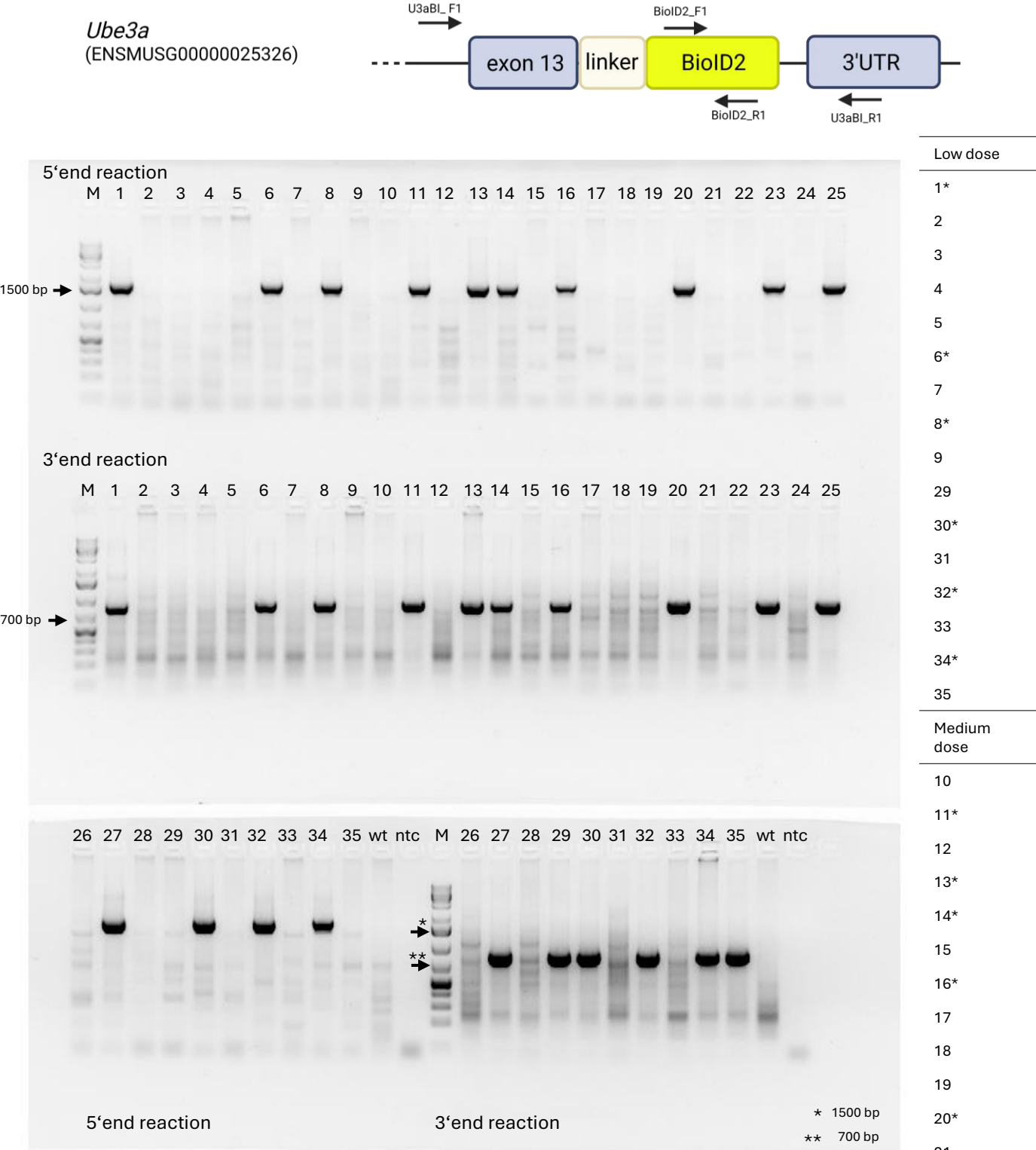


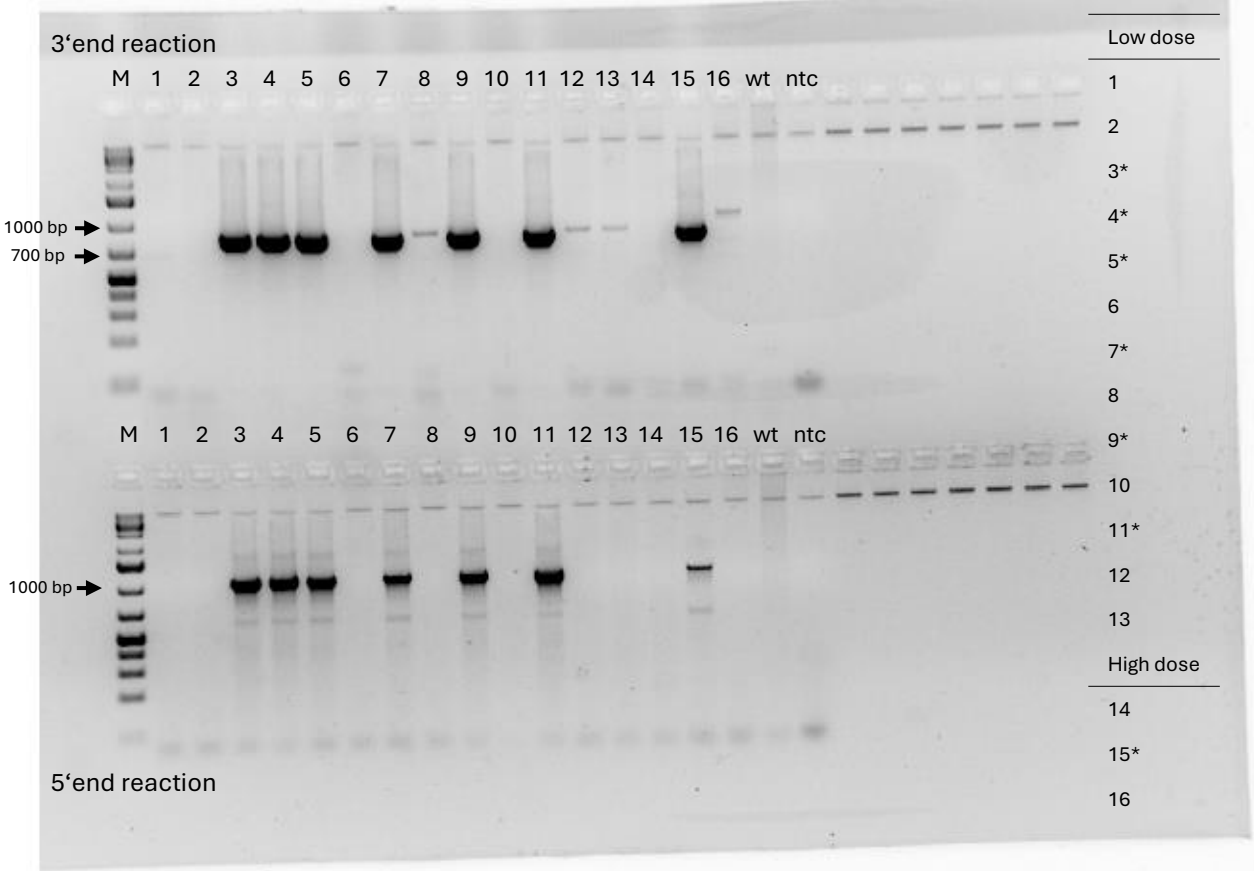
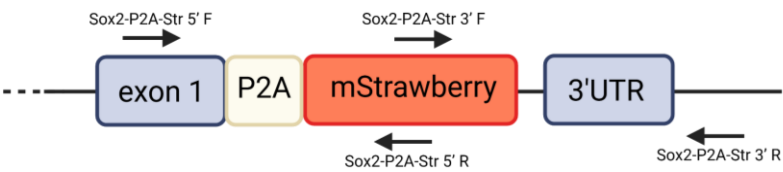
A) C57BL/6NCrI-Ube3a^{em2}(BioID2)Ccpcz



5'reaction	Primers sequence (5'>3')	Product size (bp)
U3aBI_F1	CCATATTCCATACACGCAAGCAG	1672
BioID2_R1	TCGCCAGGTACAGCATC	
3'reaction	Primers sequence (5'>3')	Product size (bp)
U3aBI_R1	CCAATGAAGAAGGGAGGCAC	863
BioID2_F1	ACCCAGGAGAGACTGAAGG	

