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A method for stable transgenesis of radial glia lineage in rat neocortex by *piggyBac* mediated transposition

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Abstract

Methods that combine lineage tracing with cellular transgenesis are needed in order to determine mechanisms that specify neural cell types. Currently available methods include viral infection and Cre-mediated recombination. *In utero* electroporation (IUE) has been used in multiple species to deliver multiple transgenes simultaneously into neural progenitors. In standard IUE, most plasmids remain episomal, are lost during cell division, and so transgenes are not expressed in the complete neural lineage. Here we combine IUE with a binary *piggyBac* transposon system (PB-IUE), and show that unlike conventional IUE, a single embryonic transfection of neocortical radial glia with a *piggyBac* transposon system results in stable transgene expression in the neural lineage of radial glia: cortical neurons, astrocytes, oligodendrocytes, and olfactory bulb interneurons. We also developed a modular toolkit of donor and helper plasmids with different promoters that allows for *shRNA*, bicistronic expression, and transgenesis in subsets of progenitors. As a demonstration of the utility of the toolkit we show that transgenesis of epidermal growth factor receptor (EGFR) expands the number of astrocytes and oligodendrocytes generated from progenitors. The relative ease of implementation and experimental flexibility should make the *piggyBac* IUE method a valuable new tool for tracking and manipulating neural lineages.

Keywords

In utero electroporation; *piggyBac*; Radial glia; Lineage; Astrocytes; Oligodendrocytes

1. Introduction

In utero electroporation (IUE) is an efficient method for delivering multiple plasmid DNAs into CNS progenitors *in vivo* (Saito and Nakatsuji, 2001; Tabata and Nakajima, 2001). While an effective method for delivering multiple plasmids with high efficiency into the same progenitor, it suffers from a disadvantage relative to retrovirus or transgenic reporter lines in that it does not result in labeling of an entire lineage. IUE has become widely used in studies of neuronal migration in developing neocortex, and is ideal for labeling pyramidal neurons of defined regions and layers of neocortex (Bai et al., 2003; Manent et al., 2009). In order to label a particular cell type with IUE, the time and location of transfection is varied based on the birthdate and site of generation of that cell type desired to be labeled (LoTurco et al., 2009). Birthdating by IUE is likely caused by plasmid loss or inactivation upon cell division, because viruses applied at the same time label an entire lineage that includes neurons and glia (LoTurco et al., 2009).

A possible non-viral solution to the loss or inactivation of episomal plasmid in IUE is the use of DNA transposon systems such as the *piggyBac* transposon to drive genomic integration of transgenes. Such systems have been used successfully for efficient and stable transgene delivery in multiple cell types (Ding et al., 2005; Wilson et al., 2007; Woltjen et al., 2009; Yoshida et al., 2010). The typical transposon system involves a transgene from a donor plasmid and a helper plasmid that expresses a transposase. The donor plasmid must contain terminal repeats flanking the transgene of interest for transposition into the genome to occur (Cadinanos and Bradley, 2007). If the helper plasmid does not contain the terminal repeats necessary for DNA transposition, then as with any episomal plasmid, expression of the transposase is lost, and without further transposase expression transgenes are stably integrated into the genome.

Currently there are two well established transposon systems adapted for use in mammalian cells: *Sleeping Beauty* (SB) and *piggyBac* (PB) (VandenDriessche et al., 2009). *PiggyBac* was originally isolated from the genome of the cabbage looper moth *Trichoplusia ni* (Cary et al., 1989; Elick et al., 1996; Fraser et al., 1995). Compared to *Sleeping Beauty*, *piggyBac* has a more precise “cut and paste” mechanism (Fraser et al., 1996; Yusa et al., 2009), higher transposition efficiency (Wu et al., 2006) and larger cargo capacity (Ding et al., 2005; Lacoste et al., 2009). *PiggyBac* has been used to generate iPS cells (Lacoste et al., 2009; Woltjen et al., 2009; Yusa et al., 2009), in gene therapy models (Wilson et al., 2007), and for neuronal development studies in *Drosophila* (Schuldiner et al., 2008) and chicken (Lu et al., 2009).

