# Transfection on early chick embryo with METAFECTENE PRO

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## *Introduction:*

Gene transfection is certainly the critical technique on the study of developmental biology because it opens the door on genetic study in embryo. Chick embryo is good model for studying cell migration, differentiation, and signal transduction during development because early chick embryo allows being accessed and manipulated easily. So far viral and non-viral mediated transfection was used for gene transfection. Viral transfection to embryo comes cross several barriers, such as safety, high expense and complicated procedure although the advantage is high efficiency. Thus, most of chick embryo laboratory including ours utilize a non-viral transfection way - electroporation to deliver gene into the cells of interested region in chick embryo at moment. However, another problem can not be avoided for electroporation experimenters, which the embryo will be damaged to some extent, especially for younger chick embryo. The experimenters have to equilibrium the electroporation intensity and harming embryo as less as possible when electroporation is carried out because electroporation itself will damage embryo if too strong and simultaneously the transfection efficiency will be too low if electroporation is not strong enough. Thus, the development of new efficient, harmless transfection for embryo is highly desired. METAFECTENE PRO could be one of good candidates for this aim.

## *Materials and methods:*

Embryo in vitro incubation:

Un-incubated fresh Brown Leghorn chick embryos (Henry Stewart, Lincolnshire) were prepared with early chick (EC) cultures [1], and then were incubated at 37$^{o}$C in vitro until to the required stage (HH0 & HH4).

Transfection with METAFECTENE PRO:

We used GFP as a marker for transfection efficiency estimation of Metafectene Pro transfection reagent (Biontex). The first of all, the mixture of pEGFP-N1 (1mg DNA/ml) and Metafectene Pro transfection reagent with the ratio of 1:10 was incubated at room temperature for 15-20min. The mixture was dropped on the HH0 or HH4 chick embryo on EC culture (ventral side of embryo on top), and then incubate the embryo overnight at 37$^{o}$C. For further visualization of GFP-transfected cells with Metafectene Pro some embryos were fixed after transfection 15 hours in 4% paraformaldehyde in PBS and processed for cryostat sectioning. The labeled embryos and sections were photographed using a Nikon fluorescence dissecting microscope equipped with a Nikon DXM1200 digital camera.

***Results and discussion:***

Transfection with mixture of different ratio of pEGFP-N1 (1mg DNA/ml) and Metafectene Pro reagent was tested firstly. The ratio rank is 1:1, 1:10 and 1:100. The transfection efficiency are not different between the mixture ratio at 1:1 and 1:10 while no embryo cells was transfected at the ratio of 1:100 of pEGFP-N1 (1mg DNA/ml) and Metafectene Pro reagent (data not shown). Thus, the experiments were carried out in the following experiment at the mixture ratio of 1 μl pEGFP-N1(1mg DNA/ml):10 μl Metafectene Pro reagent.
To investigate cell migration or gene regulation during chick embryo gastrulation, we need to transfer some genes into chick embryo as early as possible. As shown in Fig 1 cartoon, the mixture of pEGFP-N1 and Metafectene Pro reagent was dropped on the top of un-incubated chick embryo (HH0), and incubated at 37°C overnight. GFP-positive cells accumulated in the foreside of chick HH4 embryo (Fig 1B, C, E, F) after overnight incubation. The reason is that whole epiblast sheet migrate forward during the formation of chick primitive streak [2], in another word, the epiblast cells were even labeled at beginning before primitive streak form, and they moved forward as the epiblast migrate as embryo development.

