Tissue Microdissection and Degenerate Oligonucleotide Primed-Polymerase Chain Reaction (DOP-PCR) Is an Effective Method to Analyze Genetic Aberrations in Invasive Tumors

Yuichi Hirose,^{*§} Kenneth Aldape,^{‡§}
Michelle Takahashi,^{‡§} Mitchel S. Berger,^{*§} and
Burt G. Feuerstein^{†§¶}

From the Departments of Neurological Surgery* and Lab
Medicine,[†] the Division of Neuropathology,[‡] the Brain Tumor
Research Center,[§] and the Cancer Genetics Program,[¶] University
of California-San Francisco, San Francisco, California

We amplified various amounts of DNA derived from frozen SF210 and U251NCI human glioblastoma cells, carried out comparative genomic hybridization (CGH) using degenerate oligonucleotide primed-PCR (DOP-PCR) products as test probes, and compared results to analyses performed with probes prepared by standard nick translation. Next we extracted DNA from hematoxylin-eosin (HE)- and methyl green (MG)-stained, microdissected sections of formalin-fixed and paraffin-embedded U251NCI cells, amplified and labeled it by DOP-PCR, and subjected it to CGH. Finally, we used the same methods in multiple samples from a single human mixed glioma tissue. DOP-PCR products from 50 pg to 250 ng of DNA were equally effective in generating the same CGH profiles as the standard method. DOP-PCR products from microdissected pieces of MG-stained cells were effective probes for CGH, but HE-stained samples were not desirable. As the proportion of HE-stained sample increased, CGH profiles deteriorated. DOP-PCR products from microdissected pieces of MG-stained paraffin sections of glioma tissue produced CGH profiles compatible with their histological features. CGH performed with DOP-PCR products from microdissected paraffin blocks allows for the accurate investigation of the cytogenetic characteristics from invasive tumors and of cytogenetic heterogeneity within neoplastic tissue. (*J Mol Diag* 2001, 3:62–67)

To evaluate the genetic aberrations in an invasive tumor, the normal tissue adjacent to the tumor must be carefully removed. Tissue microdissection based on microscopic observation is one way to accomplish this. Recently, degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) has been reported to be a good

method to produce a representation of small amounts of DNA.^{1–6} The method allows analysis of less than 1 ng of DNA. These studies suggest that DOP-PCR is useful for amplifying DNA from microdissected tissue to use for comparative genomic hybridization (CGH). CGH produces a map of relative DNA copy number as a function of chromosomal location by comparing the hybridization of test and reference DNA to metaphase chromosomes.^{7–9} The results are consistent with fluorescence *in situ* hybridization (FISH) and flow cytometry when DNA taken directly from the test and the reference is nick translated.¹⁰ We have also found that CGH data are compatible with spectral karyotyping, and that conventional karyotyping of complex chromosome aberrations can be unreliable (Pellarin M, Shapiro J, Feuerstein BG, unpublished data). However, it is not clear whether DOP-PCR products of DNA from microdissected tissue faithfully represent each chromosomal abnormality in a sample. To develop a method of cytogenetic investigation in microdissected tissues of known histology, we first studied limits on the amount of DNA which could be reproducibly amplified by DOP-PCR for CGH analysis. Ten to 250 pg of DNA from human glioblastoma cell lines SF210 and U251NCI were used to generate DOP-PCR products. CGH profiles from samples ≥ 50 pg were nearly identical to profiles generated from nick translated probes without DNA amplification. Ten-picogram samples produced profiles of poorer quality. We also found that tissue microdissection using formalin-fixed, paraffin-embedded cell pellets of U251NCI and DOP-PCR produced faithful CGH profiles. This report confirms that DOP-PCR produces a faithful enough representation of small amounts of DNA (≥ 50 pg) to allow the use of microdissected tissue for CGH. We also discuss sample conditions which can affect CGH results.

Supported in part by the National Institutes of Health grants CA13525 and CA 64898, and by funds from the National Brain Tumor Foundation and the Farber Foundation.

