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Construction and Application of a Full-Coverage, High-Resolution, Human Chromosome 8q Genomic Microarray for Comparative Genomic Hybridization

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Background: Array-based comparative genomic hybridization (aCGH) enables genome-wide quantitative delineation of genomic imbalances. A high-resolution contig array was developed specifically for chromosome 8q because this chromosome arm is frequently altered in many human cancers.

Methods: A minimal tiling path contig of 702 8q-specific bacterial artificial chromosome (BAC) clones was generated with a novel computational tool (BAC Contig Assembler). BAC clones were amplified by degenerative oligonucleotide primer (DOP) polymerase chain reaction and subsequently printed onto glass slides. For validation of the array DNA samples of gastroesophageal and prostate cancer cell lines, and chronic myeloid leukemia specimens were used, which were previously characterized by multicolor fluorescence in situ hybridization and conventional CGH.

Results: Single and double copy gains were confidently demonstrated with the 8q array. Single copy loss and high-level amplifications were accurately detected and confirmed by bicolor fluorescence in situ hybridization experiments. The 8q array was further tested with paraffin-embedded prostate cancer specimens. In these archival specimens, the copy number changes were confirmed. In fresh and archival samples, additional alterations were disclosed. In comparison with conventional CGH, the resolution of the detected changes was much improved, which was demonstrated by an amplicon of 0.7 Mb and a deletion of 0.6 Mb, both spanned by only six BAC clones.

Conclusions: A comprehensive array is presented, which provides a high-resolution method for mapping copy number alterations on chromosome 8q. © 2004 Wiley-Liss, Inc.

Key terms: genomic array; comparative genomic hybridization; 8q contig; cancer; chromosomal aberrations; deletion; gain; amplification

Comparative genomic hybridization (CGH) is widely used for whole-genome analysis of copy number changes that may reflect important events in carcinogenesis, such as the activation of oncogenes or the inactivation of tumor suppressor genes. However, CGH is characterized by a limited ability to detect copy number gains and losses smaller than 10 Mb. Recently, the technique of array-based CGH (aCGH) was introduced to overcome this and other limitations associated with conventional CGH (1,2). In conventional CGH versus aCGH, differently labeled reference and test DNAs are cohybridized onto glass slides containing metaphase chromosomes versus arrayed DNA probes, respectively, as a matrix for CGH evaluation. Array-based CGH uses an array of mapped genomic clones for targeted and genome-wide copy number analyses. Using a small set of clones along chromosome 20, several alterations in breast cancer were found that had not been detected previously (2). Subsequently, an array consisting of overlapping bacterial artificial chromosome (BAC) and Phage 1 (P1) clones was used to map amplicon boundaries

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across 20q13.2, resulting in the identification of CYP24 as a candidate oncogene (3). To enable genome-wide measurement of DNA copy number changes, an array comprising more than 2,400 BAC and P1 clones was assembled (4). This array, which has an average resolution of 1.4 Mb, has been used for a variety of studies including copy number analysis of micrometastatic tumor cells, gastric carcinoma, and archival prostate cancer specimens (5–7). In the latter study it was demonstrated that array CGH can be applied to archival tissue with high-resolution detection of deletions and amplifications (6). Recently, genome-wide aCGH with 1-Mb resolution has been described (8). Region-specific arrays proved an attractive way of tracking down oncogenes and tumor suppressor genes in genomic areas previously identified to be of interest. For chromosome 22 a full-coverage, high-resolution array was constructed that consisted of 480 clones (9). Similarly, a region-specific array was realized for chromosome 8q21-24 that consisted of 166 overlapping BAC clones (10). The same group recently reported the construction of a contig BAC array comprising the whole human genome (11). Further, an array for B-cell non-Hodgkin lymphoma research has been constructed, in which gene-specific clones were combined with region-specific clones (12). Other clinical applications for array CGH include differential diagnosis of renal cancer, lung cancer, liposarcoma, and dermatofibrosarcoma protuberans and analysis of subtelomeric chromosome rearrangements for the diagnosis of mental retardation (9,13–16). The use of cDNA arrays for CGH has been reported (17–20). This method has allowed high-resolution analysis with the opportunity to compare genomic imbalances with RNA expression levels (19,21). In addition, the application of oligonucleotide arrays, originally designed to detect single-nucleotide polymorphisms, was evaluated to assess DNA copy number changes (22). The advantages of aCGH over conventional CGH are the ability to detect small regions of amplification or loss and a higher resolution to detect closely spaced aberrations. In addition, because aCGH involves hybridization to a set of mapped sequences, the analysis is highly simplified and the possibility exists to link genetic changes immediately to specific markers and genes (1,2).

Conventional CGH analysis has shown recurrent gain of the long arm of chromosome 8 in a wide range of cancers, including lung, breast, colorectal, and prostate cancers and acute and chronic myeloid leukemias (23–26). Moreover, gain of 8q has been associated with poor clinical outcome in, for instance, prostatic adenocarcinoma (27–29) and breast cancer (30). Apart from the amplicon at 8q24, in which region c-myc resides, amplification at other sites, such as 8q22-23 and 8q21, have been reported. This suggests the presence of more than one target gene at 8q (10,31,32). Therefore, we constructed and tested a high-resolution genomic microarray for chromosome 8q. The following questions were addressed: Is it possible to accurately detect genomic gains and losses of different sizes? Is it feasible to analyze archival, formalin-fixed, specimens?

