

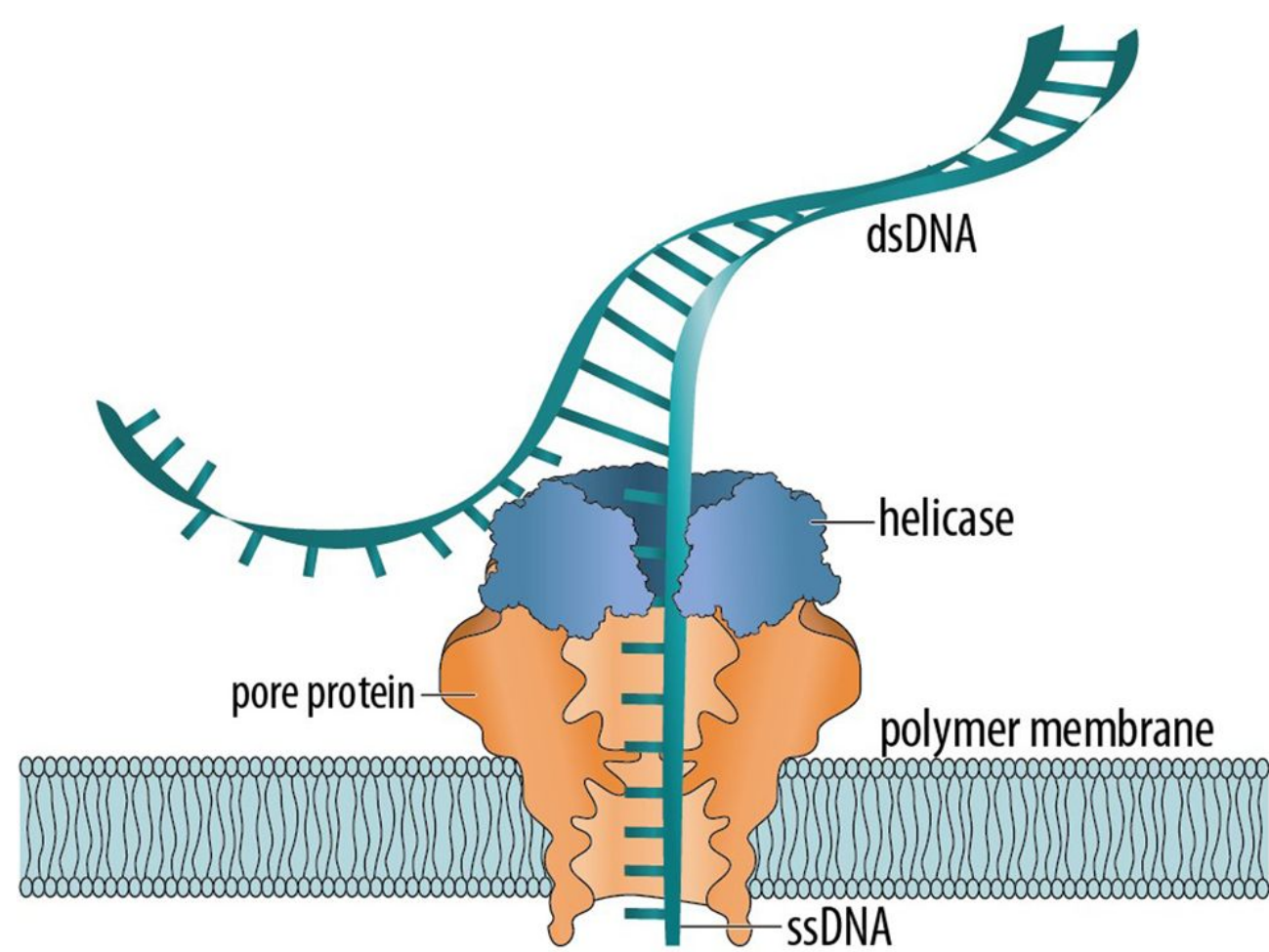
Whole Genome Sequencing of *Rhinichthys cataractae* Using MinION Technology



