Genotyping protocol for ATX (Enpp2) mice

A neomycin selection cassette has been inserted upstream of exon 1, and the neo cassette and exons 1 and 2 are flanked by loxP sites yielding the conditional knock-out mouse for ATX, named ATX neo allele [B6-Enpp2tm1Vart/Flmg].

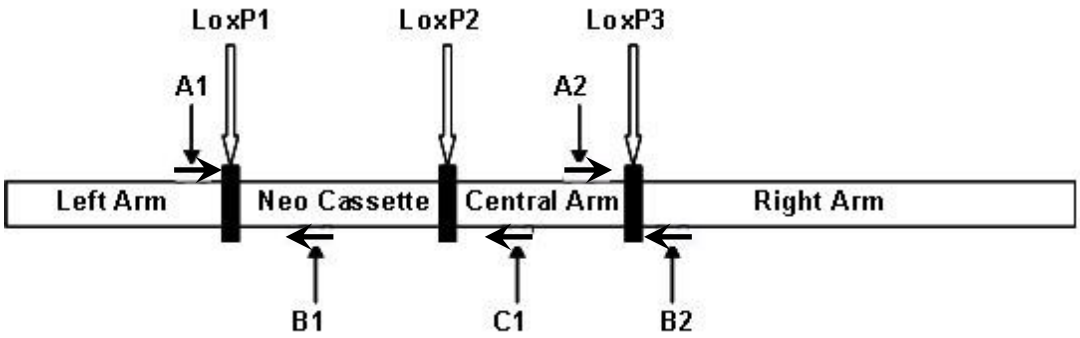
The ATX floxed allele [B6-Enpp2tm1.2Vart/Flmg] was derived from the Enpp2tm1Vart/Flmg, where the neomycin selection cassette was excised by Tmx-induced R26CreER^{T2}-mediated recombination.

The ATX defloxed allele [B6-Enpp2tm1.1Vart/Flmg] was derived from the Enpp2tm1Vart/Flmg. Upon CMV-Cre-mediated recombination, exons 1 and 2 together with the neomycin selection cassette were excised to generate the knock-out allele for ATX.

A. Genotyping protocol for the conditional knock-out ATX neo mice

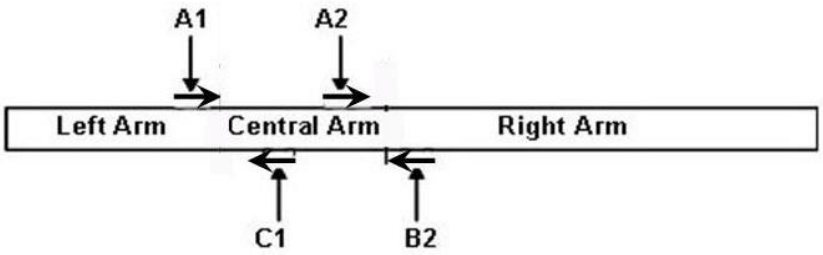
The PCR strategy design for the genotyping of conditional ATX neo and wt mice is shown schematically in the figures below.

ATX^{neo} Mouse



A1/B1: 457bp
A1/C1: 2256bp
A1/B2: 3582bp
A2/B2: 553bp

ATX$^{+/+}$ (WT) Mouse

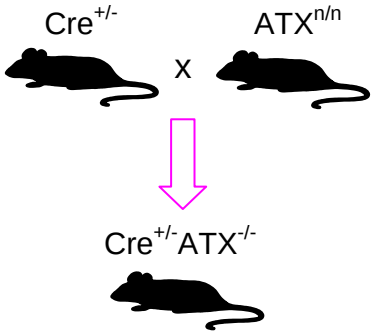


A1/C1: 223bp

A1/B2: 1456bp

A2/B2: 460bp

Upon crossing of an ATX$^{n/n}$ mouse with a mouse expressing the Cre recombinase (Cre$^{+/-}$), the tissue specific knock-out for ATX mouse is generated (deletion of ATX at the tissue where the cre is expressed).



