Thorsten Stuhmer Protocol for the electroporation of brain slices
March 2000

preparation of tissue:

Dissect the embryos in cold Krebs buffer, when removing the brains of early embryos take care that the ventral side (hypothalamus) remains as intact as possible, as that will be much better for the coherence of the slices in later steps. Keep tissue on ice.

10 x Krebs buffer stock solution:

1l	0.5l		
73.6g	36.8g	NaCl	1.26M
1.87g	0.935g	KCl	25mM
1.66g	0.83g	NaH_2PO_4 x H_2O (or 1.44g/0.72g anhydr.)	12mM
2.44g	1.22g	$MgCl_2$ x $6H_2O$	12mM
3.68g	1.84g	$CaCl_2$ x $2H_2O$	
	25mM		

0.5l 1 x Krebs for slices: 50 mls stock solution diluted with cold sterile H_2O, 1g glucose (dextrose) and 1.05g $NaHCO_3$ added, sterile filtred. pH after filtration should be about 7.5-7.6

sterile Krebs for the slices:
to 50ml of the above-filtred buffer add:
- -0.5 ml 1M HEPES
- -0.5 ml PenStrep
- -100 µl 50 mg/ml gentamycin

sectioning:

Prepare 5% low-melting point agarose in 1xPBS, keep at 42°C. To embed the brains, fill agarose into a tissue embedding mold, transfer the brain with as little buffer as possible, gently stir the lot with a glassrod, so that the agarose can properly contact the tissue. Be careful not to tear or squeeze the brain (especially in E14.5 and younger embryos). Keep on ice until the agarose has solidified, then trim away the edges so that a small block suitable for sectioning is obtained. The block should not be too high, as this may lead to less precise cutting. Glue the block(s) onto a mount, adjust the vibratome and cut sections of 200 or 250 microns in ice cold Krebs buffer. The slices are best handled with a pair of spatulas, which provide good support to the tissue when it is transferred between dishes. Transfer the sections into the cold Krebs buffer for slices, and after 10 minutes transfer the slices onto a polycarbonate membrane, floating on MEM in a culture dish (Whatman Nucleopore Track-Etch membranes (Fisher special order number NC9656875, 42$ for a package of 100 filters)). At this point the slice is well supported and can easily be moved around. Keep in a 37°C incubator for two hours or until the start of the electroporation

medium with serum: -43.5 ml minimal essential medium
-5 ml fetal calf serum
-1 ml 25% glucose
-1 ml PenStrep

electroporation:

We use the following setup: a BTX electroporation system (Electro Square Porator T820). The machine is set to low voltage mode, charging voltage at 100-150V, delivering 1 or 2pulses of 5ms duration each. The electrode system is a custom made set of two solid platinum electrodes, one of which remains fixed in a glass petri dish, whereas the other can be freely moved (models CUY700-1 and CUY700-2 from Protech International, Inc., San Antonio, Texas; www.protechinternational.com; this is the supplier for the U.S.A., the electrodes are actually manufactured by TR Tech Co. in Tokio, Japan (FAX: 813-3944-6196), but must be bought through Protech. The price is about 1500$ for the pair. The company offers an electroporator as well, claiming it is the bees' knees. And about another 10K).

Electroporator from BTX Technologies
http://www.btxonline.com
ECM 830 Square Wave Electroporator

Electrodes from Protech International
Dennis McCarthy <protech@texas.net>
http://www.protechinternational.com

Petri Dish electrode CUY701P20E (6mm x 6mm)
Electrode CUY191-3 (3mm x 3mm)

Agarose Gel Casting System
http://www.bio-rad.com
Mini-PROTEAN II System

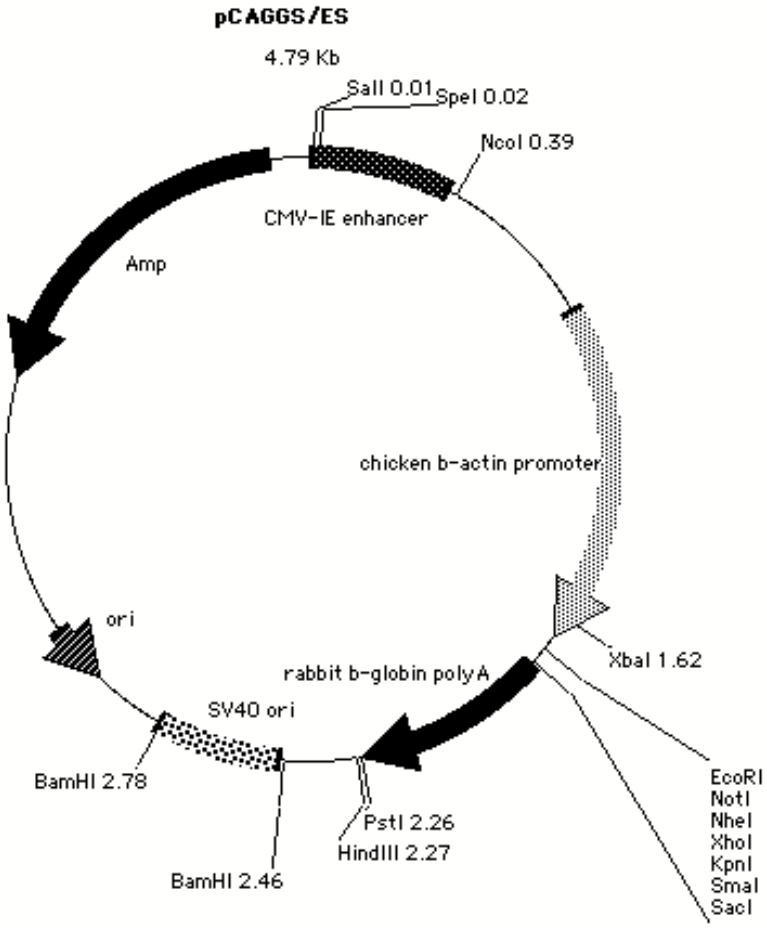
All our genes are cloned into the expression vector pCAGGS, where a chicken β-actin promoter drives expression. This plasmid appears to be very suitable for strong expression in mouse nervous tissue (and seems to work fine in chicken too). I use CsCl purified plasmids and try to keep the concentration above 5mg/ml, so that they can be mixed and still are fairly highly concentrated. I like to initially resuspend them in a little water, and adjust to the final concentration with Krebs buffer.

