Impact of Sample Condition on DNA Identification of Birdstrike Remains

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Smithsonian Institution National Museum of Natural History

Washington, D. C.

Bird Strike Committee USA Annual Meeting, August 2021

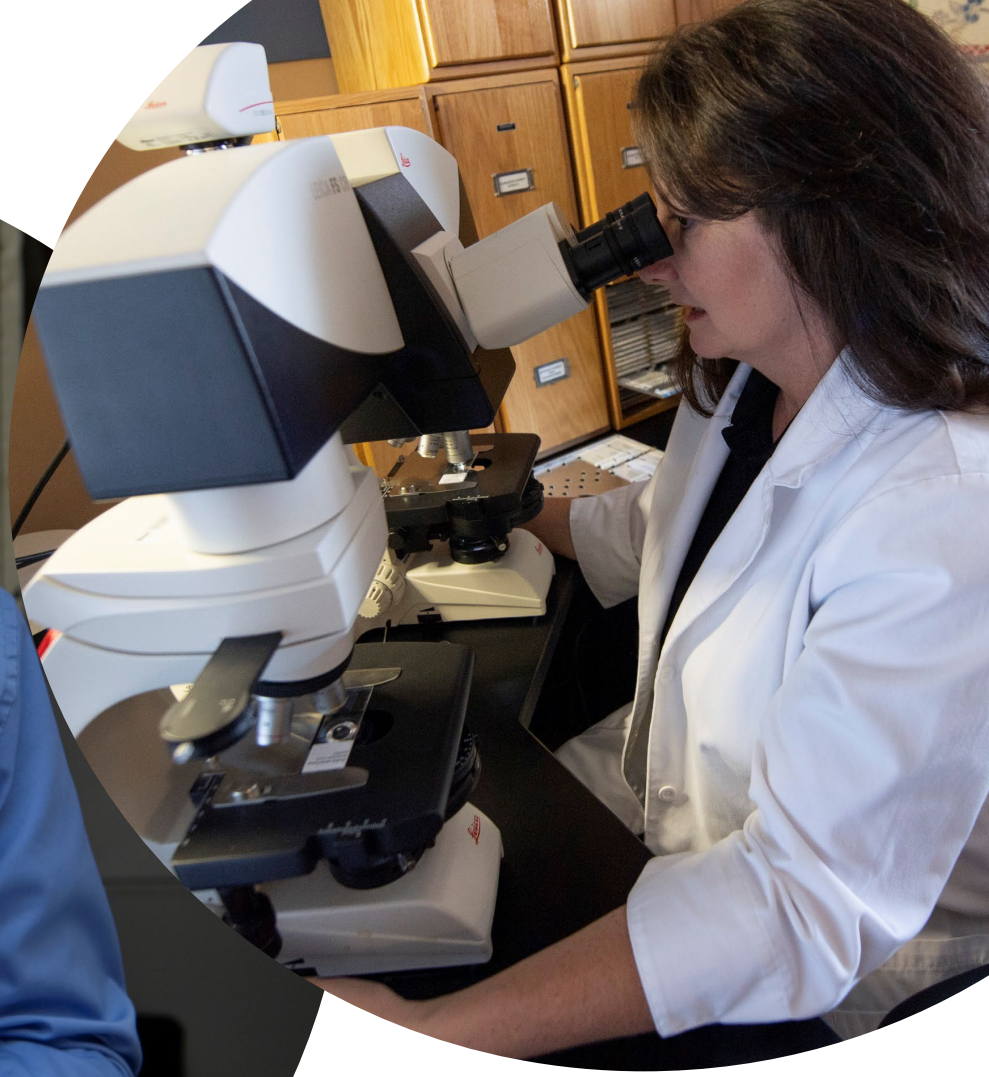
The Feather ID Lab

- We are a team of five, located at the Smithsonian National Museum of Natural History.
- We identify birdstrike samples from FAA, Air Force, Navy, engine manufacturers .
- Without identification you can’t effectively reduce birdstrikes, we’re here to help!
- Want to learn more? Come to our Panel Discussion Thursday Aug. 19 @ 11am

Identification Methods

DNA Barcoding

Whole Feather



Feather Microstructure

~ 70% of remains received by the Feather Lab are analyzed using DNA barcoding

DNA Barcoding Background

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   - It has enough variation that it can differentiate most organisms to species level.
   - DNA must be removed and purified from the tissue, then Polymerase Chain Reaction (PCR) is used to find and copy just that small region of DNA from a given sample.

DNA Barcoding Background

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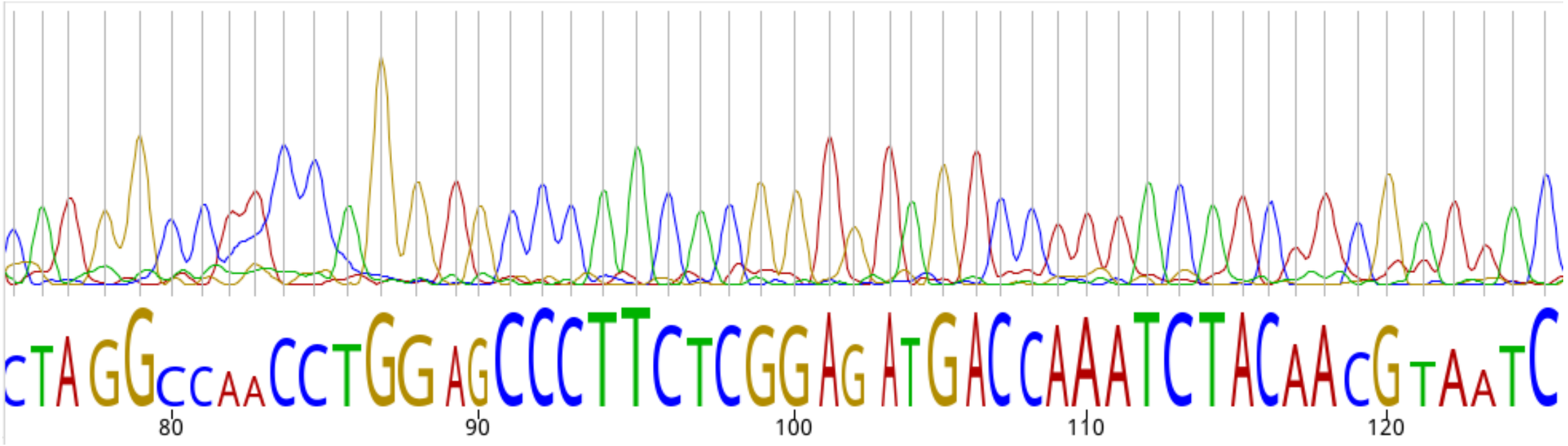
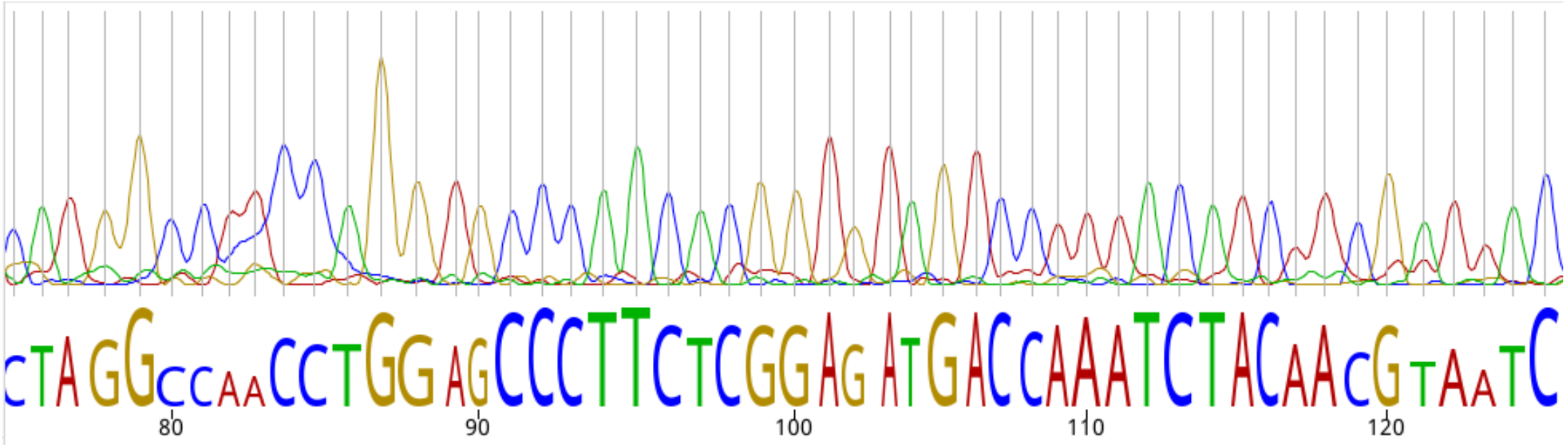
   - It relies on accurately reading the bases in that small section of DNA and identifying how many match that same small section of a set of reference sequences
   - Voucher sequences exist for ~60% of all bird species globally.



