

# Stream Typing with Parallel Methodologies

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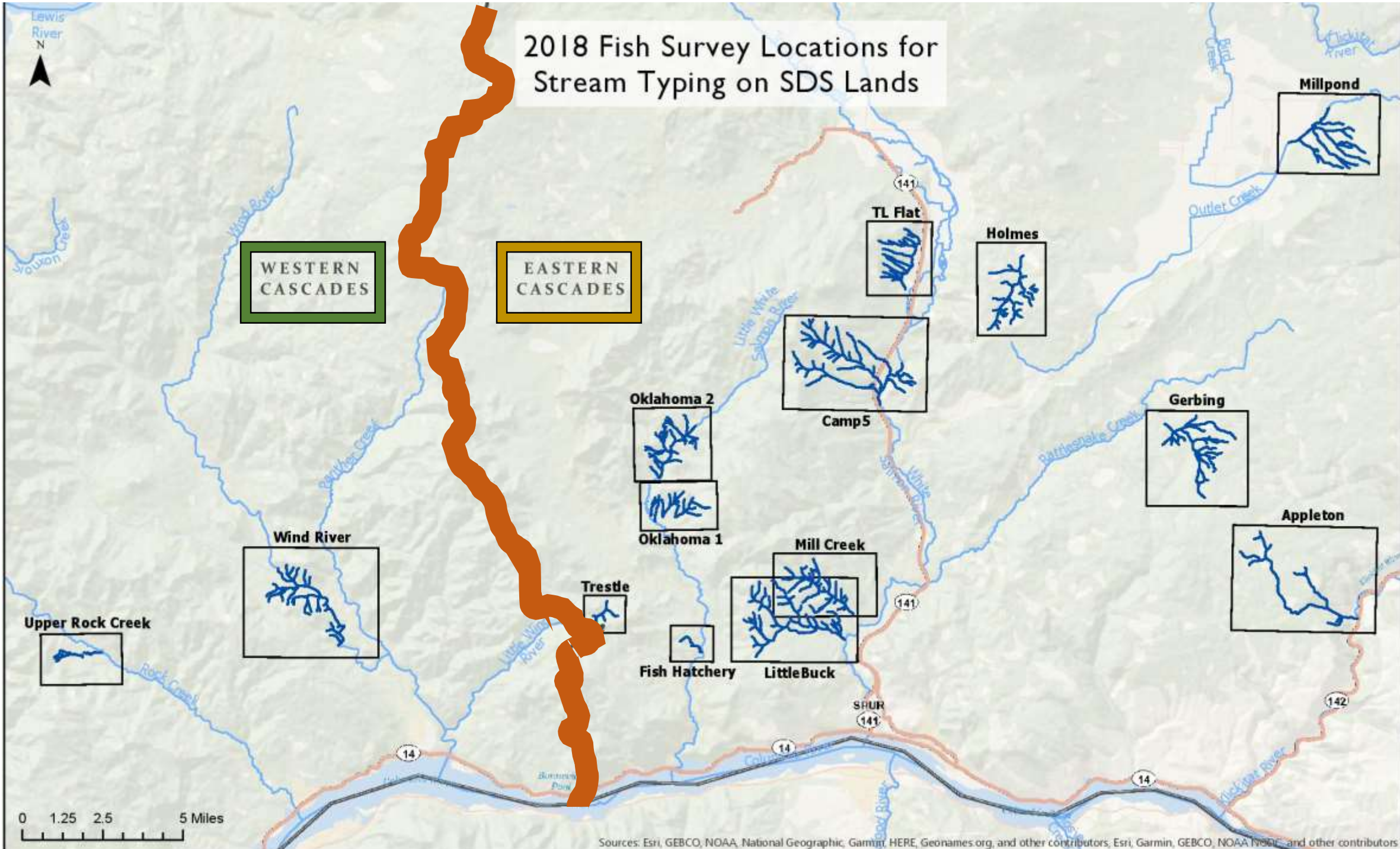
Scott Blankenship



# Summary

- Questions:
  - Can eDNA augment/replace e-fishing?
  - eDNA false negative rate?
    - sensitivity?
  - eDNA false positive rate?
    - specificity?
- Approach
  - Parallel methods
  - WDNR Protocol Fish Survey
    - e-Fishing
    - habitat survey
  - qPCR analyses of eDNA
    - species-specific

# 2018 Fish Survey Locations for Stream Typing on SDS Lands

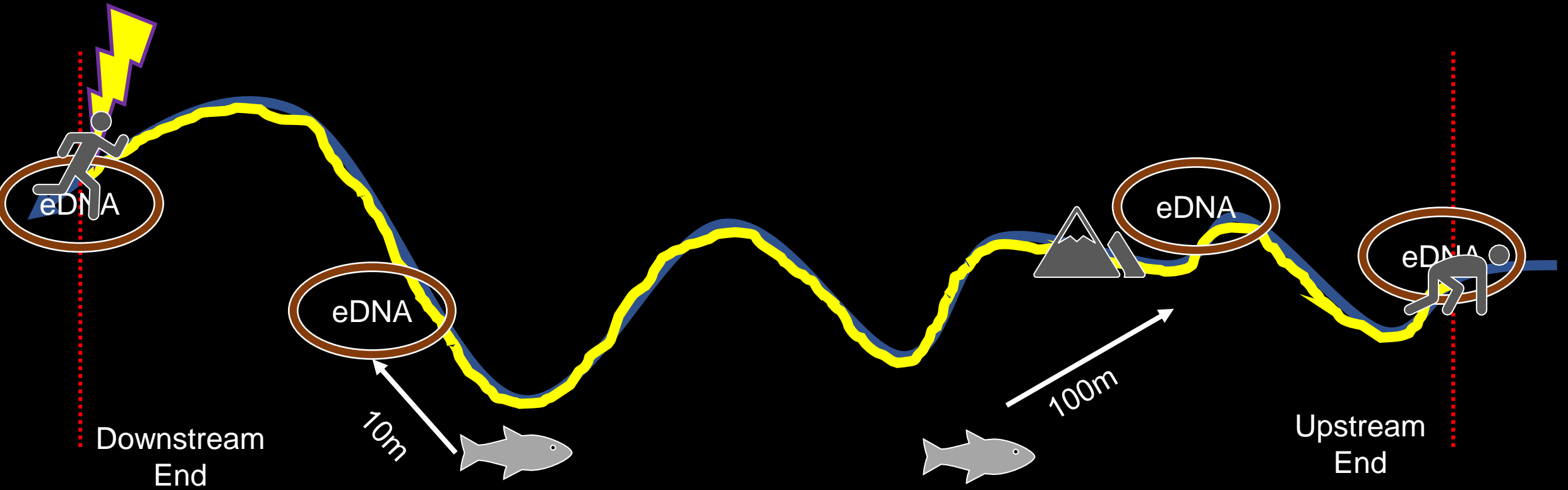


Sources: Esri, GEBCO, NOAA, National Geographic, Garmin, HERE, Geonames.org, and other contributors; Esri, Garmin, GEBCO, NOAA, and other contributors

# Survey Methods

- Field Operations:
  - conduct protocol survey
    - in-channel physical habitat survey
    - protocol fish survey
      - *backpack e-fishing*
  - eDNA water samples

# Survey Methods



# Lab Methods

- Analyze eDNA samples using qPCR
  - Target most abundant spp
    - *Oncorhynchus mykiss*
    - *O. clarkii*
    - *Salvelinus fontinalis*
  - qPCR is specific
  - qPCR is sensitive

