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Hi-C based translocations detection in human single cells

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Motivation and Aim: More than 10 million people have already been born from *in vitro* fertilization procedure [1]. The number keeps growing as the reproductive health of the population deteriorate and increase the effectiveness of assisted reproductive technologies in overcoming fertility disorders for some categories of patients. Carriers of balanced translocations often suffer from infertility, recurrent miscarriages, and the birth of children with severe developmental disorders. In addition, some offspring with a balanced karyotype will be carriers of translocations, which will lead to the same reproductive problems in the future [2]. In this connection, the need for an effective method of analyzing the embryo is obvious. The preimplantation genetic testing detects unbalanced translocations whereas balanced translocations remain unnoticed. The aim of the study is to adapt single cell Hi-C for detection balanced and unbalanced translocations.

Methods and Algorithms: For low-input Hi-C, cells was collected manually with a microscope control and fixed with 2 % formaldehyde for 10 minutes. We made following modifications of the previously published protocol [3]: fixed and lysed cells were embedded in low melting agarose, chromatin ends biotinylation and subsequent target ligation products enrichment were used, and the whole genome amplification was abandoned. The libraries for NGS were prepared using the KAPA HyperPrep kit.

Results: We evaluated the possibility of translocations detection using low-input Hi-C. Dozens of iPSC iP10-2 cells with a translocation between chromosomes 5 and 10 (46,XX,t(5;10)) were collected and Hi-C libraries were prepared. The generated contact heatmaps displayed the reciprocal translocation between chromosomes 5 and 10 (Fig. 1), which was previously confirmed by cytogenetics – t(5;10)(q11.2;q11.2)dn and by the population Hi-C. Then we analyzed peripheral blood lymphocytes from patient P47, who, had balanced translocations between chromosomes 8, 11, 20 and 21 (46,XY,t(8;11;20;21)). Four Hi-C libraries were prepared from the P47 lymphocytes. 8 cells were taken for each experiment, is comparable to the number of cells in a biopsy for preimplantation genetic testing. Despite the shallow sequencing (less than 500 thousand reads) of the prepared libraries, we detected t(8,11) translocations (Fig. 2). If 4 replicas were combined, we were able to detect the remaining rearrangements.

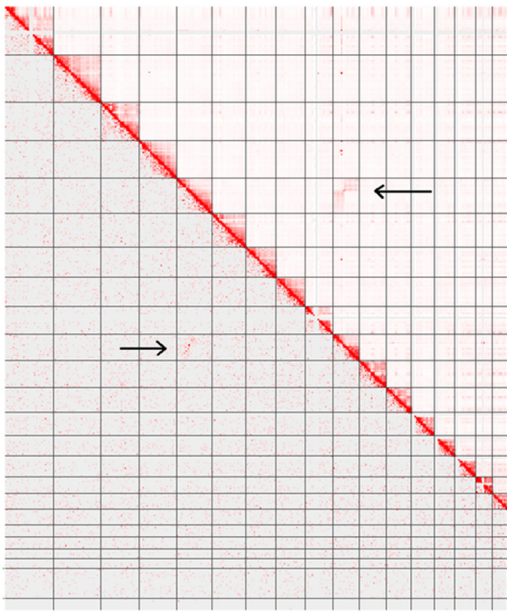

Fig. 1. The Hi-C heatmaps of 2.5 million iP10-2 cells above the diagonal and dozens of iP10-2 cells – below

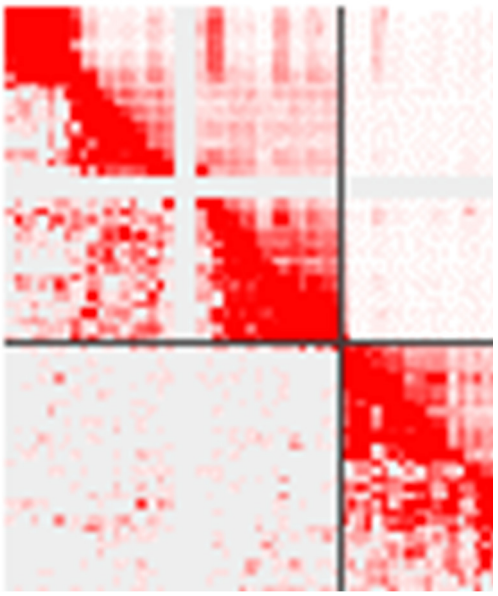

Fig. 2. The Hi-C heatmaps of P47 lymphocytes. Above the diagonal is population Hi-C data, below – 8 cells

Conclusion: Our low-input Hi-C protocol detects balanced reciprocal translocations. In addition, we believe that this method can be proposed for preimplantation genetic testing of structural chromosomal rearrangements to identify both unbalanced and balanced translocations in human embryos.

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