Sequence Results

- Sequencer uses spectrophotometry to read the base identity at each position and confidence in that identification.



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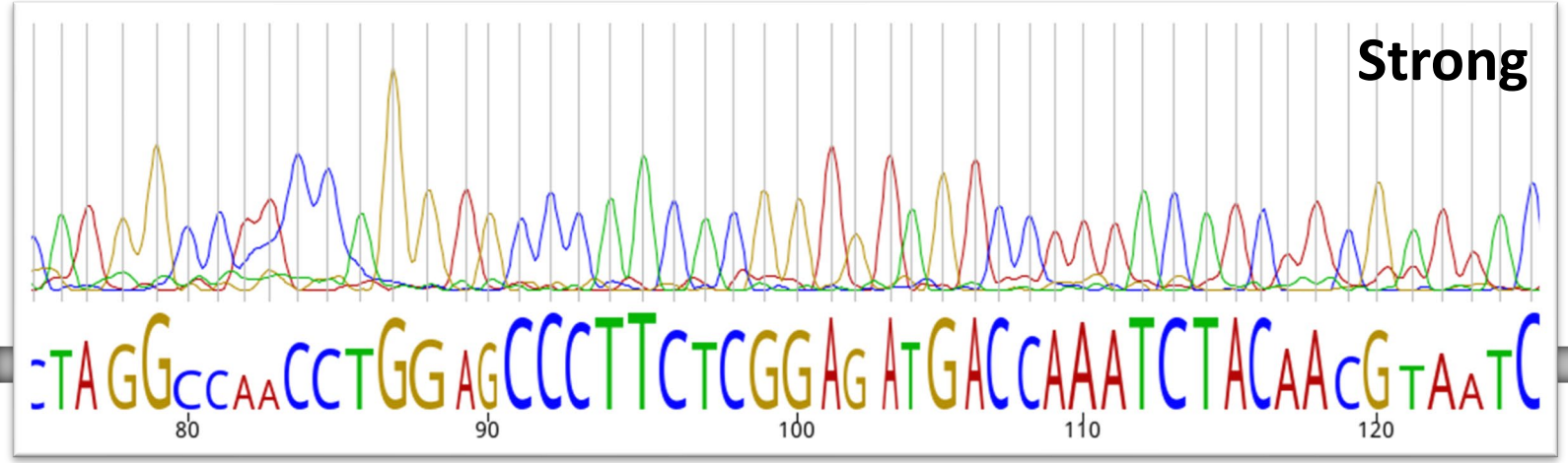
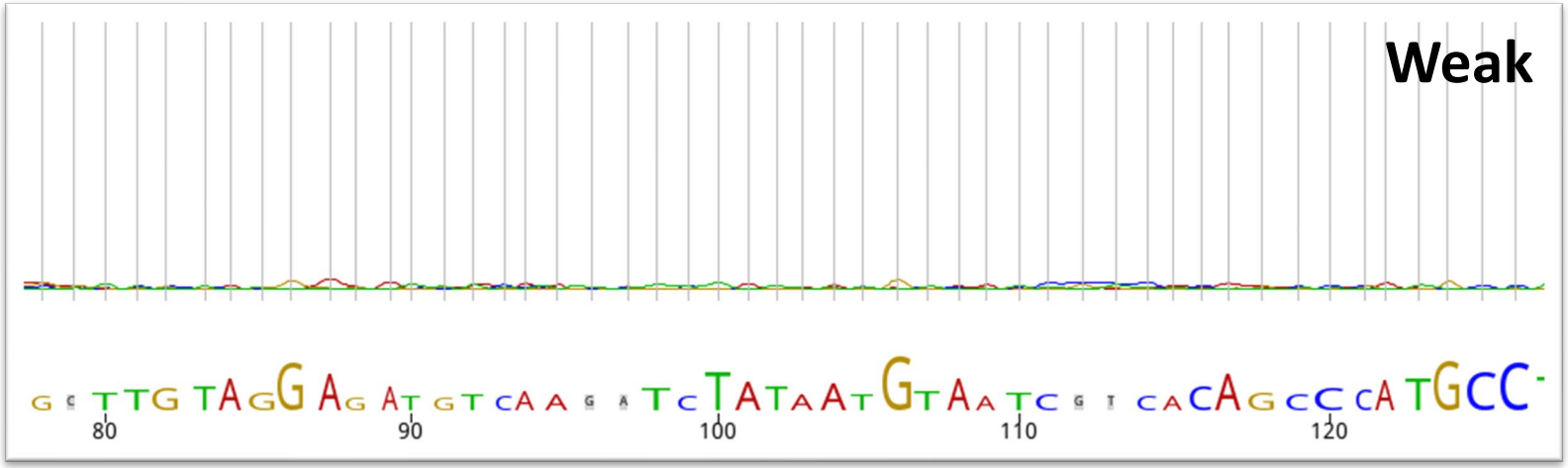
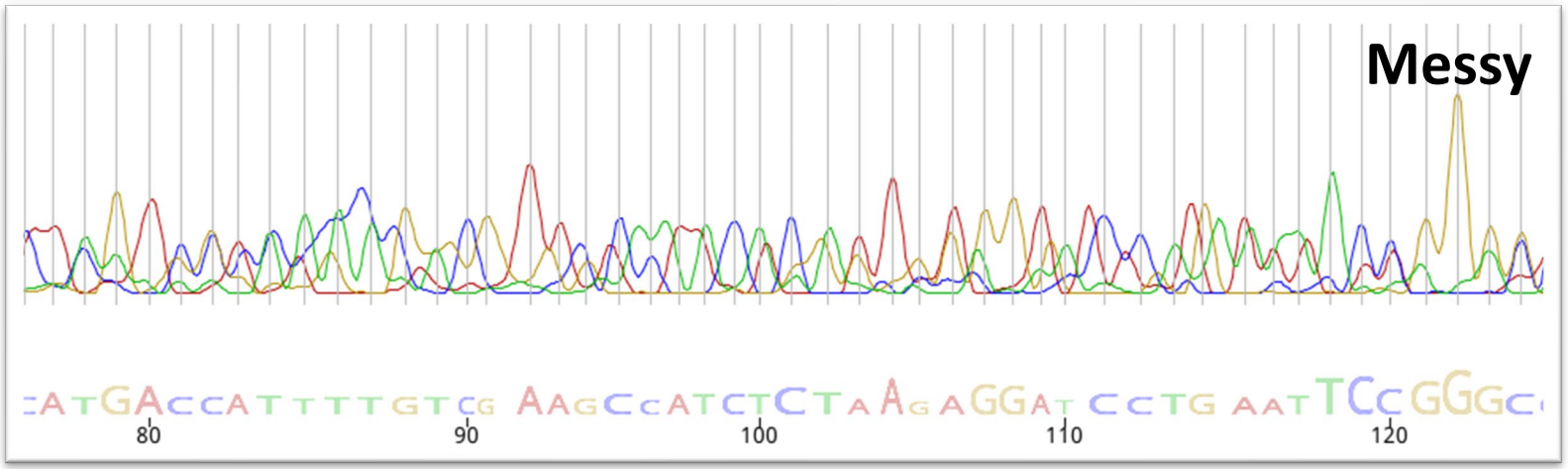
- The quality of the sequence read can greatly affect the ability of the matching algorithm to find a sequence match.







