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Analysis of *Pax6* Contiguous Gene Deletions in the Mouse, *Mus Musculus*, Identifies Regions Distinct from *Pax6* Responsible for Extreme Small-Eye and Belly-Spotting Phenotypes

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FILE S1

Extended Material and Methods, and Results

Deletion analysis by array comparative genomic hybridization (array-CGH): An array of 836 overlapping BACs covering Chr 2 from position Mb 3 to Mb 181 was analysed to characterise the *Pax6* deletions. The complete tile path array clone set and detailed information are available at the Sanger website (www.ensembl.org). This array was made and array CGH was performed essentially as described (Chung et al 2004, Genome Res 14, 188-196).

Briefly, reference and test genomic DNAs were labelled with Cy3-dCTP or Cy5-dCTP using random-primer labelling with a BioPrime DNA Labeling Kit (Invitrogen, Carlsbad, CA). Un-incorporated nucleotides were removed on sephadex G-50 columns (GE Healthcare BioScience Corp., Piscataway, NJ). Labelled reference and test DNAs were combined, mixed with 250 µg of mouse Cot-1 DNA (Invitrogen) and yeast tRNA (1.2 mg), and precipitated. The samples were resuspended in ULTRAhyb hybridization buffer (Ambion/Applied Biosystems, Austin, TX), denatured at 70° for 10 min, and incubated at 37° for 60 min. The labelled genomic DNA was added to the slide with a coverslip and was placed in a Slidebooster hybridisation station (Advalytix, Concord, MA) at 37° for 24 hours. Slides were washed in PBS/0.1% Tween 20 for 10 min at room temperature, in 0.1XSSC for 30 min at 55°, and in PBS for 10 min at room temperature and then dried by centrifugation at 150 × *g* for 5 min.

Hybridised microarrays were scanned using a Packard Biochip ScanArray 5000XL scanner (Packard BioScience, Berkshire, UK). We performed all CGH experiments in a fluorochrome-reversed pair of two-color hybridizations. For all data points, we obtained quadruple measurements derived from duplicate spots on arrays from both hybridizations. Spot positions were automatically located with Bluefuse software (BlueFuse for microarrays, BlueGnome Ltd, Cambridge, UK). Data transformations were performed using the same software. The $\log_2$ ratio of the signal from mutant relative to the signal from wildtype was normalised using the block lowess function. Some spots were excluded based on quality control (QC) values or excessive variability between replicates. Spots with a QC value (defined by Bluefuse) below 0.3 were excluded. Spots in replicates within an array, with a SD greater than 0.2, were excluded. Spots among the replicates of the dye swap experiments with a SD greater than 0.4 were also excluded. Copy number variations were called using a CGH smoothing algorithm (Jong et al 2004, Bioinformatics 20, 3636-3637). The $\log_2$ ratio thresholds for calling copy number variation were -0.3 and 0.3.

Deletion analysis by FISH cytogenetics: BAC clones carried in E. coli from the RP23 and RP24 libraries were used in the FISH experiments. BACs mapping to the *Pax6* region were identified from the Ensembl database (Build 35.5, www.ensembl.org) and were purchased from CHORI BacPac Resources (Oakland, CA). A continuous series of overlapping BACs were selected for the region of about 1 Mb around the *Pax6* locus. For mapping of the breakpoint at the distal region of the *Pax6*^{12Neu} deletion an initial series of BACs was selected with 1 Mb distances between neighboring BACs. After narrowing down the breakpoint to 1 Mb further contiguous overlapping BACs were selected for FISH.

A standard DNA extraction from BACs was done by alkaline lysis. About 1 µg of each DNA was labeled either with BiotindUTP or with Digoxigenin-dUTP by a standard nick translation procedure (90 min, 15°). Probe length was analyzed on a 1% agarose gel. The probes showed the optimal average length of about 300 bp after nick translation. About 100 ng from two alternatively labeled BAC DNAs were co-precipitated together with 1 μg mouse Cot-1 DNA (Roche Biochemicals) and dissolved in 10 μl hybridization buffer (50% formamide, 2xSSC, 10% dextransulfate). The DNA was applied to the chromosomes fixed on a slide, mounted with a cover slip and sealed with rubber cement. Probe DNA and chromosomes were denatured together at 72° for 3 min on a hot plate, and hybridized over night at 37° in a wet chamber.

After hybridization the cover slips were carefully removed and the slides were washed in 2xSSC for 8 min. Slides were then incubated at 70° in 0.4xSSC/0.1% Tween for 1 min and then washed shortly in 4xSSC/0.1% Tween/5% BSA at room temperature. Probe detection used an anti-Digoxigenin antibody coupled with rhodamin and Avidin-FITC, respectively. Incubation with the antibody and Avidin was for 30 min at 37° in 4xSSC/0.1% Tween/5% BSA followed by washes in 4xSSC/0.1% Tween two times for 10 min. Slides were then stained in DAPI (4',6-Diamidino-2-phenylindole) for 10 min. For microscopy the slides were mounted in antifade solution (Vectashield).

In situ hybridization signals were analyzed on a Zeiss Axioplan II microscope. Each image plane (blue, green and red) was recorded separately with a b/w CCD camera (SenSys, Photometrics). Chromosomes and FISH signals were then displayed in false colors and images were merged on the computer. Camera control, image capture and merging were done with SmartCapture X software (Digital Scientific, Cambridge, UK).

Deletion analyses with polymorphic SNP sites: We identified a series of SNP or insert/deletion sites which are polymorphic between the strains C3H/HeJ and C57BL/6EL and surround the 5' and 3' deletion breakpoints. Briefly, we retrieved the NCBI sequence data of Chr 2 regions defined by a series of BAC ends surrounding the breakpoints as indicated from the array-CGH and FISH results, and designed primers to sequence the BAC ends from genomic DNA of both strain C3H/HeJ and C57BL/6El. Sites polymorphic between the strains were then sequenced from *Pax6*^{11Neu} and *Pax6*^{12Neu} heterozygotes, in which the deletion mutations were carried over both a wildtype bearing Chr 2 from strain C57BL/6El and a wildtype bearing Chr 2 from strain C3H/HeJ. A site was determined to be not deleted if a double peak at the position of the polymorphic site was observed in the sequence from the mutation heterozygote carried over the wildtype bearing Chr 2 from strain C57BL/6El. A polymorphic site was shown to be deleted if a single peak was observed in the sequence results for the mutation carried over both the C57BL/6El and C3H/HeJ wildtype bearing Chr 2, and the allele observed at the site always corresponded to the allele from the wildtype bearing Chr 2 in the heterozygote construct. The deletion analysis using BAC end sequences above yielded a more accurate localisation of the deletion breakpoints.

