# Buckley lab SIP pipeline overview: from soil sampling to MiSeq sequencing

## Pipeline steps and references to the specific protocols:

* Soil sampling
  + [soil\_sampling\_protocol](../sampling/soil_sampling_protocol.html)
* Microcosm setup
  + Ashley's priming experiment setup
    - [SIP\_microcosm](../microcosm/SIP_microcosm.html)
  + Nick's full cycle pilot exp. setup
    - [SIP\_fullCyc\_pilot\_microcosm](../microcosm/SIP_fullCyc_pilot_microcosm.html)
* Microcosm headspace CO2 measurements (via GC/MS)
  + [CO2\_batch\_run](../GCMS_operation/CO2_batch_run.html)
* Nucleotide extraction
  + [DNA\_RNA\_extraction\_Protocol](../nucleotide_extraction/DNA_RNA_extraction_Protocol.html)
* **If DNA:** CsCl fractionation
  + [CsCl\_fractionation](../CsCl_fractionation/CsCl_fractionation.html)
* **Else if RNA:** CsTFA fractionation
  + [RNA\_SIP](../RNA_SIP/RNA_SIP.html)
* Nucleotide quantification:
  + [Picogreen](../nucleotide_conc/picogreen.html)
* Nucleotide sample concentration
  + [speed-vac](../speed-vac/speed-vac.html)
* Pippin Prep
  + [Pippin\_prep](../Pippin_prep/Pippin_prep.html)
* Fraction nucleotide quantification:
  + [picogreen](../nucleotide_conc/picogreen.html)
* MiSeq library prep:
  + [Illumina\_barcoding\_protocol](../library_prep/Illumina_barcoding_protocol.html)

## Others

* Cellulose farming
  + Cellulose production
    - [CelluloseProductionProtocol](../cellulose_farming/CelluloseProductionProtocol.html)
  + Cellulose grinding:
    - [CelluloseGrindingProtocol](../cellulose_farming/CelluloseGrindingProtocol.html)
* Plant stimulant
  + [Substrate\_Additions\_MicrobSuccession](../plant_stimulant/Substrate_Additions_MicrobSuccession.htm)

## Workflows

### Bulk DNA sequencing of the 16S rRNA gene

* [Nucleotide extraction](../nucleotide_extraction/DNA_RNA_extraction_Protocol.html)
* [Sephadex column clean-up](http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences/27533001)
* [Nucleotide quantification via Picogreen](../nucleotide_conc/picogreen.html)
* PCR with barcoded primers targetting a fragment of the 16S rRNA gene
  + See robot method: qPCR\_wWorklist\_altdispense.med
* [SequalPrep Normalization](https://www.lifetechnologies.com/order/catalog/product/A1051001)
* Library pooling, then concentrating via [speed-vac](../speed-vac/speed-vac.html)
* [Gel extraction](../gel_extraction/gel_extraction.html)
* [Nucleotide quantification via Picogreen](../nucleotide_conc/picogreen.html)
* **Optional:** Concentrating via [speed-vac](../speed-vac/speed-vac.html)

### Microcosm -> gradient fractionation -> 16S rRNA gene sequencing

* [Nucleotide extraction](../nucleotide_extraction/DNA_RNA_extraction_Protocol.html)
* [PippinPrep](../Pippin_prep/Pippin_prep.html)
* [Nucleotide quantification via Picogreen](../nucleotide_conc/picogreen.html)
* [CsCl\_fractionation](../CsCl_fractionation/CsCl_fractionation.html)
  + Including desalting
* [Nucleotide quantification via Picogreen](../nucleotide_conc/picogreen.html)
* PCR with barcoded primers targetting a fragment of the 16S rRNA gene
  + See robot method: qPCR\_wWorklist\_altdispense.med
* [SequalPrep Normalization](https://www.lifetechnologies.com/order/catalog/product/A1051001)
* Library pooling, then concentrating via [speed-vac](../speed-vac/speed-vac.html)
* [Gel extraction](../gel_extraction/gel_extraction.html)
* [Nucleotide quantification via Picogreen](../nucleotide_conc/picogreen.html)
* **Optional:** Concentrating via [speed-vac](../speed-vac/speed-vac.html)