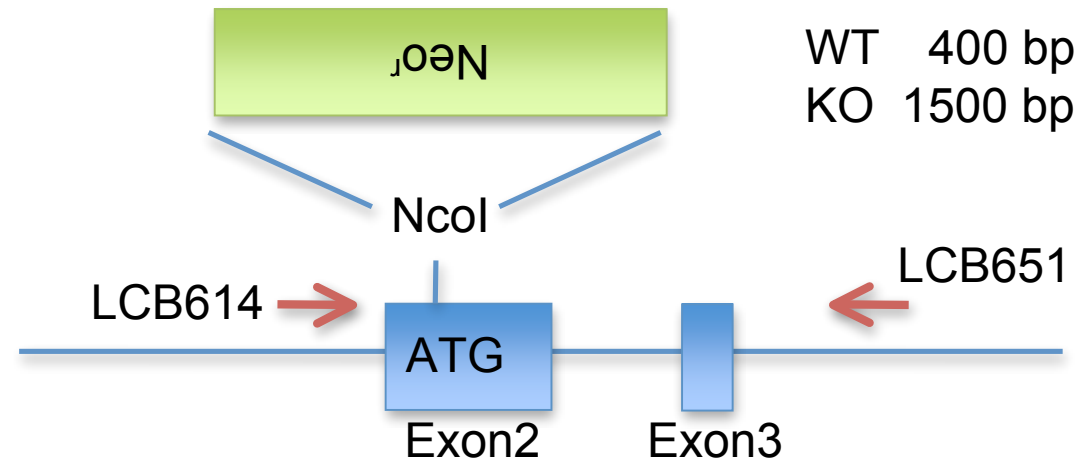
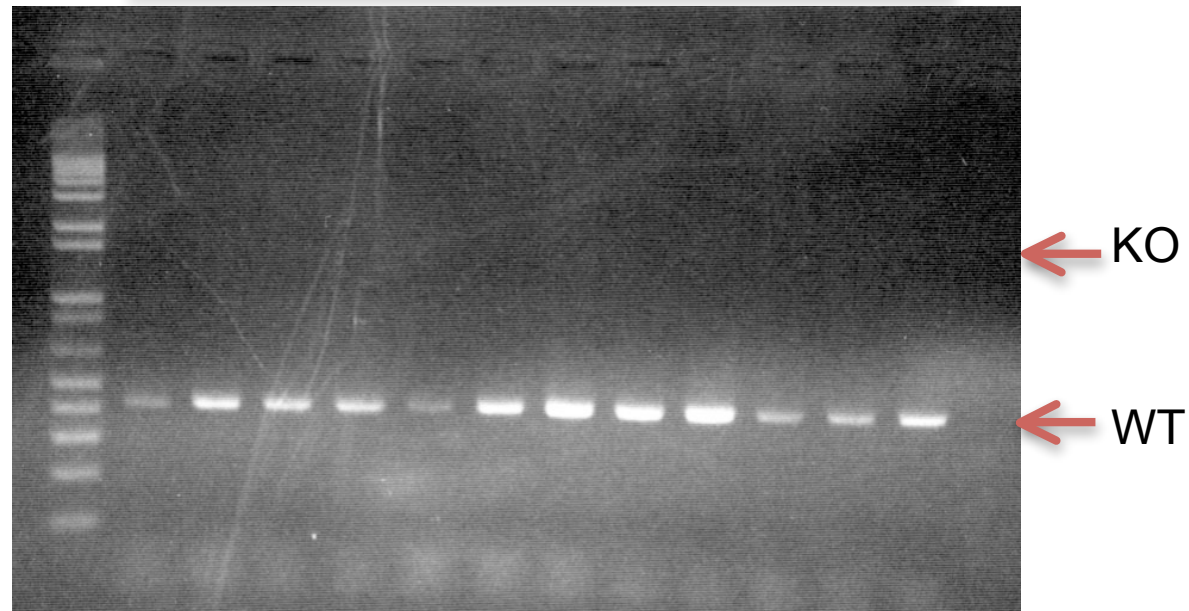


p53 KOマウス作製法



Roche TaqでのPCR

Pups from p53^{+/-} x WT



p53遺伝子の配列

ここにNeoカセット挿入

Genomic sequence of the p53 gene with annotations for Neo cassette insertion, restriction sites, and exons.

