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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to a mammalian haploid embryonic stem cell and methods for the production of the mammalian haploid embryonic stem cell. In an additional aspect, the invention also relates to a cell culture comprising the mammalian embryonic haploid stem cell and a stem cell line obtainable by proliferation of the stem cell. In a further aspect, the invention relates to the use of the haploid embryonic stem cells in genetic screening.

BACKGROUND OF THE INVENTION

[0002] Genetic screening is an important tool to identify new genes or mutant alleles of known genes that underlie biological processes. Increasingly stem cells, particularly embryonic stem cells, have been used in these screens. Stem cells are characterised by two important properties, which are of value in genetic screening. Firstly, they have an ability to proliferate in an undifferentiated state for prolonged periods of time. Secondly, stem cells are pluripotent. This means that they are capable of differentiating into any cell of the mesoderm, ectoderm or endoderm. As a result, pluripotent cells can develop into any cell of the body.

[0003] It is at present however, very difficult to screen for recessive mutations in mammalian cells. The main reason is that mammalian cells are diploid, meaning that each cell has two copies of each gene. As a result, the phenotypic traits of heterozygous recessive mutations are masked by the second copy of the gene.

[0004] A solution to screen for recessive mutations is to use a haploid mammalian embryonic stem cell. Haploid stem cells are stem cells that in contrast to diploid stem cells possess only one copy of each gene. As a result, the phenotypic traits of recessive mutations are essentially unmasked, and as a result the underlying genes can be easily identified and studied.

[0005] Haploid embryonic stem cells have been obtained from fish as described in Yi and Hong (2009). In this paper, the authors developed haploid embryonic stem cell lines from the medaka fish (*Oryzias latipes*).

[0006] However, notably, at present, no such cells have been obtained from mammals and fish haploid embryonic stem cells can not be used as a substitute. Unlike placental mammals, fish are oviparous animals and have no placentation. As a result, embryonic development differs substantially from fish to mammals. Furthermore, key features such as sex determination and dosage compensation are also regulated differently and implantation, placentation and genomic imprinting are all absent in Medaka fish.

[0007] The use of a near-haploid tumour cell line for genetic screening has been described by Carette et al., (2009). In this paper, insertional mutagenesis was used to generate null alleles in a human cell line haploid for all chromosomes except chromosome 8. Therefore, the cell line described in this paper was not a true haploid. Furthermore, as the cell line carried genetic rearrangements and a

transformed phenotype, its usefulness in genetic screening in developmental system is limited.

[0008] A number of reports have also found near-haploid cells in a variety of human tumours, Nonetheless, except for one case, no near-haploid cell lines have been derived from these tumours (Sukov et al., 2010).

[0009] Finally, Kaufman et al. (1983) describe the production of pluripotent cell lines from haploid embryos from parthenogenetically activated oocytes. However, the cell lines even at early passages of the cells were diploid not haploid.

[0010] Accordingly, there exists a need to produce mammalian haploid embryonic stem cells. Such cells would enable genetic screening for recessive mutations in a developmentally relevant context. For example, it is envisaged that such cells would be an important tool in identifying new genes involved in signalling pathways, developmental decisions and cell cycle regulation. Moreover, there is a need to produce haploid stem cells without tumour-derived mutations, genomic rearrangements or oncogenes. Stem cells with such characteristics are obtained when cells are derived from tumour cells. Accordingly, there exists a need to develop mammalian embryonic stem cells with a normal karyotype.

SUMMARY OF THE INVENTION

[0011] According to a first aspect of the present invention, there is provided a mammalian haploid embryonic stem cell, wherein the stem cell is pluripotent, capable of proliferation, and can maintain a haploid karyotype during proliferation in culture. After haploid ES cells have been established they can be maintained in a wide range of conditions. Haploid ES cells can be cultured in standard ES cell medium conditions usually including LIF and fetal calf serum or Knock-out serum replacement (Invitrogen; 10828028) or chemically defined media using 2i inhibitors.

[0012] In certain embodiments, the haploid ES cells appear to have a smaller size than diploid ES cells.

[0013] In a preferred aspect, the stem cell can maintain a haploid karyotype for at least 15, preferably 20, and more preferably at least 25 passages of the stem cell. A passage is the degree of subculturing of a cell.

[0014] A further aspect of the invention provides a method for the production of a mammalian embryonic stem cell, wherein the method comprises the following steps -

- activating isolated oocytes *in vitro* to produce haploid embryos;
- culturing the activated embryos to the 8-cell, morula or blastocyst stage;
- removing the zona pellucida;
- isolating the inner cell mass of the activated embryos; and
- further culturing the inner cell mass to derive a haploid embryonic stem cell.

[0015] The method may further comprise the step of flow sorting based on DNA content, to isolate

haploid ES cells.

[0016] The *in vitro* activation may comprise incubation in M16 medium, strontium chloride and EGTA. In a further preferred embodiment, the incubation time is no more than 5 hours, preferably 1 to 3 hours. M16 medium may be obtained from Sigma; Cat. no. M7292. Potential other means for oocyte activation include activation with an electric pulse and by using calcium ionophores such as ionomycin.

[0017] In a further aspect, the cultured activated embryo is preferably cultured with kinase inhibitors. The kinase inhibitors may be selected from threonine/tyrosine kinase inhibitors and/or glycogen synthase kinase 3 β inhibitors. In a preferred embodiment, the kinase inhibitors are PD0325901 and CHIR99021 (CHIR99021 from Stemgent Catalog #04-0004; PD0325901 from Stemgent Catalog #04-0006). The culture will typically include fibroblast feeder cells.

[0018] Other culturing methods may include:

1. a) High glucose DMEM medium (PAA, Cat. No. E15-009) or Knockout™ D-MEM (Invitrogen, Catalog# 10829018) with 15% KnockOut™ Serum Replacement (Invitrogen, Catalog# 10828028) supplemented with Glutamin (Invitrogen), beta mercapto ethanol (Sigma), penicillin-streptomycin (Invitrogen), non-essential amino acids (Invitrogen) and 500 units per millilitre recombinant mouse LIF (home made).
2. b) It is possible that conditions as a) but with 15% fetal calf serum (PAA) instead of 15% KnockOut™ Serum Replacement would also be effective.

[0019] Potential other culture methods which may be suitable include:

- c) Serumfree medium with BMP and LIF (commercially available from Millipore, ESGRO Complete PLUS Clonal Grade Medium, Millipore Cat No. SF001-500P)
- d) iSTEM media (Stem Cells, Inc, Cat. No. SCS-SF-ES-01), this is a commercial formulation of the 2i media but does not contain LIF.
- e) A combination of 2i culture conditions and KnockOut™ Serum Replacement (Invitrogen) such as the one used in Hanna et al, 2010, PNAS, 107(20):9222-9227

[0020] The zona pellucida is preferably removed using acidic Tyrode's solution (Millipore; MR-004-D). Oocytes are incubated in Acidic Tyrode's solution until zona pellucida is dissolved. This usually takes 30 seconds up to one minute.

[0021] In a further aspect, the trophectoderm layer of the blastocyst is preferably removed using specific antibodies (immunosurgery) prior to isolation of the inner cell mass. For example, after removal of the zona embryos are incubated with Anti-Mouse Serum antibody produced in rabbit (Sigma, M5774). After washing the trophectoderm layer is lysed using complement (rat serum, home made or commercial such as Complement sera from guinea pig, Sigma S1639). Immunosurgery is not required when ES cells are derived using 2i conditions. The procedure is described in Manipulating the

Mouse Embryo, A LABORATORY MANUAL, Second Ed., Hogan, B. et al., 1994, Cold Spring Harbour Laboratory Press, ISBN 0-87969-384-3).

[0022] The cells of the inner cell mass may be cultured using either fibroblast feeders in serum-free media, cytokines and kinase inhibitors or using high glucose media supplemented with serum or a serum replacement and cytokines. In a more preferred aspect, the serum-free media is N2B27 medium. The cytokine is preferably LIF (Leukemia Inhibitory Factor). In another embodiment the high glucose media is DMEM and the serum replacement is KnockOut™ Serum Replacement (KSR).

[0023] Cell growth may be monitored by microscopy and cell clumps are dissociated using trypsin (0.25%, Invitrogen; 15090046) or Accutase (Invitrogen; A110501). The inner cell mass outgrowth and embryonic stem cells grow as a colony morphology. Haploid embryonic stem cells are obtained when many colonies are obtained in the culture dish which grow rapidly. The cells are passaged every 3 to 4 days, or every 2 to 3 days when growth speeds up. We believe that haploid ES cells are already present at the first passage, but it takes about 4 to 5 passages to expand haploid ES cells from a single ICM to a confluent 6 well sized dish with about 3×10^6 cells.

[0024] Haploid stem cells may be further be enriched from the culture using cell sorting. Preferably, living cells are stained. Any type of cellular DNA stain may be used. Preferably, the stain may be a fluorescent stain such as a Hoechst stain (Hoechst 33342 at a concentration of 1 microgram per millilitre). Haploid cells can be sorted using a cell sorter, preferably a fluorescence-activated cell sorter. Potential other DNA stains include VYBRANT® DYECYCLE™ STAINS for living cells (Invitrogen, Molecular probes), DRAQ5® (4084, Cell Signaling Technology) and Nuclear-ID™ Red DNA Stain (ENZ-52406, ENZO LIFE SCIENCES INTERNATIONAL, INC.) Other enrichment methods may include separation by cell size such as elutriation or subcloning from single cells.

[0025] An additional aspect of the invention provides a cell culture comprising the mammalian haploid embryonic stem cells. The culture conditions for maintaining haploid ES cells include all conditions for derivation (above). Culture in DMEM with 15% serum and LIF has also been successful for maintaining haploid ES cells.

[0026] A further aspect of the invention provides a mammalian haploid embryonic stem cell line obtained by proliferation of the mammalian embryonic stem cell. The embryonic stem cell line is pluripotent, capable of proliferation and has a stable haploid karyotype during proliferation.

[0027] Another aspect of the invention provides the use of the mammalian embryonic stem cell line in genetic screening. In a preferred embodiment, the genetic screen is a genetic forward screen. We have successfully performed a forward genetic screen in the haploid ES cells as follows. A pool of haploid ES cells is exposed to a mutagen such as an insertional vector. Cells containing mutations can be selected for example using a selection marker of the insertional mutagen. The pool of mutant haploid cells can optionally now be diploidized as mutations will be in a homozygous configuration. Selection of desired mutations can be achieved by appropriate metabolic conditions, reporter genes combined with cell sorting or by phenotypic characteristics including cell adhesion and cell surface markers. We have performed a pilot screen as a proof of principle experiment for genes involved in the mismatch repair pathway. In this screen a Piggybac transposon derived vector was used as the insertional mutagen. Selection for cells with mutations in genes required for mismatch repair was achieved using 2-amino-6-mercaptapurine (Sigma, 6-TG), a substance that is toxic to cells proficient

in mismatch repair. The gene mutations were identified by a Splinkerette PCR strategy. This screen identified the two known autosomal mismatch repair genes Msh2 and Fanci (see below) and demonstrates the principle application of haploid mouse ES cells for genetic screening.

[0028] Potential other mutagens include: a) Genetrap vectors such as attenuated viruses; b) Chemical mutagens; c) Physical methods for mutagenesis such as ionizing irradiation.

[0029] Potential other methods for identifying mutations include: a) Reporter gene activity combined with cell sorting; b) Reporter gene activity combined with antibiotic resistance; c) Cell surface molecules combined with appropriate methods for cell isolation such as antibody based; d) Metabolic conditions; e) Exposure to biological agents such as pathogens for instance for identifying host cell factors or receptors for viruses.

[0030] Potential other methods for gene identification include: a) 5 prime race for identification of trapped genes; b) Genome wide expression profiling; c) DNA sequencing; d) directed screening for mutations in specific genes or gene sets such as by DNA sequencing or PCR.

[0031] A benefit of using mouse ES cells is the availability of a large number of genetic modifications that have been introduced into the mouse genome and specific mutations can be readily generated. Therefore, genetic modification of haploid ES cell lines or derivation of haploid ES cells lines from genetically modified mouse strains can be utilized to enable complex screens or make screens more specific. Potential uses are (1) the incorporation of sensitizing mutations for finding synthetic lethal combinations of genes for uncovering parallel or redundant pathways and (2) the introduction of appropriate selection markers such as reporter genes for pathway activity that enable or support the isolation of desired mutations.

[0032] Potential other uses for haploid ES cells include:

1. a) Haploid ES cells can be introduced into mice. One way of doing this is by diploidizing them in culture to obtain parthenogenetic diploid ES cells. These can be introduced via blastocyst injection, morula aggregation or 8-cell injection into chimeric mice. Germline transmission of parthenogenetic mouse ES cells has been reported and presents a route for introducing mutations identified in a screen into mice for further study.
2. b) Haploid ES cells can be used for cell fusion. Two different haploid cells can be combined to give a diploid cell. Similarly, triploid cells result from fusion of a haploid ES cell with a diploid cell. Potential partners for fusion with haploid ES cells are other haploid ES cells for instance to combine two genetic modifications. This could be useful for testing genetic interactions. Other potential applications could be the fusion of naturally haploid cells such as gametes with haploid ES cells to derive diploid cells.
3. c) Haploid ES cells can be used to derive differentiated cells. Haploid ES cells can differentiate but in the course of differentiation they mostly become diploid.

[0033] In a preferred aspect, the mammal is a rodent, preferably a mouse (*Mus musculus*). In a more preferred aspect, the strain of the mouse is CBA/B6. However, a number of different strains of mouse can be used. This would allow different genetic modifications to be introduced, such as selectable marker genes.

[0034] Potential other mouse strains include:

1. a) inbred mouse strains such as 129Sv or C57/BL6
2. b) outbred mouse strains - We have successfully derived haploid ES cells from a mixed background containing a genetic modification of the Xist gene locus (Xist2LOX).
3. c) Genetically modified mouse strains such as strains carrying gene deletions or transgenes. Especially, mouse strains containing reporter genes (including antibiotic resistance or GFP) such as Oct4 or Rex1 promoter driven GFP reporter strains could be used for screening. In addition reporters for cell signalling pathways could be introduced from the mouse strain such as a TOPflash reporter for Wnt signalling pathway activity. In addition mutations for sensitizing the genetic background can be introduced by deriving haploid ES cells from mice carrying gene deletions. An important aspect of haploid mouse ES cells is the ability to realize the potential of a large number of preexisting mouse mutations that could be utilized to screen for specific components in mammalian signaling, cell cycle, and metabolic pathways.

[0035] ES cells with similar properties as mouse ES cells have been described from rats and rabbits (Telugu et al, 2010, The International Journal of Developmental Biology 54: 1703-1711). The method of the present invention could be applied to these mammals.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The present invention will now be described, by way of example only, with reference to the accompanying drawings, in which

Figure 1 shows a strategy for the derivation of the mammalian haploid embryonic stem cells.

Figure 2 shows the characterisation of a haploid mouse embryonic stem (ES) cell line. Figure 2a shows three representative pictures of metaphase spreads of a haploid mouse ES cell line showing a haploid set of 20 chromosomes. DNA was stained with DAPI. Figure 2b shows a FACS analysis of fixed ES cells after DNA staining with propidium iodide. Position of haploid (1 n), diploid (2n), and tetraploid (4n) DNA content is indicated. The haploid line (upper panel) shows a predominantly 1n DNA content with some G2 cells after DNA replication showing as 2n. The contamination with diploid cells is low as judged from the 4n peak corresponding to G2 cells. A diploid control ES cell line (lower panel) shows a 2n G1 peak and a 4n G2 peak.

Figure 3 shows immunofluorescence staining of haploid and control diploid ES cells for detecting Nanog, a marker of pluripotent ES cells, and Gata4, an early ES differentiation marker. Colonies of both haploid and control diploid ES cells express the pluripotency marker Nanog but do not express markers that are indicative of differentiation.

Figure 4 shows analytic flow profiles after DNA staining with PI for 129Sv derived diploid control embryonic stem cells, the haploid H129-1 ES cell line at passage 6 and at passage 12 after two rounds of sorting the 1 n peak.

Figure 5 shows derivation of haploid ESCs. Flow analysis of DNA after PI staining of (a) diploid control ESCs, (b) haploid ESC line HAP-1 at passage 7 (p7) and (c) HAP-1 (p11) after sorting at p7. (d)

Colony morphology of haploid ESCs (HAP-1). (ef) Chromosome spreads of HAP-3 (e) and HAP-1 (f), (Scale bar = 10 μ m). (g) CGH analysis of HAP-1 and HAP-2 ESCs and control male CBA kidney DNA. Relative copy number is plotted at 200 kb resolution using a log₂ scale. Genomic positions indicated by blue bars (top) are enlarged at 40 kb resolution in (h); CBA control (black), HAP-1 (red) and HAP-2 (green).

Figure 6 shows expression analysis of haploid ESCs. (a) Immunofluorescence shows Nanog protein (red) in haploid (HAP-1) and diploid ESCs, and Gata4 (green) in differentiated cells (Scale bar = 10 μ m). (b) Expression of pluripotency markers in haploid and diploid (set to 1) ESCs by real time PCR. Error bars represent standard deviation (n=3). (c) Scatter plot showing log₂ transformed average expression values from gene expression profiles of 3 haploid (HAP-1, HAP-2 and HTG-1) and three diploid J1 ESC lines for 45,001 probe sets (r is the Pearson correlation coefficient; red lines indicate 2-fold up- and down-regulation). (d) Diagram of more than 2-fold up- and down-regulated genes in haploid ESCs.

Figure 7 shows developmental potential of haploid ESCs. (a) GFP marked haploid HAP-2 ESCs (p18) contribute to chimeric embryos at E12.5. 6 out of 9 embryos showed GFP contribution. A GFP negative embryo is shown as a control (below). (b) Representative flow analyses of DNA content of all cells (above) and GFP positive cells (below) extracted from a chimeric E12.5 embryo are shown. All 6 embryos gave similar results. (c) Live born chimeric mice were obtained from GFP marked HAP-2 ESCs. (d) Chimeric mice obtained from injection of HAP-1 ESCs into C57BL/6 blastocysts (black) show coat colour contribution from the ESCs (agouti).

DETAILED DESCRIPTION OF THE INVENTION

Example 1

Oocyte activation

[0037] Oocytes were isolated in the morning from mouse oviducts after hormonal induction of ovulation. Ovulation was induced by intraperitoneal injection of PMS and hCG 48 hours later (this superovulation procedure is described in *Manipulating the mouse embryo*, ISBN 0-87969-384-3). A potential other method for obtaining unfertilized oocytes could be through natural matings with vasectomised males.

[0038] Activation of oocytes was carried out by incubation in M16 medium to which 5 mM (millimolar) SrCl₂ and 2 mM EGTA were added. Incubation times of 1 to 3 hour were found to be efficient. Shorter incubation led to lower activation rates whereas 5 hour incubation appeared to affect subsequent development. After activation oocytes were cultured in in groups of 50 using microdrop culture. For this 80 microliter drops of M16 medium were overlayed with mineral oil. All culture media were preequilibrated and incubation was at 37°C, 5% CO₂ in a tissue culture incubator (SANYO). Blastocyst stage embryos were obtained at day 3 to 4 and used for ES cell derivation.

[0039] Activation of freshly ovulated oocytes *in vitro* is mediated by induction of Calcium (Ca) waves. This can be achieved by prolonged culture in medium without Ca. The addition of Strontium (Sr) has been used to increase activation rates and enhance the development of diploid parthenogenetic embryos (Bianchi *et al.*, 2010). Complexing the Ca with EGTA has made it possible to use preformulated media in combination with Sr for efficient activation of mouse oocytes (Kishigami and Wakayama, 2007). Similar protocols are established based on permeabilization of the oocyte membrane for Ca by ionomycin treatment. These protocols are more relevant for other mammalian species including human where Sr does not lead to efficient oocyte activation.

ES cell derivation in chemically defined medium

[0040] For derivation of ES cells in chemically defined medium 8-cell embryos were cultured for two days in M16 supplemented with the following inhibitors: 1 μ M PD0325901 and 3 μ M CHIR99021 (M16-2i). 2i is a composition that enhances the efficiency of ES cell line derivation and thereby reduces the number of embryos required (see, for example, Nature. 2008 May 22;453(7194):519-23. The ground state of embryonic stem cell self-renewal. Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, Cohen P, Smith A).

[0041] This step expanded the inner cell mass of the blastocyst. The zona pellucida was then removed with acidic Tyrode's solution (Hogan *et al.*, 1994). Optionally, the trophectoderm was removed by immunosurgery. Isolated inner cell masses or embryos were then cultured on mitotically inactivated embryonic fibroblast feeders in chemically defined N2B27 medium containing above 2i inhibitors and LIF (N2B27-2i plus LIF). LIF is a cytokine that inhibits embryonic stem cell differentiation and stimulates stem cell growth. Outgrowths were passaged repeatedly every 3 to 4 days until ES cell lines emerged. In 2i ES cell lines emerged after the first passage.

[0042] ES cell culture in defined medium with signal inhibition (Ying *et al.*, 2008) can be used to derive ES cells from recalcitrant mouse strains (Nichols *et al.*, 2009) and rats (Buehr *et al.*, 2008). Recently, similar approaches have led to a culture of an embryonic stem cell state from humans that have similar properties with mouse ES cells (Hanna *et al.*, 2010).

ES cell derivation in standard conditions

[0043] For ES cell derivation the zona pellucida was removed from morula and blastocyst stage embryos with acidic Tyrode's solution (Hogan *et al.*, 1994). After removal of the trophectoderm by immunosurgery (Hogan *et al.*, 1994) the inner cell mass was explanted and cultured in high glucose DMEM supplemented with fetal bovine serum or serum replacement (KSR) and LIF. Outgrowths were passaged every 3-4 days until ES cell lines were obtained.

Enrichment for cells with 1n content using cell sorting

[0044] HOECHST 33342 stained living cells were sorted using a DAKO MoFlo high speed sorter. Cells with a haploid (1 n) DNA content were selected. Diploid cell lines (2n) were used as controls. For

analysis the FlowJo Flow Cytometry Analysis Software was used (Tree Star Inc.).

[0045] Additionally, ES cell lines can be subcloned.

Characterization of haploid ES cell lines

[0046] DNA content was investigated by analytical FACS (Fluorescence Activated Cell Sorting). Karyotypes were identified by metaphase chromosome spreads. Haploid ES cell lines display a characteristic colony morphology and growth rate comparable to diploid mouse ES cells. In addition these cells express markers of mouse ES cells (Fig. 3).

Forward genetic screen to identify genes involved in mismatch repair.

[0047] This proof of principle screen was designed according to a screen performed previously (Genome Res. 2009 Apr;19(4):667-73. Epub 2009 Feb 20. A piggyBac transposon-based genome-wide library of insertionally mutated Blm-deficient murine ES cells. Wang W, Bradley A, Huang Y).

[0048] 5×10^6 haploid ES cells were co-transfected with a gene trap cassette embedded in a PiggyBac vector and a vector carrying the PiggyBac transposase gene. Integration of the gene trap cassette into a transcribed locus brings a puromycin resistance cassette under control of the endogenous promoter. This allows selection for successful integration of gene trap cassettes into the genome using puromycin (Sigma; P8833) at a concentration of 2 microgram per millilitre. The pool of puromycin resistant haploid ES cells was used for further screening. Cells were seeded at a density of 1.5×10^6 per 15cm plate and selection for mutations in MMR genes was performed using 2 μ M, 2-amino-6-mercaptapurine (Sigma, 6-TG). Selection was initiated 24h after plating and continued for 8d. Colonies were picked and expanded before analysis. PiggyBac integrations were mapped by Splinkerette PCR. (Mikkers, H., et al (2002) High-throughput retroviral tagging to identify components of specific signaling pathways in cancer. Nat. Genet. 32:153-159.) Results are shown in table 2.

Conclusion

[0049] The above results describe the successful production of a mouse haploid embryonic stem cell. As discussed previously, to date there has been no prior description of a mammalian haploid cell and cell line. Indeed, it was previously believed that the derivation of such cells was impossible. Moreover, the above-described methods allow haploid embryonic stem cells to be derived from unfertilised oocytes. As a result, such cells do not contain tumour derived mutations, genomic rearrangements or oncogenes. We therefore describe a means for deriving embryonic stem cells with a normal haploid karyotype. Haploid ES cells provide a platform for highly efficient forward genetic screening in mammalian cells. Further, we believe that haploid ES cells are maintained most purely if they are sorted every few passages. Haploid ES cells also have a tendency to diploidize, which could enable the generation of developmental models.

Table 1 - Derivation of haploid mouse ES cell lines

derivation protocol	genetic background	Oocytes activated	Nr of blastocysts	ES cell lines obtained	ES cell lines with haploid contribution	max. haploid contribution (%)
2i / immunosurgery	CBA/B6	132	30	27	6	40
2i	CBA/B6	22	10	5	1	15
2i	mixed	50	32	3	3	60
KSR / immunosurgery	CBA/B6	273	48	22	6	10

Table 2 - Forward genetic screen in haploid ES cells to identify genes involved in mismatch repair

genes identified	
cell line number	known MMR genes
1	Msh2
2	Fanci
3	Msh2
novel MMR candidates or false positives	
4	Zfp462, Lypdl, nlg1, Slc44a3
5	Acdb6, Lypdl, nlg1
6	Usp14, At11, 7SK
7	Wdr40a, Sorbs2

Example 2**Derivation of haploid embryonic stem cells from the 129Sv inbred mouse strain**

[0050] 140 oocytes were collected from superovulated 129Sv female mice and activated using strontium chloride and EGTA in M16 medium for 2 hours. Embryos were cultured in M16 medium until the blastocyst stage. 13 blastocysts were obtained, the zona was removed and the inner cell masses were cultured in 2i medium in the presence of LIF and BSA. From a total of 10 embryonic stem cell lines obtained, 3 showed a substantial content of cells with a haploid genome equivalent. The haploid cell content was estimated between 40% and 60%. Sorting of the haploid 1 n peak allowed the establishment of pure haploid 129Sv mouse embryonic stem cells cultures. Figure 4 shows analytic flow profiles after DNA staining with PI for 129Sv derived diploid control embryonic stem cells, the haploid H129-1 ES cell line at passage 6 and at passage 12 after two rounds of sorting the 1 n peak.

Example 3

[0051] Most animals are diploid but haploid-only and male-haplo species have been described ¹. Diploid genomes of complex organisms limit genetic approaches in biomedical model species such as in mice. To overcome this problem experimental induction of haploidy has been used in fish ^{2,3}. In contrast, haploidy appears less compatible with development in mammals ^{4,5}. Here we describe haploid mouse embryonic stem cells and show their application in forward genetic screening.

[0052] Experimentally induced haploid development in zebrafish has been utilized for genetic screening ². Recently, haploid pluripotent cell lines from medaka fish have also been established ³. In contrast to fish, haploidy is not compatible with development in mammals. Although haploid cells have been observed in egg cylinder stage parthenogenetic mouse embryos ⁶ the majority of cells in surviving embryos become diploid. Previous attempts to establish pluripotent stem cell lines from haploid embryos have resulted in the isolation of parthenogenetic embryonic stem cells (ESCs) with a diploid karyotype ⁴. These studies reported the development of apparently normal haploid mouse blastocysts with a defined inner cell mass (ICM) ^{4,5}. In order to investigate the haploid ICM, we cultured haploid mouse blastocysts in chemically defined medium with inhibitors of mitogen activated protein kinase kinase (MEK) and glycogen synthase kinase 3 (GSK3). This 2i medium ⁷ has previously been used for isolating ESCs from recalcitrant mouse strains ⁸ and rats ⁹ and may help to maintain certain characteristics of early mouse epiblast cells ^{10,11}.

[0053] We generated haploid mouse embryos by activation of unfertilized oocytes isolated from superovulated B6CBAF1 hybrid female mice using strontium chloride. After culture in M16 medium 30 blastocysts (22 %) were obtained from 132 activated oocytes and used for ESC derivation. After removal of the zona and trophectoderm, ICMs were cultured in gelatinized 96 well dishes in 2i medium in the presence of LIF. 27 ESC lines were obtained (93 %). Individual ESC lines were expanded and their DNA content was analysed by flow analysis using diploid ESCs as controls (Fig. 5ab). In 6 ESC lines, at least 10 % of the cells had a haploid DNA content and the proportion of haploid cells could reach a conservatively estimated 60 % (Fig. 5b). Further enrichment was achieved by flow sorting of cells with a haploid DNA content after staining with HOECHST 33342 (Fig. 5c). This allowed expansion of haploid ESC lines for over 35 passages.

[0054] We further tested the requirements for deriving haploid mouse ESCs (Table 3). These experiments showed that removal of the trophectoderm by immunosurgery was not essential. Haploid ESCs could also be established using DMEM medium supplemented with Knockout Serum Replacement (KSR) and LIF showing that derivation without kinase inhibitors is possible (Table 3 and Suppl. Fig. 1). We further succeeded in isolating haploid ESCs from the 129Sv inbred mouse strain and two genetically modified mouse lines. In the latter several alleles had been bred to homozygosity and maintained on a mixed genetic background for several generations (Table 3, and Suppl. Fig. 2). In summary, we derived 25 haploid ESC lines in 7 independent experiments. Haploid ESC cultures could also be maintained on feeders in serum containing DMEM supplemented with LIF.

