MilliTrace™ Constitutive GFP Reporter Neural Stem Cell Lines

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Abstract

GFP (green fluorescent protein) is a common molecular tool used to monitor and track cells both *in vitro* and *in vivo*. It is especially helpful in stem cell research, where it makes cells easily identifiable for transplantation and co-culture studies. Millipore has developed two primary rodent neural stem cell (NSC) lines, adult rat hippocampal NSCs and embryonic mouse cortical NSCs, that express humanized *mulleri* GFP (hmGFP) under the control of the constitutive chicken actin promoter. These constitutive GFP reporter NSC lines are multipotent, exhibit normal karyotypes, and are suitable for a variety of applications.

Introduction

Multipotent self-renewing cells with the capacity to differentiate into neurons, astrocytes and oligodendrocytes have been isolated and propagated extensively from many species. Unlike most human NSC cultures, rodent NSCs are able to undergo prolonged self-renewal and can thus accommodate long-term passaging without an apparent impact on their chromosomal stability. Monolayer cultures of NSC lines isolated from two different regions of the rodent brain have been established at Millipore and are readily propagated in a defined, serum-free medium. Millipore's adult rat hippocampal and embryonic mouse cortical NSCs have been maintained in culture for over 25 passages and have been shown to respond similarly to neuronal and astrocytic differentiation cues. Using a combination of retinoic acid and forskolin over a course of four days, both rat and mouse NSCs can be readily differentiated into approximately 80% neurons and 20% oligodendrocytes, while exposure to bone morphogenic protein 4, LIF, and serum will preferentially direct differentiation to astroglial lineages.

As a tool to monitor the behavior of stem cells in the host tissue environment or niche (i.e. transplantation studies) and in the methodical dissection of the contribution of neighboring cells on the fate of NSCs (i.e. co-culture studies), GFP-labeled NSCs enjoy several advantages over other labeling technologies. First, GFP is non-invasive and can be easily visualized. Lineage determination of non-labeled NSCs suffers from the disadvantage of being an end-point analysis – cells must be fixed before immunocytochemical staining can be performed. In contrast, GFP can label live cells. Other cell labeling methods such as Brdu and $DiI_{[MCI][a2]}$ are problematic as the dyes tend to possess short half-lives, can be progressively diluted as the cells divide, and could also undergo cellular processing. GFP-labeled cells are compatible with other analyses such as ICC, IHC, flow cytometry, confocal microscopy, and fluorometric assays.

Methods and Results

MilliTrace Constitutive GFP Reporter Rodent NSC lines were generated as follows from Millipore's Adult Rat Hippocampal NSCs and Mouse Embryonic Cortical NSCs. First, adult rat hippocampal NSCs and mouse embryonic cortical NSCs were cultured as monolayers on poly-L-ornithine and laminin-coated tissue culture flasks in rat and mouse NSC expansion medium respectively. A plasmid containing human *mulleri* GFP (hmGFP)

protein under the control of the constitutive chicken actin promoter was then transfected into both types of primary rodent NSCs. Stable cell lines were selected by puromycin over a period of four weeks. Pooled lines were FACS analyzed and over 95% of cell population in both rat and mouse GFP NSC lines were verified to express high levels of GFP (Figure 1A, B).

Both rat and mouse GFP reporter NSC lines displayed normal karyotype as assessed by chromosomal spread (>90% of cells display normal 42 and 40 chromosomes, respectively). Thus, GFP fluorescence (Figure 1A) and karyotype (Figure 1C) of the two rodent NSC were stable and retained over prolonged passage (>10 passages).

MilliTrace Constitutive GFP Reporter Rodent NSCs displayed the immunochemical staining characteristics of NSCs. Under proliferative culture conditions, most cells were positively stained for nestin and SOX2 (Figures 2A-F and 4A-F) and negatively stained for the neuronal and astrocyte restricted lineage markers, βIII-tubulin and GFAP, respectively (data not shown).