MATERIALS AND METHODS

Cell Lines and Clinical Specimens

Cell lines JROECL 33, OACM4.1X, and OACM5.1C were derived from adenocarcinomas of the gastroesophageal junction (33,34). OACM4.1X originated from gastric cardia cancer and JROECL 33 and OACM5.1C from esophageal adenocarcinomas. Cell line PC3 was derived from a bone metastasis of a prostate carcinoma (35). Chronic myeloid leukemia (CML) cases 1 and 2 were bone marrow aspirates from patients who had CML/blast crisis, and cell lines MC3 and MEG-01 were derived from CML cases (26). CML samples were obtained from the Royal Free & University College London School of Medicine.

Formalin-fixed, paraffin-embedded specimens were obtained from four patients with prostate cancer. A fresh-frozen sample was used from a patient with gastric cardia adenocarcinoma. The prostate cancer and gastric cardia specimens were obtained from surgical resections performed in the Erasmus Medical Center.

Minimal Tiling Path Contig Construction

The contig of minimally overlapping BAC clones was assembled across the entire long arm of chromosome 8 by using a BAC Contig Assembler computational tool (36). The tool uses information from The Institute for Genomic Research (TIGR) BAC end sequence resource and the normal genome sequence generated by the University of California at Santa Cruz (UCSC) group and is presently publicly available on the Internet (http://www.tigr.org/, http://genome.ucsc.edu, http://shark.ucsf.edu/gc/site.html). The contig spanned the entire 8q region, which corresponded to 42700001 to 143874322 coordinates of the June 2002 freeze of the UCSC genome assembly and was built of clones from the RPCI-11 library. Control clones (n = 189) were chosen from all chromosomal locations, including chromosome 8p. Gaps in the contig array amount to 8.2 Mb of 8q (97.7 Mb). In addition to a large gap of 997 kb near the start of the tiling path contig (8q11), 11 gaps of 200 to 700 kb are positioned at the following cytogenetic bands: 8q13, 8q21, 8q23, and 8q24. Gaps smaller than 200 kb include 26 gaps between 50 kb and 200 kb and 42 gaps smaller than 50 kb. DNA from target clones was obtained from BACPAC resources, Children's Hospital Oakland Research Institute (Oakland, CA, USA).

Array Preparation

To prepare DNA for spotting on the array, amplification with degenerative oligonucleotide primer (DOP) polymerase chain reaction (PCR) was performed in a 96-well format, as described by Hodgson et al. (37) with minor modifications (38). Briefly, a water control in each 96-well format was included to ensure the absence of contaminating DNA. PCR was performed on 40 to 400 ng of BAC DNA in a reaction volume of 100 μl. The final PCR mix contained 3 mM $MgCl_2$, 5 U Taq polymerase (New England Biolabs, Beverly, MA, USA), 200 μM dNTPs, 1× PCR buffer, and 1.5 μM 5′ amine-modified DOP primer (5′-

CCGACTCGAGNNNNNNATGTGG-3′; Epoch Biosciences, San Diego, CA, USA). The amine group in the primer allowed the PCR products to be covalently linked to the amine-reactive slide surface. PCR cycling conditions consisted of an initial denaturation of 3 min at 94°C, 25 cycles consisting of 30 s at 94°C, a 37°C to 72°C linear ramp of 10 min, and 1 min at 72°C followed by a final elongation step of 7 min at 72°C.

PCR product from each reaction was checked on agarose gel (0.8%), with the products ranging from 0.2 to 5 kb. PCR products were precipitated with ethanol and sodium acetate, air dried, and resuspended in 12.5 μl spotting solution (20% dimethylsulfoxide in water). Samples were then transferred to 864-well, round-bottom polypropylene plates (Whatman, Maidstone, UK) and printed in quadruplicate onto CMT-GAPS2–coated slides (Corning, Corning, NY, USA) by using a custom DNA arraying device developed at the University of California San Francisco Cancer Center. All replicates were printed onto the same subarray, with two sets of two adjacent replicates being separated by seven rows. Center-to-center spacing on the print was 175 μm. Two arrays were printed per slide, each covering an area of 12 × 12 mm.

Hybridization to Microarrays

Array-based CGH was performed as described previously (6,39). Four hundred nanograms of test DNA (xenograft or tumor) and reference genomic DNA (Promega, Madison, WI, USA) was labeled with indocarbocyanine (Cy3) and indodicarbocyanine (Cy5; Amersham Pharmacia Biotech, Piscataway, NJ, USA), respectively, according to a modified random priming protocol from the BioPrime labeling system (Invitrogen, Carlsbad, CA, USA). Briefly, random primer (random DNA octamers) was added to the DNA to a final concentration of 300 ng/μl. After denaturation for 10 min at 100°C, the sample was put on ice and dNTPs (dATP, dGTP, and dCTP, final concentration 200 μM; dTTP, final concentration 50 μM), cyanine dye-labeled dUTP (Cy3-dUTP or Cy5-dUTP, final concentration 40 μM), and 40 U of Klenow fragment (40 U/μl) were added to a final volume of 25 μl. The reaction mixture was incubated overnight (o/n) at 37°C. Reference and test DNAs for each array were then pooled. Unincorporated nucleotides were removed using microspin columns according to the recommendations of the manufacturer (Amersham Pharmacia Biotech). The mixture of labeled reference and test DNAs was precipitated in the presence of 60 μg Cot-1 DNA (Roche, Basel, Switzerland) and resuspended in 50 μl of hybridization solution that contained 50% formamide, 10% dextran sulfate, 2× standard saline citrate (SSC), 4% sodium dodecylsulfate, and 10 μg/μl yeast tRNA. Probes were denatured for 10 min at 74°C, after which pre-annealing of the Cot-1 DNA took place for 60 min at 37°C.