# Sample summary

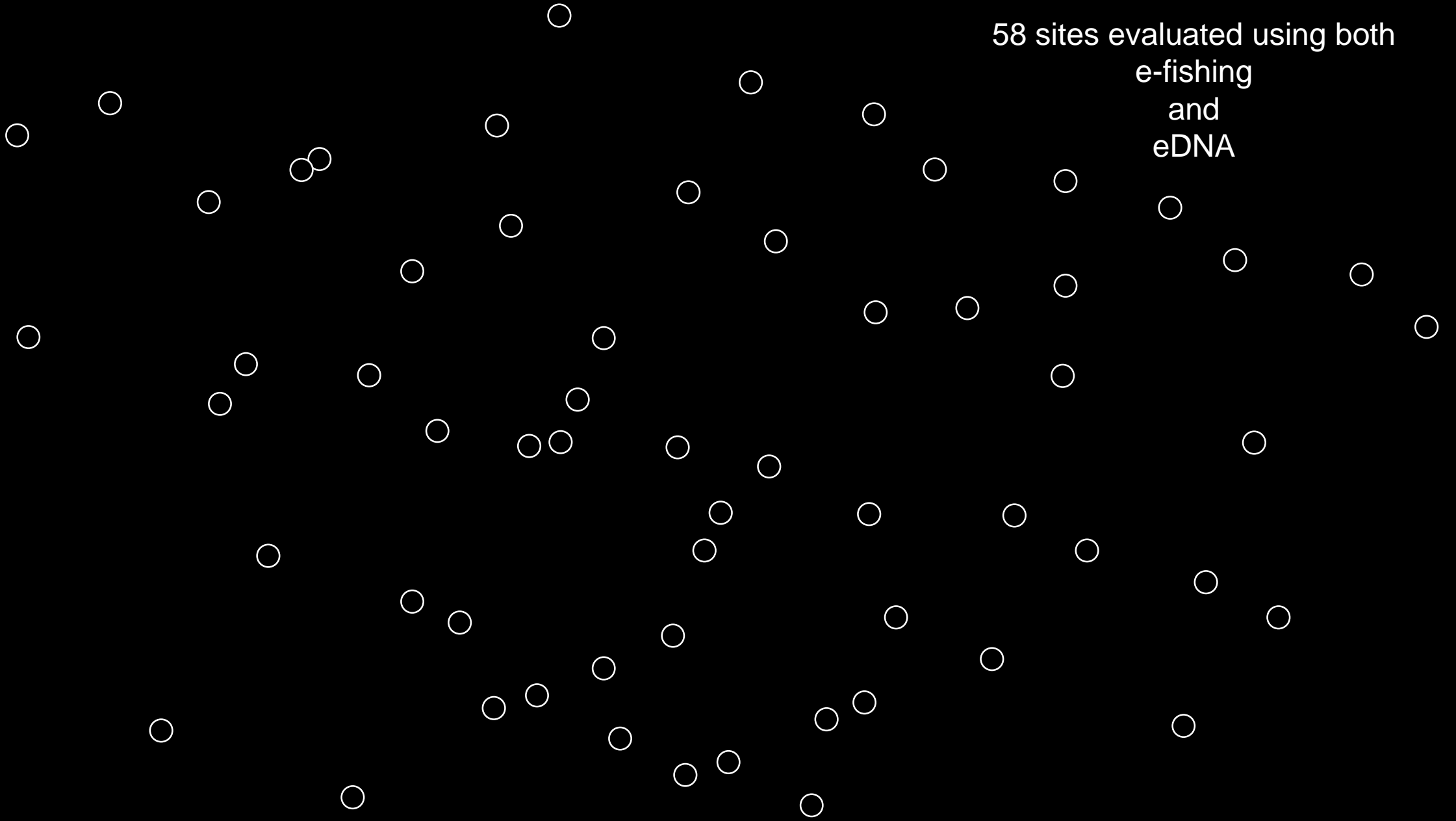
- 105 samples collected from 58 sites, including:
  - 16 un-replicated sites
    - (16 sites x 1 sample per site) = 16 samples
      - *Retain all 16 samples*
  - 42 replicated sites that returned identical results each rep
    - (38 sites x 2 samples per site) +  
(2 sites x 3 samples per site) +  
(2 sites x 4 samples per site) = 90 samples
      - *Retain 1x per site for 42 samples*
- ***So, all analyses based on n=58***