Interpreting Sequences

- Weak signals may be interpretable if they are clean.

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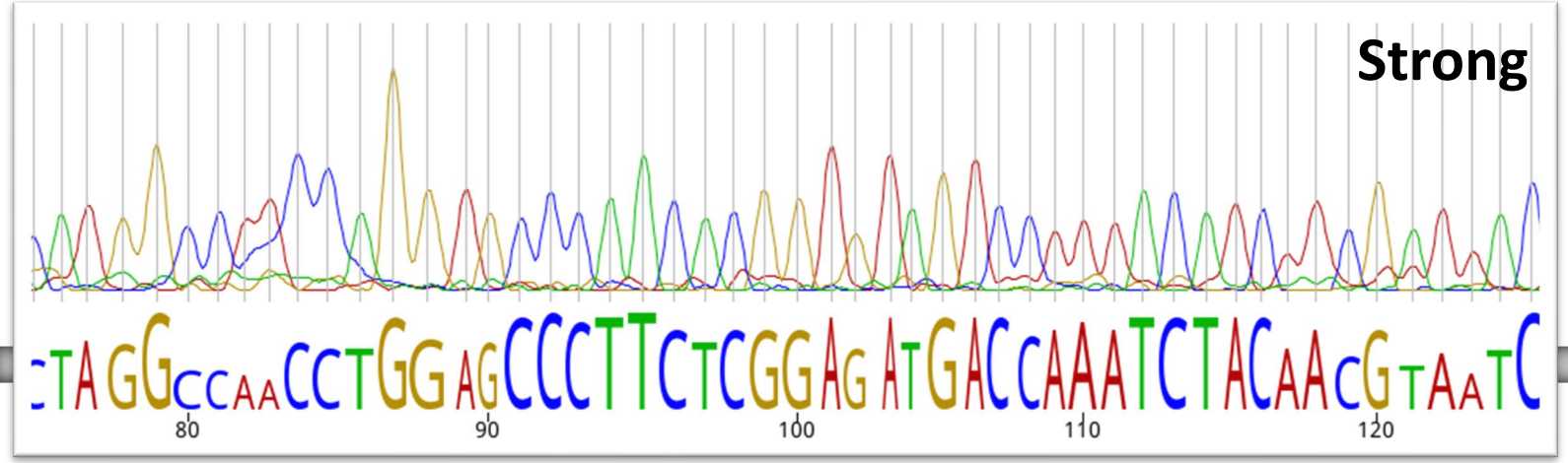

