

Array-CGH

An innovative screening method in molecular cytogenetics

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Introduction

Microarray-based comparative genomic hybridization (array/matrix-CGH) is an improvement of conventional comparative genomic hybridization (CGH, Kallioniemi et al. 1992) and becomes more and more important as an innovative screening method in molecular cytogenetics. In combination with fluorescence in situ hybridization (FISH), new chances of an improved and more precise approach are developing within the scope of tumor cytogenetics and pre- and postnatal genetic analysis. Further applications of array-CGH and FISH are polar body and preimplantation genetic analysis.

Matrix-CGH/ array-CGH was developed in 1997 by Solinas-Toldo et al. at the group of Peter Lichter (DKFZ, Heidelberg, Germany). A grid of well-defined, genomically mapped DNA fragments (i.e. BAC clones, cDNA, oligonucleotides) immobilized on a glass surface replaces condensed metaphase chromosomes as hybridization target (Figure 1). Compared to conventional cytogenetic methods, the resolution of copy number analysis could be increased dramatically thereby. Array-CGH allows automated analysis of submicroscopic chromosomal imbalances such as microdeletions and microduplication/-amplifications. The resolution of the analysis is dependent on the number and distance of fragments represented on the array. Currently whole genome arrays with resolutions ranging from 1Mb to 70kb are available. These high resolutions permit not only the detection of submicroscopic genomic imbalances, but also precise breakpoint determination of genomic aberrations. Therefore array-CGH can complement the standard cytogenetic methods (chromosome analysis, subtelomere analysis, FISH) in prenatal, postnatal and tumorcytogenetic analysis. The main requirements for cytogenetic applications are standardized and maximal reproducible experimental conditions with the highest possible sensitivity. Automation of hybridization and washing to a sensible degree guarantees standardized, user independent experimental conditions with minimal variation, whilst offering an uncomplicated setup, ease of use and maintenance-free operation. In addition the assay time is considerably reduced by the automation, making the implementation of array-CGH in the daily laboratory work feasible and calculable.

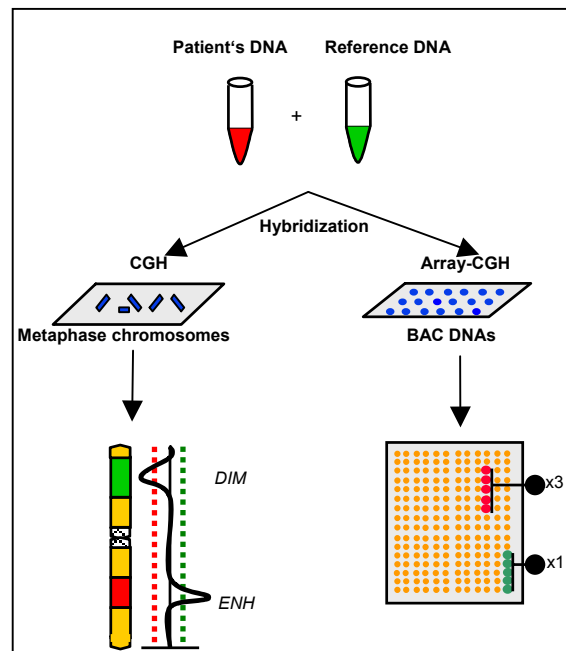


Figure 1: Comparison of conventional CGH and array-CGH. Metaphase chromosomes as hybridization targets are replaced by a grid of BAC-DNAs. “DIM” (CGH), “x1” (array-CGH) refers to a deletion in the patient’s DNA. “ENH” (CGH), “x3” (array-CGH) refers to a duplication in the patient’s DNA. Adapted with changes from Tönnies et al. (2002).

Method

To get an overview of the specific steps during an array-CGH experiment refer to the flow chart (Figure 2). The applied methods are adapted from protocols developed at the Max Planck Institute of Molecular Genetic with slight modifications concerning instruments and the sample amount (http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics/Protocols.html). Patient’s DNA and reference DNA (300-1000ng) are fluorescently labeled with Cy3 and Cy5, respectively, using a random priming protocol (e.g. Bioprime Array CGH Labeling Kit, Invitrogen). DNA concentration prior to labeling and efficiency of fluorescent dye incorporation is precisely measured using the NanoPhotometer™ (Implen, Munich, Germany). Differentially labeled DNAs are precipitated with CotI-DNA and dissolved in hybridization buffer. Prior to hybridization the arrays are treated with blocking solution. After a short denaturation step, preannealing is carried out at 42°C for 2 hours. In

the SlideBooster™ Hybridization Station (supplied by Implen, Munich, Germany) 8 arrays can be hybridized in parallel with one unit (extension up to 32 arrays possible) under standardized conditions (42°C, 24h, pulse 5_5).