[0055] Haploid ESCs exhibited a typical mouse ESC colony morphology (Fig. 5d). Chromosome spreads showed 20 chromosomes corresponding to the haploid mouse chromosome set (Fig. 5ef). For further characterizing the genetic integrity we performed comparative genomic hybridization (CGH) of 4 haploid ESC lines and control DNA from the CBA strain and the mixed transgenic mouse line from which HTG-1 and HTG-2 ESCs were derived (Fig. 5gh and Suppl. Fig. 3 and 4). Copy

number variations (CNVs) that were detected in the genome of haploid ESC lines were also present in the strains of origin (Suppl. Table 1). Albeit some CNVs appeared haploid ESC specific, inspection of the actual signals (Suppl. Fig. 4) suggested that these CNVs were also present in the CBA or HTG control DNAs but not detected with the threshold applied. CNVs between the C57BL6 and CBA strain of mice were consistent with a previously reported analysis¹². Taken together these data show that haploid ESCs maintained an intact haploid genome without amplifications or losses.

[0056] On the molecular level, haploid ESCs expressed pluripotency markers including *Oct4*, *Rex1*, *Klf4*, *Sox2* and *Nanog* (Fig. 6ab). Genome-wide expression analysis showed a high correlation (Pearson correlation coefficient $r=0.97$ over all genes) between haploid ESCs and control diploid male ESCs (Fig. 6c and Suppl. Fig. 5). In haploid ESCs 279 and 194 genes were more than 2-fold up- or down-regulated ($p<0.05$), respectively (Suppl. Table 2). Among these, 99 X-linked genes were overexpressed and 4 Y-linked genes were lost in haploid ESCs consistent with different sex chromosome constitutions (Fig. 6d). Thus, haploid ESCs largely maintain a mouse ESC transcription profile.

[0057] This prompted us to investigate the developmental potential of haploid ESCs. For this we introduced a piggyBac transposon vector for expressing green fluorescent protein (GFP) into HAP-2 ESCs. Flow sorting of cells for GFP fluorescence and DNA staining with Hoechst 33342 yielded a haploid ESC population that expressed GFP at high level showing that a haploid genome content was maintained during the transfection procedure (Suppl. Fig. 6). GFP marked haploid ESCs contributed substantially to chimeric embryos when injected into C57BL/6 blastocysts (Fig. 7a). The great majority of GFP positive cells extracted from chimeric embryos had a diploid DNA content (Fig. 7b) indicating that haploid ESCs contributed extensively to development after diploidization. We also obtained 2 male and 2 female live born chimeras with a substantial contribution from haploid ESCs (Fig. 7c). These mice developed normally with apparent coat colour chimerism. Similar results were obtained with the HAP-1 and HTG-2 ESCs (Fig. 7d and Suppl. Fig. 7a). Furthermore, the diploid fraction of HAP-2 ESCs at passage 31 could be differentiated into Nestin positive cells following a neural *in vitro* differentiation protocol¹³ (Suppl. Fig. 7b). Taken together these findings demonstrate that haploid ESCs maintain a wide differentiation potential. These chimeric mice have transmitted the GFP transgene from the GFP transgenic HAP-1 haploid embryonic stem cell line to two of their offspring. This is evidence for the ability of introducing mutations or genetic modifications into mice via haploid ES cells.

[0058] To investigate the utility of haploid ESCs for genetic screening we performed a pilot screen for mismatch repair genes following a previously published strategy¹⁴. For this, 5×10^6 haploid ESCs were co-transfected with a gene trap piggyBac transposon vector (Suppl. Fig. 8a) and a plasmid for expressing an optimized piggyBac transposase¹⁵. Gene trap insertions were selected with puromycin. A pool of 1×10^7 cells was then cultured in the presence of 2-amino-6-mercaptopurine (6-TG) which is toxic to mismatch repair proficient cells. After 8 days 20 6-TG resistant colonies were isolated and the integration sites were mapped using Splinkerette PCR¹⁶. Of 7 clones analysed we identified two independent insertions in *Msh2* and one in *Hprt* (Suppl. Fig. 8b). *Msh2* is a known mismatch repair gene and *Hprt* is required for converting 6-TG into a toxic metabolite¹⁴. Thus, identification of mutations in autosomal genes was possible suggesting a potential for haploid ESCs in forward genetic screening in mammals.

[0059] The difficulty in obtaining haploid ESC lines in previous attempts might be explained by

aberrant gene regulation such as aberrant dosage compensation and genomic imprinting. However, diploid ESCs from mouse and human parthenogenetic embryos have been established^{17,18}. Misregulation of X inactivation has been observed to some extent in haploid mouse embryos⁵ and has also been shown to reduce the efficiency of producing cloned mice¹⁹. Thus, it is conceivable that X inactivation is initiated aberrantly in haploid embryos during some ESC derivation procedures. Direct capture of naive pluripotent cells from ICM outgrowths as accentuated by the use of 2i conditions¹¹ could have contributed to the success of our study.

[0060] Previously, near-haploid cells have been observed in human tumours (for literature review see²⁰) and a near-haploid human tumour derived cell line has been described^{21,22}. These tumour cells carry genomic rearrangements and mutations that might stabilize the haploid genome. An interesting aspect of haploid ESCs is their developmental potential. We have observed rapid diploidization when haploid ESCs differentiate. The resulting diploid parthenogenetic cells can contribute to development²³. It is interesting to speculate whether differentiated haploid lineages can be generated perhaps through suppression of X inactivation and whether it is possible to derive haploid human ESCs.

Methods summary

[0061] For the derivation of haploid ESCs mouse oocytes were activated in M16 medium as described²⁴. ESC culture in chemically defined 2i medium has been described previously^{7,8}. Cell sorting for DNA content was performed after staining with 15 µg/ml Hoechst 33342 (Invitrogen) on a MoFlo flow sorter (Beckman Coulter) selecting the haploid 1n peak. For analytic flow profiles cells were fixed in ethanol, RNase treated, and stained with propidium iodide (PI). For karyotype analysis cells were arrested in metaphase with demecolcine (Sigma). After incubation in hypotonic KCl buffer cells were fixed in methanol-acetic acid (3:1) and chromosome spreads were prepared and stained with DAPI. RNA was extracted using the RNeasy Kit (Quiagen). Transcription profiles were generated using Affymetrix GeneChip 430.2 arrays. Sample preparation, hybridization, and basic data analysis were performed by Imagenes (Berlin, Germany). Further analysis was performed using the Genespring GX software (Agilent). For CGH analysis genomic DNA was isolated from haploid ESC lines and hybridized to NimbleGen 3x720K whole-genome tiling arrays by Imagenes (Berlin, Germany) using C57BL/6 kidney DNA as a reference. For chimera experiments GFP labelled HAP-1 (p29), HAP-2 (p18) and HTG-2 (p23) ESCs were injected into C57BL/6 host blastocysts. Live born chimaeras were analysed for expression of GFP at postnatal day 2. Genetic screening was performed following a previously published strategy²⁵. In brief, HAP-1 ESCs were co-transfected with 2 µg piggyBac transposase expression vector¹⁵ and 1µg piggyBac gene trap vector (Suppl. Fig. 8) using Lipofectamine 2000 (Invitrogen). Selection for transposon insertions was performed using 2 µg/ml puromycin for 8 days. 1×10^7 puromycin resistant ESCs were plated in two 15 cm dishes and mutations in mismatch repair genes were selected using 0.3 µg/ml 6-TG (Sigma). piggyBac integration sites in seven 6-TG resistant clones were mapped by Splinkerette PCR¹⁶.

References for example 3

[0062]

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Table 3: Derivation of haploid mouse ESC lines

Exp. No	derivation protocol	Names of ESC lines used in study	genetic background	Oocytes activated	number of blastocysts	ESC lines obtained	ESC lines with haploid contribution (max. % haploid before sorting)
1	2i / immunosurgery	HAP-1 to 6 †	B6CBAF1	132	30	27	6 (>60%)
2	2i	HAP-7	B6CBAF1	22	10	5	1 (>15%)
3	2i	HTG-1 to 3 ‡	mixed TG*	50	32	3	3 (>90%)
4	KSR / immunosurgery	HAP-8 to 13	B6CBAF1	273	48	22	6 (>10%)
5	2i	H129B6-1 to 5	129B6F1	250	37	8	5 (>10%)
6	2i	HTX-1	mixed TG**	70	11	1	1 (>40%)
7	2i	H129-1 to 3	129Sv	140	13	10	3 (>60%)

† Contribution to chimeric mice was confirmed for the HAP-1 and HAP-2 haploid ESC lines.

‡ Contribution to chimeric mice was confirmed for the HTG-1 haploid ESC line.

* Derived from *ROSA26^{nlsrtTA} LC1 Xist^{2LOX}* homozygous female mice.

** Derived from *ROSA26^{nlsrtTA} tetOPXist* homozygous female mice³⁰.

Methods for example 3**Derivation of haploid ESCs**

[0063] Oocytes were isolated from superovulated females and activated in M16 medium using 5 mM strontium chloride and 2 mM EGTA as described ²⁴. Embryos were subsequently cultured in M16 or KSOM medium microdrops covered by mineral oil. Under these conditions around 80 % of oocytes reached the 2-cell stage on the next morning. Thereafter development of preimplantation embryos was variable with a large number of embryos showing unequal sized blastomeres or unusual embryo

morphology. Removal of the zona, immunosurgery for removal of the trophectoderm and ESC derivation was performed as described previously ^{7,8}. ESCs were cultured in chemically defined 2i medium plus LIF as described ^{7,8} with minor modifications. 2i medium was supplemented with non essential amino acids and 0.35% BSA fraction V. Culture of ESCs on feeders was performed as previously described ²⁶. Knockout serum replacement (KSR) was obtained from Invitrogen. Cell sorting for DNA content was performed after staining with 15 µg/ml Hoechst 33342 (Invitrogen) on a MoFlo flow sorter (Beckman Coulter). The haploid 1n peak was purified. Diploid cells did arise in cultures to various extents in all ESC lines. Periodic purification by flow sorting every four to five passages allowed us to maintain cultures containing a great majority of haploid ESCs in all cases. Analytic flow profiles of DNA content were recorded after fixation of the cells in ethanol, RNase digestion, and staining with propidium iodide (PI) on a Cyan analyser (Beckman Coulter). For karyotype analysis cells were arrested in metaphase using demecolcine (Sigma). After incubation in hypotonic KCl buffer cells were fixed in methanol-acetic acid (3:1) and chromosome spreads were prepared and stained with DAPI. Immunostaining was performed as described ²⁷ using Nanog (Abcam; 1:100), Oct4 (Santa Cruz; 1:100), Nestin (Developmental Studies Hybridoma bank, Iowa City; 1:30) and Gata4 (Santa Cruz; 1:200) antibodies.

Microarray analysis

[0064] RNA from biological triplicates of diploid ESCs and three independently derived haploid ESCs (HAP-1 p21, HAP-2 p24, HTG-1 p11) was extracted using the RNeasy kit (Quiagen). Gene expression analysis on Affymetrix GeneChip 430 2.0 arrays was performed by Imagenes Ges.m.b.H. (Berlin, Germany). Additional gene expression profiles of neural progenitors, mesodermal progenitors and mouse embryonic fibroblasts (MEF) were obtained from a previously published dataset (GEO accession number GSE12982 ²⁸). The data was analysed using Genespring GX software (Agilent Technologies). Data were normalized using the RMA algorithm. Lists showing differentially regulated genes (> 2 fold change; p < 0.05) are provided in Suppl. Table 2. p-values were established by an unpaired t test followed by FDR adjustment by the Benjamini Hochberg method. Hierarchical clustering was performed based on the Euclidean distances and complete linkage analysis. The relatedness of transcription profiles was determined by calculating the Pearson correlation coefficient (r). DNA samples for comparative genomic hybridization (CGH) experiments were extracted and sent to Imagenes Ges.m.b.H. (Berlin, Germany) for CGH analysis using NimbleGen 3x720K mouse whole-genome tiling arrays with an average probe spacing of 3.5 kb. Adult male C57BL/6 kidney DNA was used as a reference. A genomic overview of these analyses is presented in Fig.5g and Suppl. Fig. 3 at 200kb resolution and selected zoomed in regions at 40 kb resolution. The complete data set at 40 kb resolution is included in Suppl. Fig. 4.

Accession of datasets

[0065] Gene expression and CGH data sets can be accessed as the GEO reference series GSE30879 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30879>). This series includes the GSE30744 (Expression analysis of haploid and diploid ES cells in 2i medium) and the GSE30749 (CGH analysis of haploid ES cells) data sets.

Quantitative gene expression analysis

[0066] RNA was extracted using the RNeasy kit (Quiagen) and converted into cDNA using the Quantitect reverse transcription kit (Quiagen). Real time PCR was performed on a StepOnePlus machine (Applied Biosystems) using the Fast Sybr green master mix (Applied Biosystems) and previously published primers ²⁷. The ddCt method was used for quantification of gene expression. Expression levels were normalized to L32 ribosomal protein mRNA and values in diploid control ESCs were set to 1.

Embryo analysis

[0067] Haploid ESCs were co-transfected with a piggyBac vector carrying a CAG-GFP-IRES-hygro transgene and a piggyback transposase expression plasmid. Stable integrants were selected using 150 µg/ml Hygromycin for 7 days. The haploid fraction of HAP-1 (p29), HAP-2 (p18) and HTG-2 (p23) GFP positive cells were purified by flow sorting (Suppl. Fig. 6). GFP labelled ESCs were expanded and injected into C57BL/6 host blastocysts which were transferred to recipient females. Embryos were analysed at E9.5 and E12.5. Dissociation to single cells was performed by incubation in 0.25% Trypsin/EDTA for 15 min. Prior to PI staining cells were fixed in 4% PFA and permeabilized in PBS/0.25% Triton X-100. Live born chimeras were analysed at postnatal day 2 (P2) for expression of GFP using UV illumination. Images were obtained using a Canon Powershot S5 IS camera with a FHS/EF-3GY2 filter (BLS). All mouse experiments were conducted in accordance with institutional guidelines of the University of Cambridge. All necessary UK home office licenses were in place.

Gene Trap screen

[0068] The screen was performed based on a previously published protocol ²⁵. 5×10^6 HAP-1 ESCs were co-transfected with 2 µg piggyBac transposase plasmid ¹⁵ and 1 µg piggyBac gene trap vector (Suppl. Fig. 8a) using Lipofectamine 2000. piggyBac insertions into expressed genes were selected with 2 µg/ml puromycin for 8 days. 1×10^7 ESCs corresponding to approximately 5,000 puromycin resistant colonies were then plated onto two 15cm dishes. Selection for mismatch deficient integrants was performed using 0.3µg/ml 6-TG (Sigma). 20 colonies were picked and piggyBac integration sites of seven clones were identified by Splinkerette PCR and mapped using iMapper ²⁹.

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HAPLOIDE EMBRYONISKE PATTEDYRESTAMCELLER

PATENTKRAV

1. Haploid embryonisk pattedyrestamcelle, hvor stamcellen er pluripotent, i stand til at proliferere og i stand til at bevare en haploid karyotype under proliferation i dyrkning i mindst 15 passager.
- 5 2. Stamcelle ifølge krav 1, hvor cellen kan proliferere, når den dyrkes i betingelser, der er udvalgt fra:
 - a) N2B27 kemisk defineret medium med LIF- og 2i-hæmmere;
 - b) med 15 % KnockOut™ serumerstatning tilsat glutamin, beta-mercaptoethanol, penicillin-streptomycin, ikke-essentielle aminosyrer og 500 enheder pr. milliliter rekombinant muse LIF; og/eller
 - c) DMEM-medium med højt glucoseindhold eller Knockout™ D-MEM med 15 % fotalt
- 10 kalveserum tilsat glutamin, beta-mercaptoethanol, penicillin-streptomycin, ikke-essentielle aminosyrer (Invitrogen) og 500 enheder pr. milliliter rekombinant muse-LIF.
3. Stamcelle ifølge krav 1 eller 2, hvor proliferationsbetingelserne endvidere indbefatter et fibroblastfodelag.
4. Haploid embryonisk pattedyrestamcelle ifølge et hvilket som helst af kravene 1 til 3, hvor
- 15 stamcellen kan indeholde en haploid karyotype i mindst 20 passager.
5. Haploid embryonisk pattedyrestamcelle ifølge et hvilket som helst af kravene 1 til 4, hvor pattedyret er *Mus musculus*.
6. Haploid embryonisk pattedyrestamcelle ifølge krav 5, hvor stammen er CBA/B6.
7. Fremgangsmåde til fremstilling af den haploide embryoniske pattedyrestamcelle ifølge et hvilket
- 20 som helst af kravene 1 til 6, hvor fremgangsmåden omfatter følgende trin:
 - aktivering af isolerede oocytter *in vitro* for at fremstille haploide embryoer;
 - dyrkning af de aktiverede embryoer til blastocyststadiet;
 - fjernelse af zona pellucide;
 - isolering af de aktiverede embryoers indre cellemasse;
 - 25 - yderligere dyrkning af den indre cellemasse og
 - berigelse af de dyrkede celler for at udlede en haploid embryonisk stamcelle.
8. Fremgangsmåde ifølge krav 7, hvor:
 - a) de aktiverede embryoer dyrkes med kinasehæmmere; eller
 - b) den indre cellemasse dyrkes ved anvendelse af fibroblastfødere i et serum-frit medium,
- 30 LIF og kinasehæmmere eller ved anvendelse af et medium med højt glucoseindhold tilsat serum eller en serumerstatning og LIF;

hvor kinasehæmmerne omfatter hæmmere af threonin/tyrosinkinaser og glycogensyntasekinase 3β.
9. Fremgangsmåde ifølge krav 7, hvor aktivering *in vitro* omfatter inkubation i M16-medium, strontiumchlorid og EGTA, fortrinsvis hvor inkubationsperioden ikke er længere end 5 timer, fortrinsvis 1
- 35 til 3 timer.
10. Fremgangsmåde ifølge krav 7, der endvidere omfatter fjernelse af trophectodermen ved immunkirurgi før isolering af den indre cellemasse.
11. Fremgangsmåde ifølge krav 7, hvor

- 2 -

- a) zona pellucida fjernes ved anvendelse af sur tyrode-opløsning; eller
 - b) passage af cellerne af den indre cellemasse hver 3. til 4. dag, indtil den haploide embryoniske stamcellelinje er opnået.
12. Fremgangsmåde ifølge krav 7, hvor pattedyret er *Mus musculus*, fortrinsvis hvor stammen er CBA/B6.
13. Anvendelse af den haploide embryoniske pattedyrestamcelle ifølge et hvilket som helst af kravene 1 til 6 i genetisk screening
14. Cellekultur, der omfatter den haploide embryoniske pattedyrestamcelle ifølge et hvilket som helst af kravene 1 til 6.
- 10 15. Haploid embryonisk pattedyrestamcellelinje, der kan opnås ved proliferation af stamcellen ifølge et hvilket som helst af kravene 1 til 6.

DRAWINGS

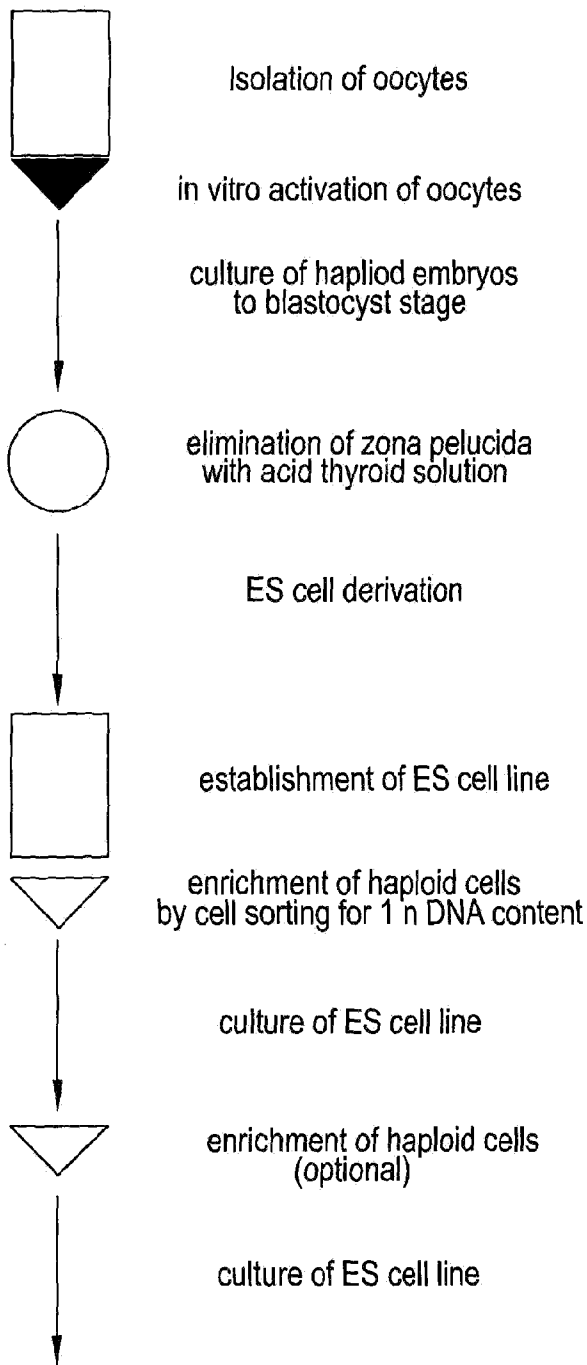


Figure.1

Strategy for the derivation of haploid mouse embryonic stem (ES) cell lines

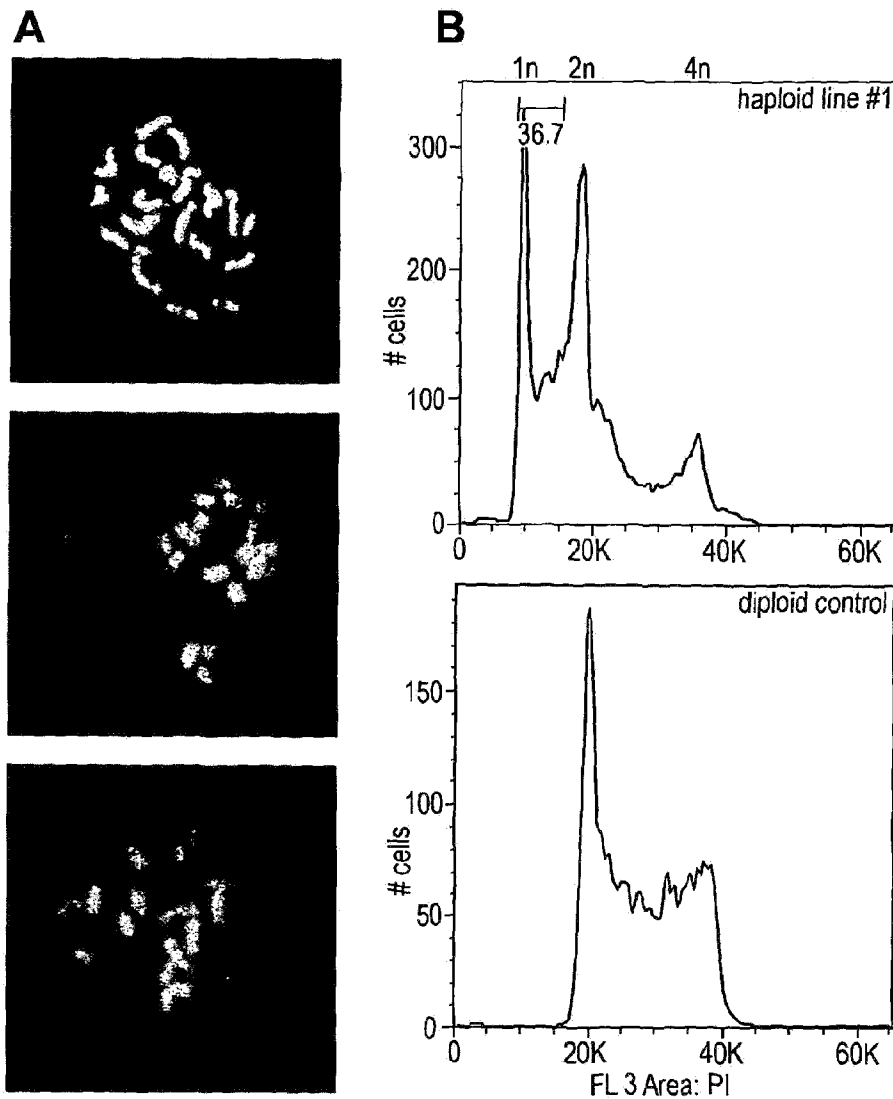


Figure.2

Characterization of a haploid mouse embryonic stem (ES) cell line

A) Three representative pictures of metaphase spreads of a haploid mouse ES cell line showing a haploid set of 20 chromosomes. DNA was stained with DAPI.

B) FACS analysis of fixed ES cells after DNA staining with propidium iodide. Position of haploid (1n), diploid (2n), and tetraploid (4n) DNA content is indicated. The haploid line (upper panel) one shows a predominantly 1n DNA content with some G2 cells after DNA replication showing as 2n. The contamination with diploid cells is low as judged from the 4n peak corresponding to G2 cells. A diploid control ES cell line (lower panel) shows a 2n G1 peak and a 4n G2 peak.

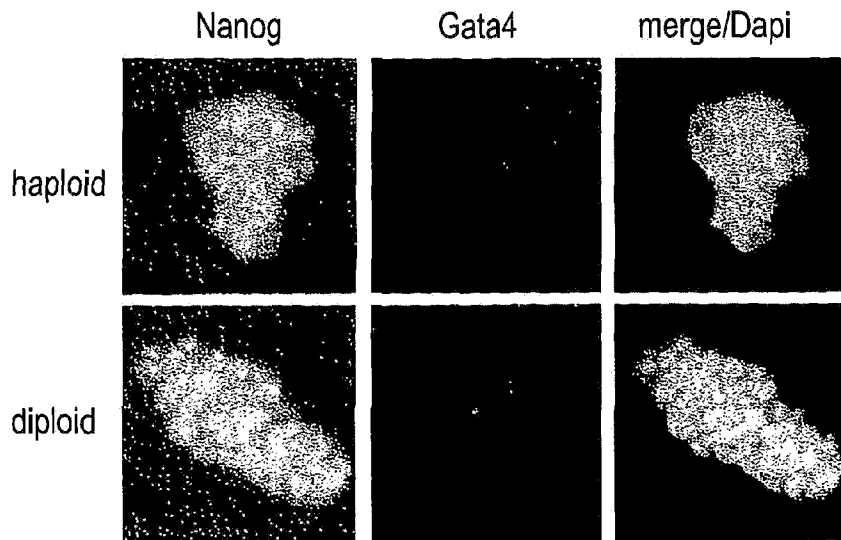


Figure.3

Characterization of a haploid mouse embryonic stem (ES) cell line

Immunofluorescence staining of haploid and control diploid ES cells for detecting Nanog, a marker of pluripotent ES cells, and Gata4, a marker of early ES differentiation marker. Colonies of both haploid and control diploid ES cells express the pluripotency marker Nanog but do not express markers that are indicative of differentiation.

