Gene Targeting in Human Cells Without Isogenic DNA

Targeted homologous recombination (gene targeting) is widely used in murine embryonic stem (ES) cells as the method of choice for genetic ablation. Soon after the method became established (1, 2), it was demonstrated that the frequency of homologous recombination was adversely affected by the presence of vector-target mismatches (3). To optimize the efficiency of gene targeting, it therefore became common practice to construct vectors from “isogenic” DNA.

Recent improvements in methodology have opened the door to gene targeting in somatic cells (4). This approach can be of considerable value in the study of human cells, especially in cases where animal models may not adequately represent human physiology (5). Single-nucleotide polymorphisms in the human genome occur at a frequency of one per 500 to 1000 bp (6). In contrast to ES cells, where the scientific community has adopted a single inbred genetic background for gene targeting, a large number of human cell lines is currently in common use. A requirement for isogenic DNA would be a significant impediment because it would necessitate the customization of all vectors for each individual cell line. Furthermore, as a result of outbreeding of most human populations and the consequent heterozygosity of human cells, a strict requirement for isogenicity would necessitate the construction of chromosome (allele)-specific vectors.

Because gene targeting in human somatic cells is rapidly gaining acceptance (7), we have compiled gene targeting data available to date (Table 1). The presented data set is comprised of 23 independent experiments performed in six different laboratories; eight different genes were targeted in seven cell lines.

Table 1. Gene targeting frequencies in human cells.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Gene targeted</th>
<th>Cell line targeted</th>
<th>Source of DNA</th>
<th>Selection</th>
<th>Frequency</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ki-ras</td>
<td>DLD-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Calu-1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>G418, DT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7/24</td>
<td>(8)</td>
</tr>
<tr>
<td>2.</td>
<td>p21&lt;sup&gt;WAF1&lt;/sup&gt;</td>
<td>HCT116&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Calu-1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>G418, DT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8/93</td>
<td>(9)</td>
</tr>
<tr>
<td>3.</td>
<td>p21&lt;sup&gt;WAF1&lt;/sup&gt;</td>
<td>HCT116&lt;sup&gt;e&lt;/sup&gt;</td>
<td>G418</td>
<td>37/100</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>HCT116&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Hygro</td>
<td>5/20</td>
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<td>5.</td>
<td>DLD-1</td>
<td>DLD-1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>G-418</td>
<td>5/157</td>
<td>(11)</td>
<td></td>
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<tr>
<td>6.</td>
<td>DLD-1</td>
<td>LF1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>G418</td>
<td>1/135</td>
<td></td>
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<td>7.</td>
<td>p53</td>
<td>LF1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Hygro</td>
<td>3/20</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td>LF1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>G418</td>
<td>1/87</td>
<td>(12)</td>
<td></td>
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<tr>
<td>9.</td>
<td></td>
<td>LL1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>G418</td>
<td>4/33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Smad4</td>
<td>HCT116&lt;sup&gt;j&lt;/sup&gt;</td>
<td>G418</td>
<td>1/600</td>
<td>(13)</td>
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<tr>
<td>11.</td>
<td></td>
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<td>Hygro</td>
<td>1/940</td>
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<td>12.</td>
<td>Rb</td>
<td>TK6&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>(14)</td>
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<td>13.</td>
<td></td>
<td>TK6&lt;sup&gt;6&lt;/sup&gt;</td>
<td>histidinol</td>
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<td>4/300</td>
<td>(15)</td>
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<tr>
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<td>Hygro</td>
<td>2/254</td>
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<td></td>
<td>HaCaT&lt;sup&gt;k&lt;/sup&gt;</td>
<td>G418</td>
<td>5/44</td>
<td>(16)</td>
<td></td>
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<tr>
<td>17.</td>
<td>Rb</td>
<td>LL1&lt;sup&gt;l&lt;/sup&gt;</td>
<td>G418</td>
<td>2/10</td>
<td>(19)</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td></td>
<td>LF1&lt;sup&gt;l&lt;/sup&gt;</td>
<td>G418</td>
<td>5/33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td></td>
<td>LF1&lt;sup&gt;l&lt;/sup&gt;</td>
<td>G418</td>
<td>2/103</td>
<td>(20)</td>
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<tr>
<td>20.</td>
<td></td>
<td>LF1&lt;sup&gt;l&lt;/sup&gt;</td>
<td>G418</td>
<td>4/37</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>KARP1</td>
<td>HCT116&lt;sup&gt;g&lt;/sup&gt;</td>
<td>G418</td>
<td>3/24</td>
<td></td>
<td></td>
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<td>22.</td>
<td></td>
<td>HCT116&lt;sup&gt;g&lt;/sup&gt;</td>
<td>G418</td>
<td>1/7</td>
<td>(22)</td>
<td></td>
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</tbody>
</table>