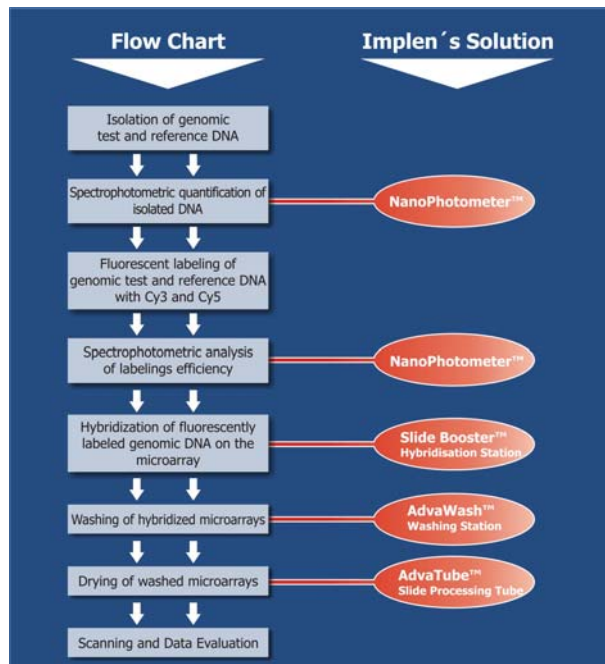


Figure 2: Flow Chart for the array-CGH process. Implen offers solutions for every single step speeding up the whole process due to automation and standardization.

75 µl hybridization solution has been used with LifterSlips™ (supplied by Implen, Munich, Germany) for a slide surface of 25 x 60 mm. Subsequent to hybridization the arrays are washed in the AdvaWash™ Universal Slide Washing Station (supplied by Implen, Munich, Germany). The slides are dried by centrifuging prior to scanning (AdvaTube™, supplied by Implen, Munich, Germany). The resulting relative fluorescence intensities are measured by a computer scanning Microarray scanner (Figure 3). Following the initial image analysis the genomic profile is calculated using an appropriate analysis software like CGHPRO (Chen et al., 2005). Losses (deletions) and gains (duplications) are detected if the genomic profile exceeds predefined thresholds (\log_2 ratio probe/reference -0.3 and 0.3, respectively). If desired, array-CGH results can be confirmed by fluorescent in situ hybridization (FISH) using BAC DNA as a probe. BAC arrays with a resolution of 1 Mb were used for the comparative study between conventional cytogenetic methods and array-CGH. The arrays comprise clones from the 1 MB Sanger set. The clones are courtesy of Nigel Carter, Wellcome Trust Sanger Centre. The arrays have been printed at the VUMC, Amsterdam (Cooperation of the Institute of Medical Genetic, Charité mit Anja Matthäi and Evelin Schröck, Institute for Clinical Genetic, TU Dresden and Gerrit Meijer and Bauke Ylstra, VUMC, Amsterdam).

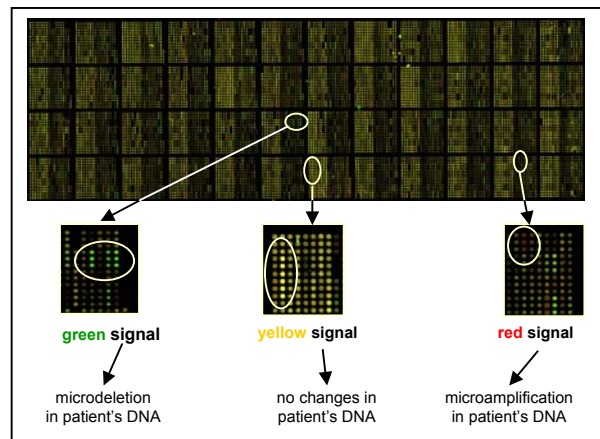


Figure 3: Scan of an array-CGH Slide. Green Signal: microdeletion in patient's DNA. Hybridization only by the green labeled reference DNA. Red Signal: microamplification in patient's DNA. Hybridization only by the red labeled patient's DNA. Yellow Signal: no changes in patient's DNA. Red and green labeled DNA is hybridizing.

