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Oxford Nanopore sequencing and library construction

Forked from Oxford Nanopore sequencing and library construction

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

BGI GIGA 1 more workspace

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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.

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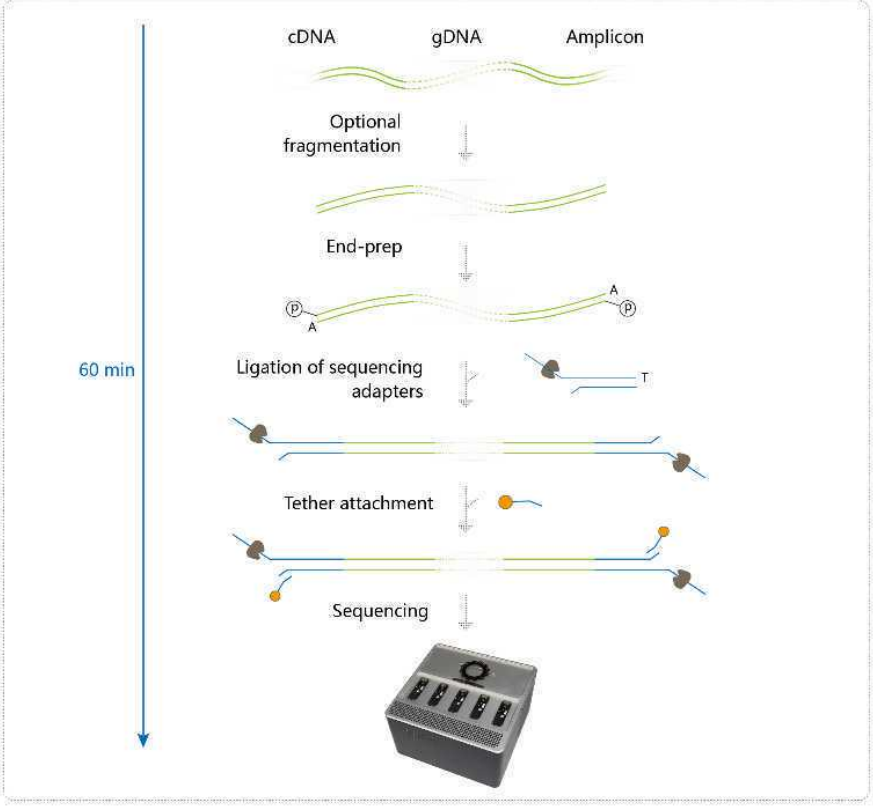
1 ONT Library preparation and Quality Control

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 (LSK109, Oxford Nanopore Technologies).

1.4 Finally, DNA library was quantified by Qubit.



Library construction process

2 DNA Sequencing

2.1 A certain concentration and volume of DNA library was loaded onto a flow cell- PromethION cell R9.4.1, which was then transferred to Nanopore PromethION sequencer for real-time single molecule sequencing.

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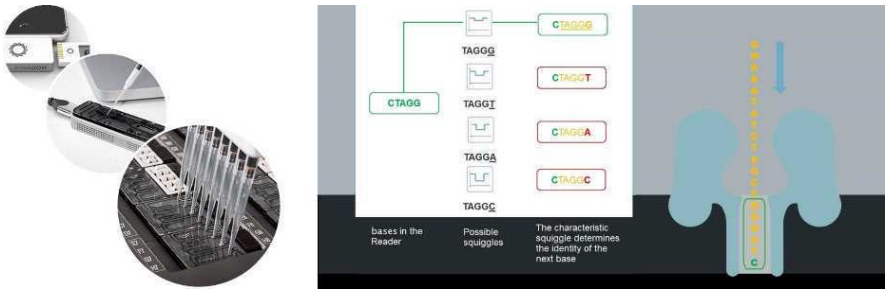

Nanopore single molecule real-time sequencing

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