DNA extraction (Qiagen DNeasy 96)

- Input: pellets from 200 uL T0 mixed inocula, or 500 uL matured culture in DW96s
- Output: 100 uL of DNA in PCR plates

Table 1: Materials.

Items	Usage	Amount
PBS	dissolve pellets	36 mL
proteinase K	DNA extraction	2.5 mL
buffer AL	DNA extraction	20 mL
ethanol	DNA extraction	20 mL
buffer AW1	DNA extraction	50 mL
buffer AW2	DNA extraction	50 mL
buffer AE	DNA extraction	10 mL
DNeasy96 plate	DNA extraction	1
S-block or DW96	collect flow-through	1
PCR plate	save DNA	1
1000uL tips box	dispense	3
200uL tip box	dispense	4
20uL tip box	dispense	1

☐ Thaw pellet plates at room temperature.
☐ Dissolve pellets in 180μL of PBS and transfer to DNeasy microtube plates.
☐ Add 25μL proteinase K and 200μL Buffer AL. Seal the plate with caps and mix by vortexing. Incubate at 56C for 30 min.
☐ Add 200μL pure ethanol. Seal the plate with caps and mix thoroughly by vortexing.
☐ Assemble the DNeasy 96 plates on top of DW96s. Mark the DNeasy 96 plates.
☐ Use mP1000 set at 625μL to transfer the lysate of each sample from DW96s to each well of DNeasy96 plates (maximum 900μL).
☐ Seal DNeasy96 with AirPore film. Use vacuum to collect the flow-through.
☐ Add 500μL of Buffer AW1 to each sample.
☐ Seal DNeasy96 with AirPore film. Use vacuum to collect the flow-through.
☐ Add 500μL of Buffer AW2 to each sample.
☐ Seal DNeasy96 with AirPore film. Use vacuum to collect the flow-through.
☐ Label two PCR plates. Place DNeasy96s on these PCR plates.
☐ Add 100μL Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPore Tape Sheets (provided). Incubate for 1 min at room temperature (15–25°C). Centrifuge for 2 min at 6000 rpm.
☐ Cover the PCR plates with aluminum foil and save it in -20C freezer.

PCR with master mix in 96-well plate

Table 2: Materials.

Items	Usage	Amount
PCR plates	duplicate PCR	2
50 mL faclon tube	PCR master mix	1
20uL tip box	dispense	3
200uL tip box	dispense	1

Table 3: CASEU PCR master mix.

Reagent	WellVolume	TotalVolume
ddH20	23.5	5076
5X HF buffer	10.0	2160
dNTPs (10mM)	1.0	216
Phusion	0.5	108
Total	35.0	7560

Table 4: CASEU PCR reagents with master mix.

Reagent	WellVolume	TotalVolume
PCR master mix	35	7560
27F primer (3uM)	5	1080
1492R primer (3uM)	5	1080
teamplate DNA polymerase	5	1080

- Label 2 PCR plates with naming convention like **T0 C P2 PCR** and **T3 C P2 PCR**.
- Make **1080 uL** of 3uM of each primer (32.4 uL 100 uM stock + 1047.6 uL ddH2O).
- Premix the PCR reagents (total 7.56 mL) in a 50 mL falcon tube.
- Use mP200 set at **35 uL** to dispense 35 uL of PCR master mix into each well of 2 PCR plates. This premix can stay at room temperature.
- Right before starting the PCR reaction, use mP20 set at **5 uL** to add primers.
- Use mP20 set at **5 uL** to add DNA. Cover the PCR plates with clear PCR films.
- Use the program "CASEU" in "16S" folder. See the table below for PCR cycle.
- Store PCR plates in -20C freezer.

Table 5: CASEU PCR reagents.

Reagent	WellVolume
ddH2O	23.5
5X HF buffer	10.0
dNTPs (10mM)	1.0
27F primer (3uM)	5.0
1492R primer (3uM)	5.0
Phusion polymerase	0.5
template DNA	5.0
Total	50.0

Table 6: CASEU PCR cycle.

Step	Temperature	Duration
Initial denaturation	98 C	30 seconds
Amplification (30 cycles)	98 C	30 seconds
	50 C	30 seconds
	72 C	90 seconds
Final extension	72 C	10 minutes
Storage	4 C	Forever

SequalPrep PCR product cleanup and normalization

Input: duplicate PCR plates

Output: 20 uL 1.25 ng/uL DNA in PCR plate clean_normalized_PCR

Table 7: Materials.

Items	Usage	Amount
PCR plate	PCR_mix, unbound_DNA, chean_PCR	3
SequalPrep plate	PCR cleanup and normalization	1
SequalPrep binding buffer	binding	2 mL
SequalPrep wash buffer	wash	5 mL
SequalPrep elution buffer	elution	2 mL
20uL tip box	dispense	4
200uL tip box	dispense	2

- Thaw the 2 PCR plates and spin down.
- Label 3 PCR plates with the naming convention like **T0 C P2 PCR_mix**, **T0 C P2 unbound_DNA**, and **T0 C P2 chean_PCR**. Same for T3.
- Mix 20uL of each replicate PCR into each well of a skirted PCR plate PCR_mix (40 uL in total).
- Add 20uL SequalPrep Binding buffer to each well of SequalPrep plate.
- Add 20uL mixed PCR product into each well of SequalPrep plate.
- Cover with PCR lid, vortex 30 sec, and centrifuge briefly.
- Incubate for 1 hour at room temperature. Note: extra incubation time will not improve yield but will not decrease it either. Overnight incubations are fine if necessary.
- Transfer unbound DNA to fresh skirted PCR plate unbound_DNA, being careful not to touch the sides of the wells. Discard or foil and save at -20C for up to 30 days and reuse for further SequalPrep.
- Add 50 uL SequalPrep Wash buffer to the SequalPrep plate containing bound DNA and pipet up and down twice to mix. Vortex.
- Remove buffer from wells by pipetting. Tap plate on paper towel gently to remove remaining buffer. Spin down the plate.
- To elute, add 20 uL SequalPrep Elution buffer to each well.
- Cover with lid, vortex 30 sec, and centrifuge briefly.
- Incubate at room temperature for 5 minutes.
- Elute DNA from each well into a new skirted PCR plate clean_normalized_PCR and store at -20C.

Genewiz Sanger sequencing prep

Input: 20 uL normalized DNA 1.25 ng/uL in clean_normalized_PCR

Output: DNA with primer in a PCR plate for sequencing

Table 8: Materials.

Items	Usage	Amount
PCR plate	sequencing	1
27F 5uM primer	CASEU primer	0.5 mL
20uL tip box	dispense	1

- Label 1 PCR plates with the naming convention **T0 C P2 seq** and **T3 C P2 seq**.
- Make 500 uL 5uM 27F primer stock (25 uL 100 uM primer stock + 475 uL ddH2O).
- Add **5 uL** of each primer to the PCR plate seq.
- Add **10 uL** of normalized DNA to each well of the PCR plate seq.
- For <48 samples, Label tubes with tube ID from Genewiz online ordering system, which will have your initials and sample number. Write these codes on the sides of tubes. Note: For >48 samples, arrange the samples vertically (in columns).
- Store the PCR seq plates in a box.
- Drop off the box at Genewiz West Campus pickup location: ISTC room 366.