Neo Cassette Insertion: Indicated by a red arrow pointing to the sequence between positions 6310 and 6400.

Restriction Sites:

- >NcoI:** Located at position 6510.
- >SstI:** Located at position 6550.

Exons:

- Exon 2:** Highlighted in a red box, spanning positions 6510 to 6590.
- Exon 3:** Highlighted in a red box, spanning positions 6810 to 6890.

Other Annotations:

- LCB614:** A blue box spanning positions 6370 to 6400.
- p53 WTKO-Rv2:** A purple box spanning positions 6710 to 6780.
- LCB651:** A blue box spanning positions 6910 to 6940.

Sequence details (positions 6310-6400):

```
6310 TGGTTACCTC 6320 TGCTCAGCCC 6330 CCGGCTTCTG 6340 TCCTCCATGT 6350 TCCTGGGGGA 6360 ATCCCTACAC 6370 TTTCAGAATT 6380 TAATTTCCCT 6390 ACTGGATGTC 6400 CCACCTTCTT
6410 ACCAATGGAG 6420 ACGAGTCGGG 6430 GGCCGAAGAC 6440 AGGAGGTACA 6450 AGGACCCCCT 6460 TAGGGATGTG 6470 AAAGTCTTA 6480 ACCTACAG 6490 GGTGGAAGAA
```

Sequence details (positions 6510-6590):

```
6510 CAGATGACTG 6520 CCATGGAGGA 6530 GTCACAGTCG 6540 GATATCAGCC 6550 TCGAGCTCCC 6560 TCTGAGCCAG 6570 GAGACATTTT 6580 CAGGCTTATG 6590 GAAACTGTGA 6600 GTGGATCTTT
6610 GTCTACTGAC 6620 GGTACCTCCT 6630 CAGTGTCAAG 6640 CTATAGTCGG 6650 AGCTCGAGGG 6660 AGACTCGGTC 6670 CTCTGTAAAA 6680 GTCCGAATAC 6690 CTTTGACACT 6700 CACCTAGAAA
```

Sequence details (positions 6710-6780):

```
6710 TAAATCCTGC 6720 GGGGCGGGGT 6730 GGCGGGGGGG 6740 GGGGGGGACT 6750 GCAGGGTCTC 6760 AGAAGTTTGA 6770 GGTCATCATT 6780 GACTACATA
```

Sequence details (positions 6810-6890):