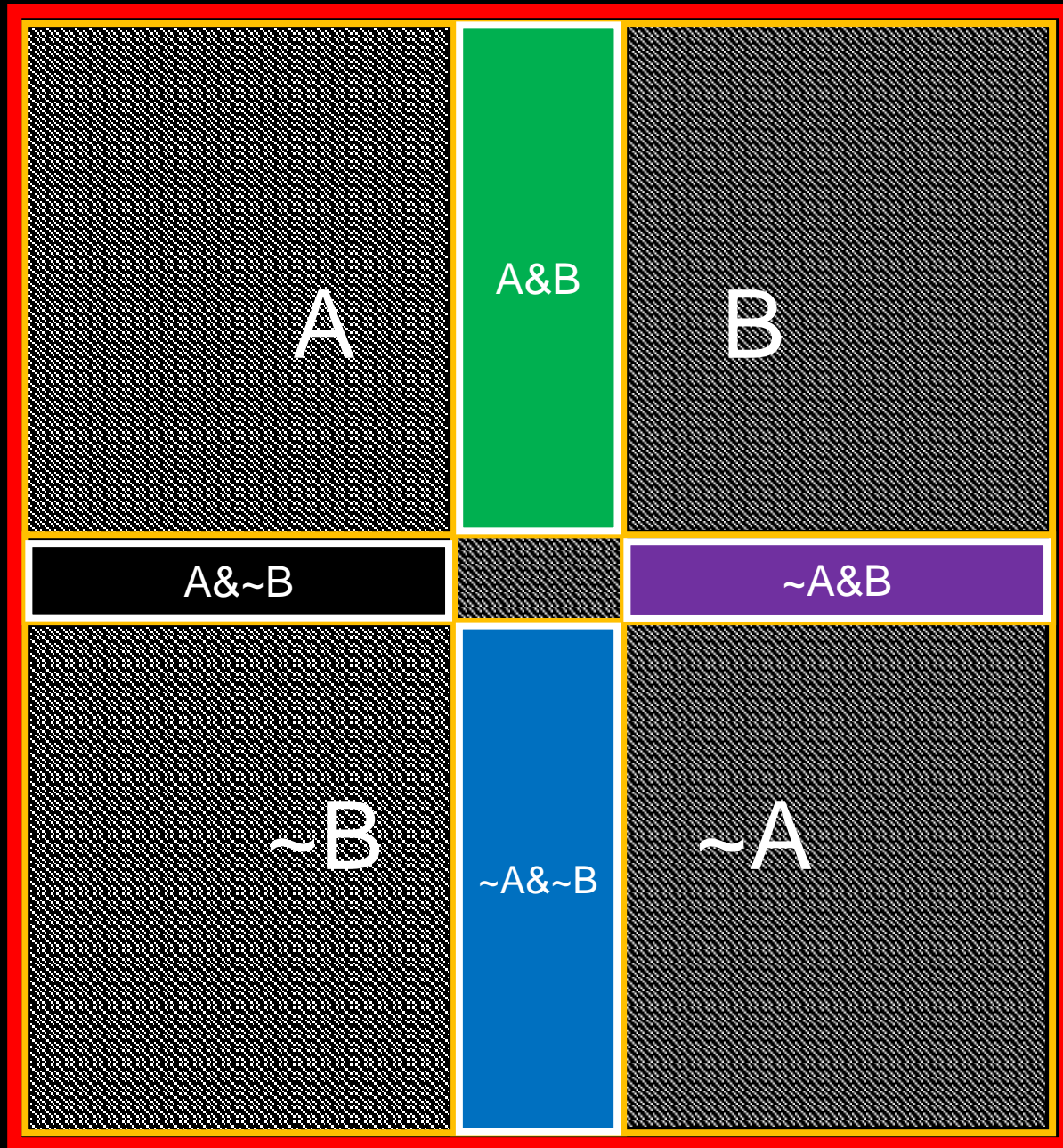
# Results

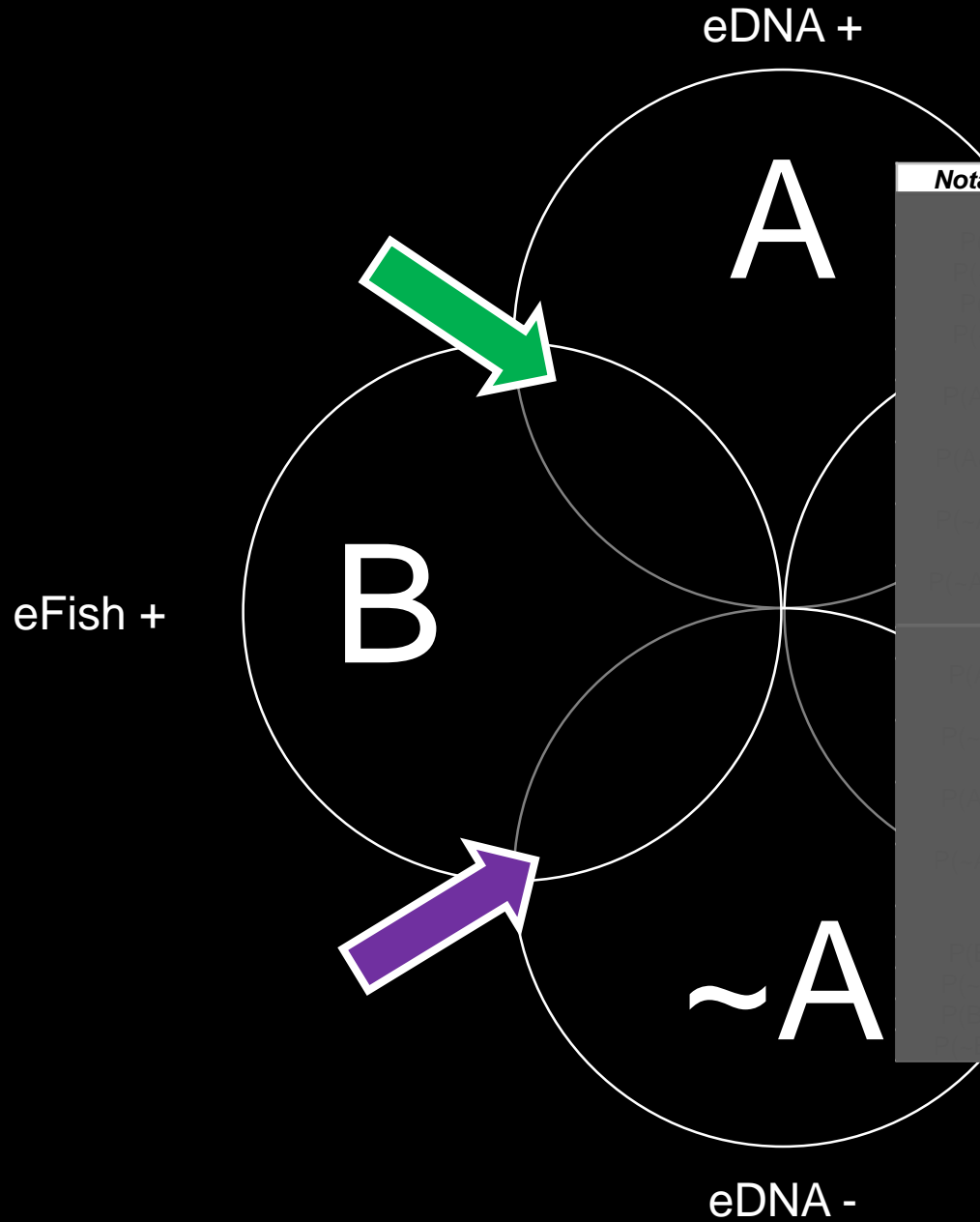
eDNA versus e-fishing



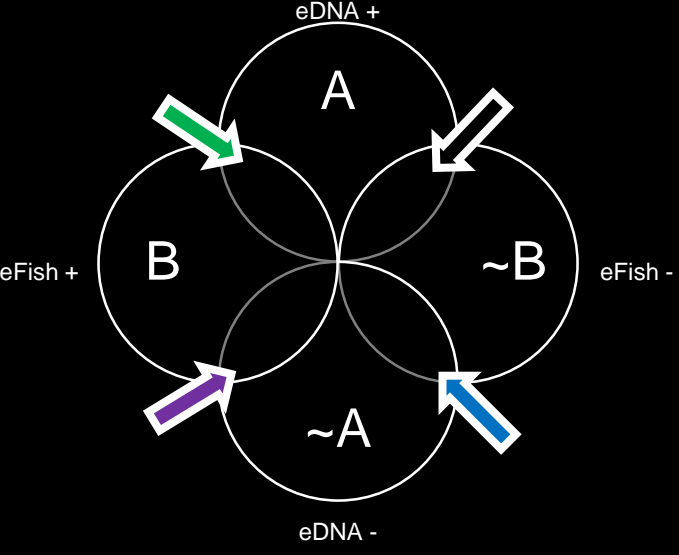
58 sites evaluated using both  
e-fishing  
and  
eDNA

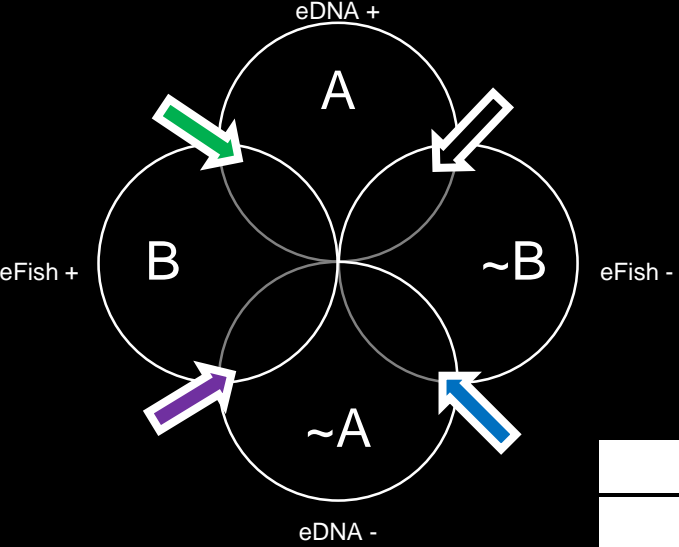






| <i>Notation</i> | <i>Explanation</i> |
|-----------------|--------------------|
|                 |                    |
|                 |                    |