Accepted for publication February 21, 2001.

Address reprint requests to Burt G. Feuerstein, Cancer Genetics Program, Box 0808, UCSF, San Francisco, CA 94143-0808. E-mail: feuer@cc.ucsf.edu.

Materials and Methods

DNA Extraction

SF210 and U251NCI human glioblastoma cells were grown in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum and non-essential amino acids. Genomic DNAs from these cells and leukocytes from a normal male donor were extracted using standard protocols.¹¹ DNA concentration was measured with a fluorometer (Hoefer Scientific, San Francisco, CA) using Hoechst 33258 (Sigma, St. Louis, MO).

U251NCI was trypsinized and centrifuged in a polyethylene tube. The cell pellet was fixed in 10% neutral buffered formalin and processed in paraffin. The paraffin block was cut into sections 5 μ m thick, deparaffinized, and stained with hematoxylin and eosin (HE; Fisher Scientific, Pittsburgh, PA) or methyl green (MG, Sigma). MG was used at 1% concentration in distilled water, sections were immersed for 15 to 60 seconds, washed in water for 1 to 5 minutes (depending on how blue/green the tissue was), and air-dried. Small (2 mm $\times$ 2 mm) and large (6 mm $\times$ 6 mm) amounts of tissue were microdissected and scraped with a 22-gauge needle. The tissue was incubated in 60 μ l 1 $\times$ PCR buffer (Roche, Indianapolis, IN) containing 0.5% Tween 20 and 0.5 mg/ml proteinase K (Life Technologies, Inc., Rockville, MD) for 3 days at 55°C. Proteinase K was added twice a day (1 μ g per 2.5 μ l sample). After 3 days' incubation, proteinase K was inactivated by heating for 10 to 15 minutes at 95°C.

We also extracted DNAs from microdissected MG-stained pieces of formalin-fixed, paraffin-embedded sections of mixed oligo-astrocytoma in the same manner. The regions for microdissection were marked according to consecutive HE-stained sections. Aliquots of these crude DNA extracts were subjected to DOP-PCR.

DOP-PCR

DOP-PCR amplification was performed in two phases using SF210 and U251NCI genomic DNA as templates. We used amounts ranging from 1 pg to 250 ng in 1 μ l volume or 1 μ l DNA extract from microdissected specimens. In the first phase (low stringency reaction), 1 μ l of sample was added to 4 μ l of buffer A (2.5 μ l of 600 μ mol/L dNTPs (dATP, dCTP, dGTP, and dTTP; Roche), 0.5 μ l of 10 μ mol/L DOP primer (5'-CCGACTC-GAGNNNNNNATGTGG-3', where N = A, C, G, or T)¹ and 1 μ l of 5 $\times$ Sequenase Reaction Buffer (Amersham, Cleveland, OH). The reaction was carried out using 5 cycles of 30°C for 5 minutes, 37°C for 2 minutes, and 96°C for 2 minutes, adding 0.65 units Sequenase at every 30°C step. The first phase product was subjected to the second phase reaction in 1 $\times$ PCR buffer (Roche) with dNTP (200 μ mol/L final concentration), DOP primer (1.4 μ mol/L final concentration) and 2.5 units of *Taq* polymerase (Roche). PCR conditions were as follows: 95°C for 5 minutes, 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes, followed by a final extension at 72°C for 5 minutes.

Test (cell line) DNA was labeled with a PCR reaction using 5 μ l of DOP-PCR product in 1 $\times$ PCR buffer with dNTP (100 μ mol/L final concentration), DOP primer (1.4 μ mol/L final concentration), Mg (4.5 mmol/L final concentration), digoxigenin (DIG)-11-dUTP (50 μ mol/L final concentration; Roche) and 2.5 units of *Taq* polymerase. PCR conditions were as follows: 95°C for 10 minutes, 25 cycles at 94°C for 70 seconds, 56°C for 70 seconds, and 72°C for 3 minutes, followed by a final extension at 72°C for 10 minutes. Reference (normal) DNA was amplified from 50 ng normal male DNA and labeled as described above except that fluorescein isothiocyanate-dUTP (DuPont Inc., Wilmington, DE) was used instead of DIG-dUTP.

