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BIT595_Ligation Sequencing Kit_Individual Project_Birchler De Allende

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

The Oxford Nanopore Ligation Sequencing Kit is a versatile tool for generating long-read sequencing data across a wide range of organisms. This protocol outlines the general steps for library preparation and sequencing, with specific considerations for different sample types and the option for duplex sequencing.

The kit is designed to prepare high molecular weight DNA for nanopore sequencing, capable of generating read lengths from a few kilobases to over 100 kilobases. The main steps include DNA repair, end-prep, adapter ligation, and clean-up, followed by loading onto a flow cell for sequencing.

A key feature of this kit is its compatibility with duplex sequencing. Duplex sequencing involves reading both strands (template and complement) of a single DNA molecule through the same nanopore. This technique significantly improves sequencing accuracy, potentially reaching Q30 (99.9%) for a single double-stranded DNA molecule. To optimize for duplex sequencing, ensure successful ligation of sequencing adapters to both ends of the DNA strands and aim for a flow cell loading of 10-20 fmols.

Intro and Overview

1 The Oxford Nanopore Ligation Sequencing Kit is a versatile tool for generating long-read sequencing data across a wide range of organisms. This protocol outlines the general steps for library preparation and sequencing, with specific considerations for different sample types and the option for duplex sequencing.

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When working with different sample types, consider the following:

1. For animal samples:
   - Use gentle extraction methods to preserve DNA integrity
   - Consider using nuclease inhibitors if working with tissue samples

2. For plant samples:
   - Pay special attention to removing polysaccharides and secondary metabolites during DNA extraction
   - Use CTAB-based or specialized plant DNA extraction kits

3. For cellular samples (e.g., bacteria, cell cultures):
   - Optimize lysis conditions to maximize DNA yield while minimizing shearing
   - For gram-positive bacteria, enzymatic pre-treatment may be necessary

Regardless of the sample type, the key to successful long-read sequencing is starting with high-quality, high molecular weight DNA. Avoid excessive pipetting or vortexing throughout the protocol unless stated to maintain DNA integrity.

2 **Before you begin, you must**

1. Extract DNA using kit of your choice, check for quality using Tapestation and Qubit
2. Check kit has all equipment and reagents. Also check for any reagents required that do not come in the Ligation Sequencing Kit
3. Check that you flow cell has the correct number of available pores for quality sequencing

3 **Main Steps:**

Library Prep:

1. DNA repair and end-prep - 35 min
2. Adapter ligation and clean-up - 20 min
3. Priming and loading the flow cell - 5 min

Sequencing and Data Analysis:

1. Basecalling
2. Flow cell reuse
3. Downstream Analysis and Bioinformatics

DNA Repair and End Prep

4 1. Prepare the NEB reagents in accordance with manufacturer's instructions, and place on ice.

2. In a 0.2 ml thin-walled PCR tube, mix the following:
Between each addition, pipette mix 10-20 times.

	Reagent	Volume
	DNA	47 µl
	NEBNext FFPE DNA Repair Buffer v2	7 µl
	NEBNext FFPE DNA Repair Mix	2 µl
	Ultra II End-prep Enzyme Mix	3 µl
	Total	**60 µl**

Adapter Ligation and Clean-up

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Priming and Loading the Flow Cell

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Basecalling

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Flow Cell Reuse

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Downstream Analysis and Bioinformatics

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Citations:

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