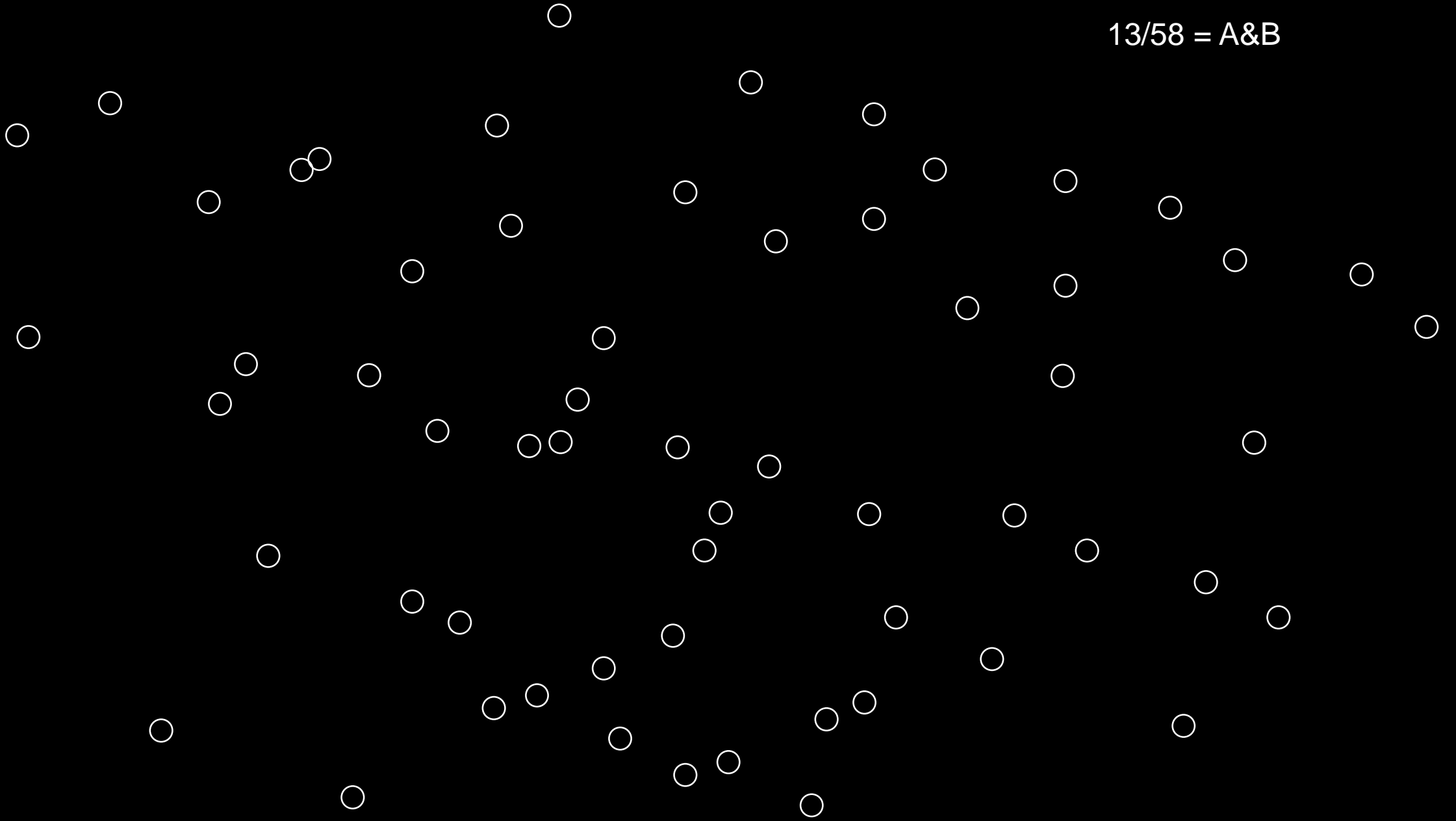


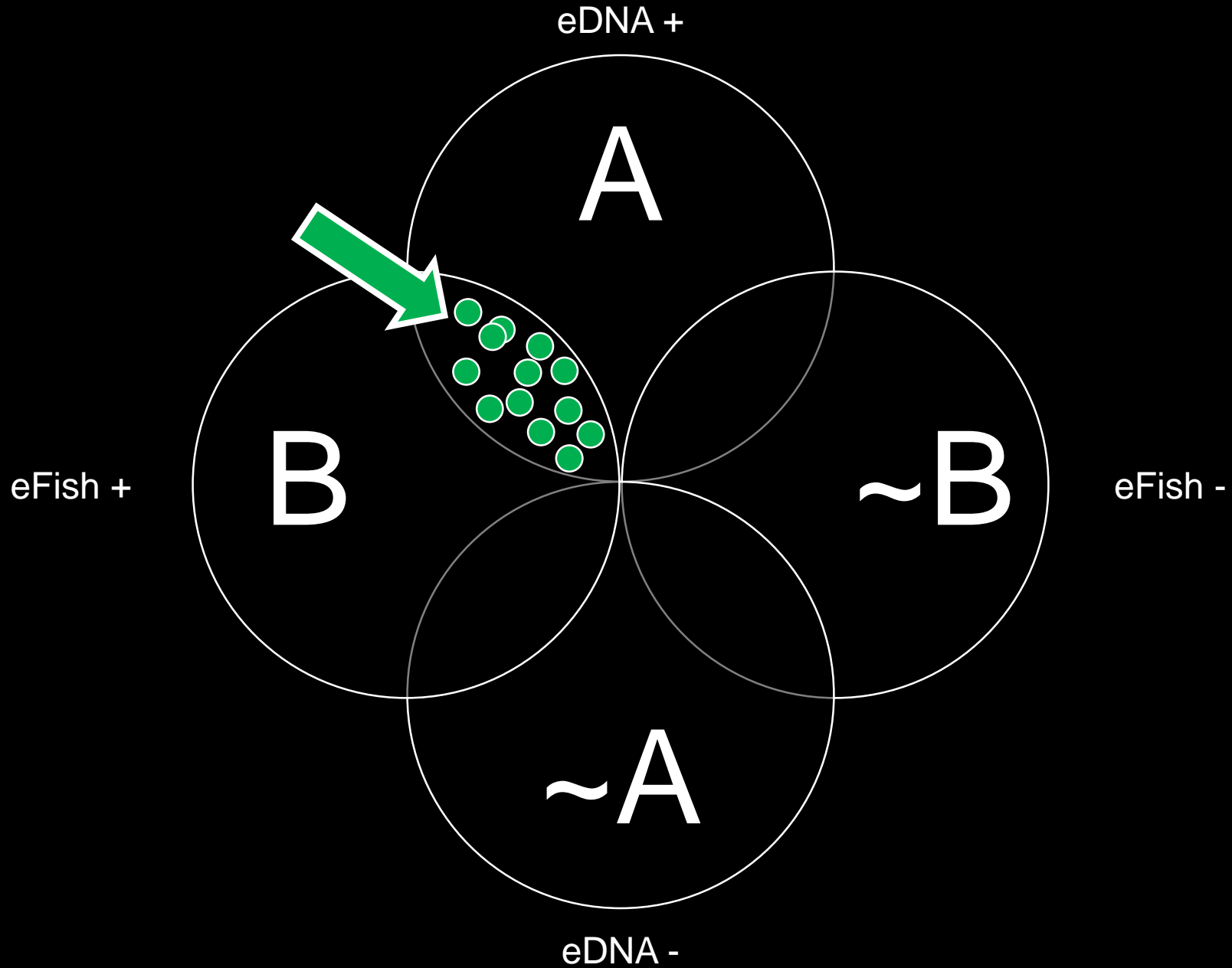


4-Fold Table ->

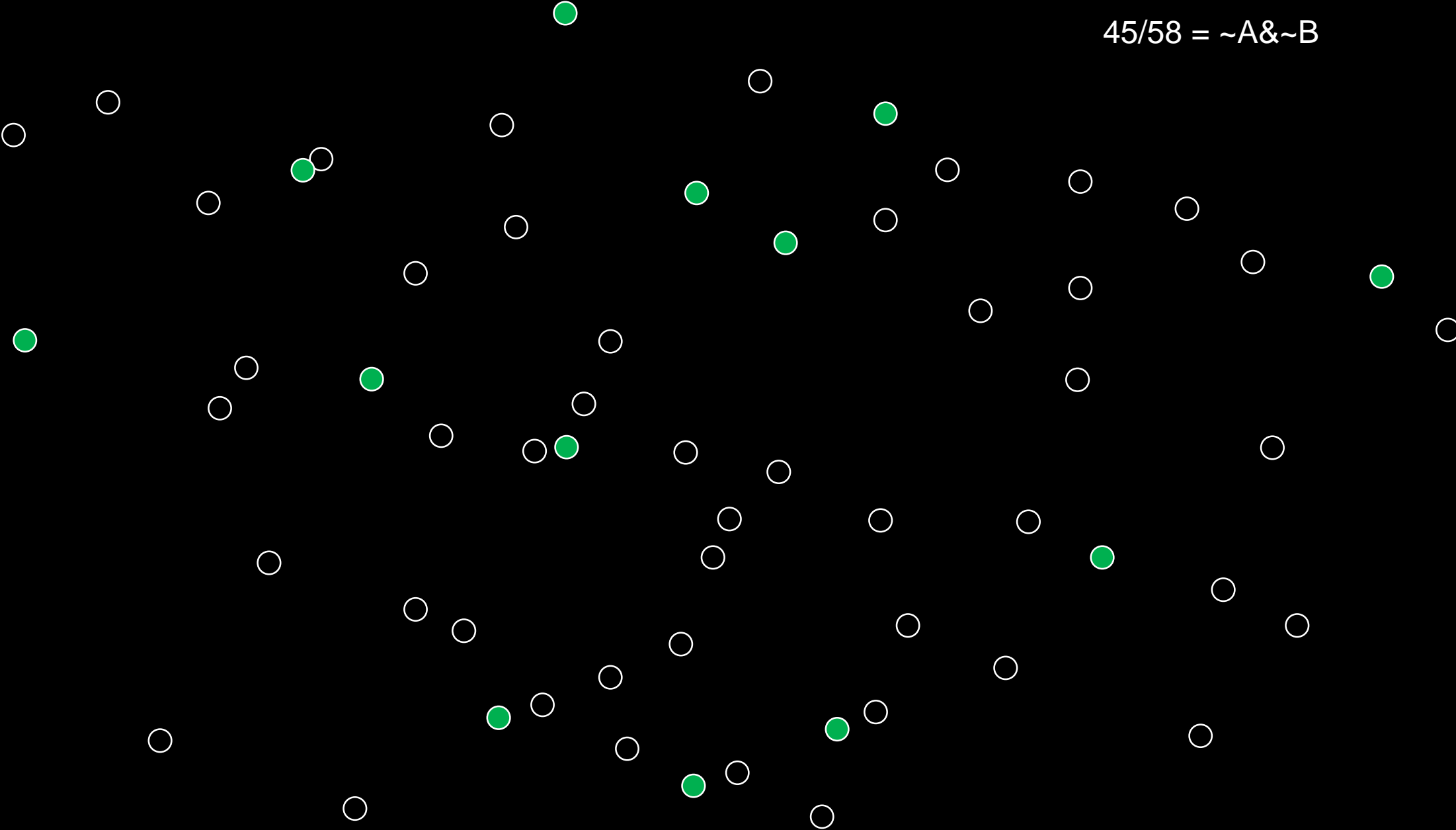
|              |                    | <i>A</i>       | <i>~A</i>      |              |
|--------------|--------------------|----------------|----------------|--------------|
|              |                    | <i>qPCR: +</i> | <i>qPCR: -</i> | <i>Total</i> |
| <i>B</i>     | <i>Protocol: +</i> | 13             | 0              | 13           |
| <i>~B</i>    | <i>Protocol: -</i> | 0              | 45             | 45           |
| <i>Total</i> |                    | 13             | 45             | 58           |