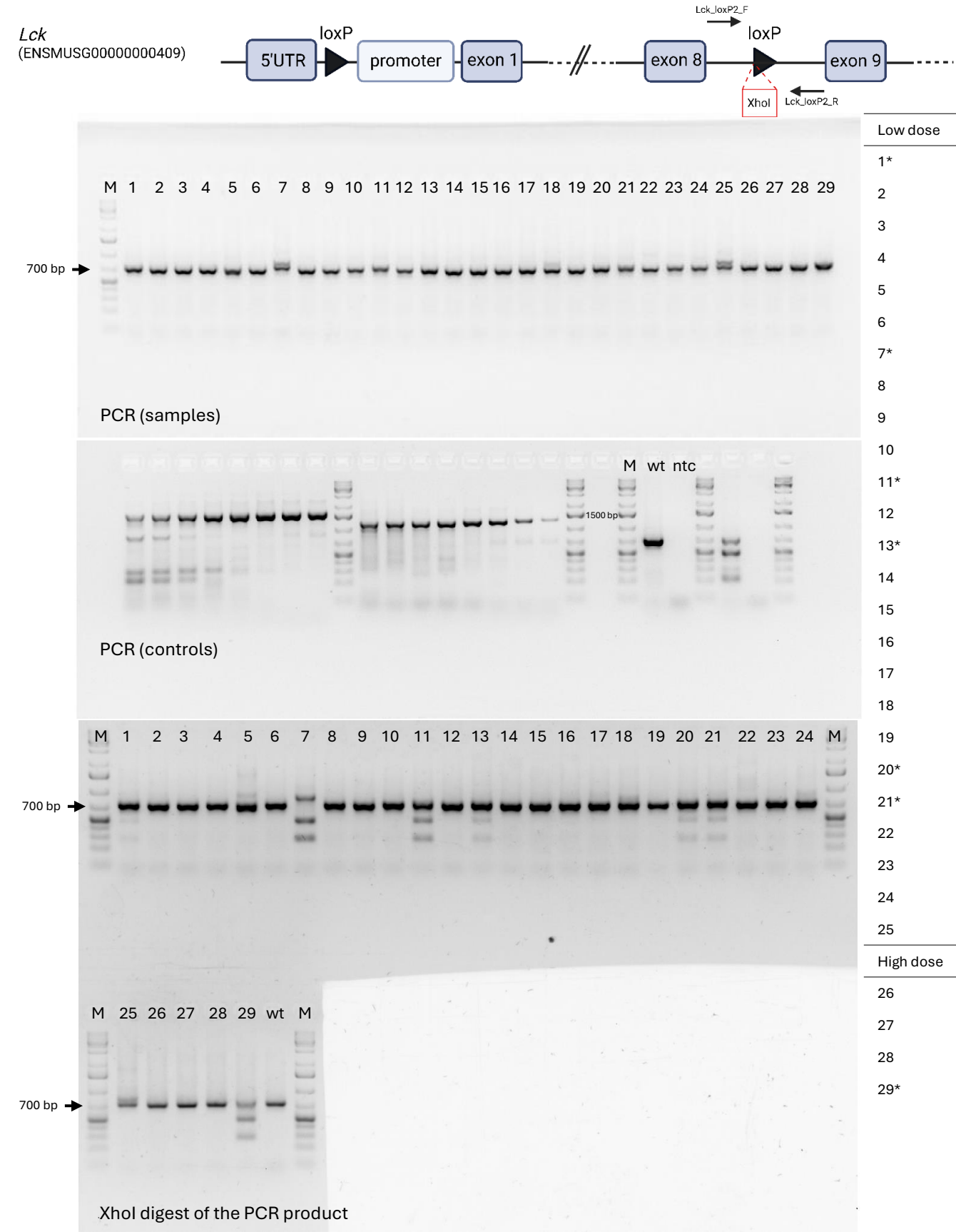
B) C57BL/6NCrI-Sox2^{em1}(P2A-mStrawberry)Ccpcz

Sox2
(ENSMUSG00000074637)



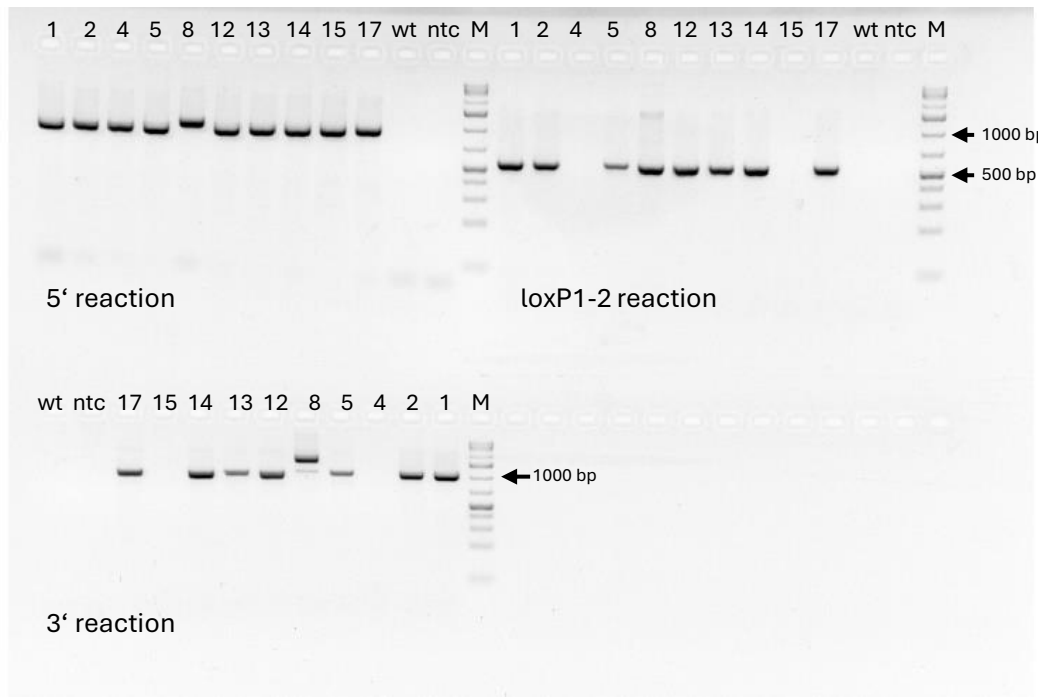
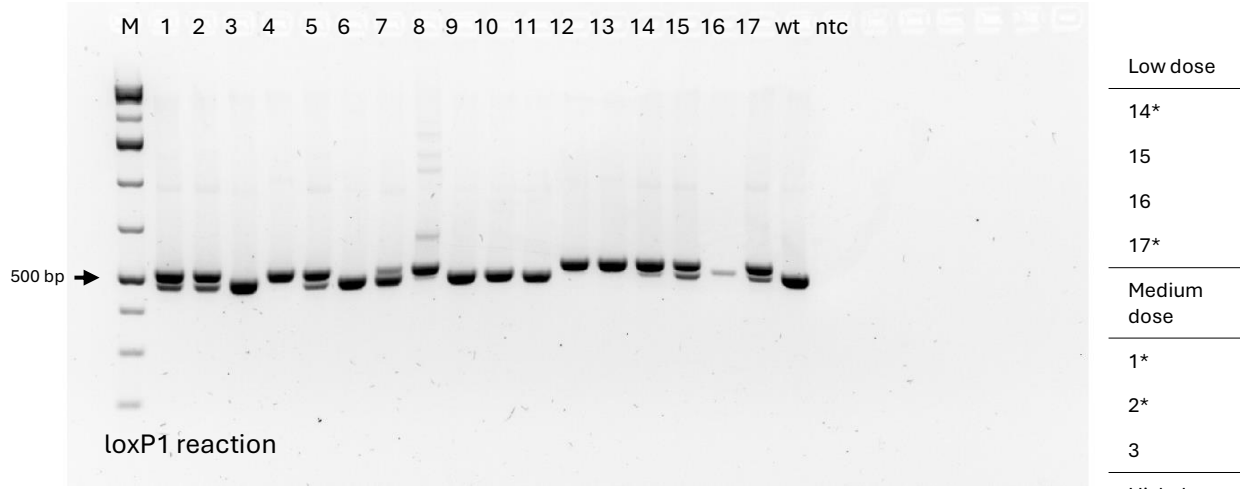
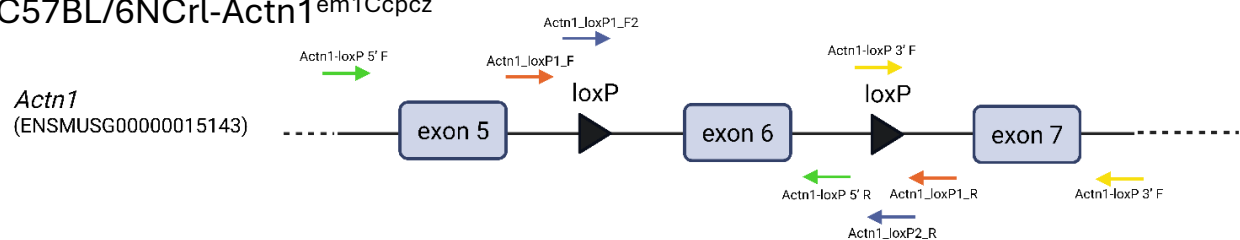
3'reaction	Primers sequence (5'>3')	Product size (bp)
Sox2-P2A-Str 3' F	GCAGAAGAAGACCATGGGCT	836
Sox2-P2A-Str 3' R	CCCAGCAAGAACCCTTTCCT	
5'reaction	Primers sequence (5'>3')	Product size (bp)
Sox2-P2A-Str 5' F	TAAGTACACGCTTCCCGGAG	1088
Sox2-P2A-Str 5' R	AGCCCATGGTCTTCTTCTGC	

C) C57BL/6NCrI-Lck^{em2Ccpcz} (loxP insertion)



