APPENDIX 2: PROCESSING STANDARD MUSEUM SAMPLES

Protocol adjustments for museum specimens:

Use the "MMYT" DNA extraction protocol below. The "MMYT" method yields more DNA than Phenol-chloroform and Qiagen. See Derkarabetian et al (2019). Phenol-chloroform may work better if samples are preserved in another preservative, like Urea buffer. Do not sonicate/fragment the samples, unless the specimen is recent (<20 years) and you have gel-verified that it does need sonication. Adjust sonication time only if needed.

During library prep:

- Throughout: do 2.5X bead cleanups
- Step 1.14: use the entire product in library prep
- Step 3.2: use 5 uM stubs
- Step 3.4: ligate for 45-60 minutes
- Step 5.3: amplify for 18 cycles (rarely 20)
- Post hybridization amplification for 18 cycles

During post-library prep pooling: you can include fewer samples per column (e.g. 6 per columns), giving you more reads per sample. Depends on sequencing machine though; you don't need to do it with if using the NovaSeq (so much data).

If you have a lot of primer in the post hyb amp pool (usually ~135 bp peak seen in the TapeStation), you can do a ~1.8x bead cleanup to get rid of the primers.

"MMYT" Protocol

Adapted by Ligia Benavides, from: Man-Ying Tin, M. et. al. PLOS one May 2014. Vol. 9, Issue 5.

DNA Extraction Buffer

50 grams guanidine isothiocyanate
5.3 ml of 1M Tris-HCl ph 7.5
5.5 ml of 0.2M EDTA
10.6 ml of 20% Sarkosyl
1 ml B-mercaptoethanol
Bring volume up to 50 ml with water

DAY 1

1. Place tissue in a 1.5ml Eppendorf with 200µl of extraction buffer

2. Incubate at 55C overnight

DAY 2

3. Prepare silica beads:
   3.1. Re-suspend G-Biosciences Silica Magnetic Beads thoroughly by pipetting or vortex
   the vial.
   3.2. Transfer adequate amount of beads into a clean tube (20µl). Can do multiple samples in single tube.
   3.3. Place the tube on the magnetic stand for 30-60 seconds.
   3.4. Discard the supernatant by aspiration with a pipette.
   3.5. Remove the tube from magnetic stand.
   3.6. Add 100 µl Elution Buffer (10mM Tris-HCl, 1mM EDTA, pH8.0) or ddH2O and re-suspend the beads by pipetting or vortex.
   3.7. Place the tube on the magnetic stand for 30-60 seconds.
   3.8. Discard the supernatant, and then remove the tube from the magnetic stand.
   3.9. Repeat steps 6-8 twice
   3.10. Add 20µl ddH2O
4. Add lysate to tube with silica beads. Centrifuge extraction if needed.
5. Add 200µl 100% ethanol to lysate + beads
6. Gently mix and incubate in a rotary mixer for 15 minutes
7. Place tubes in a magnetic stand for ~5 minutes
8. Discard supernatant
9. Pipette 200µl of PE buffer and incubate in a rotary mixer for 10 minutes.
10. Repeat 7-9 two times
11. Remove supernatant
12. Keeping tubes on the magnetic stand, air dry beads for 30-45 minutes
13. Remove tubes from magnet and elute beads in 40µl of buffer EB (or Qiagen AE buffer) or water (select amount of buffer according to amount of tissue used).
14. Incubate for 10 minutes at 55C
15. Place tubes on magnet, wait until liquid is clear and remove supernatant to a new labeled tube.