

Sensitive detection of chromatin co-associations using enhanced chromosome conformation capture on chip



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Introduction

Chromosome conformation capture (3C) is a powerful technique for analyzing spatial chromatin organization in vivo. Technical variants of the assay ('4C') allow the systematic detection of genome-wide co-associations with bait sequences of interest, enabling the nuclear environments of specific genes to be probed. We describe enhanced 4C (e4C, enhanced chromosome conformation capture on chip), a technique incorporating additional enrichment steps for bait-specific sequences, and thus improving sensitivity in the detection of weaker, distal chromatin co-associations. In brief, e4C entails the fixation, restriction digestion and ligation steps of conventional 3C, with an optional chromatin immunoprecipitation (ChIP) step to select for subsets of chromatin co-associations, followed by bait enrichment by biotinylated primer extension and pull-down, adapter ligation and PCR

amplification. Chromatin co-associations with the bait sequence can then be assessed by hybridizing e4C products to microarrays or sequencing. The e4C procedure takes approximately 1 week to go from tissue to DNA ready for microarray hybridization.

Protocol

Please use the link below to access the protocol.

http://www.babraham.ac.uk/pjl_pages/fraser/protocols.html

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