Mar 16, 2021

# Oxford Nanopore sequencing and library construction

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.btcwnixe

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ABSTRACT

The humpback puffer,Tetraodon palembangensis, is a species of poisonous freshwater pufferfish mainly distributed in Southeast Asia (Thailand, Laos, Malaysia and Indonesia). The humpback puffer has many interesting biological features, such as inactivity, tetrodotoxin production and body expansion. Here, we reported the first chromosome-level genome assembly of the humpback puffer. The genome size is 362 Mb with ~1.78 Mb contig N50 and ~15.8 Mb scaffold N50. Based on the genome, ~61.5Mb (18.11%) repeat sequences were identified, 19,925 genes were annotated, and 90.01% of these genes could be predicted with function. Finally, a phylogenetic tree of ten teleost fish species was constructed, which suggests that humpback puffer and *T. nigroviridis* shared a common ancestor at 18.1 MYA and diverged from *T. rubripes* at 45.8 MYA. The humpback puffer genome will be a valuable genomic resource to illustrate possible mechanisms of tetrodotoxin synthesis and tolerance.

DOI

dx.doi.org/10.17504/protocols.io.btcwnixe

PROTOCOL CITATION

Rui Zhang 2021. Oxford Nanopore sequencing and library construction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btcwnixe

COLLECTIONS

**Protocols for "Chromosome-level genome assembly of the humpback puffer, Tetraodon palembangensis"**



CREATED

Mar 16, 2021

LAST MODIFIED

Mar 16, 2021

PROTOCOL INTEGER ID

48246

PARENT PROTOCOLS

Part of collection

Protocols for "Chromosome-level genome assembly of the humpback puffer, Tetraodon palembangensis"

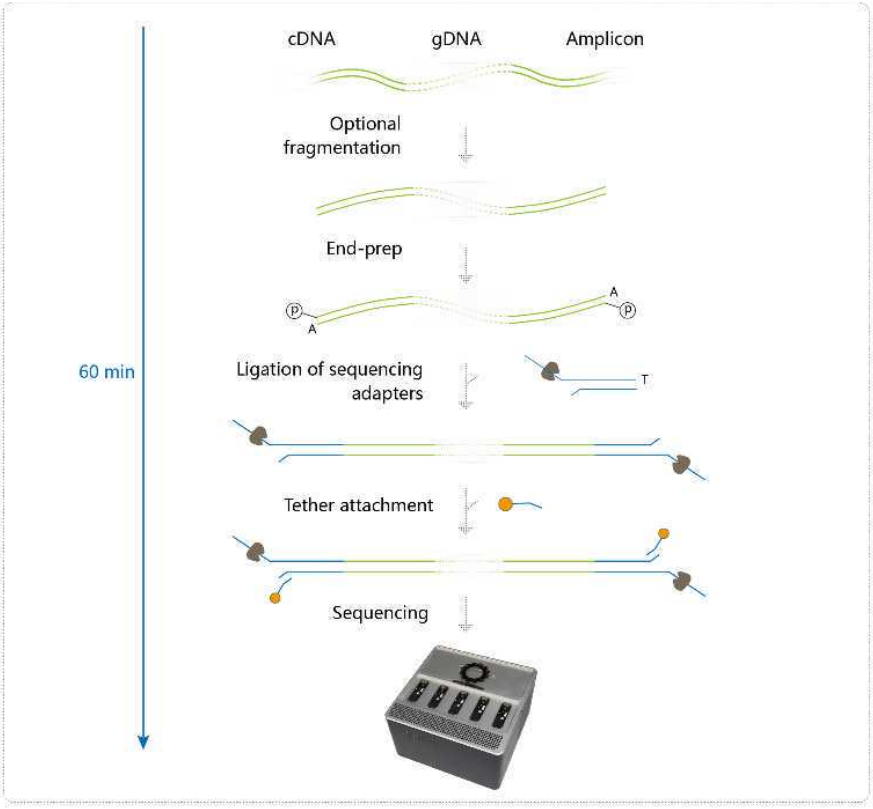
## 1 ONT Library preparation and Quality Control

1.1 After obtaining the qualified DNA, the large size fraction was selected by automated gel electrophoresis (BluePippin).

1.2 Next, the DNA was treated with the end-repair/dA tailing module.

1.3 After purification, adapter ligation was performed using ligation sequencing kit ({{kit}}, Oxford Nanopore Technologies).

1.4 Finally, DNA library was quantified by Qubit.



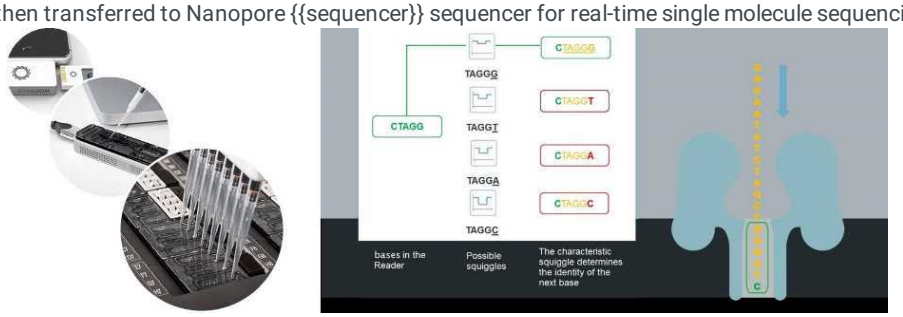

Library construction process

## 2 DNA Sequencing



**Citation:** Rui Zhang (03/16/2021). Oxford Nanopore sequencing and library construction. https://dx.doi.org/10.17504/protocols.io.btcwnixe

2.1 A certain concentration and volume of DNA library was loaded onto a {{cell_num}} flow cell, which was then transferred to Nanopore {{sequencer}} sequencer for real-time single molecule sequencing.




Nanopore single molecule real-time sequencing



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