Here we report a novel application of *piggyBac* mediated transgenesis in neocortical progenitors in rat by *in utero* electroporation (PB-IUE). This method, unlike standard IUE, can be used to successfully label the complete lineage of neural progenitors: neurons, astrocytes, oligodendrocytes and olfactory bulb interneurons. We additionally developed a *piggyBac* plasmid toolkit that allows for *shRNA*, bicistronic expression and transgenesis in subsets of neural progenitors. The relative ease of implementation and inherent flexibility of a plasmid-based system should make this method valuable to many interested in marking and manipulating neural lineages in the CNS for rats and other species for which Cre reporter lines are not available.

2. Methods

2.1. Plasmids

Both the 3' and the 5' *piggyBac* terminal repeats (3'TR and 5'TR) were amplified from pZGs (Wu et al., 2007). To make pPBCAG-eGFP, the 3'TR was cloned into pCAG-eGFP (Matsuda and Cepko, 2007) using *Sall* and *SpeI* sites and the 5'TR was cloned into the same vector using *PstI* and *HindIII* sites. For construction of pPBCAG-mRFP, the eGFP cassette in pPBCAG-eGFP was replaced by mRFP cassette from pCAGGS-mRFP (Manent et al., 2009) using *XbaI* and *BglII* sites. pCAG-PBase was constructed by replacing eGFP with PBase sequence (Wu et al., 2007) in pCAG-eGFP using *EcoRI* and *NotI* sites. pGLAST-PBase was made by inserting PBase downstream of GLAST promoter provided by Dr. D.J. Volsky (Kim et al., 2003). To make pPBCAG-EGFR, human wild type EGFR was PCR amplified from EGFR WT (Greulich et al., 2005) (Addgene plasmid 11011), and inserted into *KpnI* and *NotI* sites of pPBCAG-eGFP.

2.2. Construction of a *piggyBac* toolkit

To make pPBCAG-CFP, CFP sequence was amplified from CMV-Brainbow-1.0 (Livet et al., 2007), (Addgene plasmid 18721) and replaced the eGFP cassette in pPBCAG-eGFP using *EcoRI* and *NotI* sites. For construction of pNestin-PBase, pGFAP-PBase and

pTalpa1-PBase, PBase coding sequence was directly inserted downstream of the rat Nestin promoter, a gift from Dr. Steven Goldman (Roy et al., 2000), mouse GFAP promoter, a gift from Dr. Vijay Sarthy (Kuzmanovic et al., 2003) and Talpa1 promoter, a gift from Dr. Albert Ayoub and Dr. Pasko Rakic (Gal et al., 2006), respectively. For construction of pPBGFAP-eGFP, pPBDCX-eGFP, pPBCamKII-eGFP and pPBMBP-eGFP, CAG promoter in pPBCAG-eGFP was replaced with mouse GFAP promoter provided by Dr. Vijay Sarthy (Kuzmanovic et al., 2003), mouse DCX promoter, a gift from Dr. Qiang Lu (Wang et al., 2007), rat MBP promoter, a gift from Dr. Robin Miskimins (Wei et al., 2003), and CamKII promoter (Chow et al., 2010), (Addgene plasmid 22217), respectively. For construction of bicistronic donor plasmid pPBCAG-eGFPT2amRFP, T2A sequence *gagggcaggg gaagtctact aacatgcggg gacgtggagg aaaatcccg ccca* was added to the 3'-end of eGFP coding sequence using standard PCR method and then eGFP T2A was inserted into the *XbaI/EcoRI* sites of pPBCAG-mRFP. For future cloning into the T2A plasmid, *EcoRI/BglII* sites can be used to replace mRFP with gene of interest. Construction of pPB-mU6pro was achieved by inserting *piggyBac* terminal repeats into the mU6pro vector (Yu et al., 2002). 3'TR was inserted into the *NotI* site and 5'TR was inserted into the *PstI/HindIII* sites of mU6pro. *shRNA* sequence can be cloned into the *XbaI/BbsI* sites.

