CHROMOSOME PAINTING IN BARLEY – A NEW MILESTONE IN CYTOGENETICS OF CEREALS

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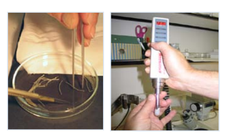
Background

Chromosome painting is an appealing technique, which significantly increased the attractiveness of cytogenetics. It is not only a technique of basic research, but an important tool of clinical cytogenetics, which helps to uncover serious diseases and save human lives. The history of this technique covers several decades, has gone through changes, diversified a lot and was adapted to current demands. The principle of the method is generating fluorescently labeled probes from whole chromosomes, which are used for fluorescence *in situ* hybridization on metaphase chromosomes and interphase nuclei. The technique was originally developed for analysis of human cells. The attempts to utilize chromosome painting with composite chromosome probes in plants failed, mainly due to the presence of dispersed repeats. Here we describe a novel approach suitable for chromosome painting in plants with large genomes. The method relies on the ability to prepare chromosome painting probes composed mainly from low-copy coding sequences, which are obtained after gene capture from chromosomes isolated by flow cytometric sorting.

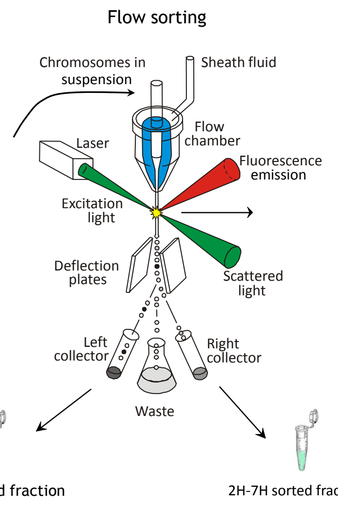
Material and Methods

Flow cytometric chromosome sorting

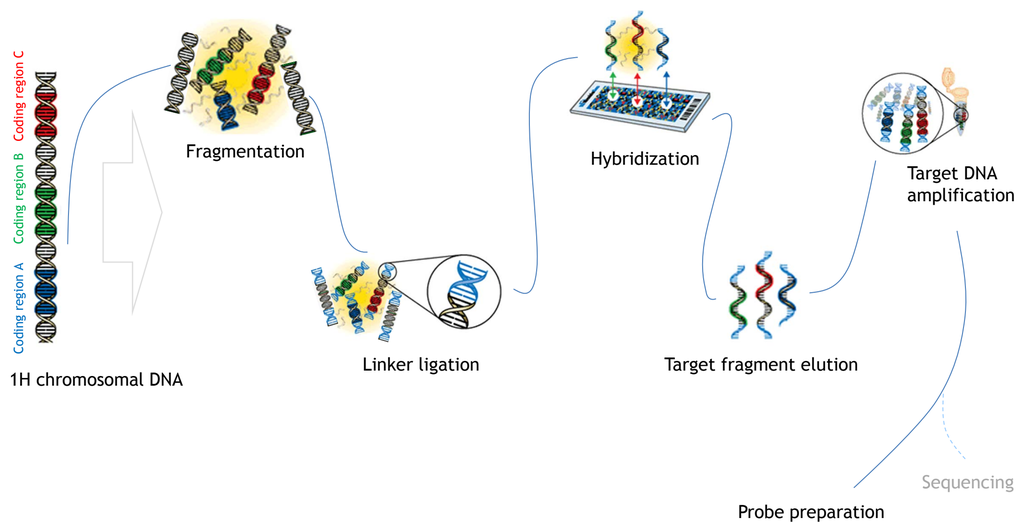
We synchronized cell cycle in root-tip meristems of barley cv. „Barke“ seedlings to accumulate mitotic cells in metaphases.



Chromosomes were extracted from the root tips into the liquid suspension.



Gene capture



Probe preparation and FISH

Painting probe

1. LM-PCR (ligation mediated PCR)
   (Reamplification of captured DNA using ligated linkers)
2. PCR labeling
   (Incorporation of Texas Red labeled dUTPs)
3. Probe purification