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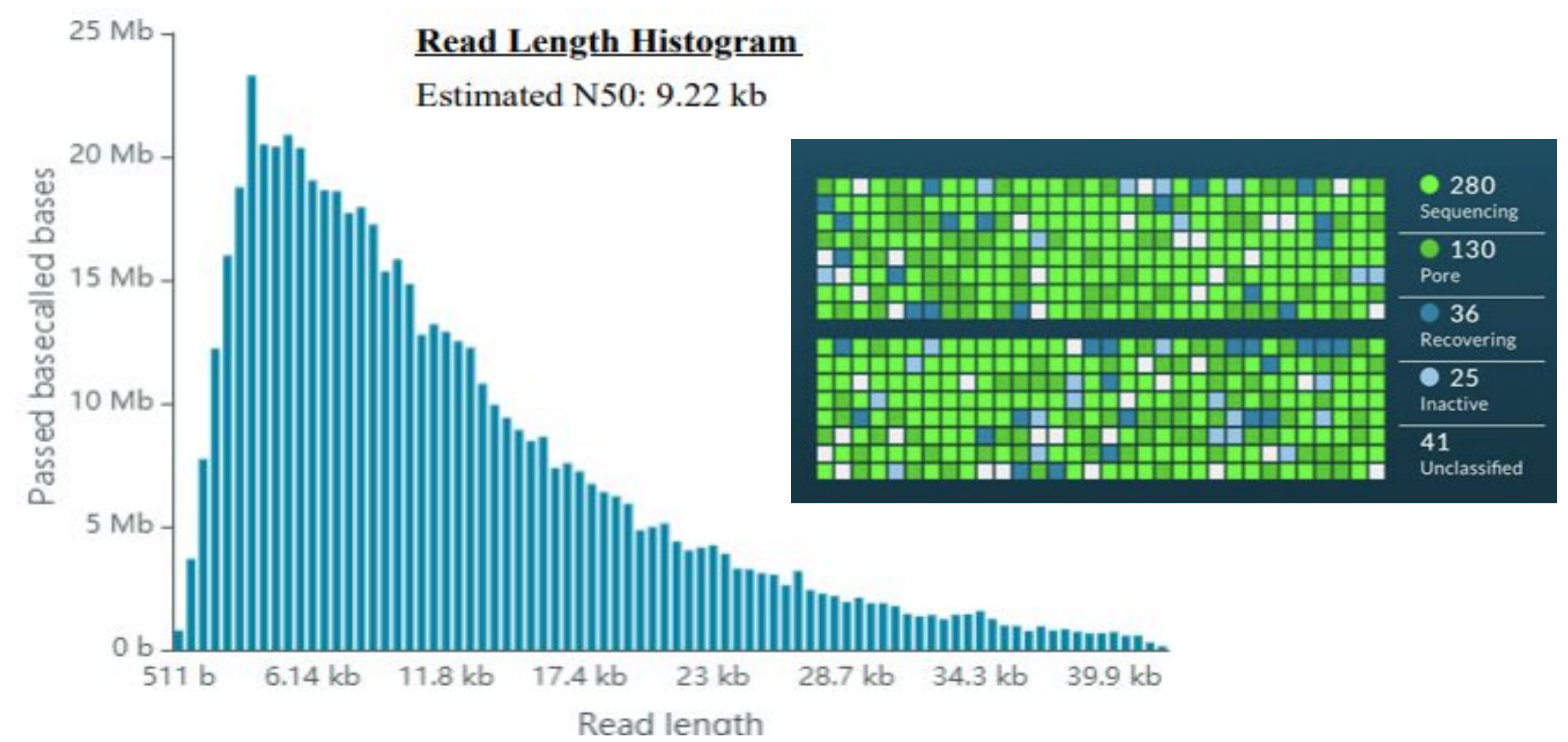
BACKGROUND

- Longnose dace, *Rhinichthys cataractae*, are riffle-dwelling minnows native to freshwater systems in Connecticut
- Whole genomic sequencing of non-model organisms is very limited--the closest sequenced relative to Longnose dace, the Zebrafish, *Danio rerio*, diverged 150 million years ago (Tan et. al.)
- A sequenced genome will allow us to study the effects of anthropogenic and climatic factors on the genetic structures of fish populations
- MinION technology provides a rapid, long-read, lower-cost, portable alternative to Illumina sequencing

