Title: Preparation of protein lysates from FFPE tissues.

Date: 29 January 2020
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I. Chemical Reagents and Recommended Sources

1. Xylene (Acros, 422685000)
2. Ethanol (EtOH) (Decon Labs, 2701)
3. Ammonium bicarbonate (NH4HCO3) (Fisher, A643-500)
4. RapiGest SF surfactant (Waters, 186001861)
5. Water, HPLC grade (Fisher, W5-1)
6. Phosphatase Inhibitor Cocktail 3 (Sigma, P0044)
7. Phosphatase Inhibitor Cocktail 2 (Sigma, P5726)
8. Protease Inhibitor Cocktail (Sigma, P8340)
9. pH strips (MColorpHast 1.09535.0001, range 0-14)

II. Reagent Preparation for FFPE Cell Lysis and Protein Digestion

A. 50mM NH4HCO3, pH 8.0. Must be made fresh weekly:
   1. Add at least 10mg NH4HCO3 for every 5 samples to be processed to a 15mL Falcon tube.
   2. Add HPLC water to a final NH4HCO3 concentration of 3.953mg/mL.
   3. Mix well.
   4. Test pH should be 8.0 by paper test strip.
   5. Label and store at 4°C.

B. 50mM NH4HCO3, pH 8.0 with inhibitors. Must be made fresh weekly:
   1. Transfer at least 1mL 50mM NH4HCO3 for every 10 samples to be processed to a labeled 15mL Falcon tube.
   2. Add 1% Phosphatase Inhibitor Cocktail 3.
   3. Add 1% Phosphatase Inhibitor Cocktail 2.
   4. Add 1% Protease Inhibitor Cocktail.
   5. Mix well.
   6. Label and store at 4°C.
0.2% (w/v) RapiGest in 50mM NH4HCO3, pH 8.0. **Must be made fresh daily:**
1. To one 1mg vial of RapiGest, add 500µL of 50mM NH4HCO3, pH 8.0 with inhibitors.
2. Mix until dissolved.
3. Store on wet ice until use.

## V. Deparaffination, Rehydration and Resolubilization Protocol of FFPE Samples

### A. Deparaffination with xylene (Repeat 3x)
1. Place slides in gray 24 slide holder.
2. Add 150mL xylene to three tubs. Xylene may be recycled from previous experiments.
3. Place slides in tub for 3 min.
4. Drain & blot excess solution and move slides to next tub.

### B. Rehydration of FFPE samples
1. Add 150mL 100% EtOH to two tubs. 100% EtOH may be recycled from previous experiments.
2. Place slides in tub for 3 min.
3. Drain & blot excess solution and move slides to next tub.
4. Add 150mL 85% EtOH to two tubs. 85% EtOH may be recycled from previous experiments.
5. Place slides in tub for 3 min.
6. Drain & blot excess solution and move slides to next tub.
7. Add 150mL 70% EtOH to a tub and 150mL H2O to another tub. 70% EtOH and H2O must be fresh.
8. Place slides in tub for 3 min.
9. Drain & blot excess solution and move slides to next tub.

### C. Resolubilization
1. Add 100 µL 0.2% RapiGest in 50 mM NH4HCO3.
2. Incubate at 95 °C with mixing at 1000 rpm for 30 minutes.
3. Cool on ice for 5 min.
4. Sonicate 3x in cup horn probe (filled with ice water) at 50% power for 30 s, incubate on ice for at least 30 s between each sonication step.
5. Incubate at 80 °C with mixing at 1000 rpm for 120 minutes.
6. Cool on ice for 5 min.
7. Add 100 µL 50 mM NH4HCO3, pH 8.0.
8. Sonicate 3x in cup horn probe (filled with ice water) at 50% power for 30 s, incubate on ice for at least 30 s between each sonication step.

### D. Storing samples until digestion
1. Cool samples on ice for 5 min.
2. Store samples at -80°C until the day of digestion.
3. On day of digestion, thaw samples on ice for 5 min.
4. Vortex samples for 10-15 s.
5. Centrifuge at 20000 rcf for 10-20 min at 4°C.