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There are no mistakes, only happy accidents: Deviation from phage amplification protocol yielded high titers of the novel Actinobacteriophage OldNelly (EA1)

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As part of the 2023-2024 SEA-PHAGES program, freshmen students from Drexel University used the host *Microbacterium foliorum* NRRL B-24224 to isolate 34 novel bacteriophages. Among these, one phage was generated in a slightly unconventional manner. A soil sample was collected from a garden along a nearby Drexel University building in Philadelphia, PA. The subsequent isolated phage was named OldNelly. During amplification, an unintentional deviation was made from the standard procedure while trying to increase the overall lysate volume. Instead of properly flooding a webbed plate with phage buffer, the plate was flooded with a previously collected lysate of the same phage - a technique resembling “lysate double-dipping”. As a result of this protocol deviation, the resulting augmented lysate yielded almost a four-fold increase in titer compared to the original lysate, and recovered a high DNA concentration with minimal contamination ratios. Having demonstrated positive results from the flooding with lysate protocol, it was replicated by other lab groups whose lysates were below the required titer concentration of 5x10^9 pfu/mL to proceed. As expected, they also were able to increase their titer concentration considerably, past the minimum titer threshold for archiving and DNA extraction. Subsequently, OldNelly and 5 other phages (following standard protocol) were sent to be sequenced at The Pittsburgh Bacteriophage Institute using Illumina Sequencing: OldNelly (subcluster EA1), Pharpay (cluster EF), PHISB (cluster EB), Phiderman (subcluster EA1), SoilGremlin (subcluster EA1), and Delphidian (subcluster EA1). The latter 2 were submitted to the genome exchange and have been adopted to be annotated by 2 other institutions. There appeared to be no irregularities with generating a genome or during bioanalysis, which showed that OldNelly is a lytic phage, with 99% similarity to other archived EA1 subcluster phages. Collaboratively, the several sections of our cohort worked to produce and ensure accurate genome annotation. While the technique did not appear to introduce contamination in the case of OldNelly, further extensive studies can be performed to be certain whether phage purity is being affected. We propose that this technique can be widely adopted to significantly help other students in the SEA-PHAGES community, as well as further expand the existing phage archive. Additionally, students have begun planning independent projects which may focus on OldNelly. Examples of possible studies include to test UV (ultra-violet) coupled with temperature deviation or dye exposure (with or without UV), exposures to Zinc and Iron, nicotine, and EDTA. These will lend greater insight into its and other phages’ behavior and morphology.