GFP-labeled rodent NSCs are multipotent: they can be readily differentiated into neurons or astrocytes using Millipore's differentiation kits (Figures 3A-F; Fig 5A-F). Collectively, these results demonstrate the capacity of rodent NSCs to stably and constitutively express GFP while still retaining the stem cell properties of self-renewal and multipotentiality.

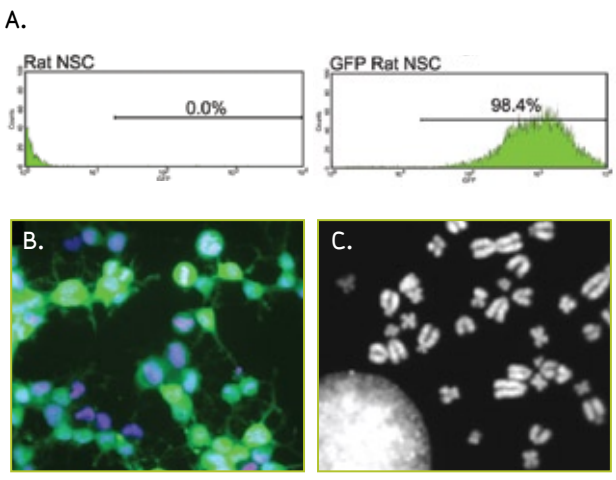

Figure 1. MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal and Mouse Cortical (data not shown) Neural Stem Cells stably express the green fluorescent protein (Figures 1A, B) and maintain an apparent stable karyotype over prolonged passages (>10 passages, Figure 1C). Representative FACS analysis and chromosomal spread of stable constitutive GFP adult rat hippocampal (Figure 1A, B) and mouse cortical neural stem cells (data not shown).

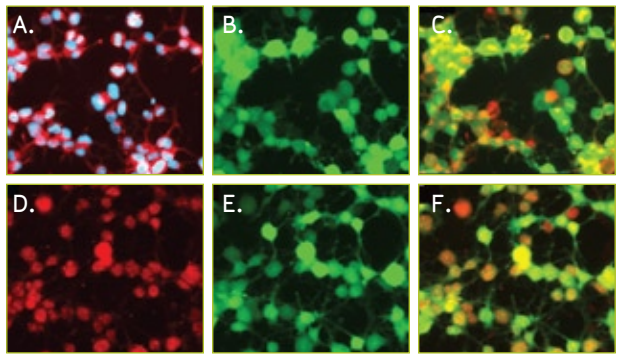

Figure 2. MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal NSCs express NSC markers, nestin (A, C) and SOX2 (D, F). Nuclei of the cells were visualized with DAPI (blue). The transcription factor is co-localized with the GFP staining in the nucleus. Majority of cells are GFP-positive (B, C, E, and F).

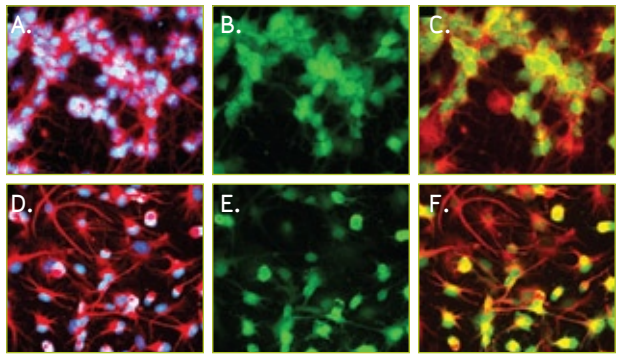

Figure 3. MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal NSCs are multipotent. Using Rodent Neuron Differentiation Kit, NSCs can differentiate into neurons (βIII-tubulin, A, C). Using Rodent Astrocyte Differentiation Medium, rat NSCs can differentiate into astrocytes (GFAP, D, F). Majority of cells are GFP-positive (B, E).

