Supplementary information

Supplementary Figure Legends

Supplementary Figure S1. Strategy for construction of 21HAC1 in DT40 cells.

(a) The wild type locus of AP001657, the 5'HPRT-loxP-Hyg-TK targeting construct, and the targeted allele are shown. Targeting constructs were linearized and electroporated into DT40 (hChr. 21) cells. Homologous recombination was confirmed by PCR and Southern blot analyses. PCR analysis was performed using the 21cen<1>2L and PGKr1 primers indicated by arrowheads. Southern blotting was performed using 5' probe and 3' probe. The probes used for Southern blotting are indicated. (b) Southern blotting for confirmation of the correct targeted recombination. Genomic DNA was digested with SphI to detect the correct fragments of 11.5 kb (wild type) or 6.1 kb (targeted allele) (arrowheads) using 5'-probe, and those of 11.5 kb (wild type) or 6.0 kb (targeted allele) (arrowheads) using 3' probe. (c) The wild type locus of AL163201, the pBS-TEL/Δp Puro targeting construct, and the targeted allele, are shown. Targeting constructs were linearized and electroporated into DT40 (hChr.21-loxP) cells. Homologous recombination was confirmed by PCR using the Pcen1L and PuroI primers indicated by arrowheads. (d) The wild type locus of AP001657, the pBS-TEL/Δq HisD targeting construct, and the targeted allele, are shown. Targeting constructs were linearized and electroporated into DT40 (hChr.21-loxPΔp) cells. Homologous recombination was confirmed by PCR using the q4L and SK23 primers indicated by arrowheads.

Supplementary Figure S2. Strategy for insertion of a desired gene into the HAC in DT40 cells.

(a) The wild type locus of AP001657, the I-EGFP-I-Bsd targeting construct, and the targeted allele, are shown. Targeting constructs were linearized and electroporated into DT40 (21HAC1) cells. Homologous recombination was confirmed by PCR using the bsdF / #21cenG6R and EGFPL / EGFPR primers indicated by arrowheads. (b) The wild type locus of AP001657, the DsRed-neo targeting construct, and the targeted allele, are shown. Targeting constructs were linearized and electroporated into DT40 (21HAC2) cells. Homologous recombination was confirmed by PCR using the nDsRed-L / 21Red-2R and nDsRed-L / nDsRed-R primers indicated by arrowheads.

Supplementary Figure S3. Cre-loxP mediated gene insertion with reconstitution of the HPRT gene. The plasmid vector, I-EGFP-I-loxP-3'HPRT, was inserted into the loxP site of 21HAC1 in CHO cells by Cre expression. Targeted insertion was confirmed by PCR using the TRANSL1 / TRANSR1 and the EGFPL / EGFPR primers indicated by arrowheads.

Supplementary Figure S4. Strategy for construction of 21HAC4 and insertion of the desired gene

(a) The 5'HPRT-loxP-Hyg-TK inserted locus of AP001657, the 3'neo-loxP-Bsd-TK targeting construct, and the targeted allele, are shown. Targeting constructs were linearized and electroporated into DT40 (21HAC1) cells. Homologous recombination was confirmed by PCR using the #21CEN <1>2L and KJneo primers indicated by arrowheads. (b) A schematic diagram of the insertion of RPCI-6 PAC containing the desired gene into the HAC vector is shown. For example, the PAC vector, RP6-127C8, containing the human HPRT gene, was inserted into the loxP site of 21HAC4 in CHO cells by Cre expression. Targeted insertion was confirmed by PCR using the CMV586 / Neo817 and the HPRT2L /

HPRT2R primers indicated by arrowheads.

Supplementary Figure S5. PFGE and Southern blotting of the 21HACs

Southern blots of PFGE size-separated BamHI restriction fragments, hybridized with a human chromosome 21-derived α-satellite probe detected the integrity of the 21HACs in DT40 (21HAC1), DT40 (21HAC2) and DT40 (21HAC4). DT40 was used as negative control.

Supplementary Figure S6. Purification of ES cells without HAC, and FISH analyses

(a, b) FISH analyses for E14 (21HAC2) cells and GCV resistant E14 (21HAC2) cells. Digoxigenin-labeled human COT-1 DNA (red) was used to detect the HAC. An arrow indicates 21HAC2 and the inset shows an enlarged image of 21HAC2. (c) Chromosomal analyses for GCV resistant E14 (21HAC2) cells. 20 metaphases were analyzed and scored.

Supplementary Table S1. Primers and oligos used in the TAR cloning

Supplementary Table S2. Primer list for genomic PCR analyses