Supplementary Materials for

Derivation and maintenance of mouse haploid embryonic stem cells

Elling, Woods et al.

Supplementary Figure 1

Supplementary Data files 1 to 4

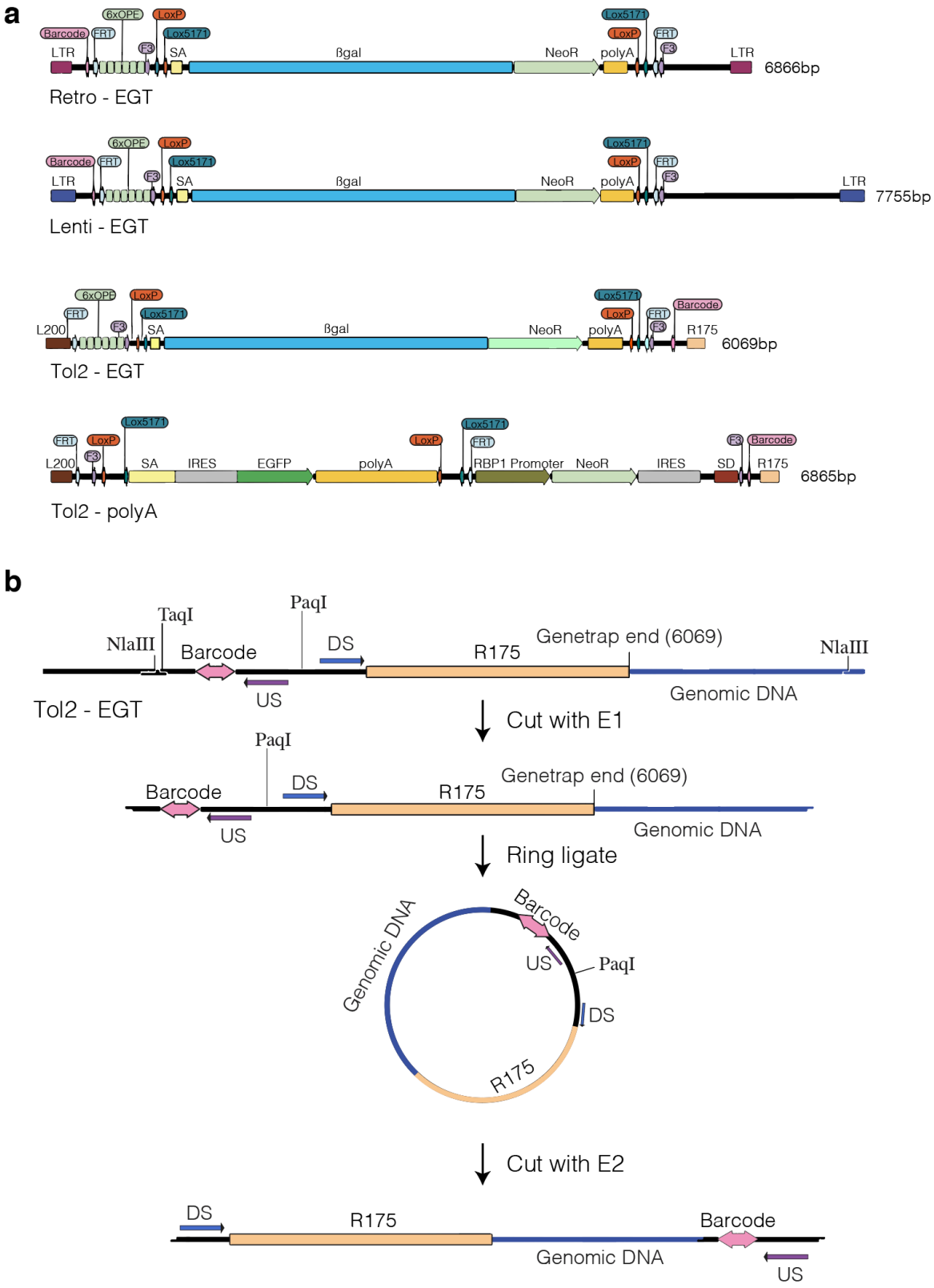
Derivation and maintenance of mouse haploid embryonic stem cells

Ulrich Elling[1]*, Michael Woods[2,3]*, Josep V. Forment[4], Beiyuan Fu[3], Fengtang Yang[3], Bee Ling Ng[3], Jose R. Vicente[2], David J. Adams[3], Brendan Doe[3], Stephen P. Jackson[5], Josef M. Penninger[1,6#] and Gabriel Balmus[2#]

1. Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna Biocenter (VBC), Dr. Bohr Gasse 3, Vienna, Austria.
2. UK Dementia Research Institute at University of Cambridge and Department of Clinical Neurosciences, University of Cambridge, CB2 0AH, UK.
3. Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK.
4. DNA Damage Response Biology, Oncology Innovative Medicines, Astra Zeneca, Cambridge CB4 0WG, UK
5. The Wellcome Trust CRUK Gurdon Institute and Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK.
6. Department of Medical Genetics, Life Science Institute, University of British Columbia, Vancouver, Canada.

* These authors contributed equally to this work.

