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The *Terminator* mouse is a diphtheria toxin–receptor knock-in mouse strain for rapid and efficient enrichment of desired cell lineages

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Biomedical research often requires primary cultures of specific cell types, which are challenging to obtain at high purity in a reproducible manner. Here we engineered the murine Rosa26 locus by introducing the diphtheria toxin receptor flanked by loxP sites. The resultant strain was nicknamed the *Terminator* mouse. This approach results in diphtheria toxin–receptor expression in all non-Cre expressing cell types, making these cells susceptible to diphtheria toxin exposure. In primary cultures of kidney cells derived from the *Terminator* mouse, over 99.99% of cells were dead within 72 h of diphtheria toxin treatment. After crossing the *Terminator* with the podocin-Cre (podocyte specific) mouse or the Ggt-Cre (proximal tubule specific) mouse, diphtheria toxin treatment killed non-Cre expressing cells but spared podocytes and proximal tubule cells, respectively, enriching the primary cultures to over 99% purity, based on both western blotting and immunostaining of marker proteins. Thus, the *Terminator* mouse can be a useful tool to selectively and reproducibly obtain even low-abundant cell types at high quantity and purity.

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An enormous amount of research is currently conducted using immortalized cell lines that have been isolated from the organs of various mammalian species and maintained in culture for long periods. These cell lines provide a ready platform for the *in vitro* dissection of signaling pathways, metabolic cycle analysis, cell proliferative and survival responses, channel function, and so on. However, this approach is continuously confounded by two problems: (1) immortalized cells exhibit altered proliferative and apoptotic responses as a result of either abnormal gene expression (SV40, e.g.) or selection (MDCK cells, e.g.); and (2) continuous cell culture typically leads to significant epigenetic and phenotypic changes and the dysregulated expression of many genes when compared with the *in vivo* expression profile of the parent cell type.[1–3]

These problems can be partially overcome using primary cultures of freshly isolated cells. However, these cells are hard to be efficiently isolated in large numbers with high purity, and attempts to expand primary cultures to obtain large numbers of cells are frequently unsuccessful or lead to either replicative senescence or the selective expansion of abnormal clonal populations. Low-abundant cell types are particularly difficult to be purified and analyzed in primary culture.

Generation of the *Terminator* mouse

To overcome these problems and provide rapid and efficient isolation of large numbers of primary cells of any type, we have developed a mouse in which the diphtheria toxin (DT)–receptor (*DTR*) gene is knocked into the Rosa26 locus, thus ubiquitously expressed, but can be turned off by expression of the Cre recombinase[4] (Figure 1a). By mating this mouse, nicknamed the *Terminator* mouse (*Rosa-DTR^{flox}*), to an appropriate Cre strain, DTR expression is permanently removed from cells that express Cre at any time before DT selection. Upon Cre-mediated recombination, GFP expression is designed to be turned on, driven by the Rosa26 promoter (Figure 1a).[4] After gene targeting in embryonic stem cells, a total of eight clones were identified that carried the correctly modified Rosa26 locus and two were subjected to blastocyst microinjection to establish chimeras. Four male chimeras were generated and three had germ line

