

Report for the three months visiting work

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I would like to appreciate the SABRE Placement Project to support funding to me to visit Roslin Institute from Sep. 28 2008 to Dec. 28 2008. Thanks for Carol Telford and Toine Roozen' helps. I also would like to express my deep gratitude to Dr. Helen Sang, she gave me direction with my project, and I will remember all members' kindness and help in her lab. Thank Dr. Denis Headon and Dr. Megan Davey give me helps for in situ hybridization. I learned more than what I expected, so it is that a good chance to train in Helen's lab, and to study in RI.

Aims

1. To learn the technologies involved in production of transgenic chickens using lentiviral vectors. (a) Production of high titre lentivirus vector (b) production of transgenic chicks by injection of embryos and embryo culture.
2. To investigate the expression of the chicken SOBP gene in feather follicles of wildtype and silkie chicken embryos.

1. Technologies for generation of transgenic chickens

I have successfully learned all the methods involved in generation of transgenic chickens. The steps involved are:

1. Plasmid sub cloning
2. Culture and transfection of 293T cells, with the vector plasmid and packing plasmids.
3. Collection of packaged virus produced by the transfected cells plus concentration by centrifugation.
4. Titration of the virus on D17 and HT1080 cells.
5. Preparation of needles and microinjection of the virus into stage X chick embryos and initial incubation in host shells.
6. Transfer of manipulated embryos at 3 days of incubation to large host shells.
7. Monitoring of chicks to hatch.

Results

I used the two plasmid system to package the lentivirus vector plenti6/CAG-myr-GFPcppt, in which the report protein GFP is expressed using the CAG promoter. The plasmids were transfected into 293T cell using FuGene with a ratio of plasmids of GFP: psPAX2:vsv-G of 1:2:0.8. The virus was titred on HT1080 and D17 cells by serial dilution on cells 48 hours after plating. The titre on HT1080 cells was estimated as 10^7 .

14 chick embryos were injected with the virus. By day three of incubation one had died, one developed as twins and one was broken during transfer to the larger host shell. The remaining 11 were incubated and 10 hatched. DNA was extracted from the chorioallantoic membrane of individual hatched chicks and screened by PCR (Adrian Sherman). One chick was identified as approximately 1% transgenic. We are going to collect blood samples for DNA to confirm this result.

Discussion

The titre of the virus produced was lower than other virus preparations generated at the same time by Feifei Song. This may have been because the 293T cells were close to confluent or because they partially detached when the culture medium was changed. The survival of injected embryos to hatched when the culture medium was changed. The survival of the embryos after injection of virus was very high, showing that the microinjection technique was good.

The survival of injected embryos to hatch was excellent, so the virus preparation was not toxic to the embryos and the embryo manipulations did not affect survival. Helen's lab has perfect conditions to incubate, hatch and maintain the chicken. I have successfully learned all the steps in the process for generation of transgenic chickens.

2. Analysis of expression of the chicken SOB_P gene by *in situ*

hybridization

Procedures of whole-mount *in situ* hybridization chicken embryos as follow:

1. Artificial insemination of White Leghorn and Silkies chicken (staff)
2. Collect and incubate fertile eggs
3. Order and make solution
4. Preparation of DNA template to produce RNA probes
5. Synthesis of a DIG-labelled RNA probe
6. Collect the embryos
7. *in situ* hybridization
8. Take photos

Results

I got two plasmids, including cDNA of *Sobp* from Liu Weiwei, *csobp*, *sobp-2*. Two pairs of primers for PCR amplifying were designed. I got four segments from two different templates, so I got two segments long 574bp, 655bp for every template. I inserted each one into pCR-TOPO4 vector and sequenced the plasmids. I got the coincident sequences from plasmid *csobp*.

I used a plasmid, including 574bp of cDNA sequence *Sobp*. I got another

plasmid which included a segment of cDNA of *Shh* from Megan Davey, Roslin Institute. And then synthesized DIG-labelled RNA probes after linearing each one.

I collected 6 chick embryos for each day 8,10,12,14 separately for White Leghorn and Silkies. I stored embryos within 100% MeOH in -20 degree after dehydrating.

I just done day6 and day 8 embryos on the first time, the results show us sobp express in day 8 embryos of White Leghorn for the first time, but signal is weaker, positive control (shh) expressed in feather follicle at this stage. No signal have been observed from sobp negative control at any stage and shh in day 6.

I did *in situ* hybridization for embryos day 8, 10 12, 14 for the second time. Pictures showed us that there are signals both *Sobp* negative control and positive at E10、 E12 and E14 in both White Leghorn and Silkie. But for day 8, It is easier to say the differences between White Leghorn and Silkie. Shh work well at all control.

Discussion

On the agarose gel, probe for shh is very good, anti-sense and sense (negative control) appeared many bands. That may be caused by secondary construction in the RNA or degraded during running the gel for long time. But most bands focus on one band when run short time. The first results for ISH showed us that those probes can work. For the second time, both sobp negative and positive were found signals. That may be caused by blocking, put too long to make color develop or antisense does express some other transcripts

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