We identified BAC sequences which covered the critical regions of the deletion breakpoints (i.e. one end sequence deleted and the other end sequence not deleted), sequenced regions across each of these BACs from strains C3H/HeJ and C57BL6/J, and identified sites polymorphic between the inbred strains. For the *Pax6*^{13Neu} mutation we sequenced regions across a series of BACs covering the *Pax6* gene and the neighboring 5' and 3' regions to identify polymorphic sites between the strains C3H/HeJ and C57BL/6. Sites polymorphic between the strains were then sequenced from *Pax6*^{11Neu}, *Pax6*^{12Neu} and *Pax6*^{13Neu} heterozygotes, in which the deletion mutations were carried over both a wildtype bearing Chr 2 from strain

C57BL/6El and a wildtype bearing Chr 2 from strain C3H/HeJ, and assessed as being deleted or not deleted as above.

Explanation of the results for the deletion analyses with polymorphic SNP sites: The proximal and distal breakpoints of the *Pax6*^{11Neu} deletion were localized by first sequencing for polymorphic sites contained within BAC end sequences over the Chr 2 region Mb 104.83 – 105.76. The proximal breakpoint was shown to be in the region Chr 2 Mb 104.93 – 104.96 defined by the BAC RP23-8C14 for which the 5' end sequence was not deleted and the 3' end was deleted (data not shown). By sequencing for a series of polymorphic sites across the sequence defined by the BAC RP23-8C14, the proximal breakpoint could be more accurately localized to a 634 base region between the polymorphic sites at position 19,723 and 20,357 (Table S4). The distal breakpoint of the *Pax6*^{11Neu} deletion was shown to be within the Chr 2 region Mb 105.43 – 105.76 defined by the BAC RP23-431C3 for which the 5' end was deleted and the 3' end was not deleted (data not shown). By sequencing for a series of polymorphic sites contained within the genomic sequence defined by the BAC RP23-431C3, the distal breakpoint was localized within a 462 base region between the polymorphic sites at positions 132,729 and 133,191 (Table S5).

The proximal and distal breakpoints of the *Pax6*^{12Neu} deletion were localized by sequencing for polymorphic sites contained within BAC end sequences over the Chr 2 region Mb 105.12 – 112.54. The proximal breakpoint was shown to be within the Chr 2 region Mb 105.30 – 105.46, defined by the BAC RP23-444H3 3' end sequence not deleted (position of the most 3' SNP site was at 179692) and the BAC RP23-290H11 3' end sequence deleted. We sequenced for a series of polymorphic sites contained within the sequence defined by the BAC RP23-290H11 and determined that the most 5' polymorphic site to be deleted was at position 2397 (Table S6). The sequencing results 5' to this site were problematical and apparently due to unspecific amplification products. By BAC alignment and inspection of the region in the Ensembl database we could conclude that the proximal deletion breakpoint is contained within a 2548 base region defined by the polymorphic site in RP23-444H3 at position 179692 (not deleted) and the polymorphic site at position 2397 in RP23-290H11 (deleted). The results to localise the distal breakpoint of the *Pax6*^{12Neu} deletion from the FISH cytogenetics and array-CGH were contradictory. The FISH cytogenetic analysis indicated that the genomic sequence defined by the BAC RP23-336F11 was deleted whereas the array-CGH results indicated this region to be not deleted. We analysed polymorphic sites over the sequence defined by this BAC and results indicated that the *Pax6*^{12Neu} deletion covered all polymorphic sites analysed (Table S7). An analysis of polymorphic sites in the neighboring distal genomic region defined by the BAC RP23-35G10 indicated that all polymorphic sites were not deleted (Table S8). By BAC alignment and inspection in the Ensembl database the distal breakpoint of the *Pax6*^{12Neu} deletion could be localised within a 9275 base sequence defined by the last polymorphic site in the BAC RP23-336F11 shown to be deleted (position 210,658) and the first polymorphic site in the BAC RP23-35G10 shown to be not deleted (position 9,097).

The proximal breakpoint of the *Pax6*^{13Neu} deletion was localized by sequencing for a series of polymorphic sites across the genomic sequences defined by the BACs RP23-290H11 (Chr 2 Mb 105.25 – 105.46) and RP23-431C3 (Chr 2 Mb 105.43 – 105.76). All polymorphic sites in the genomic region defined by the BAC RP23-290H11 were shown to be not deleted (data not shown). The proximal breakpoint was shown to be in the sequence defined by BAC RP23-431C3 within a 583 base region between the polymorphic sites at positions 76,948 and 77,531 (Table S9). The distal breakpoint of the *Pax6*^{13Neu} deletion was localized to a 511 base region in the genomic sequence defined by BAC RP23-146D23 (Chr 2 Mb 105.58 – 105.78) between the polymorphic sites at positions 148,218 and 148,729 (Table S10).

