PPY SLiCE extract preparation protocol (adapted from Zhang 2012):

Preparation of SLiCE Extract

1) Acquire CelLytic™ B Cell Lysis Reagent (Sigma, B7435).

2) Prepare sufficient chloramphenicol stock and streptomycin stock to produce LB agar plates (10 μg/mL streptomycin and 12.5 μg/mL chloramphenicol), 1 L of 2X YT media (10 μg/mL streptomycin), and LB (10 μg/mL streptomycin and 12.5 μg/mL chloramphenicol).

3) Prepare sufficient L-(+)-arabinose (Sigma, A3256) to induce at 0.2% (weight/volume) in 50 mL.

4) Prepare 1 L of 2X YT media as follows:

	16	g	Bacto-tryptone
+	10	g	Bacto-yeast extract
+	5	g	NaCl
+			base to pH 7.2 (1 M NaOH or 1 M KOH)
+	_____		ddH_20 to 1 L
	1	L	

Autoclave to sterilize. Once cooled, under sterile conditions add streptomycin to 10 μg/mL. Store at 4 C.

5) Streak PPY glycerol stock or fresh culture on an LB agar plate (10 μg/mL streptomycin and 12.5 μg/mL chloramphenicol). Incubate at 37 C overnight.

6) Inoculate 1 colony into a 50 mL tube containing 25 mL 2X YT with 10 μg/mL streptomycin. Incubate at 37 C with heavy shaking (~338 rpm) overnight.

7) Measure OD600 of the overnight culture.

Note on OD600: Use extra media as blank. Ensure culture is evenly mixed when taking OD600 samples. Take out OD600 samples from cultures under aseptic conditions (flame, positive airflow cabinet) Measure OD600 within the photometric linear range for your spectrophotometer (~0.1-1.0, variable by machine). Dilute sample ~1/10 (with media), measure OD600, and calculate OD600 of culture.

8) Using a sterile 250 mL or greater baffled flask, dilute the overnight culture to 0.03 OD600 using 2X YT media with 10 μg/mL streptomycin. Shake at 37 C at ~338 rpm until culture reaches OD600 of 5-5.5.