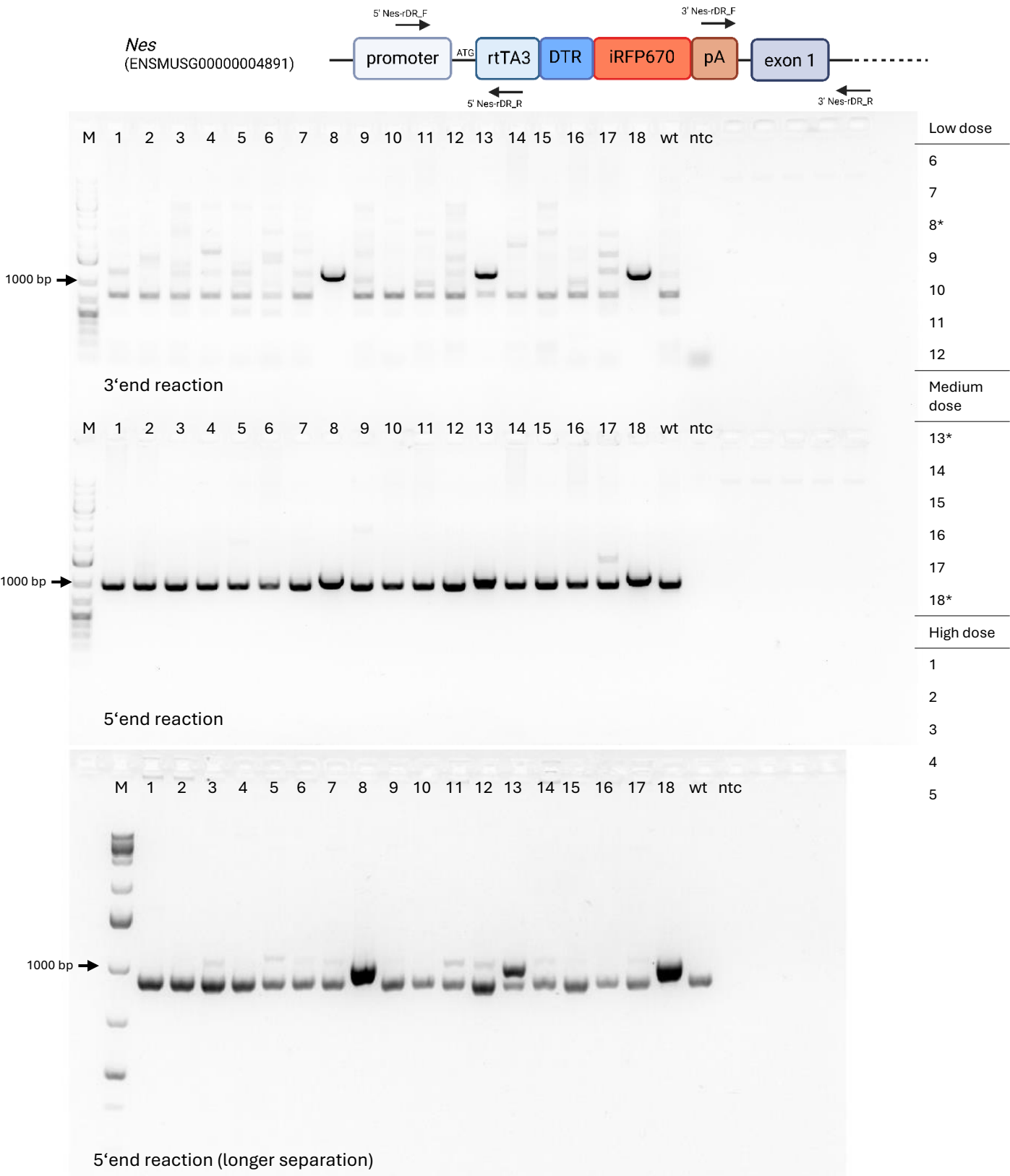
PCR reaction	Primers sequence (5'>3')	Product size (bp)
Lck_loxP2_F	TTGCTGACAAGCCTGATGAGC	713 (wt), 753 (+loxP)
Lck_loxP2_R	TGGGAACATCCCTAGGTCACAA	
XhoI digestion	WT product – no digestion, Lck cKO loxP2 – 274 + 479 bp fragments	

D) C57BL/6NCrI-Actn1^{em1Ccpcz}



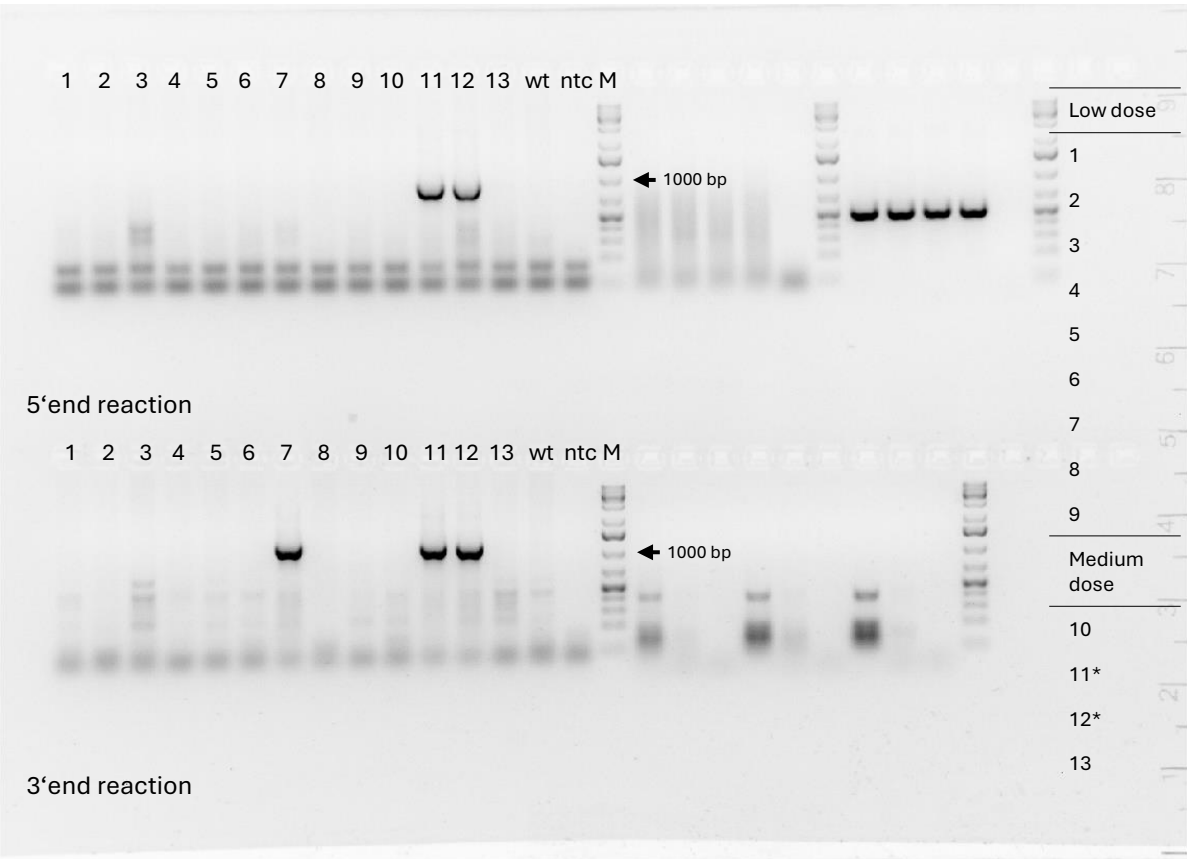
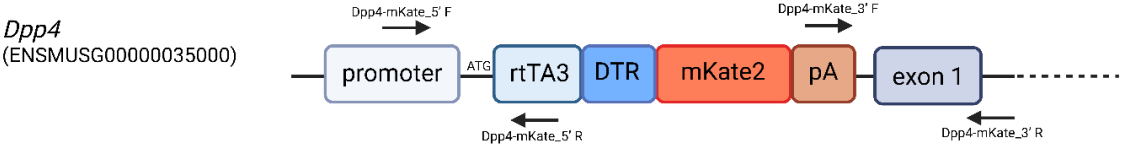
loxP1 reaction	Primers sequence (5'>3')	Product size (bp)
<u>Actn1_loxP1_F</u>	ACACTGTCAAGACCCCAAG	420 (wt)/524 (loxP1)
<u>Actn1_loxP1_R</u>	TGTAGCTGGTCCATAAGGCCAA	
5' reaction	Primers sequence (5'>3')	Product size (bp)
<u>Actn1-loxP 5' F</u>	AGTTCACAGATCTGAACACACG	874
<u>Actn1-loxP 5' R</u>	ATTGAGCAAGAGAGGACAGC	
3' reaction	Primers sequence (5'>3')	Product size (bp)
<u>Actn1-loxP 3' F</u>	TGTTACCCAGCGATAACTTCG	1060
<u>Actn1-loxP 3' F</u>	ACATATGAGACAGTCTGTGAC	
loxP1-2 reaction	Primers sequence (5'>3')	Product size (bp)
<u>Actn1_loxP1_F2</u>	GCTTTAGTGAACGATAACTTCG	559
<u>Actn1_loxP2_R</u>	CGGGATGTGCAATAACTTCGT	

