To: Philip Steindel, PhD

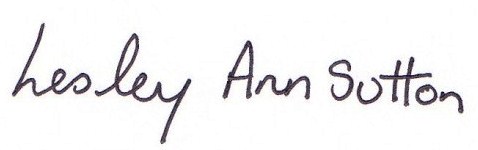
Review Editor, JoVE Publications

Stockholm, 14th May 2018

Dear Dr. Steindel,

We thank you for your favorable appraisal of our manuscript (JoVE57787) entitled “AN ERIC PRACTICAL GUIDE FOR IMMUNOGLOBULIN GENE SEQUENCE ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA: FROM PATIENT MATERIAL TO SEQUENCE INTERPRETATION”. We are resubmitting with the requested formatting modifications including our responses to the editorial comments.

Sincerely,



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**Editorial comments:**

**1.** I have edited this to Jove format (imperative tense with notes; consolidating some shorter steps and splitting up some longer ones); however, there are still issues with the selections for filming. For the protocol section of the video (what the highlighting is for), we will only be able to film concrete steps as done in the lab (e.g., most of section 2) or use of software with a graphical user interface (GUI; e.g., most of 6). Vague steps like choosing primers or deciding on a substrate will not be able to be filmed (implications of these choices can be discussed in the results section of the video; no highlighting will be necessary in that section of the manuscript, though).

**Reply:** The sections that are to be included in the film are highlighted in yellow in the revised version and, in brief, cover the following process:

Section 1: Material requirements, collection and storage.

Section 2: Ficoll gradient separation

Section 3: Nucleic acid extraction

Section 4: Amplification and sequencing of IGHV-IGHD-IGHJ rearrangements - preparation of the mastermix and the gel, clonal assessment and purification of the PCR product(s)

Section 6: Sequence analysis

2. There are still necessary details missing for the particular protocols you intend to show; see below.

1. [4.3.2.1](http://4.3.2.1): How are primers fluorescently labeled (which fluorophores, where on the molecule, etc.)?

**Reply:** The following text ‘Custom 5´- labeled primers are fluorescently labeled oligos with a choice of dyes on the 5’ end. Examples of reporter dyes include 6-FAM, HEX, NED or TET.’ has been added to the appropriate section at lines 282-283 (highlighted in green).

2. 4.3.3: How long to run the gel and what settings?

**Reply:** The following text ‘It is recommended that PCR products are allowed to migrate on the gel for sufficient time so that monoclonal samples can be separated from the polyclonal background and the DNA size marker can separate adequately. Factors such as the size and thickness of the gel can affect migration so no single setting (voltage and time) can guarantee an optimal result. That said, setting the voltage at 80V for 45 minutes is a good starting point as it minimizes the risk of the sample migrating too quickly and ‘running’ off the gel.’ has been added to the appropriate section at lines 287-293 (highlighted in green).

3. 4.3.4: How should the gel be imaged?

**Reply:** The following text ‘An intercalating agent such as Ethidium bromide (EtBr) or less hazardous and more sensitive commercially available DNA stains can be used for the visualization of DNA on acrylamide gels using UV excitation or blue-light.’ has been added to the appropriate section at lines 298-300 (highlighted in green).

4. 4.4.4: This is not the protocol I found for ExoSAP-IT (2 μL of a single reagent per 5 μL product; see <https://www.thermofisher.com/order/catalog/product/78201.1.ML>); also, 0.05 μL is a very small amount to reliably add.

**Reply:** At our center we follow the guidelines provided and optimized by the genome center where sequencing is performed. Admittedly, 0,05 ul is a very small volume however we prepare a mastermix of the reagents and from this mix dispense to all reactions. Nevertheless, to avoid confusion for the reader we have modified the text (lines 317-320) to read as follows: **‘**To perform Sap/Exo clean-up, follow the manufacturer’s instructions regarding the specific volumes to use, however a typical reaction may contain 1 μl Sap, 0. 5 μl Exo and 2 μl 5x incubation buffer per 5-10 μl PCR reaction. Mix by gently vortexing each sample and incubate on a PCR block at 37oC for 30 minutes. Inactivate the enzymes by heating to 85oC for 15 minutes.’

5. 5.2.9 (formerly 5.3.16): What exactly is supposed to be repeated here? The original 5.3.11 and 5.3.12 instruct the user to centrifuge and discard supernatant, but there doesn’t seem to be any supernatant to discard at this point (maybe some residual).

**Reply:** The relevant sentences (lines 367-371) have been clarified and read as below:

5.2.7. Add 100 μl of 70% ethanol (-20oC) and gently mix.

5.2.8. Centrifuge at 14,000 rpm for 10 minutes (4oC). Discard the supernatant.

5.2.9. Repeat steps 5.2.7 and 5.2.8.

6. 5.2.11: What is the ‘sequencing plate’? How is the sequencing done (e.g., sent off elsewhere; an in-house machine, etc.)?

**Reply:** The relevant section (lines 376-380) has been expanded and now reads as follows: Transfer the sample to a sequencing plate, cover with strip caps or tape seals, load to the machine and perform Sanger sequencing. The sequencing plate is usually a 96-well, v-bottom, full-skirt PCR plate compatible with the sequencer. The plates may be barcoded depending on whether the sequencing is to be performed in-house, at a commercial company or at an academic sequencing center/platform.