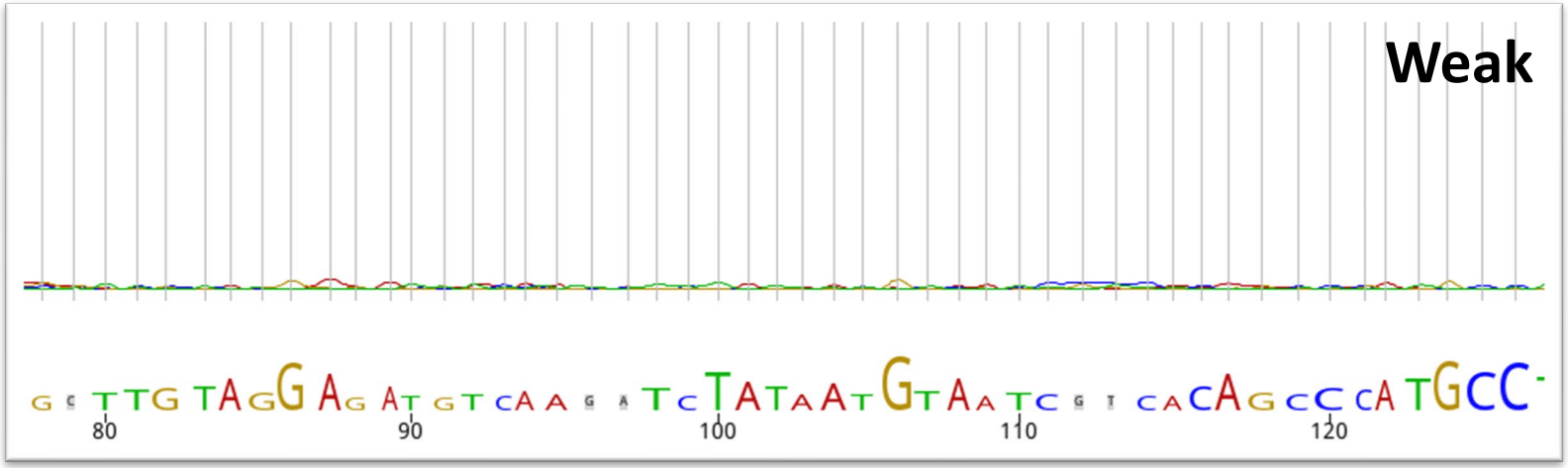

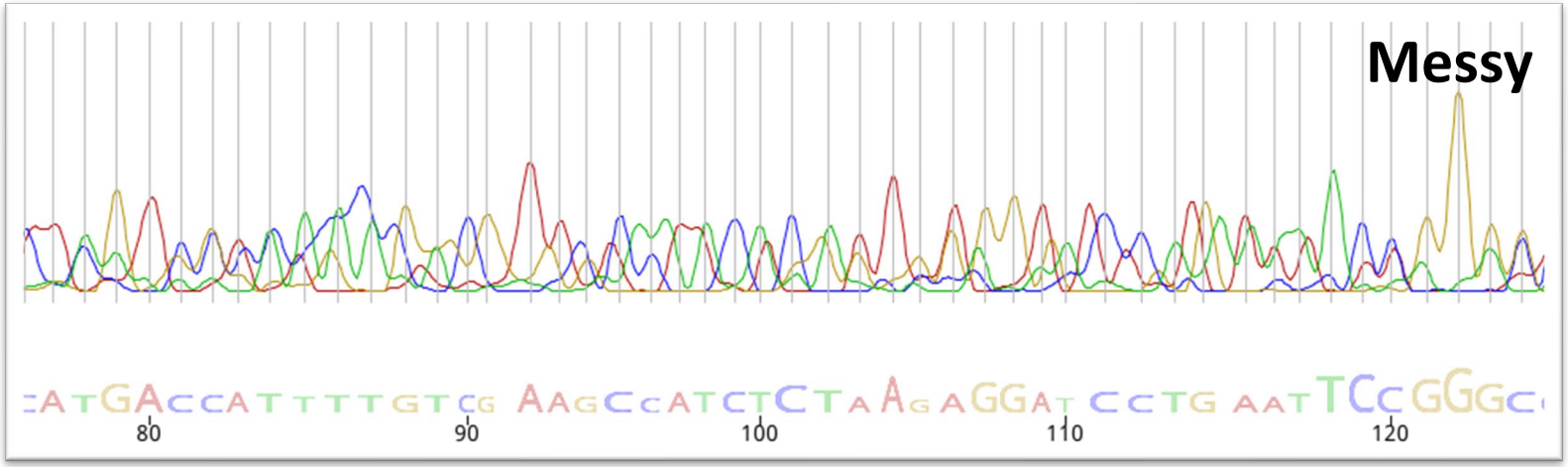

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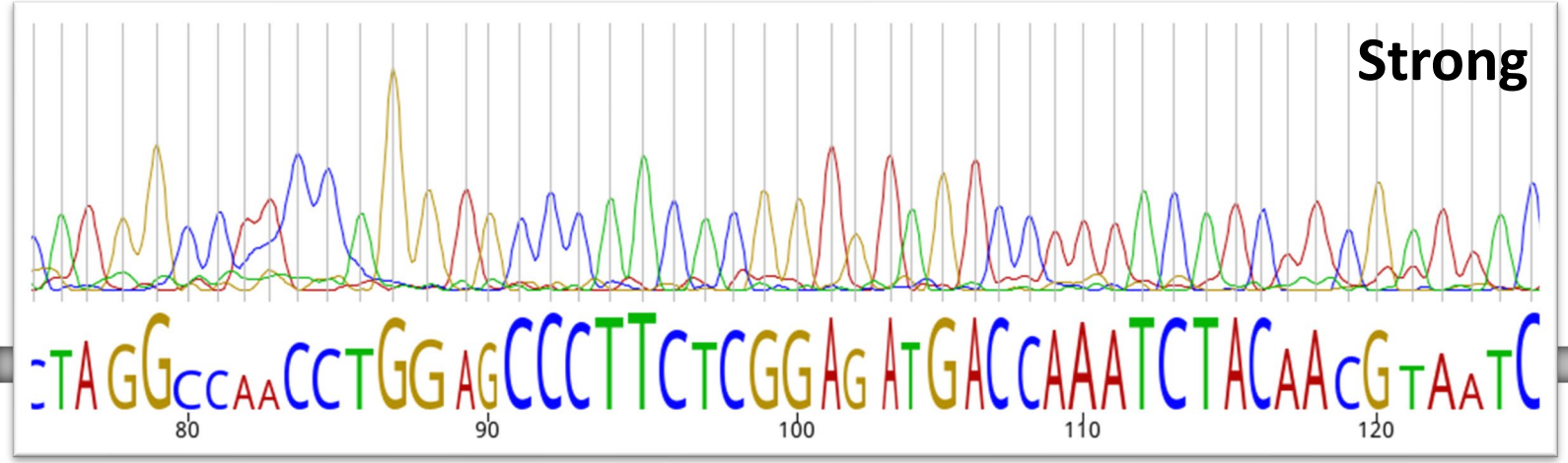
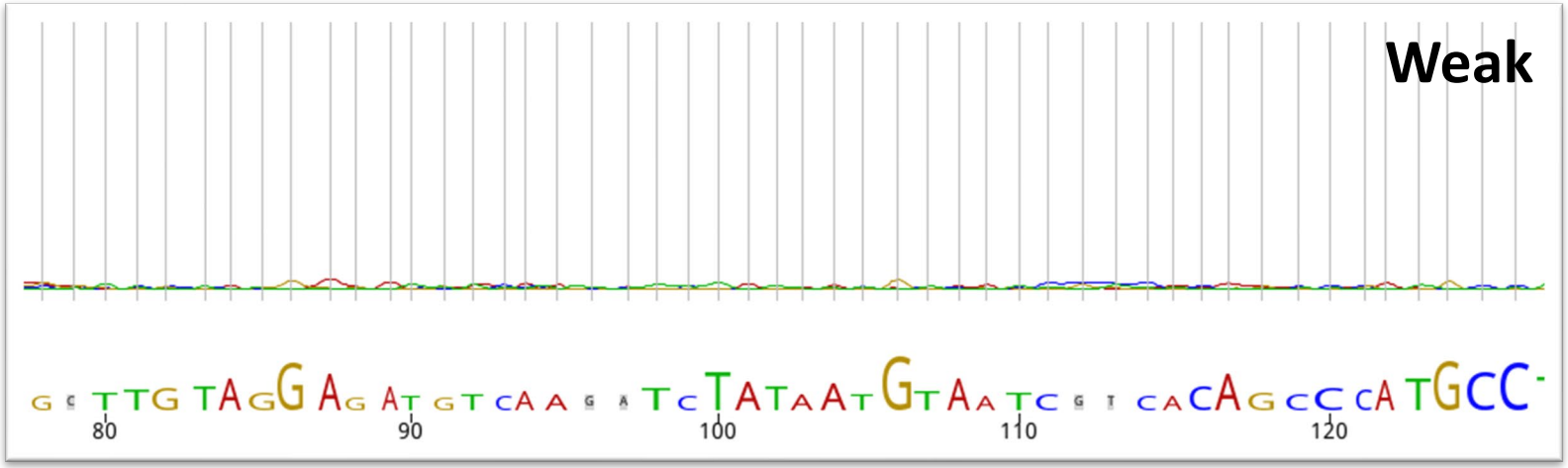
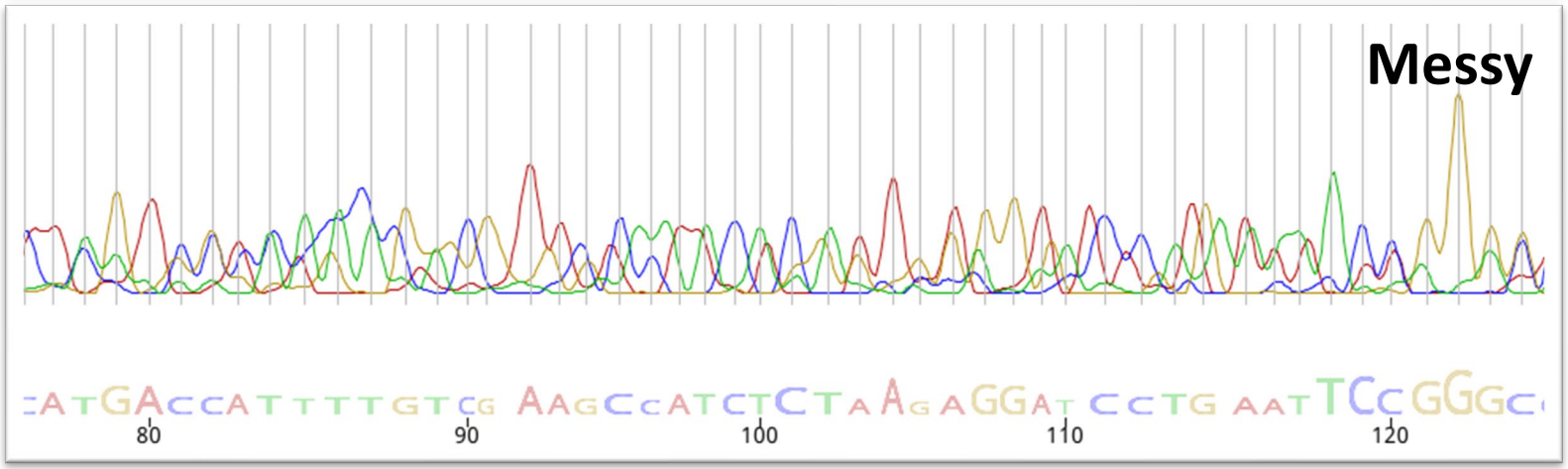






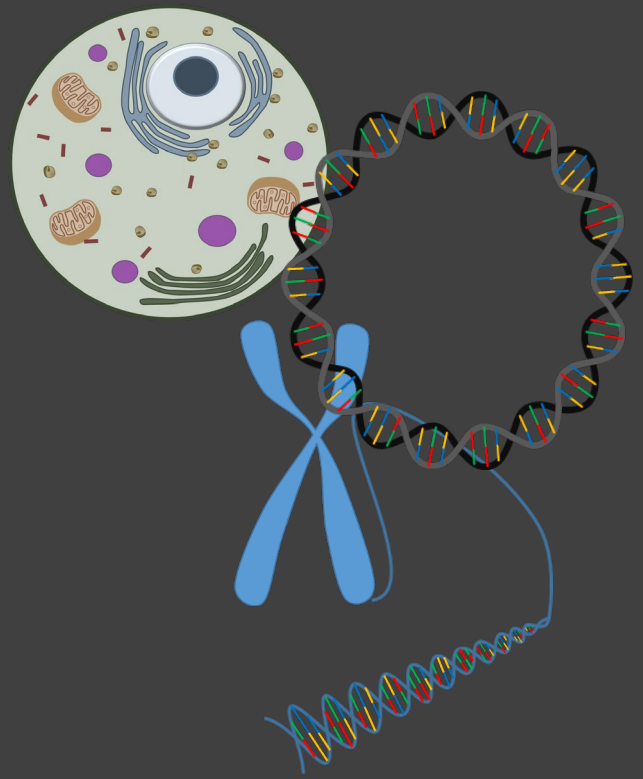
- Weak signals may be interpretable if they are clean.
- Messy signals often cannot be interpreted even if they are strong.
- Like a Captcha, sometimes the human eye can interpret portions of messy sequences better than the computer, or crop only the best quality regions.