Table 1 footnotes:

<sup>a</sup> DLD-1, human colorectal adenocarcinoma cell line, ATCC CCL 221.
<sup>b</sup> Calu-1, epidermoid lung carcinoma cell line, ATCC HTB 54.
<sup>c</sup> DT (diphtheria toxin) was used as a negatively selectable marker. However, the positive selection in this vector, as in all the vectors shown in this table, was of the “promoterless” design (4).
<sup>d</sup> HCT116, human colorectal adenocarcinoma cell line, ATCC CCL 247. This cell line is deficient in mismatch repair. It was initially chosen because of evidence that isogenic DNA is not required in mismatch repair-deficient murine cells (9). The fact that HCT116 does not behave differently in gene targeting experiments than mismatch repair-proficient cells is further indication that isogenic DNA is not required in human cells.
<sup>e</sup> LF1, human embryonic lung fibroblasts cell strain (5).
<sup>f</sup> LL1, human embryonic skin fibroblast cell strain. First-trimester human-skin fibroblasts were established in culture as indicated (5). Cell strains LL1 (skin) and LF1 (lung) were obtained from different embryos.
<sup>g</sup> DNA was extracted from early passage LL1 cells and a library was prepared in the lambda phage vector DASH II (Stratagene, La Jolla, California).
<sup>h</sup> TK6, human keratinocyte cell line (17).
<sup>i</sup> Human peripheral lymphocytes (18).
<sup>j</sup> HCT116, human embryonic lung fibroblast cell strain. First-trimester human-skin fibroblasts were established in culture as indicated (5). Cell strains LL1 (skin) and LF1 (lung) were obtained from different embryos.
<sup>k</sup> Gene targeting data available to date (Table 1). The presented data set is comprised of 23 independent experiments performed in six different laboratories; eight different genes were targeted in seven cell lines.
lines, two of which were nonimmortalized fibroblast cell strains. Six different sources of DNA were used for vector construction, and only one of the 23 experiments used isogenic DNA (experiment 9). The best represented target is the p53 gene, which has been targeted in four different cell lines using the same targeting vector.

The data (Table 1) reveal many examples of high-efficiency gene targeting using nonisogenic DNA. Because more than 100 kb of genome-vector homology have been scanned in these experiments, it is highly unlikely that all these sequences were identical, especially because the bulk of the targeting vectors are comprised of intronic and intergenic regions. Also, in the seven cases where knockouts were pursued to homozygosity (lines 1-2, 3-4, 5-6, 7-8, 11-12, 13-14, and 15-16), the two gene copies were targeted with similar frequencies. We therefore conclude that there is no apparent bias for isogenic DNA in human gene targeting.

While the mechanistic basis for the different gene targeting behavior of murine and human cells remains to be investigated, the practical ramifications are clear: one vector can be used to target many human cell lines. We thus envision the rapid emergence of a library of tested and optimized gene targeting vectors that will be available for widespread genetic analysis in the large number of human experimental cell systems.

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References and Notes

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