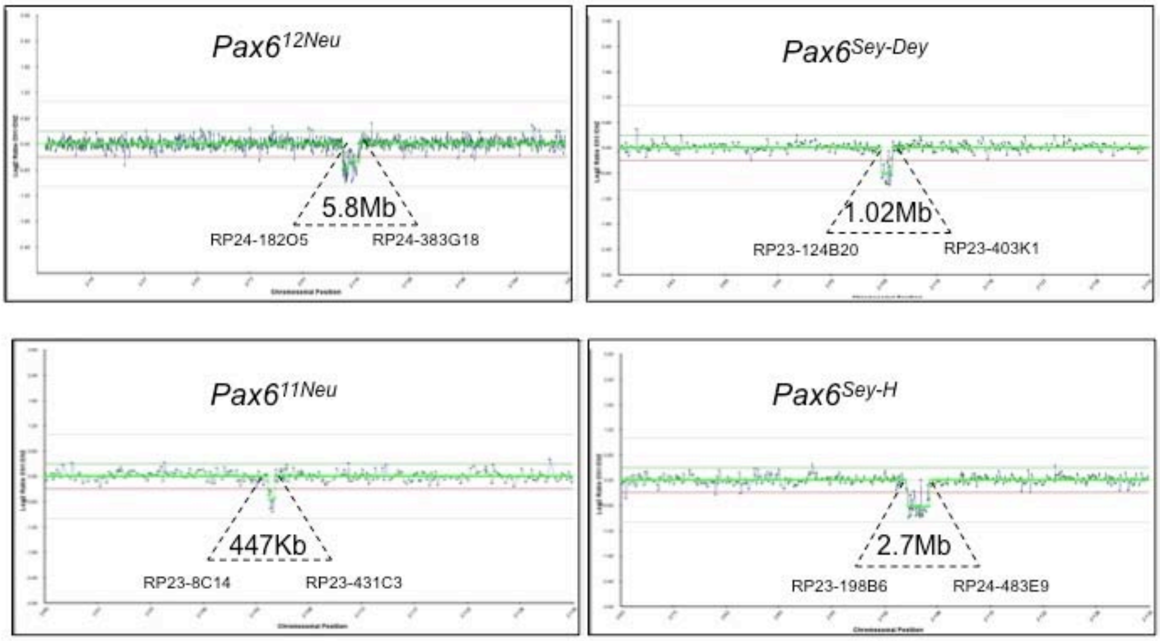

FIGURE S1.—CGH plots of the $\log_2$ of the ratio of the hybridization signal of genomic DNA to mouse Chr 2 BACs from heterozygous *Pax6* deletion mutants relative to the hybridization signal from C3H wildtype. For all four *Pax6* deletion mutants analysed a distinct region surrounding the *Pax6* Chr 2 region displayed a series of overlapping BACs with $\log_2$ ratios lower than the copy number call threshold of -0.3. The *Pax6*^{12Neu} deletion was estimated to be the longest (~5.8 Mb) and the *Pax6*^{11Neu} deletion the shortest (~ 450 kb) of the four deletions analysed.

TABLE S1

Deletion analysis of the mouse *Pax6*[11Neu], *Pax6*[12Neu] and *Pax6*[13Neu] mutations for *Pax6* and flanking Chr 2 microsatellite markers

Marker	Location[a]	*Pax6* Deletions[b]		
		Pax6[11Neu]	*Pax6*[12Neu]	*Pax6*[13Neu]
D2Mit42	104.41	not deleted	not deleted	not deleted
D2Mit387	105.06	Δ	not deleted	not deleted
D2Mit44	105.11	Δ	not deleted	not deleted
D2Mit442	105.16	Δ	not deleted	not deleted
D2Mit185	105.30	Δ	not deleted	not deleted
Pax6	105.47	Δ	Δ	Δ
D2Mit128	106.12	not deleted	Δ	not deleted
D2Mit100	106.34	not deleted	Δ	not deleted
D2Mit58	108.06	not deleted	Δ	not deleted
D2Mit480	109.75	not deleted	Δ	not deleted
D2Mit482	110.89	not deleted	Δ	not deleted
D2Mit163	111.10	not deleted	Δ	not deleted
D2Mit207	111.99	not deleted	not deleted	not deleted

[a] Ensembl build 42 Chr 2 location (Mb).

[b] Δ = deleted.

TABLE S2

FISH cytogenetic analysis of Chr 2 in homozygous wildtype or heterozygous *Pax6[11Neu]* and *Pax6[12Neu]* mouse mutants

BAC ID	Start (Mb)[a]	End (Mb)[a]	*Pax6* Alleles[b]		
			WT	*Pax6[11Neu]*	*Pax6[12Neu]*
RP23-60E18	3.000	3.245	+	+	+
RP23-74F20	3.890	4.105	+	+	+
RP23-394K13	104.237	104.404	+	+	+
RP24-313N13	104.367	104.523	+	+	+
RP23-450K1	104.504	104.674	+	+	+
RP23-198B6	104.647	104.844	+	+	+
RP23-232D1	104.831	104.983	+	+	+
RP24-388E5	104.978	105.138	+	Δ	+
RP24-362E10	105.257	105.426	+	Δ	Δ
RP24-371A16	105.291	105.472	+	Δ	Δ
RP24-325K21	105.428	105.608	+	Δ	Δ
RP23-146D23	105.578	105.774	+	+	Δ
RP24-387K4	105.799	105.954	+	+	Δ
RP23-50K11	105.957	106.156	+	+	Δ
RP23-306B16	106.445	106.653	+	nd	Δ
RP23-467P7	106.538	106.718	+	nd	Δ
RP23-416L9	106.664	106.857	+	+	Δ
RP23-35K10	107.692	107.915	+	nd	Δ
RP23-206C1	109.046	109.241	+	nd	Δ
RP23-239G1	110.648	110.835	+	nd	Δ
RP23-9A5	110.830	111.071	+	nd	Δ
RP23-303N16	110.862	111.036	+	nd	Δ
RP23-336F11	111.163	111.376	+	nd	Δ
RP23-35G10	111.304	111.506	+	nd	+
RP23-88C15	111.506	111.774	+	nd	+
RP24-405C9	111.625	111.796	+	nd	+
RP23-353B17	111.713	111.921	+	nd	+
RP23-63C10	111.904	112.156	+	nd	+
RP23-208E23	112.072	112.282	+	+	+

[a] Ensembl build 42 Chr 2 location (Mb).

[b] + = not deleted; Δ = deleted; nd = not determined.

TABLE S3

Log$_2$ of the CGH hybridization signals to mouse Chr 2 BACs of genomic DNA from heterozygous *Pax6* deletions relative to C3H wildtype