2.3. Animals

Pregnant Wistar rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and maintained at the University of Connecticut vivarium. Animal gestational ages were determined and confirmed during surgery. Both male and female embryos were used. All procedures and experimental approaches were approved by the University of Connecticut IACUC.

2.4. In utero electroporation

In utero electroporation was performed as previously described (Bai et al., 2003; Ramos et al., 2006). Briefly, rats were anesthetized with a mixture of ketamine/xylazine (100/10 mg/kg i.p.). Metacam analgesic was administered daily at dosage of 1 mg/kg s.c. for 2 days following surgery. To visualize the plasmid during electroporation, plasmids were mixed with 2 mg/ml Fast Green (Sigma). In all conditions, pPBCAG-eGFP, pPBCAG-mRFP, pPBCAG-EGFR, pCAG-eGFP and pCAG-mRFP were used at the final concentration of 1.0 µg/µl, while pCAG-PBase and pGLAST-PBase were used at the final concentration of 2.0 µg/µl. Electroporation was performed at embryonic day 13 or 15 (E13 or E15). During surgery, the uterine horns were exposed and one lateral ventricle of each embryo was pressure injected with 1–2 µl of plasmid DNA. Injections were made through the uterine wall and embryonic membranes by inserting a pulled glass microelectrodes (Drummond Scientific) into the lateral ventricle and injecting by pressure pulses delivered with a Picospritzer II (General Valve). Electroporation was accomplished with a BTX 8300 pulse generator (BTX Harvard Apparatus) and BTX tweezerrodes. A voltage of 65–75 V was used for electroporation. Hippocampal electroporation was performed as previously described (Navarro-Quiroga et al., 2007).

2.5. Immunohistochemistry

Animals were deeply anesthetized with isoflurane and perfused transcardially with 4% paraformaldehyde/PBS (4% PFA). Brain samples were post fixed overnight in 4% PFA and sectioned at 65 µm thickness on vibratome (Leica VT 1000S). Sections were processed as free-floating sections. After blocking in PBS containing 5% of normal goat serum (Sigma) and 0.3% Triton X-100 (Sigma) for 1 h at room temperature, tissue sections were incubated with primary antibodies overnight at 4°C in the blocking solution. The following primary antibodies were used: mouse anti-GFP (1:1000, Molecular Probes), rat anti-DsRed (1:1000,

Molecular Probes), rabbit anti-Ki67 (1:1000, Novus Biologicals), mouse anti-GFAP (1:200, Chemicon), mouse anti NG2 (1:500, Chemicon), mouse anti-CC1 (1:200, Santa Cruz). Tissue sections were washed in PBS, incubated with the appropriate secondary antibodies (all Alexa Fluor in 1:200, Invitrogen,) for 2h at room temperature (Alexa Fluor 488 anti-mouse IgG, Alexa Fluor 488 anti-rabbit IgG, Alexa Fluor 568 anti-mouse IgG, Alexa Fluor 568 anti-rabbit IgG, Alexa Fluor 647 anti-rabbit IgG, Invitrogen) and washed in PBS. In some tissues, nuclei were labeled with TOPRO-3 (Molecular Probes) and 4-6-diaminodino-2-phenylindole (DAPI, Invitrogen). Images were acquired on either a Leica TCS SP2 confocal system or Stereo Investigator (Microbright Field) with the HAMAMATSU digital camera C10600. Montage images were taken using the virtual slice function of Stereo Investigator (Microbright Field).

2.6. Neighbor analysis and statistics

For neighbor analysis virtual slices were taken using Stereo Investigator (Microbright Field). The virtual slices were divided into $250\ \mu\text{m} \times 250\ \mu\text{m}$ grids. Distances between cells with the same color were measured within the grid and was analyzed by Neurolucida Explorer (Microbright Field). Neighbor analysis was performed both in control condition and EGFR over expression condition. To statistically compare changes in the distance distributions in control condition and EGFR over expression condition determined by neighbor analysis, the Kolmogorov-Smirnov test was performed (http://www.physics.csbsju.edu/stats/KS-test.n.plot_form.html). To compare ratios of labeled cells that were astrocytes or neurons, a one-way analysis of variance (ANOVA) was performed by KaleidaGraph version 4.0 (Synergy Software 2006). A confidence interval of 95% ($p < 0.05$) was required for values to be considered statistically significant. All data are presented as standard error of the mean (SEM).