Most common recombination: LoxP1-3 deletion

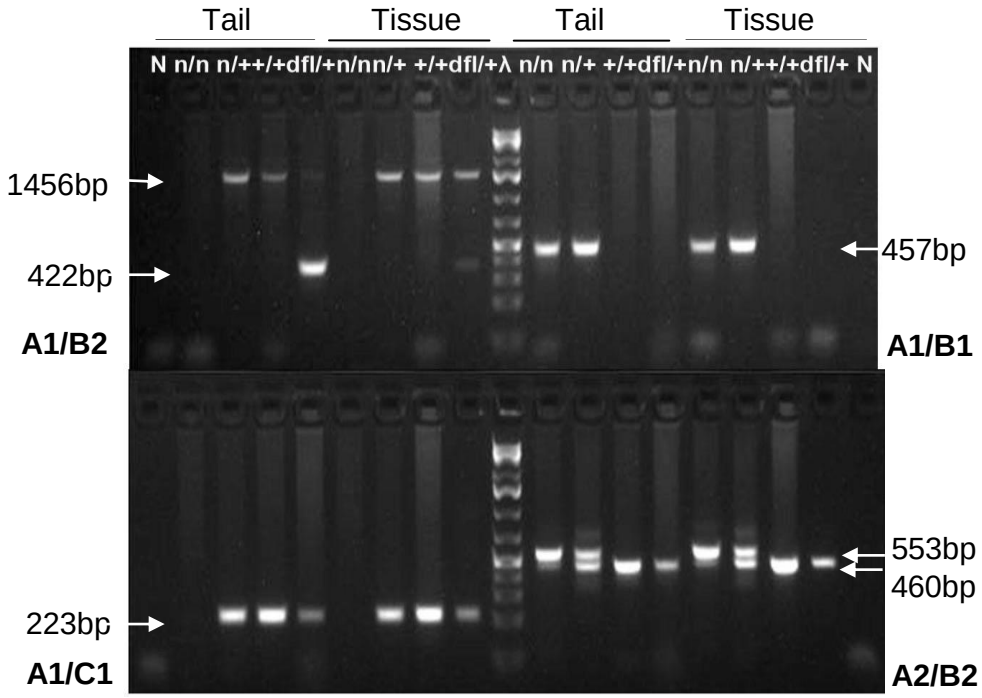
- All PCR products from the different PCR primer sets are shown in the table below.

A1/B1		A1/C1	
ATX WT/	no product	ATX WT/	223 bp
ATX neo/	457 bp	ATX neo/	2256 bp
ATX FL/ (1-2 del)	no product	ATX FL/ (1-2 del)	~276 bp
ATX neo/ (2-3 del)	457 bp	ATX neo/ (2-3 del)	no product
ATX dFL/ (1-3 del)	no product	ATX dFL/ (1-3 del)	no product
A1/B2		**A2/B2**	
ATX WT/	1456 bp	ATX WT/	460 bp

ATX neo/	3582 bp	ATX neo/	553 bp
ATX FL/ (1-2 del)	~1600 bp	ATX FL/ (1-2 del)	553 bp
ATX neo/ (2-3 del)	2363 bp	ATX neo/ (2-3 del)	no product
ATX dFL/ (1-3 del)	422 bp	ATX dFL/ (1-3 del)	no product

Genotyping PCR products using A1/B1/C1 primers and mouse tail DNA.

PCR products using the different sets of primers |A1/B2, A1/B1, A1/C1, A2/B2 and tissue DNA for the verification of recombination.



2% agarose gel electrophoresis in 1x TBE, 30 min at 120V

- Primer set A1/B1 yields a PCR product of 457 bp corresponding to the neo allele.
- Primer set A1/C1 yields a PCR product of 223 bp corresponding to the Wild-type (WT) allele.
- Primer set A1/B2 yields a PCR product of 422 bp corresponding to the recombined allele and a PCR product of 1456 bp that corresponds to the Wild-type (WT) allele.

- Primer set A2/B2 yields PCR products of 460 bp and 553 bp corresponding to the Wild-type (WT) and neo alleles, respectively.
- The PCR for the genotyping of the ATX neo and wt alleles is performed with 50-200 ng DNA, 0.25mM dNTPs, 0,5uM of primer A1, 0,3uM of primer B1, 0,25uM of primer C1, a custom made Taq DNA polymerase and 1,5 mM $MgCl_2$ at a final volume of 20ul. A typical pipetting scheme for a single reaction is the following: 1 ul DNA, 2 ul A1 (from 5 pmol/ ul), 1,2 ul B1 (from 5 pmol/ ul), 1ul C1 (from 5 pmol/ ul), 2 ul of 10 x PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C), 1% Triton X-100), 2 ul dNTPs (from 2,5 mM each), 1,2 ul $MgCl_2$ (from 25mM), 0,4 ul Taq and 9,2 ul Gibco H_2O.
- The PCR for the verification of recombination is performed with 50-200 ng DNA, 0.25mM dNTPs, 0.25uM of each primer, a custom made Taq DNA polymerase and 1.75 mM $MgCl_2$ at a final volume of 20ul. A typical pipetting scheme for single reactions is the following: 1 ul DNA, 1 ul A1/A2, 1 ul B1/C1/B2, 2 ul of 10 x PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C), 1% Triton X-100), 2 ul dNTPs from 2,5 mM each, 1,4 ul $MgCl_2$ from 25mM, 0,4 ul Taq and 11,2 ul Gibco H_2O.
- PCR reactions are performed using the same Thermocycler conditions: 1 cycle at 94 °C for 3 min, 30 cycles of 94 °C for 2 min, 59 °C for 1,5 min, 72 °C for 1,5 min and 1 cycle of 72 °C for 5 min.
- Primer sequences:

