Multiplexed identification of nucleic acids in single chromatin complexes

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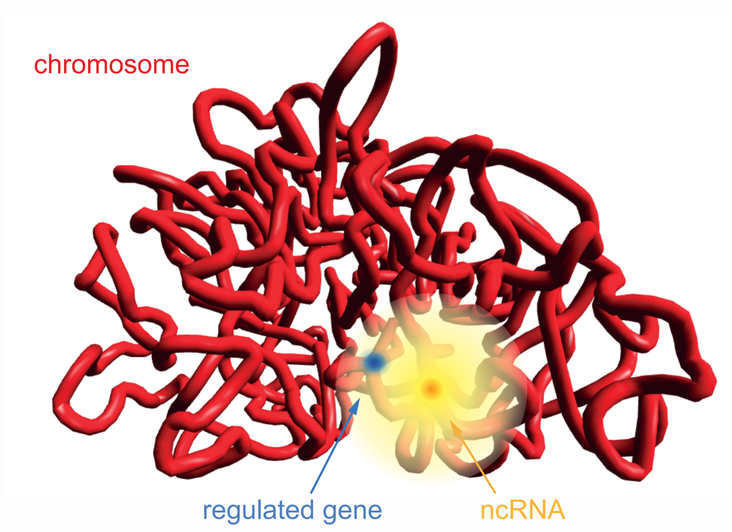
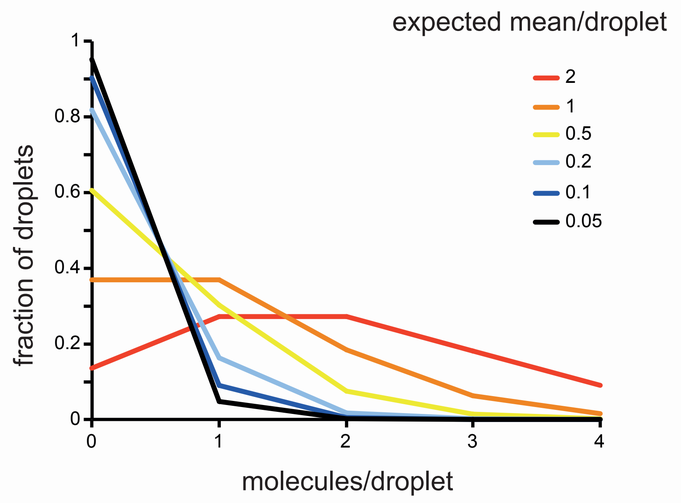
Background

Spatial proximity between regulatory and gene-encoding DNA, as well as transcription factor occupancies on chromatin serve as correlates of gene expression. Furthermore, RNA molecules have recently been shown to associate with and modulate the expression of genes for example through the regulation of chromatin modifications. Such regulatory RNAs are expressed and accumulate as point sources in the nuclear space, in contrast to protein factors, imported from the cytoplasm. Therefore local 3D chromatin structure likely affects target specificity of such RNAs. Here, we present a novel method that identifies genomic loci and interacting nuclear RNAs in the context of chromatin structures.



