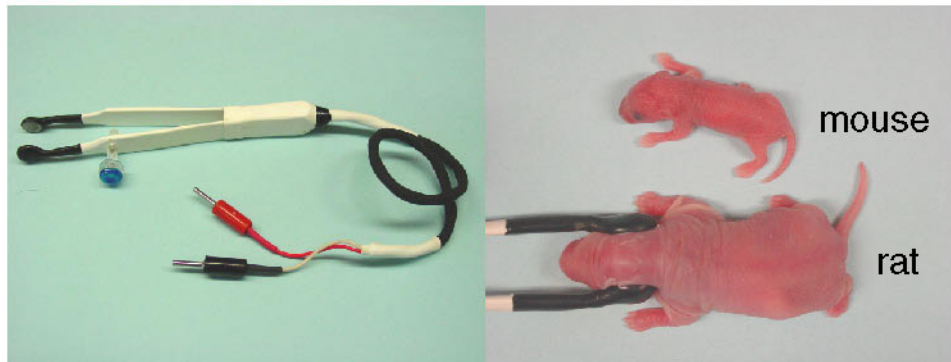
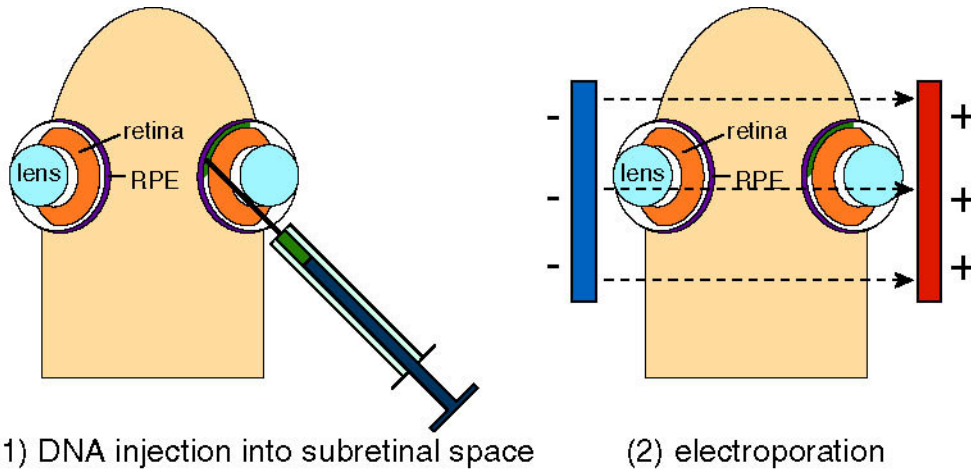