Why Didn’t it Work?!

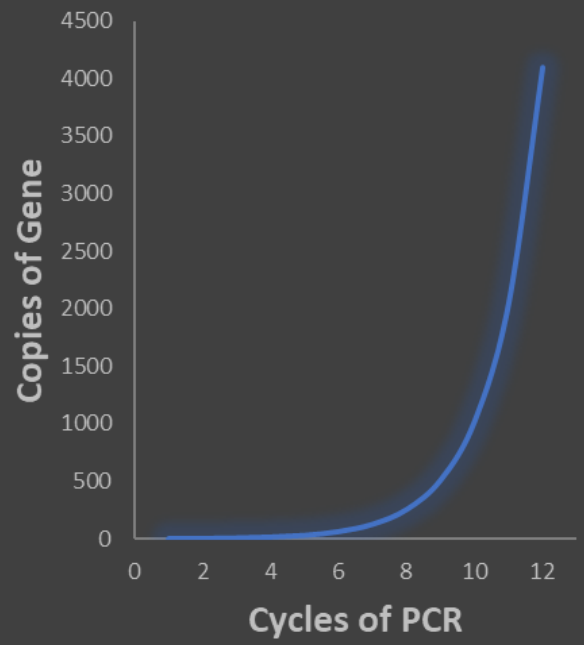
- Many steps in the process where sequence quality can be impacted

Sample Selection

DNA Extraction



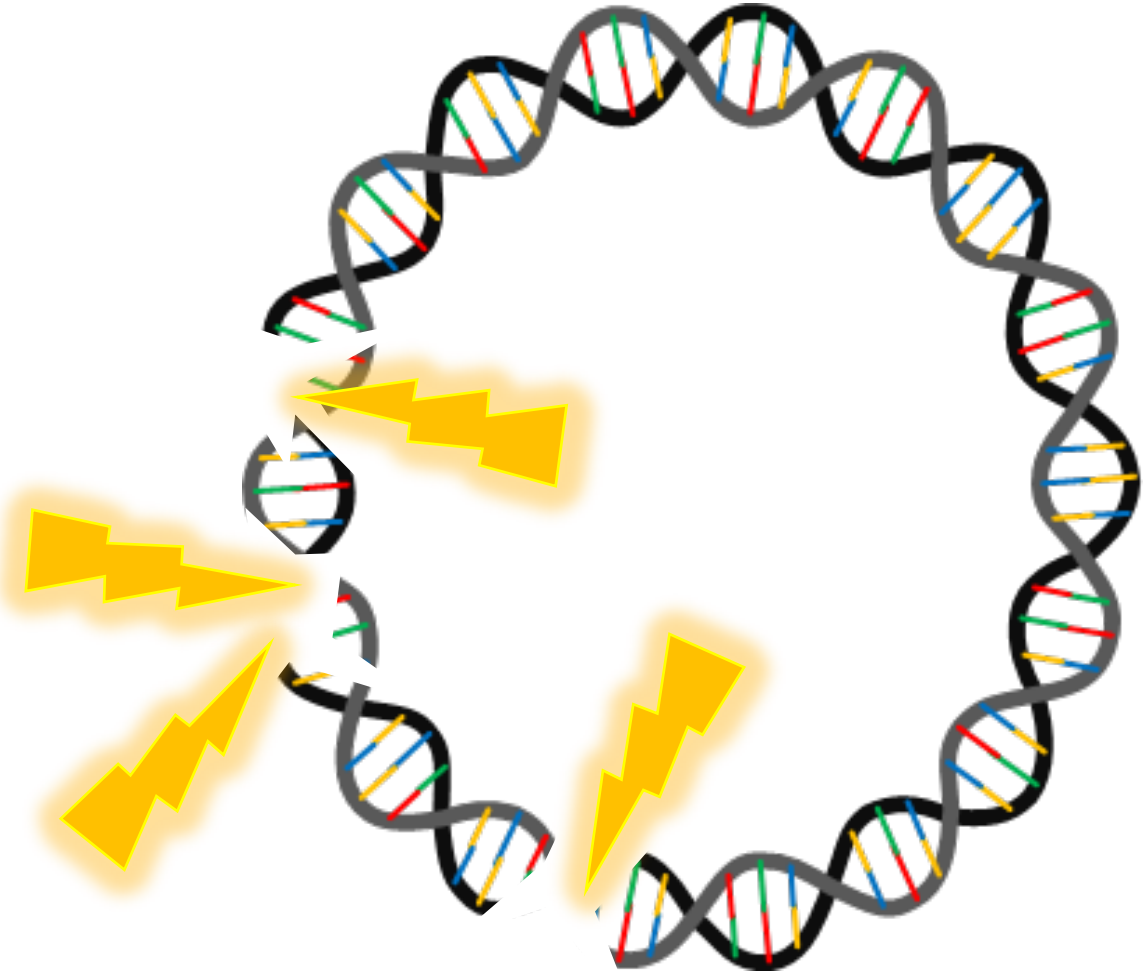
Fragment Selection and Amplification



Fluorescent Fragment Labeling

Bad Data In = Bad Data Out

- Rot, mold, and irradiation can cause DNA damage in original tissue sample.
- We noticed that rotten, moldy, and irradiated samples rarely amplified, so we set out to generate some numbers to test our observation.



Methods

Dataset includes 18,163 samples (Feb. 2019 – July 2021)

Feathers	Feather only, no tissue or blood present in sample.
Tissue/Blood	At least ½ a lentil sized dry material, alcohol wipe, or FTA card available in sample.
Irradiated	Sample subjected to harmful irradiation process by US Postal Service.
Moldy	Mold present on sample upon arrival.
Minimal	Minute amounts of tissue fragments, insect parts, or debris.
Not Visible	No visible tissue, blood, or biological material in sample.
Other	Does not fall into another category, ex. Hair, bone, fiber, plant, or non-biological.
Rotten	Visibly decomposing or smelly tissue.

