# High-throughput phospholipid extraction from soil protocol

**NOTE: this is a work-in-progress**

* Based on Buyer and Sasser 2012. Applied Soil Ecology
  + [link](http://www.midi-inc.com/pdf/Rapid_PLFA_Extraction.pdf)

## Equipment needed

* sonication bath
* tube rotator
* speed-vac
  + swinging bucket cfg-evaporator?
  + freeze drier?
* SPE plate
* 1.5 ml glass vials
* 1 ml vials
* 96-well Multi-Tier Micro Plate System
  + for 1.5 ml and 1 ml vials
* Agilent Ultra 2 column
  + 25 m long × 0.2 mm internal diameter × 0.33 m film thickness
* GC-FID
  + To get PLFA concentrations

## reagents needed

* K2HPO4
* phosphatidylcholine
  + internal standard
* 1:1 chloroform:methanol
* KOH
* methanol
* toluene
* acetone
* 5:5:1 methanol:chloroform:H2O
* transesterification reagent
* acetic acid
* hexane

## existing protocols

* [Blaser](http://nature.berkeley.edu/soilmicro/methods/BalserPLFA.pdf)
* [wsu](http://vegetables.wsu.edu/scri/PLFA_protocolsOnly.pdf)

## Paying for the service

* [Ward Labs](http://wardlab.com/FeeSchedule/WardLabs_FeeSchedule_Web.pdf#page=4)

## Cornell groups doing PLFA

* [Kao-Kniffin & Zhu](http://link.springer.com/article/10.1007%2Fs00248-013-0254-8)

## GC columns used in other studies

* [Mohanty et al., 2006](http://aem.asm.org/content/72/2/1346.full.pdf+html)
* [link](http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2007.01466.x/full)
  + CP-Sil 5CB-fused silica capillary column
    - 60 m × 0.32 mm inner diameter; 0.25 μm film thickness
* [link](http://aem.asm.org/content/72/2/1346.full.pdf+html)
  + polar capillary column
    - SGE, BPX-70;50 m by 0.32 mm by 0.25m

### GC-MS used

* [link](http://www.sciencedirect.com/science/article/pii/S0038071709004209)
* [link](http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2007.01466.x/full)

## transforming PLFA concentrations to cell numbers

* [link](http://link.springer.com/article/10.1007%2FBF00384433)
  + 1.4 x 10^-17 mol of bacterial PLFA per cell
* [link](http://www.sciencedirect.com/science/article/pii/016864969500046D)
* Comparison of chloroform fumigation-extraction, phospholipid fatty acid, and DNA methods to determine microbial biomass in forest humus
  + CFE\_flush = 3.2(total\_PLFA) - 1287

## converting [DNA] to biomass C

* Relationship between dsDNA, chloroform labile C and ergosterol in soils of di􏰈erent organic matter contents and pH
  + [link](http://www.sciencedirect.com/science/article/pii/S0038071799002102)
  + dsDNA = 0.477 \* chloroform-labile C

# Alternative phospholipid extraction from soil protocols

* [link](http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0115775#s2)
* PFLA methods review
  + [link](http://www.sciencedirect.com/science/article/pii/S0016706109000548)