E) C57BL/6NCrl-Nes^{em1}(rtTA3,DTR,iRFP670)Ccpcz



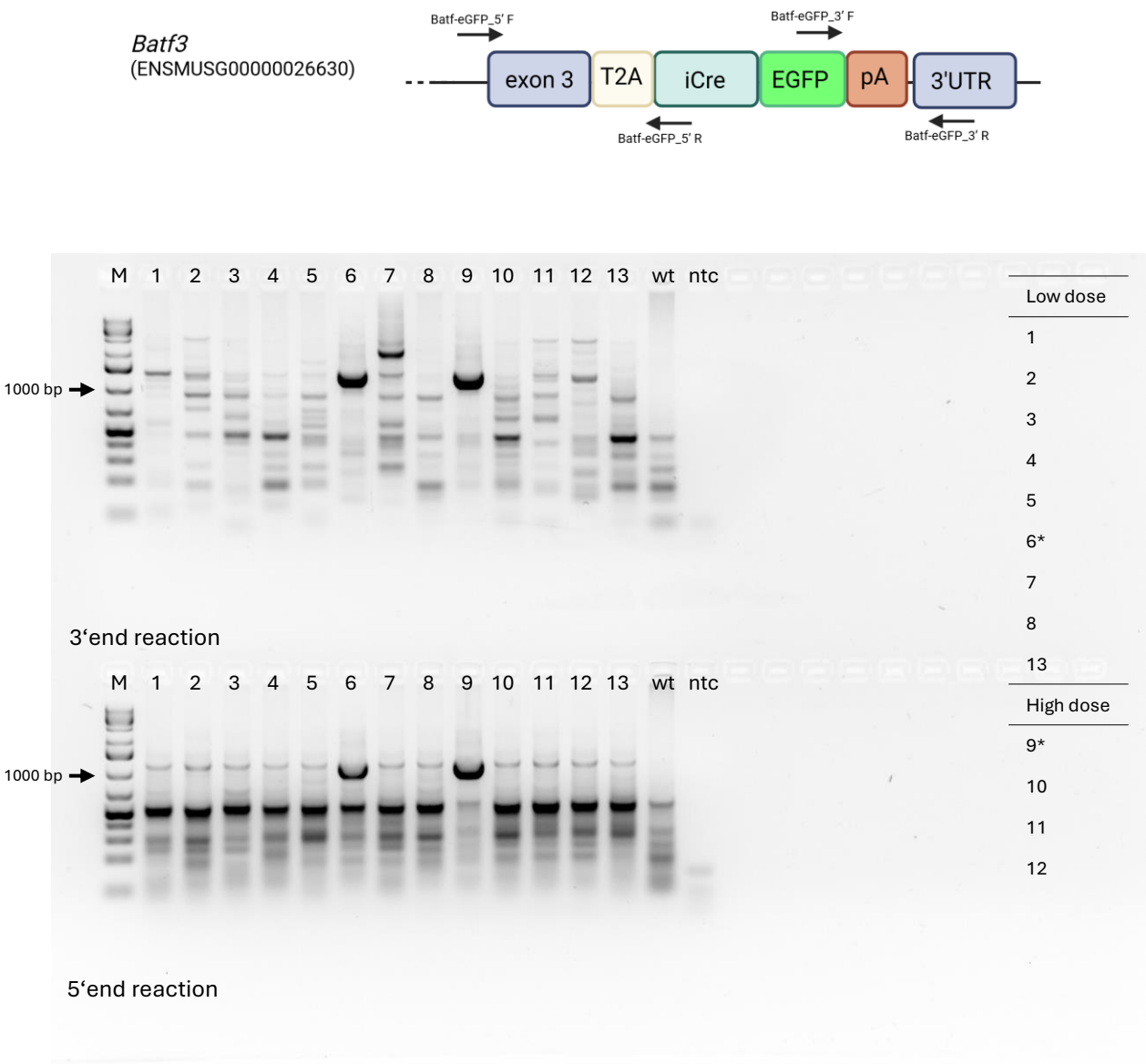
3'reaction	Primers sequence (5'>3')	Product size (bp)
3' Nes-rDR_F	TGCGGGGTCTATTGGGAAC	1154
3' Nes-rDR_R	CTGAAGGTCTCTTGCCATCCT	
5'reaction	Primers sequence (5'>3')	Product size (bp)
5' Nes-rDR_F	AGCCGCGTAACTTCTTCACT	1026
5' Nes-rDR_R	AACTCCCAGCTTTTGAGCGA	

F) C57BL/6NCrl-Dpp4^{em1}(rtTA3,DTR,mKate)Ccpcz



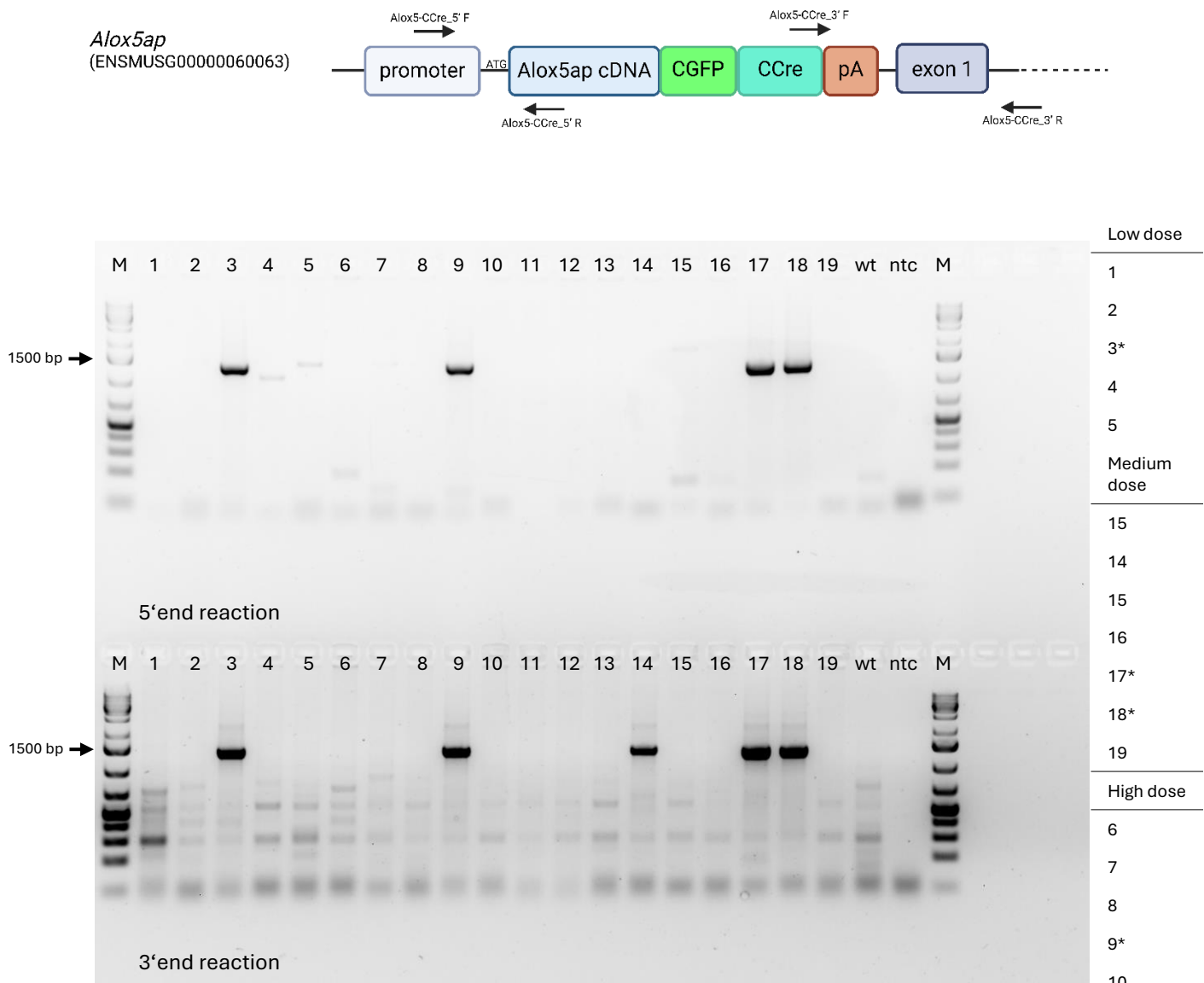
3'reaction	Primers sequence (5'>3')	Product size (bp)
Dpp4-mKate_3' F	CTGCGGGGTCTATTGGGAAC	1213
Dpp4-mKate_3' R	TGGGTCTTCAAAAGCTGCCT	
5'reaction	Primers sequence (5'>3')	Product size (bp)
Dpp4-mKate_5' F	CCTCTCAGGGAAGGGGACAAG	938
Dpp4-mKate_5' R	GAAGTGGGGGCATAGAATCGG	

G) C57BL/6NCrI-Batf3^{em1(iCre,eGFP)}Ccpcz



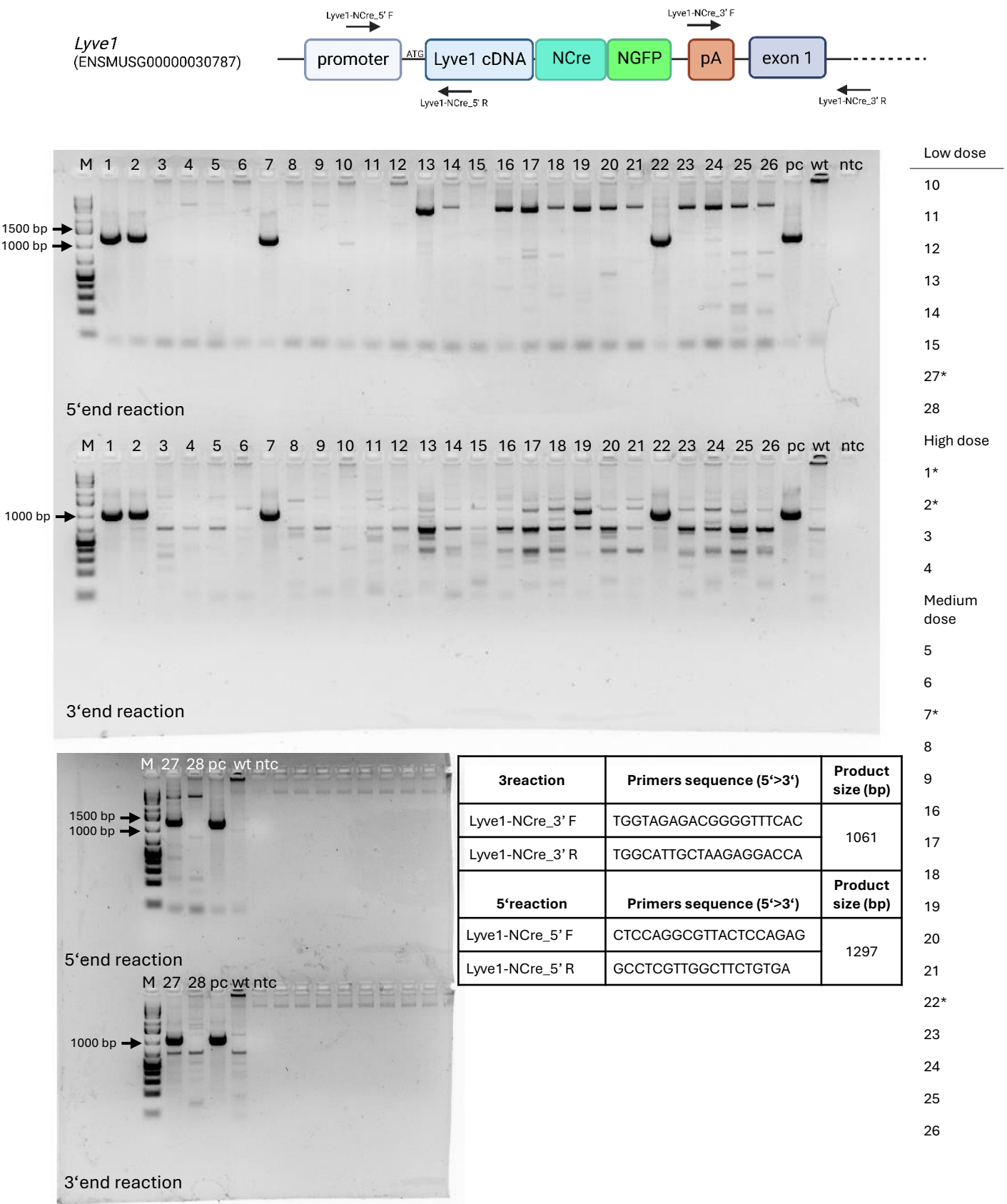
3'reaction	Primers sequence (5'>3')	Product size (bp)
Batf-eGFP_3' F	CACATGGTCCTGCTGGAGTT	1350
Batf-eGFP_3' R	TGCAAGAAGAATGGGCACCT	
5'reaction	Primers sequence (5'>3')	Product size (bp)
Batf-eGFP_5' F	GAACTTGTAAGGGCGAGGGA	1103
Batf-eGFP_5' R	ATCAGAGGTGGCATCCACAG	