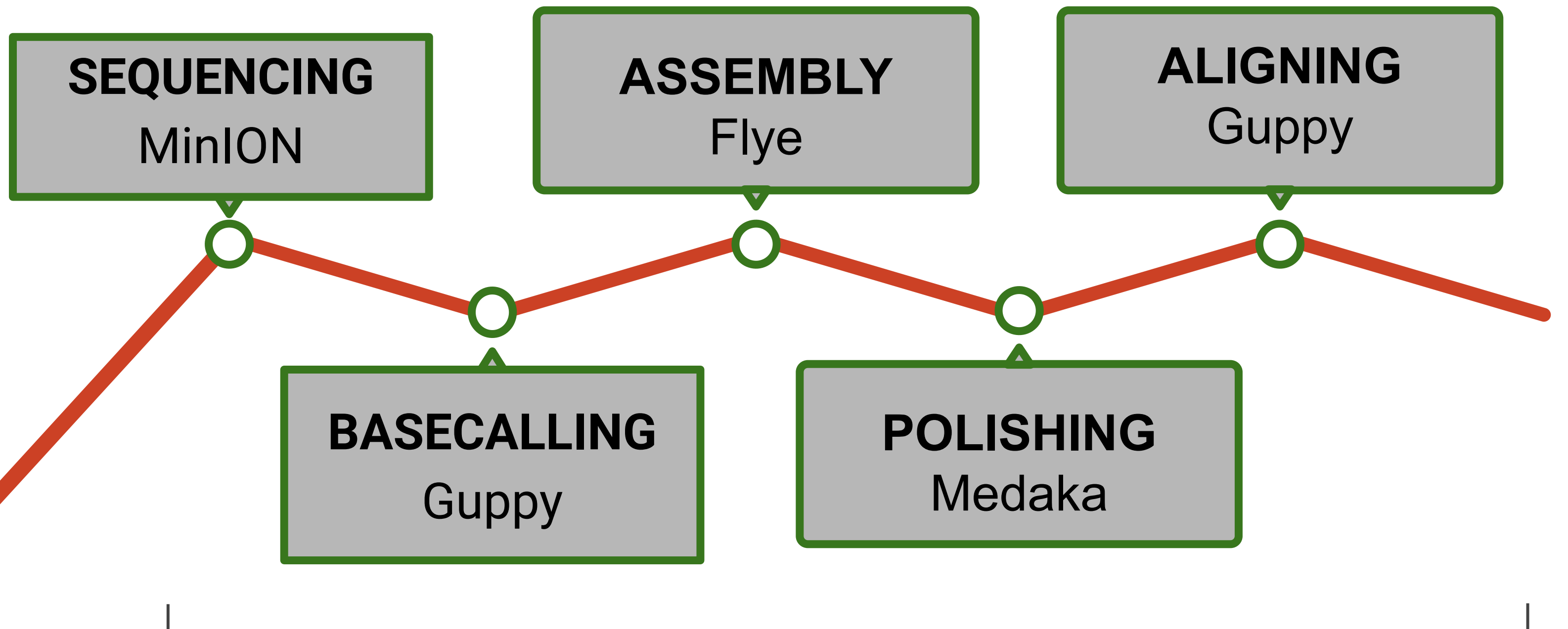
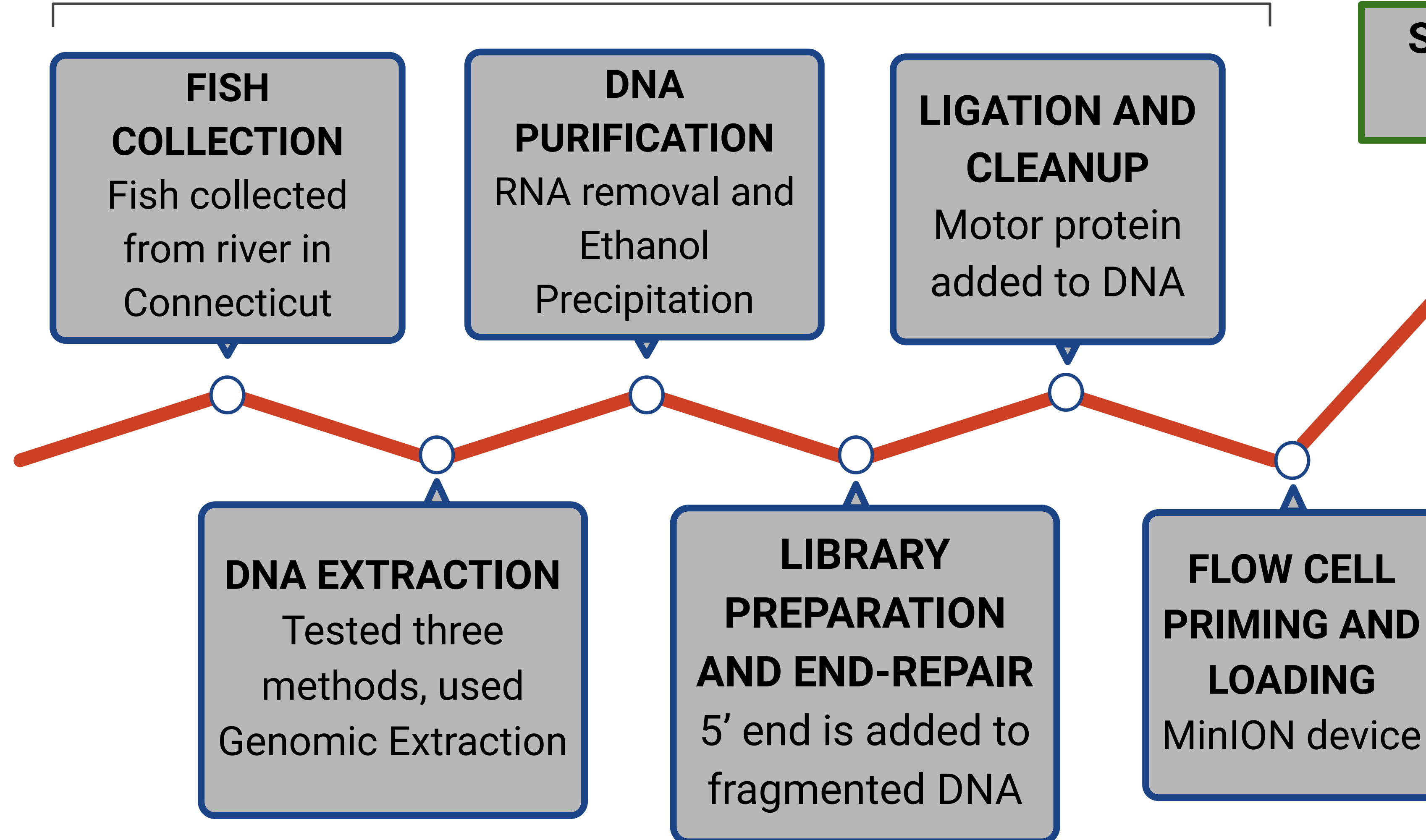


