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Extraction of Ultralong Genomic DNA from Human Cells for Oxford Nanopore Sequencing

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We use this protocol and it's working

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Abstract

This is a condensed version of the manufacturers protocol for the use of the NEB Monarch Genomic DNA extraction kit for Tissue (T3060). It is specifically adapted for the extraction of genomic DNA from cultured human cells upstream of ultralong-read nanopore sequencing. Changes recommended by Oxford Nanopore Technologies for use with their SQK-ULK114 library preparation and sequencing kit have been incorporated with some minor changes. Some language is taken directly from the manufacturers' protocols. First-time users should read the original protocols from the manufacturers in their entirety before using this protocol, and have them on-hand for reference.

This protocol accompanies Sidhwani et al. (2025) "Histone H3 lysine methyltransferase activities control compartmentalization of human centromeres". It is reflective of the precise procedures used in the long-read sequencing experiments featured in this publication.



Introduction

- 1 This protocol is adapted from the manufacturer's instructions for the NEB Monarch Genomic DNA extraction kit for Tissue (T3060; <https://www.neb.com/en-us/products/t3060-monarch-hmw-dna-extraction-kit-for-tissue>). This is an adaptation of that protocol designed for the extraction of DNA from cultured human cells, upstream of ultralong nanopore DNA sequencing with the SQK-ULK114 kit from Oxford Nanopore Technologies. Changes suggested by ONT (<https://nanoporetech.com/document/ultra-long-dna-sequencing-kit-sqk-ulk114>) have been added, and the protocol has been condensed for clarity. First-time users should read the linked protocols from the manufacturers in their entirety before using this protocol, and have them on-hand for reference.

This protocol accompanies Sidhwani et al. (2025) "Histone H3 lysine methyltransferase activities control compartmentalization of human centromeres".

Protocol

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