**Sample weighing cheat sheet**

* Weigh the following weights into the vials, +/- 0.1mg
  + Fish standard: 3
  + Coral host: 8
  + Symbiont: 4

**Acid Hydrolysis Cheat sheet**

* Set acid hydrolysis oven to 110˚C and put aluminum blocks in
* Set up reactivap with one needle
* Label glassware for 6N HCl
* Add 0.5mL – 1mL 6N HCl (enough to cover sample fully)
* Flush tubes with N2
* Tighten caps
* Put in oven, check caps after 5 mins
* *20-24 hour incubation*
* Set up reactivap with enough needles and turn to 80˚c
* Cool vials in freezer
* Optional step: filter samples into new tubes (can’t be hot!!)
* Blow-down reagent until samples are dry about 30 mins
* Store in freezer

**AA Derivatization Protocol Notes**

1. Prep

* Thaw standard + Norleu
* Check gas
* Prep glassware for DCM, TFAA
* Make vials for standards
* Turn on reactivap for 60-80˚C & set up enough needles
* Turn on heating block to 110˚C

1. Prep standard & reagents

* Add 200uL of standard to the AA standard vial
* Add 15uL of Norleu to all samples EXCEPT the AA vial

|  |  |  |
| --- | --- | --- |
| N samples | Iso | Ace |
| 8 | 7.5 | 1.5 |
| 16 | 15 | 3 |
| 14 | 12.5 | 2.5 |

* Evaporate under N2
* Make acidified isopropanol (5:1 Iso:Ace, 1ml/sample)
  + Get isopropanol + acetyl chloride
  + Combine (iso first) + PUT IN FREEZER to cool

1. Esterification

* Add 1mL of acidified isopropanol in each sample
* Add a N2 environment
* 110˚C for 1 hr
* Check caps after 5 mins
* When done, cool in freezer
* Turn heating block down to 100˚C

|  |  |
| --- | --- |
| N samples | Total each of DCM and TFAA |
| 8 | 5 mL |
| 16 | 10 mL |

* Blow down samples @ 60˚C for ~20 mins

1. Acylation

* Prep TFAA & DCM
* Add 500ul DCM to each sample
* Add 500uL TFAA to each sample
* Add N2 environment
* Heat at 100˚C for 15 min
* Check caps after 5 mins
* Move to freezer

1. Clean up

* Evaporate extra chemicals
* Turn off N2
* Clean glassware & hood

**P-buffer cheat sheet**

* Prep beakers for p-buffer & chloroform
* Prep 2x new vials for every sample
* Blow off DCM/TFAA at ROOM TEMP!!
* Add 2mL p-buffer & 1mL chloroform to each sample vial
* Vortex
* Centrifuge 5 min, 600 rcf
* Transfer bottom layer to new vial
* Add 1ml chloroform to first vial
* Vortex
* Centrifuge 5 min 600 rcf
* Transfer bottom layer to new vial
* Freeze samples for at least 30 mins to freeze p-buffer
* Transfer liquid portion to new vials
* Evaporate samples AT ROOM TEMP
* Repeat acylation step:
* Prep TFAA & DCM
* Add 500ul DCM to each sample
* Add 500uL TFAA to each sample
* Add N2 environment
* Heat at 100˚C for 15 min
* Check caps after 5 mins
* Move to freezer

**Taking cuts for your run**

* Only do this once ready to run through the GC
* Prep tiny vials
* Prep reactivap
* Take a cut (250ul) of all the samples into the tiny vials
* Blow down tiny vial samples AT ROOM TEMP
* Add Ethyl acetate to each tiny vial
  + Coral/sym samples: 40ul
  + AA standard: 50ul
  + Fish standard: 60ul
* Use the ethyl acetate to pick up sample and transfer into teeny tiny vial
* Cap & run!