13/58 = A&B

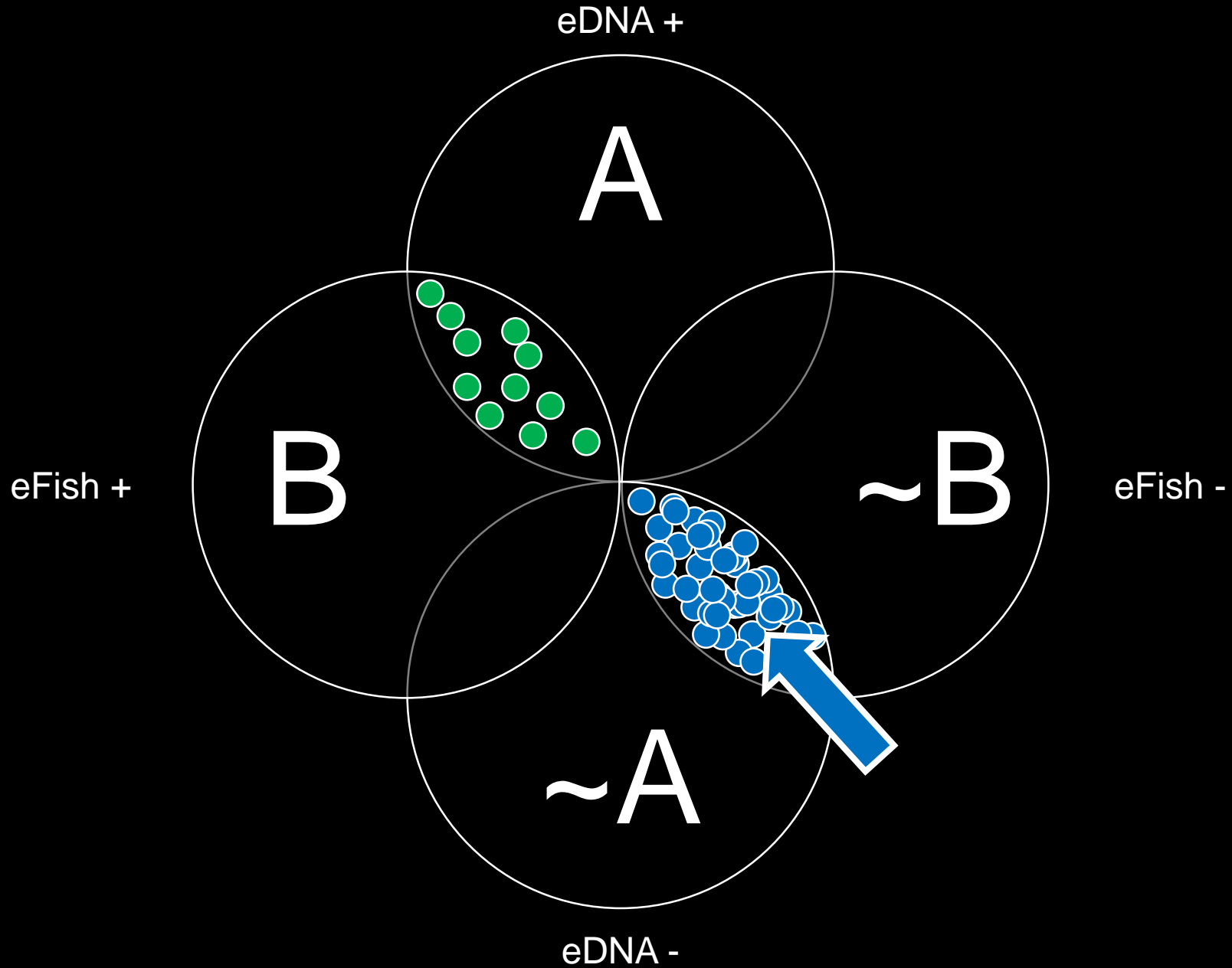




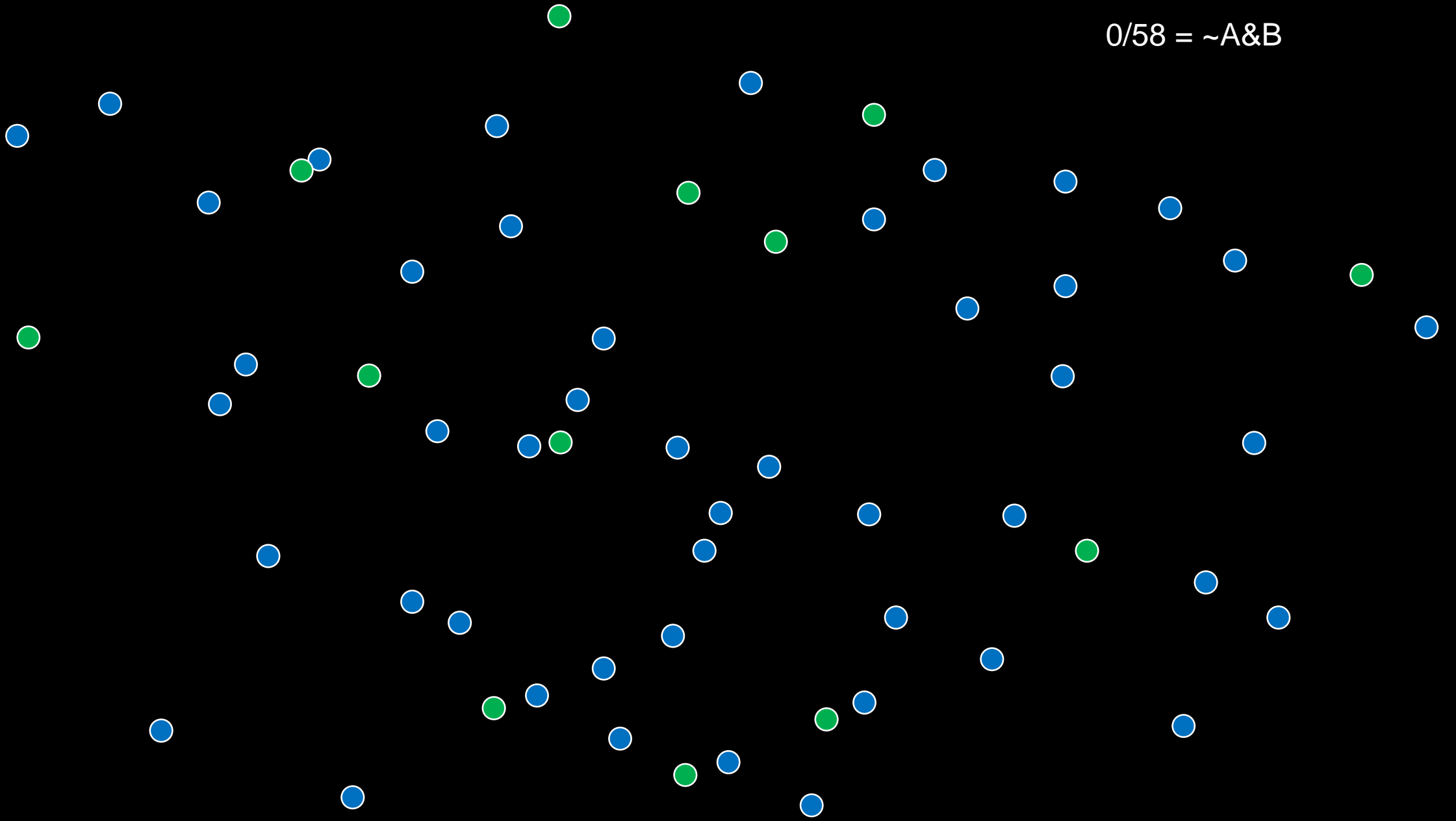
45/58 =  $\sim A \& \sim B$

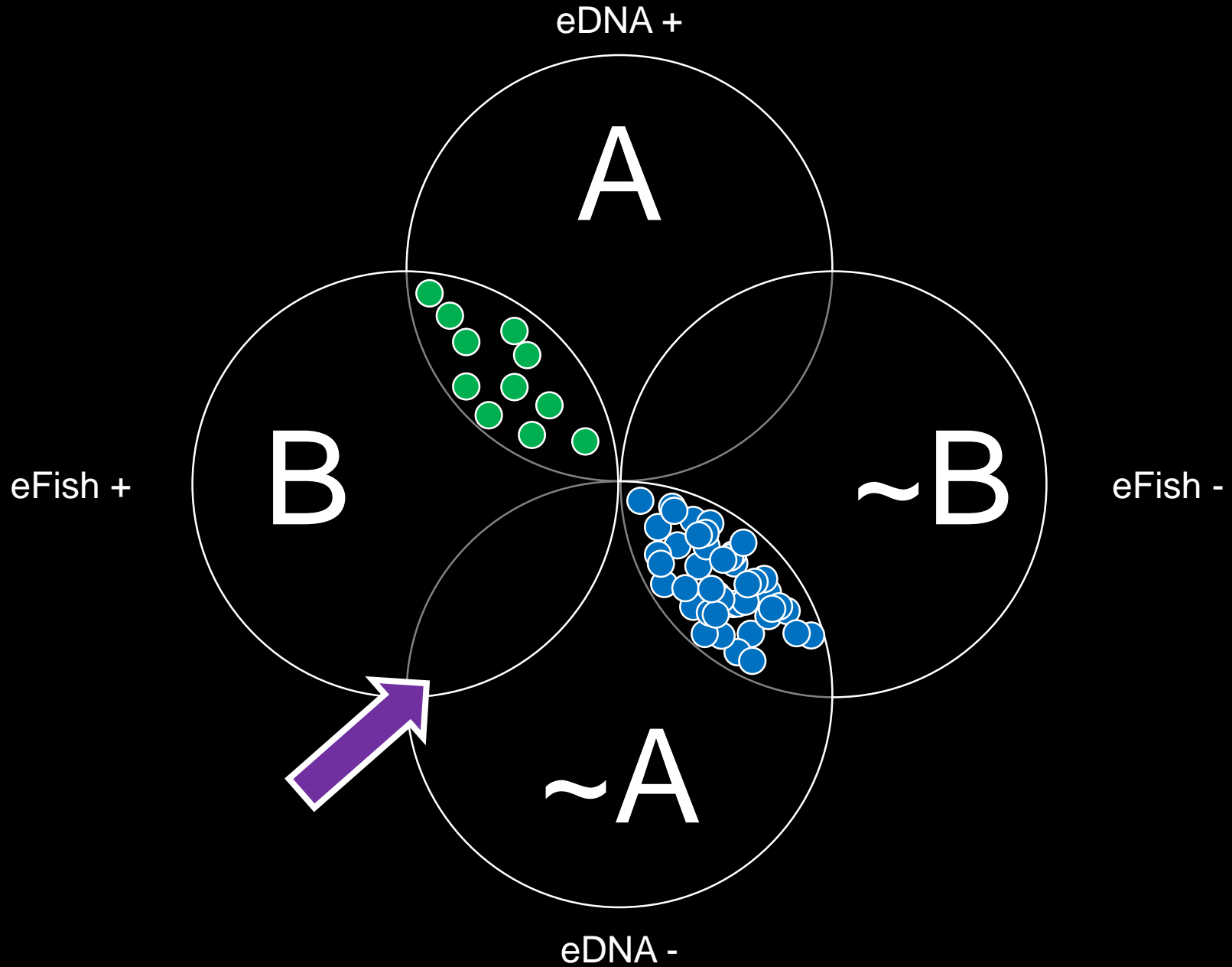




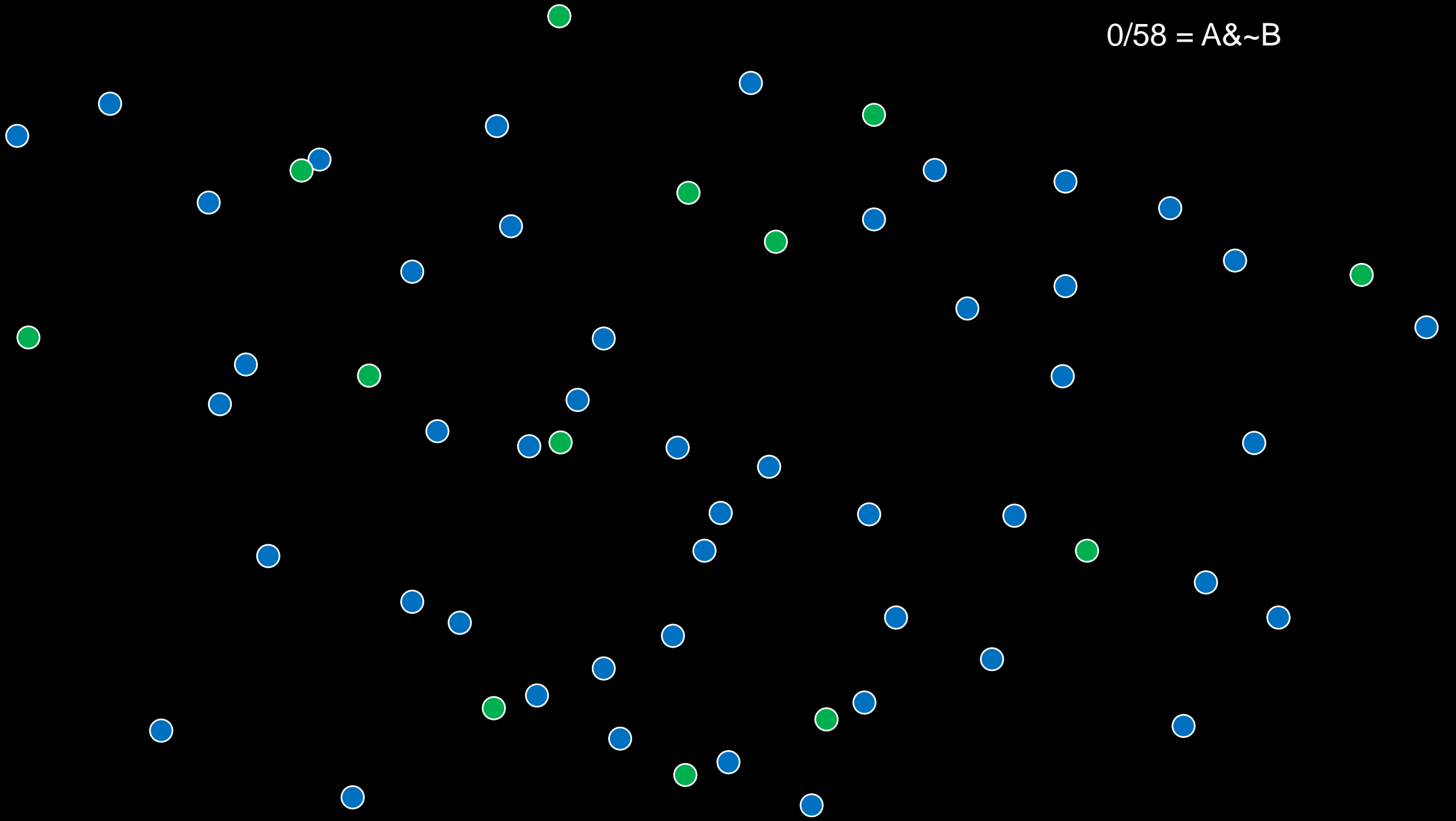


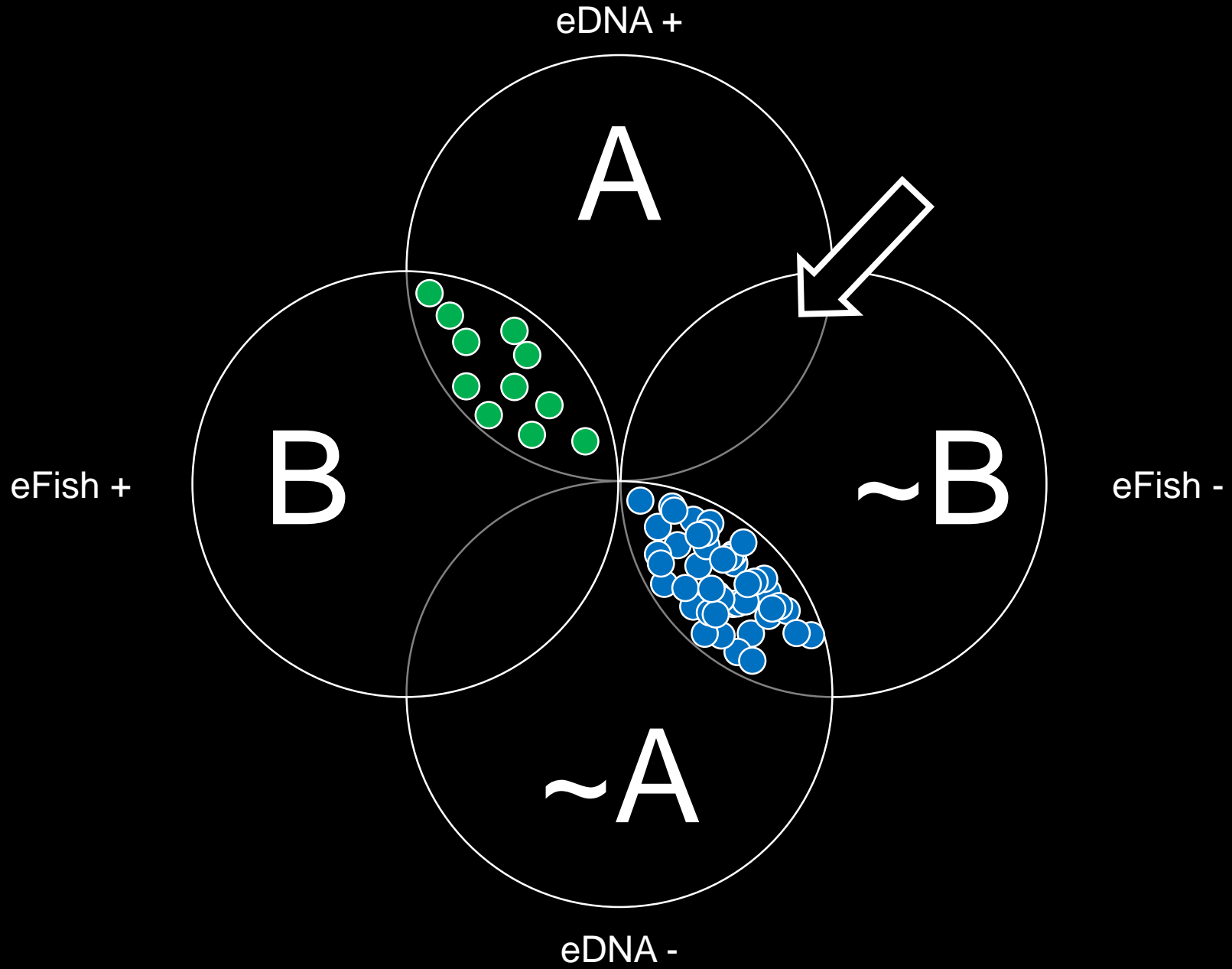
0/58 = ~A&B



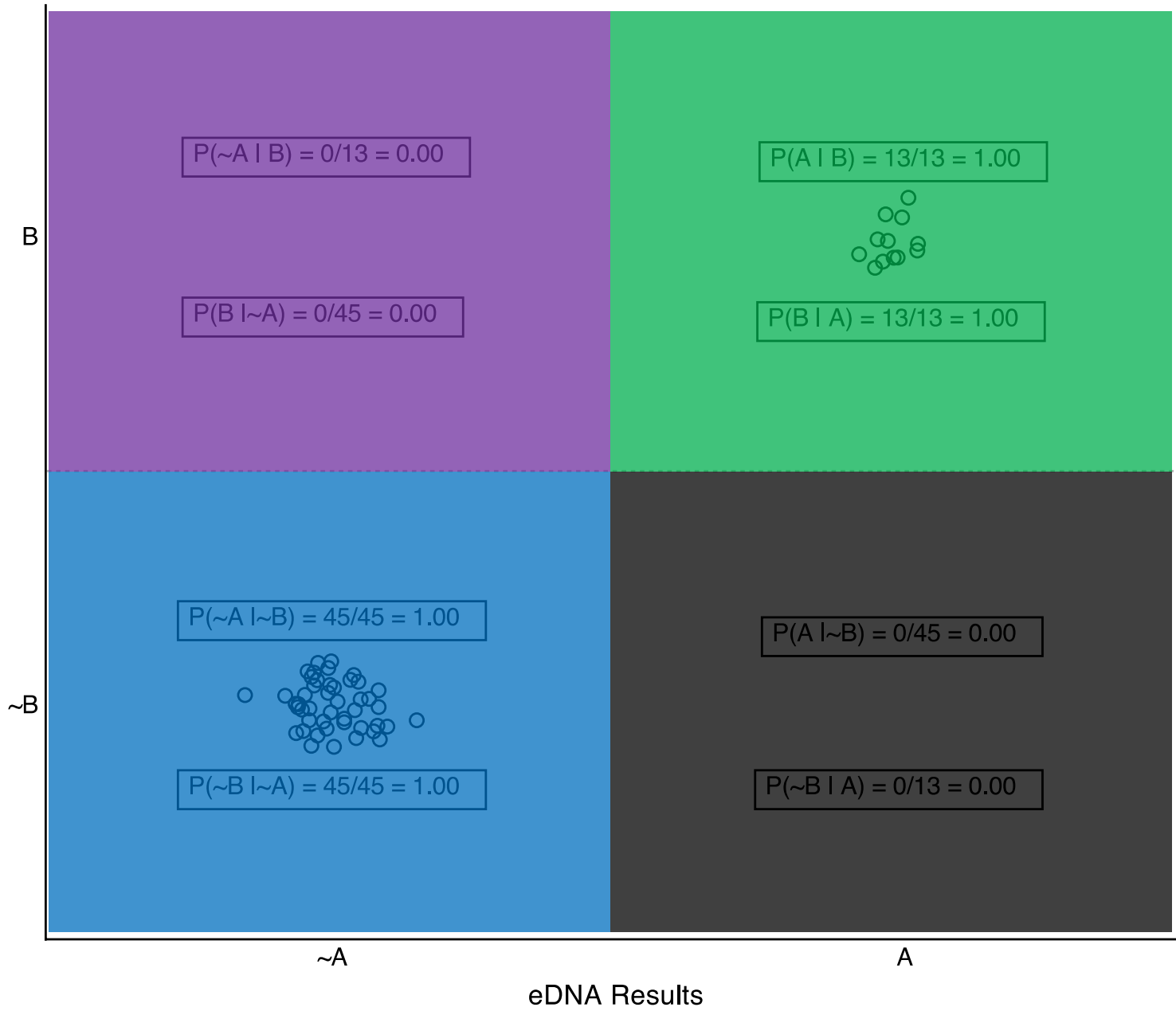


0/58 = A&~B





Protocol Survey Results



4-Fold Table ->

|              |             | A       | ~A      |       |
|--------------|-------------|---------|---------|-------|
|              |             | qPCR: + | qPCR: - | Total |
| B            | Protocol: + | 13      | 0       | 13    |
| ~B           | Protocol: - | 0       | 45      | 45    |
| <b>Total</b> |             | 13      | 45      | 58    |

58/58 = 100% agreement

Joints ->

| Notation              | Value  | Explanation  |
|-----------------------|--------|--|
| $P(A \& B)$           | 22.41% | Probability of BOTH a pos qPCR result and having fish (appropriate positive)       |
| $P(A \& \sim B)$      | 0.00%  | Probability of BOTH a pos qPCR result and not having fish (inappropriate positive) |
| $P(\sim A \& B)$      | 0.00%  | Probability of BOTH a neg qPCR result and having fish (inappropriate negative)     |