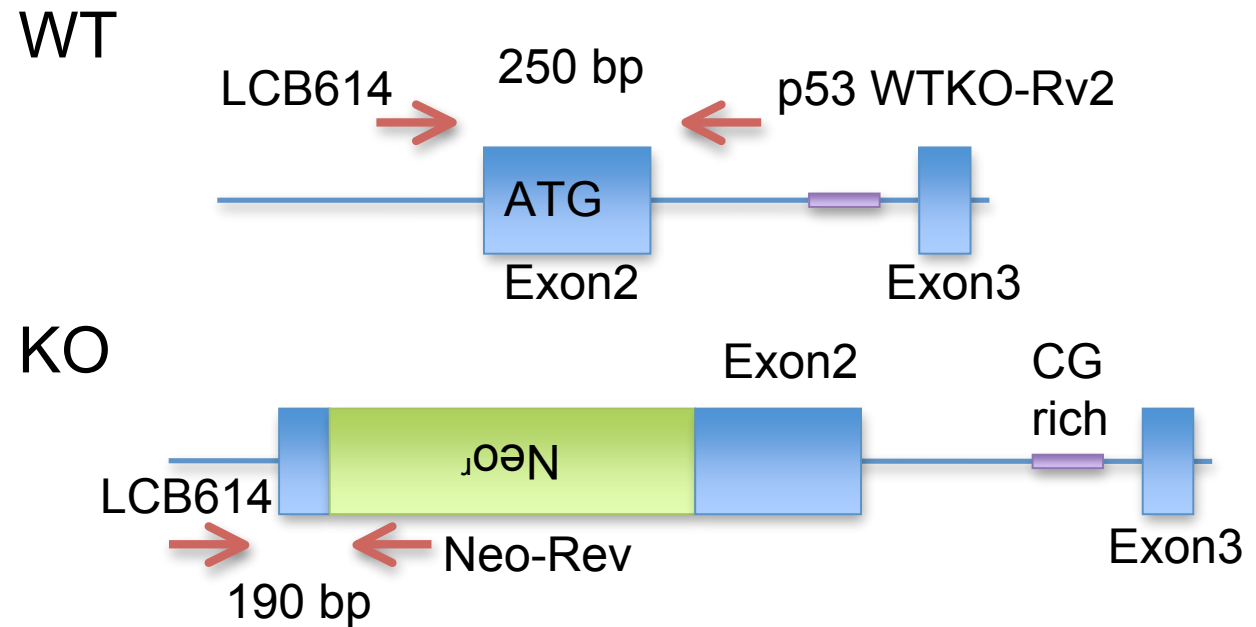
```
6810 GATAAGTGAG 6820 ATTCTGTCTT 6830 CAAAAAATCG 6840 AAGGAAATCA 6850 GGAACAACT 6860 CTCTGCTCTT 6870 GTTTTCCAGA 6880 CTTCTCCAG 6890 AAGATATCCT 6900 GGTAAGGCCC
6910 CTATTCACCT 6920 TAAGACAGAA 6930 GTTTTTTACC 6940 TTCCTTTAGT 6950 CTTGATTGA 6960 GAGACGAGAA 6970 CAAAAGGTCT 6980 GAAGGAGGTC 6990 TTCTATAGGA 7000 CCATTCCGGG
```

Sequence details (positions 6910-6940):

```
6910 AGAGCAGAAA 6920 GGGACTTGGG 6930 CTTTGGTGTG 6940 GGGCTGGTAG 6950 GCTGAGAACA 6960 CAGTCCTGAG 6970 GGTTCTTCTT 6980 TGTCCCA 6990 CA 7000 CCTCACTGCA
7010 TCTCGTCTTT 7020 CCCTGAACCC 7030 GAAACCACAA 7040 CCGGACCATC 7050 CGACTCTTGT 7060 GTCAGGACTC 7070 CCAAGAAGAA 7080 ACAGGGTGG 7090 TGTCCGTAGT 7100 GGAGTGACGT
```

GC-richな配列。これが邪魔してるので、外してprimer設定

新たに設定したprimerの位置



PrimerとPCRの条件

Primer

LBC614

(理研より情報提供)

p53 WTKO-Rv2;

5'-ACACCCAACACCATACCATGT-3'

Neo-Rev;

5'-CTTCCTCGTGCTTTACGGTATC-3'

PCR

94°C

2 min

94°C

20 sec

60°C

20 sec

cycles

x34

72°C

40 sec

72°C

2 min

Template DNA
 2-5 mm tail + 600μl 50mM NaOH
 ↓
 100°C, 1h
 ↓
 + 50μl 1M Tris-HCl(pH8.0)で中和
 ↓
 軽くvortex > template DNA

DNA		1	
X10 buffer		2.5	
dNTP		0.4	
primer	LCB	0.2	
	Neo-Rev	0.2	※もっと減らせるかもしれません
	WTKO-Rv2	0.2	
Taq		0.2	
DDW		15.8/ 25μl	

