAATGTGTACTCCAGAATTAAGC  
AAAATAATAGATTTGAGGCACACAACTCCTCTCCCTGCAGATTC

Ninja (GFP) sequence (pre-flp)

CTAGGTGCAAAATCCCCAACGACCTGAAACAGAAAGTTATGAACCACAAAGGTGAGTAC  
TAAGTACCGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGACCGGGAAGTTCC  
TATCCGAAGTTCCTATTCTTCAAATAGTATAGGAACTTCGAATTCTGCAGATATCCAG  
CACAGTGGCGGCCGCTAGCATCGTTACGCGTGAAGTTCCTATTCCGAAGTTCCTATTCT  
CTAGAAAGTATAGGAACTTCGGATCCGTACTIONCACCTTGTAATGTCAGGGCCGTTCAA  
CCCTTGTCATCGTCGTCCTTGTAAGTACGATCCCGCATGTTGCAAAGTTGTACACAGCTGG  
GGAAAAAAGGGACAGGATAAGTATGACATCATCAAGGAAACCCTGGACTACTGCGCC  
CTGGGCCGAAGTTCCTATACTATTTGAAGAATAGGAACTTCGGAATAGGAACTTCAAG  
CTTGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAA  
CCATTATAAGCTGCAATAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAG  
GTTTCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAT  
GGCTGATTATAAGCTTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGA  
ACTTCGGCGCGCCGACAGCAACTGCATGGGGGCTGGGATGGGCATAAGAATAAATGTC  
TATGTGGACAGCCTTAACCACTTCAGCCATGACCTCTATGTATGTTTCTAACCCACAG  
GGAGTTTATCAGTTTAAATCCGTTGAGTTTGACGCAGCCGCAGACCTGTCTATCTGTGC  
AAGATCC

Ninja (GFP) sequence (after-flp)

CTAGGTGCAAAATCCCCAACGACCTGAAACAGAAAGTTATGAACCACAAAGGTGAGTAC  
TAAGTACCGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGACCGGGAAGTTCC  
TATCCGAAGTTCCTATTCTTCAAATAGTATAGGAACTTCGGGCCAGGGCGCAGTAGT  
CCAGGGTTCCTTGATGATGTCATACTTATCCTGTCCCTTTTTTTTTCCCCAGCTGTGTA  
CAACTTTGCAACATGCGGGATCGACTACAAGGACGACGATGACAAGGGTTTGAACGGCC  
CTGACATTTACAAGGTGAGTACGGATCCGAAGTTCCTATACTTTCTAGAGAATAGGAACT  
TTCGGAATAGGAACTTCGGCGCGCCGACAGCAACTGCATGGGGGCTGGGATGGGCATA  
AGAATAAATGTCTATGTGGACAGCCTTAACCACTTCAGCCATGACCTCTATGTATGTTT  
CTAACCCACAGGGAGTTTATCAGTTTAAATCCGTTGAGTTTGACGCAGCCGCAGACCT  
GTCTATCTGTCTGAAGATCC

NINJA pre-FLP: 784 bp  
NINJA post-FLP: 442 bp

Ninja GFP DNA detect F AAATCCCCAACGACCTGAAAC  
Ninja GFP DNA detect R CTGAAGTGGTTAAGGCTGTCCA

PCR for NINJA genotyping:

New Rosa26 WT F        CCCTCTTCCCTCGTGATCTG  
New Rosa26 WT R        ACATAGTCTAACTCGCGACACTG  
New Rosa26 Ninja R     GTCGAGGTGCCCCGAAGGAC

WT band: 378bp  
Ninja band: 280 bp

Kapa Taq protocol:  
KAPA master mix  
95°C 3:00  
(95°C 0:15  
60°C 0:15  
72°C 0:15) x 35 cycles  
72°C 1:00  
12°C hold

PCR for typing recombination of regulatory module (Ninja(C)  
or after Cre):

New Rosa26 WT F        CCCTCTTCCCTCGTGATCTG  
New Rosa26 Ninja R     GTCGAGGTGCCCCGAAGGAC  
New NinjaAftrCre R     GAATCTGCAGGGAGAGGAGTT

Rosa 26 WT F  
New Rosa26 Ninja R  
R26 Ninja after Cre R

Ninja band: 280 bp  
Ninja after Cre: 694bp

Kapa Taq protocol:  
KAPA master mix  
94°C 3:00  
(94°C 0:30  
58°C 0:30  
72°C 1:30  
72°C 5:00) x 35 cycles  
12°C hold

PCR for typing recombination of neoantigen module:

Ninja GFP DNA detect F    AAATCCCCAACGACCTGAAAC  
Ninja GFP DNA detect R    CTGAAGTGTTAAGGCTGTCCA

Ninja (F) or after Flp:

- 1) Ninja GFP F
- 2) Ninja GFP R

Ninja pre-Flp: 784 bp

Ninja post-Flp: 442 bp

Kapa Taq protocol:

KAPA master mix

94°C 3:00

(94°C 0:30

58°C 0:30

72°C 1:30

72°C 5:00) x 35 cycles

12°C hold