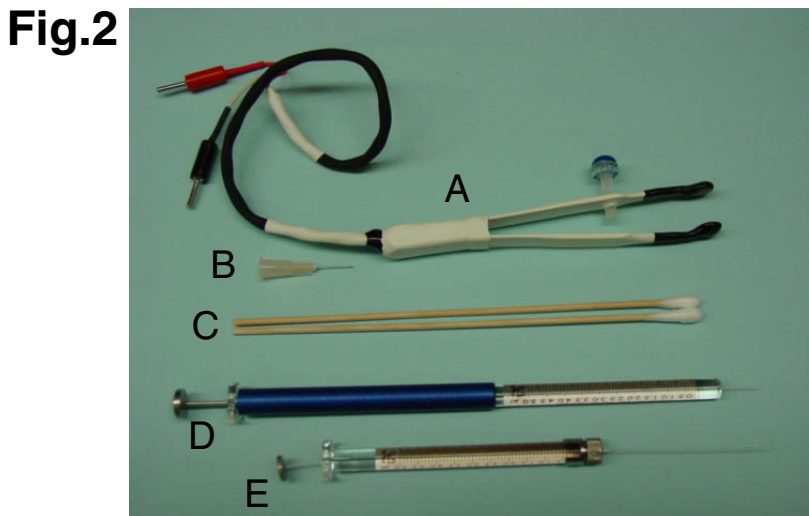
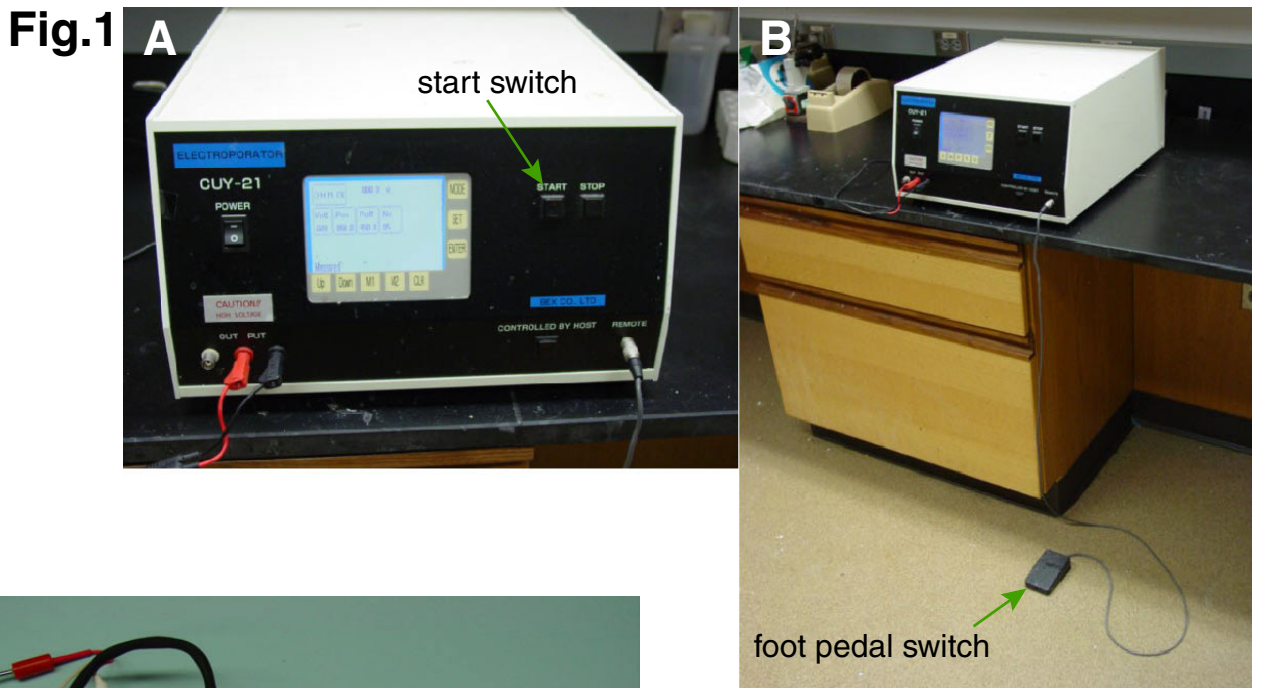
Protocol for *in vivo* electroporation into mouse/rat retina Ver. 1.0

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Materials:

- Square pulse electroporator CUY21 (Nepagene, Japan, Fig.1).
We confirmed that another square pulse electroporator ECM830 (BTX) also works, although it is a lower model and does not have the function to display the current measurements.
Because both of your hands are occupied to hold the animal and the electrodes, respectively, it is recommended to use a foot pedal switch (Fig.1B).
- Tweezer-type electrodes (BTX, model 520, 7mm diameter, Fig.2A).
Model 522 (10mm diameter) works as well.
- Disposable 30G1/2 needle (Becton Dickinson #5106, Fig.2B).
- Cotton swab (Fig2.C).
- Hamilton injection syringe with a 33G blunt end needle (#0159666, Fig2.D).
or Hamilton injection syringe with a 32G blunt end needle (#87931, Fig2.E).
(It is important to use a blunt end needle (point style 3) instead of a sharp beveled needle.)
- DNA solution 5 μ g/ μ l in PBS.
- 1% fast green in H₂O (filtered and store at RT). Add 1/10 volume of 1% fast green to DNA solution as a tracer (final 0.1%).
- 70% ethanol.
- PBS.