H) C57BL/6NCrl-Alox5ap^{em1}(CCre, CGFP)Ccpcz

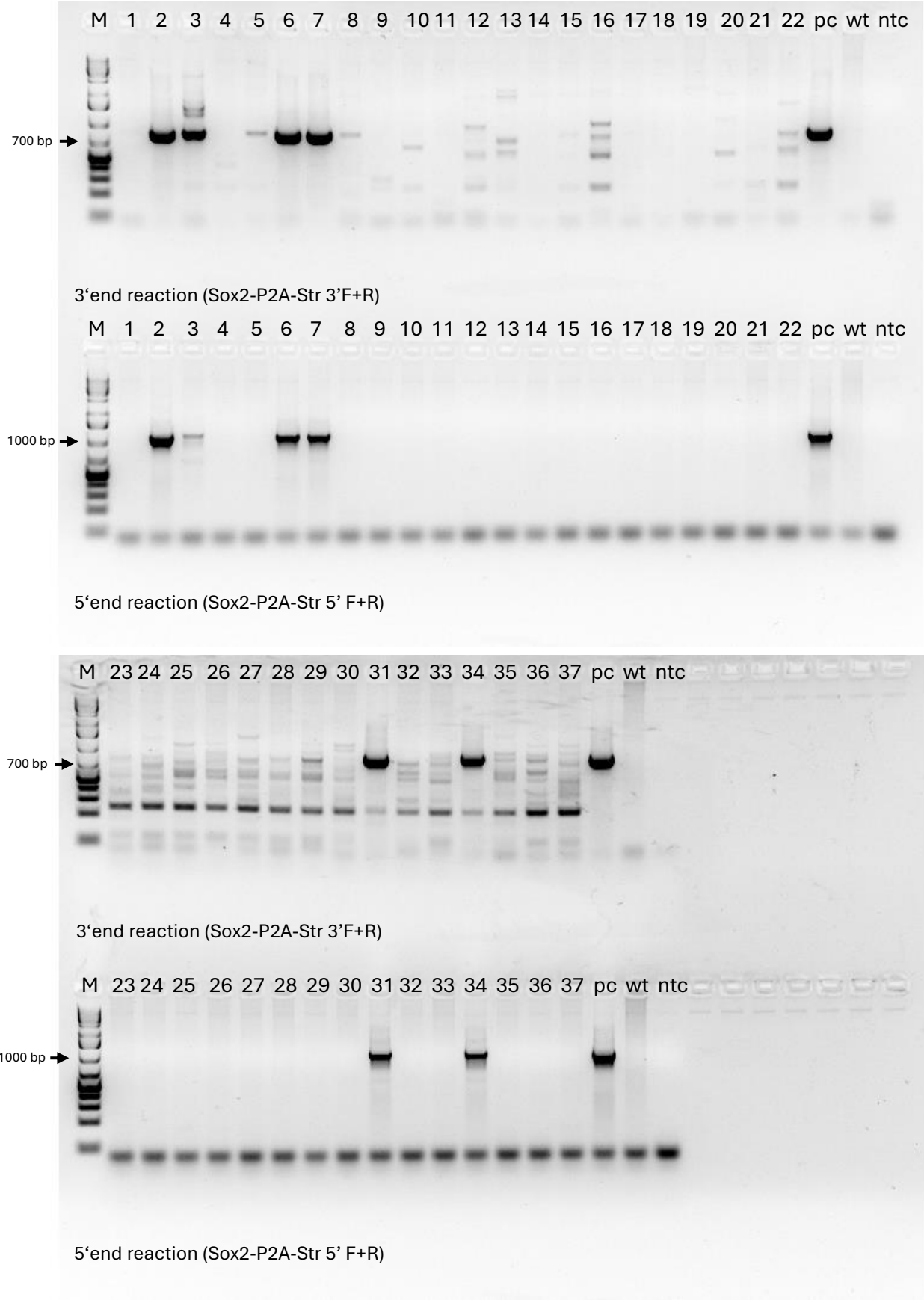


3'reaction	Primers sequence (5'>3')	Product size (bp)
Alox5-CCre_3' F	CTCGAGGATGGGGACTGATG	1358
Alox5-CCre_3' R	ACTGTGTCAAAGGGCTAGGA	
5'reaction	Primers sequence (5'>3')	Product size (bp)
Alox5-CCre_5' F	CCTGCCCTCTTCTGTAAT	1340
Alox5-CCre_5' R	GGCAAAGAACGCATTCTGGA	

I) C57BL/6NCrI-Lyve^{em1}(NCre, NGFP)Ccpcz

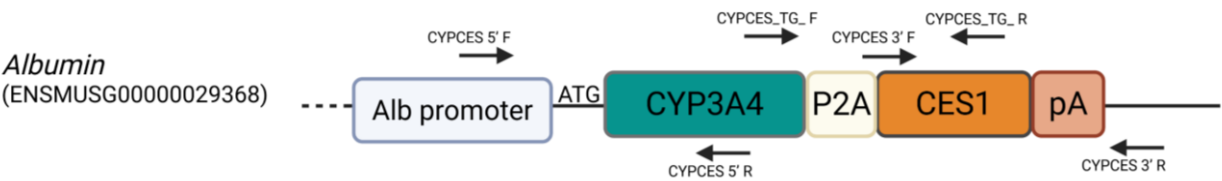


J) C57BL/6NCrl-Sox2^{em1}(P2A-mStrawberry)Ccpcz

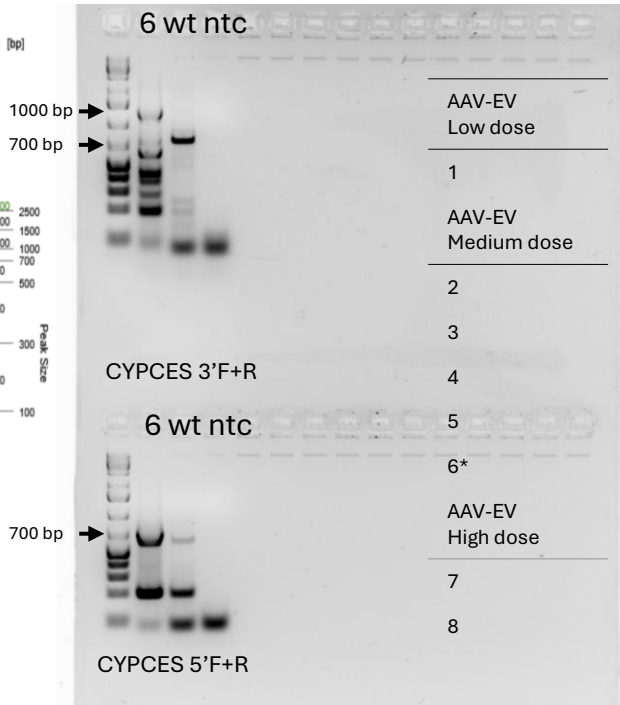
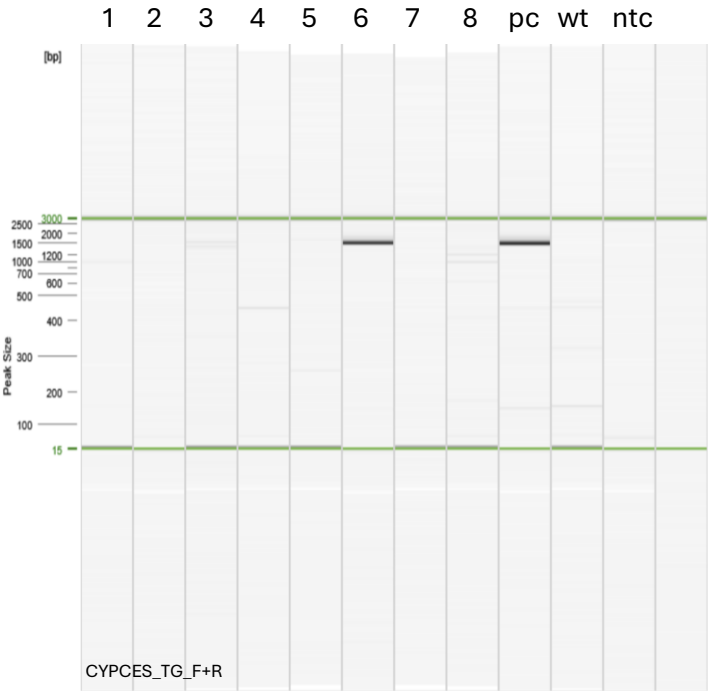