RESULTS

- We developed and fine-tuned an extraction protocol for high molecular weight genomic DNA and a MinION sequencing protocol for *R. cataractae*
- We developed a bioinformatic pipeline for sequencing, polishing, and aligning genomes
- We have four sequencing runs that yielded >14Gb of reads with N50>4Kb, and reads >50,000Kb in length



Molecular Procedure



Computational Procedure

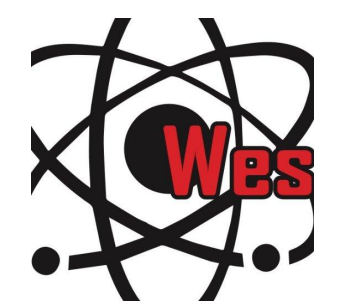
Procedural Problems	Our Solutions
1. Low DNA concentrations	1. Used a high-yield genomic extraction technique
2. Pellets from DNA extraction not fully dissolved in final buffer solution	2. Stored DNA in TE buffer at 4°C for >1 week to allow for dissolution
3. Low recovery of high molecular weight DNA	3. Used wide-bore pipet tips when handling DNA
4. MinION sequencing preferred processing of small length DNA fragments	4. Utilized a selective DNA recovery protocol to eliminate small-length fragments prior to sequencing

NANOPORE SEQUENCING

- Oxford Nanopore MinION, a type of 3rd generation sequencing technology, is a portable device that applies nanopore sequencing technology to DNA analyses
- The device has nanoscopic pores, formed by yeast membrane proteins, through which DNA strands are guided by motor proteins
- As a laser beam is passed through the pores, sensors detect absorption of radiation, corresponding to the absorption spectra of each nucleic acid
- An algorithm then interprets the output and assigns a nucleotide (A, G, C, or T)

ACKNOWLEDGEMENTS

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FUTURE DIRECTIONS

- The ability to sequence genomes of non-model organisms will elucidate the genetic basis of human and climatic impacts of fishes in the wild
- Examine the characteristics and differences between *R. cataractae* and *R. atratulus* genomes (including DNA methylation)

References: TAN, M., & ARMBRUSTER, J. W. (2018). Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi). *Zootaxa*, 4476(1), 6-39. <https://doi.org/10.11646/zootaxa.4476.1.4>