CGH

CGH was performed according to Mohapatra et al.⁹ with some modifications. For standard CGH experiments, sample and normal DNAs were labeled with fluorescein isothiocyanate-dUTP and Texas Red-dUTP (DuPont), respectively, by nick translation using DNA polymerase I (Life Technologies). Two hundred nanograms each of labeled tumor and reference DNAs and 20 μ g of human Cot-1 DNA (Life Technologies) were ethanol-precipitated and dissolved in 50% formamide, 10% dextran sulfate. The probe mixture was denatured and hybridized to normal metaphase spreads (Vysis Inc., Downers Grove, IL). For CGH experiments using DOP-PCR products, 40 μ l DIG-labeled test probe, 20 μ l biotin-labeled reference probe, and 20 μ g human Cot-1 DNA were precipitated in ethanol, dissolved, denatured, and hybridized as above. After unhybridized probes were washed, the metaphase spread was incubated in blocking buffer (1% bovine serum albumin in 4 $\times$ SSC) at room temperature for 5 minutes, followed by 1 μ g/ml rhodamine-conjugated anti-DIG-antibody (Roche) in blocking buffer at room temperature for 45 minutes. The preparations were washed and counterstained with 4,6-diamino-2-phenylindole (DAPI) in antifade solution. Red, green, and blue images were acquired with a Quantitative Image Processing System (QUIPS), and the ratios of fluorescence intensity along the chromosomes were quantitated as described by Piper et al.¹² A relative gain was scored when the mean green:red ratio was above 1.2 and relative loss was scored when the mean green:red ratio was below 0.8. Chromosomal copy number aberrations (CNAs) were not scored at or near the centromeres. Amplifications were scored only when visual inspection revealed a bright and discrete signal confined to a subchromosomal region. Hybridizations produced from DOP-PCR products were more granular than those produced from nick translated probes. If signals were too dim, the hybridization was repeated. If ratios were simultaneously altered at chromosomes 1p, 19, and 22, the experiment was repeated as described in Mohapatra et al.¹⁰

Results

CGH profiles of SF210 human glioblastoma cell line by nick translation, showed gains of 3p16-pter, 5p, 5q31.3-

Figure 1. Ratio profiles from DNA prepared from unfixed SF210 human glioblastoma cells. The x-axis represents the position along the chromosome (p arm to the left and q arm to the right). The centromeres are marked by a crosshatch on the x-axis. The y-axis represents normalized test/reference fluorescence intensity ratios (copy number aberrations). The mean and SD of the fluorescence intensity ratios for the indicated chromosomes are shown. All chromosomal aberrations in experiments using DNA extracted without amplification and labeled by nick translation (**A**) were seen in those with DOP-PCR product derived from 50 pg (**B**) to 250 ng DNA (data not shown). However, experiments performed with DOP-PCR product from 10 pg DNA (**C**) did not show gain on chromosomal arm 6p (arrowheads), and ratio profiles were of poor quality (note the large standard deviations). There were no aberrations on chromosomes not shown in the figure.

qter, 6p23-pter, 11q13-22 and 16q22.2-qter; amplification of 14q31-qter; and losses on 2, 4p, 4q11.2-28, 4q32-qter, 7p, 9p21, 12q12-21, 15q25-qter (Figure 1A). DOP-PCR products derived from 50 pg to 250 ng DNA produced equivalent CGH profiles (compare Figure 1A to Figure 1B). We found that DOP-PCR product from as little as 10 pg DNA could produce a CGH profile; however, the latter profiles were not faithful representations of the results recognized in the nick translation experiment. For example, Figure 1C shows CGH profiles produced by products from 10 pg DNA; gains on chromosomal arms 3p and 6p were not detected clearly and the quality of the profiles were generally poor (note the large standard deviations).

