

Changing a bird beak into a Snout

Condensed by Diego Garrido from

Bhullar, Bhart-Anjan S., et al. "A molecular mechanism for the origin of a key evolutionary innovation, the bird beak and palate, revealed by an integrative approach to major transitions in vertebrate history." *Evolution* (2015)

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Equipment:

Fine forceps

Surgical Needles (tungsten needles made by flame-sharpening tungsten wire, mounted into handles)

Pipette

Petridish

Parafilm

Chemicals

Formate-derivatized ion exchange beads AG1-X2

from BioRad

DMSO

SU5402 (A fibroblast growth factor receptor (FGFR) inhibitor)

Phosphate-buffered saline

The chick eggs are incubated at 38 degrees Celsius and 50-80% humidity.

1.) At Embryonic Day 2, 4 mL of albumin was removed using a syringe in order to lower the yolk and make the embryos ready for manipulation.

2.) a.) On HH stage 18, 20 ul of a 1 mM DMSO solution of SU5402 (296.32 ug per mL of DMSO) was put in a droplet of fluid on a piece of Parafilm within a small plastic petri dish. Dried beads were then added to it, and left at room temperature for 3 hours. The beads were then transferred to a small tube containing PBS and rinsed in this way twice.

b.) The surfaces of the eggs were reinforced using packing tape and oval windows ~2 cm in diameter were cut in them with small scissors just prior to manipulation. Fine forceps are used to remove the external membrane from the surfaces of the embryos in the region of manipulation.

c.) The beads were introduced to the eggs using a pipette. Using the surgical needles, superficial slits were cut precisely in the midline of the frontonasal prominence and beads implanted within them such that the beads were held in place in the epithelium and did not fall out or fall inward to the mesenchyme or brain cavity. The needles were sterilized after each operation, and excess beads were lifted out of the egg with using needles and forceps

After operation, eggs were resealed with tape and placed back into the incubator after a resting period of approximately 15 minutes at room temperature.

Of the embryos treated with 1 mM su5402, 13/18 showed phenotypes and 4 of these were strong phenotypes as depicted

