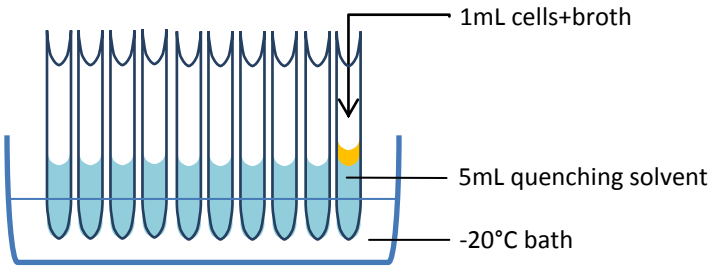
Yeast extraction protocol. West Coast Metabolomics Center, Fiehn Laboratory, UC Davis. August 2013.

Quenching Solvent

Treatment A: 3:2 methanol : water at - 20°C (1:3 sodium chloride:ice)

Extraction Solvent

Solvent 1: 3:3:2 acetonitrile : isopropanol : water at -20°C



Quenching

1. Add 5mL of quenching solvent to each 15mL tube, and let sit for at least 30 minutes in ice bath to get to temperature.
2. Add 1mL of cell culture (OD600=1.5) to tube, cap, and gently invert several times.
3. Centrifuge 1,000xg for 5 minutes.
4. Remove supernatant, and store supernatant and cell pellet in -80C until further use.

Extraction

1. Add 0.5mL of extraction solvent to tube, gently pipet to remove all cells, transfer cells to 2mL eppendorf tube. Repeat for a total of 1mL extraction solvent + cells in 2mL eppendorf tube.
2. Add 2 small stainless steel grinding beads to eppendorf tube
3. Use the GenoGrinder to grind for 3 minutes at 1,250 rpm.
4. Centrifuge at 14,000xg for 5 minutes.
5. Transfer supernatant to a fresh 2mL eppendorf tube.
6. Add 1mL of extraction solvent to tube containing cell pellet + beads, and repeat steps 3 and 4.
7. Collect supernatant, and combine with supernatant collected in step 5. Total volume of extracted sample will be approximately 2mL.
8. Dry down 50uL of extracted sample in 1.5mL eppendorf tube for GC-TOF analysis.
9. Store backups in -20 or -80C.