*Note: Vigorous shaking and aeration are necessary to reach OD600 5-5.5. Measure OD600 over time and calculate doubling time to project endpoint. If doubling takes 40 minutes this will be ~5 h 20m, but growth will slow toward the end. If culture begins to plateau in stationary phase, begin arabinose induction. Cell lysate will function, but at potentially lower ligation efficiency. Obtaining OD600 5-5.5 before induction is ideal.*

9) Induce by adding 0.2% L-(+)-arabinose to the culture. Continue shaking at 37 C at ~338 rpm for 2 hours to express λ prophage protein Red. Measure final OD600.

10) Transfer the remaining ~48 mL of culture into two 50 mL centrifuge tubes. Pellet by centrifugation at 5,000 x g for 20 minutes at 4 C. Decant/aspirate the supernatant.

11) Wash each pellet with 50 mL dd$H_2O$. Pellet by centrifugation at 5,000 x g for 20 minutes at 4 C. Decant/aspirate the supernatant. Measure the wet weight of each pellet.

12) Resuspend each cell pellet (~0.25 g each) in 300 μL CelLytic™ B Cell Lysis Reagent (Sigma, B7435). Briefly vortex and spin down. Transfer the resuspended cells into a low-binding 1.5 mL tube, and incubate at room temperature for 10 minutes to allow lysis to occur.

13) Centrifuge cell lysates at 20,000 x g for 2 minutes at room temperature to pellet insoluble cell debris. Remove the supernatants from the cell debris into a single 1.5 mL tube, being careful not to disturb cell debris.

14) Mix the cell extract with equal volume of 100% glycerol. Dispense into 40-60 μL aliquots in low-binding 0.5 mL tubes. Label appropriately as PPY SLiCE extract.

15) Store the PPY SLiCE extract at -20 C for <2 months, or at -80 C for long term storage. Aliquots can be thawed on wet ice and refrozen up to 10 times without significant loss of activity.

**Maintaining the PPY Strain**

1) Inoculate 1 single colony of the PPY strain from an LB agar plate (10 μg/mL streptomycin and 12.5 μg/mL chloramphenicol) into 5 mL LB (10 μg/mL streptomycin and 12.5 μg/mL chloramphenicol) and incubate at 37 C at 200-338 rpm overnight.

2) In a sterile tube add equal volumes of PPY culture and 20% autoclaved glycerol. Mix and store at -80 C.