

Protocol for GFP chicken neural crest cell grafting and back-grafting

Neural tube and neural crest grafting

Fertile chicken eggs, obtained from commercial sources, and transgenic GFP chicken eggs, obtained from The Roslin Institute, University of Edinburgh were incubated at 37°C and staged according to the Hamburger and Hamilton developmental tables or according to the number of pairs of somites formed. For chick^{GFP}-chick grafting, the neural tube and associated neural crest cells (NCC), adjacent to somites 2-6 inclusive, was microsurgically removed *in ovo* from normal wild-type chick embryos at the 9–11 somite stage (ss) of development (embryonic day 1.5) using a microscalpel. The neural tube was cut transversely at the level of somites 2 and 6, and then the scalpel was used to gently separate the neural tube from the connective tissue on either side. On removal, the notochord was visible as a thin white line running underneath the centre of the neural tube.

The ablated region of neural tube and was replaced with equivalent tissue obtained from chick^{GFP} embryos at the same stage of development as previously described for quail-chick grafting (Burns and Le Douarin, 1998). The chick^{GFP} embryo was removed from the egg and the trunk neural tube adjacent to somites 2-6 inclusive was cut out using dissecting scissors. This tissue was then placed in 2% pancreatin and excess tissue (somites, notochord, connective tissue) removed using dissecting needles. The cleaned chick^{GFP} neural tube was transferred into DMEM medium, then placed using a mouth pipette into the ablated neural tube region in the correct anterior to posterior orientation. Dissecting needles were used to move the chick^{GFP} neural tube into position in the host embryo.

Grafts of this size provided extensive labelling of the neural crest-derived ENS combined with good embryo survival. Following grafting, eggs were returned to the incubator, and embryos allowed to develop up to a further 7 days such that GFP-positive NCC colonised the

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host embryo.

GFP-positive NCC were visible in the host tissue without immunohistochemistry, even post-fixation in 4% paraformaldehyde.

Gut and lung neural crest back-grafting

For back-transplantations, small segments of gut or lung containing GFP-positive NCC were removed from E5.5-6.5 chicks and back-transplanted into the vagal (neural tube ablated) region of a second host chick embryo at E1.5.

A chimeric embryo that had received a neural tube from a GFP embryo was harvested at E5.5 and the gut and lungs dissected out. Under a fluorescent dissecting stereomicroscope, small pieces of gut and lung containing a high density of GFP-positive NCC were cut out and placed in DMEM medium. E1.5 wild-type host embryos were prepared for back-grafting by vagal neural tube ablation, described above. A piece of gut or lung from the chimeric donor was placed into the ablated region of the neural tube. The egg was then sealed, returned to the incubator, and allowed to develop further until harvesting at E3.5-8.5.

Based on the protocol from Freem et al. Lack of organ specific pre-specification of vagal neural crest cells as shown by back-transplantation of transgenic GFP chicken tissues.

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