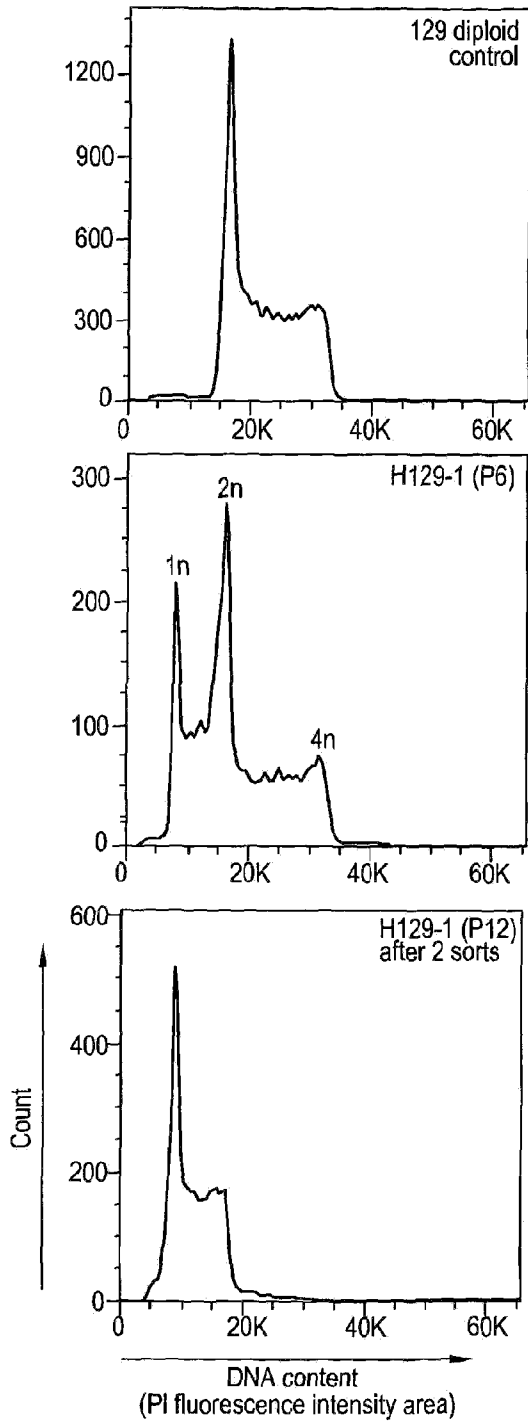


Figure.4

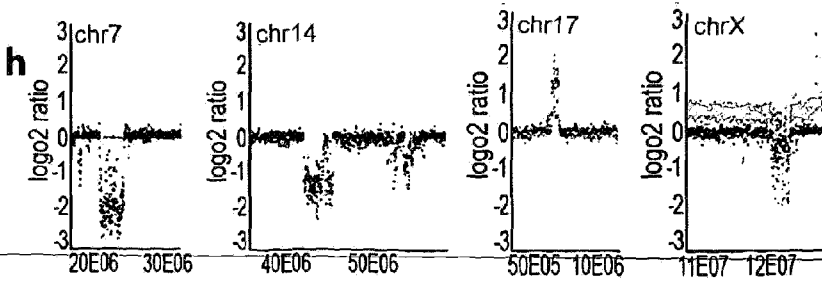
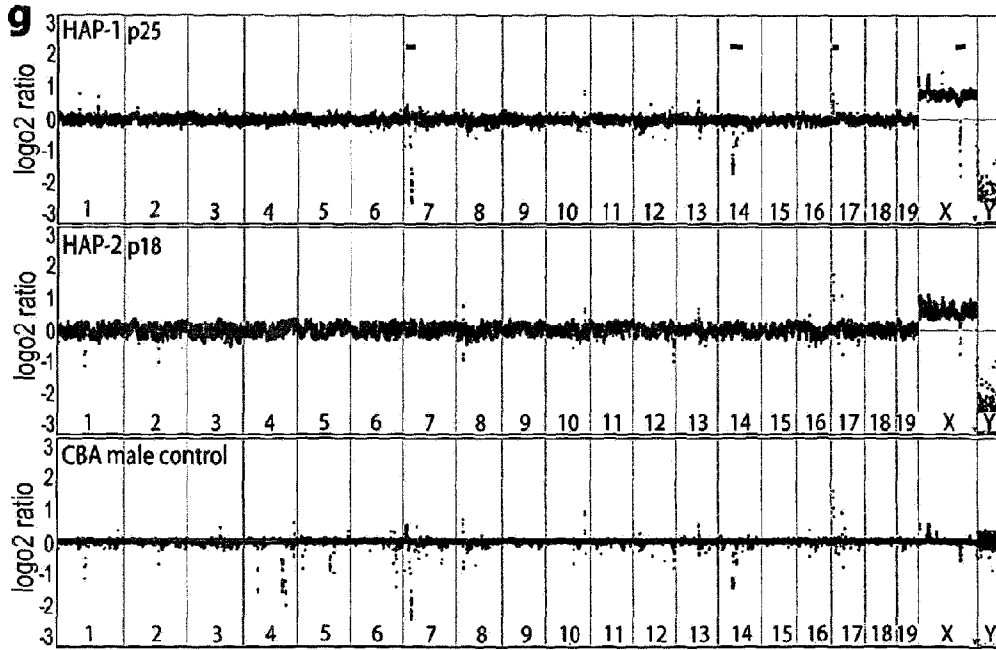
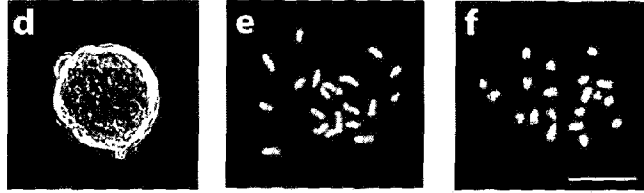
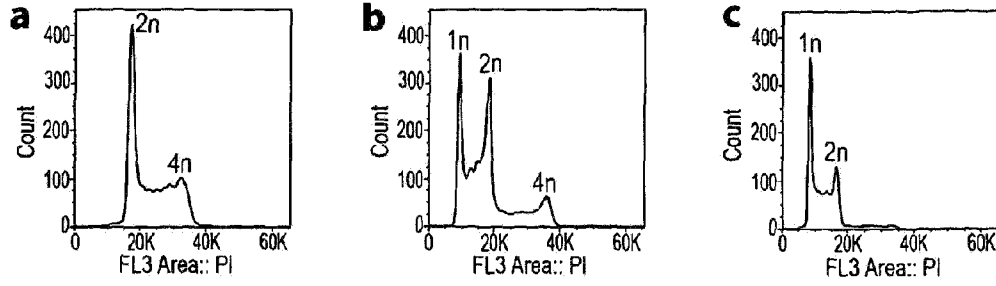


Figure 5

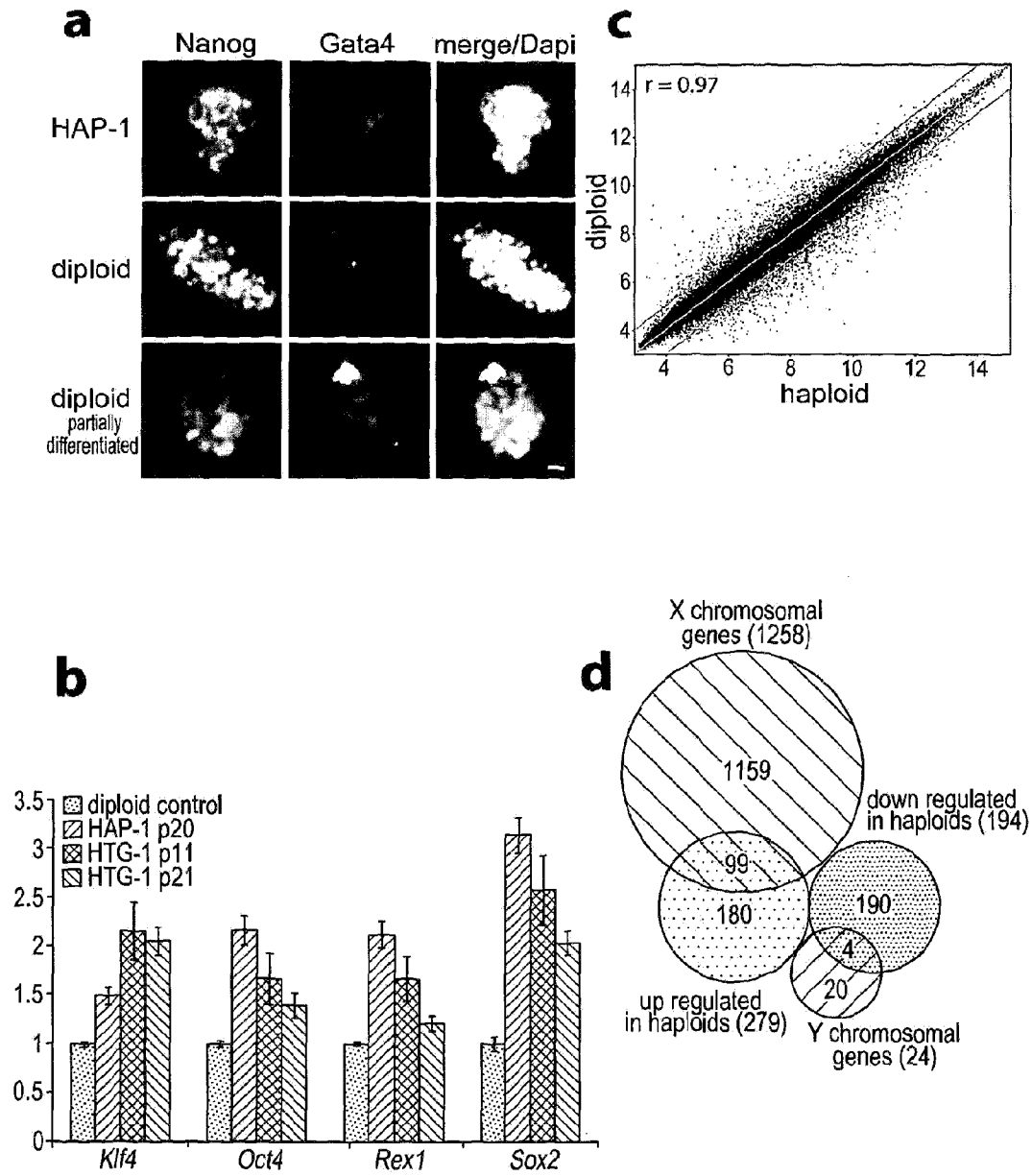


Figure.6

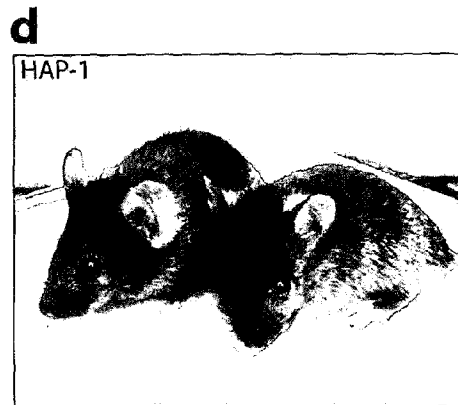
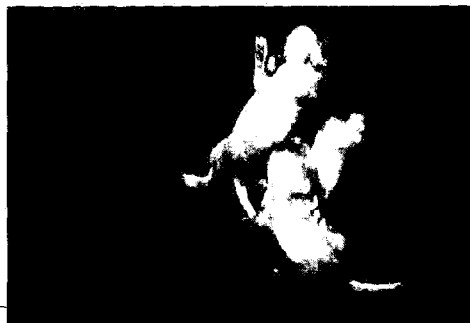
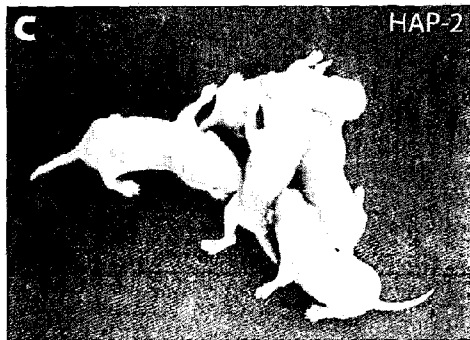
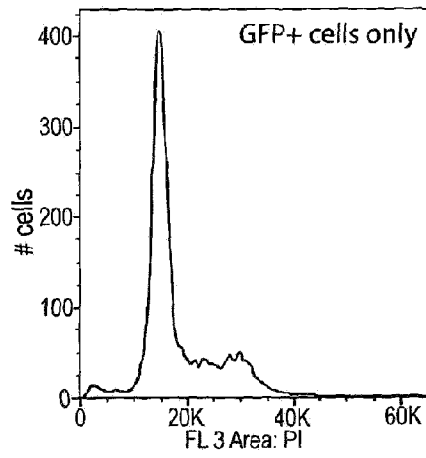
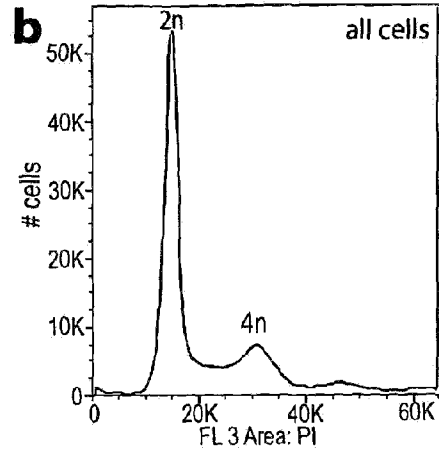
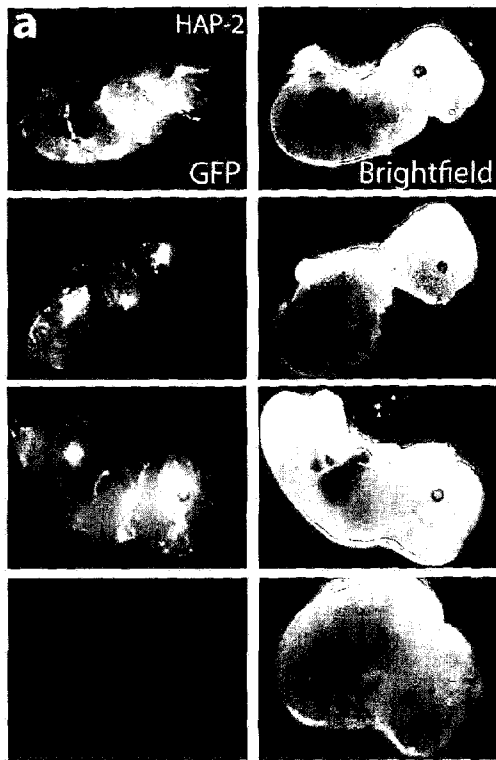
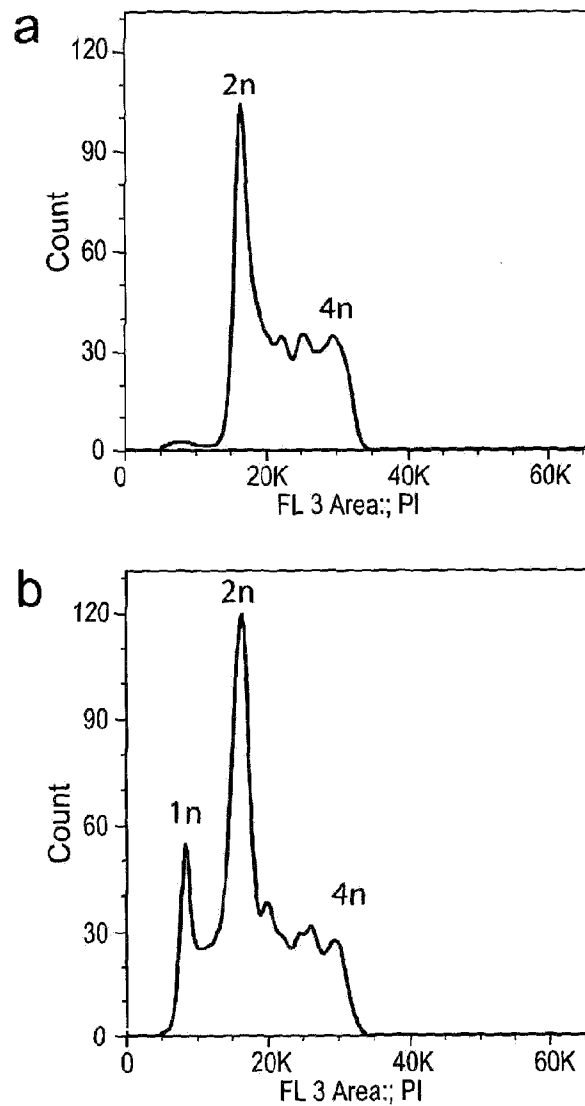
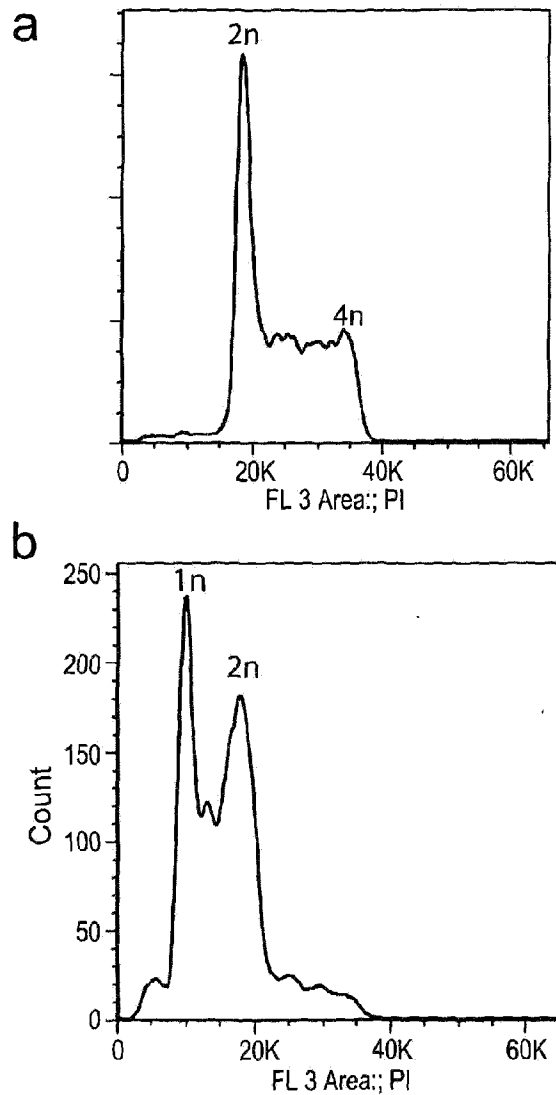


Figure 7

SUPPLIMENTARY FIGURES**Supplementary Figure 1 - Derivation of CBA/B6 hybrid haploid ES cells in KSR**

(a) A flow profile of DNA content after PI staining recorded from diploid control shows a 2n and 4n peak.

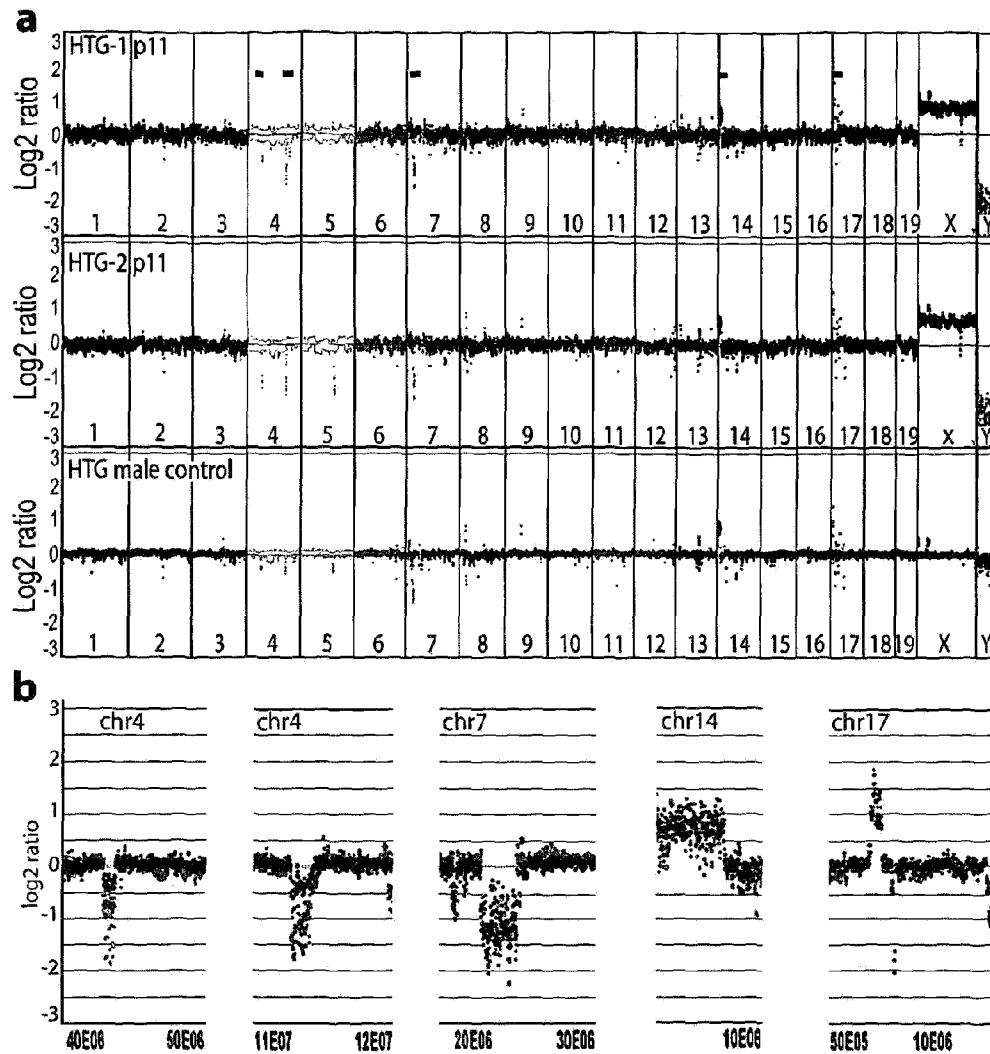
(b) A flow profile of DNA content of an ES cell line (HAP-9, p8) derived from haploid blastocysts using Knockout Serum Replacement shows an additional 1n peak.



Supplementary Figure 2 - Derivation of haploid ES cells from a mixed genetic background in 2i medium

(a) A flow profile shows the DNA content of a diploid control ES cell line after PI staining.

(b) A flow profile of a haploid ES cell line (HTG-1, p8) derived from blastocysts with a mixed genetic background shows a nearly pure haploid cell population with 1n and 2n peaks but no 4n peak.



Supplementary Figure 3 - CGH analysis of HTG haploid ES cells.

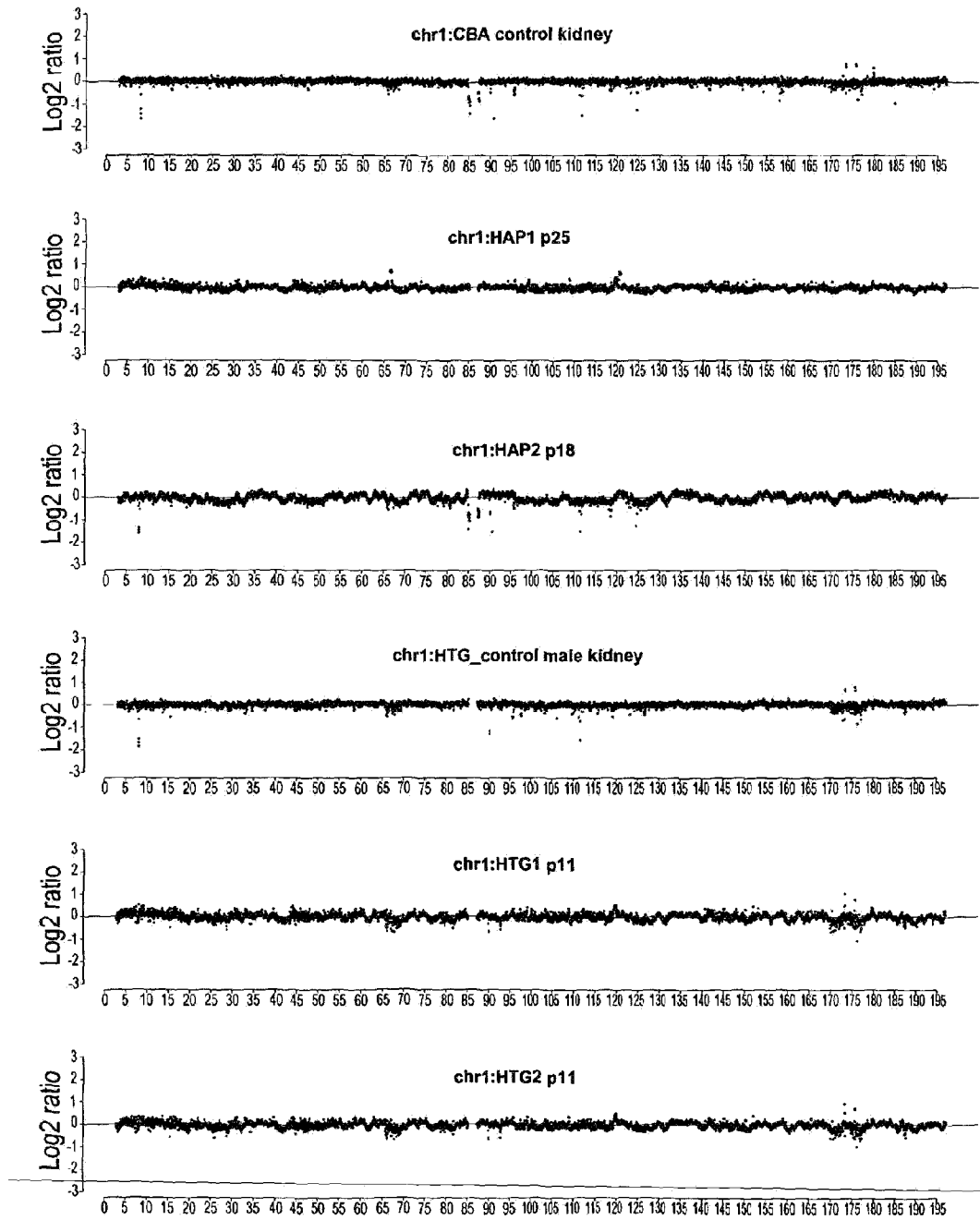
(a) Genomic overview of copy number variations (CNVs) in 200kb resolution in HTG-1 and HTG-2 haploid ES cells and control male kidney DNA from the mixed strain background of which the ES cells were derived. Average values of log₂ ratios are plotted using somatic C57BL6 DNA as a reference. Blue bars on top indicate the positions of regions enlarged in panel b.

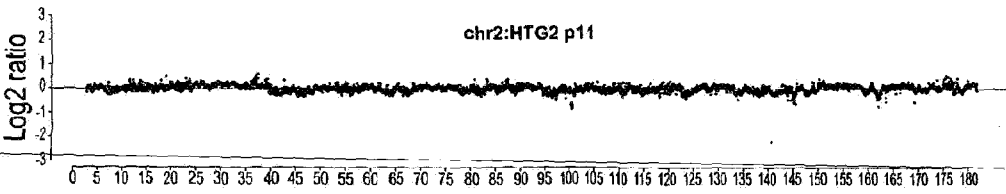
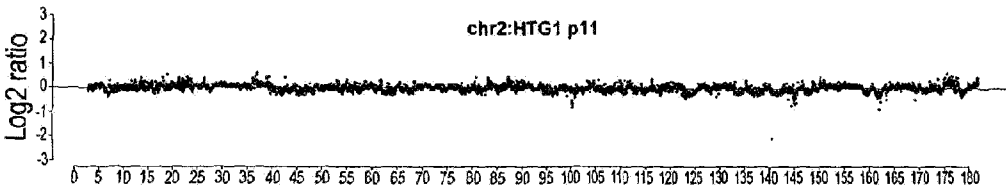
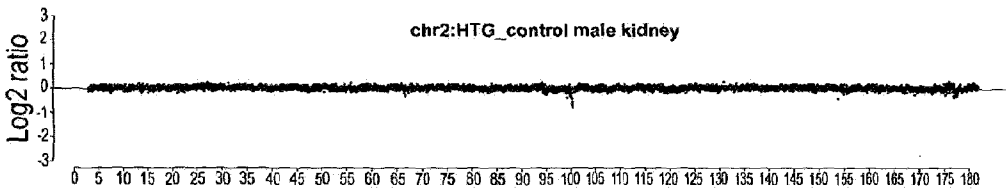
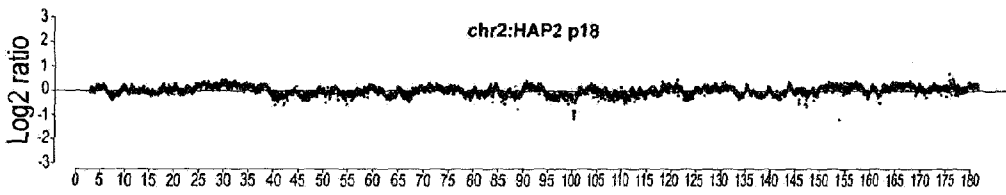
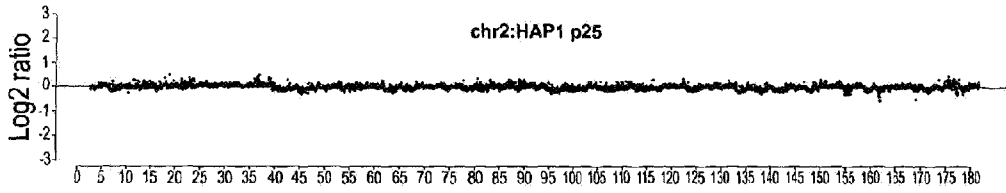
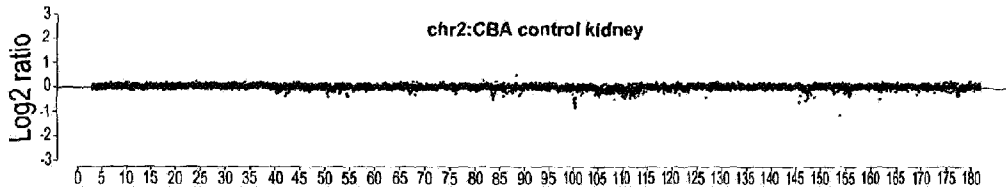
(b) Zoom in views of regions with CNVs on chromosomes 4, 7, 14 and X are shown.