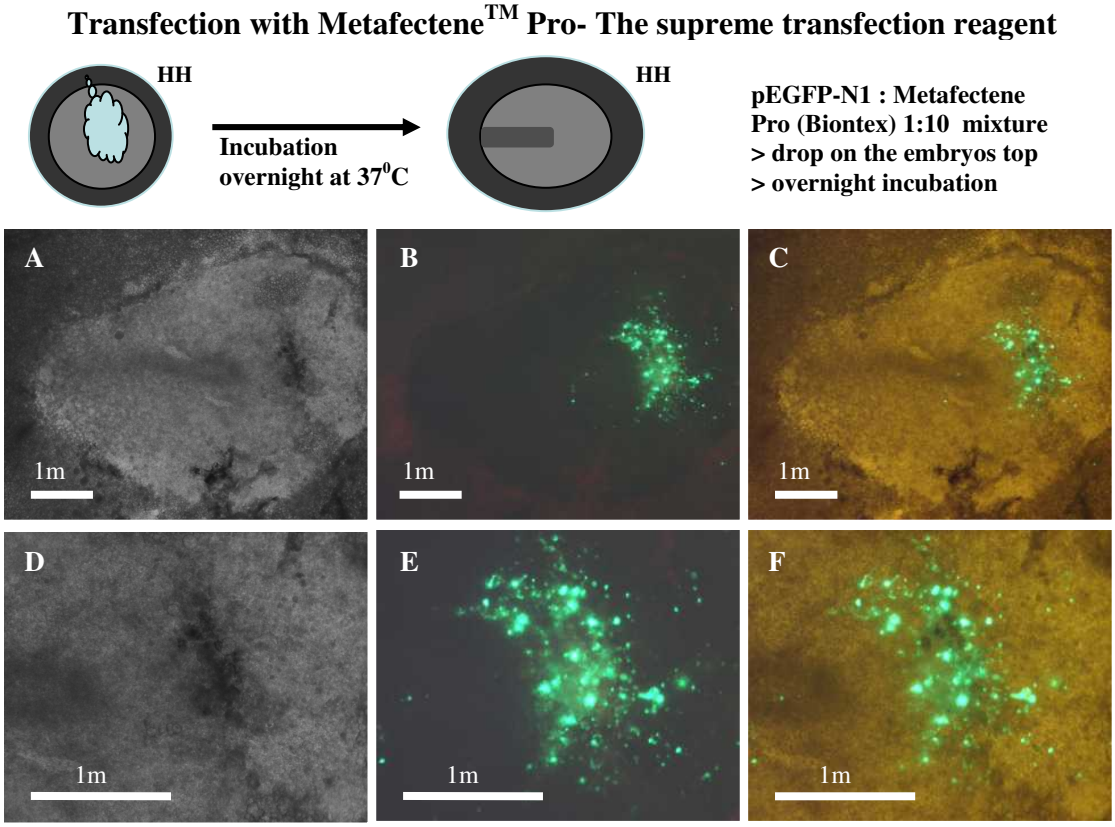


*Fig 1. HH0-HH4 chick embryo transfection with Metafectene Pro reagent. A: bright-field photo after overnight transfection with Metafectene Pro at HH0 while D is the 2.6x higher magnification than A in the same embryo. B and D: fluorescence photos (E at higher magnification). C and F: merge images from bright field and fluorescence (F at higher magnification). The scale bars = 1mm*

For some experiment we need to transfect chick embryo at HH4. In Fig 2A & B the chick embryo was photographed after 20-Hour incubation since transfection with the mixture of pEGFP-N1 and Metafectene Pro reagent at HH4 chick embryos. We could see that GFP-positive cells were distributed in whole embryo, and we confirm that the cells located in mesoderm layer via cross section (Fig 2A1-3, Fig

2B1-3). It suggests that the labeled cells with Metafectene Pro reagent are healthy and migrate normally. Possibly the DNA-Metafectene reagent didn't diffuse efficiently into epiblast at HH4 embryo as it did in HH0 embryos, so not too many labeled cells were found comparing to HH0 embryo transfected with Metafectene Pro reagent.