Methods:

DNA injection

There are two injection methods (Fig.5). One is to inject DNA without opening the eyelid (method 1). This is a kind of “blind injection”, but causes less damage to the animal. This method works well especially for rat pups. The other one is to inject DNA after cutting the future edge of the eyelid (method 2). In this method, you can see the tip of the injection needle through the lens and can check if DNA is correctly injected into the subretinal space. If you have no experience of subretinal injection, try the latter method for the first time.

Method 1 (injection without opening the eyelid)

All the procedures are done under a dissection microscope.

1. Anesthetize newborn pups on ice for several minutes (Fig.3).
It is possible to do the operation without anesthetization. But you should anesthetize the animals to reduce their pain.
2. Clean the eyelid with 70% ethanol using a cotton swab (Fig.5B).
By wetting the eyelid, you can easily recognize the shape of an eyeball and the future edge of the eyelid.
3. Make a small incision in sclera near the cornea through the skin using the tip of a sharp 30-gauge needle (Figs.4, 5C, 7A).
4. Carefully insert an injection needle into the eyeball through the incision until you feel slight resistance (Figs. 5E, 7C). When you feel resistance, the tip of the injection needle is located in the subterinal space between RPE and retina.
Note that even a blunt end needle can easily pierce the sclera behind the retina if you further push it.
5. Slowly inject DNA (5 μ g/ μ l) containing 0.1% fast green into the subretinal space (Figs.5F, 7D). For rat newborn pups, we inject 0.5-1.0 μ l of DNA (less than 1 μ l), and for mouse newborn pups, we inject 0.3-0.5 μ l of DNA (less than 0.5 μ l). If DNA is correctly injected, the eyeball becomes green (Fig. 5G).

Method 2 (injection after opening the eyelid)

1. Anesthetize newborn pups on ice for several minutes (Fig.3).
 2. Clean the eyelid with 70% ethanol using a cotton swab (Fig.5B).
 3. Carefully cut the future edge of the eyelid using the tip of a sharp 30-gauge needle (Figs.4, 5H).
Cutting the area shown in yellow in Fig. 4 occasionally causes bleeding, which makes injection difficult. Therefore it is better not to cut these areas.
 4. Expose the eyeball by pulling down the skin (Fig.5I).
 5. Make a small incision in sclera near the cornea using the tip of a sharp 30-gauge needle (Fig.5J).
 6. Insert an injection needle into the eyeball through the incision until you feel resistance (Fig. 5K).
You can see the inserted needle through the lens (Fig.6).
 7. Slowly inject DNA into the subretinal space (Fig.5L).
- If you correctly inject DNA into the subretinal space, the dye spreads within a relatively small area (not in the entire retina), and you can see “green” and “non-green” areas in the retina by slightly rotating the injected animal (Figs.7D, 8).
 - If you inject DNA into the vitreous, fast green spreads more rapidly and uniformly in the eyeball (Fig.7E).
 - If you inject DNA outside the retina, the eyeball does not become green (Fig.7F).

