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Gif-sur-Yvette, July, 10th, 2016

Jaydev Upponi

Science Editor

[JoVE](#)

One Alewife Center, Suite 200

Cambridge, MA 02140

Dear Editor,

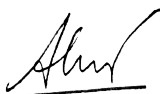
We are sending you herewith the revised version of our manuscript entitled "Combining *in ovo* electroporation and heterospecific grafting to unravel the role of *Six1*, *Six2*, *Six4* genes in cephalic neural crest for head development".

The present article describes how we combine xenograft between avian embryos and *in ovo* electroporation for multiple gene silencing and rescue, to uncover the complementary roles exerted by *Six* genes in the cephalic neural crest cells for vertebrate encephalogenesis.

Here is the 'point by point' list of changes addressed in the present manuscript.

Hoping the article in the present format will be suitable for publication in JoVE.

Sincerely yours,



Dr. Sophie Creuzet

Principal investigator at CNRS

Group leader *Neural Crest: Development & Evolution*

Editorial comments:

• **NOTE: Please download this version of the Microsoft word document (File name: 54180_R3_050316) for any subsequent changes. Please keep in mind that some editorial changes have been made prior to peer review pro**

• Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

• **Formatting:**

- Please include a space between 1.2.1 and 1.2.2.
- Please include spaces between all numbers and units.
- Please use the correct formatting for the materials table, and please include company and catalog number for all items.

• **Grammar:**

- Please copyedit the manuscript for numerous grammatical errors. Such editing should be performed by a native English speaker and is required prior to acceptance.
- Line 56 – “combine xenograft between”
- Line 72 – “on stage- and tissue-specific RNAi strategy”
- Line 74 – “knocked-down”
- Avoid the term “perfectly” to describe these techniques, as this is not an objective assessment.
- 1.3.1 – “made up with glass”
- 1.3.2 – “microscapel”
- 2.1.4 – “so that as the blastoderm”
- 2.2 note, 2.2.1 – “Sequences” aren’t dispersed or transferred, but nucleic acid molecules or nucleic acids are.
- 2.2.5 – “blow” – do you mean “inject”? If mouth pipetting is used, this should be specified.
- 2.2.11 note – What does “it” refer to?
- 2.3.5 – “the heterospecific transfected”
- Line 343 – “well tractable”
- Line 349 – “negative charged”
- Line 350 – “towards cathode”
- The discussion is difficult to understand due to numerous errors.

• **Additional detail is required:**

- Is a stereomicroscope used for any of these procedures? Please specify if so.
- 1.3.1 – Please clarify “under the limit of capillarity.” How is the tip stretched?
- 2.1.3 – How is a hole made in the egg?
- 2.1.5 – Where is the opening made? If this is the opening referred to in 2.1.4, this detail should be found in 2.1.4.
- 2.3.4 – Please clarify “Cut out transversely”.
- 2.3.6 – What is “a flexible plastic pipe”? Are inhaling and blowing done by mouth?

• **Results:** Please include scale bars. There are none visible. Panels M, O & Q should have their own scale bars. What staining is shown in M, O, Q?

• **Discussion:** Please discuss any modifications/troubleshooting that can be performed as well as the future applications of the protocol.

• If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from...” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

• JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

• **IMP:** Please copyedit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

rcgarcez 10/7/16 23:49

Commentaire: included

rcgarcez 10/7/16 23:49

Commentaire: included

rcgarcez 10/7/16 23:49

Commentaire: new section added

rcgarcez 10/7/16 23:49

Commentaire: All text was submitted to a professional reviewer of American English

rcgarcez 10/7/16 23:49

Commentaire: add information (213-214)

rcgarcez 10/7/16 23:49

Commentaire: text changed to become more obvious

rcgarcez 10/7/16 23:49

Commentaire: added

rcgarcez 10/7/16 23:49

Commentaire: text changed to become more obvious

rcgarcez 10/7/16 23:49

Commentaire: text changed to become more obvious

rcgarcez 10/7/16 23:49

Commentaire: yes, the inhaling and blowing done by mouth

rcgarcez 10/7/16 23:49

Commentaire: SOPHIE

rcgarcez 10/7/16 23:49

Commentaire: 2 new paragraphs have been added?

rcgarcez 10/7/16 23:49

Commentaire: SOPHIE, are all originals?

rcgarcez 10/7/16 23:49

Commentaire: Some references are very old and do not have DOI.

•NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

In this paper, authors combine hetero-specific tissue grafting and electroporation in chicken embryo to evaluate the roles of six genes in head development. The idea is well conceived and the technique will be very useful in a variety of experiments using chicken embryo as a model. A clearly and vividly illustrated video procedure will be useful to many who use chicken embryo as an experimental model, and encourage young students to get interested into developmental biology.

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

rcgarcez 10/7/16 23:49

Commentaire: Ok!

Reviewer #2:

Manuscript Summary:

The manuscript well explain the need of cell labeling, and up- and down regulation of the gene of interest, and show the xenografts transplantation between quail and chick embryos, and up- and down-regulation of Six genes in the cephalic neural crest cells. By this method, the authors could show hierarchy among Hoxa2, Noggin and Six genes by single and total silencing Six1, Six2 and Six4 genes, and Noggin genes by their dsRNA. The method is very useful for the researchers who use chick embryos.

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

rcgarcez 10/7/16 23:49

Commentaire: Ok!

Reviewer #3:

Manuscript Summary:

The protocol is nicely described and may have some importance for other labs (although electroporation has been described in JOVE several times previously, but not combined with grafting).

Major Concerns:

-The section of REPRESENTATIVE RESULTS should be modified, as the 2 first paragraphs are not relevant to the described protocol and representative results, and only the last paragraph discusses the representative results. The first paragraphs are more relevant to the Introduction.

-The first parts of the current Introduction are too general and long and and may be edited to become more focused to the relevant published method.

-The Discussion should discuss the prones and cones of the current method as compared to bilateral electroporation of NCCs in an embryo without grafting the tissue to another, as in such a case the manipulated NCCs will also invade a non-electroporated head tissue. Also, in my view a unilateral electroporation will be more informative with the grafting method since the non-manipulated side of the grafted tissue can serve as an internal control for grafting effects versus electroporation effects. This should be also discussed.

Minor Concerns:

1 details regarding the preparation or purchase of the triplet electrode system is needed.

rcgarcez 10/7/16 23:49

Commentaire: Disagree, these paragraphs just present the most relevant results that the group obtained with this technique

rcgarcez 12/7/16 16:49

Commentaire: I find it important to keep the text as it is. It is our writing style. We believe it is important to contextualize historically and experimentally the methodology presented.

Additional Comments to Authors:

N/A