BAC	Start[a]	End[a]	*Pax6* Deletions[b]			
			Pax6^{12Neu}	*Pax6*^{11Neu}	*Pax6*$^{\text{Sey-H}}$	*Pax6*$^{\text{Sey-Dey}}$
RP23-394K13	104.24	104.41	0	0	0	0
RP24-334I20	104.37	104.51	0	0	0	0
RP23-124B20	104.40	104.63	0	0	0	-0.61
RP23-189F6	104.51	104.70	0	0	0	-0.61
RP23-198B6	104.65	104.84	0	0	-0.63	-0.61
RP23-232D1	104.83	104.98	0	0	-0.63	-0.61
RP24-86J23	104.84	105.04	0	0	-0.63	-0.61
RP23-8C14	104.93	105.18	0	-0.56	-0.63	-0.61
RP24-460B2	104.98	105.24	0	-0.56	-0.63	-0.61
RP23-444H3	105.09	105.33	0	-0.56	EXC	-0.61
RP23-247F16	105.12	105.30	0	-0.56	-0.63	-0.61
RP24-182O5	105.23	105.46	-0.48	-0.56	-0.63	-0.61
RP23-290H11	105.25	105.46	-0.48	-0.56	-0.63	-0.61
RP23-431C3	105.41	105.59	-0.48	-0.56	-0.63	-0.61
RP23-403K1	105.44	105.65	-0.48	0	-0.63	-0.61
RP24-244B3	105.58	105.77	EXC	0	EXC	0
RP23-146D23	105.59	105.74	-0.48	0	-0.63	0
RP24-86A17	105.67	105.89	-0.48	0	-0.63	0
RP24-225B15	105.70	105.87	-0.48	0	-0.63	0
RP23-245K23	105.76	105.98	-0.48	0	-0.63	0
RP23-191A18	105.92	106.16	-0.48	0	EXC	0
RP23-50K11	105.96	106.16	-0.48	0	-0.63	0
RP23-415G15	106.05	106.25	-0.48	0	-0.63	0
RP23-173C23	106.09	106.29	-0.48	0	-0.63	0
RP23-253D15	106.17	106.38	-0.48	0	-0.63	0
RP24-461O8	106.22	106.35	-0.48	0	-0.63	0
RP23-300H23	106.25	106.51	-0.48	0	-0.63	0
RP24-100A4	106.26	106.48	-0.48	0	-0.63	0
RP23-140I23	106.45	106.63	EXC	0	-0.63	0
RP23-306B16	106.45	106.65	-0.48	0	-0.63	0
RP23-148F7	106.58	106.77	-0.48	0	-0.63	0
RP23-416L9	106.66	106.86	-0.48	0	-0.63	0
RP23-12N7	106.67	106.87	EXC	0	EXC	0
RP23-359M23	106.77	106.95	-0.48	0	-0.63	0
RP23-417K1	107.02	107.22	-0.48	0	-0.63	0
RP24-369N16	107.21	107.36	-0.48	0	-0.63	0
RP24-294K11	107.26	107.42	-0.48	0	-0.63	0

RP24-483E9	107.38	107.59	-0.48	0	-0.63	0
RP23-35J22	107.41	107.62	EXC	0	0	0
RP23-454N6	107.63	107.79	-0.48	0	0	0
RP23-193A4	107.69	107.92	-0.48	0	0	0
RP24-71G5	107.79	108.03	-0.48	0	0	0
RP24-496D7	107.82	108.00	EXC	0	0	0
RP23-266F4	108.12	108.40	EXC	0	0	0
RP23-176P20	108.14	108.30	-0.48	0	0	0
RP23-444A4	108.28	108.46	-0.48	0	0	0
RP23-226E14	108.38	108.57	-0.48	0	0	0
RP24-170C23	108.41	108.72	-0.48	0	0	0
RP24-135N15	108.42	108.58	-0.48	0	0	0
RP23-405K7	108.61	108.80	-0.48	0	0	0
RP23-469G4	108.76	108.93	-0.48	0	0	0
RP23-450P5	108.88	109.07	-0.48	0	0	0
RP23-206C1	109.05	109.24	-0.48	0	0	0
RP23-248F18	109.14	109.38	-0.48	0	0	0
RP23-70G24	109.25	109.49	-0.48	0	0	0
RP23-393E8	109.40	109.67	-0.48	0	0	0
RP23-207C2	109.52	109.73	-0.48	0	0	0
RP23-184O20	109.68	109.90	-0.48	0	0	0
RP23-431J22	109.75	109.96	-0.48	0	0	0
RP23-300M9	109.90	110.13	-0.48	0	0	0
RP24-331J23	110.41	110.56	EXC	0	0	0
RP23-48D8	110.45	110.68	-0.48	0	0	0
RP23-239G1	110.65	110.84	-0.48	0	0	0
RP23-92H5	110.73	110.91	-0.48	0	0	0
RP24-383G18	111.09	111.27	-0.48	0	0	0
RP23-336F11	111.16	111.38	0	0	0	0
RP23-35G10	111.30	111.51	0	0	0	0

[a] Ensembl build 42 Chr 2 location (Mb).

[b] EXC denotes excluded. See Material and Methods.

TABLE S4

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-8C14 (AL672153) to localise the proximal breakpoint of the *Pax6^{11Neu}* deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6^{11Neu}* -/+
10 239	C	A	C/A
10 366	A	G	A/G
after 12 146	-	insG	het
12 218	T	A	T/A
12 232	G	T	G/T
12 246	C	T	C/T
12 258	T	Δ	het
12 338	T	A	T/A
12 420	T	G	T/G
12 423	A	T	A/T
12 427	A	TGA	het
12 437	G	A	G/A
12 456	G	T	G/T
12 473	T	G	T/G
12 490	A	G	A/G
12 546	A	G	A/G
14 267	C	A	C/A
after 14 312	-	insCCGAA	het
14 418	A	C	A/C
14 524	T	C	T/C
16 281	T	Δ	het
16 503	C	T	C/T
16 511	G	A	G/A
16 531	A	T	A/T
16 560	T	G	T/G
16 621	G	A	G/A
16 639	A	G	A/G
18 754-55	CA	AG	het
18 821-22	CC	AA	het
18 825	T	C	T/C
18 855	G	T	G/T
18 898	C	T	C/T
18 968	A	G	A/G
18 992	A	C	A/C
19 107	G	A	G/A
19 129	T	A	T/A
19 268	T	C	T/C

19 360	C	T	C/T
19 723	T	C	T/C
20 357	T	A	T
20 359	T	C	T
20 947	T	G	T
21 156	G	A	G
21 250	C	G	C
25 208	C	G	C
after 25 244	-	insCTA	-
25 264	C	A	C
after 25 300	-	insTGA	-
25 318	C	T	C
25 332	T	G	T
25 523	A	G	A
25 578	A	C	A
25 631	A	G	A
30 365	A	T	A
30 436	T	C	T
after 30 579	-	insA	-
30 627	G	A	G
30 649	G	A	G
35 147	T	C	T
35 172	G	A	G
35 173	C	Δ	C
35 334	G	A	G
after 35 337	-	insACAG	-

[a] A *Pax6*[11Neu] heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. Non-deleted polymorphic sites (positions 10,239 to 19,723) were identified as carrying the alleles from both C3H/HeJ and C57BL/6. Deleted polymorphic sites (positions 20,357 to 35,337) carry only the C57BL/6 allele. The deleted sites were confirmed by performing the same analysis of a *Pax6*[11Neu] heterozygote constructed over a wildtype bearing Chr 2 from strain C3H/HeJ. In this analysis all deleted sites yielded the C3H/HeJ allele (data not shown).