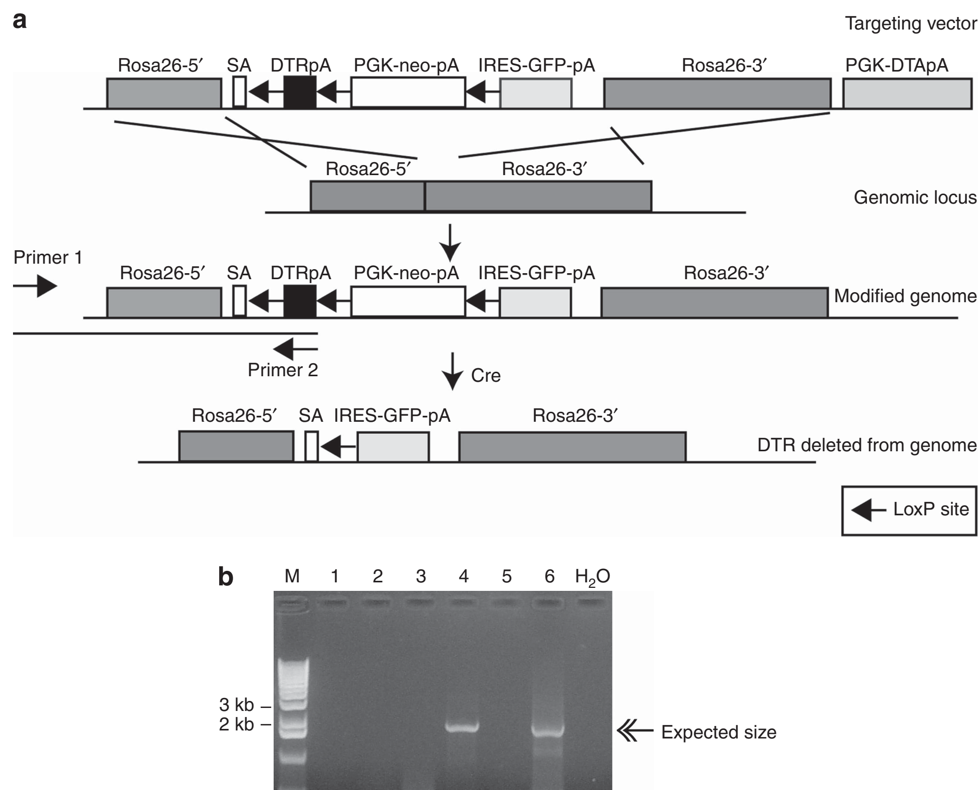

Figure 1 | Generation of the *Terminator* mouse. (**a**) The targeting vector was constructed to contain the diphtheria toxin–receptor (DTR)-polyA fragment flanked by a pair of loxP sites. PGK-Neo-polyA fragment was placed between the DTR and the IRES-GFP-polyA to serve as a positive selection marker during embryonic stem (ES) targeting; (**b**) long-template PCR to screen for clones that have undergone proper homologous recombination. See (**a**) for primer location. An example of six ES clones is shown, with no. 4 and no. 6 exhibiting successful recombination. SA, splicing acceptor sequence.

transmission. The *Terminator* mouse can be genotyped by a pair of primers that amplify the Rosa26 promoter and the inserted DTR, with the sense primer complementing a genomic area outside of the targeting vector (Figure 1b). The *Terminator* mice are viable, fertile, and healthy (>14 months) without any macroscopic or microscopic abnormalities observed (data not shown).

DTR is ubiquitously expressed

Multiple organs including the brain, heart, intestine, kidney, liver, lung, pancreas, skeletal muscle, gonads, and spleen were isolated and subjected to western blotting. DTR is detected in all tested organs from the *Terminator* mouse, whereas wild-type mice do not have detectable levels of DTR (Figure 2a, and data not shown).

To test the sensitivity of cells isolated from the *Terminator* mouse to DT, kidneys were minced, digested in collagenase, and the resultant cells seeded onto multiple 10 cm dishes. DT (100 ng/ml) was added to the culture medium starting on day 2 and cultures washed every 48 h to remove dead cells. Whereas the wild-type kidney cells remained healthy after DT treatment, the vast majority of kidney cells from the *Terminator* (*Rosa-DTR^{flox}*) mouse had detached within 24 h after DT treatment. By 72–96 h, the treated dishes had fewer than 10 viable cells/dish remaining, whereas the untreated dishes were nearly confluent (Figure 2b and c). This suggests that the DTR is expressed by nearly all renal cells in the *Terminator* mouse, but that rare cells do survive DT selection, even in the absence of Cre expression.

Primary culture of podocytes using the *PODOCIN-CRE;ROSA-DTR^{flox}* mouse

To test our hypothesis that the *Terminator* mouse can assist in the enrichment of primary cultures of a particular type of cells, primary cultures were examined from whole kidneys of *Podocin-Cre;Rosa-DTR^{flox}* double transgenic mice.[5] In the absence of DT treatment, whole-kidney cultures exhibited a typical mixed phenotypic picture (Figure 3a). DT treatment resulted in death of many of the adherent cells by 96 h, and the surviving cells exhibited typical podocyte features (Figure 3a). Western blot analysis of surviving cells revealed enriched expression of the podocyte marker Wt1 and downregulation of the proximal tubule marker megalin in the DT-treated samples (Figure 3b). Immunofluorescence revealed that $>99\%$ of the cells that survived DT treatment exhibited Wt1$^+$ expression, whereas untreated cultures have $\sim 22\%$ WT1$^+$ cells (Figure 3c, d). Podocyte enrichment was further confirmed by quantitative reverse transcriptase (RT)–PCR analysis of Wt1 and podocin expression (Figure 3e and f).

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