AAV Medium dose
1
2
3*
4*
AAV-EV Low dose
5
6*
7*
8
9
10
11
12
13
14
15
16
35
36
37
AAV-EV Medium dose
17
18
19
20
21
22
29
30
31*
32
33
34*
AAV-EV High dose
25
26
27
28

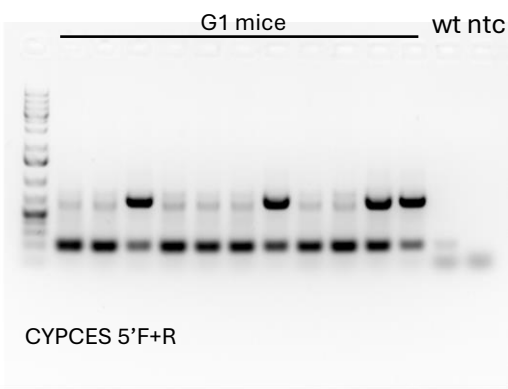
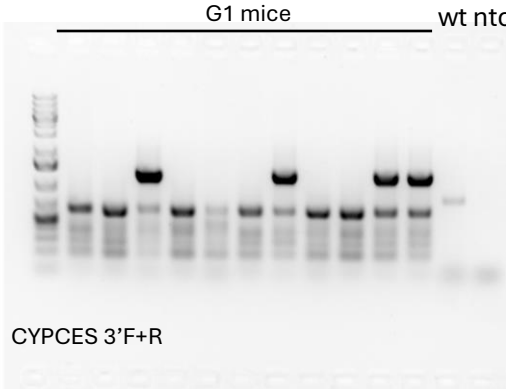
K) C57BL/6NCrI-Alb^{em6}(Cyp3a4,P2A,Ces1)Ccpcz



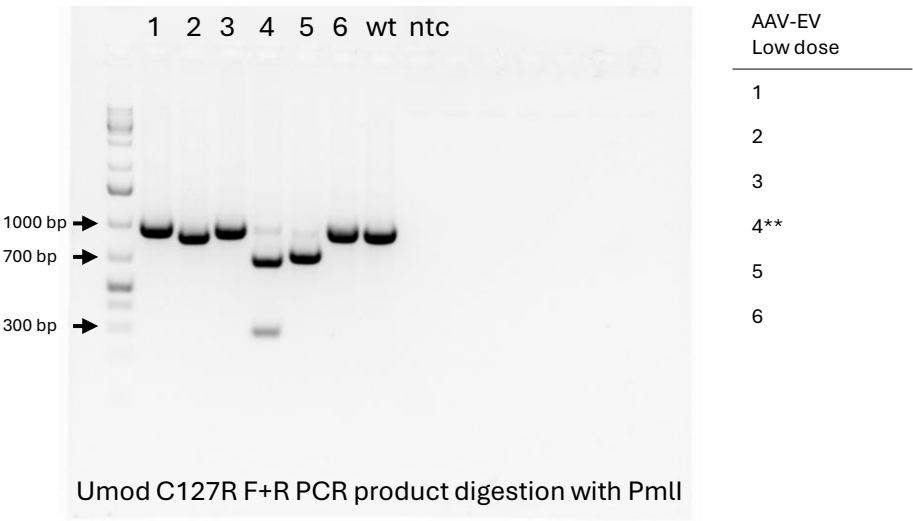
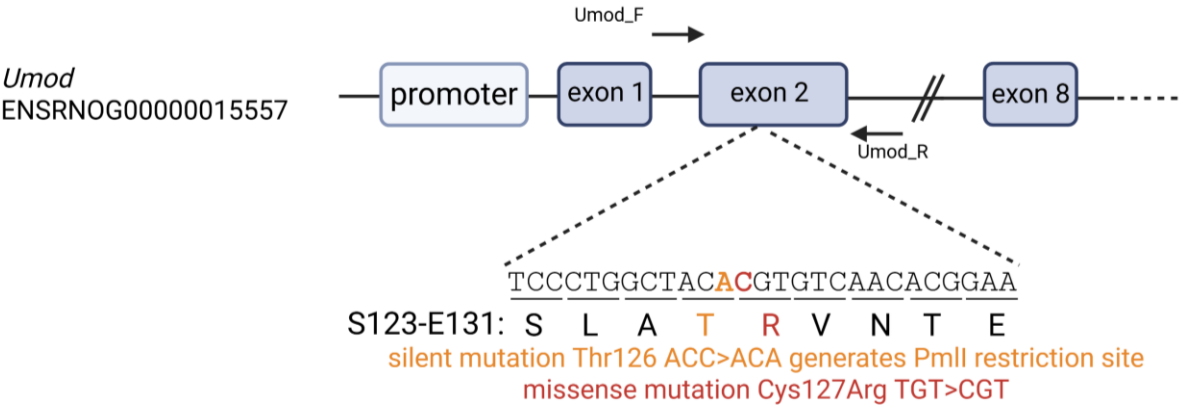
3'reaction	Primers sequence (5'>3')	Product size (bp)
CYP3ES 3'F		836
CYP3ES 3'R		
5'reaction	Primers sequence (5'>3')	Product size (bp)
CYP3ES 5'F	CACCCCGAGAAAGAGGTTCA	689
CYP3ES 5'R	GCCAAGTCTGGGATGAGAGC	
transgene	Primers sequence (5'>3')	Product size (bp)
CYP3ES_TG_ F	CAAGGGATGGCACCGTAAGT	1420
CYP3ES_TG_ R	GCTCCAGCATCTCTGTGGTT	



G1 genotyping



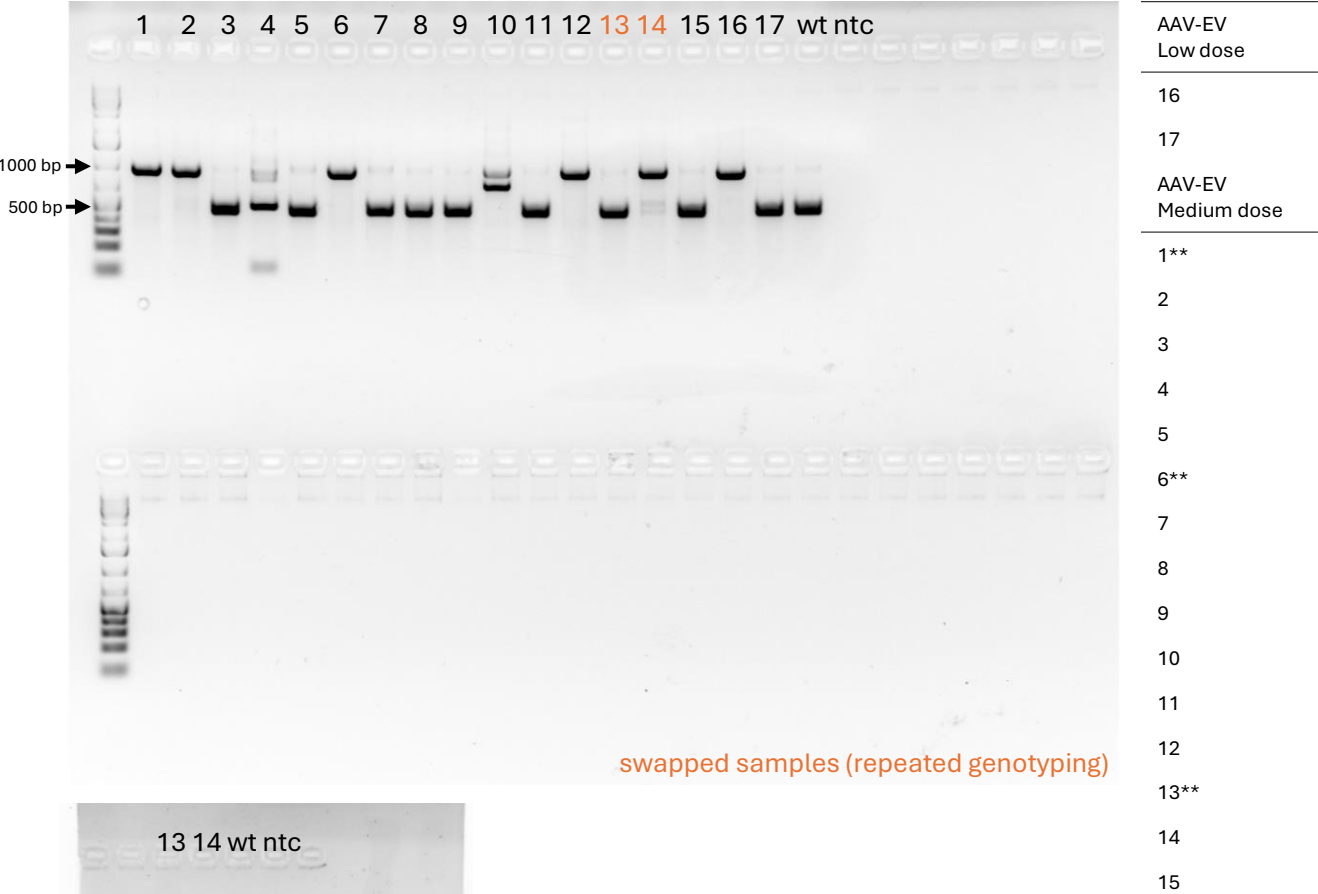
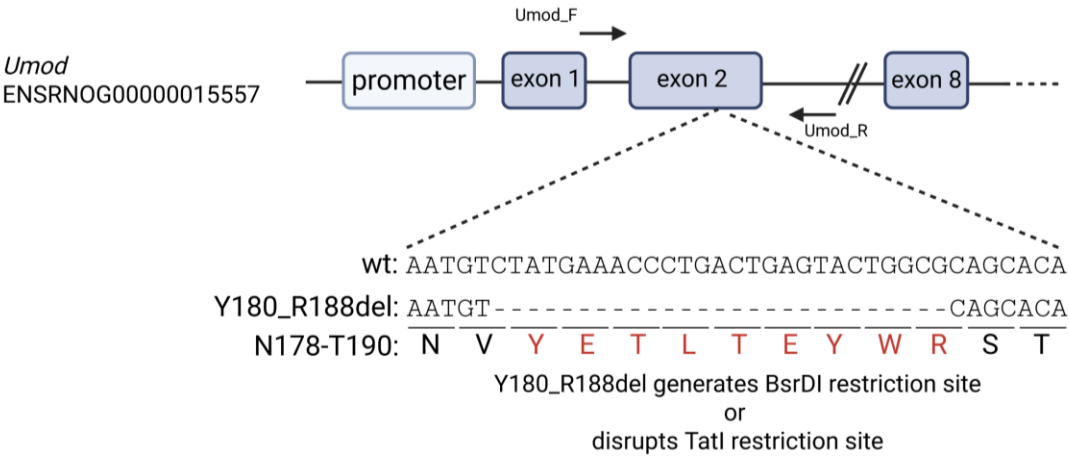
L) SD-Umod^{em1(C126R)Ccpcz}



