SPRI Bead Extraction:
Version 6 (13 July 2020)

Notes:

- This protocol has been optimized using nucleated avian blood stored on lysis buffer.
- Beads MUST be at room temp and well mixed
    - Set out from fridge ~30 mins prior to starting
    - Also a good time to set out TLE (elution buffer) if that's what you'll use
- You must prepare FRESH 80% EtOH. This is critical because if too low and the DNA will elute off the beads, and too high you are likely to over-dry due to evaporation.
- Set the incubator to 37C while you're getting set up.

Digestion:

1. Use wide-bore 1,000uL pipette to wrangle ~200-400uL starting material into a new 1.5uL Eppendorf
    a. Be sure you're using low-bind tubes!
    b. May have to "cut" the blood blob by closing cap

2. Add 30uL of 20 mg/mL Prot-K and 20uL 10X Queen's Lysis buffer
    a. Vortex for 5 seconds to mix and flash spin to collect any drops

3. Incubate at 66C / 250 RPM overnight in the shaking incubator

DNA Isolation:

1. Combine **200uL of digest with 300uL SpeedBeads**
    a. MUST BE 1-2X beads than digest.
    b. Save extra digest product in case extraction fails
    c. Mix by pipetting with WIDE BORE tips, spin down only if necessary
    d. Let tubes sit for **5 mins. at room temperature**

2. Place **tubes on magnet for 15-30 mins** or until the solution is clear
    a. Be patient! All your DNA is binding to the magnet right now so give it time!
    b. **<u>While you wait, make the 80% EtOH and TLE aliquot</u>**.

3. **Remove and dispose supernatant** without disturbing the beads.
    a. You CAN KEEP the supernatant at this point and do another round if needed

4. **Wash 1:** Add 700uL of **FRESH** 80% EtOH to the tube
    a. **T**his volume must be greater than volume of beads + digest solution
    b. Let it sit for 1 min
        i. Use a stopwatch if it's only a couple samples, but if you have ~10+ it will be over a minute before you get back around
    c. Remove and dispose EtOH without disturbing the beads.

5. **Wash 2:** Add 800uL of 80% EtOH to the tube
    a. Let it sit for 1 min.
    b. Remove and dispose EtOH without disturbing the beads.
    c. Take tubes off the magnet and flash spin (5 secs) to pellet beads
    d. Place back on magnet and use 200uL pipette to remove remaining EtOH

6. **Add 200uL of TLE buffer** to each tube
    a. Mix by flicking or gentle vortexing, flash spin if necessary
        i. Need beads and DNA to disassociate, but don't want to shear DNA
        ii. I found higher volume of TLE helps beads disassociate

7. **Incubate at 37C for ~15 mins**.
    a. Keep tubes open so any EtOH carryover from the wash steps can evaporate
    b. Use this time to make qubit buffer if that's next

8. Place **tubes back on magnet for ~10 mins** or until solution is clear
    a. VERY IMPORTANT! The solution MUST be clear. If the DNA solution is highly concentrated this could take a while… Be patient!

9. **Remove supernatant (aka liquid gold**) and place into a new, labelled tube!
    a. Cross fingers that everything worked ;)