Preparing Plastic:

1. Hole punch circles of plastic to use in degradation assay. Can use whatever hole punch you have around.

2. Wash plastic in 1% SDS

3. Wash plastic in 20% ethanol

4. Wash plastic in dI H20.

5. Dry plastic overnight for multiple days or under a vacuum for one night. Do not heat to dry as this will change the properties of the plastic.

Plastic Degradation Assay:

1. Take a washed plastic disc and weigh it on a microbalance three times. Mass is the average of all three readings.

2. Take pH 8.0 bicine buffer (volume can be whatever you choose but want enough to cover the plastic thoroughly) and add enzyme so that the final concentration of the solution is 200nM. We've been doing this step in culture tubes.

3. Incubate for 3 days at the enzyme's optimum temperature (DuraPETase's I believe is 40C).

4. After 3 days, take your plastic disc out of solution (save the supernatant!) and repeat the Preparing Plastic protocol listed above.

5. Weigh dried plastic in triplicate and average those numbers for the final mass.

6. Take supernatant and filter through 0.2 micron syringe filters.

7. Test supernatant via HPLC.