  A1 (F): 5'- CGCATTTGACAGGAATTCTT -3' (20bp, T_m=54,6°C)

  B1 (R): 5'- ATTTGTCACGTCCTGCACGA -3' (20bp, T_m=60°C)

  C1 (R): 5'- ATCAAAATACTGGGGCTGCC -3' (20bp, T_m=58°C)

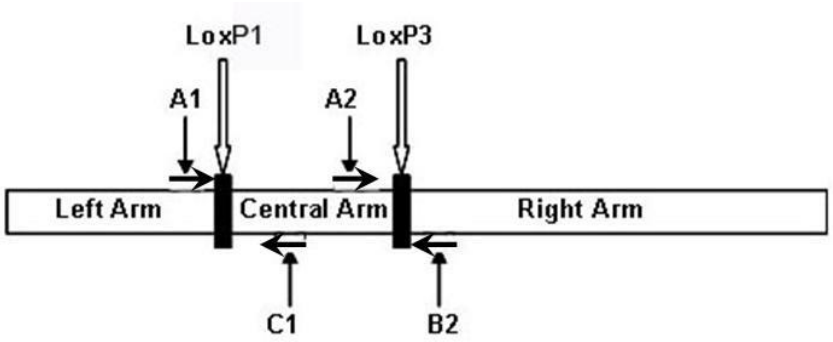
  A2 (F): 5'- CGAATCTCTCCGATCACTAC -3' (20bp, T_m=55°C)

  B2 (R): 5'- TACACAACACAGCCGTCTCA -3' (20bp. T_m=59°C)

B. Genotyping protocol for the conditional knock-out ATX floxed mice

The PCR strategy design for the genotyping of conditional ATX floxed and wt mice is shown schematically in the figures below.

ATX^{floxed} Mouse

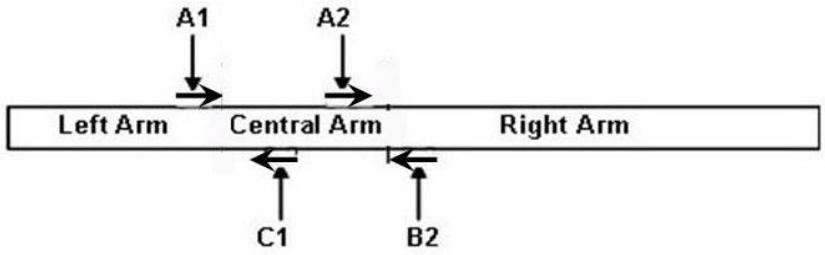


A1/C1: 276bp

A1/B2: 1600bp

A2/B2: 553bp

ATX$^{+/+}$ (WT) Mouse

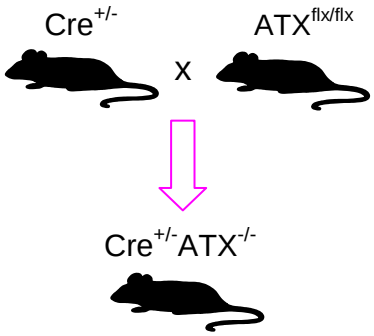


A1/C1: 223bp

A1/B2: 1456bp

A2/B2: 460bp

Upon crossing of an ATX$^{flx/flx}$ mouse with a mouse expressing the Cre recombinase (Cre$^{+/-}$), the tissue specific knock-out for ATX mouse is generated (deletion of ATX at the tissue where the cre is expressed).