TABLE S5

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-431C3 (AL512589) to localise the distal breakpoint of the *Pax6[11Neu]* deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6[11Neu]* -/+
91 061	C	G	C
91 066-67	CT	Δ	CT
91 094	C	T	C
91 919	C	G	C
after 91 926	-	insCAT	-
91 964-65	TG	CA	TG
91 970	T	C	T
91 978	C	T	C
91 999	C	T	C
after 92 003	-	insGTA	-
92 063	C	T	C
92 152	A	G	A
92 305	T	C	T
92 516	C	T	C
after 92 684	-	insA	-
94 007	G	A	G
99 941	A	G	A
100 015	A	G	A
100 059	T	G	T
100 155	C	T	C
106 672	C	A	C
106 813	G	A	G
115 885	G	C	G
115 913	T	G	T
115 975	A	Δ	A
116 120	A	G	A
after 131 084	-	insC	-
132 213	C	T	C
132 729	A	G	A
133 191	A	G	A/G
133 213-14	AA	CC	het
133 451	G	A	G/A
after 134 264	-	insCTGCTG TGTCTCC	het
134 309	T	C	T/C
134 369	C	T	C/T
134 373	T	C	T/C
135 168	A	G	A/G

135 429	C	T	C/T
135 440	G	A	G/A
140 496	C	T	C/T
140 503	G	A	G/A
140 540	G	A	G/A
140 591	C	G	C/G
140 627	C	T	C/T
146 363	T	G	T/G
156 688	T	C	T/C
156 732	G	A	G/A
156 742-43	CC	Δ	het
158 721	G	A	G/A
158 835-38	ATGT	Δ	het
163 247	T	C	T/C
163 284	G	A	G/A
169 253	T	C	T/C

[a] A *Pax6[11Neu]* heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. Non-deleted polymorphic sites (positions 133,191 to 169,253) were identified as carrying the alleles from both C3H/HeJ and C57BL/6. Deleted polymorphic sites (positions 91,061 to 132,729) carry only the C57BL/6 allele. The deleted sites were confirmed by performing the same analysis of a *Pax6[11Neu]* heterozygote constructed over a wildtype bearing Chr 2 from strain C3H/HeJ. In this analysis all deleted sites yielded the C3H/HeJ allele (data not shown).

TABLE S6

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-290H11 (AL512582) to localise the proximal breakpoint of the *Pax6[12Neu]* deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6[12Neu]* -/+
2 397	T	C	T
2 481	T	C	T
2 505	G	A	G
2 509	A	T	A
2 606	G	A	G
2 636	A	C	A
2 674	T	C	T
2 691	C	T	C
2 755	-	insT	-
2 781	G	A	G
2 814	C	A	C
2 823	G	T	G
3 060	T	C	T
3 066	T	G	T
3 177	T	C	T
3 307	T	A	T
3 354	G	A	G
5 205	G	A	G
5 240	T	C	T
5 329	G	T	G
5 342	T	A	T
5 432	T	C	T
6 298	A	G	A
6 306	G	A	G
6 348	A	G	A
6 434-37	ACCA	Δ	ACCA
6 459	T	C	T
6 576	G	C	G
6 652	C	T	C
6 990	A	G	A
7 038	A	G	A
after 7 083	-	insC	-
7 168	G	C	G
7 189	C	A	C
10 503-04	TA	Δ	TA
10 551	C	A	C
10 573	T	C	T

10 591	G	A	G
10 699	C	T	C
20 334	T	A	T
20 349	G	C	G
30 435	A	G	A
30 552	A	G	A
30 566	A	C	A
30 841	A	G	A
50 424	-	ins	-
60 433	A	G	A
80 623	G	A	G
80 643	G	A	G
80 982	G	A	G
91 031	C	T	C
101 071	T	C	T
101 215	G	T	G
101 217	A	G	A
101 228	G	Δ	G
101 314	A	G	A
101 340	C	A	C
101 464-65	TT	GC	TT
101 505	T	C	T
101 535	T	C	T
101 546	A	G	A
110 876	G	A	G
110 944	A	G	A
110 995	G	T	G
111 020	G	C	G

[a] A *Pax6^{12Neu}* heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. Deleted polymorphic sites carry only the C57BL/6 allele, starting from position 2397 through to the last 3' site at position 111,020. The deleted sites were confirmed by performing the same analysis of a *Pax6^{12Neu}* heterozygote constructed over a wildtype bearing Chr 2 from strain C3H/HeJ. In this analysis all deleted sites yielded the C3H/HeJ allele (data not shown).

TABLE S7

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-336F11 (AL731699) to localise the distal breakpoint of the *Pax6*[12Neu] deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6*[12Neu] -/+
21 399	A	C	A
21 491	G	C	G
21 589	A	G	A
31 103	T	G	T
31 139	G	A	G
31 167	A	G	A
31 215	G	Δ	G
109 911	T	A	T
110 134	T	C	T
110 153	A	C	A
118 585	T	A	T
118 656	T	A	T
after 118 669	-	insA	-
after 118 884	TTT	Δ	TTT
118 963	A	G	A
141 170	A	G	A
after 141 402	-	insAC	-
after 168 829	-	insA	-
176 859	T	Δ	T
176 889	A	G	A
177 071	C	G	C
177 126	G	T	G
177 230	C	A	C
177 338	A	T	A
184 798	A	T	A
184 877	T	C	T
184 981	T	G	T
184 987	A	G	A
185 036	A	G	A
185 045	A	C	A
185 084	T	A	T
185 335	T	C	T
185 344	G	A	G
193 779	A	T	A
193 789-90	GT	TC	GT
193 957	G	A	G
193 993	C	T	C

