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siRNA Immunological Fishing Training (SIFT) Experience as a Novel Research Education Tool for Students Studying Immunology †

Diana M. Elizondo, Temesgen E. Andargie, Dineeta S. Kubhar, Karis M. Marshall, Aamir M. Zariwala, Clarence M. Lee, and Michael W. Lipscomb*
Department of Biology, Howard University, Washington, DC 20059

INTRODUCTION

The siRNA Immunological Fishing Training (SIFT) experience was created in response to: (i) the Vision and Change initiative (1) and (ii) works by Kuldell et al. on RNA interference (RNAi) methods employed in undergraduate student research (2). The SIFT research education experience is largely built on the immunological genome project (3) and knockdown strategies as a means to investigate novel genes in immune cells. The chief goal of SIFT is to provide students with a training experience that strongly exposes them to meritorious studies in immunology.

In our approach, the students' independent research project is centered on characterization of novel genes uniquely and/or strongly expressed in dendritic cells (DC); notably, similar approaches can evaluate other immune cell subsets (i.e., T cells, B cells, or macrophages) using methods described in this report. Using the immunological genome project database (3–5), students work with the instructor(s) to *sift* through expression profile datasets and identify a novel gene for mentored-independent investigations. After candidate genes are identified, students then use RNA interference (RNAi)-mediated approaches to knockdown their chosen gene and characterize the underpinning role. The innovation lies largely in providing students with the ability to search wide-screen expression profiles in immune cells and concomitantly investigate functional outcomes experimentally through RNAi-mediated approaches, thereby bridging bioinformatics studies with wet-lab experimentations. The activities provide students exposure to modern investigative approaches and an exciting means to engage in research education, as well as reinforcing concepts learned in the classroom. Success of the SIFT experience has been realized with a recent peer-review publication (6).

*Corresponding author. Mailing address: 415 College St. NW, Department of Biology, Howard University, Washington, DC 20059. Phone: 202-806-7939. E-mail: michael.lipscomb@howard.edu.
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PROCEDURE

We have divided the SIFT experience into six major sections (Appendix 1). A flow chart is available in Appendix 2 and an instructional guide in Appendix 3, which also contains: (i) suggested experimental approaches (along with coursework integration), (ii) a timeline, (iii) biosafety guidelines, (iv) anticipated costs, (v) examples of student-generated datasets, (vi) materials and methods, and (vii) references.

In Section 1, the instructors work hands-on with the students to explore the immunological genome project's database (www.immgen.org). This microarray databank, provided as a public resource, was created as a large-scale effort to provide a repository of gene expression profiles in immune cells (3). Students can explore gene expression profiles and regulatory networks in various immune cell subsets (4, 5). This exposes students to the breadth of bioinformatics and provides an avenue for them to use their learned knowledge of cell biology to evaluate functional profiles. Under the instructor's guidance, each student identifies one or two novel genes highly expressed in DC and that have not been well characterized in the literature.

In Section 2, students learn to isolate bone marrow cells (from mice) to generate DC *in vitro*; alternative approaches can be used to isolate or generate other immune cells. All research described should be approved by IACUC and performed in BSL2 conditions following the ASM guidelines for biosafety in teaching laboratories. After successful *in vitro* preparation of DC, students next perform RNA isolation, cDNA preparation, and PCR analyses to confirm mRNA expression of their specific gene. This exposes and trains students in the use of NCBI Primer-BLAST to design DNA primers and perform gel electrophoresis. After confirmation of expression, students transition into Section 3, where they generate lysates and perform western blots to assess protein expression (of their candidate gene).

For Section 4, students are trained to design target specific siRNA oligonucleotides using online design tools (i.e., www.sirnawizard.com). Importantly, students are also taught the science behind RNAi and other methods of knockdown used for research studies. Next, they treat the DC with siRNA and measure successful knockdown (by western blot and flow cytometry). For Section 5, students

investigate altered fates of DC upon gene-specific knockdown. Immunophenotypic analyses include: a) measuring changes in maturation/differentiation markers (by flow cytometric analyses) and cytokine profiles (by enzyme-linked immunosorbant assays). Students learn to correctly use readout indices to qualify and quantify the presence of target molecules. They begin to understand differential expression patterns and relate altered profiles to immune governing responses.

Finally, T cell responses are evaluated in Section 6. These studies utilize naive CD4+ T cells isolated from OT-II transgenic mice. Studies employ knockdown vs. control DC to evaluate cognate T cell responses, which include: (i) early activation (i.e., CD69, CD25, CD62L changes); (ii) proliferation (i.e., CFSE dilution); and (iii) T helper-associated cytokine profiles. In conclusion, students have an exciting opportunity to well characterize the role of their candidate gene and measure potential alterations in governing of immune responses.

CONCLUSION

The promise of the SIFT experience as an educational tool for preparing students for careers in biological research enterprise is vibrant. The authors have found that being able to take a project from inception-to-completion serves to expand and reinforce students' interests in the sciences by providing a sense of ownership, motivation, and continuity.

The approach also provides opportunities for laboratories to screen candidate genes of interest while concomitantly exposing young trainees to the wealth that science has to offer. With the breadth of transcriptomics datasets, SIFT can be applied to other biological disciplines (i.e., neurobiology, cancer biology, and plant sciences). In conclusion, we highly recommend educators employ the elements described in this report as an innovative tool to excite, expose, engage, and train students in immunology.

SUPPLEMENTAL MATERIALS

Appendix 1: Table illustrating measurable objectives
Appendix 2: Flowchart of the SIFT experience
Appendix 3: Instructional guide

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