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PacBio Isoseq samples preparation

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PacBio RNA



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We use this protocol and it's working

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Abstract

This protocol described how to prepare samples for PacBio Isoseq

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.