HARVEST and RNA ISOLATION
Name_____
Date_____
Check One:

☐ wells 1-6 (hrs 1-6) ☐ wells 1-6 (30min, 7, 8, 10, 12hrs)

Consumables:
RNeasy mini kit (Qiagen, cat# 74104)

STEP 1

- ☐ Remove RNeasy mini kit (Qiagen, cat# 74104)
- ☐ Ensure station is clean: pipettes, bench, and gloves sprayed with RNase free then EtOH, tubes are labelled
- ☐ Add 10uL β-mercaptoethanol to 1mL Buffer RLT. The mixture can be stored @ RT for 1 month
  **For 6 samples add 30uL β-ME to 3mL Buffer RLT (only 2.1mL needed)
- ☐ Wash cells twice with 3mL PBS
- ☐ add 350uL Buffer RLT with β-ME into wells
- ☐ use a syringe to pipette the cells up and down at least 5 times while rinsing the wells
- ☐ transfer cells by pipetting to rnase/dnase free tubes on ice
  ****SAFE STOPPING POINT** - snap freeze cells and stored @ -80C

Name_____
Date_____
Check One:

☐ wells 1-6 (hrs 1-6) ☐ wells 1-6 (30min, 7, 8, 10, 12hrs)

Consumables:
RNase free DNase set (Qiagen cat#79254)
RNeasy mini kit (Qiagen, cat# 74104)

STEP 2

**If cells were frozen, thaw on ice before proceeding to next step
**remove RNase free DNase set, keep on ice.
**If not already done, add 4 volumes of ethanol (96–100%) to RPE as indicated on the bottle to obtain a working solution
**If not already done dissolve the lyophilized DNase I (1500 Kunitz) in 550 µl of the RNase- free water. (If new, remove the silver tab and use a syringe to add water). Mix gently by inverting the vial. Do not vortex. Keep on ICE.

☐ Mix RDD and DNaseI as follows (Do NOT vortex):
*Freeze the RNase free DNase set after reconstituting the DNaseI

	vol/sample (uL)	**6**
RDD	70	441
DNaseI	10	63
total	**80**	**510**

# of samples (5% excess built in)

☐ Add 350uL of 70% EtOH to each sample. Pipette up and down to mix
☐ Transfer 700uL of sample to a Rneasy mini spin column placed in a 2mL collection tube
☐ Centrifuge for 15s @10,000 RPM. Discard flow-through
☐ Add 350uL RW1 to the RNeasy spin column. Centrifuge for 15s @10,000 RPM. Discard flow-through
☐ Pipette 80uL RDD/DNaseI mix to each sample, be sure to cover but not disturb the membrane
☐ Incubate on bench for 15min@RT
☐ After 15min, add 350uL RW1. Spin 15sec @10,000 RPM. Discard flow-through
☐ Add 500uL buffer RPE to spin column. Centrifuge for 15s @10,000 RPM. Discard flow-through
☐ Add 500uL buffer RPE to spin column. Centrifuge for 1min @10,000 RPM. Discard flow-through
☐ Centriguge at full speed for and additional 2min to dry the membrane
☐ Place tbe RNeasy spin column in a new 1.5mL collection tube
☐ Add 30uL Rnase free water to the membrane without disturbing the membrane
☐ Allow tubes to sit with lids closed @RT for 1min.
☐ After 1min. Spin tubes for 1min @ 15,000RPM
☐ Place tubes on ice. Check on Nanodrop.
*if not proceeding to next step, samples can be stored at -80C
☐ Create sample plate map

- [ ] Plate out 20ng/uL in a volume of 55uL on ICE (only 1ug in 50uL is needed for lib prep)
- [ ] Check quality of RNA using Agilent RNA screen tape