To confirm the sensitivity of DOP-PCR CGH, we performed CGH experiments by nick translation and DOP-PCR using DNA extracted from another human glioblastoma cell line, U251NCI. CGH profiles using nick-translated probes showed gains of 1p, 3q23-qter, 7p21-pter, 9p, 15q, 17p, and 17q25; amplification of 10q26; and losses of 2q32.3-35, 4q32-qter, 10p, 13q31-34, and 18q (Figure 2A). The results of experiments using DOP-PCR products from 50 pg to 250 ng DNA were similar (Figure 2B) to profiles derived from nick-translated products. DOP-PCR product derived from 10 pg DNA were not (Figure 2C). In both series of experiments, fluorescence intensity on metaphase spreads produced by the amplified test probes was dim when DOP-PCR products were derived from less than 1 ng DNA (data not shown). When products

Figure 2. Ratio profiles from DNA prepared from unfixed U251NCI human glioblastoma cells. All chromosomal aberrations in experiments using DNA extracted without amplification and labeled by nick translation (**A**) were seen in those with DOP-PCR product from 50 pg (**B**) to 250 ng DNA (data not shown). Experiments performed with DOP-PCR product from 10 pg DNA (**C**) did not show gains on chromosomal arm 9p (arrowheads), and ratio profiles were of poor quality (note the large standard deviations). There were no aberrations on the chromosomes not shown in the figure.

were derived from 10 pg or less, signal intensity was too dim to give reproducible results.

We also performed CGH using DOP-PCR product from normal DNA as a test probe and normal DNA labeled by nick translation as a reference and found there was no distinguishable gain or loss in the profile (data not shown). These results were obtained with experiments using DOP-PCR products derived from DNA ranging from 50 pg to 250 ng, and they are in agreement with the study by Kuukasjärvi et al.⁴

Because products derived from very small amounts of DNA (50 pg, corresponding to 5–10 normal human cells) reproducibly yield sensitive and specific CGH profiles, we tested the efficacy of DOP-PCR CGH using microdissected formalin-fixed, paraffin-embedded cell pellets of U251NCI cells stained with HE and MG. Crude DNA extract of MG-stained tissue was a homogeneous blue solution (color intensity depended on the tissue content in the buffer), and those of HE-stained tissue were in two phases; one was a pink solution and the second was a purple precipitate. The former was separated from the latter by brief centrifugation and used as crude DNA extract. Agarose gel electrophoresis of DOP-PCR products from these tissues suggested DNAs could be amplified although their size was smaller than products amplified from fresh cellular DNA (Figure 3). These DOP-PCR products were used as probes for CGH experiments. DOP-PCR products from small amounts (2 mm × 2 mm) of both MG- and HE-stained samples produced CGH profiles equivalent to those produced by nick translation (Figures 4 and 5). On the other hand, larger (6 mm × 6 mm) HE-stained samples (ie, a more concentrated sample) produced unfaithful CGH profiles (Figure 5, A and B), even though the DOP-PCR product sizes were adequate (Figure 3, lane 11). Increasing the Mg²⁺ concentration for the second

Figure 3. Agarose gel electrophoresis of DOP-PCR products. Five microliters of each DOP-PCR product were applied to 1.2% agarose gel. M, ϕ X 174/*Hae*III. The sizes of the molecular weight markers are indicated at the left. **Lanes 1-6:** DOP-PCR product from 10 pg (1), 100 pg (2), 1 ng (3), 10 ng (4), 50 ng (5), and 250 ng (6) of DNA from unfixed U251NCI cells. Size of DOP-PCR products ranged from approximately 400 to 1500 bp, and amplification was better when template DNA was 1 ng or more. **Lanes 7-11** show DOP-PCR product from DNA extracted from fixed, embedded, and microdissected piece of MG-stained (7 and 8) and HE-stained (9-11) U251NCI cell pellet. DNA was extracted from smaller (2 mm $\times$ 2 mm, lanes 7 and 9) and larger (6 mm $\times$ 6 mm; lanes 8, 10, and 11) pieces in the same amount of buffer (60 μ l) and amplified by DOP-PCR. DNA from the larger HE piece was amplified with (lane 10) or without (lane 11) increased magnesium. Size of DOP-PCR products from fixed and microdissected samples (apparently <1000 bp) was smaller than that amplified from freshly prepared DNA.

phase of DOP-PCR improved the CGH profile (Figure 3, lane 10; profile data not shown).