Fig.3



cutting these areas occasionally causes bleeding

Fig.4

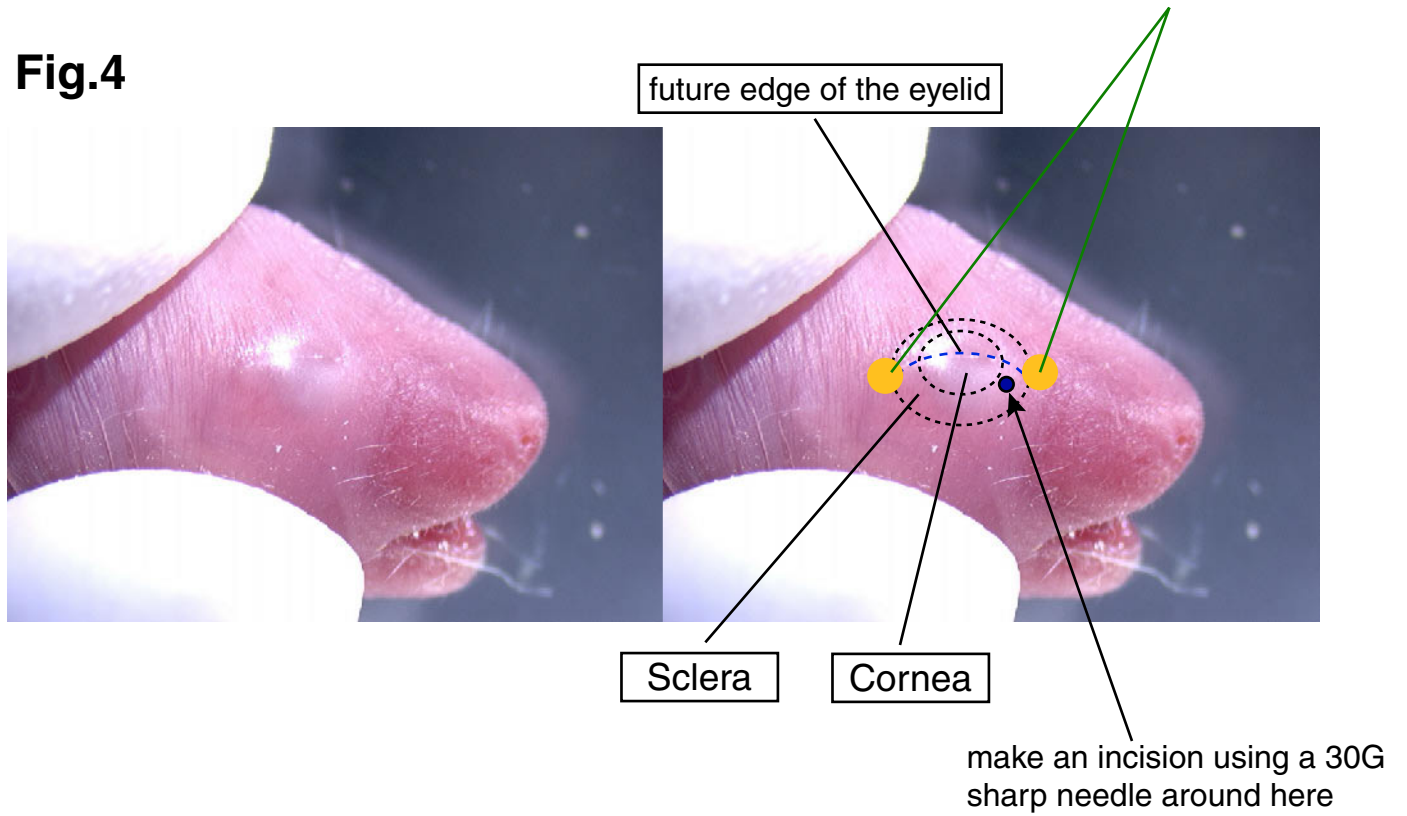
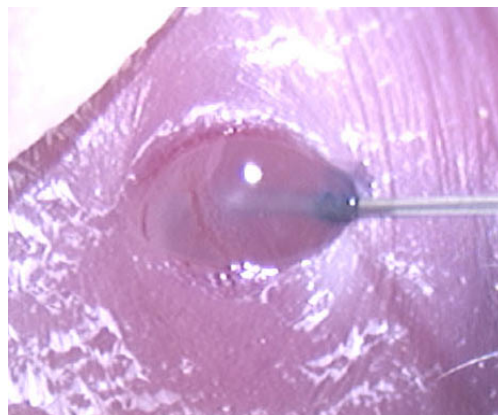