| $P(\sim A \& \sim B)$ | 77.59% | Probability of BOTH a neg qPCR result and not having fish (appropriate negative)   |

Conditionals,  
based on fish  
status ->

| Notation           | Value   | Explanation   |
|--------------------|---------|---|
| $P(A B)$           | 100.00% | Probability of a pos qPCR result, given fish (sensitivity)                |
| $P(\sim A B)$      | 0.00%   | Probability of a neg qPCR result, given fish (False Negative, TYPE I)     |
| $P(A \sim B)$      | 0.00%   | Probability of a pos qPCR result, given no fish (False Positive, TYPE II) |
| $P(\sim A \sim B)$ | 100.00% | Probability of a neg qPCR result, given no fish (specificity)             |

Test  
diagnostics

Conditionals,  
based on test  
results ->

| Notation           | Value   | Explanation                                     |
|--------------------|---------|---|
| $P(B A)$           | 100.00% | Probability of fish, given pos qPCR result      |
| $P(\sim B A)$      | 0.00%   | Probability of no fish, given pos qPCR result   |
| $P(B \sim A)$      | 0.00%   | Probability of fish, given negative qPCR result |
| $P(\sim B \sim A)$ | 100.00% | Probability of no fish, given neg qPCR result   |

Test  
interpretation

# For those who love $p$ -values

- Cramér's  $V$  ( $\varphi_c$ ) test of strength of association
  - Ranges in strength from 0-1
  - Results
    - Effect strength of 0.95
  - Interpretation
    - Very strong association between e-fishing and eDNA results
- 2-tailed Fisher's exact test of independence
  - Results
    - $N= 58$ ,  $DF=1$ ,  $p<0.0001$
  - Interpretation
    - qPCR results significantly differed among eDNA result categories



# Conclusions

- Questions:
  - If we assume e-fishing always detects truth, what are false positive and negative rates associated with eDNA?
    - qPCR Results and backpack e-fishing found same results ***in all cases***
      - ***i.e., qPCR was 100% accurate***
    - Compared to e-fishing, qPCR returned zero false negatives, zero false positives
  - If qPCR +
    - 100% observed probability of fish detection using backpack e-fishing
  - If qPCR –
    - 100% observed probability of no fish detection using backpack e-fishing

# Conclusions

- Questions:
  - e-fishing is current standard; possible to augment/replace with eDNA?
  - ?



Questions?  
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