Correspondence should be addressed to GB (gb318@cam.ac.uk) or JMP (Josef.Penninger@imba.oeaw.ac.at)


a
6xQPE
LoxP
Barcode
FRT
Lox5171
LTR
F3
SA
βgal
NeoR
polyA
LTR
6866bp
Retro - EGT
7755bp
Lenti - EGT
L200
R175
6069bp
Tol2 - EGT
IRES
EGFP
RBP1 Promoter
SD
6865bp
Tol2 - polyA
b
TaqI
PaqI
NlaIII
DS
US
R175
Genetrap end (6069)
Genomic DNA
Tol2 - EGT
Cut with E1
Ring ligate
Cut with E2

Supplementary Figure 1. **(a)** Schematic representation of the gene trap vectors as presented in Elling et al. 2017[1] and on the Haplobank website (www.haplobank.at). Retroviral enhanced gene trap (Retro-EGT; related sequence provided as Supplementary Data file 1). Lentiviral enhanced gene trap (Lenti-EGT; related sequence provided as Supplementary Data file 2). Tol2 autonomous transposon enhanced gene trap (Tol2-EGT; related sequence provided as Supplementary Data file 3). Tol2 autonomous transposon polyadenylation enhanced gene trap (Tol2-polyA-EGT; related sequence provided as Supplementary Data file 4). **Abbreviations used**: LTR, long terminal repeat; 6xOPE, six osteopontin enhancer elements; FRT/F3, heterotypic improved flippase target sequences; LoxP/Lox5171, heterotypic target sequences for the Cre-recombinase; SA, splice acceptor; βgal, β-galactosidase; NeoR, neomycin phosphotransferase fusion gene; polyA, bovine growth hormone polyadenylation sequence; L200/R175, left and right *Tol2* transposon elements; IRES, internal ribosome entry site; EGFP, enhanced green fluorescent protein; RPB1, DNA-directed RNA polymerase II subunit rpb1; SD, splice donor. **(b)** Schematic representation of library preparation of gene trap vectors integration site. Tol2 – EGT is shown as example. Following fragmentation of the genome with enzyme 1 (E1, NlaIII in the example), the gene trap end containing the barcode and a genomic DNA portion is circularized (ring ligation). Prior to PCR amplification, linearization with enzyme 2 (E2, PaqI in the example) is needed. Each integration site can be mapped by using two different E1 enzymes. The genomic region is then amplified by PCR using US and DS primers.

Supplementary Data file 1. Sequence file for the retroviral construct (Retro-EGT) presented in Supplementary Figure 1a.

Supplementary Data file 2. Sequence file for the Lentiviral-EGT construct (Lenti-EGT) presented in Supplementary Figure 1a.

Supplementary Data file 3. Sequence file for the transposon construct (Tol2-EGT) presented in Supplementary Figure 1a.

Supplementary Data file 4. Sequence file for the transposon construct (Tol2-polyA-EGT) presented in Supplementary Figure 1a.

References

1. Elling, U. *et al.* A reversible haploid mouse embryonic stem cell biobank resource for functional genomics. *Nature* **550,** 114 (2017).

Supplementary **Video 1.** Removing cumulus oocyte complex from ampulla.

Supplementary **Video 2.** Identification and isolation of sub-viable (pathogenic) embryos.

Supplementary **Video 3.** Zona pellucida removal (denuding) – first 20 seconds.

Supplementary **Video 4.** Zona pellucida removal (denuding) – making sure zona is gone up to 70

Supplementary Figure 1 Gene trap vectors and library preparation.

(a) Schematic representation of the gene trap vectors as presented in Elling et al. 20171 and on the Haplobank website (www.haplobank.at). Retroviral enhanced gene trap (Retro-EGT; related sequence provided as Supplementary Data file 1). Lentiviral enhanced gene trap (Lenti-EGT; related sequence provided as Supplementary Data file 2). Tol2 autonomous transposon enhanced gene trap (Tol2-EGT; related sequence provided as Supplementary Data file 3). Tol2 autonomous transposon polyadenylation enhanced gene trap (Tol2-polyA-EGT; related sequence provided as Supplementary Data file 4). Abbreviations used: LTR, long terminal repeat; 6xOPE, six osteopontin enhancer elements; FRT/F3, heterotypic improved flippase target

sequences; LoxP/Lox5171, heterotypic target sequences for the Cre-recombinase; SA, splice acceptor; βgal, β-galactosidase; NeoR, neomycin phosphotransferase fusion gene; polyA, bovine growth hormone polyadenylation sequence; L200/R175, left and right Tol2 transposon elements; IRES, internal ribosome entry site; EGFP, enhanced green fluorescent protein; RPB1, DNA-directed RNA polymerase II subunit rpb1; SD, splice donor. (b) Schematic representation of library preparation of gene trap vectors integration site. Tol2 – EGT is shown as example. Following fragmentation of the genome with enzyme 1 (E1, NlaIII in the example), the gene trap end containing the barcode and a genomic DNA portion is circularized (ring ligation). Prior to PCR amplification, linearization with enzyme 2 (E2, PaqI in the example) is needed. Each integration site can be mapped by using two different E1 enzymes. The genomic region is then amplified by PCR using US and DS primers.

Supplementary Data: Four sequences for gene trap cassettes harbouring disruptive splice acceptor sites.