Fig.6



A high magnification view of Fig.5K

Fig.5

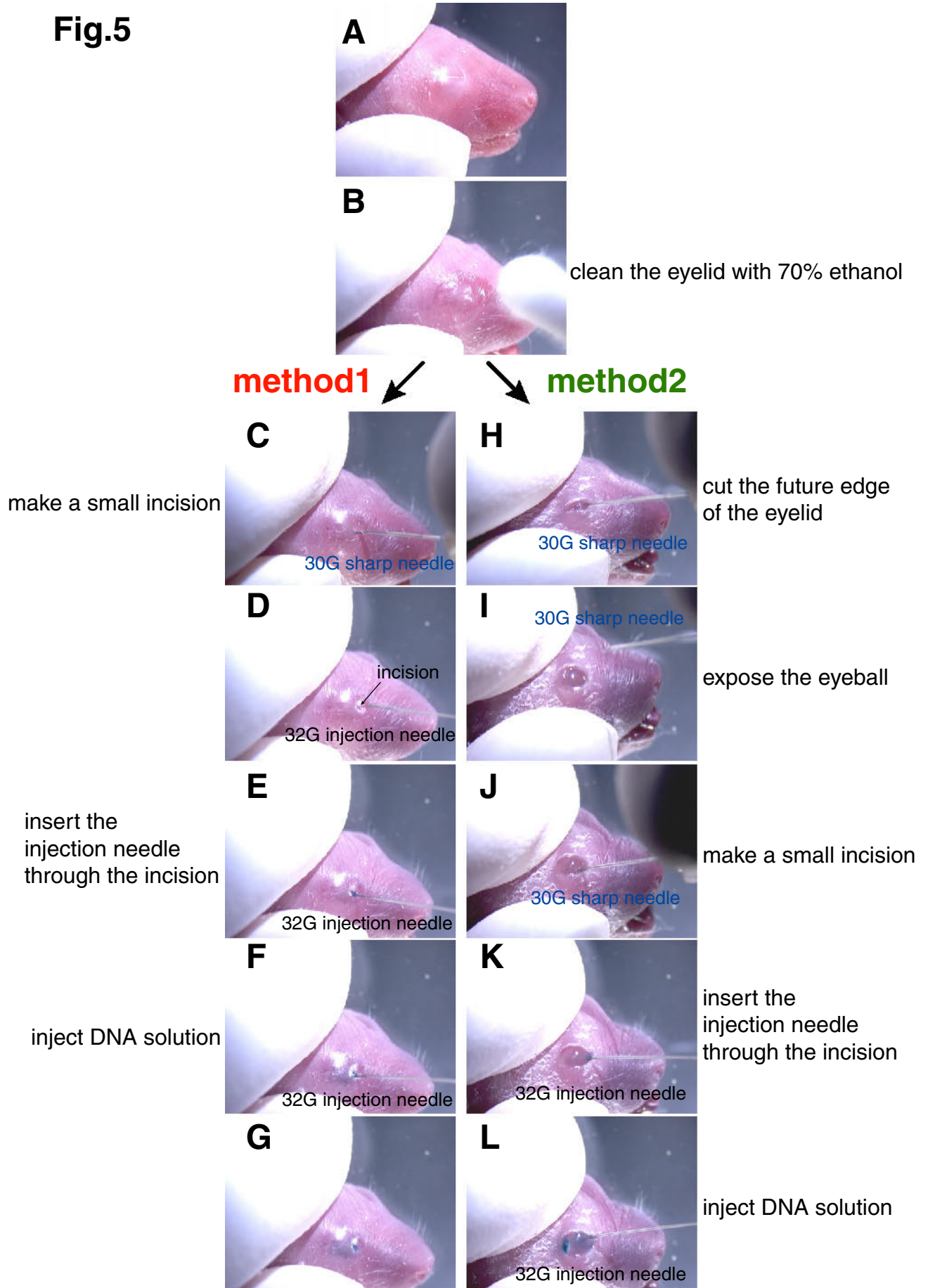


