Assaying the cultures:

1. Pipet 5 ml from 2 separate flasks into two 15 ml conicals (do in hood)
2. Spin in megafuge 425g for 2' (stop brake when rpm is around 100)
3. Aspirate supe, resuspend worms in 15 ml M9 and repellet
4. Aspirate M9 and using cutoff tip pipet worm slurry onto a glass slide and look at the worms

Initiating the cultures:

It is important to have clean worms for biochemistry so I usually first axenize (i.e., bleach) them. To do this, mix 5 µl of 2M NaOH and 5 µl of Bleach on the edge of a small seeded plate. Pick 10 adult hermaphrodites into the pool of liquid. Place the plates at 24.5C for 3 days - this will give you adults to seed large plates.

Growing worms in liquid culture:

Day 1

Seed large plates with 15 cleaned N2s you will need 3 plates per 500 ml of liquid culture-put the plates at 20C (4 days),[if at 16C (5 days) if at 24.5 (3 days)}. I would initially start 1-1.5 liters of culture. Streak out some OP50-1 onto a LB plate with streptomycin.

Day 2

Take out the OP50-1 plate and store in the cold room.

Day 3

Start a 100 ml overnight of OP50-1 in LB

Day 4

Start overnight cultures of OP50-1 in LB. You need 1 liter of culture per 500 ml of C. elegans culture.

Day 5

Spin down bacteria in sterile 1 liter bottles. Resuspend the bacteria from each liter of culture in 500 ml of S complete medium (see recipe sheet) and pour into a sterile 2 liter glass flask.
Rinse 3 plates of N2 sequentially with 10 ml of sterile M9. Chase with an additional 5 ml of M9. Collect in a 15 ml falcon tube. Spin worms in the clinical centrifuge for 2 minutes at 1000 rpm to pellet. Resuspend the worms in 10 ml of M9 buffer and inoculate this into the flask containing the resuspended bacteria. Shake the cultures at 20°C at 230 rpm.

Days 6-8

Follow the growth of the cultures carefully and harvest when desired. The cultures will take about 3-3.5 days depending on the age of the worms on the plates you inoculate with-and the desired state of the final culture.
To harvest, we pour the cultures into 500 ml cylinders and let the worms settle for 4 hours to overnight in the cold room.

Harvesting Large Scale Cultures & Embryo Isolation:

To harvest 6x500 ml liquid culture:

1. Sterilize 3x1L bottles by washing with 70% ethanol and allowing them to dry in the laminar hood.
2. Transfer cultures into 1L bottles in the hood.
3. Pellet worms at 200g in 8.1000 rotor for 5' with brake on SLOW
4. Aspirate using a cutoff sterile 5 ml pipet and transfer slurry to 6-8 50 ml conicals (try to aim for 2 conicals per liter culture) (NOTE: Alternative is to settle embryos in 0.5L cylinders in coldroom for 4 hours)
5. Pellet in megafuge at 150g for 3'; stop brake when ~100 rpm
6. Aspirate supe, collate into 2 conicals and wash 2X with ice-cold M9 as above.

7. Bring loose worm pellet in each conical up to 20 ml with ice-cold M9 & store on ice; make 4 x 50 ml conicals, each containing ~30 ml cold M9 and put these on ice before starting bleaching process
8. Add bleach stock to the water/NaOH mixture to make 2X Bleach Solution (10 ml 4M NaOH; 5 ml water; 25 ml bleach stock); mix by inversion and pour into tube with worms until liquid level in conical is 40-45 ml; START TIMER!!
9. Vigorously mix bleaching solution with worms by shaking and vortexing tube horizontally while moving it from left to right; at ~2' 30"-3' after bleach addition pour into 50 ml syringe with 25g needle and push through; repeat passage through syringe 2X more (total of 3 passes)
10. Vortex some more and assay quickly under dissection scope using a cutoff tip; at the latest by 7' after bleach addition split the mixture into two 50 ml conicals on ice containing ~30 ml M9; mix by inversion
11. Rapidly collect embryos by centrifugation at 425g for 3' in megafuge; stop brake when ~100 rpm
12. Aspirate bleach and wash embryos with ice-cold M9 for a total of 4X; after first wash pool embryos into 1 conical.

