Dr Renaud Vincentelli

AFMB UMR 7257

Campus de Luminy

Case 932

163, avenue de Luminy

13288 MARSEILLE Cedex 09 France

renaud.vincentelli@afmb.univ-mrs.fr

Marseille, 29th of July 2013.

Dear JoVE editors,

Please find enclosed a manuscript entitled “High throughput cloning, quantitative expression screening and analytical affinity purification of disulfide-rich proteins and peptides in *E. coli*”, as previously requested by JoVE editor Nandita Singh. The protocol describes a redox-active fusion strategy for the expression of disulfide-bonded targets at the small scale to identify constructs yielding soluble protein after affinity purification (and optional cleavage). The expression of disulfide-bonded proteins and peptides in *E. coli* is still considered challenging, and we hope that with these protocols, the expression of such proteins will become less daunting and more accessible to a wider range of researchers. We currently use the protocols herein for the expression of disulfide-rich venom peptides as part of the FP7 European VENOMICS Project and representative results are provided for targets from this project.

We believe that this work fits very well with the video format of JoVE. The high throughput protocols described in this article utilize robotics and the video will better demonstrate the simplicity and eloquence of the techniques than several pages of text explaining the various steps and details of the procedures.

Of the four authors contributing to this manuscript, all have been involved in the previous experimental work allowing the development and compilation of the protocols herein. HN and MB cloned the destination plasmids; NS and RV designed and supervised the high throughput experiments; NS, HN, MB and RV have performed the high throughput recombinant expression screening experiments; HN and MB have performed scale-up validation of the expression and purification results at small-scale. NS and HN have performed mass spectroscopy experiments; All authors have analyzed data and contributed to the writing of the manuscript. The final manuscript (and the accompanying video to be produced by JoVE) has been approved by all authors, as well as the associated VENOMICS Consortium.

We suggest the following people for the peer review of this article:

|  |  |
| --- | --- |
| Imre Berger  [iberger@embl.fr](mailto:iberger@embl.fr) | European Molecular Biology Laboratory, France |
| Joop Van den Heuvel  Joop.VandenHeuvel@helmholtz-hzi.de | Helmholtz-Zentrum für Infektionsforschung, Germany |
| Ray Owens  ray@strubi.ox.ac.uk | Oxford Protein Production Facility, UK |
| Glenn F. King  [glenn.king@imb.uq.edu.au](mailto:glenn.king@imb.uq.edu.au) | Institute for Molecular Bioscience, Australia |
| Bill Gillette  gillettew@mail.nih.gov | Frederick National Laboratory for Cancer Research, USA |
| Jean-Denis Pedelacq  [Jean-Denis.Pedelacq@ipbs.fr](mailto:Jean-Denis.Pedelacq@ipbs.fr) | Institute of Pharmacology and Structural Biology, France |

Thank you for taking the time to consider our submission.

Sincerely,

Natalie J. Saez and Renaud Vincentelli