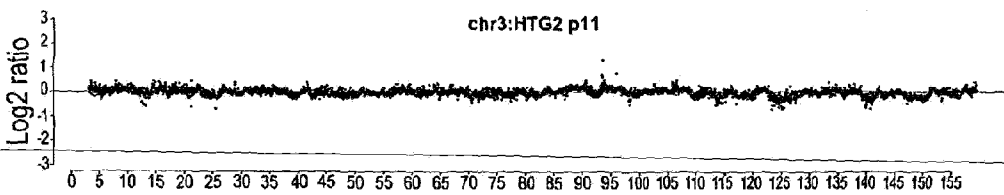
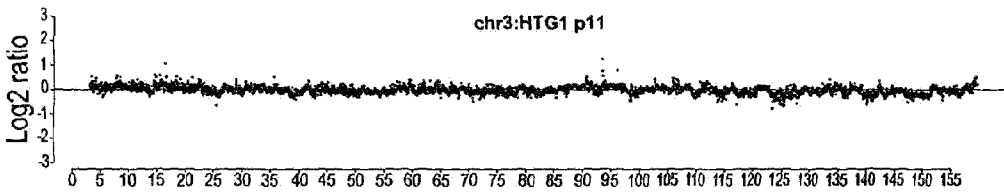
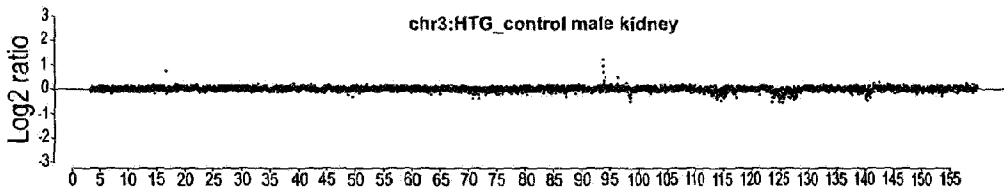
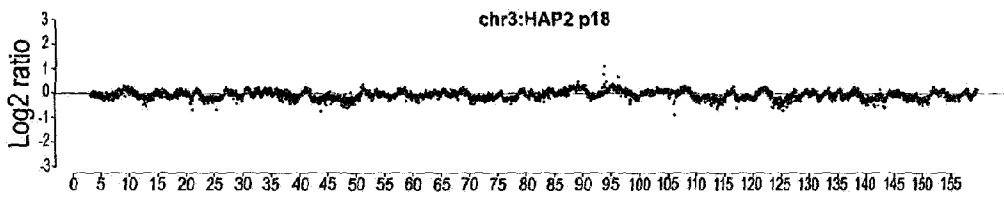
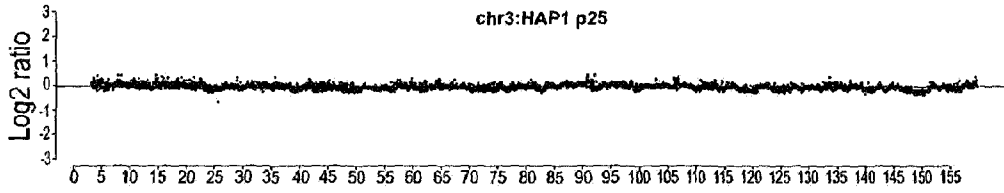
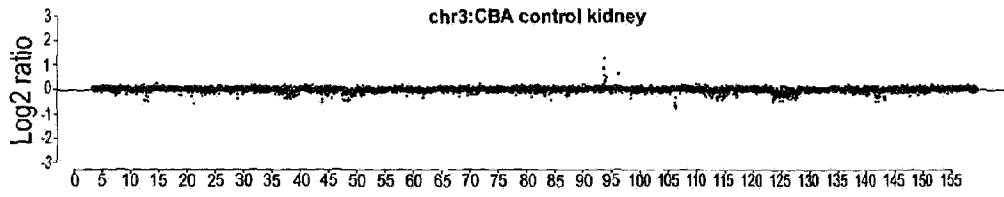
Signals from somatic kidney DNA from HTG genetic background mouse strain (black), HTG-1 (green) and HTG-2 (red) ES cells are overlaid and shown at 40 kb resolution. The positions of CNVs overlap indicating they are likely resulting from genomic variation between the strain of origin and C57BL6 mouse strains.

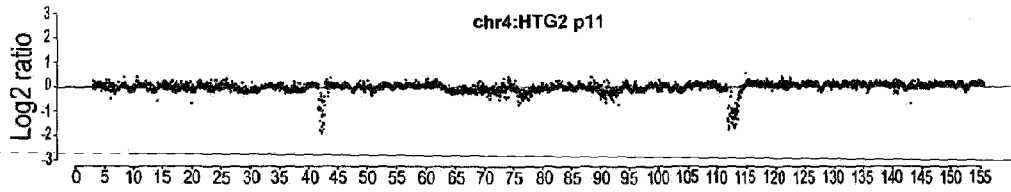
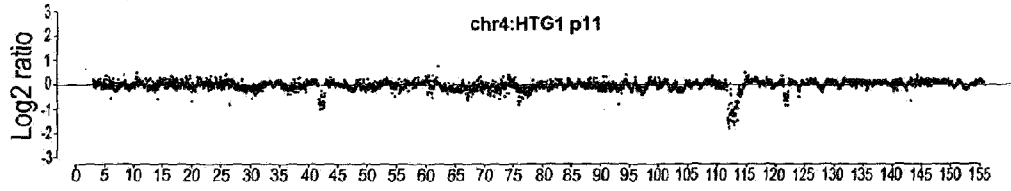
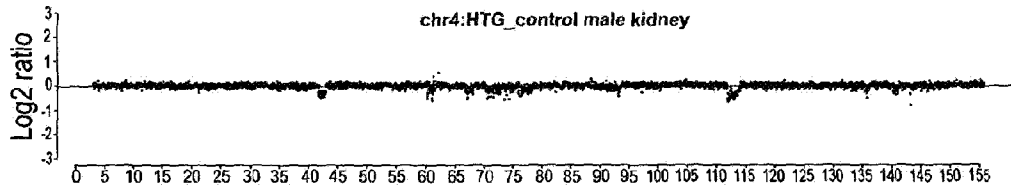
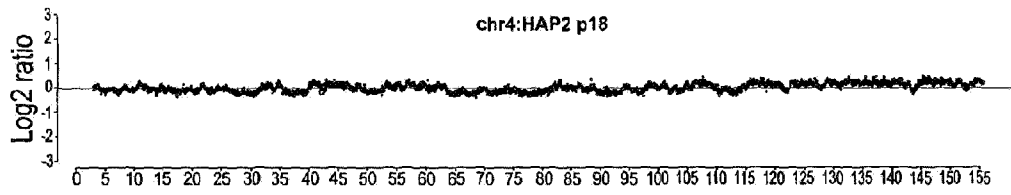
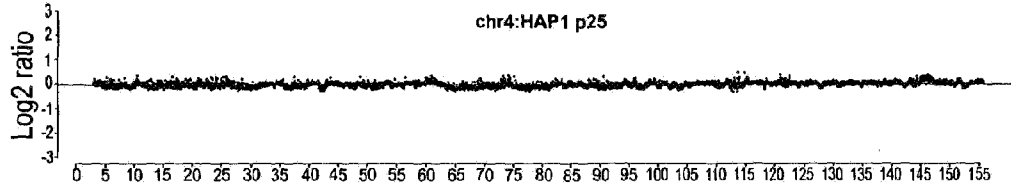
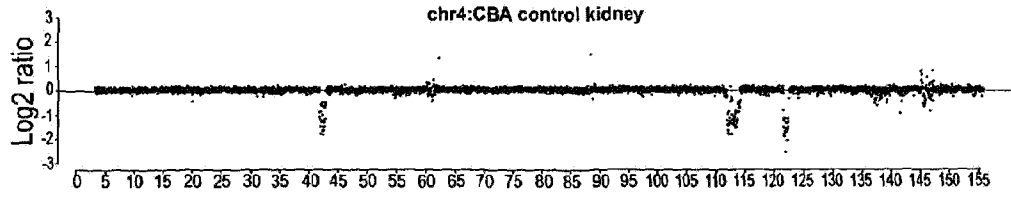
Supplementary Figure 4 - CGH analysis of haploid ES cells

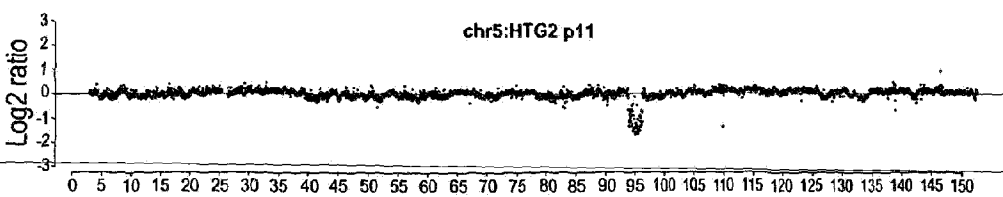
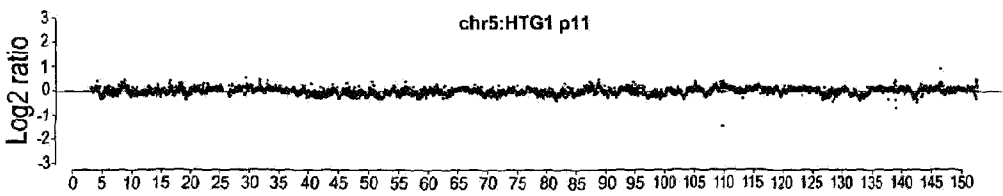
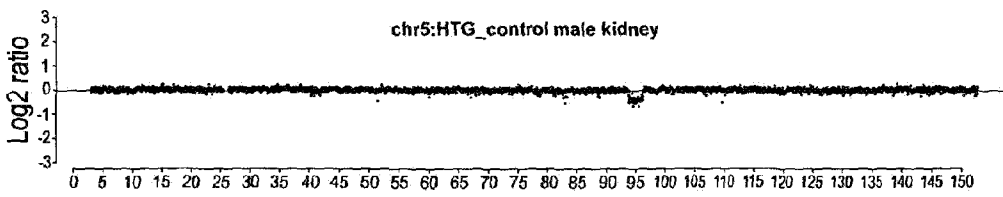
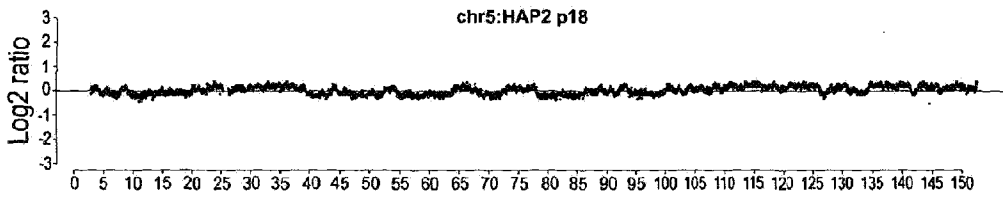
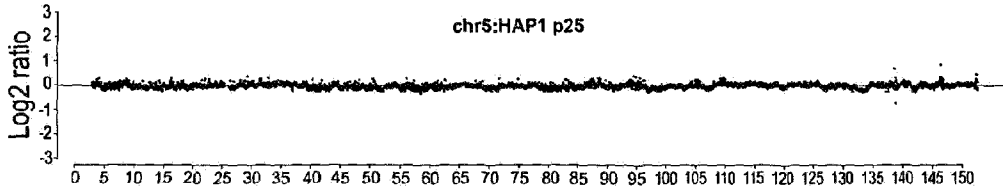
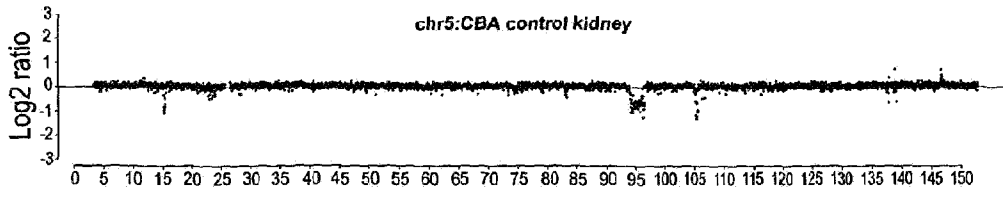
CGH profiles of HAP-1, HAP-2, HTG-1 and HTG-2 haploid mouse ES cells are shown along genomic coordinates. Kidney DNA from a CBA male mouse and from a male of the transgenic mixed background strain from which HTG ES cells were derived were analysed as controls. All hybridizations were performed using a C57BL6 male DNA reference. Average values of log₂ ratios are plotted at a 40 kb resolution.

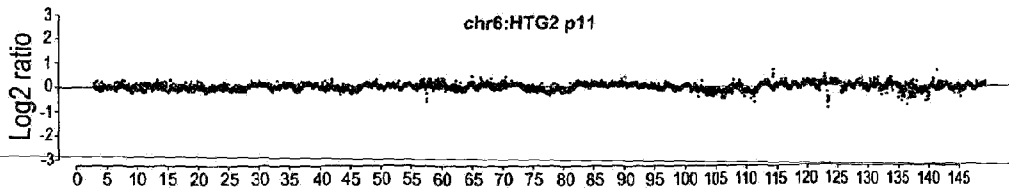
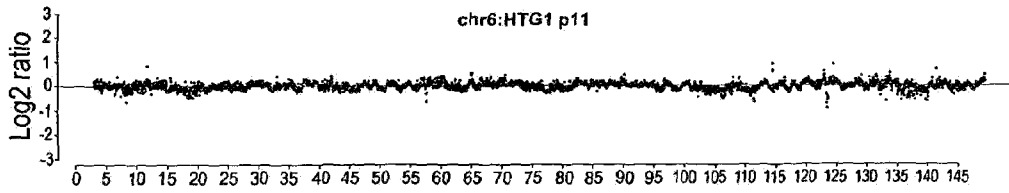
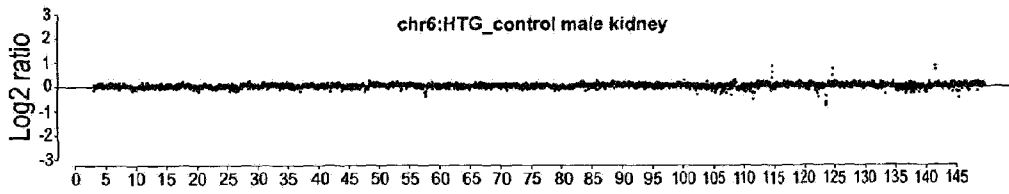
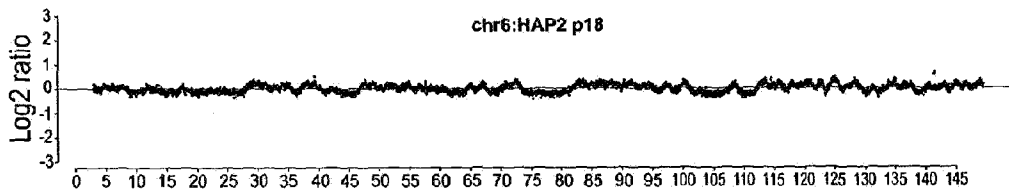
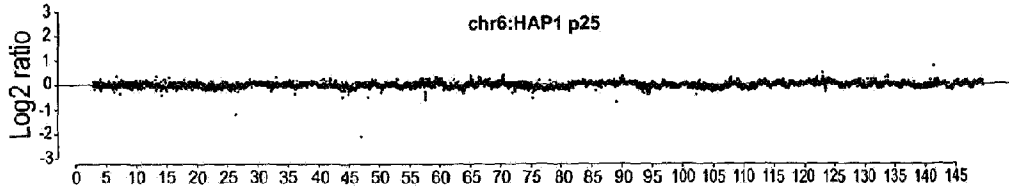
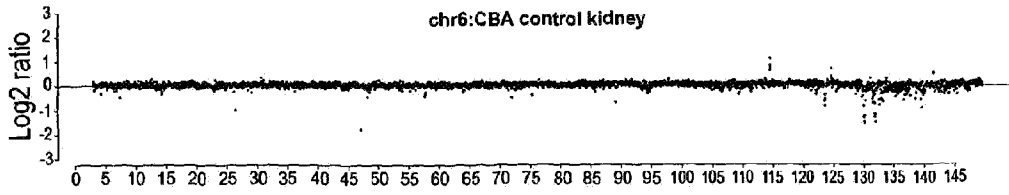


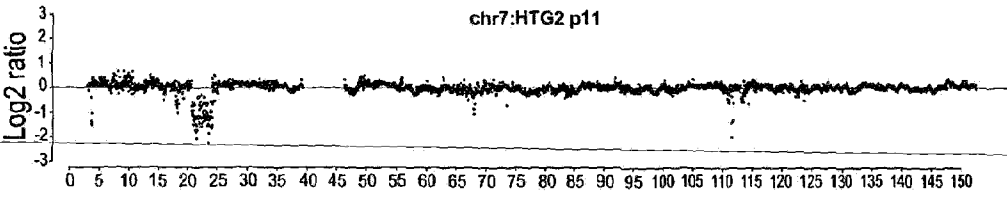
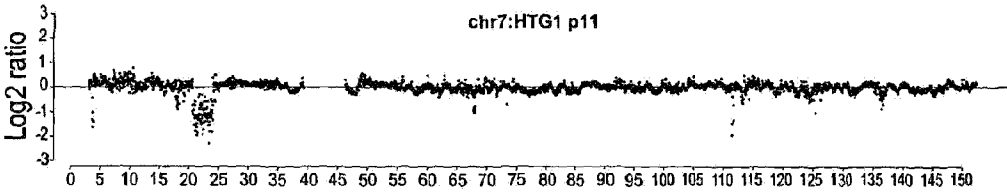
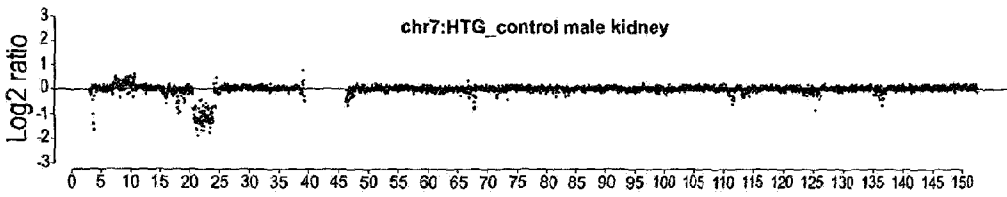
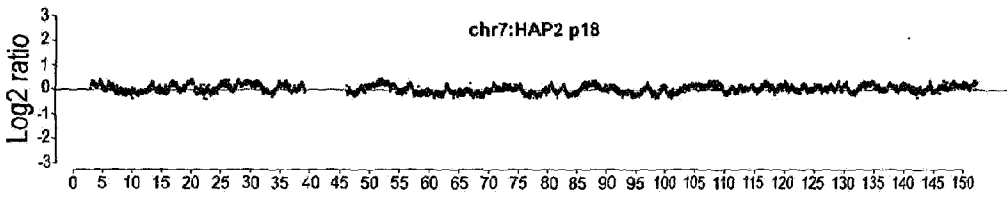
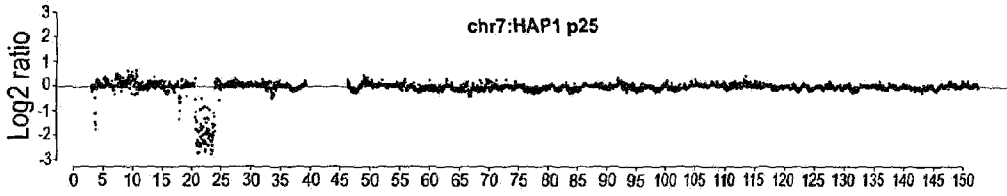
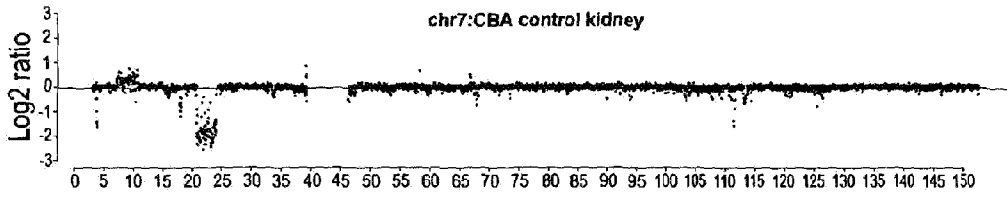


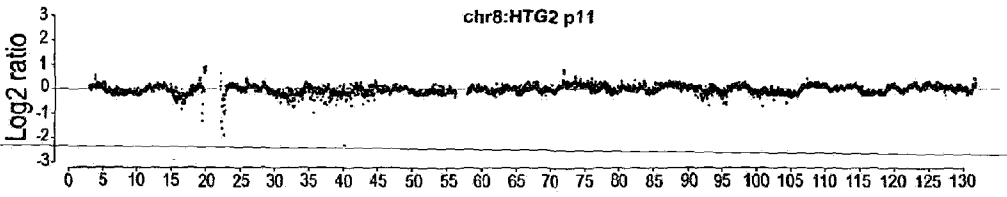
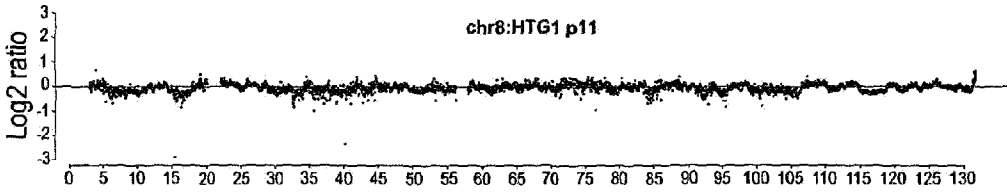
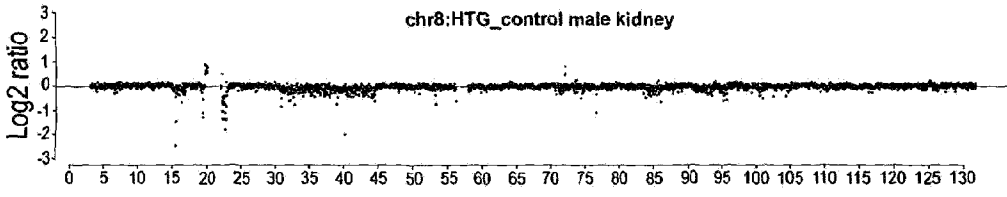
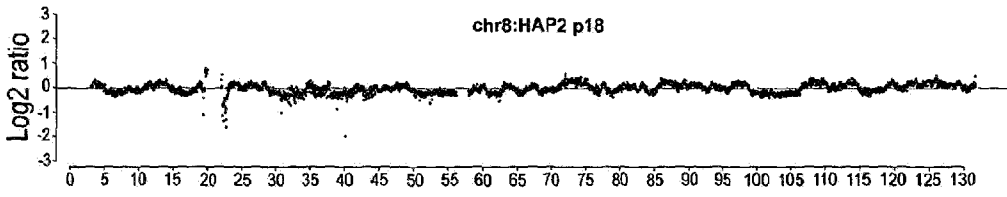
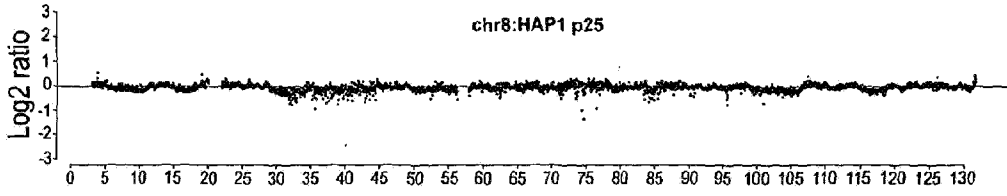
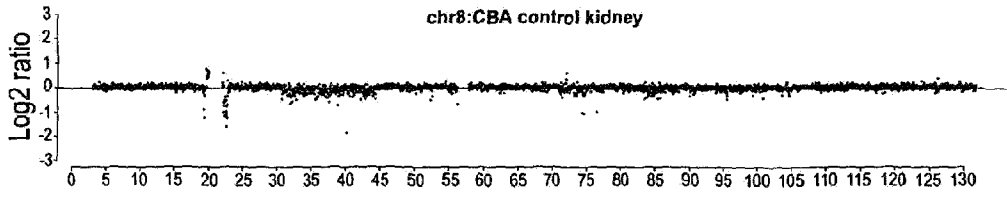


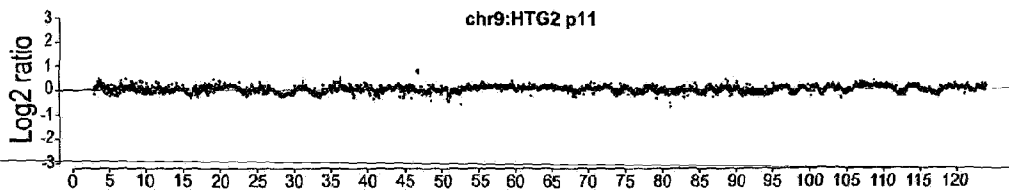
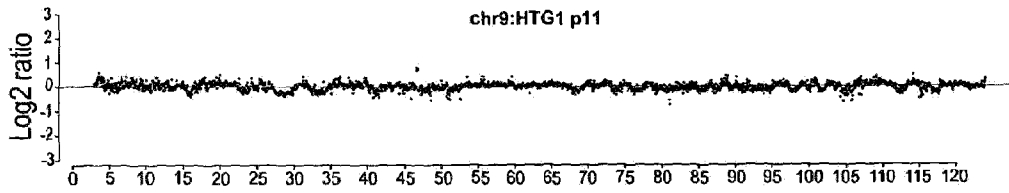
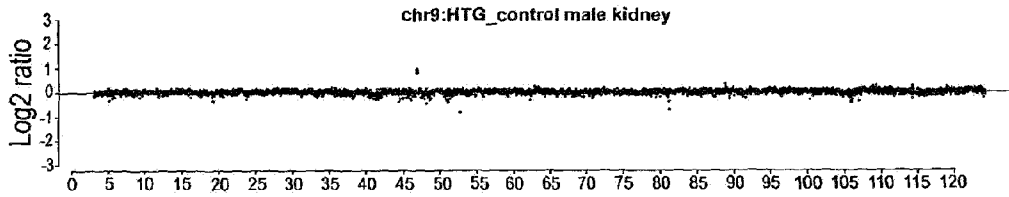
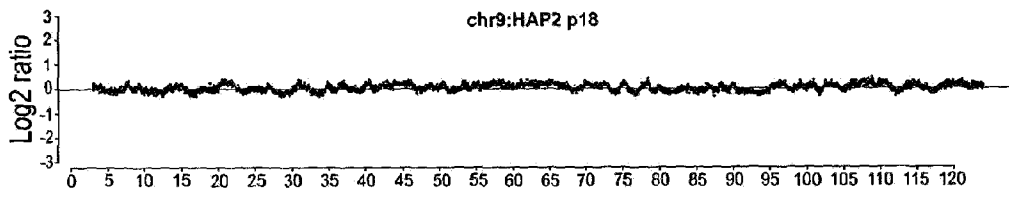
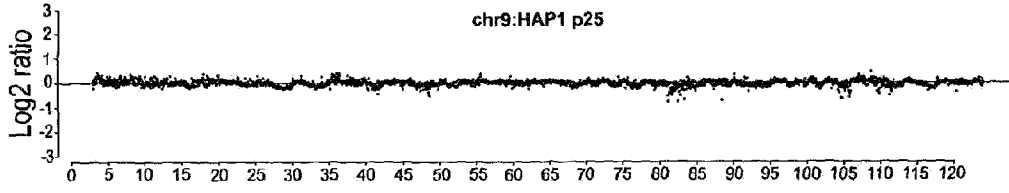
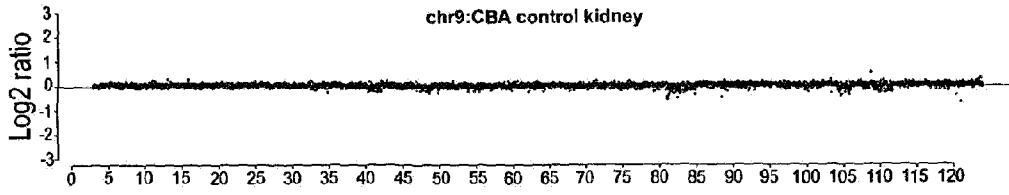