Repeat steps 8-12 for the second tube of worms that was stored on ice

13. For extract preparation, wash embryos 1X with 50 ml H100, transfer into 15 ml conical and repellet embryos before resuspending in lysis buffer (see later for details)
14. For hatching starved L1s, put embryos derived from 750 ml -1L original culture volume onto 5 large unseeded NGM plates; resuspend this amount of embryos in ~25 ml M9 and put ~ 5 ml per plate; incubate ON on rocker at RT - next day you should see a mass of starved L1s

Seeding New Cultures Using Starved L1s:

2 Days Before
Start 50 ml ON of OP50-1 in LB + 50 µg/ml streptomycin
Day Before
Start 6 x 1L cultures of OP50-1 in LB + 50 µg/ml streptomycin
Day of

1. Sterilize 6 1L centrifuge bottles by washing with 70% ethanol and putting in laminar flow hood.
2. Harvest bacterial cultures at 4000 rpm for 10' with MAX brake in 8.1000
3. Remove supe as thoroughly as possible and put bottle in hood
4. Make Complete S-basal in hood by adding trace metals, potassium citrate and divalent cation stocks to S-basal bottles
5. Resuspend bacterial pellets in Complete S-basal; best way to do this is add some S-basal and scrape pellet with 25 ml pipet; then pipet up and down and transfer chunks into sterile 2.8L fernbachs; there is no need to make a homogenous suspension as shaker agitation will resuspend bugs thoroughly
6. After flasks with bacterial food are ready, start processing L1s. In the hood, wash L1s off plate and collect in 50 ml conical; fill with sterile M9
7. Wash 3 x 50 ml sterile M9 (425 g 3'; stop brake when rpm ~100)--. This removes dauer pheremone.
8. Transfer to 15 ml conical and pellet; estimate volume of pellet using a separate conical, adding known amounts of water and comparing to pellet
9. Resuspend to total volume of 12 ml using sterile M9 and look at sample under dissection scope; estimate %L1s and %other crap (dead embryos/worm parts/clumps)
10. Seed each flask with equivalent of 50 µl pure L1 pellet (e.g. if pellet volume is 0.6 ml and % L1 in the resuspension is 70% then seed with 1.4 ml of the resuspended pellet) Try to avoid overseeding or cultures will starve!
11. Put flasks at 20°C at 230 rpm; best time to harvest will be ~55-60 h later although cultures should be monitored as described above; if timing is inconvenient then shift to 16°C ~40-45 hours after starting culture and this will extend harvest time to ~65-70h.

Extract Preparation:

1. Wash embryos 1X with 50 ml H100, transfer into tared 15 ml conical and repellet
2. Weigh embryos and add equal volume of Lysis Buffer and resuspend
3. Setup sonication ice/water bath and sonicate 30% amplitude for 3' total (15 s on; 45s off - after each 1' wait ~2' to chill)40% amplitude for 30s (15s on; 45s off) Save a CRUDE sample
4. Transfer crude extract to TLA100.3 tube and spin at 20,000rpm (16,500xg) for 10' at 2°C, DECEL = 5 (5000rpm to stop in 3') Save a LSS sample
5. Remove supe and respin at 50,000rpm (135,000xg) for 20' at 2°C Save a HSS sample
6. Collect supe into a tube on ice (HSS; sometimes this might need respinning if too murky)
7. Use HSS for IPs; for column runs desalt 2X over spin columns into column buffer.

Solutions needed

Lysis Buffer

(H100 + 10% glycerol)
50 mM HEPES, pH 7.4
1 mM EGTA
1 mM MgCl2
100 mM KCl
10% glycerol
0.05% NP-40

Just prior to use, to 5 ml lysis buffer, add 1 tablet mini EDTA-free Complete PI tablet

S basal (before use, add what is described in the recepie below)

1 Liter

5.9 g NaCl
50 ml of 1M potassium phosphate pH 6.0
1 ml of 5 mg/ml cholesterol (in EtOH)

2 Liter

11.8 g NaCl
100 ml 1M KPO4 pH 6.0

4 Liter

23.6 g NaCl
200 ml 1M KPO4 pH 6

Split into 12 x 500ml bottles; to each bottle add 0.5 ml 5 mg/ml cholesterol (in EtOH; should form a light cloudy precipitate)

Autoclave and store at RT

Complete S Basal

To each 500 ml bottle add:

5 ml 1M Potassium citrate, pH 6.0
5 ml Trace Metals solution
1.5 ml 1M MgCl2
1.5 ml 1M CaCl2

Trace Metals Solution

Disodium EDTA 1.86 g (5 mM)
FeSO4 7H2O 0.69 g (2.5 mM)
MnCl2 4H2O 0.20 g (1 mM)
ZnSO4 7H2O 0.29 g (1 mM)
CuSO4 5H2O 0.025g (0.1 mM)

Dissolve in 1L water; aliquot into 50 ml conicals and store in dark.

1M Potassium Citrate, pH 6.0

268.8 g tripotassium citrate
26.3 g citric acid monohydrate
water to 900 ml
Adjust pH to 6.0 using 10N KOH and bring up to 1L
Autoclave and store at RT

X Bleach Solution

10 ml 4M NaOH
5 ml water
25 ml Sigma bleach (add this just before starting bleaching of worms)