193 995-96	AG	GA	AG
193 998	C	A	C
194 080	A	G	A
194 101	G	T	G
194 146	G	A	G
194 161	G	A	G
194 173	A	G	A
194 187	A	G	A
194 237	A	Δ	A
194 251	A	G	A
194 253	A	G	A
194 263	G	A	G
194 277	G	T	G
194 285	T	A	T
194 295	C	G	C
194 319	C	T	C
194 386	A	T	A
194 427	A	G	A
194 453	G	C	G
194 467	A	T	A
194 471	A	C	A
199 322	A	G	A
199 405	A	G	A
after 199 446	ACAGAGAG AG	Δ	ACAGAGAG AG
199 572	C	G	C
after 199 603	-	insTC	-
203 787-88	CC	TT	CC
203 924	A	G	A
204 053	C	T	C
204 282	G	C	G
208 548	G	A	G
208 584	G	A	G
208 625	T	C	T
208 635	T	C	T
208 666	C	T	C
208 781	A	G	A
208 825	G	T	G
208 861	T	C	T
208 926	A	C	A
209 120	T	C	T
209 193	G	A	G
210 220	A	T	A
210 307	A	T	A
210 315	A	T	A
210 388	C	T	C

210 456	C	T	C
210 638	C	T	C
210 658	A	T	A

[a] A *Pax6[12Neu]* heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. All polymorphic sites carry only the C57BL/6 allele, indicating that the *Pax6[12Neu]* deletion covers the entire BAC RP23-336F11. The deleted sites were confirmed by performing the same analysis of a *Pax6[12Neu]* heterozygote constructed over a wildtype bearing Chr 2 from strain C3H/HeJ. In this analysis all deleted sites yielded the C3H/HeJ allele (data not shown).

TABLE S8

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-35G10 (AL732496) to localise the distal breakpoint of the *Pax6*[12Neu] deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6*[12Neu] -/+
9 097	A	G	A/G
9 098	A	T	A/T
9 115	T	G	T/G
after 9 464	-	insA	het
9 527	G	A	G/A
9 547	C	T	C/T
9 587-90	GTTT	Δ	het
after 9 850	-	insTTTG	het
9 874	A	T	A/T
9 879	A	C	A/C
14 957	A	C	A/C
15 010	G	A	G/A
15 016	T	A	T/A
15 033	G	A	G/A
15 188	G	A	G/A
15 190	G	A	G/A
15 211	A	T	A/T
after 15 444	-	insT	het
15 490	G	C	G/C
15 556	A	C	A/C
15 569	A	T	A/T
26 466	G	A	G/A
26 536	T	C	T/C
26 547	T	G	T/G
26 587	C	A	C/A
26 816	A	C	A/C
26 876	A	T	A/T
38 145	A	G	A/G
38 287	A	G	A/G
38 314	A	C	A/C
38 322	T	A	T/A
38 387	G	T	G/T
38 509	A	G	A/G
38 559	A	G	A/G
38 616	A	G	A/G
after 38 690	-	insT	het
38 729	T	Δ	het

38 792	T	C	T/C
38 840	T	A	T/A
38 873	A	C	A/C
38 910	T	G	T/G
38 923	A	C	A/C
38 963	T	Δ	het
49 608	G	A	G/A
49 647	A	G	A/G
49 649	C	T	C/T
49 682	C	T	C/T
49 871	A	Δ	het
49 925	G	A	G/A
57 790	T	A	T/A
57 791-93	TTT	Δ	het
57 802	C	A	C/A
57 819	G	T	G/T
57 927	C	T	C/T
57 937	C	T	C/T
57 954	C	T	C/T
58 095	C	A	C/A
58 108	A	G	A/G
58 116	G	A	G/A
58 143	G	A	G/A
58 175	C	T	C/T
58 201	G	A	G/A
58 237	T	A	T/A
58 239	G	A	G/A
58 318	T	C	T/C
58 347	G	A	G/A

[a] A *Pax6*^{12Neu} heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. All polymorphic sites were shown to be not deleted, starting from position 9097 through to the last 3' site at position 58,347.

TABLE S9

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-431C3 (AL512589) to localise the proximal breakpoint of the *Pax6*^{13Neu} deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6*^{13Neu} -/+
63 038	G	A	G/A
63 406	C	T	C/T
63 792	T	C	T/C
64 790	C	T	C/T
65 497	T	G	T/G
65 787	C	A	C/A
66 269	A	G	A/G
66 384	A	G	A/G
67 207	G	del	het
67 216	G	A	G/A
69 221	G	A	G/A
70 709	G	T	G/T
71 003	T	G	T/G
71 127	G	A	G/A
73 104	A	G	A/G
73 916	G	A	G/A
75 953	-	insTA	het
76 243	C	T	C/T
76 839	T	C	T/C
76 948	G	T	G/T
77 531	T	C	T
77 552	C	T	C
82 699	C	T	C
82 734	A	G	A
82 792	A	G	A
82 862	C	del	C
87 277	A	G	A
87 323	T	C	T
87 454	A	G	A
87 529	A	C	A
87 563	C	T	C
87 567	G	A	G
87 721	A	G	A
88 648/49	AA	del	AA
89 156	A	C	A
89 237	G	A	G
90 479	G	C	G
90 545	A	G	A

90 578	A	T	A
91 061	C	G	C
91 066-67	CT	Δ	CT
91 094	C	T	C
91 919	C	G	C
after 91 926	-	insCAT	-
91 964-65	TG	CA	TG
91 970	T	C	T
91 978	C	T	C
91 999	C	T	C
after 92 003	-	insGTA	-
92 063	C	T	C
92 152	A	G	A
92 305	T	C	T
92 516	C	T	C
after 92 684	-	insA	-
94 007	G	A	G
99 941	A	G	A
100 015	A	G	A
100 059	T	G	T
100 155	C	T	C
140 496	C	T	C
140 503	G	A	G
140 540	G	A	G
140 591	C	G	C
140 627	C	T	C

[a] A *Pax6*^{13Neu} heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. Non-deleted polymorphic sites (positions 63,038 to 76,948) were identified as carrying the alleles from both C3H/HeJ and C57BL/6. Deleted polymorphic sites (positions 77,531 to 140,627) carry only the C57BL/6 allele. The deleted sites were confirmed by performing the same analysis of a *Pax6*^{13Neu} heterozygote constructed over a wildtype bearing Chr 2 from strain C3H/HeJ. In this analysis all deleted sites yielded the C3H/HeJ allele (data not shown).