Preparation of the slides consisted of cross-linking the slides (UV Stratalinker, Stratagene, La Jolla, CA, USA; 2,600 × 100 μJ) and placing a dam of rubber cement around each array at least 60 min before use to allow the rubber cement to set.

The hybridization mixture (50 μl) was added to the array. A rubber gasket and a glass microscopy slide fastened to the slide provided an enclosed chamber for the hybridization. Hybridization was carried out for 48 h at 37°C on a unidirectional tilting platform (±3 rpm) placed within an incubator (Robbins Scientific, Sunnyvale, CA, USA). After hybridization, slides were washed for 15 min in 50% formamide, 2× SSC, pH 7.0, at 50°C and then for an additional 20 min in 2× SSC and 0.1% sodium dodecylsulfate at 50°C. Slides were washed in PN buffer (0.1 M sodium phosphate buffer, 0.1% NP40, pH 8.0) for 10 min at room temperature, and rubber cement dams were removed. Results from CML1, MC3, MEG-01, fresh-frozen gastric cardia adenocarcinoma, and two of the archival, formalin-fixed, prostatic cancers were based on a single hybridization. The other specimens were repeated two or three times and demonstrated a good reproducibility of the technique.

Image and Data Analysis

A ScanArray Express HT (Perkin Elmer Life Sciences, Boston, MA, USA) was used to collect 16-bit TIF images through Cy3 and Cy5 filter sets. Images were analyzed with custom software as described previously (4,40). Thus, a ratio of Cy3 to Cy5 intensities, averaged for the quadruplicate spots, was obtained per clone. Clones were excluded from analysis if the standard deviation (S.D.) of the replicate spots exceeded 0.2 or if no more than one 1 spot was available for analysis. For analysis and presentation, $\log_2$ values of fluorescence ratios were used.

Criteria for determining gains, losses, and amplifications were based on the aCGH pattern obtained from normal versus normal hybridizations. Briefly, four independent control experiments, i.e., two normal male versus normal male DNA and two normal male versus normal female DNA hybridizations (both DNAs from Promega), resulted in a mean $\log_2$ fluorescence ratio of 0.0 ± 0.14 S.D., reflecting the equal copy number in test and reference DNAs. Based on this variation in normal cells, upper and lower thresholds were chosen at 3× S.D., i.e., +0.42 and −0.42. The two normal male versus normal female DNA hybridizations also served as a primary validation of the 8q array by showing a proper sex-mismatch in the control set of clones. Nonrecurrent single BAC clone alterations could be due to suboptimal technical aspects of the hybridization procedure. Therefore, they were not considered to be genomic imbalances and were discarded. If a biological reason for sporadic single BAC changes is assumed, it is necessary to validate these findings with a different technique, such as fluorescent in situ hybridization (FISH). A high-level amplification probably representing an amplicon was defined as a distinct peak (with an arbitrary ratio > 1.5). For assessment of 8q imbalances, the control set of clones was used. This is necessary after normalization of all clone ratios on the array, 8q-specific and control clones. For example, in case of a whole arm amplification, ratios of 8q-specific clones decrease (toward 0), whereas ratios for the control clones become negative. To correct for this phenomenon, the mean ratio

of the control clones was subtracted from each 8q-specific ratio. This resulted in a proper positioning of the 8q set of clones.

Fluorescent In Situ Hybridization

FISH of fresh cell preparations and formalin-fixed and paraffin-embedded material was accomplished as previously described (41). DNA probes for bicolor FISH of the chromosome 8 centromere and 8q BAC DNA clones were labeled with Spectrum Orange and Spectrum Green, respectively, using a Nick Translation Reagent Kit (Vysis, Downers Grove, IL, USA) according to the manufacturer's directions. The following 8q BAC clones were chosen from the contig set used for the 8q array: RP11-136D18 (8q21.13, 80 Mb), RP11-280G23 (8q21.13, 81 Mb), RP11-316N10 (8q22.2, 98 Mb), RP11-7J8 (8q22.3, 101 Mb), RP11-769F16 (8q23.1, 106 Mb), RP11-1140O6 (8q23.3, 113 Mb), RP11-739E11 (8q24.22, 133 Mb), and RP11-978D20 (8q24.3, 139 Mb). Briefly, after overnight hybridization, cells were counterstained with 4′,6-diamidino-2-phenylindole in antifade solution (Vectashield, Vector, Burlingame, CA, USA).

Two investigators scored a minimum of 50 interphase cells per specimen for the centromere 8/8q BAC probe combinations on a computer screen. Images of each of the three fluorochromes were collected with an epifluorescence microscope (Leica DM, Rijswijk, The Netherlands) equipped with appropriate excitation and emission filter sets (Leica) and a cooled CCD camera (Photometrics, Tucson, AZ, USA). The green, red, and blue images were collected sequentially by changing the excitation filter with Smartcapture software (Vysis).