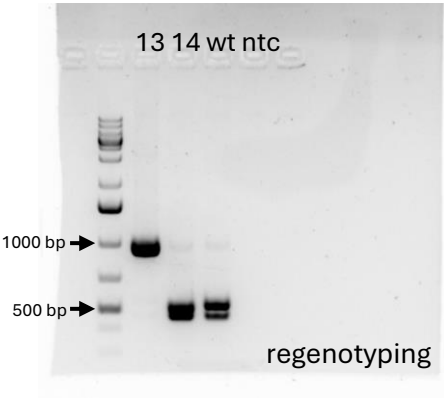
** genotype confirmed by Sanger sequencing

PCR reaction	Primers sequence (5'>3')	Product size (bp)
Umod C127R F	AGGTGTTCTGAATGCCACGA	993
Umod C127R R	ATCTCCCTCTGAGTCTCACCT	
PmlI digestion	WT product – no digestion, C127R positive product – 293 + 710 bp fragments	

M) SD-Umod^{em1}(DelY178-R186)Ccpcz



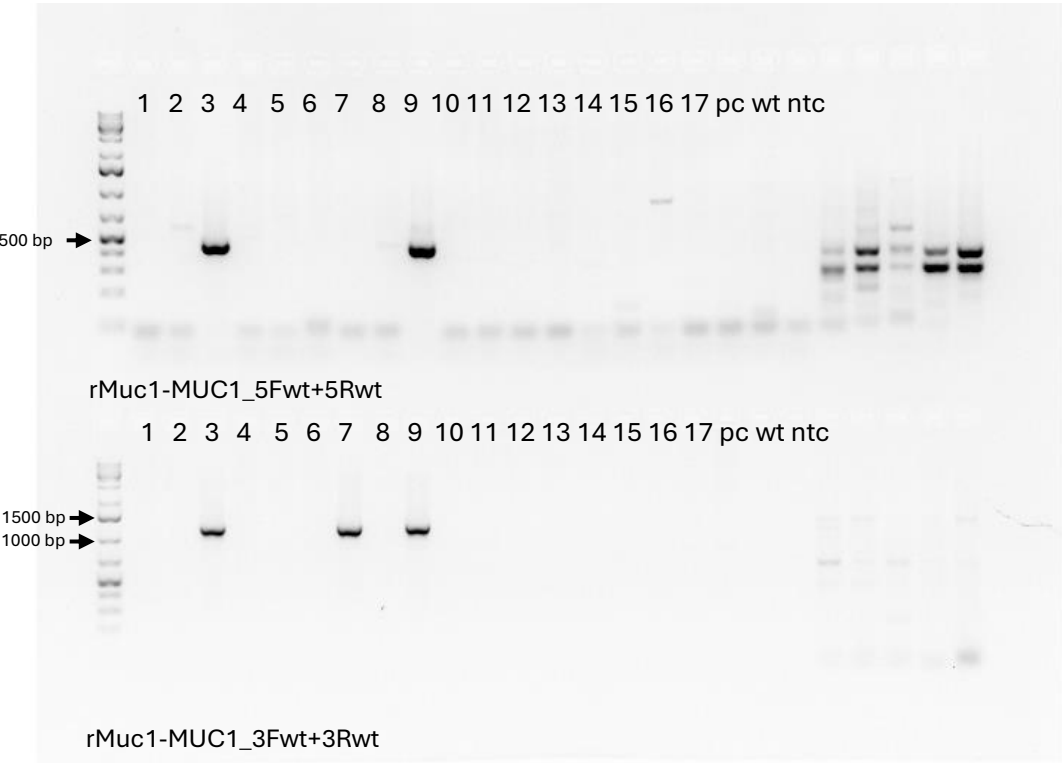
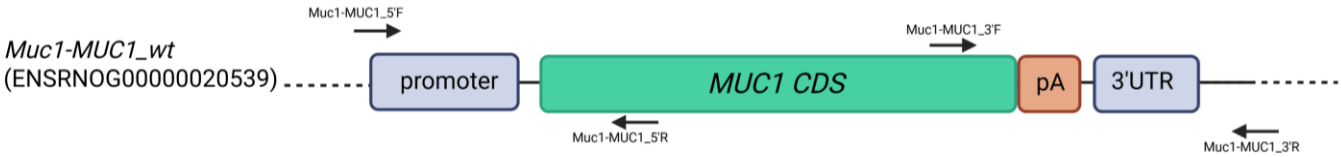
swapped samples (repeated genotyping)



** genotype confirmed by Sanger sequencing

PCR reaction	Primers sequence (5'>3')	Product size (bp)
Umod YR F	AGGTGTTCTGAATGCCACGA	993
Umod YR R	ATCTCCCTCTGAGTCTCACCT	
PmlI digestion	WT product – after TatI digestion: 458+532bp, Y180-R188 deletion positive product: 993 bp	

N) *SD-Muc1^{em1}(MUC1wt)Ccpcz*

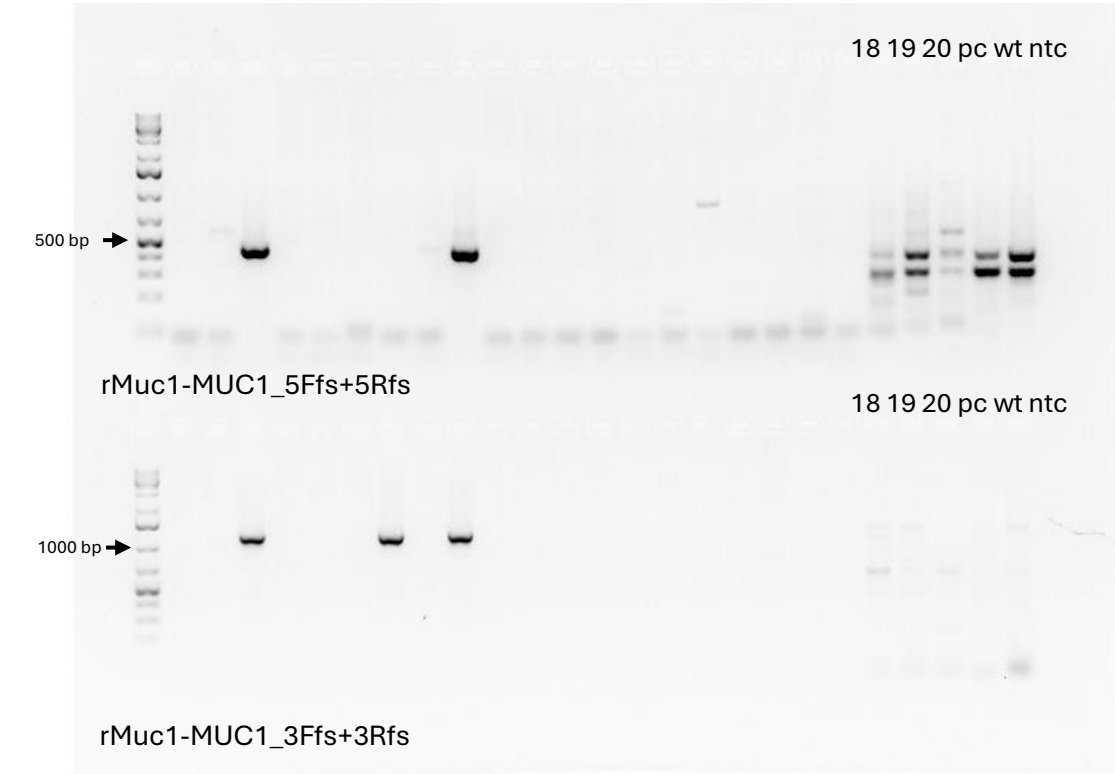
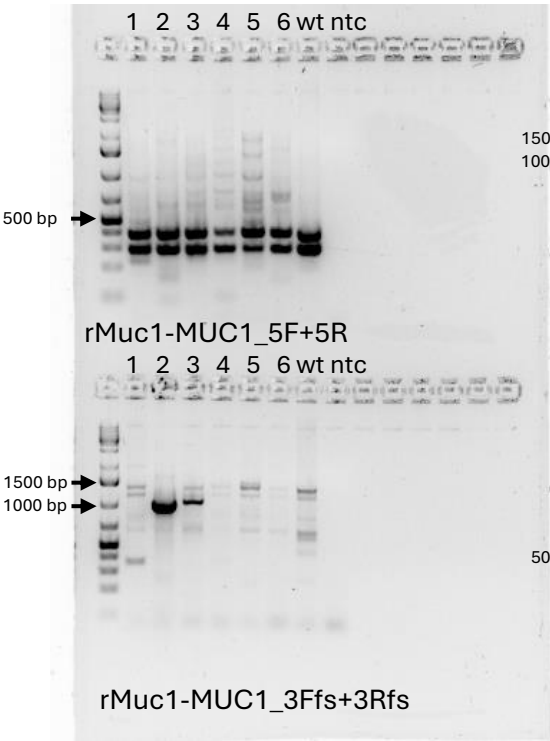
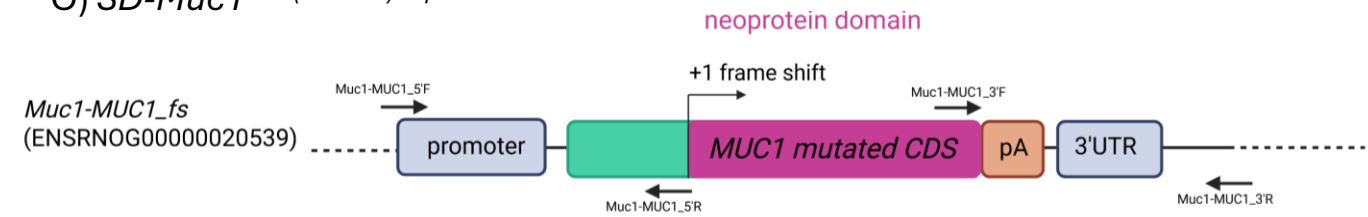


