Mar 30, 2019

In devel.

Multi-Contact 4C (MC-4C): long molecule sequencing of complex proximity ligation products to uncover local cooperative and competitive chromatin topologies

Carlo Vermeulen[1], Amin Allahyar[1], Britta A.M. Bouwman[1], Peter H.L. Krijger[1], Marjon J.A.M. Verstegen[1], Geert Geeven[1], Christian Valdes-Quezada[1], Ivo Renkens[2], Roy Straver[2], Wigard P. Kloosterman[2], Jeroen de Ridder[2], Wouter de Laat[1]

[1]Oncode Institute, Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, the Netherlands, [2]Oncode Institute, Center for Molecular Medicine, University Medical Center, Utrecht University, Utrecht, the Netherlands

dx.doi.org/10.17504/protocols.io.zmwf47e

Amin Allahyar

ABSTRACT

We present the protocol and data analysis toolbox for Multi-Contact 4C (MC-4C), a new proximity ligation method tailored to study the higher-order chromatin contact patterns of selected genomic sites. Conventional chromosome conformation capture (3C) methods fragment proximity ligation products for efficient analysis of pairwise DNA contacts. In contrast, MC-4C is designed to preserve and collect large concatemers of proximity ligated fragments for long molecule sequencing on Oxford Nanopore or Pacific Biosciences platforms, thus allowing study of multi-way chromatin interactions. By inverse PCR with primers specific for a fragment of interest (the viewpoint) and DNA size selection, sequencing is selectively targeted to thousands of different complex interactions containing this viewpoint. A tailored statistical analysis toolbox employing data-intrinsic background models then discerns whether contacts between more than two regulatory sequences are mutually exclusive or, conversely, simultaneously happening at chromatin hubs. The entire procedure can be completed in two weeks and requires access to a third generation sequencing platform.

PDF

Nature_protocols_proof.pdf

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

1