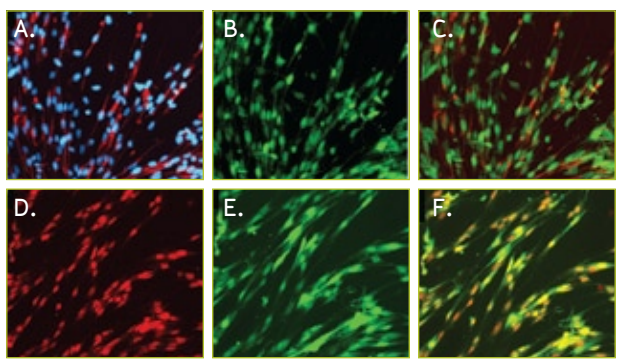

Figure 4. MilliTrace Constitutive GFP Reporter Mouse Cortical NSCs express NSC markers, nestin (A, C) and SOX2 (D, F). Nuclei of the cells were visualized with DAPI (blue). The transcription factor is co-localized with the GFP staining in the nucleus. Majority of cells are GFP-positive (B, C, E, F).

Conclusion

Reporter cell lines are valuable tools to help monitor the behavior of specific populations of cells as they proliferate, migrate, differentiate, and integrate into the cellular milieu. However, the generation and characterization of stable reporter cell lines remain challenging and time-consuming tasks with few reporter stem cell lines available commercially.

We have addressed this problem by constructing two well-characterized rodent NSC lines that constitutively express GFP under the control of the chicken actin promoter. MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal and Mouse Cortical Neural Stem Cells display the immunocytochemical staining properties of neural stem cells: they are nestin and SOX2 positive and βIII-tubulin and GFAP negative. Under conditions that favored differentiation, both GFP reporter NSCs were able to give rise to high numbers of neurons and astrocytes while expressing GFP at high levels, thus demonstrating their multipotentiality. Chromosomal spreads indicate that both rodent cell lines contain the appropriate number of chromosomes (42 for rat and 40 for mouse).

As such, these MilliTrace GFP reporter stem cell lines can be used for a variety of applications, including *in vivo* transplantation studies (i.e. GFP-expressing stem cells can be distinguished from unlabeled endogenous cells), direct co-culture studies of cell-cell interactions and contributions to the stem cell niche (GFP-expressing stem cells can be distinguished from other cells in the local environment), and also as for high-content and high-throughput screens to discover agents that affect stem cell maintenance and differentiation.

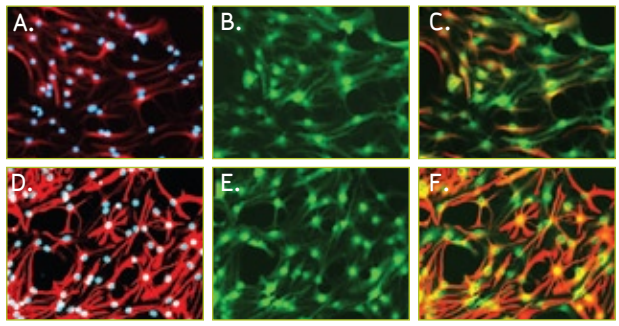

Figure 5. MilliTrace Constitutive GFP Reporter Mouse Cortical Neural Stem Cells are multipotent. Using Rodent Astrocyte Differentiation Medium majority of cells differentiate into GFAP-expressing astrocytes (D, F) while a smaller percentage of cells differentiate into βIII-tubulin-positive neurons (A, C, red). Majority of cells are GFP-positive (B, E).

Millipore Products

Description	Catalogue No.
MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cell Kit	SCR080
MilliTrace Constitutive GFP Reporter Mouse Cortical Neural Stem Cell Kit	SCR081
MilliTrace Rat Neural Stem Cell Expansion Medium	SCM040
MilliTrace Mouse Neural Stem Cell Expansion Medium	SCM041
MilliTrace Rodent Neural Stem Cell Basal Medium	SCM060
Neural Stem Cell Characterization Kit	SCR019
Rodent Neuron Differentiation Kit	SCR035
Rodent Astrocyte Differentiation Medium	SCM010
Adult Rat Hippocampal Neural Stem Cells	SCR022
Cryopreserved Mouse Cortical Neural Stem Cells	SCR029
Rat Neural Stem Cell Expansion Medium	SCM009
Mouse Neural Stem Cell Expansion Medium	SCM008

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