

# c-MycER<sup>TAM</sup> Transgene Silencing in a Genetically Modified Human Neural Stem Cell Line Grafted in Rodent Brain

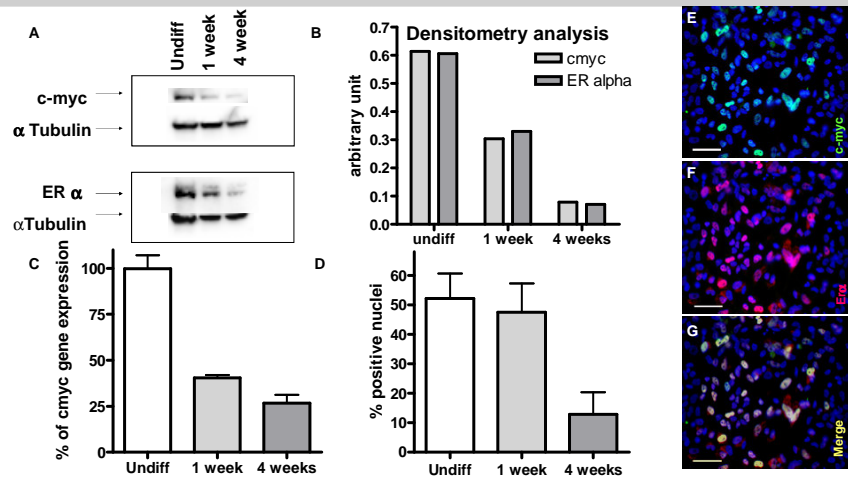
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## Introduction

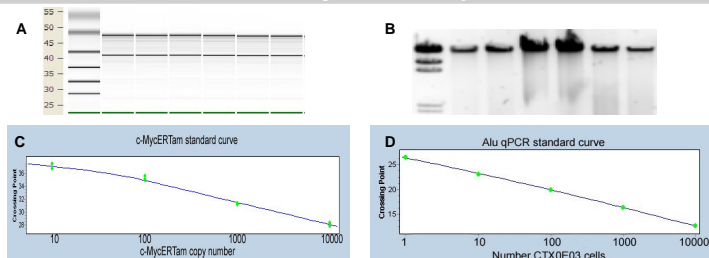
CTX0E03 is a human neural stem cell line developed for the cell therapy of chronic stroke disability. c-MycER technology, under the conditional regulation of 4-hydroxytamoxifen (4-OHT), enabled the large-scale stable banking of CTX0E03. *In* this study, we investigated the fate of this transgene following mitogen withdrawal *in vitro* and *in vivo* post intracerebral grafting in MCAo occluded rats. These data show that expression of the c-mycER transgene is considerably silenced following grafting of CTX0E03 cells into MCAo lesioned rat brain.

## In vitro characterization



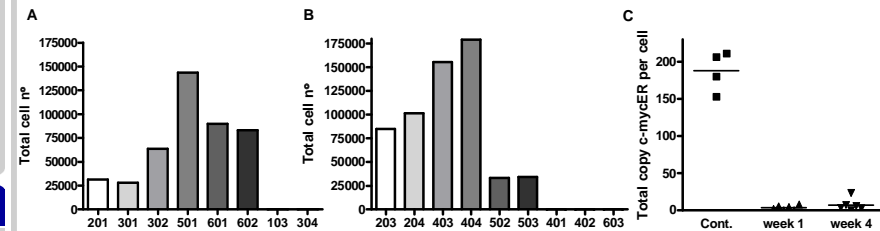
*In vitro* characterization of c-mycER expression by (A, B) western blot; (C) qRT-PCR (n=2); and (D-G) immunocytochemistry for c-myc and estrogen receptor  $\alpha$  (n=3). *In vitro*, the c-mycER transgene is reduced by ~75% at 4-weeks post-mitogen withdrawal by qRT-PCR, western blot, and ICC.

## Assay development



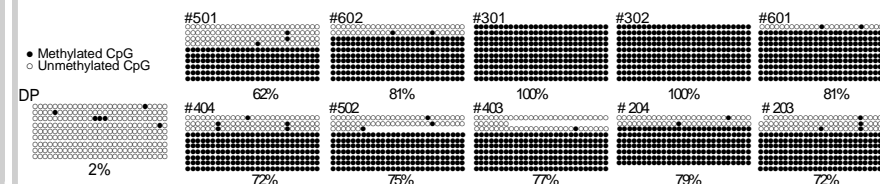
A) Virtual gel of total RNA (Agilent 2100 Bioanalyzer); (B) gel electrophoresis analysis of gDNA samples; (C) Alu qPCR; and (D) c-mycER qRT-PCR standard curves.

## In vivo characterization



CTX0E03 cells (450,000) were implanted into the brains of 12 adult rats 4-weeks post-MCAo lesion and analysed for grafted cell survival and c-mycER expression by Roche LC480. At 1-week and 4-weeks CTX0E03 cells were present in all animal brains ranging from 6.3% to 39.8% of the total injected cells. c-MycER retro-transcribed copies per CTX0E03 cell *in vivo* was calculated to be 3.29 (SEM=1.24) at 1-week and 7.08 (SEM=3.41) at 4-weeks - significantly lower than 187.88 (SEM = 13.42) copies in control cells. Total cells found at (A) 1-week and (B) 4-weeks post-implantation by Alu qPCR. Rats # 103, 304, 401, 402 and 603 are vehicle injected only control brain samples. (C) Absolute quantification of c-mycER transcript level per cell *in vivo* by c-mycER qRT-PCR.

## Transgene methylation



Methylation analysis by bisulfite sequencing of CTX0E03 c-mycER construct in tissues and control (DP). These data confirm that c-mycER silencing occurred through methylation of transgene sequence.

## Summary

- *In vitro* c-mycER silencing was confirmed by quantitative PCR, immunocytochemistry, and western blot analysis.
- The Alu qPCR assay allows the accurate quantification of CTX0E03 cell number in implanted ischemic rat brain.
- 1-week and 4-weeks post-grafting, c-mycER gene expression is significantly reduced as a result of transgene methylation.