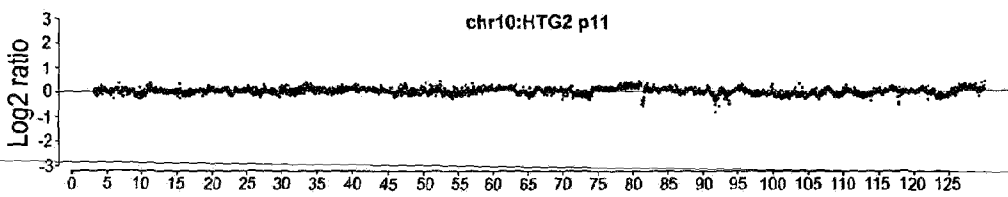
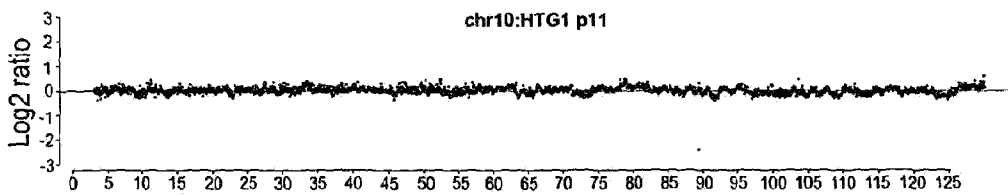
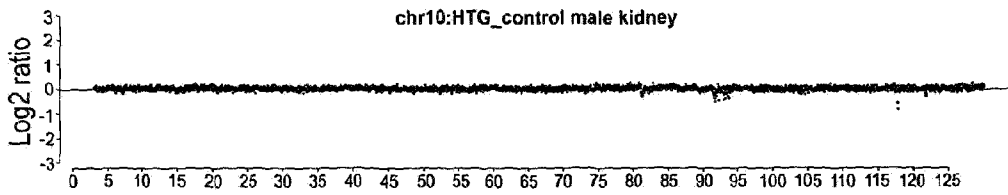
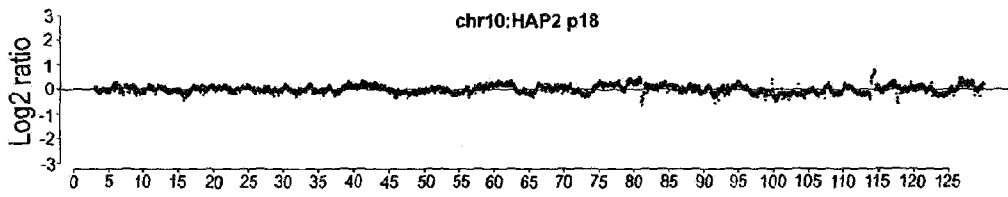
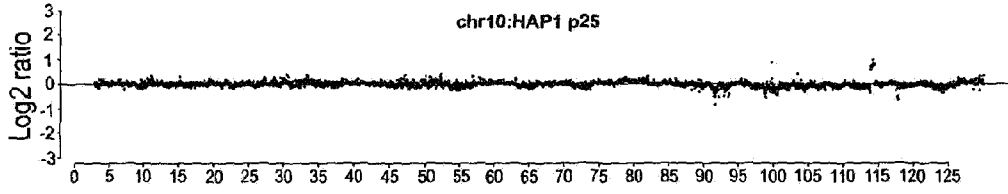
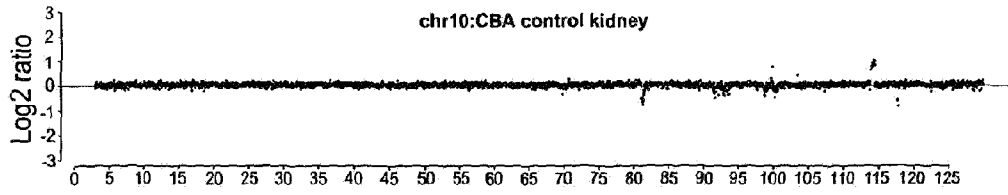


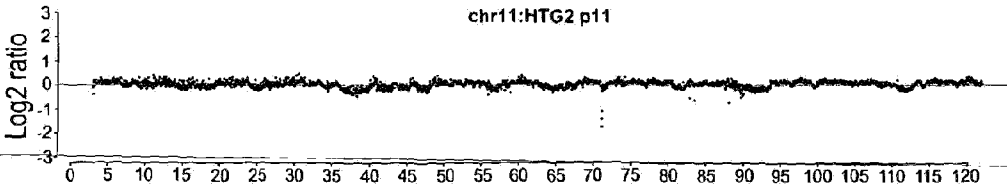
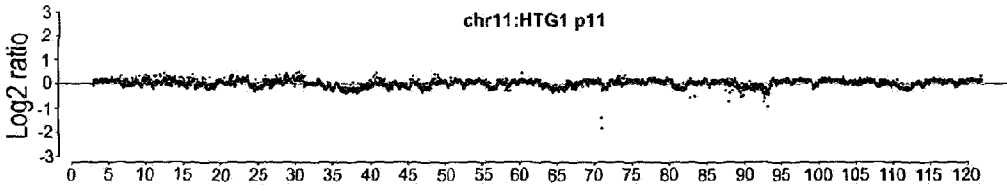
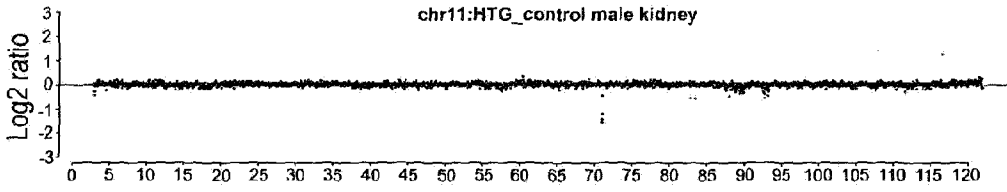
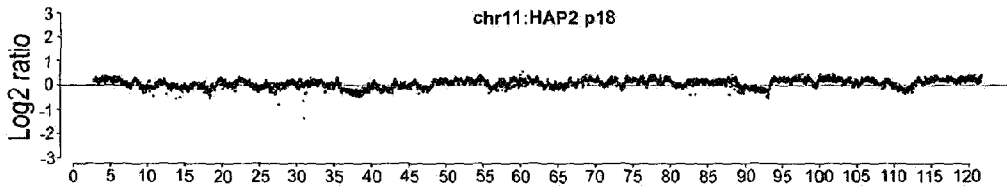
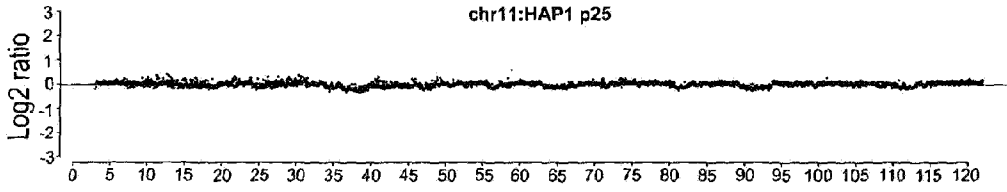
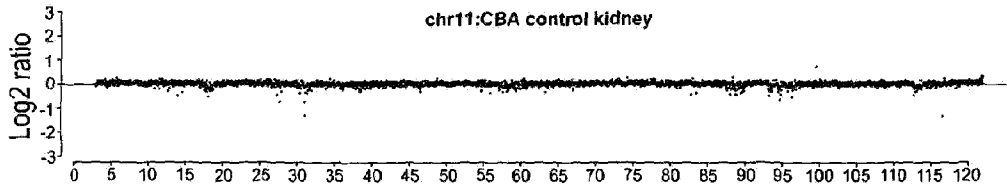


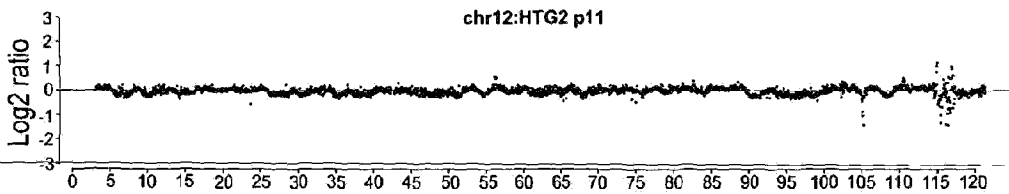
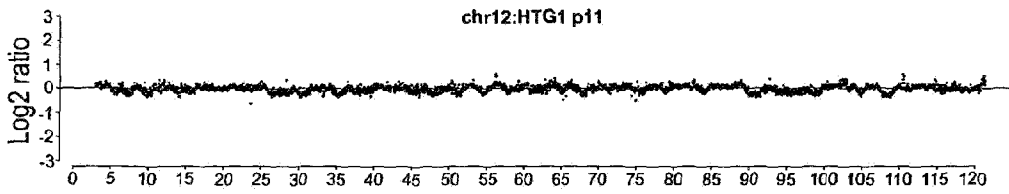
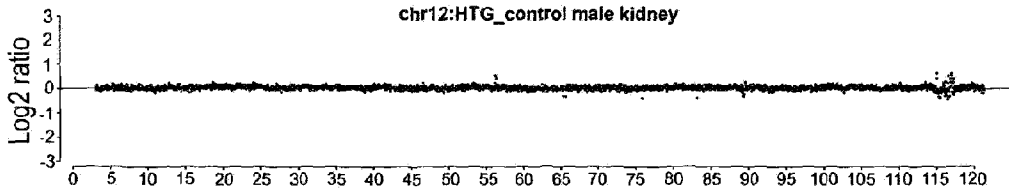
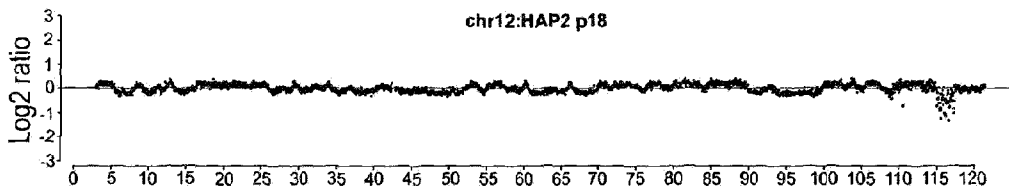
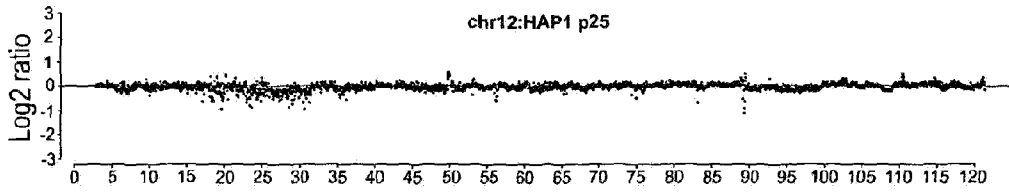
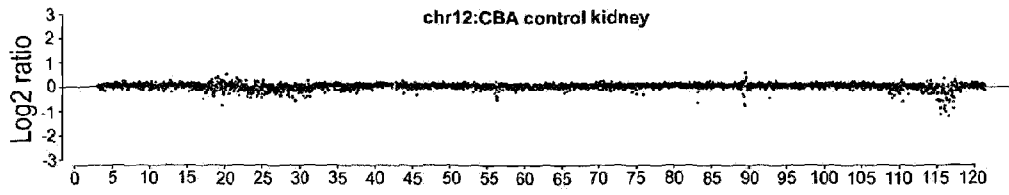


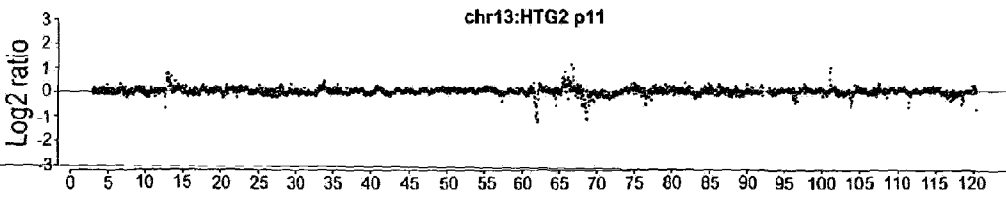
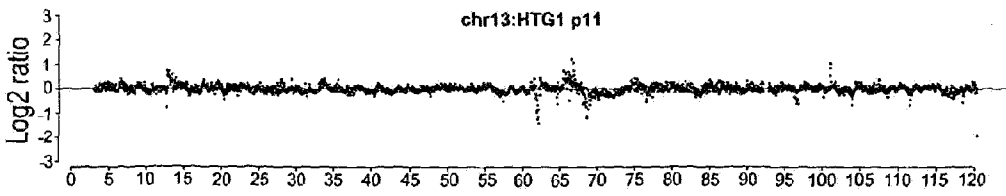
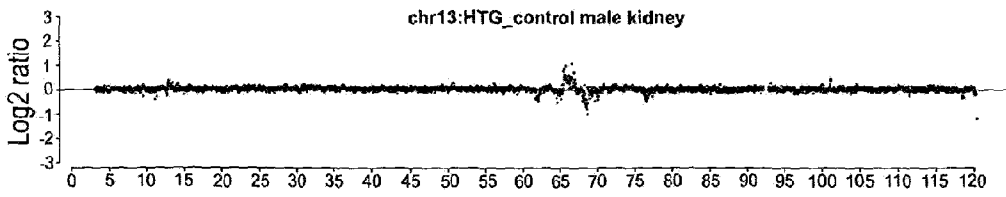
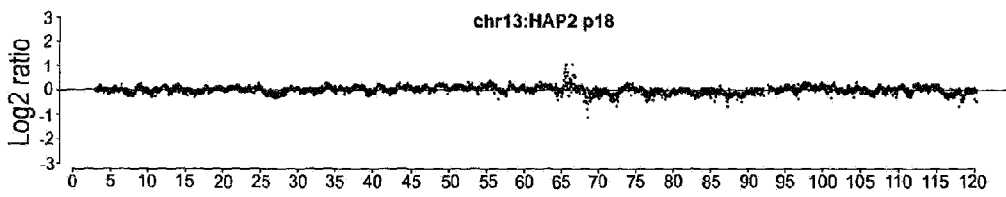
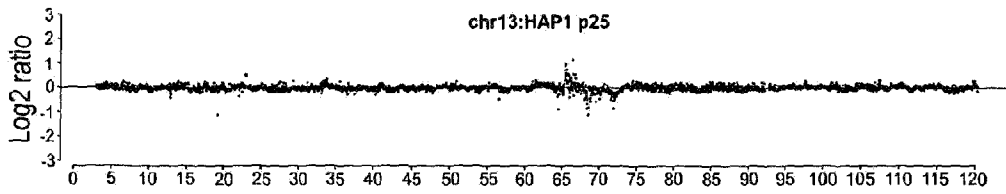
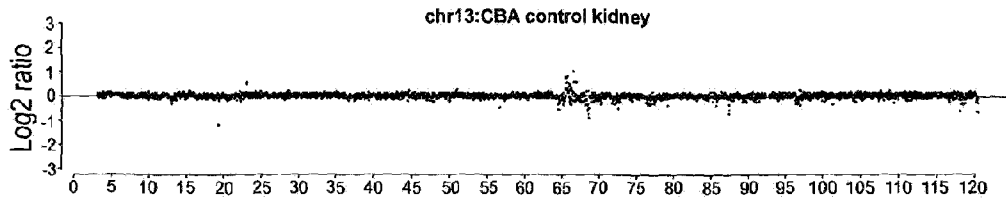


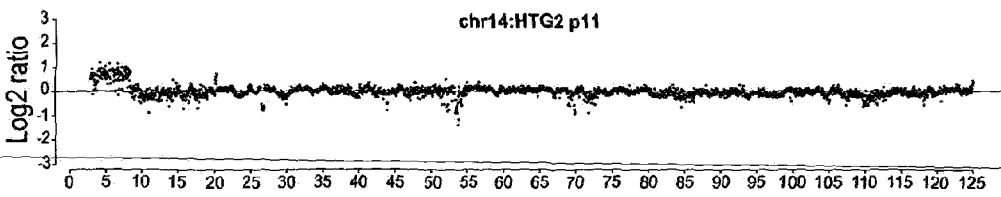
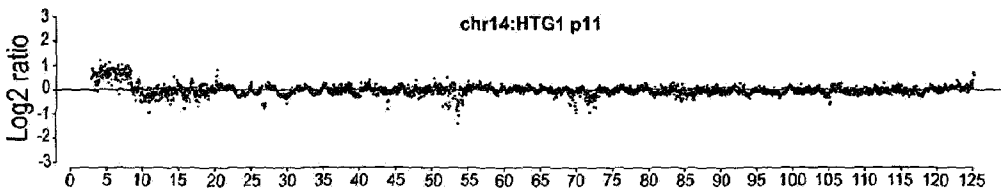
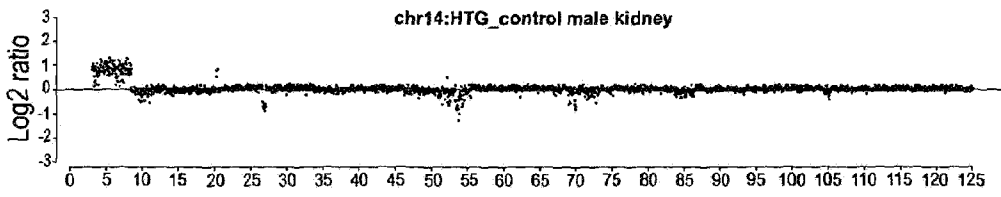
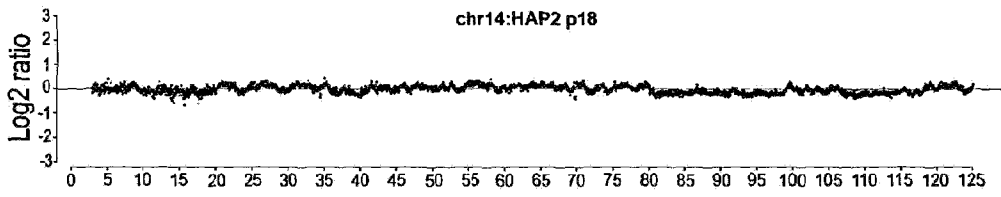
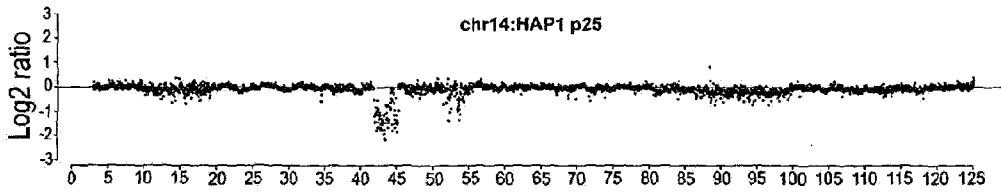
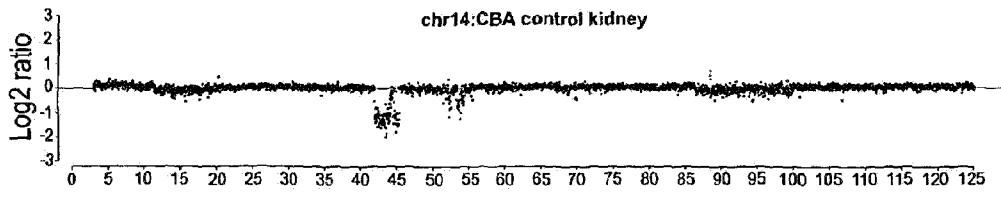


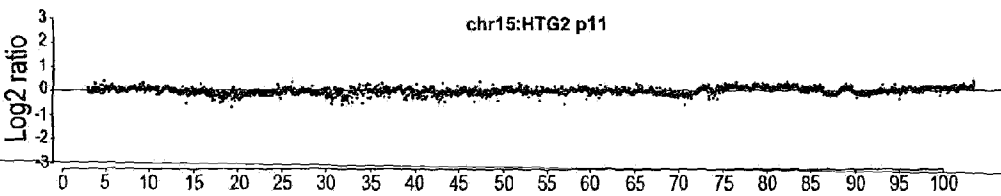
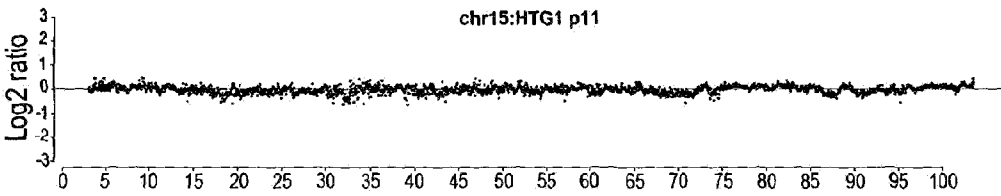
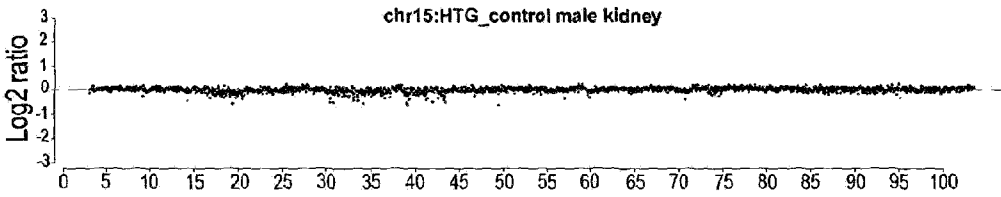
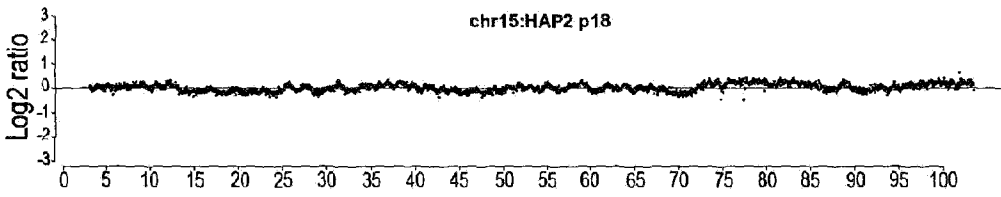
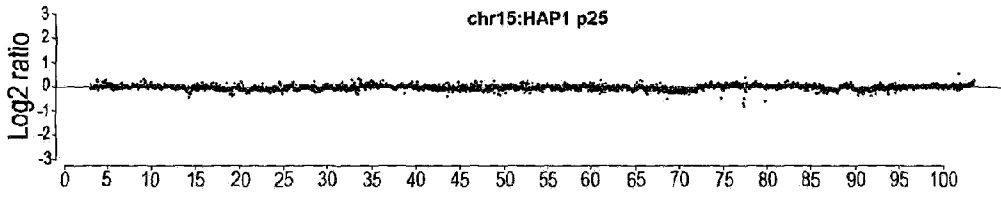
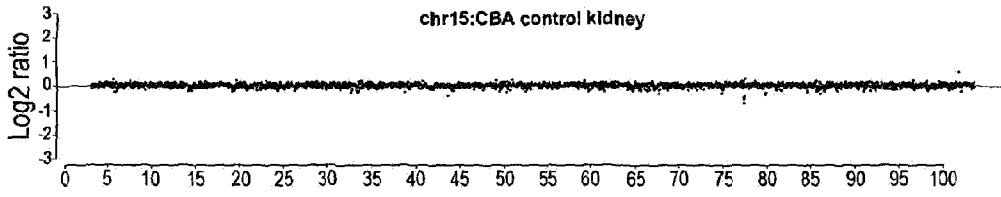


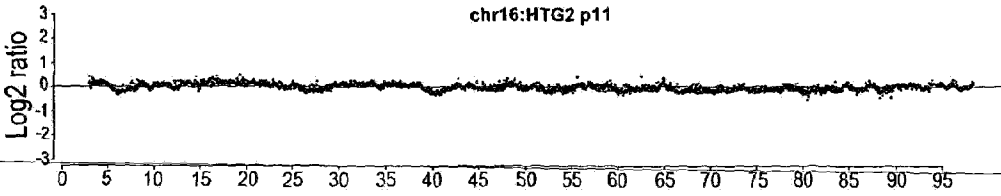
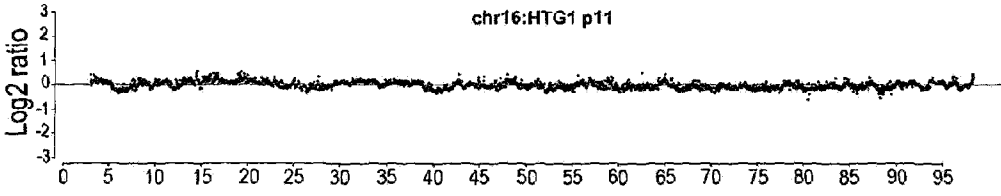
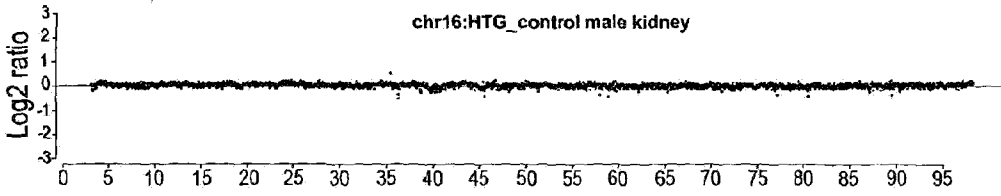
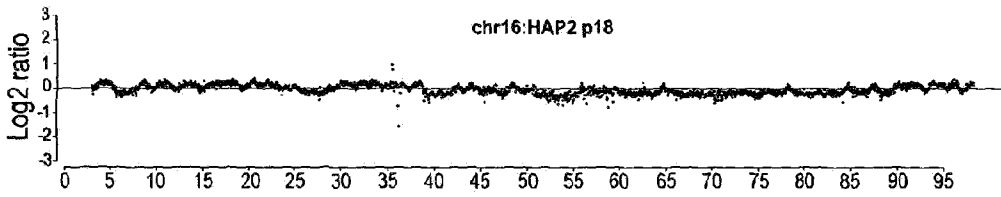
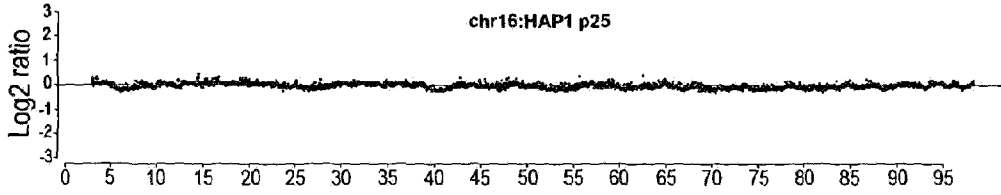
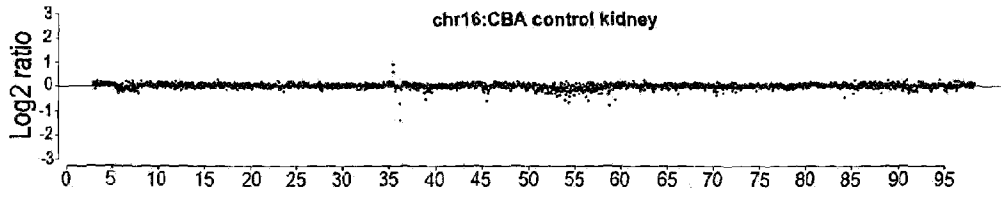


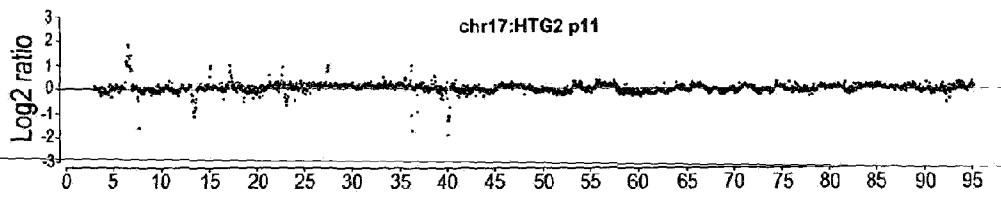
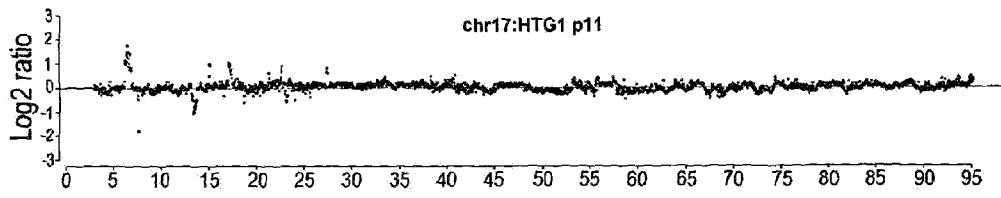
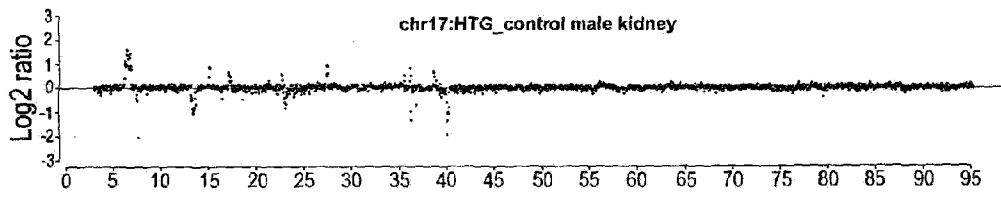
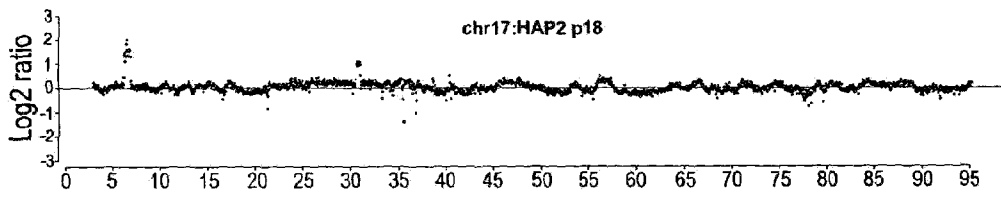
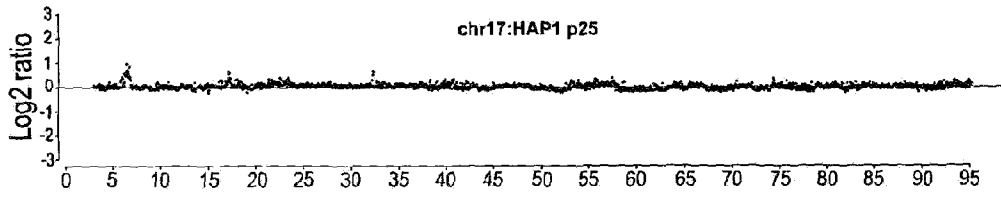
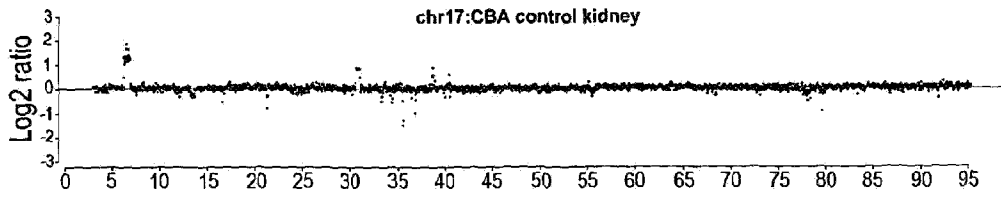


