Bead-based DNA extraction protocol for the EGL automated pipetting system (Benchsmart 96)

**Materials**

* Zymo tissue buffer ("Biofluid & Solid Tissue Buffer")
* Proteinase K (NEB)
* DTT
* RNAse A
* 80% ethanol
* EGL low ratio SPRI beads
* Nuclease-free water
* 1.2mL or 1.3mL deep-well plate
* Magnetic rack

Organize 96-well plates by species because protocol conditions may vary between species

**Protocol**

Mastermix of 120 uL buffer + 40 uL ProK + 2 uL DTT for 96 samples

***Digestion:***

1. Transfer 96 wing tissue samples to a 1.2 or 1.3 mL deep well plate
   * Record samples names on the plate\_organization datasheet as you go
   * Use sterile tweezers to move the wing punch to a Kimwipe to blot the ethanol, then transfer to well
   * Tape over rows and remove as you go to avoid accidentally adding two samples to the same well
2. Add 200 uL Zymo buffer to each well
3. Add 40 uL 20mg/mL Proteinase K. Pipette with wide tip and gently mix by stirring with pipette tip. Note that this will likely be viscous--so decrease the Benchsmart pipetting speed
4. Add 2 uL of DTT to digest the hair because it is impossible to pellet (DTT is in the little -20C in the main Sudmant lab)
5. Using thermomixer, apply heat and mixing, 55C at 1250 speed overnight

***Morning after digestion:***

1. Take out Low Ratio SPRI beads--need to equilibrate to room temperature (can take >30 min in vial)
2. **Spin down 10 minutes at 10,000 rpm** and transfer lysate to a new plate (to get rid of any undigested hair or other matter)
3. Add 20uL of RNAse A. Incubate at room temperature for (at least) 10 minutes.
4. Vigorously vortex the SPRI beads because they don't work when they're clumped
5. Apply a 1:1 volume ratio (161 uL **TWICE**) of EGLs Low Ratio SPRI beds, and slow pipetting/stirring to mix. Lysate will probably be very viscous
6. Program Benchsmart to gently tip mix on Benchsmart for 20 min (or put individual tubes on the rotator)
7. Spin down very briefly to get liquid off the sides
8. Incubate on a magnetic rack for ~20 minutes (until supernatant totally clear and pellets tight on the side of the tube). Exact amount of time required will depend on the amount of DNA in the sample
9. Spin down very briefly to get liquid off the sides–DO THIS, otherwise you’ll have drops you can’t get rid of at the end

***1st wash***

1. Use 200uL tip to aspirate out supernatant. **3 uses of Benchsmart tips**. SAVE the supernatant in case something went wrong
2. Keep tubes ON the magnetic rack
3. 200uL of 80% ethanol--Stacy doesn’t drip directly over bead pellet, instead she passively fills the tube (Benchsmart will probably do the same)
4. Incubate for ~2 minute (until clear)

***2nd wash:***

1. Use 200uL tip to aspirate out supernatant. 2 uses of Benchsmart tips
2. Keep tubes ON the magnetic rack
3. 200uL of 80% ethanol--Stacy doesn’t drip directly over bead pellet, instead she passively fills the tube (Benchsmart will probably do the same)
4. Incubate for ~2 minute (until clear)

***Elution***

1. Take tube off rack and elute in (pre-warmed) nuclease-free water (or an EDTA-free buffer) (50-100 uL); use benchsmart to tip mix x 20
2. Using thermomixer, apply heat and mixing, 55C at 1000 rpm, 1 hour
3. Incubate on magnetic stand ~5 minutes (until clear)
4. Transfer lysate solution w/ DNA to a plate and label

**Fragmentation reaction conditions**

* We won’t be able to finalize the total volume of the reaction until we know the rough total DNA yield from the Mada wing punches
* We can use the MVZ samples to narrow in on an optimal reaction time, which will probably end up being between 30 and 40 minutes (while also testing out our Benchsmart protocols)

**Additional thoughts:**

* We should add a step to save the first lysate in a plate (at least for the first few tries) just in case the DNA didn’t stick to the beads for some reason and we need to re-extract…
* Let’s just keep using water as the DNA buffer to keep it simple
* Goals to keep in mind:
  1. We want the lowest cost per sample possible so that others can apply our protocol across the large sample sizes you need for modeling analyses
  2. We want to keep in mind where/how our protocol can be applied in labs with less equipment/resources. Maybe can add notes regarding substitutes/alternative strategies

**Benchsmart notes:**

* Demo: https://www.youtube.com/watch?v=fN3ZfSEk7M4
* Maximum volume per use is 200uL (minimum is 5uL)--per pipette tip (so you can reuse the same tip multiple times, e.g., to remove a supernatant, but any modifications that reduce the volumes will be a big time saver)
* Deep-well plates in EGL stock are 1.2 or 1.3 mL. EGL tries to keep volumes under half of that, especially with any buffers that might contain surfactants and/or be foamy. Tip mixing (and vortexing) can cause some foam that can risk cross-contamination
  + They got a protocol working with 800uL max volume, but worked even better with half of that--so target total ~ 400 uL
* They don’t vortex or invert deep well plates--plates have less surface area for sealing and there is too high risk for cross-contamination; but can program the Benchsmart to tip-mix