

## Mouse Haploid Embryonic Stem Cells

Dr Anton Wutz has developed a novel method to derive haploid mouse embryonic stem cells (ESC) for the first time. Different lines are currently maintained, including cells from a range of mouse strains and transgenic lines.

### Key features:

- Haploid cells maintain an intact haploid genome without amplification or losses
- Original mouse ESC gene expression profile is maintained in haploid cells
- Haploid cells show a wide differentiation potential and can successfully contribute to development
- Utility as a forward genetic screening tool has been confirmed

### Potential uses:

- Technology can be used for gene identification by forward genetic screening
- Use haploid cells to generate homozygous mutation libraries
- Potential for introducing desired mutations into mice

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### Background

Haploid organisms, such as yeast, have been crucial models in genetic screens, due to the fact that they only have 1 copy of each gene. The diploid genome of complex organisms severely limits many genetic approaches and while induction of haploidy has been described in fish, haploidy appears less compatible with development in mammals. The work described here has developed, for the first time, a novel method to derive haploid mouse embryonic stem cells (ESC).

### Technology

In order to generate haploid mouse ESCs, unfertilized oocytes were activated and cultivated to the blastocyst stage. Following removal of the zona and trophectoderm, the inner cell mass was isolated and expanded in defined media, plus LIF. After expansion of the cells, haploid DNA content was analyzed by flow analysis and enrichment of haploid cells was achieved by flow sorting. Haploid ESC have been derived from 25 different mouse strains, including transgenic lines and cells have been expanded for over 35 passages (Figure 1).

Haploid ESCs exhibit a typical mouse ESC colony morphology and chromosome spreads showed 20 chromosomes corresponding to the haploid mouse chromosome set (Figure 1). Comparative genomic hybridization (CGH) also confirmed an intact haploid genome without amplification or losses. Haploid ESCs expressed the relevant pluripotency markers and genome-wide expression analysis showed a high correlation between haploid ESCs and conventional diploid ESCs.

We also demonstrated the developmental potential of haploid ESCs. Following labelling with GFP using a piggyBac transposon, it was shown that the haploid genome content was maintained following transfection. GFP marked haploid ESCs contributed substantially to chimeric embryos when injected into blastocysts. The cells contributed to development following diploidization and female live chimeras were obtained (Figure 2).

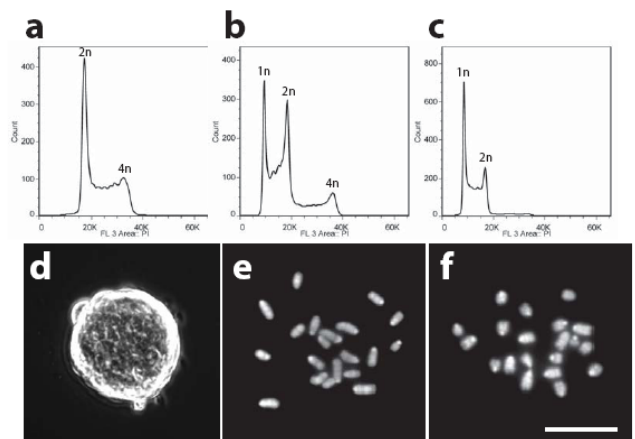
### References

Martin Leeb and Anton Wutz (2011) Derivation of haploid embryonic stem cells from mouse embryos, Nature, 2011 Sep 7;479(7371):131-4.

### Commercialisation

We are seeking a commercial partner for licensing, collaboration and development of this technology, protected by patent application GB201115343D0.

**Figure 1. Derivation of haploid ESCs.** Flow analysis of DNA content of (a) diploid control ESCs, (b) a haploid ESC line at passage 7 and (c) at passage 11 following sorting at passage 7. (d) Colony morphology of haploid ESC in culture. (e,f) Chromosome spreads of two different haploid ESC lines showing 20 chromosomes corresponding to the haploid mouse chromosome set.



**Figure 2. Developmental potential of haploid ESCs.** Live born chimeric mice were obtained from injection of GFP marked haploid ESCs into blastocysts. Green fluorescence (picture below) indicates tissues to which haploid ESCs contributed. The great majority of cells contributing to development had become diploid at this stage.