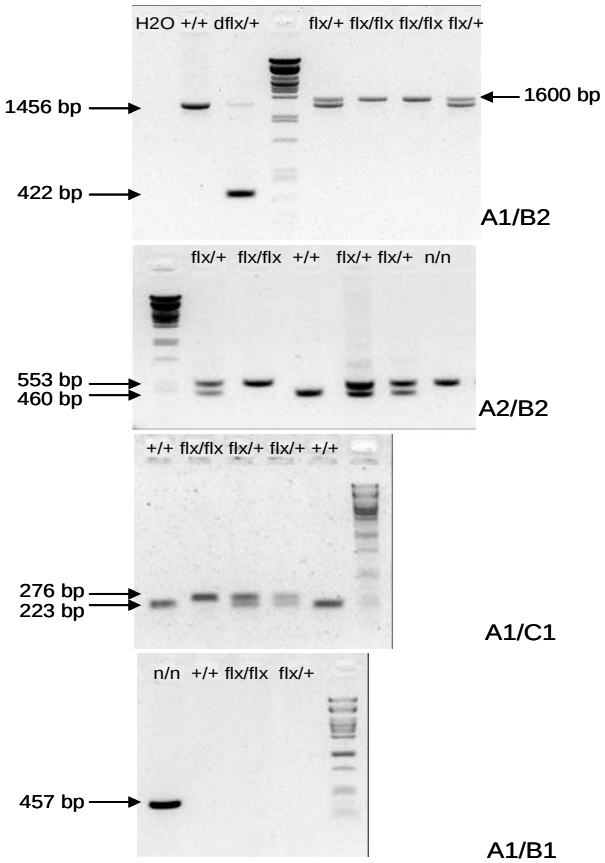


Recombination: LoxP1-3 deletion

- All PCR products from the different PCR primer sets are shown in the table below.

A1/B1		A1/C1	
ATX WT/	no product	ATX WT/	223 bp
ATX FL/ (1-2 del)	no product	ATX FL/ (1-2 del)	~276 bp
ATX neo/ (2-3 del)	457 bp	ATX neo/ (2-3 del)	no product
ATX dFL/ (1-3 del)	no product	ATX dFL/ (1-3 del)	no product
A1/B2		**A2/B2**	
ATX WT/	1456 bp	ATX WT/	460 bp
ATX FL/ (1-2 del)	~1600 bp	ATX FL/ (1-2 del)	553 bp
ATX neo/ (2-3 del)	2363 bp	ATX neo/ (2-3 del)	no product
ATX dFL/ (1-3 del)	422 bp	ATX dFL/ (1-3 del)	no product

PCR products using the different sets of primers |A1/B2, A1/B1, A1/C1, A2/B2 and tail DNA for the genotyping or tissue DNA for the verification of recombination.



2% agarose gel electrophoresis in 1x TBE, 30 min at 120V

- Primer set A1/B2 yields a PCR product of 422 bp corresponding to the recombined allele and PCR products of 1456 bp and 1600 bp corresponding to the Wild-type (WT) and floxed alleles, respectively.
- Primer set A2/B2 yields PCR products of 460 bp and 553 bp corresponding to the Wild-type (WT) and floxed alleles, respectively.
- Primer set A1/C1 yields PCR products of 223 bp and 276bp corresponding to the Wild-type (WT) and floxed alleles, respectively.
- All PCRs are performed with 50–200 ng DNA, 0.25mM dNTPs, 0.25uM of each primer, a custom made Taq DNA polymerase and 1.75 mM $MgCl_2$ at a final volume of 20ul. A typical pipetting scheme for single reactions is the following: 1 ul DNA, 1 ul A1/A2, 1 ul B2/B1/C1, 2 ul of 10 x PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C), 1% Triton X-100), 2 ul dNTPs from 2,5 mM each, 1,4 ul $MgCl_2$ from 25mM, 0,4 ul Taq and 11,2 ul Gibco H_2O.
- PCR reactions are performed using the same Thermocycler conditions: 1 cycle at 94 °C for 3 min, 30 cycles of 94 °C for 2 min, 59 °C for 1,5 min, 72 °C for 1,5 min and 1 cycle of 72 °C for 5 min.
- Primer sequences:

  A1 (F): 5'- CGCATTTGACAGGAATTCTT -3' (20bp, T_m=54,6°C)

  B1 (R): 5'- ATTTGTCACGTCCTGCACGA -3' (20bp, T_m=60°C)

  C1 (R): 5'- ATCAAAATACTGGGGCTGCC -3' (20bp, T_m=58°C)

  A2 (F): 5'- CGAATCTCTCCGATCACTAC -3' (20bp, T_m=55°C)

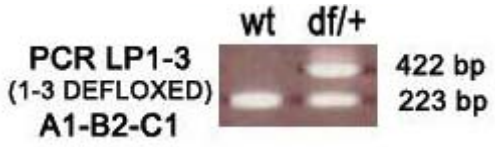
  B2 (R): 5'- TACACAACACAGCCGTCTCA -3' (20bp. T_m=59°C)