RESULTS

The minimal tiling path contig for chromosome 8q was generated with the BAC Contig Assembler software, which uses information about BAC end sequences and the normal genome sequence (36). The contig spanned the entire region and was comprised of 702 BAC clones from the RPCI-11 library. Overlapping BAC clones cover 89.5 Mb (91.7%) of a total genomic distance of 97.7 Mb (i.e., the whole long arm of chromosome 8). Thus, gaps constitute 8.2 Mb (8.3%), of which gaps smaller than 200 kb make up 3.3% of the total tiling path. Gaps larger than 200 kb occur at 12 positions (4.9 Mb; 5%) and result mostly from repeat/duplicon-rich content of genomic sequence, which hampers contig assembly with this method.

A series of experiments was performed to evaluate the 8q array for detection of copy number changes. Control hybridizations of normal male versus normal male DNA and normal male versus normal female DNA were performed in four independent experiments and resulted in a mean $\log_2$ fluorescence ratio of 0.0 ± 0.14 S.D., reflecting the equal copy number in test and reference DNA. Based on this variation in normal cells thresholds were set at $3\times$ S.D., i.e. $+0.42$ for gain and -0.42 for loss. An example of these control hybridizations is shown in Figure 1A. A series of well-characterized clinical samples and cell lines, previously analyzed by multicolor FISH (M-FISH) and conventional CGH, were used to test the ability of the array to detect alterations in copy number (26,42). Copy number changes detected by aCGH in relation to M-FISH data are listed in Table 1. These cases included CML specimens and gastroesophageal adenocarcinoma cell lines.

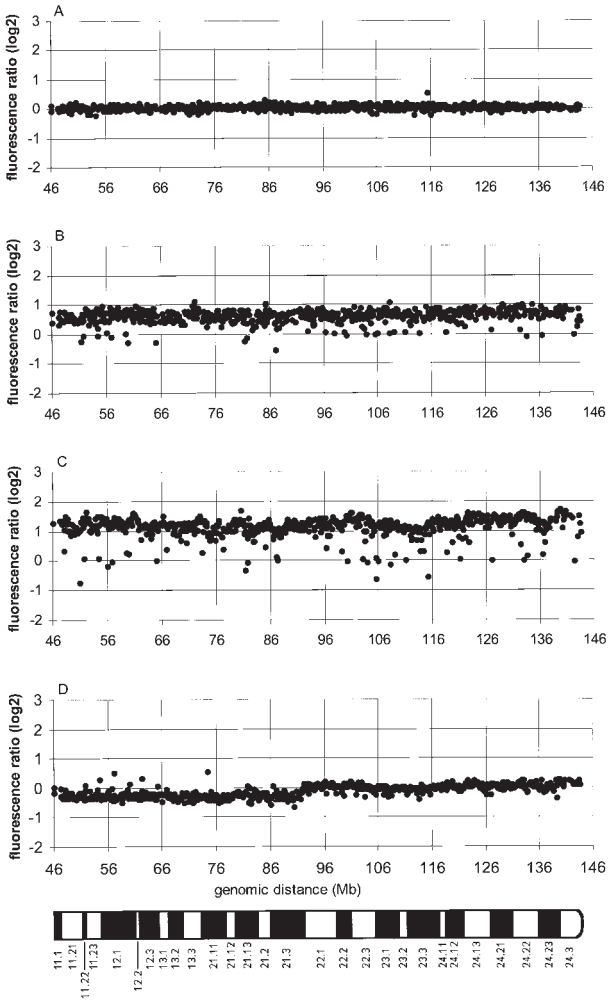

FIG. 1. Validation of the 8q array. The genomic distance (Mb) is plotted on the x axis, and the y axis shows the $\log_2$ fluorescence ratio of test and reference DNAs. **A:** $\log_2$ ratios of male versus male reference DNA at the expected value of 0. **B:** $\log_2$ ratios of CML case 1 showing a whole arm single copy gain. **C:** $\log_2$ ratios of CML case 2 showing a whole arm double copy gain. **D:** $\log_2$ ratios of a fresh-frozen gastric cardia adenocarcinoma showing a large deletion. A chromosomal ideogram of 8q is displayed at the bottom of the figure.

First, we examined the capability of the array for detection of large genomic areas with a copy number change. Single copy and double copy gains of the whole chromosome arm were detected in CML cases 1 and 2 (Table 1, Figs. 1B and 1C, respectively). The capacity of this array to delineate single copy loss was illustrated by a case of gastric cardia adenocarcinoma with deletion of 8q11.2-21.3 as previously determined by conventional CGH. The aCGH profile with single copy loss of 8q11.1-21.3 (46.1 to 91.9 Mb) is shown in Figure 1D. However, this deletion did not fully reach the lower 0.42 threshold for loss and

Table 1
*8q Array CGH Analysis and M-FISH of CML/Blast Crisis Samples, and CML, Gastric Cardia, and Esophageal Adenocarcinoma Cell Lines**