Results

In the present comparative study 15 patients with chromosomal alterations have been investigated with conventional cytogenetic methods (including CGH) and with array-CGH. The conventional cytogenetic analysis was performed at the Institute of Human Genetics, Charité Universitätsmedizin, Berlin (cooperation with Holger Tönnies). Unbalanced translocations, deletions and duplications were among the analyzed aberrations. The range of the aberration sizes was between 1.5 and 20 Mb. (Further information on the resulting karyotypes is available at Implen's homepage or via www.array-cgh.com). Every individual detected aberration could be confirmed with FISH analysis using the BAC-clones of the array-CGH as probes (Figure 4). To clarify the origin of the respective aberration, the parents of the patients have also been investigated by FISH analysis. None of the parents carried one of the detected aberrations. For that reason it can be concluded, that the patients have developed all aberrations de novo. Comparing conventional CGH and array-CGH demonstrates that all known aberrations could be detected with array-CGH using an array with a resolution of 1 Mb. The size of the aberrations and the breaking points could be defined far better by array-CGH in 73% (11 of 15) of all cases. In 2 out of 15 cases small aberrations (< 3 Mb) could be detected by array-CGH and not with conventional CGH due to the higher resolution of array-CGH. Figure 4 shows an example of this study.

The enhanced determination of the aberration size and the detection of submicroscopic genomic imbalances are two major advantages of array-CGH in comparison to conventional CGH. In addition the genotype-phenotype correlation and the identification of candidate genes for a certain phenotype can be improved by using array-CGH.

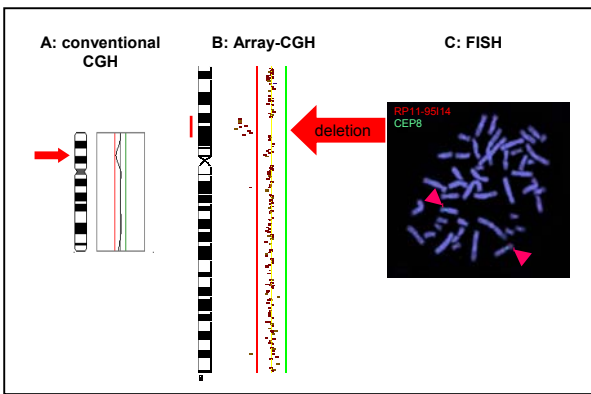


Figure 4: Interstitial deletion of chromosome 8p12-p21 (clinical case). A: Conventional CGH profile of chromosome 8. The deletion in the region 8p is indicated by the arrow. An accurate classification of the breaking points is not possible. B: Array-CGH profile of chromosome 8. The log₂ratio of patient to reference DNA is shown (CGHPRO software: Chen et al., 2005). The threshold values are marked with red and green lines (log₂ratio sample/reference -0.3 and 0.3, respectively). Ratios below or above indicate deletions or duplications, respectively. The red bar indicates the deleted region on chromosome 8p. C: Confirmation of the array-CGH results by FISH analysis. red signal: BAC-probe RP11-395114 localized at 8p21.2; green signal: control probe cep8 (Vysis, Downers Grove, IL) specific for the centromere of chromosome 8. The arrows mark chromosomes 8.

The major prerequisite for the detection of aberrations in the submicroscopic range is a maximal stable base line with minimal variation. Automation of the hybridization process with the SlideBoster™ hybridization station (supplied by Implen, Munich, Germany) guarantees this requirement with maximal reproducible hybridizations over the entire slide surface (Figure 5).



Figure 5: Array-CGH profile hybridizing two different DNAs labeled with Cy3 and Cy5 on a BAC-array with app. 36000 clones. The log₂ratio of cy3 to cy5 of the individual clones is shown (CGHPRO software: Chen et al., 2005). The threshold values are marked with red and green lines (log₂ratio sample/reference -0.3 and 0.3, respectively). The detected difference in DNA quantity corresponds to DNA copy number polymorphisms present in the normal population with currently unknown pathogenetic relevance. The picture is a courtesy of

Conclusion

Conventional cytogenetic methods have an average resolution of ~8 Mb. The use of array-CGH with a resolution of 1 Mb or higher allows the detection of submicroscopic genomic aberrations which are not detectable by conventional cytogenetics. By standardization of the protocol using the SlideBooster™ Hybridization Station and the AdvaWash™ Universal Slide Washing Station it is possible to achieve highly reproducible results. In addition, the hybridization geometry is flexible over the entire slide surface and the minimal required sample volume starts by 1 µl. The analysis does not require a background correction and the overall deviation is minimal (Figure 5). Compared to manual protocols optimization and automation of the protocol leads to a reduction of hybridization and washing times.

Applications for array-CGH

- prenatal genetic analysis
- postnatal genetic analysis
- tumorcytogenetics
(hematology, tumorpathology)
- polar body analysis
- preimplantation genetic analysis

Literature/Reviews

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