C. Genotyping protocol for the heterozygous knock-out ATX defloxed/+ mice

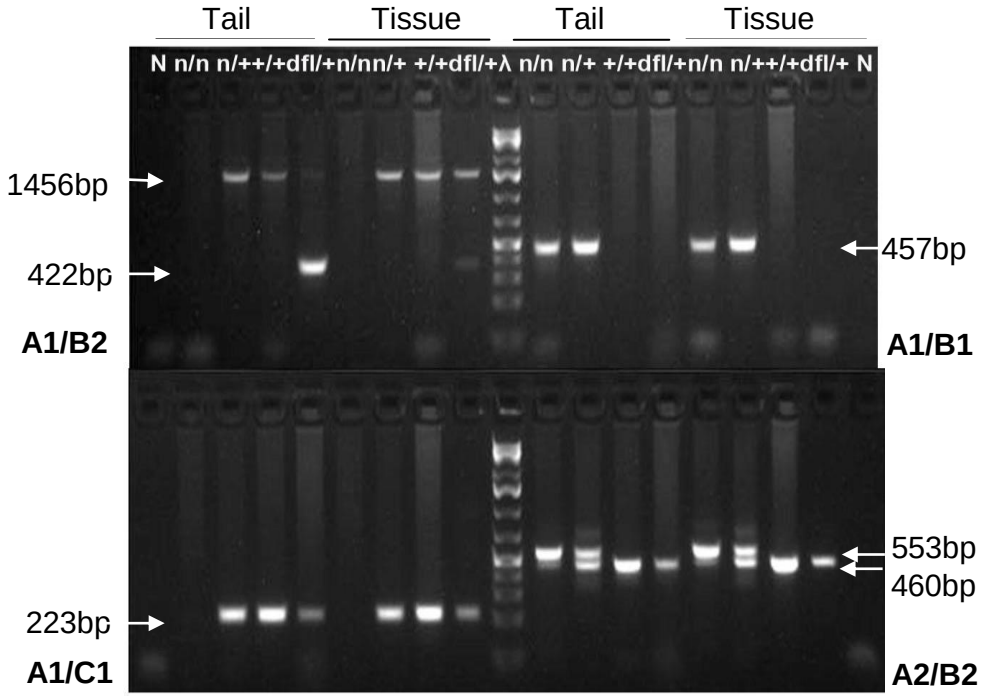
- All PCR products from the different PCR primer sets are shown in the table below.

A1/B1		A1/C1	
ATX WT/	no product	ATX WT/	223 bp
ATX dFL/ (1-3 del)	no product	ATX dFL/ (1-3 del)	no product
A1/B2		**A2/B2**	
ATX WT/	1456 bp	ATX WT/	460 bp
ATX dFL/ (1-3 del)	422 bp	ATX dFL/ (1-3 del)	no product

Genotyping PCR products using A1/B2/C1 primers and mouse tail DNA.



PCR products using the different sets of primers |A1/B2, A1/B1, A1/C1, A2/B2 and tissue DNA for the verification of recombination.



2% agarose gel electrophoresis in 1x TBE, 30 min at 120V

- Primer set A1/C1 yields a PCR product of 223 bp corresponding to the Wild-type (WT) allele.
- Primer set A1/B2 yields a PCR product of 422 bp corresponding to the defloxed allele and a PCR product of 1456 bp that corresponds to the Wild-type (WT) allele.
- The PCR for the genotyping of the ATX defloxed and wt alleles is performed with 50-200 ng DNA, 0.25mM dNTPs, 0,5uM of primer A1, 0,3uM of primer B2, 0,25uM of primer C1, a custom made Taq DNA polymerase and 1,5 mM $MgCl_2$ at a final volume of 20ul. A typical pipetting scheme for a single reaction is the following: 1 ul DNA, 2 ul A1 (from 5 pmol/ ul), 1,2 ul B2 (from 5 pmol/ ul), 1ul C1 (from 5 pmol/ ul), 2 ul of 10 x PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C), 1% Triton X-100), 2 ul dNTPs (from 2,5 mM each), 1,2 ul $MgCl_2$ (from 25mM), 0,4 ul Taq and 9,2 ul Gibco H_2O.

- PCR reactions are performed using the same Thermocycler conditions: 1 cycle at 94 °C for 3 min, 30 cycles of 94 °C for 2 min, 59 °C for 1,5 min, 72 °C for 1,5 min and 1 cycle of 72 °C for 5 min.
- Primer sequences:

  A1 (F): 5'- CGCATTTGACAGGAATTCTT -3' (20bp, T_m=54,6°C)

  B2 (R): 5'- TACACAACACAGCCGTCTCA -3' (20bp. T_m=59°C)

  C1 (R): 5'- ATCAAAATACTGGGGCTGCC -3' (20bp, T_m=58°C)