TABLE S10

Identification of non-deleted and deleted polymorphic sites within the genomic region defined by the BAC RP23-146D23 (AL590380) to localise the distal breakpoint of the *Pax6[13Neu]* deletion[a]

Site position	C57BL/6	C3H/HeJ	*Pax6[13Neu]* -/+
3 535	T	C	T
5 230	G	A	G
10 165	T	C	T
13 897	C	T	C
13 972	C	T	C
20 673	T	C	T
21 173	G	A	G
21 188	A	T	A
54 564	C	T	C
after 54 580	-	ins 19 bp	-
54 608	A	T	A
after 54 614	-	ins T	-
54 816	T	C	T
100 343	T	G	T
100 416	C	G	C
127 262	G	A	G
127 437	A	G	A
127 531	G	del	G
127 710	G	A	G
139 841	C	T	C
139 910	A	G	A
139 966	C	T	C
139 980	T	C	T
140 001	A	G	A
140 086-94	ACACACATA	del	ACACACATA
140 128	A	T	A
140 133-38	ATAAAT	del	ATAAAT
140 329	T	A	T
145 745	T	C	T
146 029	C	A	C
after 147 117	-	ins A	-
147 190	T	G	T
147 217	C	T	C
147 432	C	T	C
147 637	G	A	G
148 064	T	C	T
148 218	A	G	A

148 729	G	A	G/A
149 080	G	A	G/A
150 395	T	C	T/C
150 521	A	G	A/G
150 592	G	A	G/A
151 284	T	C	T/C

[a] A *Pax6[13Neu]* heterozygote was constructed over a wildtype bearing Chr 2 from strain C57BL/6. Non-deleted polymorphic sites (positions 148,729 to 151,284) were identified as carrying the alleles from both C3H/HeJ and C57BL/6. Deleted polymorphic sites (positions 3,535 to 148,218) carry only the C57BL/6 allele. The deleted sites were confirmed by performing the same analysis of a *Pax6[13Neu]* heterozygote constructed over a wildtype bearing Chr 2 from strain C3H/HeJ. In this analysis all deleted sites yielded the C3H/HeJ allele (data not shown).

TABLE S11

Primers used for PCR amplification and sequencing

Region	Primer pair	Sequence	Location[a]
RP23-8C14	1L	AGCCAGGTTTGGCATCTGGAG	10043
	1R	CCATTCCCTTTCCTTCTTCCAACC	10672
	2L	CACTGGTTGTCTCCAACCTCTGG	12076
	2R	TCCATCTTTGTCCCTGCATTCC	12600
	3L	GAGATGCCCTGGAAGTCATTGG	14097
	3R	GGATTTAAATGGAGGGCAGAGCC	14609
	4L	TGATGGGTACAGCAATCTTGGTTG	16190
	4R	GCCCAATGGTTGGCTGTGAGTAG	16802
	5L	TGTGAAGGTGTGGAGAGTTGGAGG	18668
	5R	TGTGCCTGGCTCCTTTCACTTAG	19147
	6L	GCTGACCGTGGAAATGTTCAAGTG	19022
	6R	CCCAGTGCTCTGTCCCAGGC	19811
	7L	CAGTGGGAGGGATGGCAACTAG	19647
	7R	AAGTCTCGGGCCTGCAAAGAC	20401
	8L	GTTTCACTCCCAGATGCCCAGC	20870
	8R	GCTGTGGGAATGGAGGCTCAAG	21313
	9L	TGGTGATTCAAATTCAGGTCCTTGG	25173
	9R	GGAGGTGAGCATCTGCCTCTGC	25636
	10L	CATTGGCACCAGGCTCTTCAC	30159
	10R	CCCTTATTGAGGGCAAGGCAGAC	30651
	11L	TCCTGGCTTTGCATCTTGGGAG	35068
	11R	TACTTCACCACTGCCGCCAAC	35520
RP23-431C3	49L	AGGAGTCAGTCCCTGGCCCTTC	62768
	49R	CTCGTGGTGTAGAGTGGCGCTG	63920
	50L	AAGAGGGTGCTAATCCACTGG	64705
	50R	GAAGTAGTGGAAGCCTGAGGGTGG	65872
	51L	CCTGGAGGCCCTCTTCGG	66195
	51R	AAGAGGCATCCTCTCTTTCGTCG	67316
	52L	TGTGTTCCCTGTCCTGTGGACTC	68148
	52R	TGCTCTTGGGTAAACCTGCTAGGC	68789
	53L	GGAGACTCAGACCTTGTGGCCTTG	69106
	53R	TCATGTCGCGAACAGATACCTCAC	69762
	54L	GAGCTGAGAGATGGACTGTGGG	70661
	54R	GATCTCACACATCTGCTCACCGC	71200
	55L	GGCGCATAATCATCGCCACTG	72907
	55R	AATTGACTCCAGGAGCCTGTGC	73878
	56L	TTTCAAAGGCAAATGTTATCCACTCC	75753
	56R	CCTAAGCCTTCCAGGATTGTACCC	77386