3. Results

3.1. PiggyBac labels cortical lineage of neural progenitors

We investigated whether *piggyBac* mediated transgenesis can label the progeny of neural progenitors and whether transgenes are stably maintained in progeny. In order to test this, we electroporated the *piggyBac* donor plasmid pPBCAG-eGFP, along with the helper plasmid pCAG-PBase, into developing rat brain at embryonic day 13 (E13) or embryonic day 15 (E15), using episomal pCAG-mRFP as transfection control (Fig. 1A). The *piggyBac* transposon system is a binary system with a helper plasmid (pCAG-PBase) providing *piggyBac* transposase (PBase), and the donor plasmid (pPBCAG-eGFP) providing the CAG-eGFP transgene between the 5' and 3' terminal repeats (TRs) of the donor plasmid.

In brains electroporated at E13, RFP labeled neurons were restricted to deep layers while GFP labeled cells were found in all layers (Fig. 1B). As shown in Fig. 1D, deep layer neurons were double labeled with GFP and RFP while the upper layer neurons were only positive for GFP. GFP positive cells in both gray and white matter were GFAP and S100B positive indicating that astrocytes, generated late during cortical development (Kriegstein and Alvarez-Buylla, 2009), can also be labeled by *piggyBac* IUE (Fig. 1E and F). When performed with pPBCAG-eGFP alone at E13, IUE labeled deep layer neurons but not upper layer neurons or astrocytes (data not shown). The absence of upper layer neurons and astrocytes in both episomal pCAG-mRFP (Fig. 1B) and pPBCAG-eGFP when transfected without the PBase expressing plasmid indicates that episomal plasmid is not maintained through multiple progenitor cell divisions.

In brains electroporated at E15, at the time when upper layer neurons are generated (Langevin et al., 2007), neurons were double labeled with GFP and RFP while astrocytes

were only positive for GFP (Fig. 1C). We also found that there were more astrocytes labeled by E15 electroporation than by E13 electroporation. At P21, E13 PB-IUE resulted in $79.8 \pm 3.3\%$ neurons and $20.2 \pm 3.3\%$ astrocytes (Fig. 1G), while at E15 there were $26.6 \pm 2.7\%$ neurons, $73.4 \pm 2.7\%$ astrocytes (one way ANOVA, $p < 0.0001$). In summary, unlike conventional IUE with episomal plasmid which only labels birthdated progeny, single embryonic transfections with the *piggyBac* system results in labeling of all cell types in the lineage of embryonic neocortical progenitor cells.

3.2. PiggyBac labels olfactory bulb interneurons and oligodendrocytes

To further demonstrate lineage labeling of neural progenitors by the *piggyBac* system, we assessed the presence of other cell types not typically labeled by standard IUE. We found GFP but RFP labeled granule cells and periglomerular cells in the olfactory bulb (Fig. 2A). GFP positive cells were positive for the neuroblast marker DCX and these cells were found in rostral migratory stream (RMS) (Fig. 2B). In the SVZ of P21 brains, we found GFP positive cells positive for both GFAP (Fig. 2C) and Ki67 (Fig. 2D). Taken together, these results show that *piggyBac* IUE applied embryonically labels stable staining of SVZ neuroblasts, migrating RMS cells, and olfactory bulb interneurons.

The next question we asked was whether *piggyBac* IUE could label oligodendrocytes. GFP positive cells showed oligodendrocyte (Fig. 2F) morphology and were positive for the oligodendrocyte marker CC1 in both gray matter and white matter. Cells co-expressing GFP and the oligodendrocyte precursor marker NG2 were also found in both white and gray matter (Fig. 2E), supporting the hypothesis that radial glia are a source of at least some oligodendrocyte precursors (Malatesta et al., 2003; Trotter et al., 2010; Ventura and Goldman, 2007).