Fig.7

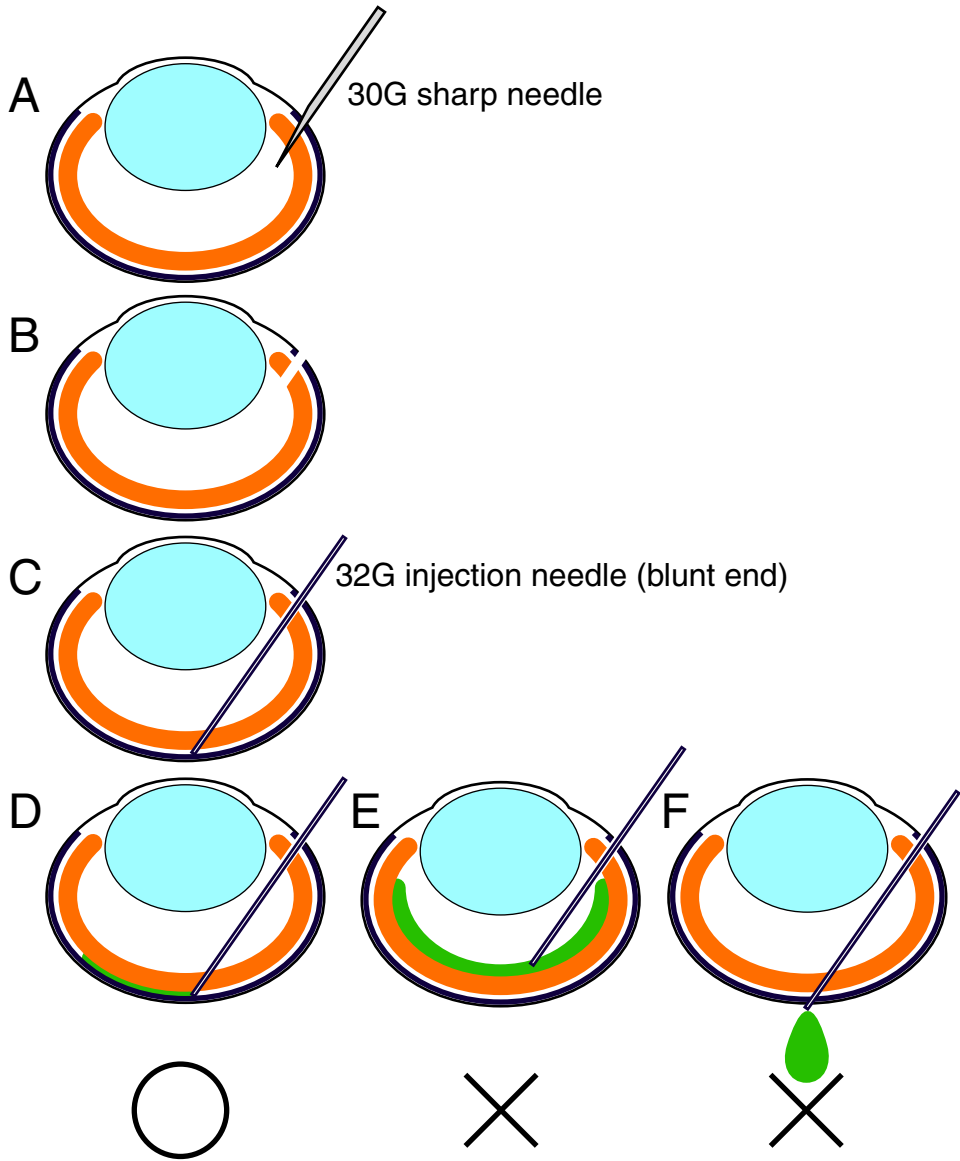
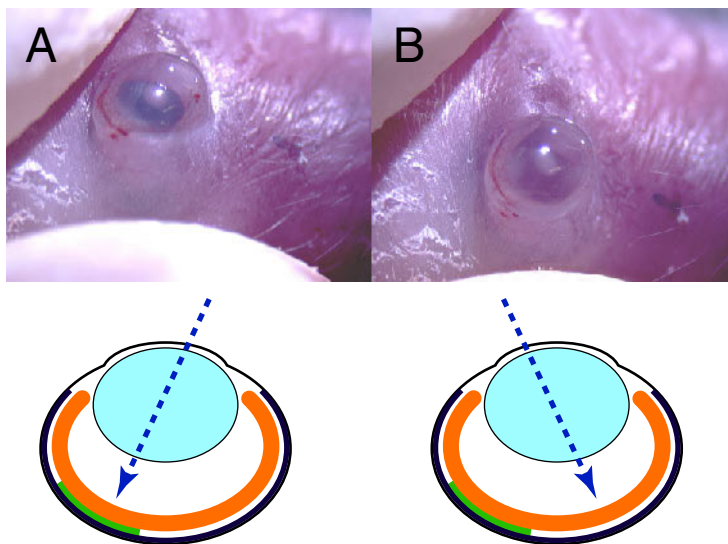