	8q aCGH			M-FISH[1]		
Cases	Gain (Mb)	Amplification (Mb)	Loss	Ploidy	Number of normal chromosome 8	Derivative chromosomes
CML 1	Whole arm, single copy (46.1–143.9)	—	—	2n	3	No structural aberrations of #8
CML 2	Whole arm, double copy (46.1–143.9)	—	—	2n	2	1 Derivative of #8 containing 2 copies of 8q (iso8q)
MC3	8q24.13-ter (125.0–143.9)	—	—	2-3n	3	1 Derivative of #1, containing the 8q24-qter region
MEG-01	8q12.3–13.2 (63.8–68.3), 21.12-21.3 (77.5–87.8), 24.13 (122.1–123.8)	8q23.3-24.12 (116.4–120.8), 24.13-24.22 (125.4–133.6)	—	2-3n	2	Derivative of #10 and Y chromosomes containing multiple copies of the 8q24-qter region
JROECL 33	8q23.1-ter (106.3–143.9)	—	—	±4n	4	2 Derivative chromosomes containing distal 8q
OACM4.1X	8q24.12-ter (118.6–143.9)	8q11.21 (49.5–50.9), 12.1-12.3 (58.4–61.7), 13.2-13.3 (67.3–70.6), 13.3-21.11 (72.6–75.7), 21.12-21.13 (78.2–80.2), 21.3 (91.0–91.7), 23.3-24.11 (114.7–117.2), 24.13-24.21 (124.2–128.9)	—	2-3n	1–2	6 Copies of 4 different derivative chromosomes containing parts of #8
OACM5.1C	8q24.13 (123.8–126.3), 24.21-24.22 (126.6–133.3), 24.23-ter (133.9–143.9)	—	—	<2n	1	3 Derivative chromosomes containing parts of #8

*Genomic distance (Mb) is presented in parentheses.
[1]M-FISH of MC3 and MEG-01 as described by Gribble et al. (26); M-FISH of JROECL 33, OACM4.1X, and OACM5.1C as described by Rosenberg et al. (42).

therefore was confirmed by loss of heterozygosity with polymorphic marker D8S273 (not shown).

Second, we tested the ability of the array to detect small(er) genome aberrations. Single copy gain of part of the 8q arm was detected in cell line JROECL 33 (Table 1, Fig. 2A). In this cell line, single copy gain was detected on 8q23.1-ter (106.3 to 143.9 Mb). Amplifications were observed in cell lines MEG-01 and OACM4.1X (Table 1, Figs. 2B and 2C, respectively). High-level amplifications found in MEG-01, located at 8q23.3-24.12 (116.4 to 120.8 Mb) and 8q24.13-24.22 (125.4 to 133.6 Mb), had genomic sizes of 4.4 Mb and 8.2 Mb, respectively. In addition to these amplifications, several gains can be seen (Table 1). In cell line OACM4.1X, eight high-level amplifications were detected at the following locations: 8q11.21 (49.5 to 50.9 Mb, for a difference of 1.4 Mb), 8q12.1-12.3 (58.4 to 61.7 Mb, 3.3 Mb), 8q13.2-13.3 (67.3 to 70.6 Mb, 3.3 Mb), 8q13.3-21.11 (72.6 to 75.7 Mb, 3.1 Mb), 8q21.12-21.13 (78.2 to 80.2 Mb, 2.0 Mb), 8q21.3 (91.0 to 91.7 Mb, 0.7 Mb), 8q23.3-24.11 (114.7 to 117.2 Mb, 2.5 Mb), and 8q24.13-24.21(124.2 to 128.9 Mb, 4.7 Mb). The most distal amplification was situated on top of a single copy gain of 8q24.12-ter (118.6 to 143.9 Mb). A deletion of only 0.6 Mb was identified at 8q22.2 (98.2 to 98.8 Mb) in prostate cancer cell line PC3 in a large region of single copy gain (8q13.1-ter; 69.4 to 143.9 Mb). In addition, a larger, proximal, single copy loss was found at 8q11.21-12.2 (48.8 to 61.6 Mb; Fig. 2D). Conventional CGH of PC3 only detected gain of 8q13-ter. In all these cases, aCGH better demonstrated the complexity of the alterations than did M-FISH and/or conventional CGH. The aCGH results were further validated by bicolor FISH analysis with a centromeric DNA probe and BAC clones from our contig array. A good concordance was seen between the $\log_2$ ratios from aCGH analysis and interphase FISH measurements for selected alterations of the four cancer cell lines depicted in Figure 2. Single copy gain in JROECL 33, amplifications in MEG-01 and OACM4.1X,