AAV-EV
Low dose

- 1
- 2
- 3*
- 4
- 5
- 6
- 7
- 8
- 9*
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17

3'reaction	Primers sequence (5'>3')	Product size (bp)
rMuc1-MUC1_3Fwt	TTTCCCATTTTCCGCCAGT	1160
rMuc1-MUC1_3Rwt	ACAGTTATGGCGCGAGTTGA	
5'reaction	Primers sequence (5'>3')	Product size (bp)
rMuc1-MUC1 5Fwt	TGGGGTACTCAAAGAGTGTTG	446
rMuc1-MUC1 5Rwt	CATTCTTCTCGGTAGAGGATGGC	

O) *SD-Muc1^{em2}(MUC1fs)Ccpcz*



AAV-EV medium dose	
1	
2	
3	
4	
5	
6	
AAV-EV low dose	
7	
8	
9	
10*	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	

3'reaction	Primers sequence (5'>3')	Product size (bp)	5'reaction	Primers sequence (5'>3')	Product size (bp)
rMuc1-MUC1_3Ffs	CTTTTATAGCGGCGCCCAAGA	1160	rMuc1-MUC1 5Ffs	TGGGGTACTCAAAGAGTGGTTG	446
rMuc1-MUC1_3Rfs	CACACCTGGCCAGCTAAGAAT		rMuc1-MUC1 5Rfs	CGTTCTTCTCGGTGGAGCTTGGC	

Additional file 8 figure: Detection of transgenic cassette in a corresponding rodent model

(A) C57BL/6NCrI-Ube3a^{em2(BioID2)Ccpcz} mouse line: site-specific insertion of Ube3a-BioID2 cassette in the genome detected at the 5' and 3' end. (B) C57BL/6NCrI-Sox2^{em1(P2A-mStrawberry)Ccpcz} mouse line: site-specific insertion of Sox2-mStrawberry cassette in the genome detected at the 5' and 3' end. (C) C57BL/6NCrI-Lck^{em2Ccpcz} mouse line: loxP insertion detected via XhoI digestion of the PCR product. (D) C57BL/6NCrI-Actn1^{em1Ccpcz} mouse line: site-specific insertion of Actn1 cKO cassette in the genome detected at the 5' and 3' end; detection of loxP1 (loxP1 reaction) and of both loxP sites on the same allele (loxP1-2 reaction). (E) C57BL/6NCrI-Nes^{em1(rtTA3,DTR,iRFP670)Ccpcz} mouse line: site-specific insertion of Nes-rtTA/DTR/iRFP670 cassette in the genome detected at the 5' and 3' end. (F) C57BL/6NCrI-Dpp4^{em1(rtTA3,DTR,mKate)Ccpcz} mouse model: site-specific insertion of Dpp4-rtTA3-DTR-mKate2 cassette in the genome detected at the 5' and 3' end. (G) C57BL/6NCrI-Batf3^{em1(iCre,eGFP)Ccpcz} mouse model: site-specific insertion of Batf3-iCre-eGFP cassette in the genome detected at the 5' and 3' end. (H) C57BL/6NCrI-Alox5ap^{em1(CCre,CGFP)Ccpcz} mouse model: site-specific insertion of Alox5ap-CCreCGFP cassette in the genome detected at the 5' and 3' end. (I) C57BL/6NCrI-Lyve^{em1(NCre,NGFP)Ccpcz} mouse line: site-specific of Lyve1-NCre-NGFP cassette insertion in the genome detected at the 5' and 3' end. (J) Genome-transgene junction genotyping of C57BL/6NCrI-Sox2^{em1(P2A-mStrawberry)Ccpcz} animals targeted with Sox-mStr construct using AAVs and distinct doses of AAV-EV vector prepared with UCF-method. (K) Genome-transgene junction genotyping of C57BL/6NCrI-Alb^{em6(Cyp3a4,P2A,Ces1)Ccpcz} animals targeted with Alb-CYP3A4-CES1 construct using AAVs and distinct doses of AAV-EV vector prepared with UCF-method. (L) SD-Umod^{em1(C126R)Ccpcz} rat line: C127R insertion detected via PmlI digestion of the PCR product and Sanger sequencing. (M) SD-Umod^{em1(DelY178-R186)Ccpcz} rat line: Y180-R188 deletion confirmed by loss of TatI restriction site and Sanger sequencing. (N) *SD-Muc1*^{em1(MUC1wt)Ccpcz} rat line: site-specific insertion of Muc1-MUC1_{wt} (human MUC1 wild-type coding sequence) cassette in the genome detected at the 5' and 3' end. (O) *SD-Muc1*^{em2(MUC1fs)Ccpcz} rat line: site-specific insertion of Muc1-MUC1_{fs} (human MUC1 coding sequence with +1 frame shift) cassette in the genome detected at the 5' and 3' end.

Positive animals are marked by an asterisk (*) in the side column, which also summarizes the number of animals per treated group. Positive animals confirmed by sanger sequencing are marked by a double asterick (**). Tables summarize used proteins for corresponding reaction.

Table: List of gRNA spacers used in rodent model generation

AAV-EV	Model	Spacer (5'-3')
Ube3a-BioID2	C57BL/6NCrl-Ube3a ^{em2} (BioID2)Ccpcz	ATCACATATGCCAAAGGATT
Sox2-mStrawberry	C57BL/6NCrl-Sox2 ^{em1} (P2A-mStrawberry)Ccpcz	CTGCCCCCTGTCGCACATGTG
Lck loxP2	C57BL/6NCrl-Lck ^{em2} Ccpcz	GGAGTGCAAATCTTCCAGGA
Actn1 cKO	C57BL/6NCrl-Actn1 ^{em1} Ccpcz	TAAGGCCTTTAGTGAACGGGT
		GCGTGACATCCCGGGTCCC
Nes-rtTA3-DTR-IRES-iRFP670	C57BL/6NCrl-Nes ^{em1} (rtTA3,DTR,iRFP670)Ccpcz	GACGCAACCCTCCATGTGCGC
Dpp4-rtTA3-DTR-H1-mKate2	C57BL/6NCrl-Dpp4 ^{em1} (rtTA3,DTR,mKate)Ccpcz	AAGGAGCCGCCCGACCATGA
Batf3-iCre-eGFP	C57BL/6NCrl-Batf3 ^{em1} (iCre,eGFP)Ccpcz	AGTGAGCTGGGGTGTCATCG
Alox5ap-CCre-CGFP	C57BL/6NCrl-Alox5ap ^{em1} (CCre, CGFP)Ccpcz	TGGCGGGCCTGAAGCAAGCA
Lyve1NCre-NGFP	C57BL/6NCrl-Lyve ^{em1} (NCre, NGFP)Ccpcz	GTAACACCAGGCTAGTGTGC
Alb-CYP3A4-CES1	C57BL/6NCrl-Alb ^{em6} (Cyp3a4,P2A,Ces1)Ccpcz	AGCCTCTGGCAAAATGAAGT
		GTTGTGATGTGTTTAGGCTA
Umod C127R	SD-Umod ^{em1} (C126R)Ccpcz	GCTGCCTTCCGTGTTGACAC
Umod Y180_R188del	SD-Umod ^{em1} (DelY178-R186)Ccpcz	GGGTTTCATAGACATTGCAG
		TACTGGCGCAGCACAGACTA
Muc1-MUC1_wt/fs	<i>SD-Muc1</i> ^{em1} (MUC1)Ccpcz	AACCCGTATGCCCGGGGTCA
	<i>SD-Muc1</i> ^{em2} (MUC1fs)Ccpcz	CTCCTACAAGTTGGCCGAAG