Next we applied *piggyBac* IUE in hippocampus. When we electroporated hippocampus at E15 with the *piggyBac* donor (pPBCAG-eGFP) and helper (pCAG-PBase) plasmids, GFP positive cells were found in CA1, CA2, CA3 and dentate gyrus. As shown in Fig. 2G, GFP positive neurons, astrocytes (GFAP positive), oligodendrocytes (CC1 positive) and oligodendrocyte precursors (NG2 positive) were present in hippocampus. In contrast, in brains electroporated with pPBCAG-eGFP alone, without the PBases expression plasmid, neurons but not astrocytes or oligodendrocytes were labeled by GFP (data not shown). Taken together, our data show that *piggyBac* IUE can be used to label the lineage of progenitors present at the surface of the ventricular zone in the neocortex, olfactory bulb and hippocampus.

3.3. A modular plasmid toolkit for piggyBac IUE

The inherent experimental flexibility of a plasmid system led us to develop additional plasmids that can be used in combination for gain- and loss-of-function studies. In addition, we developed plasmids with different promoters in order to selectively target progenitors and progeny of different types (Fig. 3). First, we produced an *shRNA piggyBac* donor plasmid (pPBmU6pro) which contains the U6 promoter to drive expression of *shRNAs* for RNAi experiments. Second, we made and tested a bicistronic eGFP-t2a-mRFP donor plasmid (pPBCAG-eGFPt2amRFP). This T2A donor plasmid can be used to express two proteins from the same transcript, and so by replacing the mRFP cassette with a gene of interest this plasmid can be useful in experiments where a “within tissue” control is desirable. For example, clones in which eGFP signal is present (*i.e.* containing the transgene for a gene of interest) can be compared to clones that are missing an eGFP signal but labeled by other fluorescent proteins. Finally, the binary nature of the *piggyBac* system makes possible independent control of the promoters in helper and donor plasmids. We have made a series of helper and donor plasmids with different promoters that can be used alone or in

combination. These include the helper plasmids pGLAST-PBase, pGFAP-PBase, pT α 1-PBase and pNestin-PBase and donor plasmids pPBGFAP-eGFP, pPBDCX-eGFP, pPBMBP-eGFP and pPBCAMKII-eGFP. Conceptually, these can be mixed in different combinations of donor and helper plasmids to integrate transgenes in subpopulations of progenitors (selected by the promoter in the helper plasmid), and have the transgene expressed in subsets of progeny (selected by the promoter in the donor plasmid). For example, by combining pGLAST-PBase with pPBGFAP-eGFP it is possible to label the astrocytes generated from GLAST positive progenitors.

3.4. EGFR transgenesis expands astrocyte clones in cortex

To demonstrate the utility of the multi plasmid *piggyBac* system in gain-of-function experiments, we transfected a *piggyBac* system mixture of plasmids containing fluorescent proteins. Transfection of three fluorescent transgenes (pPBCAG-eGFP, pPBCAG-mRFP and pPBCAG-CFP) resulted in approximately 70% of cells expressing all three fluorescent proteins, albeit at varied levels, 20% expressing 2 and 10% expressing one fluorescent protein. This relatively high co-expression rate indicated that gain-of-function experiments could be achieved in combination with two-color labeling by simply adding additional donor plasmids to the transfection mixture. For these IUE experiments we used the GLAST promoter to direct expression of transposase in radial glia at E15. In a test of this gain-of-function application we introduced a donor plasmid, pPBCAG-EGFR, which encodes epidermal growth factor receptor (EGFR). EGFR expression has been previously shown to increase the proliferation of astrocyte progenitors, and we hypothesized that increased proliferation of astrocytes or astrocyte progenitors would result in more cells in same color astrocyte groups. As shown in Fig. 4A–D the expression of EGFR increased the density of astrocytes in cortex and striatum. Within neocortex, EGFR transgenesis expanded same color astrocyte groups and this gave rise to large patches of single color groupings of astrocytes. This expansion was further demonstrated by a significant increase in the distribution of distances between same color cells within 250 μ m $\times$ 250 μ m regions (Fig. 4E). The EGFR related expansion of same color distances is consistent with previous studies showing an increase in proliferation of astrocytes following EGFR overexpression (Ayuso-Sacido et al., 2010; Burrows et al., 1997; Sun et al., 2005), and extends these results to show that clonal expansion can result in an increase in the spatial domains occupied by clonally related astrocytes. Moreover, the result supports the idea that same color groups of astrocytes are clonally related and demonstrates the gain-of-function application.