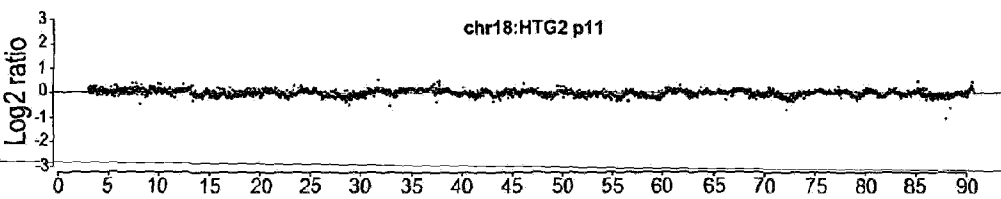
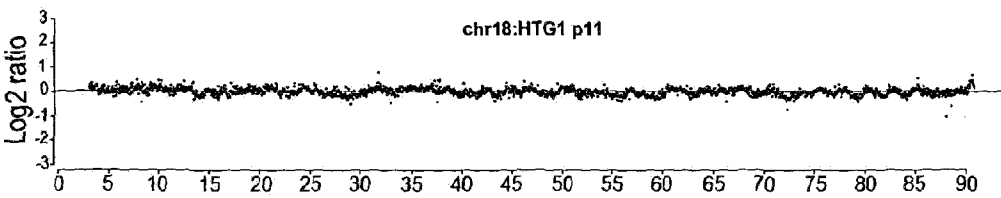
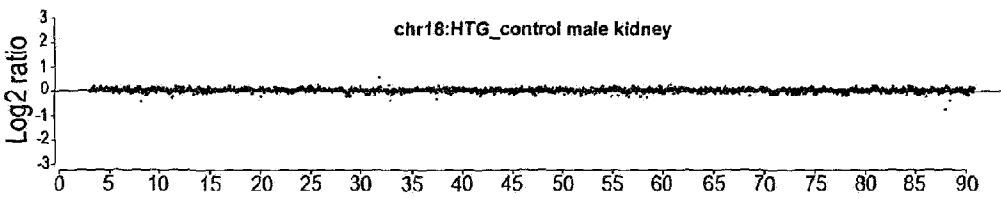
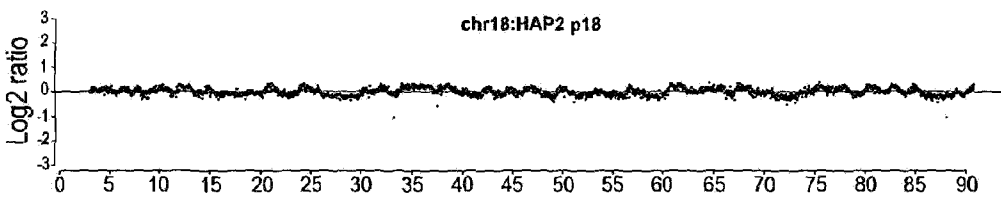
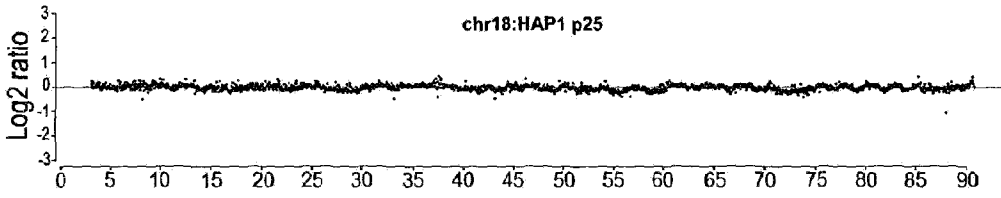
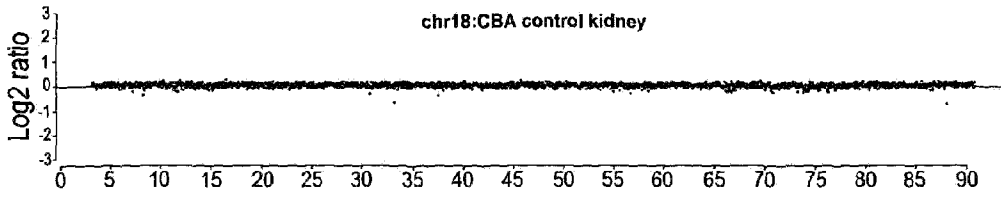


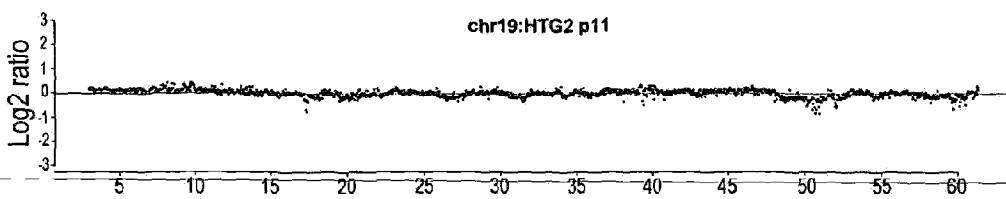
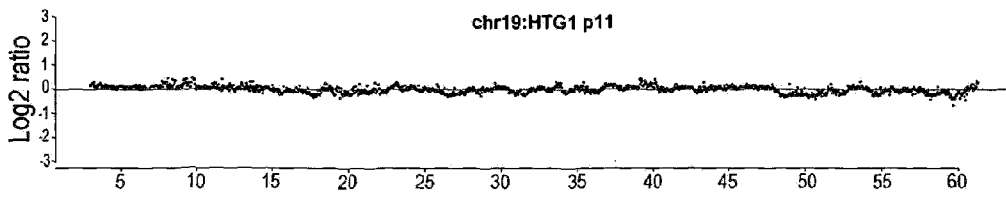
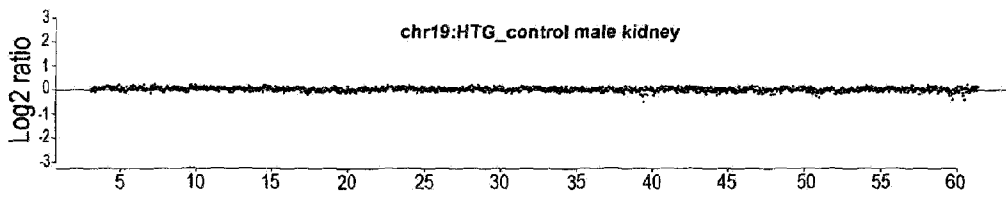
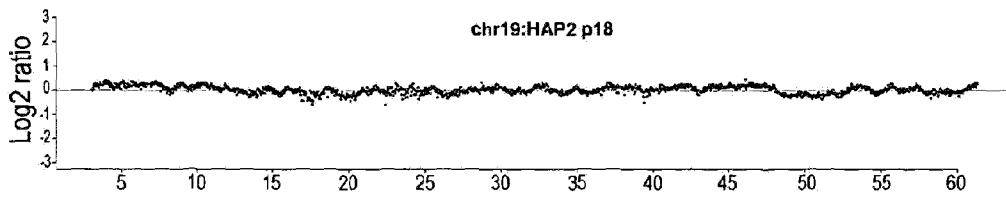
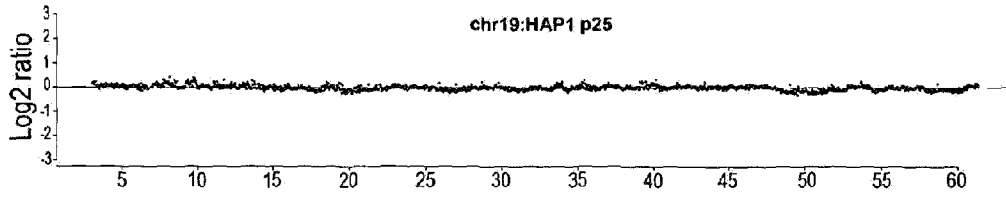
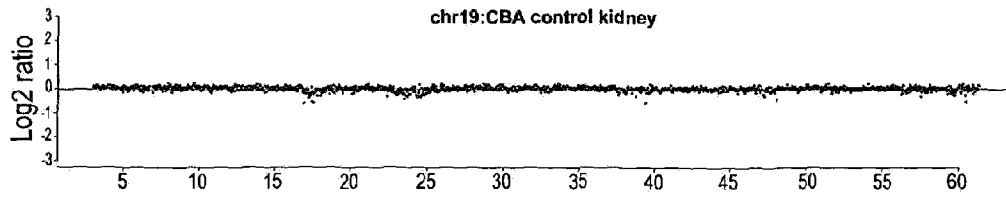


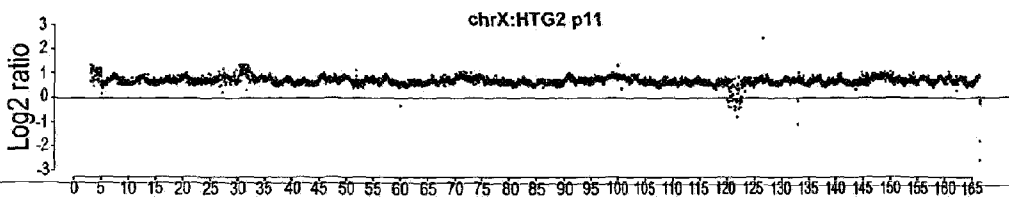
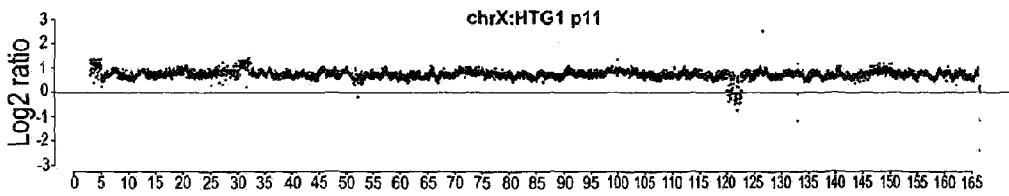
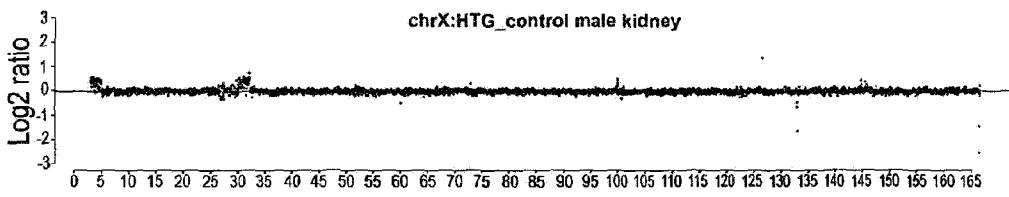
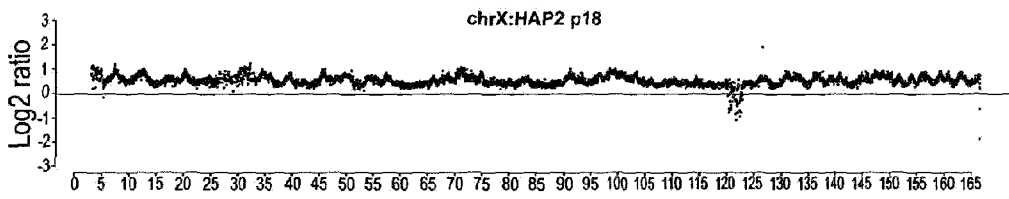
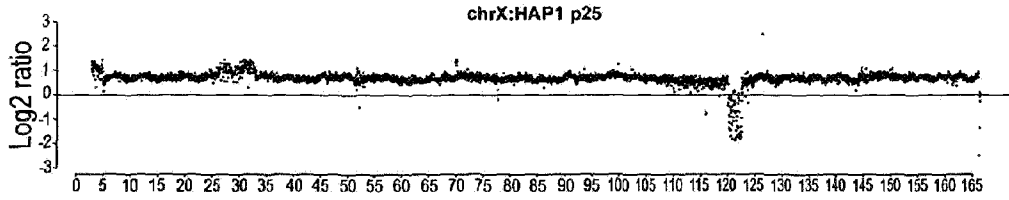
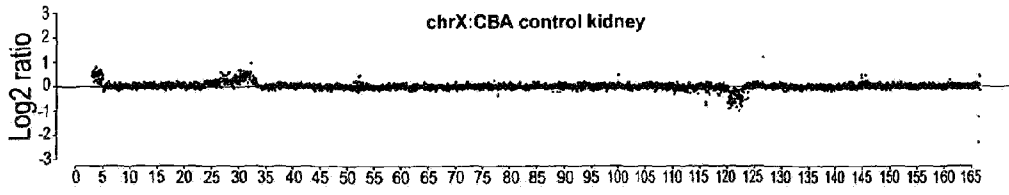


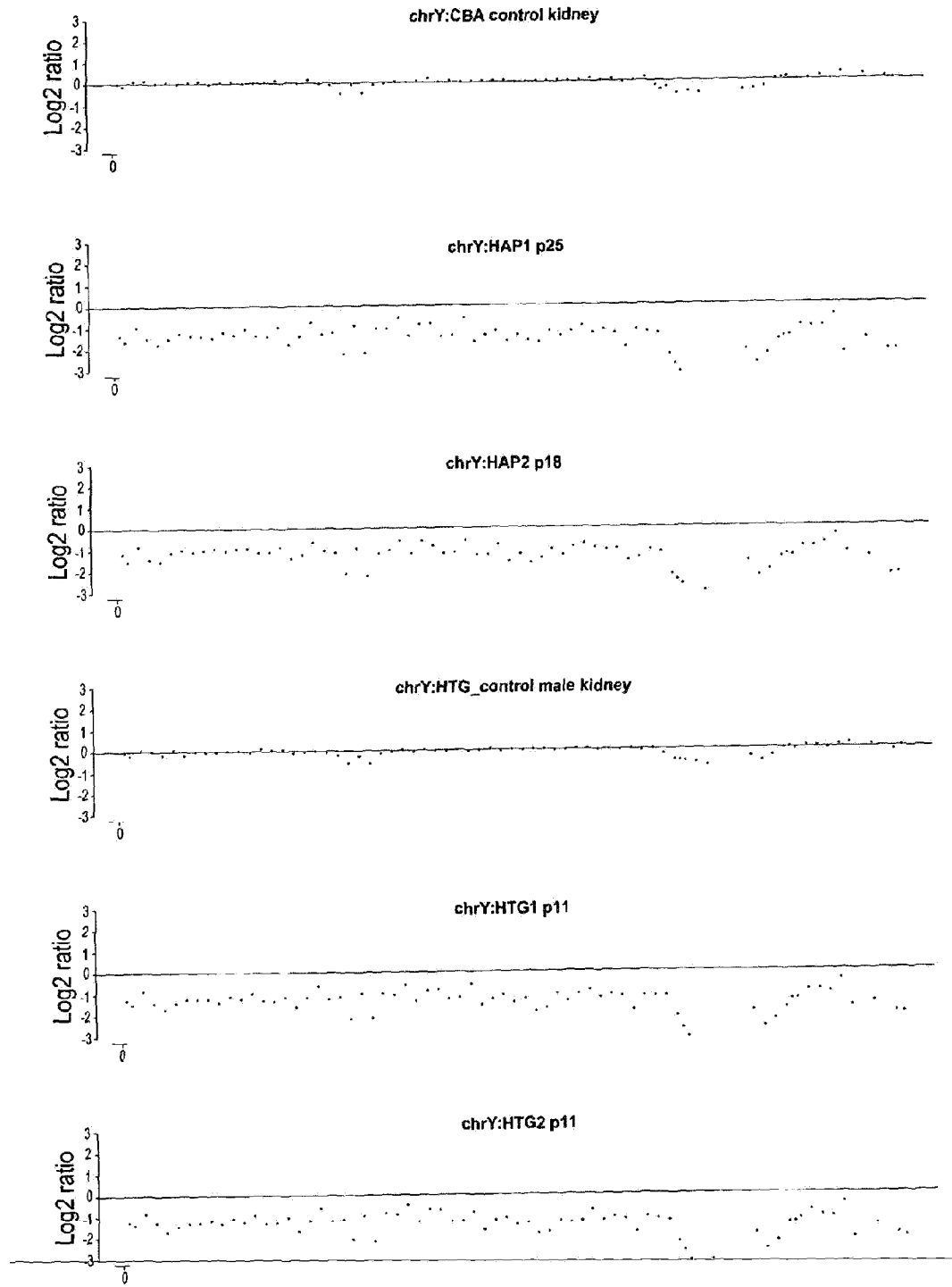


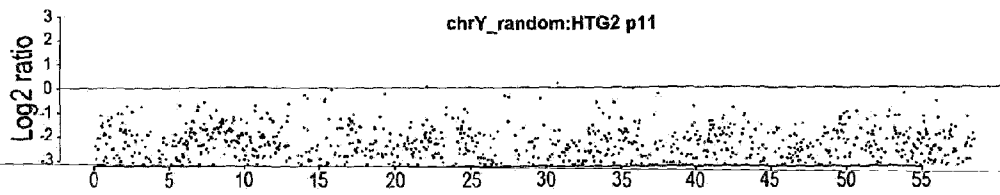
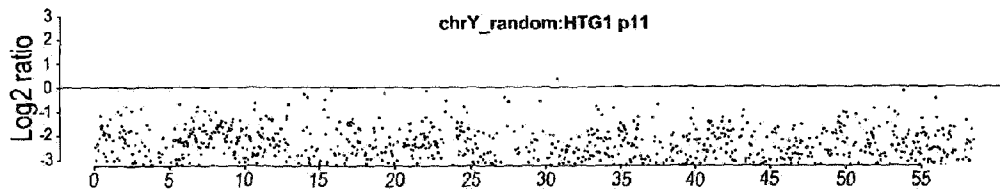
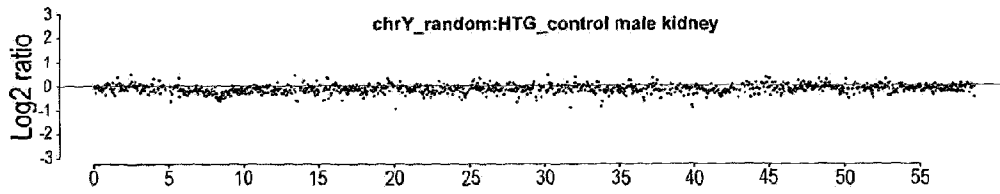
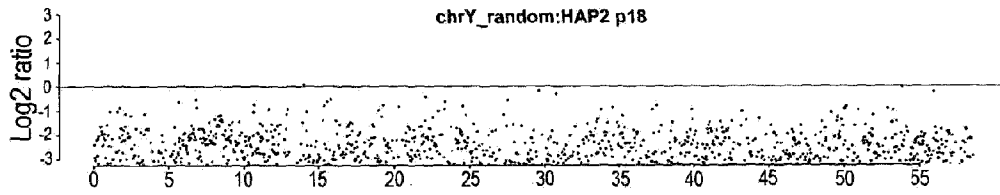
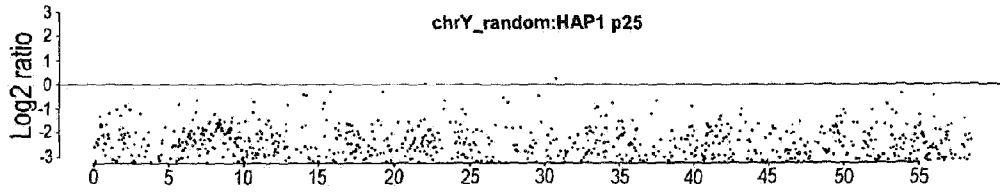
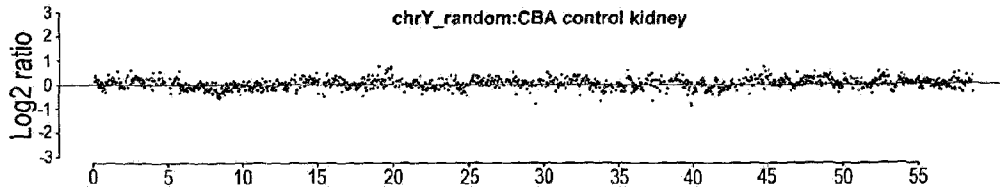


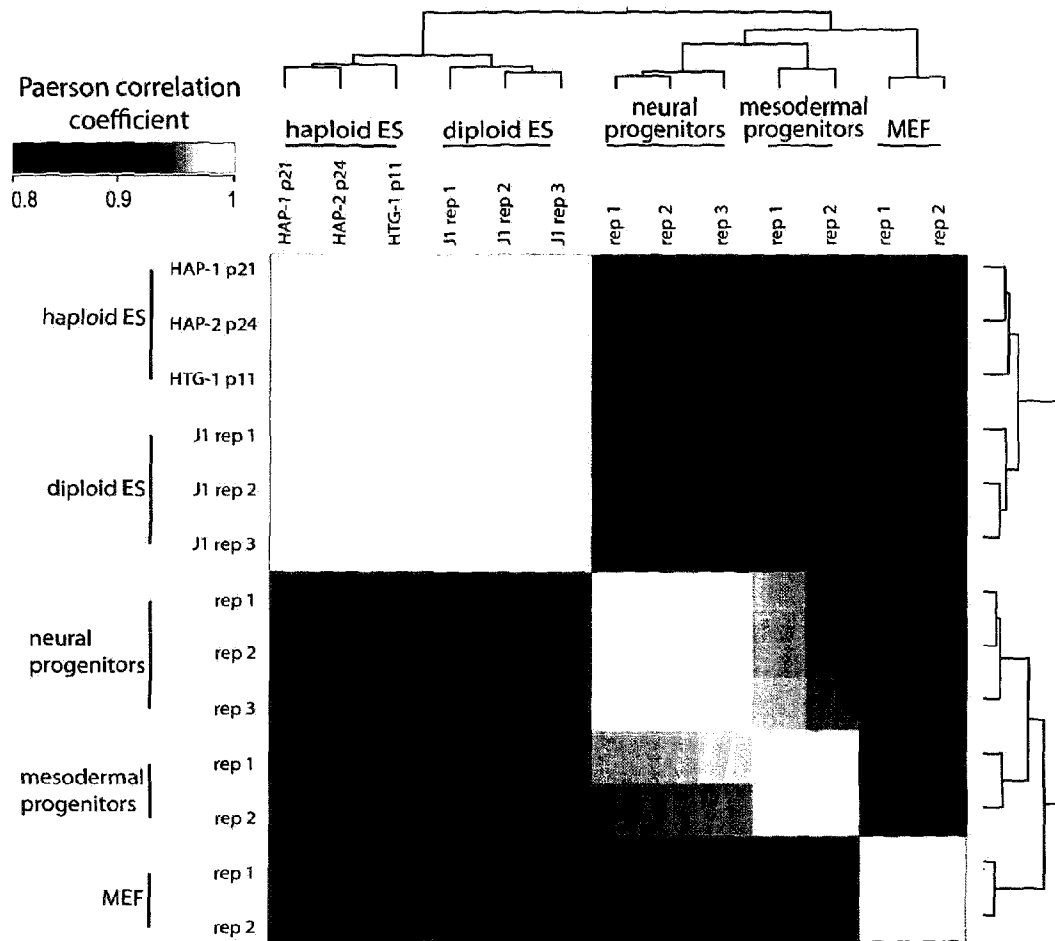






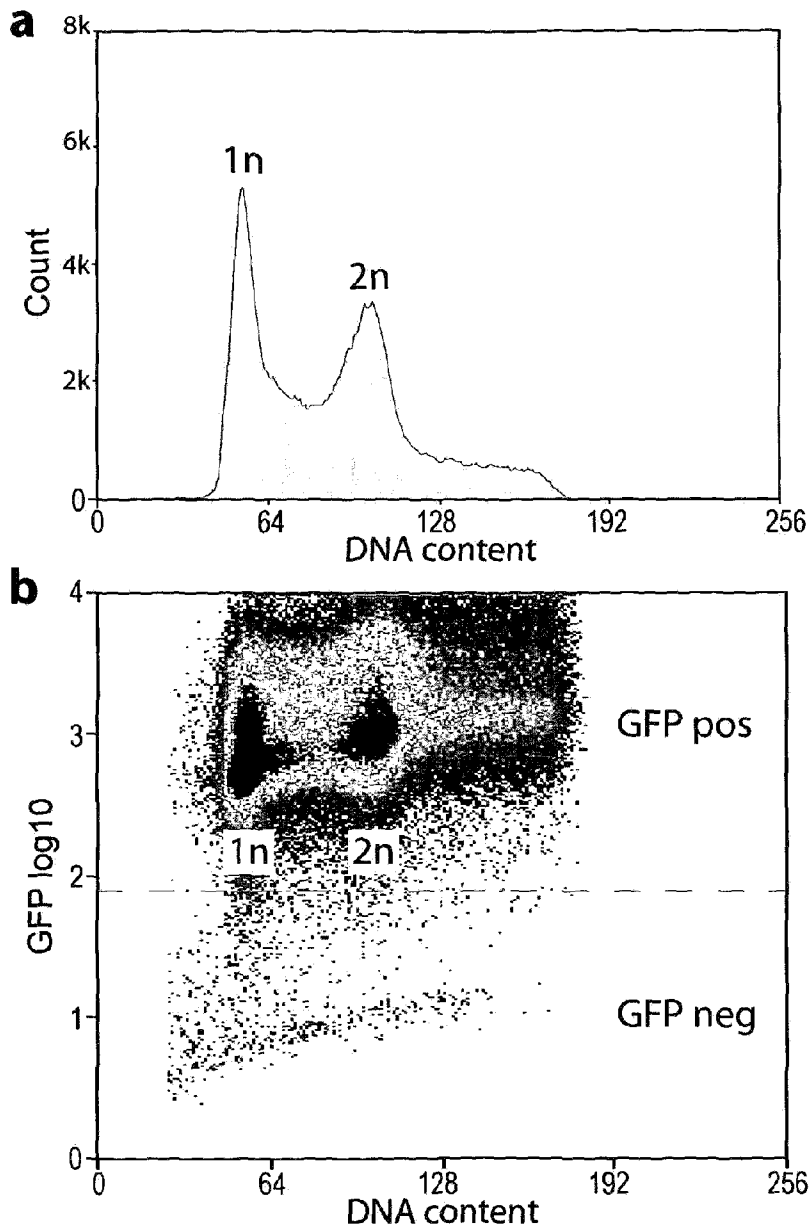




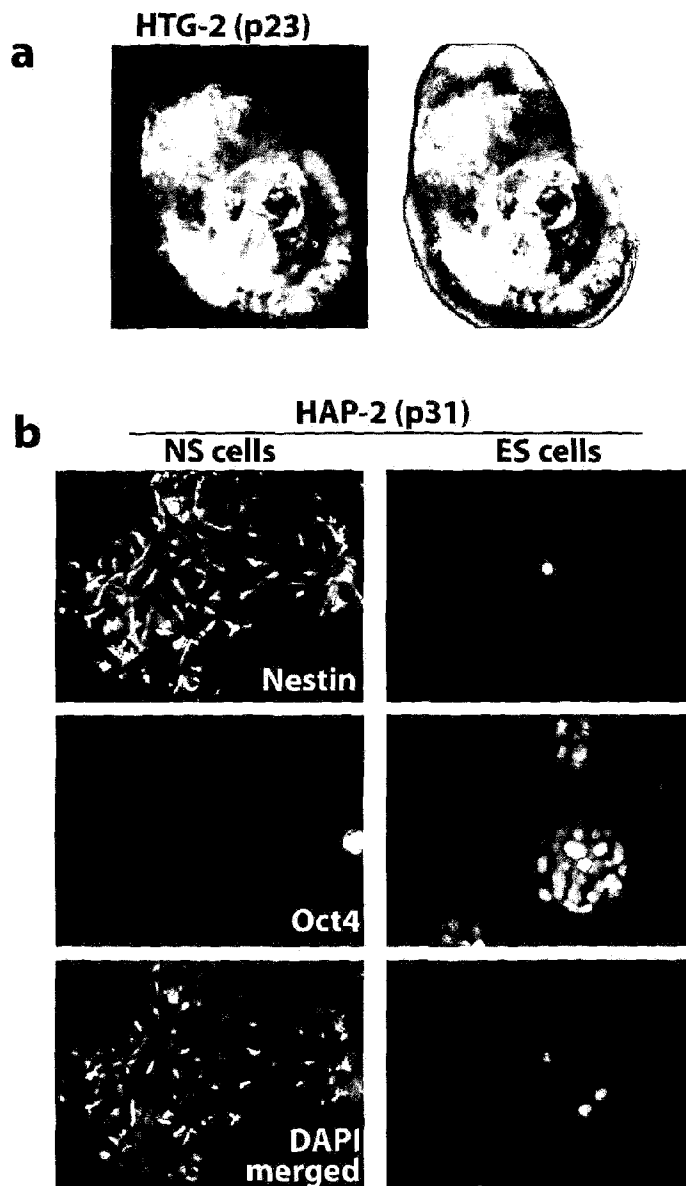


Supplementary Figure 5 - Analysis of similarity between haploid and diploid ES cells

An overview of the gene expression profiles of haploid and diploid ES cells is shown. Gene expression profiles were clustered using all genes and the Paerson correlation coefficient was calculated (indicated by red color). Three different haploid ES cell lines (HAP-1, HAP-2, and HTG-1) cluster together showing highly similar expression profiles. Gene expression of haploid ES cells is highly similar to control diploid J1 ES cells but different from mouse embryonic fibroblasts (MEF) or neural and mesodermal progenitors. The dendrogram (top and right) was generated by hierarchical clustering by Euclidean distance and complete linkage analysis (rep1, rep2, rep3 indicate biological replicates).



