MAR 15, 2024

DOI:
dx.doi.org/10.17504/protocols.io.yxmvm25j6g3p/v1

Protocol Citation: anita.adami 2024. PacBio Isoseq samples preparation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm25j6g3p/v1

Protocol status: Working
We use this protocol and it's working

Created: Feb 06, 2023

PacBio Isoseq samples preparation

anita.adami[1]

[1]Laboratory of Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, BMC A11, Lund University, 221 84 Lund, Sweden.

ASAP Collaborative Research Network

Anita Adami
Lund University

ABSTRACT

This protocol described how to prepare samples for PacBio Isoseq

Last Modified: Mar 15, 2024

PROTOCOL integer ID: 76484

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research
Grant ID: ASAP-000520
Swedish Research Council
Grant ID: 2018-02694
Swedish Brain Foundation
Grant ID: FO2019-0098
Cancerfonden
Grant ID: 190326
Barncancerfonden
Grant ID: PR2017-0053
NIHR Cambridge Biomedical Research Centre
Grant ID: NIHR203312
Swedish Society for Medical Research
Grant ID: S19-0100
National Institutes of Health
Grant ID: HG002385
Swedish Research Council
Grant ID: 2021-03494
Swedish Research Council
Grant ID: 2020-01660

RNA extraction

1 The total RNA was obtained from brain tissue samples using miRNA Easy Mini Kit (Qiagen). The manufacturer's instructions were followed step-by-step.

2 RNA samples were subsequently put on dry ice and shipped to National Genomics Infrastructure of Sweden.

Samples QC evaluation

3 At the Nation Genomics Infrastructure of Sweden, input QC of samples was performed on the Agilent Bioanalyzer instrument, using the Eukaryote Total RNA Nano kit to evaluate RIN and concentration.

Libraries preparation

4 The sample libraries were prepared as described in "Procedure & Checklist – Iso-Seq™ Express Template Preparation for Sequel® and Sequel II Systems" (PN 101-763-800 Version 02 (October 2019)) using the NEBNext® Single Cell/Low Input cDNA Synthesis & Amplification Module, the Iso-Seq Express Oligo Kit, ProNex beads (Promega) and the SMRTbell Express Template Prep Kit 2.0.

5 300 ng of total RNA was used for cDNA Synthesis followed by 12 + 3 cycles of cDNA Amplification.

6 In the purification step of amplified cDNA, the standard workflow was applied (sample is composed primarily of transcripts centered around 2 kb).

7 After purification, the amplified cDNA went into SMRTbell library construction.

8 Quality control of the SMRTbell libraries was performed with the Qubit dsDNA HS kit and the Agilent Bioanalyzer High Sensitivity kit.

9 Primer annealing and polymerase binding was performed using the Sequel II binding kit 2.0.

PacBio Isoseq sequencing

10 Finally, the samples were sequenced on Sequel II and Sequel IIe System using Sequel® II Sequencing Plate 2.0, On-Plate Loading Concentration of 110 pM, movie time 24 hours and pre-extension time 2 hours.