	57L	AAAGCAACAGATGGGCGCAGAC	77167
	57R	AACCCACCCGCTCTTCTTCCTG	77907
	58L	TTAGTGAACTCTCCGCCGTCTCTC	82134
	58R	TCGCATCTGAGCTTCATCCGAG	82998
	59L	AGTCAGAGCTGGACAGTGAGGGTC	87091
	59R	CGTGCCTTCTGTACGCAAAGGTC	87893
	60L	TCACAGTCCAATCATTTTGTGCATC	88520
	60R	CAGAGTTGGGAGTCAGTGAAGTGCC	89691
	61L	ACATGCACCCTAGAGAGATGAGCTG	90415
	61R	TCACCTAAAGCAGCGTTCTCAACC	90903
	12L	CAGGTGAATCACTCTAGGGCAGTGG	90663
	12R	TCACCATCAATAGGGTCCGAATTAAG	91298
	13L	TGTGTGTGTGTTAGCATTTTGAAGGC	91800
	13R	CCTGCTGTCCTCTAATGGGTTGTG	92410
	14L	CACCTGATGGCCTCACACTTCC	92273
	14R	GCCCAGTGTAGCATAAACACGCAC	92828
	15L	CACAAGTCACCATCATCCCATTGC	93721
	15R	TGATGGTAATGGGCTCCGTCG	94344
	63L	GGGATAGGGGATTTTCAGGGG	99781
	63R	GCAGATGCCTGAAGAGGCCAG	100244
	64L	TGCATATTCCTTGAACCTTGCTCAG	140425
	64R	GGGAGACAACCTTTGGTTGGAATC	140887
RP23-290H11	16L	GACAGCATTTGCTGTCACCTGAG	1645
	16R	GGTTCAATTGGTGGTCAACAAGTAG	2404
	17L	ACTTGTTGACCACCAATTGAACCAG	2406
	17R	AAACCCCACAAGATCATTAGATGCTG	3424
	18L	TGTAGTTTCCCTCCTTGTGTTGGAG	5133
	18R	CCTTCCTGTTTCTGACTGCACCC	5573
	19L	GGCACACATTTGTAACCACAGAACAG	6208
	19R	GGGTGGATTCTAGGAAGGGTTCAG	6836
	20L	TCCTAAATTGTGATCCCTCCCAGTG	6662
	20R	CCTCTAGGAGAGGAGCGATTGGC	7269
	21L	GCCCTGTTTCCTGGGACTCCTATC	10396
	21R	CCTTTGTAGCAGGCAACAGAAGTAACC	10872
	22L	AGGGCCCCTTGCTTTGAGG	20243
	22R	TGATTGCCTGTCAGCTACACATAAGG	20783
	23L	CCCAGGGATGTGTGACATGGAC	30412
	23R	TGTCAGGCAAAGGGTGCGAAG	30877
	24L	CCTCCTAACGCTTCCTCCCTTC	50135
	24R	CCTTGGCCAGAGGAGCAAATTC	50814
	25L	ACCTGGATGGATGTCTCCTCTCC	60057
	25R	TTCCAAGGCTCTGATACCACGG	60643
	26L	CATCCAGTGCAGTGGTCAGGTAAG	80481
	26R	CCTGAGAGGTTAGCTTTCTCTGCG	91412
	27L	GGCTCTGCCTGAAACTATTCAGATTC	100995

	27R	GGAGAGATTGAGCAGCGGGTAAC	101598
	28L	TGGATTCAAATCAGGATTCCACAGAC	110566
	28R	ACAGCTGCCAATGGAAACCCAC	111129
RP23-146D23	65L	TGCTGCATTTTGTTTCACTCACTGG	3325
	65R	TCACTCAGATGCTGGGGCAGAC	3915
	66L	CCCAGCAGCTTGCAATAGGAAC	5129
	66R	GGAGTGAATTTGAGACTGGCCTGAG	5743
	67L	GCCCGAGGGAGAGTCAGCAAG	10098
	67R	TGAGTCATGCCAGTTTGCCTTTG	10701
	68L	TGCACATGATCATTCTACTTTGGGC	13619
	68R	GCAGCCAGTTCTTCAGGCAGG	14069
	69L	ATATGGCCACTGGCGCTCAGAC	20600
	69R	GACAGCTGGGTAGGAAGCTTGGC	21275
	70L	CCCAGGGAACTCATGCACAAGG	54336
	70R	GGTCTCGTCGTCACGATTTGG	55258
	71L	TGGGAAGTGAGTCTCAACTGAGGG	100128
	71R	CAAATTTGCACAGCAATGGCC	101057
	72L	CCTCTGTCAGGGCAGGTCATCC	127105
	72R	GCCAACTCGCAGTGGCTTTAG	127839
	73L	TCCCTGTGAATTTGAGGGCAG	139734
	73R	CAGCTCCTGTGAGGATAGCACAAATG	140591
	74L	GACTGTTGGTAGCTAGAGCCCAAGG	145566
	74R	TGTTTGCAGTCCTGGACCATCC	146447
	75L	AAGGTGTGCACCACCACCGTC	146889
	75R	CCCACTGACCAATCATAAGCGG	147717
	76L	CATCCTCGCTGCGGTCCTTC	147625
	76R	CAAACTCAGCCAAACATCAAATTCC	148618
	77L	AACTCTCCAGCTCAAGGGTGGC	150477
	77R	GTCCACGGGAAGAGCGTACCAG	151094
	78L	TCACCCAGGCCCACTTTGATATG	150546
	78R	CCCTCCTGCCTAATCACCTGG	151448
RP23-35G10	42L	TGAACCATCCCTGCATCCCTG	9036
	42R	GGAACAGCCCTTTCATTCTCTTTGG	9964
	43L	TGGTGGTTTCTCGGTGACTGGAG	14888
	43R	GGGTTGTTAGGTTCTCTGGAATGCAC	15643
	44L	GGAAATGCCAGAGCCAGGAAG	26160
	44R	CACAACACGTACACAAAGCCACAG	27060
	45L	CCCAAATAGTCCAATAACCTTGTCAGC	38090
	45R	TCTTGGCTTATTGCCAAGTGATGG	38992
	46L	TGCCAAAGAACAAGGCAACCAC	49267
	46R	GCCATGAATTAGGAAGACACATGAAGG	50170
	47L	AAGGGAGAGAAAGAGGGCCAGAG	57715
	47R	TGGCACAAGTGAGTAAAGTGGGC	58403

Pax6 cDNA	80L	ATCGTAGAGCTAGCTCACAGCGG	369
	80R	TGGGCTATTTTGCTTACAACTT	593
Pax6-*Immp1L* cDNA			
Pax6	81L	CCGGTTGTCAGATCTGCTACTTCC	56
Immp1L	81R	CAGAACACATTACAACACCACCAACG	299
Pax6	82L	CTCCATCAGACCCAGGGCAATC	518
Immp1L	82R	CATCAGAAAATCTCTGGCCATTTGG	684
Pax6^{11Neu} deletion site			
RP23-8C14	83L	TCAGTGTTCCAAGGAGGGCTGT	20177
RP23-431C3	83R	TGCTGCAGACGTGCCAAAGAAC	132806
Pax6^{12Neu} deletion site			
RP23-290H11	84L	CGGTGCTTCTTAGCTCTTCAAATTTCC	1963
RP23-35G10	84R	TGCTCCAAAAACAAGGGCAGCC	6525
Pax6^{13Neu} deletion site			
RP23-431C3	85L	GTCCAGGCATCTCCGGTGTC	78621
RP23-146D23	85R	CAAACTCAGCCAAACATCAAATTCC	148618

[a] Location of the initial 5' base of the primer in the BAC, *Pax6* or the *Immp1L* cDNA sequence.