



LOMA LINDA UNIVERSITY

School of Medicine

Dr. Mary Struziak
Editorial Assistant, Developmental Biology Section
Journal of Visualized Experiments
1 Alewife Center, Suite 200
Cambridge, MA 02140

Dear Dr. Stuziak;

Thanks for the invitation to submit our protocol on validating enhancer elements via the chick bioassay approach. We are submitting our original protocol description in the manuscript entitled "A Rapid In Vivo Bioassay for Developmentally Active Enhancers" for consideration of publication in JoVE.

JoVE has published a number of protocols on *in ovo* electroporation, however, our approach assays potential regulatory sequences at various stages of development and includes a method to transfect solids organs and mesodermal tissues with technical tips to enhance targeted delivery. We believe our manuscript describing this approach to assay and validate potential regulatory sequences that function during development will be an important addition to the journals repertoire of protocols and useful for investigators using genomic tools to clarify developmental networks and pathways.

The following authors have participated in developing this protocol: **Charmaine Pira** has performed numerous experiments using this technique. **Charmaine Pira** and **Kerby Oberg** have refined and optimized the protocol. **Shelley Caltharp, Charmaine Pira** and **Kerby Oberg** originally conceived of the approach. Shelley Caltharp performed the conservation analysis on *RTN4/NOGO* locus and generated the associated reporter constructs. **Endika Haro** performed the Lmx1b ChIP-seq analysis. **Charmaine Pira, Endika Haro** and **Shelley Caltharp** generated the PRS and mPRS *ptk*-EGFP constructs.

Suggested reviewers listed below:

- 1) Marian Ros – Universidad de Cantabria – email: marian.ros@unican.es
- 2) Cathy Krull – University of Michigan – email: krullc@umich.edu
- 2) Susan Mackem – National Cancer Research Institute – email: mackemS@mail.nih.gov
- 4) Randy Johnson - UT MD Anderson Cancer center – email: rljohnson@mdanderson.org
- 5) John Fallon - University of Wisconsin – email: jffallon@wisc.edu
- 6) Marybeth Ezaki - University of Texas Southwestern – email: Marybeth.Ezaki@tsrh.org

All authors have reviewed the manuscript. We have prepared the protocol, list of reagents and equipment, and manuscript as instructed in the authors guide and look forward to your review and comments.

Sincerely,

Kerby C. Oberg, MD, PhD
Professor

Department of Pathology and Human Anatomy,
Divisions of Human Anatomy and Pediatric Pathology

A Seventh-day Adventist Institution

DEPARTMENT OF PATHOLOGY AND HUMAN ANATOMY

DIVISION OF HUMAN ANATOMY | 24760 Stewart Street, Loma Linda, California 92350

(909) 558-7602 · fax (909) 558-7950 · www.llu.edu