Supplementary Figure 6 - Stable integration of a GFP transgene into haploid ES cells
Flow profiles show the (a) DNA content and (b) DNA content combined with GFP intensity of HAP-2 ES cells transfected with a piggyBac vector for expressing GFP. The 1n / GFP positive population was purified for analysis of the developmental potential of haploid ES cells.



Supplementary Figure 7 - Developmental potential of haploid ES cells.

(a) A GFP image and merged GFP brightfield image of a chimeric E9.5 embryo from injection of GFP labeled HTG-2 ES cells (p23) into C57BL6 host blastocysts.

(b) Immunofluorescence analysis of Nestin (green) and Oct4 (white) expression in HAP-2 (p31) ES cells and HAP-2 derived neural stem (NS) cells. A merged image with DNA stained with DAPI (blue) is shown below.

Supplementary Table 1 - Segmentation table of the CGH analysis of haploid ES cells

Segmentation analysis of the CGH profiles of HAP-1, HAP-2, HTG-1 and HTG-2 haploid mouse ES cells and CBA and HTG control male somatic samples was performed using the NimbleScan software (Roche). Copynumber deviations with a cut-off log₂ value of 0.8 were determined and listed with chromosome and genomic position.

HAP1				HAP2				CBA									
CHROMOSOME	START	STOP	SIZE	DATAPOINTS	LOG2_RATIO	CHROMOSOME	START	STOP	SIZE	DATAPOINTS	LOG2_RATIO	CHROMOSOME	START	STOP	SIZE	DATAPOINTS	LOG2_RATIO
chr7	3699999	3819999	120000	43	-1.4371	chr1	8139999	8219999	80000	32	-1.15655	chr1	8139999	8219999	80000	32	-1.12563
chr7	20699999	24059999	3360000	637	-1.88153	chr2	1E+08	1.01E+08	2800000	89	-0.91674	chr3	93579999	93659999	80000	34	0.91478
chr12	89299999	89379999	80000	34	-0.89872	chr8	19339999	19379999	40000	20	-1.06806	chr4	41739999	42779999	1040000	218	-1.07415
chr13	68619999	68699999	80000	32	-1.03683	chr8	22179999	22299999	120000	41	-0.97395	chr4	1.12E+08	1.12E+08	520000	152	-1.31422
chr14	42059999	47219999	160000	54	-1.32819	chr8	22539999	22699999	160000	47	-1.10895	chr4	1.12E+08	1.14E+08	1080000	302	-1.22262
chr14	42259999	42339999	80000	29	-0.89743	chr13	68619999	68699999	80000	32	-0.82539	chr4	1.21E+08	1.22E+08	760000	190	-1.51206
chr14	42379999	42419999	40000	14	-1.37748	chr16	35499999	35539999	40000	24	0.88028	chr5	14939999	15179999	240000	65	-0.81371
chr14	42459999	42539999	80000	28	-0.84389	chr17	62599999	68999999	640000	180	1.45984	chr5	1.05E+08	1.05E+08	320000	98	-0.83343
chr14	42579999	42599999	80000	33	-1.58313	chr17	30619999	31019999	400000	124	1.01428	chr6	1.14E+08	1.15E+08	80000	21	0.80806
chr14	42699999	42859999	160000	49	-1.15406	chr17	35539999	35579999	40000	22	-1.37624	chr6	1.3E+08	1.3E+08	200000	67	-1.26461
chr14	42899999	43019999	120000	40	-1.66039	chrX	3019999	4939999	1920000	288	0.84469	chr6	1.32E+08	1.32E+08	120000	42	-1.17524
chr14	43059999	43379999	320000	92	-1.39357	chrX	6659999	8019999	1360000	391	0.85511	chr7	3699999	3819999	120000	43	-1.36942
chr14	43419999	43619999	200000	48	-1.70756	chrX	19699999	20699999	1000000	291	0.80544	chr7	20699999	24059999	3360000	637	-1.7698
chr14	43659999	43859999	200000	63	-1.0446	chrX	70699999	71699999	1000000	295	0.91882	chr7	1.11E+08	1.11E+08	40000	22	-1.44423
chr14	43899999	44099999	200000	66	-1.44165							chr8	19539999	19979999	40000	20	-1.03835
chr14	44139999	44259999	120000	38	-0.81347							chr8	22179999	22299999	120000	41	-0.87322
chr14	44539999	44579999	40000	22	-1.67539							chr8	22539999	22699999	160000	47	-0.87254
chr14	44739999	44819999	80000	33	-0.90922							chr10	1.44E+08	1.44E+08	560000	166	0.82654
chr14	44859999	45179999	320000	84	-1.32847							chr14	42059999	42219999	160000	54	-1.2489
chr14	45219999	45259999	40000	22	-1.68286							chr14	42259999	42539999	280000	71	-1.0259
chr14	45299999	45339999	40000	21	-1.2028							chr14	42579999	43379999	800000	214	-1.30787
chr14	52259999	52379999	120000	43	-0.83266							chr14	43419999	43499999	80000	18	-1.7234
chrX	1.22E+08	1.22E+08	400000	26	-1.31437							chr14	43539999	43579999	40000	20	-0.91193
chrX	1.22E+08	1.23E+08	440000	37	-1.45574							chr14	43619999	43659999	40000	20	-1.53924
												chr14	43699999	44099999	400000	119	-1.14346
												chr14	44539999	44579999	40000	22	-1.29833
												chr14	44739999	45339999	560000	149	-1.1872
												chr16	36259999	36299999	40000	23	-1.03884
												chr17	6259999	6899999	640000	180	1.33058
												chr17	30619999	31059999	440000	135	0.81562
												chr17	35539999	35579999	40000	22	-1.38383

Supplementary Table 1 - Continued

HTG1				HTG2				HTG							
CHROMOSOMI	START	STOP	SIZE	CHROMOSOMI	START	STOP	SIZE	CHROMOSOMI	START	STOP	SIZE	CHROMOSOMI	START	STOP	SIZE
chr2	1.41E+08	1.41E+08	40000	chr2	1.41E+08	1.41E+08	40000	chr1	8139999	8179999	40000	chr1	8139999	8179999	40000
chr4	1.12E+08	1.12E+08	520000	chr4	41739999	41899999	160000	chr1	90139999	90179999	40000	chr1	90139999	90179999	40000
chr4	1.12E+08	1.14E+08	1120000	chr4	41639999	42059999	120000	chr3	93579999	93659999	80000	chr3	93579999	93659999	80000
chr7	3699999	3819999	120000	chr4	42299999	42419999	120000	chr7	3699999	3819999	120000	chr7	3699999	3819999	120000
chr7	20659999	24099999	3440000	chr4	42459999	42539999	80000	chr7	20659999	24099999	3440000	chr7	20659999	24099999	3440000
chr7	1.11E+08	1.11E+08	40000	chr4	42579999	42739999	160000	chr8	19339999	19379999	40000	chr8	19339999	19379999	40000
chr11	71019999	71099999	80000	chr4	1.12E+08	1.12E+08	520000	chr8	22539999	22699999	160000	chr8	22539999	22699999	160000
chr13	61779999	62059999	280000	chr4	1.12E+08	1.14E+08	1120000	chr9	45699999	46939999	240000	chr9	45699999	46939999	240000
chr13	1.01E+08	1.01E+08	80000	chr5	93899999	96219999	2320000	chr11	71019999	71099999	80000	chr11	71019999	71099999	80000
chr17	6259999	6899999	640000	chr7	3699999	3819999	120000	chr14	3019999	3139999	120000	chr14	3019999	3139999	120000
chr17	15099999	15179999	80000	chr7	20659999	24099999	3440000	chr14	3979999	8499999	4520000	chr14	3979999	8499999	4520000
chr17	17139999	17419999	280000	chr7	1.11E+08	1.11E+08	40000	chr17	6259999	6899999	640000	chr17	6259999	6899999	640000
chrX	3019999	4939999	1920000	chr8	19339999	19419999	80000	chr17	36299999	36339999	40000	chr17	36299999	36339999	40000
chrX	30259999	32539999	2280000	chr8	22179999	22699999	520000	chr17	40059999	40299999	240000	chr17	40059999	40299999	240000
				chr11	71019999	71099999	80000	chr11	8139999	8179999	40000	chr11	8139999	8179999	40000
				chr12	1.15E+08	1.15E+08	80000	chr12	1.41E+08	1.41E+08	40000	chr12	1.41E+08	1.41E+08	40000
				chr12	1.16E+08	1.16E+08	80000	chr12	1.16E+08	1.16E+08	80000	chr12	1.16E+08	1.16E+08	80000
				chr13	61779999	62059999	280000	chr13	61779999	62059999	280000	chr13	61779999	62059999	280000
				chr13	68619999	68699999	80000	chr13	68619999	68699999	80000	chr13	68619999	68699999	80000
				chr17	6259999	6419999	160000	chr17	6259999	6419999	160000	chr17	6259999	6419999	160000
				chr17	6459999	6499999	40000	chr17	6459999	6499999	40000	chr17	6459999	6499999	40000
				chr17	6539999	6579999	40000	chr17	6539999	6579999	40000	chr17	6539999	6579999	40000
				chr17	6619999	6699999	80000	chr17	6619999	6699999	80000	chr17	6619999	6699999	80000
				chr17	6739999	6779999	40000	chr17	6739999	6779999	40000	chr17	6739999	6779999	40000
				chr17	6819999	6899999	80000	chr17	6819999	6899999	80000	chr17	6819999	6899999	80000
				chr17	13379999	13579999	200000	chr17	13379999	13579999	200000	chr17	13379999	13579999	200000
				chr17	15099999	15179999	80000	chr17	15099999	15179999	80000	chr17	15099999	15179999	80000
				chr17	27379999	27539999	160000	chr17	27379999	27539999	160000	chr17	27379999	27539999	160000
				chr17	36219999	36259999	40000	chr17	36219999	36259999	40000	chr17	36219999	36259999	40000
				chr17	36299999	36339999	40000	chr17	36299999	36339999	40000	chr17	36299999	36339999	40000
				chr17	40099999	40219999	120000	chr17	40099999	40219999	120000	chr17	40099999	40219999	120000
				chr17	40259999	40299999	40000	chr17	40259999	40299999	40000	chr17	40259999	40299999	40000
				chrX	3019999	4939999	1920000	chrX	3019999	4939999	1920000	chrX	3019999	4939999	1920000
				chrX	30259999	32219999	1960000	chrX	30259999	32219999	1960000	chrX	30259999	32219999	1960000
				chrX	1.66E+08	1.66E+08	40000	chrX	1.66E+08	1.66E+08	40000	chrX	1.66E+08	1.66E+08	40000

LOG2_RATIO_A

LOG2_RATIO_B

LOG2_RATIO_C

LOG2_RATIO_D

LOG2_RATIO_E

LOG2_RATIO_F

LOG2_RATIO_G

LOG2_RATIO_H

LOG2_RATIO_I

LOG2_RATIO_J

LOG2_RATIO_K

LOG2_RATIO_L

LOG2_RATIO_M

LOG2_RATIO_N

LOG2_RATIO_O

LOG2_RATIO_P

LOG2_RATIO_Q

LOG2_RATIO_R

LOG2_RATIO_S

LOG2_RATIO_T

LOG2_RATIO_U

LOG2_RATIO_V

LOG2_RATIO_W

LOG2_RATIO_X

LOG2_RATIO_Y

LOG2_RATIO_Z

LOG2_RATIO_AA

LOG2_RATIO_AB

LOG2_RATIO_AC

LOG2_RATIO_AD

LOG2_RATIO_AE

LOG2_RATIO_AF

LOG2_RATIO_AG

LOG2_RATIO_AH

LOG2_RATIO_AI

LOG2_RATIO_AJ

LOG2_RATIO_AK

LOG2_RATIO_AL

LOG2_RATIO_AM

LOG2_RATIO_AN

LOG2_RATIO_AO

LOG2_RATIO_AP

LOG2_RATIO_AQ

LOG2_RATIO_AR

LOG2_RATIO_AS

LOG2_RATIO_AT

LOG2_RATIO_AU

LOG2_RATIO_AV

LOG2_RATIO_AW

LOG2_RATIO_AX

LOG2_RATIO_AY

LOG2_RATIO_AZ

LOG2_RATIO_BA

LOG2_RATIO_BB

LOG2_RATIO_BC

LOG2_RATIO_BD

LOG2_RATIO_BE

LOG2_RATIO_BF

LOG2_RATIO_BG

LOG2_RATIO_BH

LOG2_RATIO_BI

LOG2_RATIO_BJ

LOG2_RATIO_BK

LOG2_RATIO_BL

LOG2_RATIO_BM

LOG2_RATIO_BN

LOG2_RATIO_BO

LOG2_RATIO_BP

LOG2_RATIO_BQ

LOG2_RATIO_BR

LOG2_RATIO_BS

LOG2_RATIO_BT

LOG2_RATIO_BU

LOG2_RATIO_BV

LOG2_RATIO_BW

LOG2_RATIO_BX

LOG2_RATIO_BY

LOG2_RATIO_BZ

LOG2_RATIO_CA

LOG2_RATIO_CB

LOG2_RATIO_CC

LOG2_RATIO_CD

LOG2_RATIO_CE

LOG2_RATIO_CF

LOG2_RATIO.CG

LOG2_RATIO_CH

LOG2_RATIO_CI

LOG2_RATIO_CJ

LOG2_RATIO_CK

LOG2_RATIO_CL

LOG2_RATIO_CM

LOG2_RATIO_CN

LOG2_RATIO_CO

LOG2_RATIO_CP

LOG2_RATIO_CQ

LOG2_RATIO_CR

LOG2_RATIO_CS

LOG2_RATIO_CT

LOG2_RATIO_CU

LOG2_RATIO_CV

LOG2_RATIO_CW

LOG2_RATIO_CX

LOG2_RATIO_CY

LOG2_RATIO_CZ

LOG2_RATIO_DA

LOG2_RATIO_DB

LOG2_RATIO_DC

LOG2_RATIO_DD

LOG2_RATIO_DE

LOG2_RATIO_DF

LOG2_RATIO_DG

LOG2_RATIO_DH

LOG2_RATIO_DI

LOG2_RATIO_DJ

LOG2_RATIO_DK

LOG2_RATIO_DL

LOG2_RATIO_DM

LOG2_RATIO_DN

LOG2_RATIO_DO

LOG2_RATIO_DP

LOG2_RATIO_DQ

LOG2_RATIO_DR

LOG2_RATIO_DS

LOG2_RATIO_DT

Supplementary Table 2 - differentially regulated genes in haploid ES cells (> 2 fold; p<0.05)

Upregulated genes in haploid vs diploid ES cells

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
1 1434068_s_at	11.99	AI662270	103814	expressed sequence AI662270
2 1416749_at	10.76	Htra1	56213	HtrA serine peptidase 1
3 1419540_at	8.46	EG434726 // Fth117 434726 /// 434727 ///		predicted gene 14458
4 1460670_at	8.39	Riok3	66878	RIO kinase 3 (yeast)
5 1419134_at	7.66	Rhbg	58176	Rhesus blood group-associated B glycoprotein
6 1436107_at	7.51	Lsm8	76522	LSM8 homolog, U6 small nuclear RNA associated (<i>S. cerevisiae</i>)
7 1427512_a_at	7.47	Lama3	16774	laminin, alpha 3
8 1450001_a_at	7.34	Ush1c	72088	Usher syndrome 1C homolog (human)
9 1456036_x_at	7.24	Gsto1	14873	glutathione S-transferase omega 1
10 1454799_at	7.01	Agpat9	231510	1-acylglycerol-3-phosphate O-acyltransferase 9
11 1424692_at	6.90	2810055F11Rik	67217	RIKEN cDNA 2810055F11 gene
12 1436355_at	6.73	Fads6	328035	fatty acid desaturase domain family, member 6
13 1454869_at	6.60	Wdr40b	245404	WD repeat domain 40B
14 1453977_at	6.54	Exoc4	20336	exocyst complex component 4
15 1437152_at	6.40	Mex3b	108797	mex3 homolog B (<i>C. elegans</i>)
16 1454737_at	6.27	Dusp9	75590	dual specificity phosphatase 9
17 1437867_at	5.95			
18 1416531_at	5.83	Gsto1	14873	glutathione S-transferase omega 1
19 1426688_at	5.80	Klhd5	232539	kelch domain containing 5
20 1450460_at	5.53	Aqp3	11828	aquaporin 3
21 1422008_a_at	5.53	Aqp3	11828	aquaporin 3
22 1421385_a_at	5.33	Myo7a	17921	myosin VIIA
23 1445281_a_at	5.28	B230311B06Rik	381914	RIKEN cDNA B230311B06 gene
24 1444416_at	5.26	Cenpa	12615	centromere protein A
25 1429308_at	5.23	Prdm16	70673	PR domain containing 16
26 1433845_x_at	5.15	Dusp9	75590	dual specificity phosphatase 9
27 1438251_x_at	4.82	Htra1	56213	HtrA serine peptidase 1
28 1449031_at	4.79	Cited1	12705	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1
29 1437409_s_at	4.78	Gpr126	215798	G protein-coupled receptor 126
30 1438238_at	4.72	2010315B03Rik	630836	RIKEN cDNA 2010315B03 gene
31 1426223_at	4.66	Ttc39c	72747	tetratricopeptide repeat domain 39C
32 1435948_at	4.40	Tmem181 // Tmem 100040525 // 77106		transmembrane protein 181C, pseudogene
33 1448596_at	4.13	Slc6a8	102857	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
34 1438842_at	4.09	Mtch2	56428	mitochondrial carrier homolog 2 (<i>C. elegans</i>); predicted gene, 100039384; predi
35 1417120_at	4.08	Miip	28010	migration and invasion inhibitory protein
36 1449036_at	4.05	Rnf128	66889	ring finger protein 128
37 1439422_a_at	4.04	Fam132a	67369	family with sequence similarity 132, member A
38 1429169_at	4.03	Rbm3	19652	predicted gene 15453, RNA binding motif protein 3
39 1424082_at	4.03	Tbc1d13	70296	TBC1 domain family, member 13
40 1429504_at	3.97	Rnpc3	67225	RNA-binding region (RNP1, RRM) containing 3

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
41 1444226_at	3.78	Foxc3	56484	forkhead box O3
42 1418681_at	3.75	Alg13	67574	asparagine-linked glycosylation 13 homolog (<i>S. cerevisiae</i>)
43 1451754_a_at	3.71	Wdr45	54636	WD repeat domain 45
44 1433844_a_at	3.60	Dusp9	75590	dual specificity phosphatase 9
45 1456804_at	3.57	EG627821	627821	predicted gene 6792
46 1437302_at	3.56	Adrb2	11555	adrenergic receptor, beta 2
47 1450843_a_at	3.55	Serpinh1	12406	serine (or cysteine) peptidase inhibitor, clade H, member 1
48 1459740_s_at	3.55	Ucp2	22228	uncoupling protein 2 (mitochondrial, proton carrier)
49 1428933_at	3.53	Hdac8	70315	histone deacetylase 8
50 1451148_at	3.52	LOC100047214 // F100047214 // 68943		similar to PTEN induced putative kinase 1
51 1422743_at	3.48	Phka1	18679	phosphorylase kinase alpha 1
52 1454009_at	3.45	1700008O03Rik	69349	RIKEN cDNA 1700008O03 gene
53 1438750_at	3.42	Atlx	22589	alpha thalassemia/mental retardation syndrome X-linked homolog (human)
54 1448599_s_at	3.39	Mip	28010	migration and invasion inhibitory protein
55 1426241_a_at	3.38	Scmh1	29871	sex comb on midleg homolog 1
56 1435069_at	3.35	BC064078	408064	cDNA sequence BC064078
57 1425601_a_at	3.34	Rlkn	20186	rhotekin
58 1430538_at	3.28	2210013O21Rik	70123	RIKEN cDNA 2210013O21 gene
59 1417116_at	3.26	Slc6a8	102857	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
60 1422823_at	3.26	Eps8 // LOC632636	13860 // 632636	epidermal growth factor receptor pathway substrate 8
61 1434092_at	3.22	Atg9b	213948	ATG9 autophagy related 9 homolog B (<i>S. cerevisiae</i>)
62 1416239_at	3.20	Ass1	11898	argininosuccinate synthetase 1
63 1455721_at	3.20	Gsp12	14853	G1 to S phase transition 2
64 1436200_at	3.19	Lonr3	74385	LON peptidase N-terminal domain and ring finger 3
65 1426466_s_at	3.19	Rps6kl1	238323	ribosomal protein S6 kinase-like 1
66 1418318_at	3.16	Rnf128	66889	ring finger protein 128
67 1435514_at	3.14	Lztl1	93730	leucine zipper transcription factor-like 1, predicted gene 6776
68 1449476_at	3.14	Rage	26448	renal tumor antigen
69 1447957_at	3.09	D7Erd128e	52222	DNA segment, Chr 7, ERATO Doi 128, expressed
70 1422711_a_at	3.08	Pnck	93843	pregnancy upregulated non-ubiquitously expressed CaM kinase
71 1417850_at	3.04	Rbf	19645	retinoblastoma 1
72 1422744_at	3.02	Phka1	18679	phosphorylase kinase alpha 1
73 1427564_at	3.02	Diap2	54004	diaphanous homolog 2 (<i>Drosophila</i>)
74 1451169_at	3.02	Nomo1	211548	nodal modulator 1
75 1448330_at	3.01	Gstm1	14862	similar to Glutathione S-transferase Mu 1 (GST class-mu 1) (Glutathione S-trans
76 1455817_x_at	3.00	Zxdb	668166	zinc finger, X-linked, duplicated B
77 1451269_at	2.99	Pdzd11	72621	PDZ domain containing 11
78 1446583_at	2.97			
79 1449119_at	2.97	Arh2	23807	ariadne homolog 2 (<i>Drosophila</i>); predicted gene 12263
80 1437369_at	2.97	Fgd1	14163	FYVE, RhoGEF and PH domain containing 1
81 1460600_at	2.96	AA414768	245350	expressed sequence AA414768
82 1453040_at	2.96	Mcart6	67062	mitochondrial carrier triple repeat 6
83 1424838_at	2.95	A330049M08Rik	230822	RIKEN cDNA A330049M08 gene
84 1450919_at	2.93	Mpp1	17524	membrane protein, palmitoylated
85 1425068_a_at	2.91	Tex264	21767	testis expressed gene 264
86 1444057_at	2.91			UBX domain protein 4
87 1434493_at	2.91	1810022K09Rik	69126	predicted gene 4540; RIKEN cDNA 1810022K09 gene
88 1421896_at	2.91	Elk1	13712	ELK1, member of ETS oncogene family
89 1422327_s_at	2.90	G6p32 // G6pdx	14380 // 14381	glucose-6-phosphate dehydrogenase 2
90 1437449_at	2.90	Rsad1	237926	radical S-adenosyl methionine domain containing 1

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
91 1444837_at	2.89			Scm-like with four rmbt domains 2
92 1417393_a_at	2.88	Fam132a	67389	family with sequence similarity 132, member A
93 1418901_at	2.86	Cebpb	12608	CCAAT/enhancer binding protein (C/EBP), beta
94 1439065_at	2.83	Rprd1a	225283	regulation of nuclear pre-mRNA domain containing 1A
95 1437435_at	2.83	1700061G19Rik	78625	RIKEN cDNA 1700061G19 gene
96 1424322_at	2.81	Apex2	77822	apurinic/aprimidinic endonuclease 2
97 1436347_a_at	2.81	5530601H04Rik	71445	RIKEN cDNA 5530601H04 gene
98 1433741_at	2.81	Cd38	12494	CD38 antigen
99 1434436_at	2.81	Morc4	75746	microorchidia 4
100 1427844_a_at	2.76	Cebpb	12608	CCAAT/enhancer binding protein (C/EBP), beta
101 1448524_s_at	2.76	Ssr4	20832	signal sequence receptor, delta
102 1428705_at	2.75	1700007K13Rik	69327	RIKEN cDNA 1700007K13 gene
103 1426863_at	2.75	Rbmx	19655	RNA binding motif protein, X chromosome
104 1417412_at	2.74	F8a	14070	factor 8-associated gene A
105 1457313_at	2.73	Ocrl	320634	oculocerebrorenal syndrome of Lowe
106 1456028_at	2.72	Maip1	17762	microtubule-associated protein tau
107 1444585_at	2.70	Adc	242669	arginine decarboxylase
108 1435218_at	2.69	Rasgef1a	70727	RasGEF domain family, member 1A
109 1439753_x_at	2.69	Six4	20474	sine oculis-related homeobox 4 homolog (Drosophila)
110 1423435_at	2.68	Gsta3	14859	glutathione S-transferase, alpha 3
111 1417357_at	2.67	Erd4	13726	emerin
112 1438671_at	2.66	Ppp2r2c	269643	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), gamma isoform
113 1418416_x_at	2.66	Gstm1	14882	similar to Glutathione S-transferase Mu 1 (GST class-mu 1) (Glutathione S-trans
114 1441229_at	2.66	D230019N24Rik	399607	RIKEN cDNA D230019N24 gene
115 1434681_at	2.66	4932441K18Rik	353170	predicted gene 8258; similar to factor inhibiting activating transcription factor 4 (f
115 1451751_at	2.66	Ddit4l	73284	DNA-damage-inducible transcript 4-like
117 1436780_at	2.65	Ogt	108155	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine
118 1436509_at	2.64	Mlec	109154	malectin
119 1438824_at	2.64	Chm	12662	similar to choroideremia, choroideremia
120 1419763_at	2.63	Nkap	67050	reproductive homeobox 3B; UPF3 regulator of nonsense transcripts homolog B
121 1448188_at	2.59	Ucp2	22228	uncoupling protein 2 (mitochondrial, proton carrier)
122 1450161_at	2.59	Ikbkg	16151	inhibitor of kappaB kinase gamma
123 1426025_s_at	2.58	Leptm5	16792	lysosomal-associated protein transmembrane 5
124 1428654_at	2.57	1200016B10Rik	66875	RIKEN cDNA 1200016B10 gene
125 1439476_at	2.56	Dsg2	13511	desmoglein 2; similar to Dsg2 protein
126 1417732_at	2.55	Anxa8	11752	annexin A8
127 1416032_at	2.54	Tmem109	68539	transmembrane protein 109
128 1418237_s_at	2.54	Col18a1	12822	collagen, type XVII, alpha 1
129 1417721_s_at	2.53	Leptm5	16792	lysosomal-associated protein transmembrane 5
130 1453375_at	2.53	4930422N03Rik	76871	RIKEN cDNA 4930422N03 gene
131 1424286_at	2.53	Ptkx	19108	protein kinase, X-linked
132 1456504_at	2.52	Zfp182	319535	zinc finger protein 182
133 1420922_at	2.52	Usp9x	22284	ubiquitin specific peptidase 9, X chromosome
134 1420433_at	2.51	Taf7l	74469	TAF7-like RNA polymerase II, TATA box binding protein (TBP)-associated factor
135 1460471_at	2.50	Ooep	67968	oocyte expressed protein homolog (dog)
136 1432026_a_at	2.50	Herc5	67138	hect domain and RLD 5
137 1427235_at	2.49	Kdm6a	22289	4lysine (K)-specific demethylase 6A
138 1455111_at	2.49	Yip6	77929	similar to Yip1 domain family, member 6; Yip1 domain family, member 6
139 1441937_s_at	2.49	LOC100047214 // F100047214 // 68943		similar to PTEN-induced putative kinase 1
140 1431856_a_at	2.48	C1orf116	72709	C1orf116 and tumor necrosis factor related protein 6