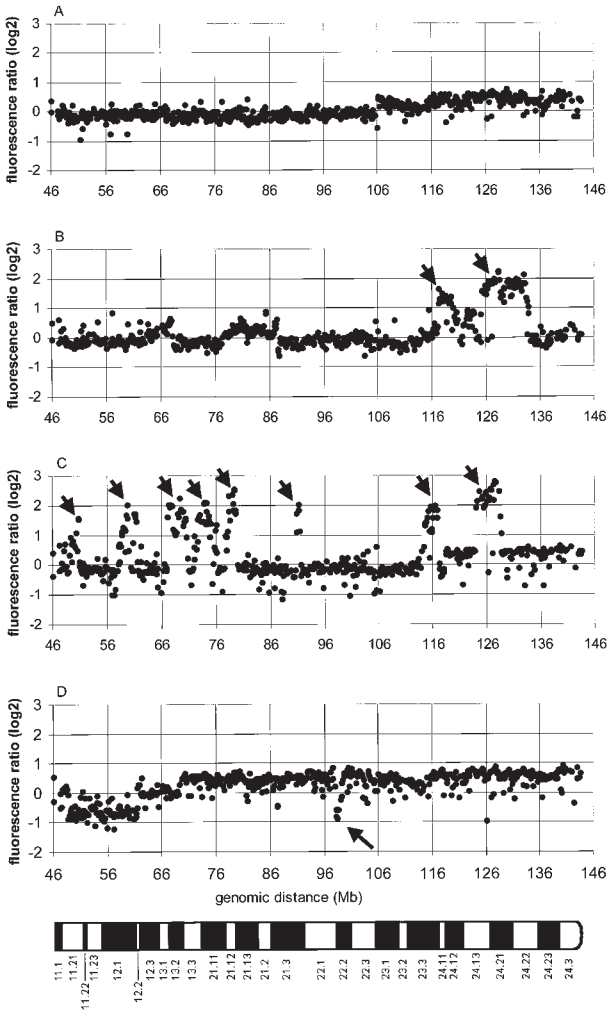

FIG. 2. Resolution of the 8q array. **A:** Log_2 ratios of JROECL 33 showing single copy gain of distal 8q. **B:** Log_2 ratios of MEG-01 illustrating a complex pattern of proximal gains and distal amplifications (arrows). **C:** Log_2 ratios of OACM4.1X showing multiple high-level amplifications (arrows). **D:** Log_2 ratios of PC 3 displaying a small single copy deletion (arrow) within a large single copy gain. A chromosomal ideogram of 8q is displayed at the bottom of the figure.

and the small deletion in PC3 were confirmed (Table 2, Fig. 3).

Third, the array was examined for aCGH with formalin-fixed, paraffin-embedded, prostate adenocarcinoma (PAC) DNAs that were previously evaluated by conventional CGH (Table 3, Fig. 4). Single copy gain of 8q13.3-ter (70.8 to 143.9 Mb) was found in PAC case 1 in addition to a single copy deletion of 8q11.1-13.3 (46.1 to 70.3 Mb; Fig. 4A). Note the sharp breakpoint at 8q13.3 spanning only a few BAC clones in Figure 4A. In PAC case 2, single copy gain was detected at 8q11.1-ter (46.1 to 143.9 Mb; Fig. 4B), in PAC case 3 at 8q11.1-24.21 (46.1 to 127.6 Mb; Fig. 4C), and in PAC case 4 at 8q12.1-12.3 (59.4 to 64.2 Mb, 4.8 Mb), 8q13.1-13.3 (66.0 to 72.0 Mb, 6.0 Mb), and 8q24.13-24.21 (124.8 to 128.0 Mb, 3.2 Mb; Fig. 4D). In most cases aCGH analysis showed more alterations or the alteration with higher resolution compared with CGH. In PAC case 1 a deletion of 24.2 Mb was found between 8q11.1 and 8q13.3, which was below the detection limit of conventional CGH. In PAC case 3 a whole arm gain was found by conventional CGH, whereas aCGH showed a distal deletion. In PAC case 4 aCGH defined three gains of limited size compared with two gains defined by conventional CGH (Table 3).

DISCUSSION

In this report we show the construction and application of a high-resolution chromosome 8q contig array for CGH. A minimal tiling path of 702 BAC clones was built by using the BAC assembler software (36), with 92% coverage over 97.7 Mb. Using this high-resolution contig array we not only detected all chromosomal aberrations, previously identified by M-FISH and/or conventional CGH, to greater resolution but also identified numerous novel genome copy number aberrations. Selected copy number changes were confirmed by interphase FISH measurements. The ability to screen formalin-fixed, paraffin-embedded tissue with this array enables the analysis of archival specimens of which no fresh material is present.

The DOP PCR used in this study has been shown to be an effective solution for generating sufficient quantities of DNA for spotting microarrays (37,43). Recently, an optimized DOP protocol and other amplification methods have been suggested as alternatives to conventional DOP PCR (8,9). Other technical improvements, such as fewer repeat sequences in target DNA and modification of target DNA to retain the repellent quality of glass surface between spots, may result in decreased background levels (9,44). In our study background can be seen as single BAC clone changes or a slightly undulating aCGH pattern, where a straight line is expected. This background may reflect the presence of repeat sequences on the array that are not or insufficiently blocked by the Cot-1 DNA (2). In

Table 2
FISH Validation of aCGH Alterations

Sample	Validated copy number event (aCGH)	8q BAC clone	Clone location (Mb)	Cen8/BAC FISH log_2 ratio	Array CGH log_2 ratio
JROECL 33	Single copy gain (8q24.3)	RP11-978D20	8q24.3 (139)	0.57	0.44
MEG-01	Low-level amplification (8q21.13)	RP11-280G23	8q21.13 (81)	0.37	0.37
	High-level amplification (8q24.22)	RP11-739E11	8q24.22 (133)	High	1.82
OACM4.1X	High-level amplification (8q21.13)	RP11-136D18	8q21.13 (80)	High	1.79
PC3	Deletion (8q22.2)	RP11-316N10	8q22.2 (98)	−0.62	−0.84