Fig.8



Electroporation

1. Soak the tweezer-type electrodes in PBS (Fig. 9), and then remove excess PBS by briefly shaking them. This step is for increasing the contact areas between the pup and the electrodes, but may be omitted.
2. Place the tweezer-type electrodes to hold the head of the pup, and slightly squeeze them (Fig. 10). The plus electrode, marked by a plastic screw (Fig. 10 arrow head), should be at the DNA-injected side, if you like to transfect DNA from the subretinal space to retina.
3. Apply five square pulses of 50 ms duration with 950 ms intervals using a pulse generator (Fig.11). For rat newborn pups, we apply 100V pulses, and for mouse newborn pups, we apply 80V pulses. We usually apply electric pulses right after DNA injection. The measured current is 0.08-0.15A.
4. Warm the operated pups (e.g. by using a heat lamp) until they recover from the anesthetic (Fig. 12), and then return them to their mother.

Fig.9



Fig.10

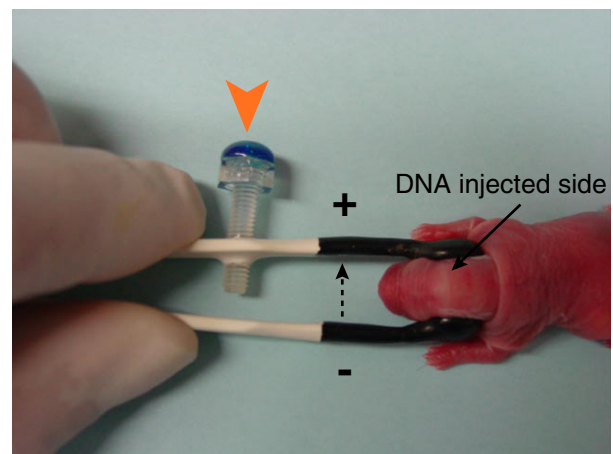


Fig.11

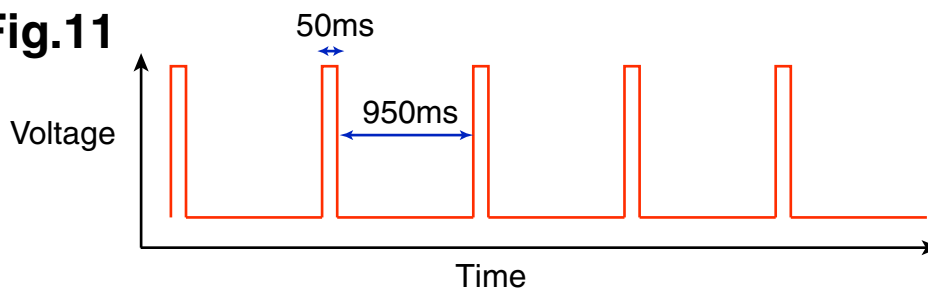


Fig.12



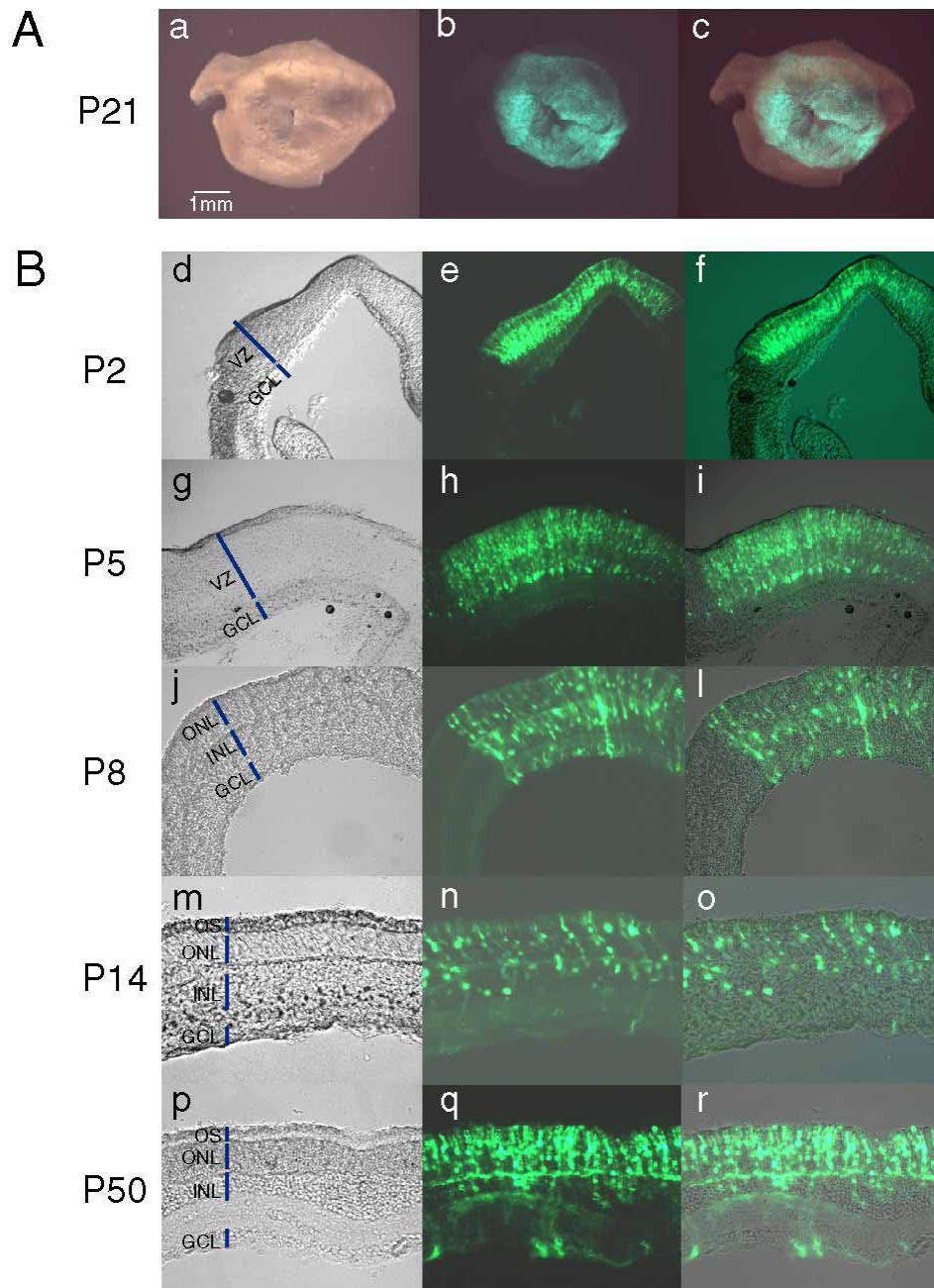


Fig.13

P0 rat retinas were *in vivo* electroporated with pCAG-GFP, and harvested at the indicated time period.

Reference Matsuda, T & Cepko C. L. *manuscript in preparation*