**FFS Protocol originally developed by Michael Yuan (UC Berkeley)**

**Modified by KAO 1/2019**

**All work done under hood with filtered tips**

**Day 1:**

**Wash 1 (~5 hours)**

1. Chop ~20mg tissue on clean parafilm and place in labeled 1.5 ml tube.
2. Soak tissue in 1.5ml GTE Buffer for 2 hrs at room temp. Pipette out liquid or move tissue with clean forceps.
3. Repeat for 2 hrs.
4. Repeat soak for 12-18 hours.

**Day 2:**

**Wash 2 (~25 min)**

1. Remove liquid or move tissue to new tube
2. Wash 1 min in 1mL 100% EtOH
3. Wash 5 min in 1mL 70% EtOH
4. Wash 10 min in water

**Digest (~10 min setup)**

1. Incubate at 55C for 12-24 hrs in a 1.5 ml tube containing:
   1. 500 ul Cell Lysis
   2. 100 ul Pro K
   3. 20 ul 1mM DTT

**Day 3 (Wednesday):**

1. Place on ice 5 min

**Extract 1 (~30 min) – UV new 1.5 mL tubes and put EtOH stock on ice!**

1. Add 200 ul PPS and invert 50 times.
2. Spin 14,000g for 5 min.
3. Pour supernatant into new 1.5 ml tube:
   1. 600 ul cold 100% EtOH
   2. 3 ul glycogen solution
4. Invert 50 times

**Day 4–5:**

1. **Incubate at -20 for 48 hours**

**Day 5:**

1. Spin 14000g for 30 min.
2. Discard supernatant
3. Add 200 ul of 70% EtOH to sample
4. Invert 50 times.
5. Spin 14000 g for 5 min
6. Drain and dry for ~3 hours
7. Re-suspend with 40–50 ul Tris