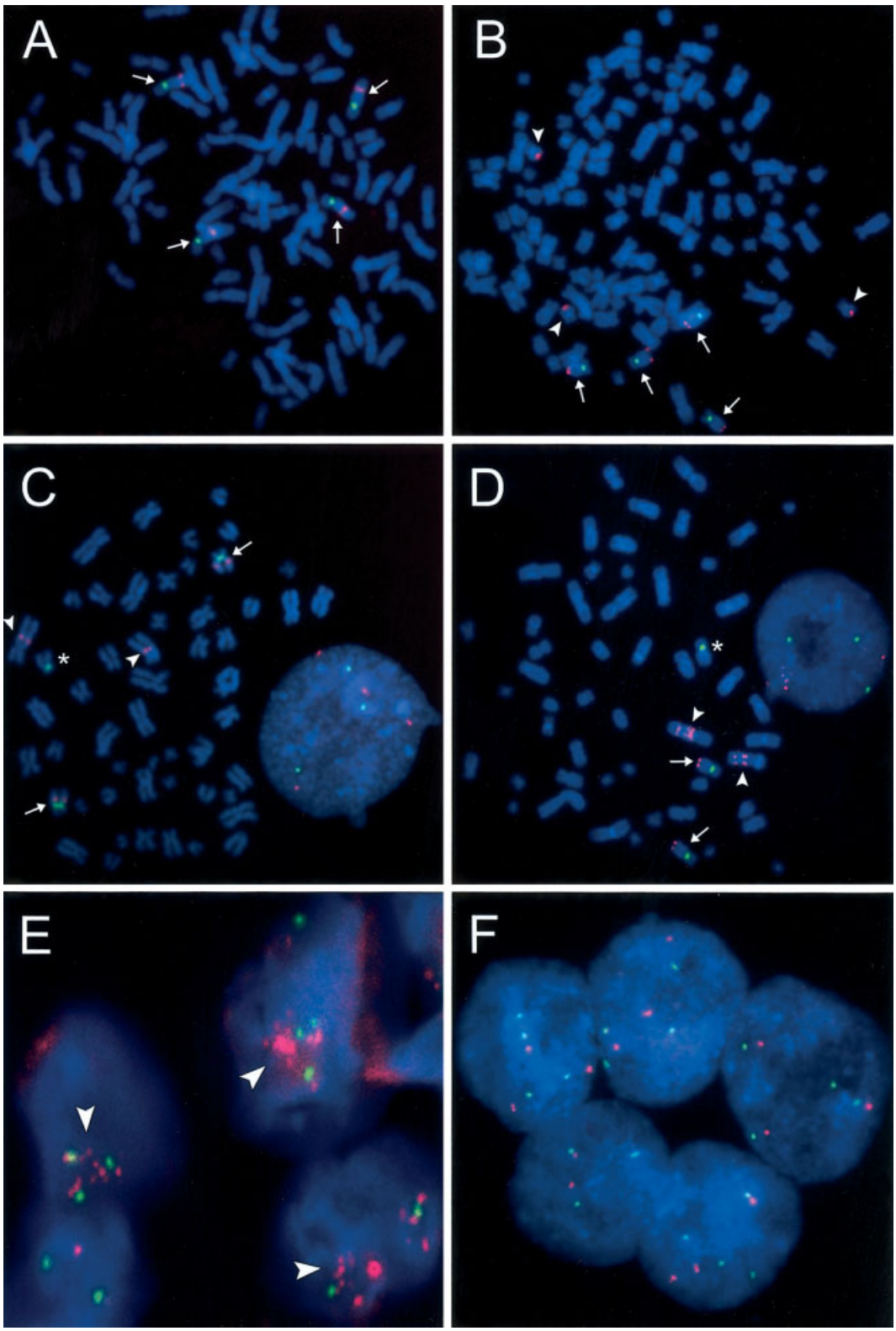

FIG. 3. Bicolor FISH with a centromeric chromosome 8–specific DNA probe (green) and 8q-specific BAC DNA clones (red) to metaphase and interphase tumor cell line cells. **A:** JROECL 33 metaphase cell showing chromosomes with four normal copies of 8q (arrows) visualized with BAC clone RP11-7J8 (8q22.3; 101 Mb). **B:** JROECL 33 metaphase cell showing four normal copies of 8q (arrows) and three derivative chromosomes harboring distal 8q (arrowheads), which are detected with BAC clone RP11-978D20 located at 8q24.3 (139 Mb); it should be noted that a mixture of metaphase cells with two or three derivative chromosomes harboring distal 8q were observed, illustrating cytogenetic heterogeneity. **C:** MEG-01 FISH to a metaphase (and interphase cell) displaying two normal 8q arms (arrows) and two derivative chromosomes (arrowheads), the latter containing extra copies of BAC clone RP11-280G23 (peak at 8q21.13; 81 Mb); in addition, a (derivative) chromosome containing only centromere 8 is present (asterisk). **D:** MEG-01, in the metaphase cell, two normal 8q arms (arrows) and two derivative chromosomes (arrowheads) are present, with the latter containing multiple copies of BAC clone RP11-739E11 (peak at 8q24.22; 133 Mb). **E:** Paraffin-embedded interphase cells of OACM4.1X showing high-level amplifications (arrowheads) of BAC clone RP11-136D18 at 8q21.13 (80 Mb). **F:** FISH with BAC clone RP11-316N10 (8q22.2; 98 Mb) to PC 3 interphase cells illustrating the small single copy deletion; compared with the green centromeric spots, fewer red BAC-related spots are seen.