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
141 1451884_a_at	2.48	Lsm2	27756	LSM2 homolog, U6 small nuclear RNA associated (<i>S. cerevisiae</i>)
142 1455188_a_at	2.48	Ppp2r3a	19054	protein phosphatase 2 (formerly 2A), regulatory subunit B', alpha; RIKEN cDNA
143 1416397_at	2.48	Mesdc1	80889	mesoderm development candidate 1
144 1430975_at	2.47	8430416G17Rik	71469	RIKEN cDNA 8430416G17 gene
145 1456716_s_at	2.47	3110002H16Rik	76482	RIKEN cDNA 3110002H16 gene
146 1449046_a_at	2.47	Josd2	66124	Josephin domain containing 2
147 1448645_at	2.46	Msl3	17692	male-specific lethal 3 homolog (<i>Drosophila</i>)
148 1435458_at	2.46	Pim1	18712	proviral integration site 1
149 1444500_at	2.46	Ahsa1	217737	AHA1, activator of heat shock protein ATPase homolog 1 (yeast)
150 1460579_at	2.45	Dnpep	13437	aspartyl aminopeptidase
151 1456609_at	2.44	Camk2n1	66259	calcium/calmodulin-dependent protein kinase II inhibitor 1
152 1434424_at	2.43	Mfsd7b	226844	major facilitator superfamily domain containing 7B
153 1452859_at	2.43	1200016B10Rik	66875	RIKEN cDNA 1200016B10 gene
154 1453091_s_at	2.43	Letmd1	68614	LETMD1 domain containing 1
155 1445634_at	2.42			microtubule-associated protein tau
156 1431774_a_at	2.41	Lym1	73919	LYR motif containing 1
157 1419309_at	2.40	Pdpn	14726	podoplanin
158 1416155_at	2.40	Hmgb3	15354	predicted gene 11805; predicted gene 8850; high mobility group box 3; similar to
159 1445241_at	2.40	Rab11fip4	268451	RAB11 family interacting protein 4 (class II)
160 1438857_x_at	2.40	Irak1	16179	interleukin-1 receptor-associated kinase 1
161 1448192_s_at	2.38	AU021838 // Prps1	19139 // 328099	mirror-image polydactyl gene 1 homolog (human); phosphoribosyl pyrophospho
162 1435452_at	2.38	LOC100047579 // T100047579 // 240661		similar to transmembrane protein 20; transmembrane protein 20
163 1436917_s_at	2.37	Gpsm1	67839	G-protein signalling modulator 1 (AGS3-like, <i>C. elegans</i>)
164 1455727_at	2.37	Zrsr2	22184	zinc finger (CCH type), RNA binding motif and serine/arginine rich 2
165 1417099_at	2.36	Ftsj1 // LOC100044	100044636 // 54632	similar to Ftsj homolog; FtsJ homolog 1 (<i>E. coli</i>)
166 1435587_at	2.36	Phk1	18679	phosphorylase kinase alpha 1
167 1457394_at	2.35	2900002K06Rik	70226	RIKEN cDNA 2900002K06 gene
168 1435051_at	2.35	Wdr44	72404	WD repeat domain 44
169 1458684_at	2.35			similar to Ss18 protein; synovial sarcoma translocation, Chromosome 18
170 1421499_a_at	2.35	Ptpn14	19250	protein tyrosine phosphatase, non-receptor type 14
171 1423228_at	2.34	B4galT6	56386	UDP-GalbetaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6; similar to B
172 1455784_at	2.34	Sac1	56546	secretory blood group 1
173 1434263_at	2.34	F630110N24Rik	73822	RIKEN cDNA F630110N24 gene
174 1419206_at	2.34	Cd37	12493	CD37 antigen
175 1448354_at	2.34	G6pdx	14381	glucose-6-phosphate dehydrogenase X-linked
176 1416865_at	2.34	Fgd1	14163	FYVE, RhoGEF and PH domain containing 1
177 1446234_at	2.34			lysine (K)-specific demethylase 6A
178 1423660_at	2.33	Ctdsp2 // ENSMUS	100043719 // 52468	predicted gene 9770
179 1434061_at	2.33	Rp2h	19889	retinitis pigmentosa 2 homolog (human)
180 1437495_at	2.33	Mbtss2 // Yy2	100073351 // 270661	membrane-bound transcription factor peptidase, site 2; similar to zinc finger, X-li
181 1434849_at	2.33	Tsply2	52808	TSPY-like 2
182 1448419_at	2.33	Pop4	66161	processing of precursor 4, ribonuclease PMRP family, (<i>S. cerevisiae</i>)
183 1451811_at	2.33	Pla2g16	225845	phospholipase A2, group XVI
184 1416052_at	2.32	Prps1	19139	mirror-image polydactyl gene 1 homolog (human); phosphoribosyl pyrophospho
185 1426955_at	2.32	Col18a1	12822	collagen, type XVIII, alpha 1
186 1426484_at	2.31	Ubxn4	67812	UBX domain protein 4
187 1423451_at	2.31	Pgmc1	53328	progesterone receptor membrane component 1
188 1417637_a_at	2.31	Hmg20b	15353	high mobility group 20 B
189 1432205_a_at	2.30	C130038G02Rik	77521	RIKEN cDNA C130038G02 gene
190 1439201_at	2.30	Usp14	59025	ubiquitin specific peptidase 14

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
191 1436541_at	2.29	2310008H09Rik	66356	RIKEN cDNA 2310008H09 gene
192 1425516_at	2.29	Ogt	108155	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine
193 1434360_s_at	2.29	LOC632664 // Ptprc	19270 // 632664	protein tyrosine phosphatase, receptor type, G; similar to protein tyrosine phospho
194 1449740_s_at	2.28	Dsg2	13511	desmoglein 2; similar to Dsg2 protein
195 1454082_a_at	2.28	Giyd2	75764	GIY-YIG domain containing 2
196 1448371_at	2.28	Mylf	17907	myosin light chain, phosphorylatable, fast skeletal muscle
197 1424236_at	2.27	Tbc1d10b	68449	TBC1 domain family, member 10b
198 1433913_at	2.27	C80913	19777	expressed sequence C80913
199 1448906_at	2.27	Ppap2b	67916	phosphatidic acid phosphatase type 2B
200 1460177_at	2.27	Cndp2	66054	CNDP dipeptidase 2 (metallopeptidase M20 family)
201 1460649_at	2.27	Irak1	16179	interleukin-1 receptor-associated kinase 1
202 1456279_a_at	2.26	Bcap31	27061	B-cell receptor-associated protein 31
203 1428357_at	2.25	2610019F03Rik	72148	RIKEN cDNA 2610019F03 gene
204 1437780_at	2.25	Fancb	237211	Fanconi anemia, complementation group B
205 1454787_at	2.25	Zdhhc9	208684	similar to zinc finger, DHHC domain containing 9; zinc finger, DHHC domain con
206 1418397_at	2.23	Zfp275	27081	zinc finger protein 275
207 1451302_at	2.23	1110012L19Rik	68618	RIKEN cDNA 1110012L19 gene; predicted gene 8512
208 1431228_s_at	2.22	4930526115Rik	75135	RIKEN cDNA 4930526115 gene
209 1450995_at	2.22	Folr1	14275	folate receptor 1 (adult)
210 1439064_at	2.22	BC030046	277154	cDNA sequence BC030046
211 1422616_s_at	2.22	Wdr54	75659	WD repeat domain 54
212 1448269_a_at	2.21	Klf13	67455	kelch-like 13 (Drosophila)
213 1423122_at	2.20	Avp1	69534	arginine vasopressin-induced 1
214 1445693_at	2.20	Araf	11836	v-rf murine sarcoma 3611 viral oncogene homolog
215 1448772_at	2.20	Ube2a	22209	ubiquitin-conjugating enzyme E2A, RAD6 homolog (S. cerevisiae)
216 1460169_a_at	2.20	Pctk1	18555	PCTAIRE-motif protein kinase 1
217 1444390_at	2.20	Prdm14	383491	PR domain containing 14
218 1448118_a_at	2.19	Ctsd	13033	cathepsin D
219 1424124_at	2.19	Mospd2	76763	motile sperm domain containing 2
220 1420725_at	2.19	Tmlhe	192289	trimethyllysine hydroxylase, epsilon
221 1450264_a_at	2.18	Chka	12660	choline kinase alpha
222 1434518_at	2.18	Phka2	110094	phosphorylase kinase alpha 2
223 1440344_at	2.18	Snord89	100217461	small nucleolar RNA, C/D box 89
224 1431044_at	2.18	Thoc1	225160	THO complex 1
225 1438843_x_at	2.17	Mtch2	56428	mitochondrial carrier homolog 2 (C. elegans); predicted gene, 100039384; predi
226 1454816_at	2.17	Rp2h	19889	retinitis pigmentosa 2 homolog (human)
227 1415956_a_at	2.17	Pctk1	18555	PCTAIRE-motif protein kinase 1
228 1429400_at	2.16	Cln5	12728	chloride channel 5
229 1424224_at	2.16	Asb8	78541	ankyrin repeat and SOCS box-containing 8
230 1453895_at	2.16	C330026N13Rik	78531	RIKEN cDNA C330026N13 gene
231 1426734_at	2.15	Fam43a	224093	family with sequence similarity 43, member A
232 1428497_at	2.15	Kdm5c	20591	lysine (K)-specific demethylase 5C
233 1423978_at	2.14	Sbk1	104175	SH3-binding kinase 1
234 1451070_at	2.14	Gdi1	14567	guanosine diphosphate (GDP) dissociation inhibitor 1
235 1456981_at	2.14	Tmc7	209760	transmembrane channel-like gene family 7; similar to Tmc7 protein
236 1455247_at	2.13	Amot1	75723	angiostatin-like 1
237 1452800_e_at	2.13	Apoa	68316	similar to Novel transmembrane domain containing protein; apolipoprotein O
238 1451299_at	2.13	Prkx	19108	protein kinase, X-linked
239 1430449_at	2.13	Kidins220	77480	kinase D-interacting substrate 220
240 1430215_at	2.13	2610019F03Rik	72148	RIKEN cDNA 2610019F03 gene

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
241 1450107_a_at	2.12	Renbp	19703	renin binding protein
242 1435860_at	2.12	Slc5a6	330064	solute carrier family 5 (sodium-dependent vitamin transporter), member 6
243 1422965_at	2.11	Agtrap	11610	angiotensin II, type I receptor-associated protein
244 1424167_a_at	2.11	Pmm1	29858	phosphomannomutase 1
245 1423662_at	2.11	Atp6ap2	70495	ATPase, H+ transporting, lysosomal accessory protein 2
246 1420909_at	2.11	Vegfa	22339	vascular endothelial growth factor A
247 1438270_at	2.11	Al846148	68229	expressed sequence Al846148
248 1427573_at	2.11	Chic1	12212	cysteine-rich hydrophobic domain 1
249 1458358_at	2.11	Pank2	74450	pantothenate kinase 2 (Hallervorden-Spatz syndrome)
250 1436905_x_at	2.11	Laptn5	16792	lysosomal-associated protein transmembrane 5
251 1421797_a_at	2.10	Snx12	55988	sorting nexin 12
252 1428334_at	2.10	Ostm1	14628	osteopetrosis associated transmembrane protein 1
253 1450038_s_at	2.10	Usp9x	22284	ubiquitin specific peptidase 9, X chromosome
254 1439631_at	2.09	Zcchc11	230594	zinc finger, CCHC domain containing 11
255 1448668_a_at	2.09	Irak1	16179	interleukin-1 receptor-associated kinase 1
256 1426410_at	2.08	Pdk3	236900	pyruvate dehydrogenase kinase, isoenzyme 3
257 1441391_at	2.07			guanine nucleotide binding protein (G protein), beta polypeptide 1-like; similar to
258 1449622_s_at	2.07	Atp6ap1	54411	ATPase, H+ transporting, lysosomal accessory protein 1
259 1426306_a_at	2.07	LOC100046560 /// \ 100046560 /// 80884		similar to melanoma antigen family D, 2; melanoma antigen, family D, 2
260 1457316_at	2.07	Mtap6	17760	microtubule-associated protein 6
261 1417442_a_at	2.06	Pex3	56535	peroxisomal biogenesis factor 3
262 1451049_at	2.05	Bcap31	27061	B-cell receptor-associated protein 31
263 1435008_at	2.05	Slc9a6	236794	solute carrier family 9 (sodium/hydrogen exchanger), member 6
264 1424106_at	2.05	1200003C05Rik	104771	RIKEN cDNA 1200003C05 gene
265 1423661_s_at	2.04	Ctdsp2 /// ENSMUS	100043719 /// 52468	predicted gene 9770
266 1435566_s_at	2.04	Araf	11836	v-raf murine sarcoma 3611 viral oncogene homolog
267 1451400_at	2.04	Gemin8	237221	similar to gem (nuclear organelle) associated protein 8; predicted gene 5455; ge
268 1428773_s_at	2.04	Bcor	71458	BCL6 interacting corepressor
269 1432158_a_at	2.04	Trappc2	66226	similar to Chain A, The Crystal Structure Of The Bet3-Trs31-Sedlin Complex; tra
270 1449916_at	2.02	Pbx4	80720	pre-B-cell leukemia homeobox 4
271 1432011_at	2.02	2900052L18Rik	76835	predicted gene 4407; RIKEN cDNA 2900052L18 gene
272 1434908_at	2.02	Scaf1	233208	SR-related CTD-associated factor 1
273 1441970_at	2.02	E430010N07Rik	399572	RIKEN cDNA E430010N07 gene
274 1429776_a_at	2.02	Dnajb6	23950	Dnaj (Hsp40) homolog, subfamily B, member 6; predicted gene 5917; predictex
275 1425525_a_at	2.01	P2rx4	18438	purinergic receptor P2X, ligand-gated ion channel 4
276 1436300_at	2.01	Dstyk	213452	dual serine/threonine and tyrosine protein kinase
277 1430780_a_at	2.01	Pmm1	29858	phosphomannomutase 1

Downregulated genes in haploid vs diploid ES cells

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
1 1417210_at	-46.94	Eif2s3y	26908	eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked
2 1443621_at	-34.01	Xaf1	327959	XIAP associated factor 1
3 1426438_at	-32.17	Ddx3y	26900	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
4 1445226_at	-23.48	BC023969	407828	cDNA sequence BC023969
5 1452077_at	-21.18	Ddx3y	26900	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
6 1427302_at	-12.31	Enpp3	209558	ectonucleotide pyrophosphatase/phosphodiesterase 3
7 1416371_at	-10.48	Apod	11815	apolipoprotein D
8 1437721_at	-9.30	Coro1c	23790	coronin, actin binding protein 1C; predicted gene 5790
9 1438833_at	-9.18	Casc5	76464	cancer susceptibility candidate 5
10 1415949_at	-8.97	Cpe // LOC100046434 // 12876	100046434	carboxypeptidase E; similar to carboxypeptidase E
11 1438933_x_at	-7.94	Rasgrp2	19395	RAS, guanyl releasing protein 2
12 1415897_a_at	-7.72	Mgst1	56615	microsomal glutathione S-transferase 1
13 1437671_x_at	-6.76	Prss23	76453	protease, serine, 23
14 1450857_a_at	-6.55	Col1a2	12843	collagen, type I, alpha 2
15 1423110_at	-6.45	Col1a2	12843	collagen, type I, alpha 2
16 1417461_at	-6.33	Cap1	12331	CAP, adenylate cyclase-associated protein 1 (yeast)
17 1426598_at	-6.23	Uty	22290	ubiquitously transcribed tetrapeptide repeat gene, Y chromosome
18 1439757_s_at	-6.02	Epha4	13838	Eph receptor A4
19 1452384_at	-5.96	Enpp3	209558	ectonucleotide pyrophosphatase/phosphodiesterase 3
20 1417462_at	-5.72	Cap1	12331	CAP, adenylate cyclase-associated protein 1 (yeast)
21 1439555_at	-5.52	Rif	109263	rearranged L-myc fusion sequence
22 1433542_at	-5.44	Inpp5f	101490	inositol polyphosphate-5-phosphatase F
23 1451260_at	-5.18	Aldh1b1	72535	aldehyde dehydrogenase 1 family, member B1
24 1460616_at	-5.11	Slc4c1	227394	solute carrier organic anion transporter family, member 4C1
25 1433783_at	-5.07	Ldb3	24131	LIM domain binding 3
26 1425538_x_at	-4.56	Ceacam1	26365	carcinoembryonic antigen-related cell adhesion molecule 1; carcinoembryonic a
27 1432075_a_at	-4.49	Tekt1	21689	tektin 1
28 1431057_a_at	-4.47	Prss23	76453	protease, serine, 23
29 1416008_at	-4.24	Satb1	20230	special AT-rich sequence binding protein 1
30 1448123_s_at	-4.16	Tgfb1	21810	transforming growth factor, beta induced
31 1421929_at	-4.10	Epha4	13838	Eph receptor A4
32 1426108_s_at	-4.09	Cacnb1	12295	calcium channel, voltage-dependent, beta 1 subunit
33 1423505_at	-4.05	Tagln	21345	transgelin
34 1435836_at	-3.90	Pdk1	228026	pyruvate dehydrogenase kinase, isoenzyme 1
35 1427630_x_at	-3.74	Ceacam1	26365	carcinoembryonic antigen-related cell adhesion molecule 1; carcinoembryonic a
36 1438403_s_at	-3.72	Malat1	72289	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
37 1429509_at	-3.72	Lsm12	268490	LSM12 homolog (S. cerevisiae)
38 1449071_at	-3.69	My7	17898	myosin, light polypeptide 7, regulatory
39 1421100_a_at	-3.62	Dab1	13131	disabled homolog 1 (Drosophila)
40 1418188_a_at	-3.52	Malat1	72289	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
41 1420989_at	-3.51	4933411K20Rik	66756	RIKEN cDNA 4933411K20 gene
42 1418189_s_at	-3.50	Malat1	72289	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
43 1423182_s_at	-3.45	Spred1	114715	sprouty protein with EVH-1 domain 1, related sequence
44 1418617_x_at	-3.44	Clgn	12745	calmegin
45 1423184_at	-3.35	Ilsn2	20403	intersectin 2
46 1444616_x_at	-3.33			C-type lectin domain family 2, member e
47 1434447_at	-3.25	Met	17295	met proto-oncogene
48 1450494_x_at	-3.24	Ceacam1	26365	carcinoembryonic antigen-related cell adhesion molecule 1; carcinoembryonic a
49 1424130_a_at	-3.23	Ptf1	19285	polymerase I and transcript release factor
50 1429021_at	-3.23	Epha4	13838	Eph receptor A4

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
51 1417780_at	-3.21	Lass4	67260	LAG1 homolog, ceramide synthase 4
52 1427311_at	-3.17	Bpif	207165	bromodomain PHD finger transcription factor
53 1416612_at	-3.17	Cyp1b1	13078	cytochrome P450, family 1, subfamily b, polypeptide 1
54 1417141_at	-3.16	Igtp	15145	interferon gamma induced GTPase
55 1456120_at	-3.15	Secisbp2l	70354	SECIS binding protein 2-like
56 1429778_at	-3.12	Opln	71648	optineurin
57 1427998_at	-3.10	Lsm12	288490	LSM12 homolog (S. cerevisiae)
58 1435679_at	-3.08	Opln	71648	optineurin
59 1421129_a_at	-3.05	Atp2a3	53313	ATPase, Ca++ transporting, ubiquitous
60 1458539_at	-2.95	R3hdm1	226412	R3H domain 1 (binds single-stranded nucleic acids)
61 1457936_at	-2.93	Mapk8	28419	mitogen-activated protein kinase 8
62 1439982_at	-2.92	Sec23ip	207352	Sec23 interacting protein
63 1424598_at	-2.87	Ddx6	13209	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
64 1450547_at	-2.87	Hps3	12807	Hermansky-Pudlak syndrome 3 homolog (human)
65 1438200_at	-2.87	Sulf1	240725	sulfatase 1
66 1450839_at	-2.85	D0H4S114	27528	DNA segment, human D4S114
67 1429183_at	-2.84	Pkp2	67451	plakophilin 2
68 1447680_at	-2.83	Anp32e	66471	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E
69 1456697_x_at	-2.80	Omf1f	23857	cyclin D binding myb-like transcription factor 1
70 1440146_at	-2.78	Vps13a	271564	vacuolar protein sorting 13A (yeast)
71 1450068_at	-2.77	Baz1b	22385	bromodomain adjacent to zinc finger domain, 1B
72 1417220_at	-2.76	Fah	14085	fumarate acetoacetate hydrolase
73 1424657_at	-2.74	Taok1	216965	TAO kinase 1
74 1448300_at	-2.73	Mgst3	69447	mitochondrial glutathione S-transferase 3
75 1449799_s_at	-2.72	Pkp2	67451	plakophilin 2
76 1433809_at	-2.70	Ddx5	13207	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5; predicted gene 12183
77 1456332_x_at	-2.69	Sox4	20677	SRY-box containing gene 19; SRY-box containing gene 4
78 1418197_at	-2.66	Ucp1	22227	uncoupling protein 1 (mitochondrial, proton carrier)
79 1436319_at	-2.65	Sulf1	240725	sulfatase 1
80 1452330_a_at	-2.63	Mxra8	74761	matrix-remodeling associated 8
81 1427456_at	-2.63	Wdfy3	72145	WD repeat and FYVE domain containing 3
82 1436311_at	-2.61	Gemin5	216766	gem (nuclear organelle) associated protein 5
83 1437117_at	-2.61	Centb1	216859	centaurin, beta 1
84 1430240_a_at	-2.60	Clgn	12745	calmegin
85 1443778_at	-2.57	Sox4	20677	SRY-box containing gene 19; SRY-box containing gene 4
86 1424703_at	-2.53	Hemk1	69536	HemK methyltransferase family member 1
87 1423066_at	-2.53	Dnmt3a	13435	DNA methyltransferase 3A
88 1416105_at	-2.52	Nnt1	18115	nicotinamide nucleotide transhydrogenase
89 1436020_at	-2.52	Zfp828	101994	zinc finger protein 828
90 1438708_x_at	-2.51	Ywhab	54401	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta
91 1448632_at	-2.50	Psm110	19171	proteasome (prosome, macropain) subunit, beta type 10
92 1427261_at	-2.49	Wwc1	211852	WW, C2 and coiled-coil domain containing 1
93 1439150_x_at	-2.49	Grip1	66790	G1 regulated TBC protein 1
94 1434140_at	-2.49	Mcl2l	17207	mcl2 transforming sequence-like
95 1453293_a_at	-2.48	2810408A11Rik	70419	RIKEN cDNA 2810408A11 gene
96 1421005_at	-2.48	Cep110	26920	centrosomal protein 110
97 1427231_at	-2.46	Robo1	19876	roundabout homolog 1 (Drosophila)
98 1443787_x_at	-2.45	Casp14	12385	caspase 14
99 1455608_at	-2.44	Scn1l	67161	sodium channel and clathrin linker 1
100 1433730_at	-2.44	Elmod2	244548	ELMO domain containing 2
101 1439995_at	-2.41	Nhedc2	97086	Na+/H+ exchanger domain containing 2
102 1421928_at	-2.40	Epha4	13838	Eph receptor A4
103 1449311_at	-2.38	Bach1	12013	BTB and CNC homology 1
104 1424658_at	-2.37	Taok1	216965	TAO kinase 1
105 1452115_a_at	-2.36	P ¹¹⁴	20972	rod-like kinase 4 (Drosophila)

Probe Set ID	Fold change	Gene Symbol	Entrez Gene	Gene Description
106 1425205_at	-2.34	Ddx19b	234733	DEAD (Asp-Glu-Ala-Asp) box polypeptide 19b
107 1427950_at	-2.34	Rnf160	78913	ring finger protein 160
108 1437248_at	-2.34	2700049A03Rik	76967	RIKEN cDNA 2700049A03 gene
109 1431215_at	-2.30	Dnajc6	72685	DnaJ (Hsp40) homolog, subfamily C, member 6
110 1418524_at	-2.28	Pcm1	18536	pericentriolar material 1
111 1455604_at	-2.27	Fzd5	14367	frizzled homolog 5 (Drosophila)
112 1443971_x_at	-2.27	Mpp7	75739	membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)
113 1453172_at	-2.27	Hspa13	110920	heat shock protein 70 family, member 13
114 1451117_a_at	-2.27	Tom11f	71943	target of myb 1-like 1 (chicken)
115 1420410_at	-2.27	Nr5a2	26424	nuclear receptor subfamily 5, group A, member 2
116 1431672_at	-2.27	9430069107Rik	77358	RIKEN cDNA 9430069107 gene
117 1418947_at	-2.27	Nek3	23954	NIMA (never in mitosis gene a)-related expressed kinase 3
118 1444749_at	-2.26			apolipoprotein D
119 1456863_at	-2.25	Epha4	13838	Eph receptor A4
120 1452497_a_at	-2.25	Nfatc3	18021	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
121 1460291_at	-2.25	Cdk6	12571	cyclin-dependent kinase 6
122 1419642_at	-2.24	Purb	19291	purine rich element binding protein B
123 1453562_a_at	-2.23	Nmra1	67824	Nmra-like family domain containing 1
124 1435155_at	-2.23	Cgn	70737	cingulin; cDNA sequence BC021767
125 1432262_a_at	-2.23	Tcd2	380712	TLC domain containing 2
126 1450093_s_at	-2.23	Zbib7a	16969	zinc finger and BTB domain containing 7a
127 1419240_at	-2.23	Tex14	83560	testis expressed gene 14
128 1455905_at	-2.21	2610507B11Rik	72503	RIKEN cDNA 2610507B11 gene
129 1449058_at	-2.21	Gli1	14632	GLI-Kruppel family member GLI1
130 1452670_at	-2.21	My19	98932	myosin, light polypeptide 9, regulatory
131 1419350_at	-2.19	Hook2	170833	hook homolog 2 (Drosophila)
132 1422546_at	-2.19	Ik3	16201	interleukin enhancer binding factor 3
133 1430530_s_at	-2.15	Nmra1	67824	Nmra-like family domain containing 1
134 1416688_at	-2.15	Snap91	20616	synaptosomal-associated protein 91
135 1447752_x_at	-2.15	Drg1	13494	developmentally regulated GTP binding protein 1
136 1426753_at	-2.14	Phf17	269424	PHD finger protein 17
137 1454849_x_at	-2.14	Clu // LOC1000461	100046120 // 12759	similar to clusterin; clusterin
138 1425608_at	-2.12	Dusp3	72349	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)
139 1449126_at	-2.11	Zfp90	22751	zinc finger protein 90
140 1416517_at	-2.10	Pnpla6	50757	patatin-like phospholipase domain containing 6
141 1437224_at	-2.10	Rtn4	68585	reticulum 4
142 1416740_at	-2.10	Col5a1	12831	collagen, type V, alpha 1
143 1428835_at	-2.09	Myh14	71960	myosin, heavy polypeptide 14
144 1456819_at	-2.09	Nrn1l	234700	neuritin 1-like
145 1430287_s_at	-2.08	Hemk1	69536	HemK methyltransferase family member 1
146 1428563_at	-2.08	Ddx10	77591	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10
147 1455998_at	-2.08	LOC667118	667118	similar to Zinc finger BED domain containing protein 4
148 1434279_at	-2.08			Fas (TNF receptor superfamily member 6)
149 1437213_at	-2.07	Nudf21	68219	nudix (nucleoside diphosphate linked moiety X)-type motif 21
150 1415973_at	-2.06	Marcks	17118	myristoylated alanine rich protein kinase C substrate
151 1434362_at	-2.06			sterol O-acyltransferase 1
152 1458361_at	-2.05	Dclre1c	227525	DNA cross-link repair 1C, P502 homolog (S. cerevisiae)
153 1436302_at	-2.05	Slc10a7	76775	solute carrier family 10 (sodium/bile acid cotransporter family), member 7
154 1434177_at	-2.05	Ece1	230857	endothelin converting enzyme 1
155 1436134_at	-2.05	Scn2b	72821	sodium channel, voltage-gated, type II, beta
156 1436893_a_at	-2.05	Mar-07	57438	membrane-associated ring finger (C3HC4) 7; similar to axotrophin
157 1428583_at	-2.04	Nufip2	68564	nuclear fragile X mental retardation protein interacting protein 2
158 1416610_a_at	-2.03	Clcn3	12725	chloride channel 3
159 1429886_at	-2.02	Rnf160	78913	ring finger protein 160
160 1447766_x_at	-2.01	Limd2	67803	similar to epithelial protein lost in neoplasm; LIM domain containing 2
161 1421142_s_at	-2.01	Foxp1	108655	forkhead box P1
162 1450644_at	-2.00	Zfc361	12192	zinc finger protein 36, C3H type-like 1