**ESC Line Information**

<table>
<thead>
<tr>
<th>Field</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Line Name</strong></td>
<td>Rosa26^{228.3TF.GFP.Cre}</td>
</tr>
<tr>
<td><strong>Parental Cell Line</strong></td>
<td>TL-1</td>
</tr>
<tr>
<td><strong>Background Strain</strong></td>
<td>129</td>
</tr>
<tr>
<td><strong>Culturing Protocol</strong></td>
<td>Std_mESC_Culture.doc</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>This cell line contains TetO-regulated genes inserted into the Rosa26^{LCA} allele by RMCE. The TetO/miniCMV promoter was placed (at -228) upstream of the putative ROSA26 transcription start site and drives the expression of a polycistrionic mRNA with transcription factors MafA, Pdx1, and Ngn3 together with a GFP-Cre fusion protein. The sequences are linked by 2A peptide cleavage sequences. In this cell line, when the effector protein rtTA is expressed, all three transcription factors and GFP-Cre will be over-expressed simultaneously upon administration of doxycycline.</td>
</tr>
</tbody>
</table>

**Genetic Alterations**

1) **RMCE Targeted Mutagenesis**

<table>
<thead>
<tr>
<th>Type of Allele</th>
<th>Cassette Acceptor</th>
</tr>
</thead>
</table>

**Targeted Gene**

Gene trap ROSA 26, Philippe Soriano

GI:R0SA26\_B6Jsor - NCBI GeneID:14910

**Targeted Allele**

targeted mutation 1 (Rosa26^tm1(LCA) - MGI:104725)

**Description of Targeting Vector**

The Rosa 26 cassette acceptor allele was created by replacing a 5.165 kb region of this gene containing exon 1 with a floxed tk-neo cassette, a puromycin-delta-thymidine kinase fusion gene driven by the mouse phosphoglycerol kinase promoter (pU-deltaTK) and a neomycin resistant gene driven by the bacterial EM7 promoter (EM7neo) flanked by minimal (34 bp) tandemly oriented lox71 and lox2272 sites.

**Targeting Vector Genbank File**

pRosa26.LCA.gb

**Recombinase-Mediated Cassette Exchange Stage**

<table>
<thead>
<tr>
<th>Type of Allele</th>
<th>Gene Replacement</th>
</tr>
</thead>
</table>

**Exchanged Cassette Gene**

Cre, GFP, Mafa (378435), Pdx1 (18609), Neurog3 (11925)

**Exchanged Cassette Allele Name**

3TF.GFP.Cre

**Description of Exchange Vector**

The pR26.228.ptight.3TF.GFP.Cre vector was made on a backbone of a basal exchange vector which contains a 5.166 kb sequence from the Rosa26 locus, Lox71.Lox2272 sites, and a flrted (flanked by FRT) Pgk-Neo cassette that is used for positive selection of ES cells after RMCE. The TetO/miniCMV promoter from the pTight vector (Clonthech) was inserted (at -228) upstream of the putative ROSA26 transcription start site, followed by a polycistrionic mRNA coding for MafA, Cre-GFP, Pdx1, and Ngn3. The sequences are linked by 2A peptide cleavage sequences.

**Exchange Vector Genbank File**

pR26.228.ptight.3TF.GFP.CRE.gb

**Citations**

Not Available
Description:
The pRosa.228.3TF.GFP-Cre vector was exchanged into the Rosa26<sup>LCA</sup> locus via Cre-RMCE, thus generating the Rosa26.228.3TF.GFP-Cre<sup>LCA</sup> allele.

Reference:
Not provided