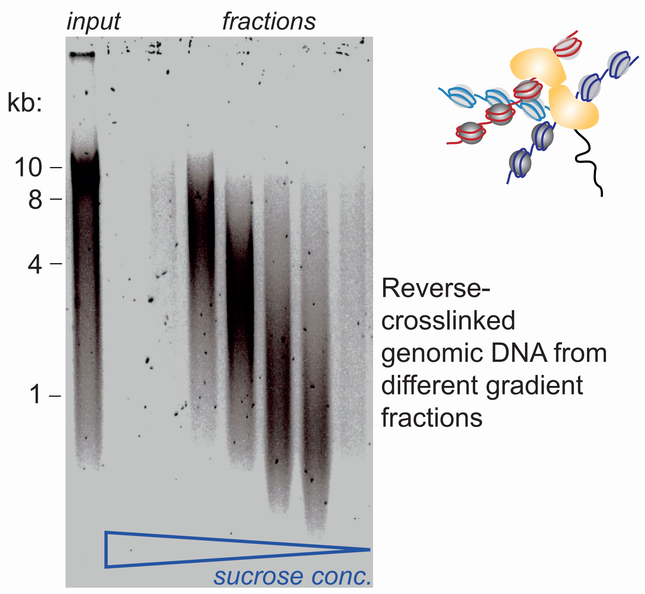
Approach

The method relies on the tagging of the set of nucleic acids contained in an individual chromatin complex with a unique DNA barcode. To tag many chromatin complexes in a highly parallel fashion, picodroplet and microfluidic systems are applied. Subsequent deep sequencing of complex-specific barcodes and the DNA and RNA molecules attached to them enables the reconstruction of the chromatin complexes and therefore of nucleic acid contacts in formaldehyde-crosslinked cell nuclei. The production of barcoded beads and the ligation of single chromatin complexes to them are both based on Poisson statistics to estimate the dilution of molecules needed in order to achieve high fractions of water-in-oil droplets containing only one molecule.



Method outline

A) High-molecular weight chromatin complex purification from sonicated formaldehyde-crosslinked nuclei by a sucrose gradient



Reverse-crosslinked genomic DNA from different gradient fractions

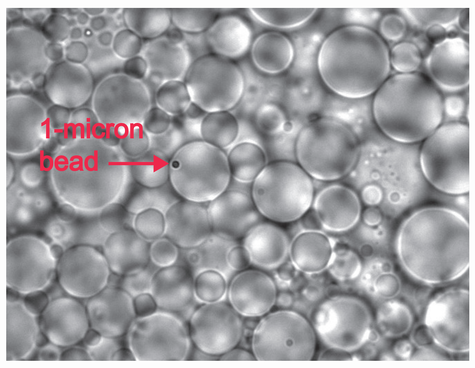
C) Encapsulation of complexes and barcode beads into water-in-oil droplets

D) Fusion of chromatin and barcode droplets (1:1) through voltage application across electrodes in microfluidic device

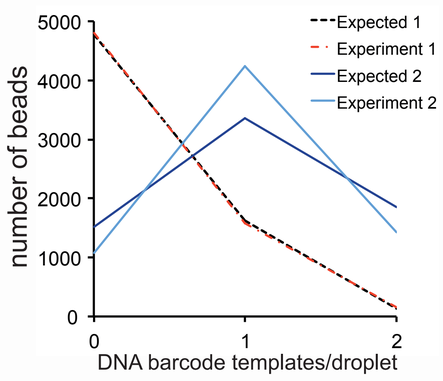
E) Barcode - chromatin ligation in fused droplets

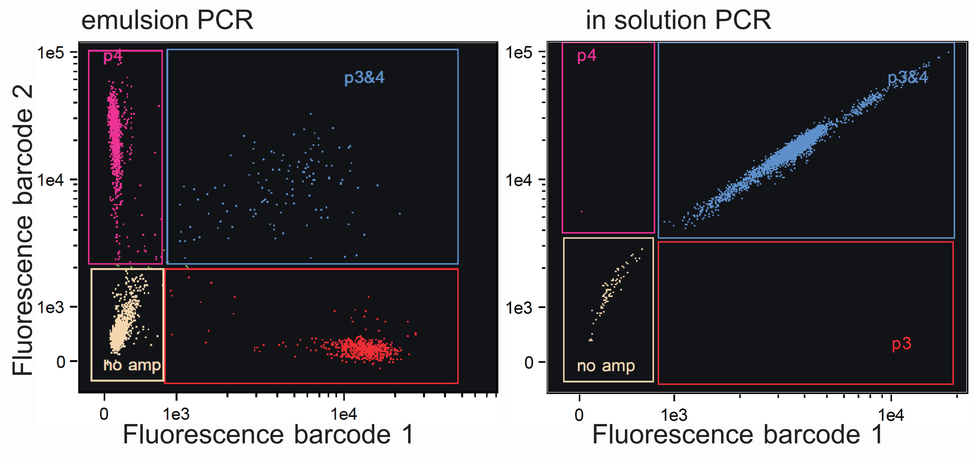
B) Random barcoding of single magnetic beads by emulsion PCR

Emulsion PCR droplet optimally contains one bead with one PCR primer immobilized, the other in solution and excess, and a single DNA template encoding a random barcode sequence.



Emulsion PCR test on beads using two different barcode templates, barcode-specific hybridisation with fluorescent probes and FACS analysis of barcoded beads





F) Bulk on bead purification, amplification of barcoded nucleic acids and deep-sequencing

G) Chromatin complex reconstruction based on shared barcodes of different RNA/DNA fragments