3.5. Patterned clonal astrocyte expansion following EGFR transgenesis

In areas outside of dorsal–lateral neocortex, EGFR transgenesis showed unique patterns of multicolor labeling that were not apparent in transfections without EGFR. Large color-defined patches of astrocytes appeared following EGFR transgenesis (Fig. 5A) in the forebrain medial wall and the striatum. Without EGFR transgenesis only scattered cells in striatum were labeled (Fig. 4A), but as shown in Figs. 4B and 5B, after EGFR transgenesis extremely large single color groups of astrocytes were found spread throughout striatum. EGFR transgenesis also expanded oligodendrocytes of single color within the anterior commissure and corpus callosum (data not shown). The number of interneurons labeled in the olfactory bulb was also increased following EGFR transgenesis. Unlike astrocytes, granule cell numbers in the olfactory bulb were expanded without any apparent clustering of color defined groups suggesting that expansion of granule cell numbers did not result in expansion of spatially restricted clonal related neurons (Fig. 5C). Astrocytes in the olfactory bulb, similar to astrocytes elsewhere, showed expansion of single color groups (Fig. 5D).

4. Discussion

In utero electroporation (IUE) is a relatively efficient method for delivering plasmid DNA into CNS progenitors *in vivo* (Saito and Nakatsuji, 2001; Tabata and Nakajima, 2001). The approach has become widely used in studies of neuronal migration in developing neocortex, and is ideal for labeling pyramidal neurons of defined regions and layers of neocortex (Bai et al., 2003; Manent et al., 2009). While an effective method, it suffers from a disadvantage, relative to retrovirus, when applied to neocortex in that it does not reliably label the entire lineage of radial glia. Here we reported that the combination of *in utero* electroporation and *piggyBac* mediated transposition (PB-IUE) successfully labels the lineage of radial glia. The method extends the application of IUE to a unique lineage labeling approach in which the “birthdated” progenies are labeled by two transgenes and subsequent progenies are labeled only by transgenes flanked by terminal repeats (Fig. 1). The utility of this type of lineage labeling is in being able to express one transgene in only the birthdated progeny (first member of a lineage near the time of transfection) but not in the subsequent progeny of the lineage. We also demonstrated that the *piggyBac* system can be readily adapted to gain-of-function applications. The modular plasmid toolkit can be used to create RNAi, bicistronic expression, and/or selected transgene integration into defined progenitors and progeny. The relative ease of implementation, and inherent flexibility of a plasmid-based system, should make this method and toolkit valuable to many interested in marking and manipulating neural lineages in the CNS.

Any method taking advantage of multiple random genomic integration events raises the risk of insertional mutagenesis altering results. In fact, insertional mutagenesis by transposases has been used for identification of cancer related genes in engineered mouse lines (Collier et al., 2005; Starr et al., 2009). These systems of mutagenesis however require constant expression of the transposase to allow for multiple transposition events, and the system we used results in *piggyBac* transposase expression that is limited to immediate progeny and not propagated into dividing members of the lineage. Moreover, we did not observe evidence of mutagenesis based on inspection of morphologies of thousands of neurons and even more astrocytes labeled with the *piggyBac* IUE method.

In conclusion, *piggyBac* mediated transgenesis approach will complement existing methods for the study of neural lineages and glial cells in brain development. The *piggyBac* toolkit shall be proven powerful in radial glia lineage labeling and manipulation.