A 1% agarose gel (which I make up with 1x Krebs buffer) is used as a conductive buffer between the electrodes and the tissue (to prevent heat damage). If a Bio-

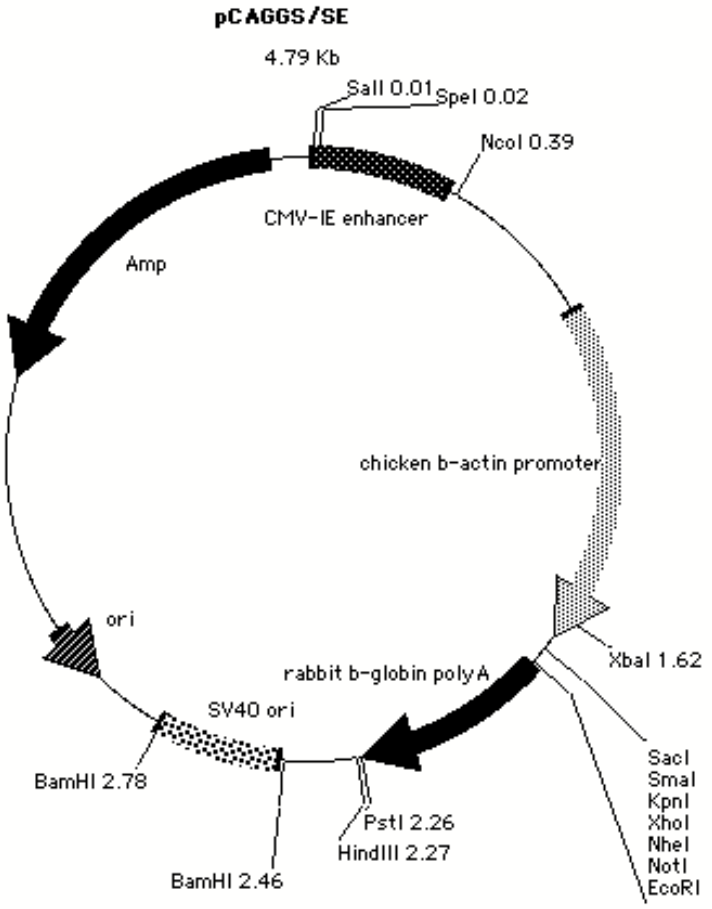
Rad Minigel system is at hand, this is very well suited to cast a gel that is strictly plane on both sides, about 2mm wide, and that can be kept fresh for hours in between the glass plates. A small block is cut from this gel to serve as a mount for the slice and placed on the bottom electrode. A filtre with a brain slice is then placed on top of the agarose mount. A tiny column of agarose (which can be conveniently made in different diameters with a set of clipped and filed syringe needles) is attached to the top electrode, a drop of plasmid solution (0.5-1µl) is transferred to the lower edge of this agarose column and the whole electrode is lowered, so that the DNA solution contacts the slice at the desired place. After the electroporation filtre and slice are transferred to a culture dish containing 1ml of Neurobasal medium and cultured at 37°C (with daily changes of the medium). I change the bottom agarose-chunk for each new slice and the much tinier top column for every electroporation. The agarose will burn somewhat where it contacts the electrodes and this can massively reduce the conductivity, especially for the small column. Occasionally check the electrodes to make sure that they are still clean.

serum-free medium:
-47.5 ml neurobasal medium
-1 ml B-27 supplement
-1 ml 25% glucose
-0.5 ml 0.2M glutamine
-1 ml PenStrep

If a GFP plasmid was introduced the protein should be visibly expressed after 10 hours.



Plasmid name: pCAGGS/ES
Plasmid size: 4.79kb
Constructed by: Jürgen Stuehmer
Construction date: March 2000
Comments/References: expression plasmid for genes in mouse brain cell suitable for electroporation. Use NheI to clone XbaI fragments, XhoI to clone SalI ends. Polylinker sequence: GAATTCGCGGCCGCTAGCAACTCGAGGTACCCCCGGGGAGCTC



Plasmid nampCAGGS/SE
Plasmid size 7.9kb
Constructed by Jorsten Stuehmer
Construction date March 2000
Comments/References: expression plasmid for genes in mouse brain cel
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ends. polylinker sequence: GAGCTCCCCGGGGGTACCTCGAGTTGCTAGCGGCCGCGAATTC