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Efficient modification of a human chromosome by telomere-directed truncation in high homologous recombination-proficient chicken DT40 cells

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ABSTRACT

Truncation of human chromosomes at desired sites by homologous recombination techniques enables functional and structural analyses of human chromosomes and development of human artificial chromosomes. However, this targeted truncation has been inefficient. We describe here an efficient method for targeted truncation in the chicken DT40 cells with a high homologous recombination rate. The human chromosome 22 was transferred into DT40 cells, where human telomeric repeat (TTAGGG)n was targeted to the *LIF* locus on the chromosome. Molecular and cytogenetic analyses showed that the predicted truncation at the *LIF* locus occurred in all of the targeted clones.

In order to produce a diverse repertoire of complete human antibodies in animals, we have been making mice with entire immunoglobulin loci by microcell-mediated transfer of human chromosomes into embryonic stem (ES) cells (1). During the course of experiments, it is suggested that the size of human chromosomes might be important for their germline-transmission. Thus, we tried to obtain a truncated human chromosome 22 where *Ig* λ gene exists by target integration of human telomeric repeat (2) into the chromosome for its germline-transmission.

Although the targeted truncation of a human chromosome has been reported, the efficiency was <0.01%, mainly due to the inefficiency of target integration of telomeric sequences (8/12 000) in addition to that of telomere truncation (1/8) (3). The chicken pre-B cell line DT40 is known to be homologous recombination-proficient (4) and enables the efficient modification of human chromosomes by gene targeting (5,6). Thus, it is expected that the efficiency of targeted truncation of a human chromosome may be raised in DT40 cells.

First, we transferred the human chromosome 22 into DT40 cells from mouse A9 cells by MMCT method (1). PCR analysis using chromosome 22-specific primers and FISH analysis with human COT1 probe confirmed the successful transfer of the chromosome 22. Next, the clones 52-18 were electroporated at 550 V and 25 μF with the plasmid construct (Fig. 1). Out of 80 clones resistant to both G418 and puromycin, eight gave 6.3 kb of PCR products containing *Sal*I sites (Fig. 1), indicating that the human telomeric repeat was integrated into the *LIF* locus at 22q12 just distal to the *Ig* λ *(7)*, at about 10% efficiency. Furthermore, PCR analysis using chromosome 22-specific primers indicated that the truncation at the *LIF* locus might occur in clones 67, 68, 328 and 343 (Fig. 2a). By FISH analysis using a probe derived from the plasmid pGK*Puro* (8), all of the eight clones underwent targeted truncation at the *LIF* locus (Fig. 2b). In clones 64, 212, 222 and 305 however, cells (<10%) with the intact chromosome 22 were also observed (data not shown), which may contribute to the above PCR results. It is possible that the timing of telomere truncation may be varied between the cells, resulting in different karyotypes within a single clone.

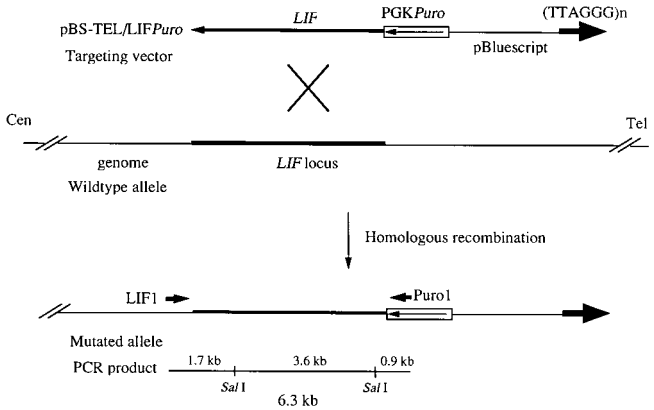

Figure 1. Strategy for targeted truncation at the *LIF* locus on the human chromosome 22 by the plasmid pBS-TEL/LIF*Puro*. Linearized pBS-TEL/LIF*Puro* is transfected by electroporation into DT40 cells. The identification of homologous recombinants was done by PCR using LIF1 primer (5′-ATGACTCTAAGGCAGGAACATCTGTACC-3′) and *Puro*1 primer (5′-GAGCTGCAAGAACTCTTCCTCACG-3′) indicated with bold arrows, followed by digestion with *Sal*I. In the targeted clones, 6.3 kb of PCR products should be amplified and digested with *Sal*I as indicated. The orientation of arrowheads in both *LIF* and PGK*Puro* represents that of their transcription.

