DNA extraction with DNeasy protocol notes for great-tailed grackles from C. Rowney
2018 – The Grackle Project – www.CorinaLogan.com

Blood + lysis buffer is in tubes

- centrifuge at full speed for 1 minute
- get together an empty 1.5mL tube for each blood sample, label with numbers that correspond to an extraction sheet

to each empty labelled tube, add:

- 20uL proteinase K (filter tip)
- 150uL PBS (nonfilter tip)
- 50uL blood + lysis (filter tip)
- 200uL Buffer AL; vortex immediately for 5s

Put this tube in thermomixer overnight at 64C, shaking at 164rpm

Next day:

- centrifuge 1.5mL tubes at full speed for 30s
- add 200uL ethanol, vortex well

- prepare spin columns by labelling with corresponding number to first day
- add all liquid, including any undigested material, from 1.5mL tube to spin column (set pipette to 650uL, use filter tips)

- centrifuge spin tubes at full speed of = or > 8000 RPM for 1 minute
    - make sure all liquid has passed through the column, if it hasn't, centrifuge again for 2-3m

- collect liquid that passes through spin column in beaker for later disposal
- blot tube dry, put spin column back in tube
- add 500uL buffer AW1 to each column
    - centrifuge spin tubes at full speed (= or > 8000 RPM) for 1m; add liquid that passes through to glass beaker for later disposal, then put spin column back in tube
- add 500uL Buffer AW2 to each spin column
    - centrifuge spin tubes at full speed (= or > 8000 RPM) for 3 minutes; column must be dry at this point, if it isn't, centrifuge for another minute
        - the liquid that passes through this can go in normal trash

- Get out one new 1.5mL tube for each sample, add spin column
- add 200uL Buffer AE (elution buffer) directly onto spin column membrane
    - incubate this at room temperature for 1m, then centrifuge spin tubes at full speed for 1m