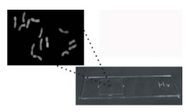
C_0t100 DNA

Unlabeled barley genomic DNA (cv. „Morex“) enriched by repetitive fraction

2H-7H blocking DNA

Unlabeled blocking DNA prepared from sorted barley chromosomes 2H-7H cv. „Barke“

Squashing of barley (cv. „Morex“) root tips



Postfixation in 4% formaldehyde solution

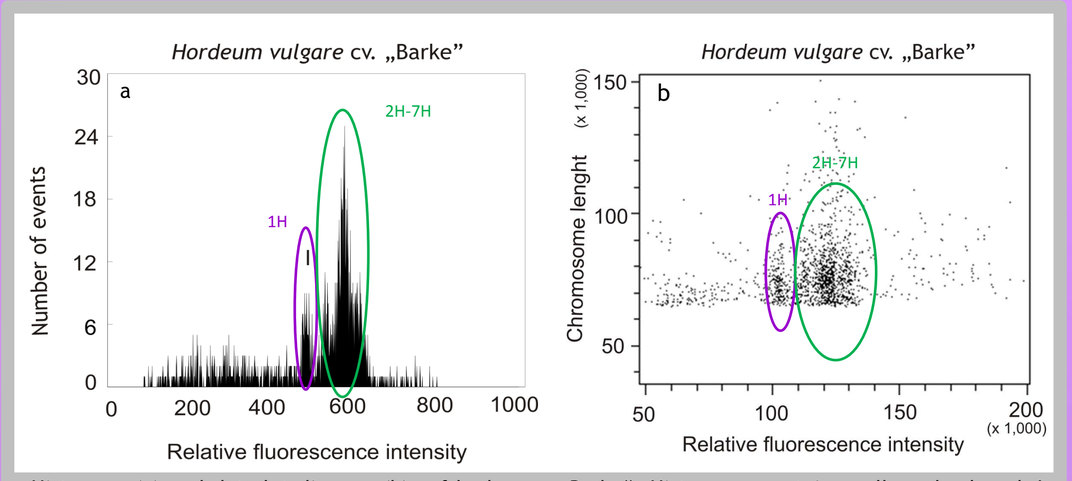
Prehybridization

Slides were incubated in cycler starting with 3 minutes denaturation at 80°C followed by decrease to 40°C by 1 °C per two minutes

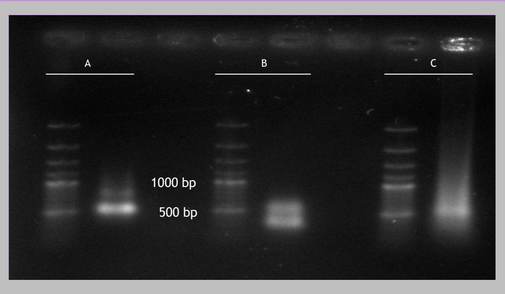
Overnight hybridization at 37°C

Stringent washing and signal imaging

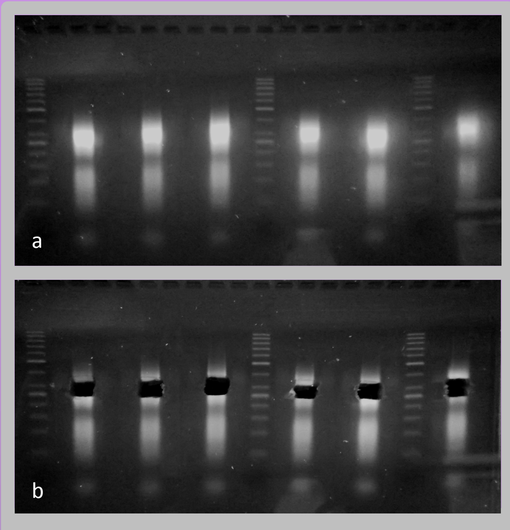
Results



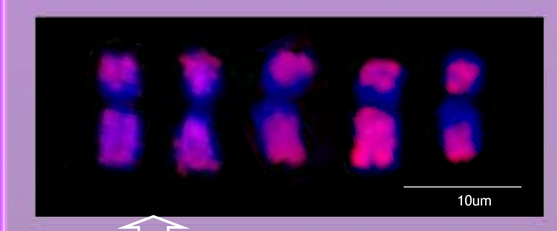
Histogram (a) and dot-plot diagram (b) of barley cv. „Barke“. Histograms contains well-resolved peak I, which comprise barley chromosome 1H. Due to the difference in DNA content, 1H chromosome can be discriminated from other chromosomes. In total, 140 000 chromosomes were sorted for one gene capture experiment.



Electrophoretic separation of: (A) reamplified copy 1 of captured DNA (B) original captured DNA of barley chromosome 1H (C) probe labeled with Texas Red.

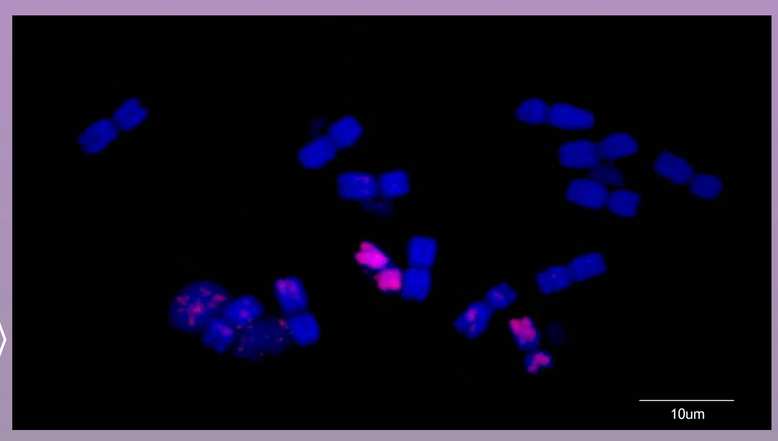


LM-PCR of captured DNA eluted from the microarray (a) and fragment size selection for library construction and chromosome painting (b).



The highly specific probe consists of coding regions and regularly provides weak or no signal in centromeric and telomeric region, which are supposed to be highly repetitive.

Chromosome painting with 1H whole chromosome probe on complete metaphase spreads of barley.



Conclusion

Here we present for the first time a protocol for chromosome painting in plants using composite chromosome probes. This advance opens avenues for the study of behavior of particular chromosomes during mitotic cell cycle, meiosis, and their organization in interphase nuclei. The ability to paint particular chromosomes provides an opportunity to study structural chromosome changes that accompanied the evolution and speciation. As chromosome sorting using flow cytometry has been described in more than 30 plant species, our new approach is not limited to barley, which was used as a model in the present study.

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