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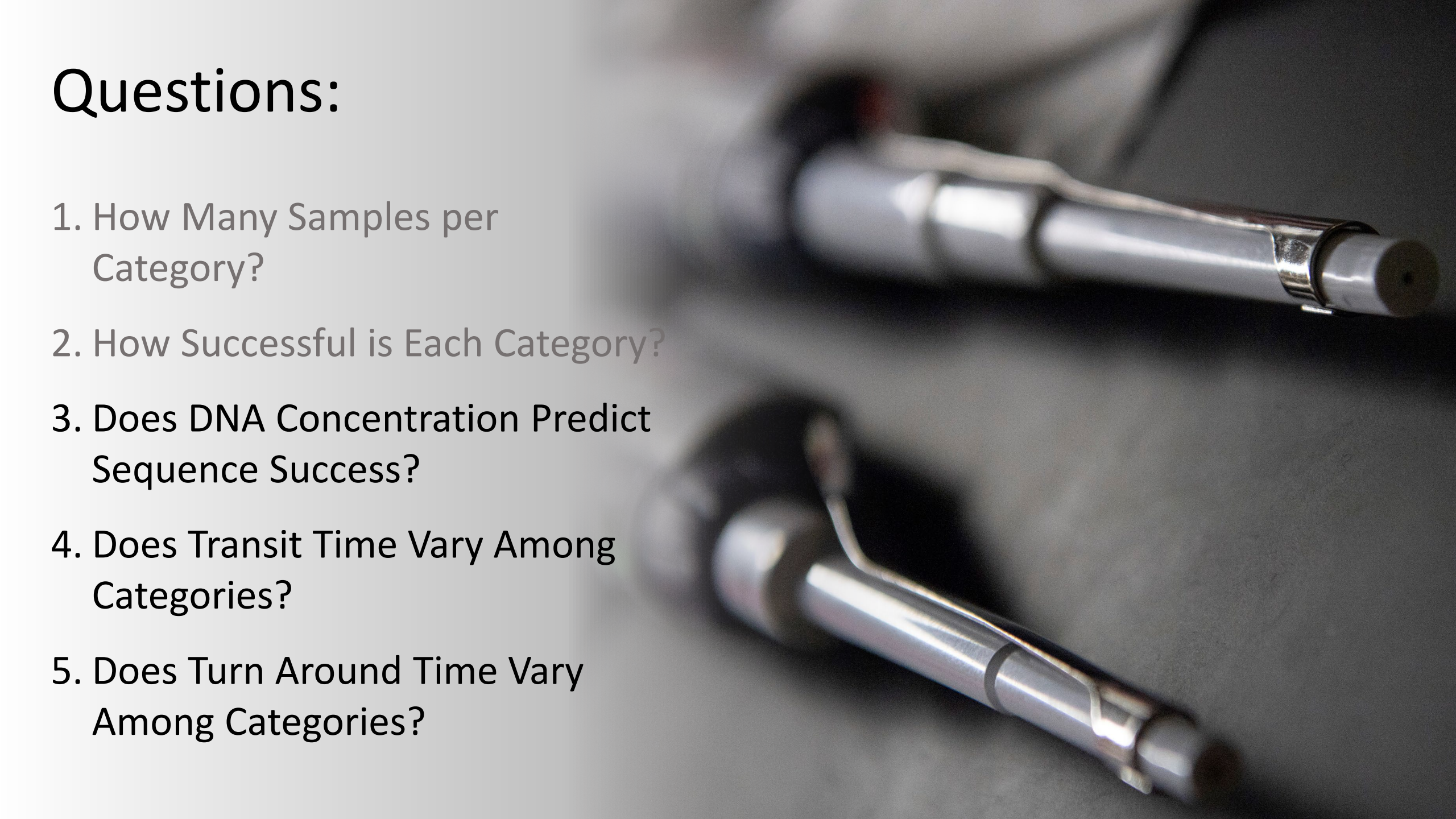
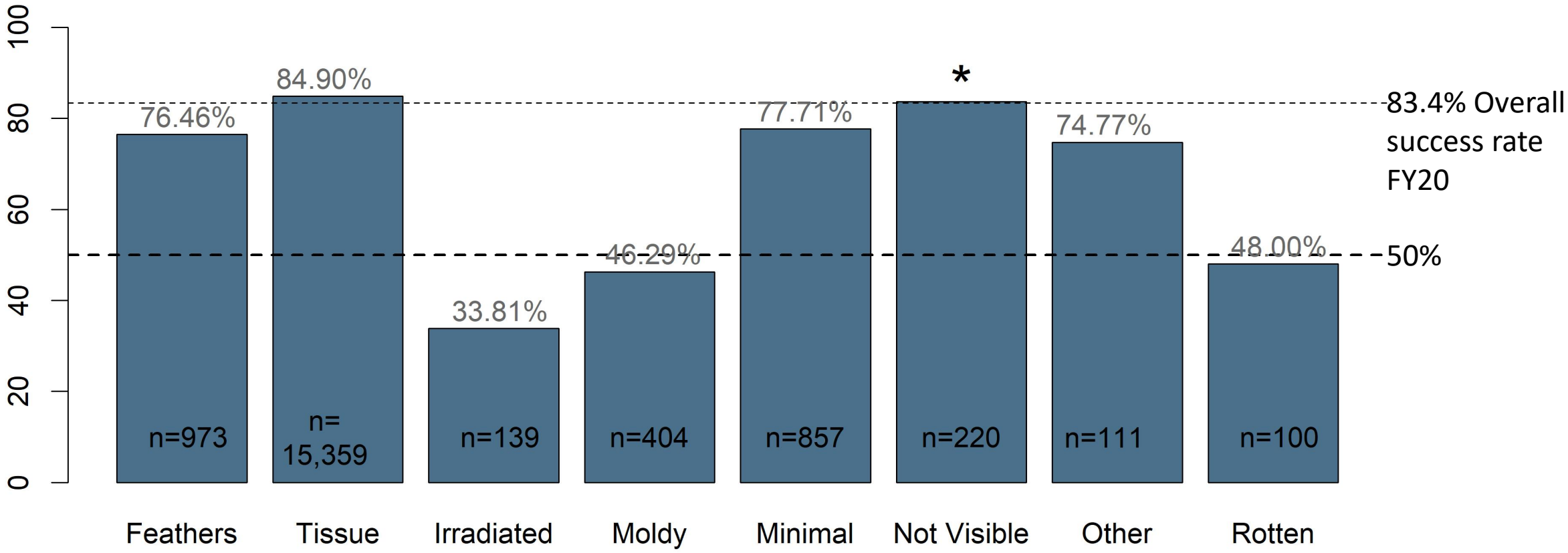
3. Does DNA Concentration Predict Sequence Success?
4. Does Transit Time Vary Among Categories?
5. Does Turn Around Time Vary Among Categories?



How many samples do we receive per category?

Quality Category	Percent	(Count)
Feathers	5.36 %	(973)
Tissue/Blood	84.56 %	(15,359)
Irradiated	0.77 %	(139)
Moldy	2.22 %	(404)
Minimal	4.72 %	(857)
Not Visible	1.21 %	(220)
Other	0.61 %	(111)
Rotten	0.55 %	(100)

