# Buckley Lab SIP protocols

## Updating non-markdown versions of protocol files

* Please don't edit the Word or html files directly!
* The markdown files (\*.md) serve as the template files for all of the other formats (eg., html or docx).
* The bash scripts (eg., md2html.sh) will convert all files ending in '.md' to a specified format.
  + Example usage: ./md2html.sh
    - This will creat a html file for each corresponding markdown file.

## Pipeline steps

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

## Others

* Cellulose farming
  + Cellulose production
    - [CelluloseProductionProtocol](./cellulose_farming/CelluloseProductionProtocol.md)
  + Cellulose grinding:
    - [CelluloseGrindingProtocol](./cellulose_farming/CelluloseGrindingProtocol.md)
* Plant stimulant
  + [Substrate\_Additions\_MicrobSuccession](./plant_stimulant/Substrate_Additions_MicrobSuccession.md)
* Soil geochemistry
  + [pH](./soil_geochemistry/soil_pH.md)
  + [organic\_content](./soil_geochemistry/soil_organic_content.md)
  + [water\_holding\_capacity](./soil_geochemistry/water_holding_capacity.md)

## Workflows

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

* [Nucleotide extraction](./nucleotide_extraction/DNA_RNA_extraction_Protocol.md)
* [Sephadex column clean-up](http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences/27533001)
* [Nucleotide quantification via Picogreen](./nucleotide_conc/picogreen.md)
* [16S rRNA amplicon library prep](./library_prep/Illumina_barcoding_protocol.md)

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

* [Nucleotide extraction](./nucleotide_extraction/DNA_RNA_extraction_Protocol.md)
* **Optional:** Concentrating via [speed-vac](./speed-vac/speed-vac.md)
* [PippinPrep](./Pippin_prep/Pippin_prep.md)
* [Nucleotide quantification via Picogreen](./nucleotide_conc/picogreen.md)
* [CsCl\_fractionation](./CsCl_fractionation/CsCl_fractionation.md)
  + Including desalting
* [Nucleotide quantification of fractions via Picogreen](./nucleotide_conc/picogreen.md)
* [16S rRNA amplicon library prep](./library_prep/Illumina_barcoding_protocol.md)
  + For automated generation of the Excel files needed for the PCR assay, see the fractionSelectFor16S-PCR.ipynb notebook in the SIPdb repo.