**Transfection with Metafectene™ Pro- The supreme transfection reagent**

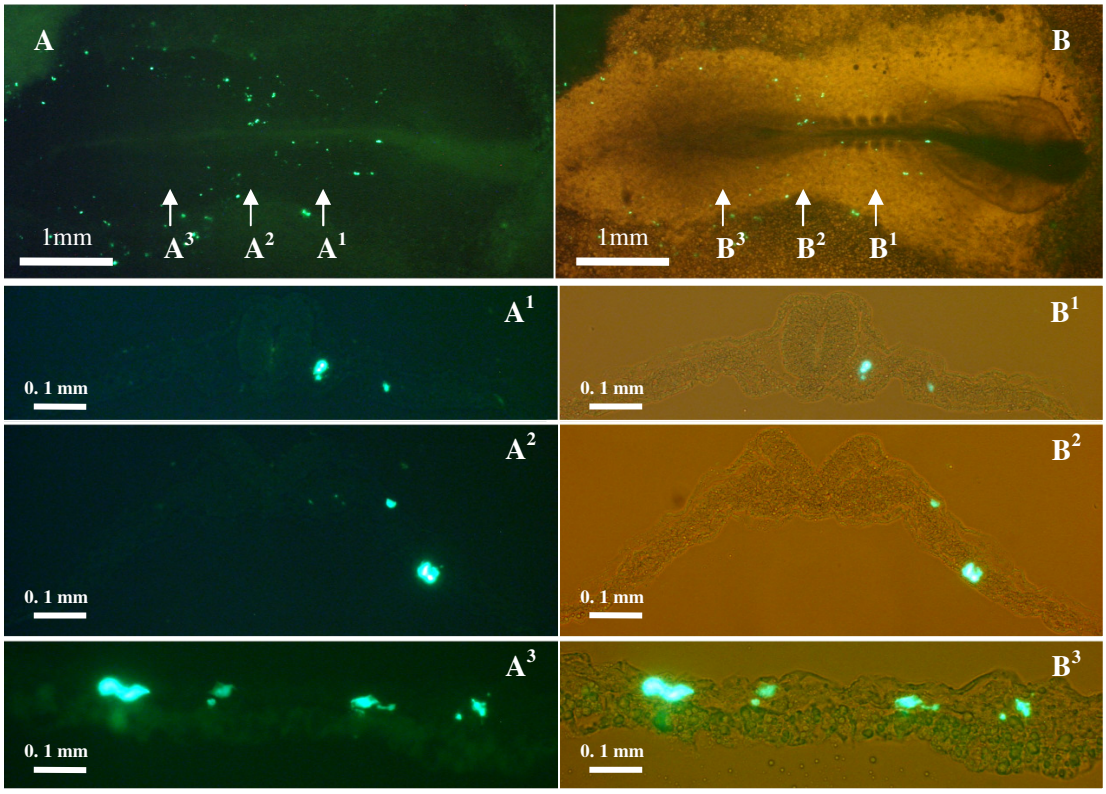


*Fig 2. HH4-HH8 chick embryo transfection with Metafectene Pro reagent. A and B: fluorescence and merger images at 15-hour incubation after transfection with Metafectene Pro respectively. A1-3 and B1-3: cryostat section at indicated position in A and B. The scale bars = 1mm or 0.1mm as indicated.*

The biggest advantage of the transfection with Metafectene Pro reagent is less damage to embryo because the physical damage for younger embryo with electroporation, which is the most powerful way to transfer gene into young chick embryos, always perplex developmental biologists. However, in order to transfect embryo more efficiently with Metafectene Pro, the effect of discovering optimums condition is still required.

***Conclusion / summary:***

Gene delivery with Metafectene Pro reagent is the sample and harmless way for developmental biologists to manipulate chick embryo transfection, especially for young chick embryo transfection.

References:

1. Chapman, J. Collignon, G.C. Schoenwolf and A. Lumsden, Improved method for chick whole-embryo culture using a filter paper carrier, *Dev. Dyn.* (2001) 220: 284–289.
2. Chuai M, Zeng W, Yang X, Boychenko V, Glazier JA, Weijer CJ. Cell movement during chick primitive streak formation. Dev Biol. (2006) 296:137-49.