2. Sequence Success per category



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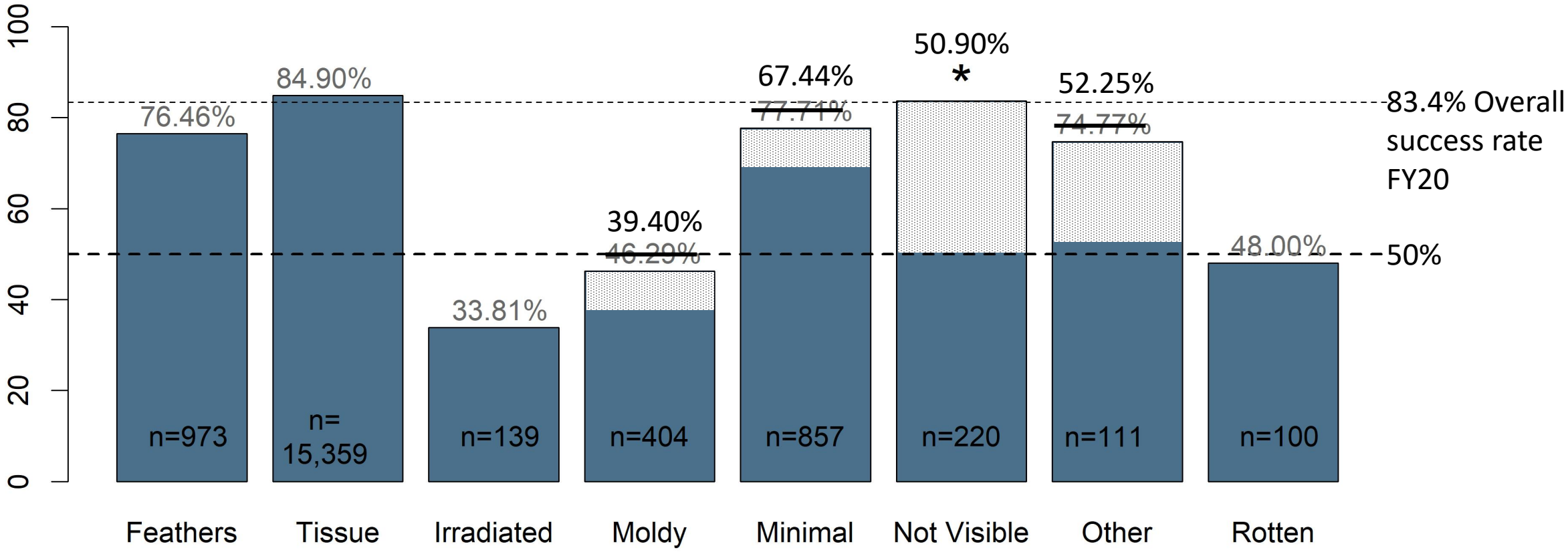
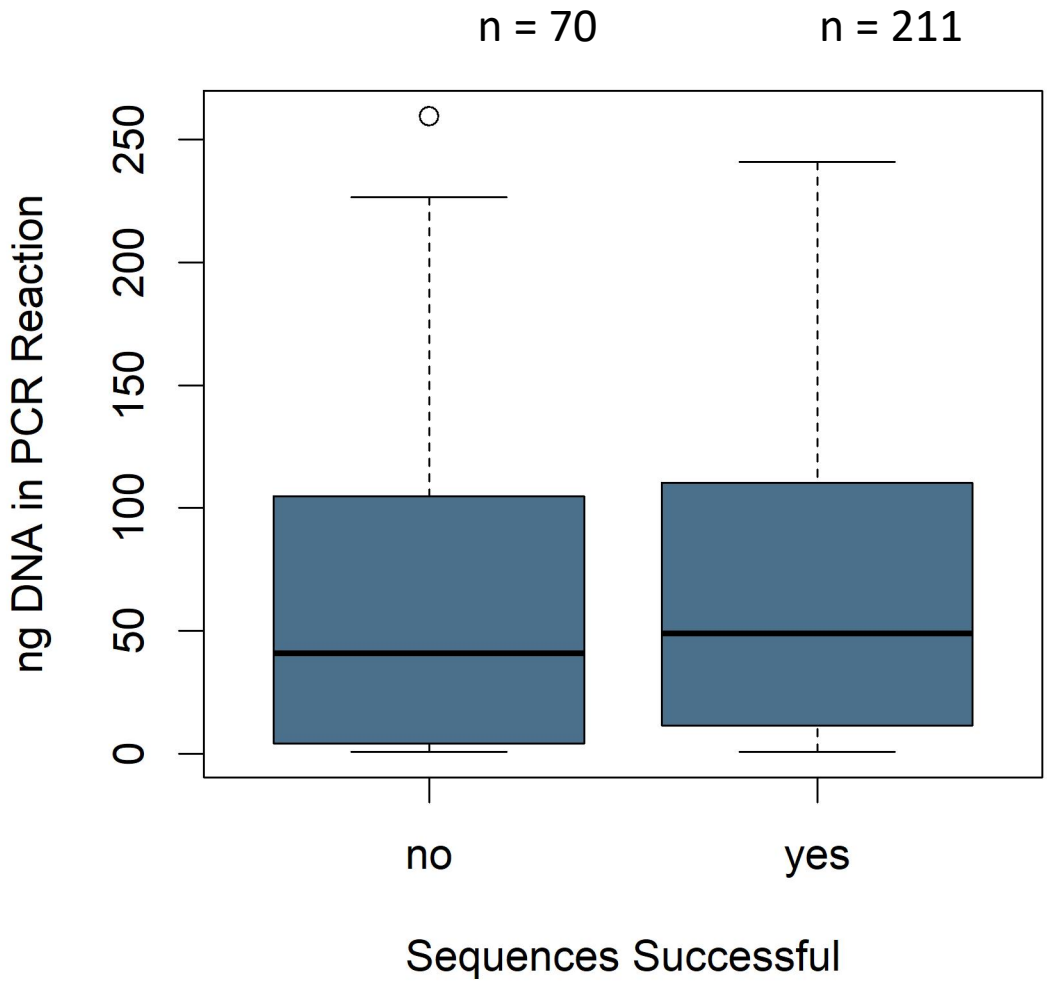


3. DNA Concentration And Sequencing Success

- Dataset = 281 samples
- No difference in DNA concentration between successful sequences and unsuccessful sequences.
- DNA quality, not quantity, or inhibitors in the reaction are the limiting factor in ability to get a sequence.

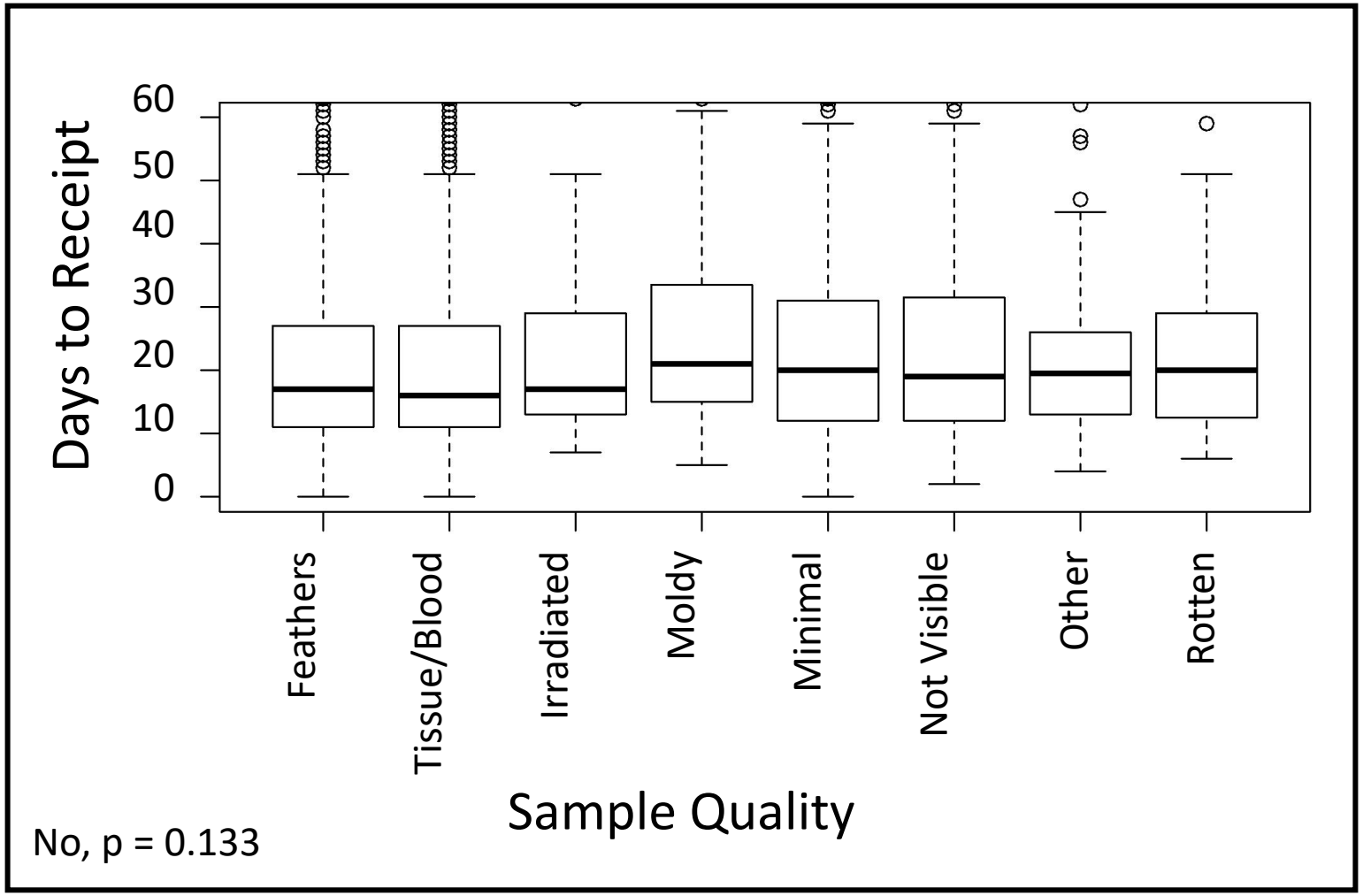


4. Transit Time By Category

- **No** statistical difference in time to receipt among sample category.

