Maternal Ancestry, Part 4
Dehybridization and Biosensor Readout

Materials
- Thermal cycler
- Mini-centrifuge
- Freezer block (1 per group)
- Micropipets and Tips (20, 200, and 1000 µL)
- Ligation Products (stored at 4 °C)
- Lateral Flow Biosensors (stored in N₂ purged dry box)
- Running Buffer
- Poly T tailed red polystyrene beads (stored at 4°C)
- 2 mL Microcentrifuge Tubes (2 per group)

Overview: Only single stranded DNA can be detected on the biosensor, so we first dehybridize the sample, and then quickly run the solution on the biosensor.

Time: ~25 minutes

Protocol

Hybridization of Samples
1. Take half of your samples, place them into the thermal cycler and run a program holding the temperature at 95 °C for 5 minutes. Use this time to do “Biosensor Setup.”
2. After dehybridization is complete, place the samples immediately in the freezer blocks and follow the instructions in “Running the Biosensor.”
3. Once you finish the first half, repeat the steps for the second half.

Biosensor Setup
1. Obtain one biosensor per tube; line them up on a clean surface.
2. Prepare a 2 mL microcentrifuge tube for each biosensor by pulling off the caps and labeling each tube with the same labels as the ligation products.
3. Add 600 µL of the running buffer to each tube.
4. Separate the beads into 24 µL aliquots. This is enough for four biosensors.

-Only 20 µL are necessary for four biosensors, but beads are inevitably lost during the sonication and pipetting processes.

Overview: Only single stranded DNA can be detected on the biosensor, so we first dehybridize the sample, and then quickly run the solution on the biosensor.

Time: ~25 minutes
Running the Biosensors

1. **Immediately before applying the samples**, sonicate the 24 µL bead aliquot for 15 seconds (count with a clock!).
   - **Sonicating can break up bead “clumping,” which would otherwise result in poor bead flow up the biosensors and subsequently weak signals.**
2. Working quickly, add 10 µL of the ligation product to the top of the biosensor’s glass fiber pad.
3. Then, add 5 µL of beads near the bottom of the pad.
   - **Add the solution slowly to prevent it from being traveling down to the lower cellulose pad and getting stuck. It is OK if the solution starts to travel up the membrane.**
4. Place the biosensor in the corresponding 2 mL microcentrifuge tube.
5. Beads will begin moving upwards within a few minutes. A full signal will develop in 15-20 minutes.
   - **Note the difference in signal between the “out of haplogroup” and “in haplogroup” samples. The difference will indicate which allele is present.**