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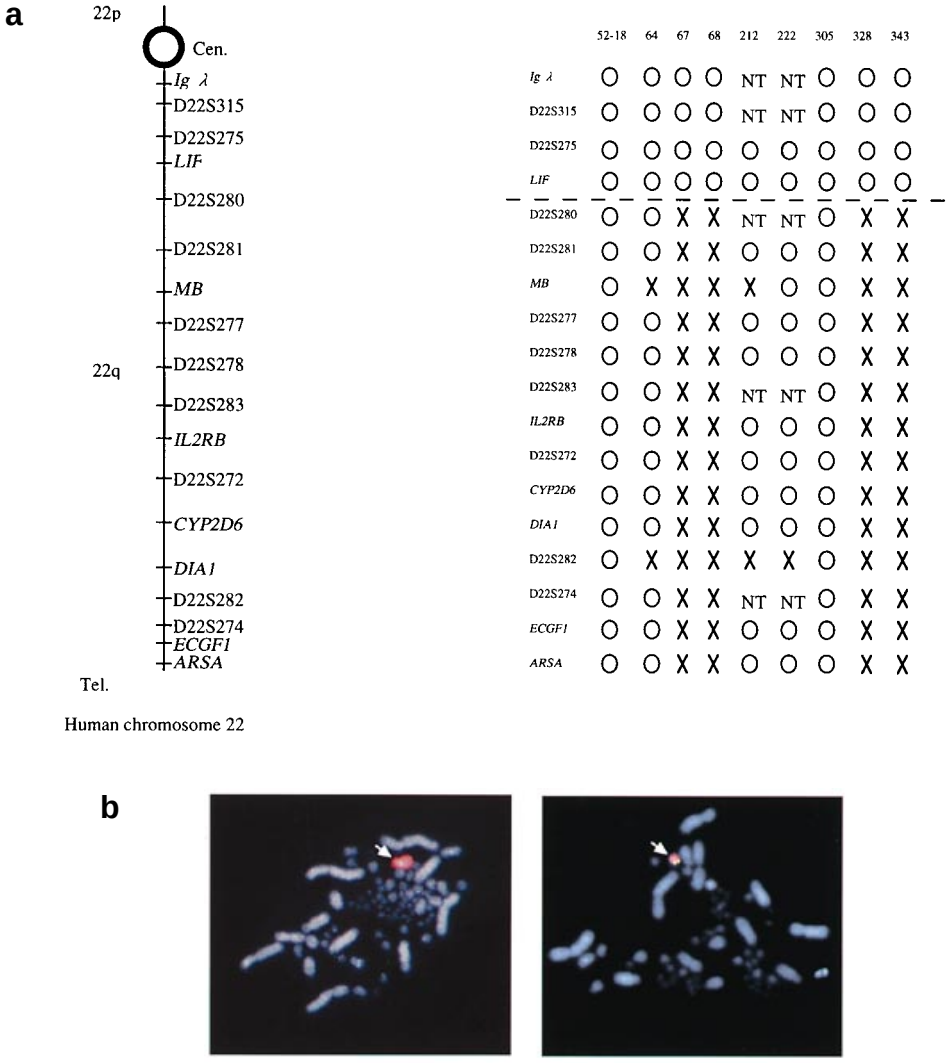

Figure 2. Molecular and cytogenetic analysis of the truncated human chromosome 22. (**a**) PCR analysis of the targeted clones using primers for the human chromosome 22-specific STS markers and genes. Their sequences are described elsewhere (1), except for both CYP2D6 (5′-CTGCGTGTGTAATCGTGTCC-3′ and 5′-TCTGCTGTGAGTGAACCTGC3′) and ECGF1 (5′-AGGAGGCACCTTGGATAAGC-3′ and 5′-TCACTCTGACCCACGATACAGC-3′). The left panel represents the approximate physical order of STS markers and genes tested, against centromere (7). In the right panel, symbols are as follows: ○, presence; ×, absence. (**b**) FISH analysis of the targeted clones with both rhodamine-labelled human COT1 probe (red) and FITC-labelled plasmid pGK*Puro* probe (green). The left panel shows the partial metaphase of clone 52-18 with the intact human chromosome 22 (red) and the right panel shows the partial metaphase of clone 68, in which the site of hybridization of pGK*Puro* is telomeric (green).

In conclusion, the predicted truncation at the *LIF* locus on the chromosome 22 was done in all of the targeted clones (8/8) in DT40 cells. Furthermore, we also observed a high efficiency of targeted truncation in the human chromosome 3 as well (M.Oshimura, manuscript in preparation).The present finding is the first indication that DT40 cells are suitable hosts for telomere-directed truncation of human chromosomes, in addition to simple gene targeting and suggests that this technology is useful for detailed gene mapping by functional assays.

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