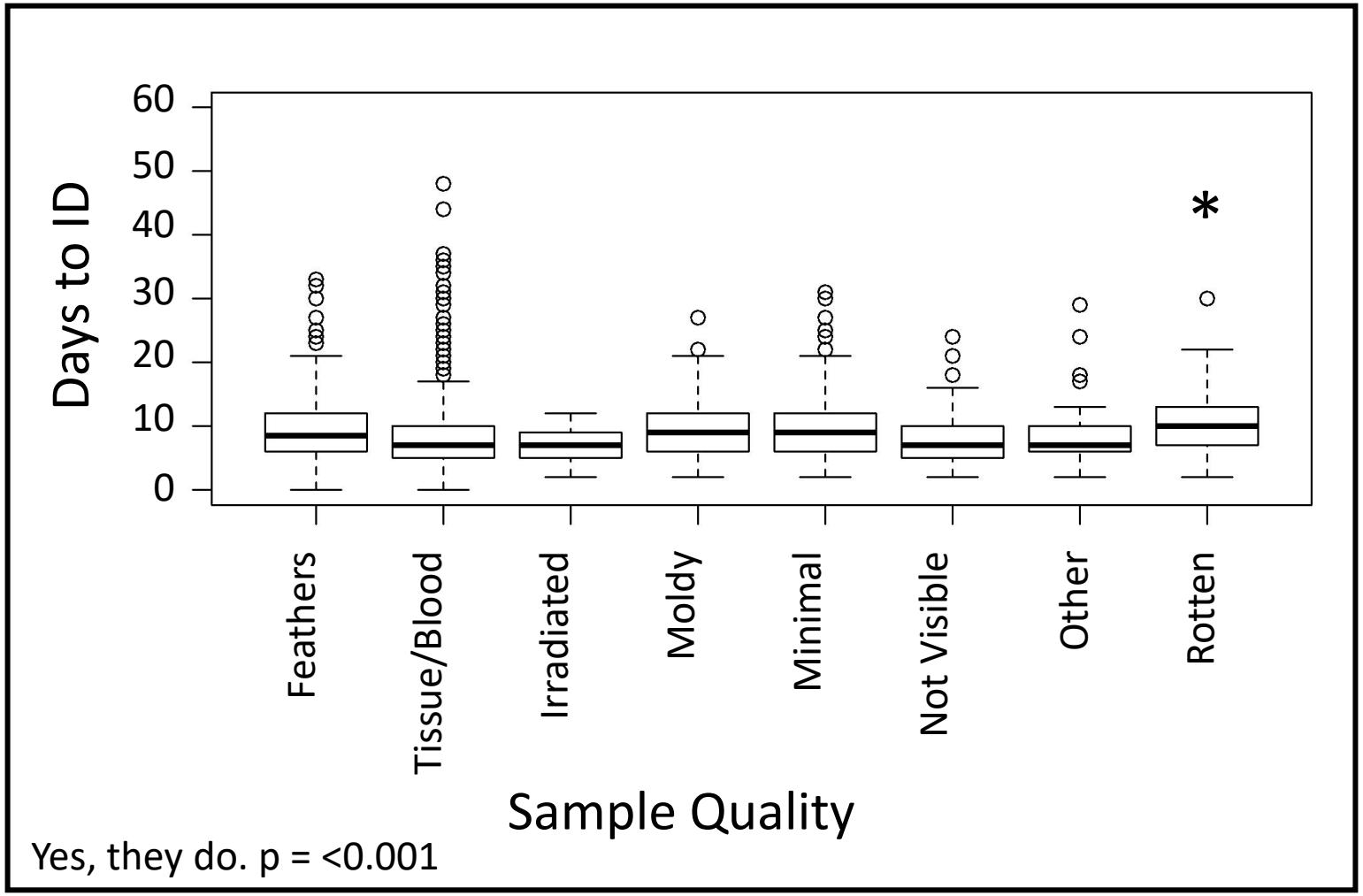
  (Kruskal-Wallis Signed Rank Comparison, p = 0.133).

- Most samples received 5-15 days after strike event (mean = 17 days).
- Lowest quality samples never reached the lab in less than 4 days.



5. Handling Time By Category

- Rotten samples take significantly longer than all other samples to ID.
- Irradiated samples take less time than Feather Samples.
- Large number of uutliers in Tissue/Blood and Feather samples due to resampling efforts.

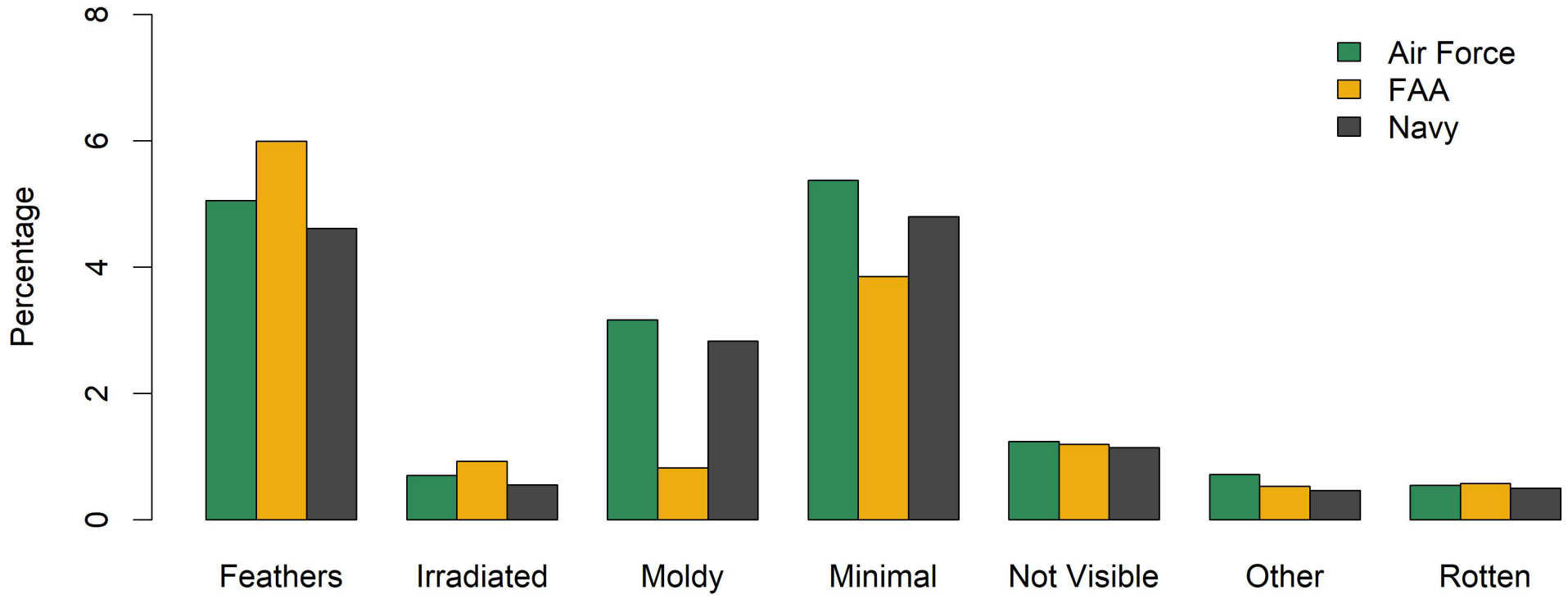


Yes, they do. p = <0.001

Poor Quality Samples are Not Agency Specific

Tissue/Blood (not pictured) is the highest category for all agencies.

Air Force = 83.21%
FAA = 86.13%
Navy = 85.11%



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- ***Help us improve your identifications and turn-around time by proper collection techniques.***

What Does this Mean for You?

When collecting remains:

Prioritize Large feathers that can be used for morphological analyses. Always include tissue or blood if available. Remember that even “Snarge” and minute amounts are useful.

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Allow the sample to dry before sealing in a re-sealable bag.

If sample cannot dry before transit it is at risk of degradation.

Thank You!

Please ask questions and join us Thursday for a Panel Discussion with the Feather ID Lab.