Table 3
*8q Array and Conventional CGH Analysis of Paraffin-Embedded Prostate Cancer Cases**

	8q aCGH			CGH	
Cases	Gain (Mb)	Amplification (Mb)	Loss (Mb)	Gain	Loss
PAC 1	8q13.3-ter (70.8–143.9)	—	8q11.1–13.3 (46.1–70.3)	8q21.3-qter	—
PAC 2	8q11.1-ter (46.1–143.9)	—	—	8q11.2-ter	—
PAC 3	8q11.1-24.21 (46.1–127.6)	—	—	8q11.2-ter	—
PAC 4	8q12.1-12.3 (59.4–64.2), 13.1-13.3 (66.0–72.0), 24.13-24.21 (124.8–128.0)	—	—	8q11.2-21.1,8q23-ter	—

*Genomic distance (Mb) is presented in parentheses.

tumor cells and cell lines, genetic heterogeneity might hamper the detection of aberrations, whereas in primary cancer samples admixture of nontumor cells will contribute to impaired evaluation. A small subset of the 8q clones used may be wrongly mapped and, in fact, be located on other chromosomes. Updates of the UCSC genome browser should result in the eventual elimination of these clones (Genome Browser UCSC; http://genome.ucsc.edu).

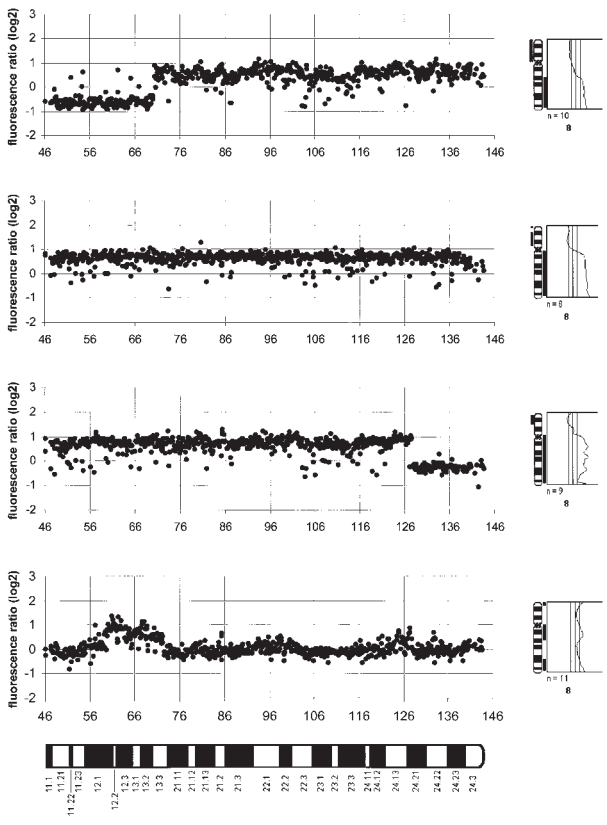

FIG. 4. Application of the 8q array on paraffin-embedded prostate adenocarcinomas (left: aCGH, right: conventional CGH). **A–D:** Log_2 ratios of prostatic adenocarcinoma cases 1 to 4 illustrating the presence of gains and losses. A chromosomal ideogram of 8q is displayed at the bottom of the figure.

This contig array was intended for elucidation of 8q amplifications. Gain of the whole arm, however, was also clearly detected. The mean ratio of a single copy gain (0.58) reflecting the ideal value for a 3/2 copy number ratio was found in CML case 1. A double copy gain, i.e., CML case 2, with an 8q isochromosome showed a value (1.1) slightly higher than the expected value of 1. This may be explained in part by the correction step used in this study. The secondary correction of the 8q set with the mean ratio of the set of control clones is necessary after the initial normalization of all of the clones on the array. However, it is unlikely that this control set yields an equal number of gains and losses in every case. For example, in some cases more deletions than gains may be found in the control set, which may result in a slight aberration of the expected ratio value. However, it must be noted that this does not affect the ability of the array to map breakpoints and amplicon boundaries. The ability to map such events with high resolution is one of the advantages of contig array CGH. In general, the resolution of minimal tiling path contig BAC arrays generated with the BAC Contig Assembler is higher than the insert size of the clones (~100 kb) (3,36). In the 8q BAC array, the average resolution was 60 kb, determined on regions with contiguous BAC clones. This value is comparable to the resolution of 75 kb on a full-coverage, high-resolution, chromosome 22 array (9). In an NF2-specific array, the limit to distinguish deletions was even decreased to 40 kb (45). The resolution for deletions was demonstrated in this study in cell line PC 3, in which a 554-kb deletion was found, defined by six clones. Similarly, a high-level amplification of only 718 kb was detected in cell line OACM4.1X.

Several gains and amplifications were seen already, suggesting involvement of specific genes. For example, amplification of 8q24.21 in cell line OACM4.1X at least in part reflects the MYC gene. MYC has been established as an oncogene, and amplification of this gene has been linked to unfavorable clinical outcome in a number of tumors (27,46,47). Amplification of MYC does not exclude the presence of other target genes at 8q. Moreover, amplifications at other bands strongly suggest the presence of other cancer-related genes (10,26,31,32,48). These may include GC84 (8q11), Elongin C (8q21), TPD52

(8q21), EIF3S3, EBAG9 (both at 8q23), PSCA (8q24.2), and PTK2 (8q24.3) (49–56). The value of contig arrays for the identification of novel oncogenes has been shown for the putative oncogene CYP24 on chromosome 20 (3). Genetic profiling by aCGH can improve tumor diagnosis, as previously demonstrated for cancer types of different histologies. However, in a diagnostic setting, FISH (with BAC DNA probes) is more appropriate to evaluate the presence of a specific genomic alteration.

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