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Abbreviations

IUE	<i>in utero</i> electroporation
PB	<i>piggyBac</i> transposon
CNS	central nervous system
GFP	green fluorescent protein
RFP	red fluorescent protein
SVZ	subventricular zone
TR	terminal repeats
EGFR	epidermal growth factor receptor
iPS	induced pluripotent stem cell
RNAi	RNA interference

Fig. 1. *PiggyBac* labels radial glia lineages. (A) Schematic representation of plasmids used. (B) Images of brains electroporated at E13. (C) Image of brains electroporated at E15. (D) Upper layer neurons were labeled with GFP but not RFP. Deep layer neurons were double labeled with GFP and RFP. (E) GFP labeled cells that were positive for S100B in both white matter and gray matter. (F) GFP labeled cells that were GFAP positive in both white matter and gray matter. (G) Quantifications of astrocyte to neuron ratio in brains electroporated at E13 and E15. Asterisk indicates significant difference (one way ANOVA, $p < 0.001$). Scale bar: 200 μm in B, 50 μm in C, 20 μm in D and E, 500 μm in F.

Fig. 2.

PiggyBac labels olfactory interneurons and oligodendrocytes. (A) Left panel, Image of P21 olfactory bulb stained with DAPI. GFP positive cells can be seen. Right panel, confocal image of GFP labeled cells in glomeruli. (B) Confocal images of DCX positive cells labeled with GFP in RMS. (C) GFAP positive cells in SVZ double labeled for GFP. (D) Ki67 positive cells in SVZ positive for GFP. (E) NG2 positive cells labeled with GFP in white matter. (F) CC1 positive cells labeled with GFP in white matter. (G) Left, images of hippocampus electroporated at E15. Right, GFAP positive, CC1 positive, and NG2 positive cells labeled with GFP in hippocampus. St: striatum; WM: white matter. Scale bar: A: 100 μ m in left panel and 10 μ m in right panel. 20 μ m in B–G: 50 μ m in left panel and 20 μ m in right panel.

Fig. 3.

PiggyBac toolkit. (A) Schematic summaries of *piggyBac* helper plasmids with five different promoters available to drive expression of PBase. (B) Schematics of the collection of *piggyBac* donor plasmids, including multi color *piggyBac* donor plasmid, donor plasmid driven by cell type specific promoters, bicistronic donor plasmid and donor plasmid for expression of *shRNAs* by the U6 promoter.

Fig. 4.

EGFR transgenesis induces spatial expansion of same colored astrocyte groups. (A) Images from a control transfected hemisphere at P27 transfected with the multicolor plasmid system without the addition of an EGFR donor plasmid. (B) Example of a hemisphere at P27 transfected with EGFR donor plasmid and the multicolor plasmid system. High magnification of control transfected cortex is shown in (C) and EGFR transfected cortex is shown in (D). (E), Cumulative probability histogram showing the distribution of same colored cell distances in 250 μm × 250 μm areas of cortex with (green line) and without (red line) transfection of EGFR donor plasmid. (Kolmogorov–Smirnov; $p < 0.0001$). Scale bar: 500 μm in A and B, 100 μm in C and D. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

EGFR induced patterns of clonal expansion in forebrain. (A) Image of an anterior section from a P27 rat brain with EGFR transgenesis. Large astrocyte groupings were observed as shown in A1. Stripes of astrocytes of same color are also noticeable. (B) Striatum with EGFR transgenesis. Large same color astrocyte groupings were apparent with some color mixing within the larger groups. B1 shows an example of large red astrocyte grouping mixing with other astrocytes. (C) Images of olfactory bulb with EGFR transgenesis. Expansion of the number of olfactory interneurons and clonal mixing were observed. Higher magnification of the olfactory ventricle, granule layer and periglomerular layer are shown in C1, C2 and C3. (D), Images of olfactory bulb with same color astrocyte clusters in the EGFR transgenesis condition. Examples of astrocytes in olfactory bulb are shown in D1 and D2. Scale bar: 100 μ m in A–D, 20 μ m in A1, B1, C1, C2 and C3, 50 μ m in D1 and D2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)