We microdissected histologically normal and neoplastic regions from MG-stained paraffin sections of human

Figure 4. Ratio profiles from MG-stained and microdissected U251NCI human glioblastoma cell pellets. DNA extracted in 60 μ l extraction buffer from both smaller (A, 2 mm $\times$ 2 mm) and larger (B, 6 mm $\times$ 6 mm) pieces produced CGH profiles similar to those by nick translation (see Figure 2A).

Figure 5. Ratio profiles from HE-stained and microdissected U251NCI human glioblastoma cell pellets. DOP-PCR product from the smaller piece (A, 2 mm $\times$ 2 mm in 60 μ l extraction buffer) produced a CGH profile similar to that by nick translation (see Figure 2A). On the other hand, the product from the larger piece (6 mm $\times$ 6 mm in 60 μ l extraction buffer) did not produce faithful CGH profiles (B). However, DOP-PCR using an increased magnesium concentration produced a faithful profile (data not shown).

oligo-astrocytoma. CGH profiles from the normal regions showed no aberration, but profiles from neoplastic regions had losses on chromosome arms 1p and 19q (Figure 6).

Discussion

Both SF210 and U251NCI cells have a variety of chromosomal aberrations, and CGH was useful in detecting each of them if ≥ 50 pg DNA was used for DOP-PCR amplification. Thus, our method is effective for investigating the whole genome by CGH. Our studies do not bear

Figure 6. Ratio profiles from fixed and microdissected human mixed oligo-astrocytoma. DNAs were extracted from histologically neoplastic (*) and normal (**) regions and amplified by DOP-PCR. **A:** MG-stained section where microdissected areas are indicated by the dotted squares. **B:** HE-stained section where areas to be microdissected are indicated by the dotted squares. **C:** CGH ratio profile from histologically normal and neoplastic regions. The former showed no aberrations, whereas the latter showed losses on chromosome arms 1p and 19q.

on the controversy concerning the effectiveness of DOP-PCR for microsatellite analysis.^{3,13,14} In the SF210 and U251NCI series of experiments, fluorescence on the metaphase spreads produced by the test probes was dim, grainier, and more patchy when <1 ng DNA was used for DOP-PCR. This suggests that the whole genome may not be amplified by DOP-PCR in a universal manner when the amount of template DNA was small (<1 ng). Another possibility is that some pieces of the genome are not included in a sample of less than 1 ng. Certainly, if the amount of template DNA is very small (<10 pg), genomic regions not included in the sample might be quite large, and aberrations (especially gains) in those regions may not be faithfully detected. However, when the sample contains DNA extracted from 5 to 10 cells (≥ 50 pg), DOP-PCR can provide appropriate probes for CGH, although it may not be suitable for experiments such as fine mapping of chromosomes or microsatellite analysis. As shown in Figure 3, the proportion of small (<100 bp) fragments in DOP-PCR products increased as the amount of template DNA decreased. These small fragments may produce unfaithful CGH profiles when unaccompanied by larger fragments. Approximately 200 cells were included in the microdissected region of mixed oligo-astrocytoma tissue that we used as sample in Figure 6. Tissue microdissection by needle can isolate cells within a region larger than 1 mm, and the number of cells in the tissue piece depends on the cellularity of the tissue. Recently, a study on CGH from a single cell was published,^{5,15} and laser-assisted tissue microdissection¹⁶ might enable us to use 10 to 20 cells in a tissue section for DOP-PCR CGH.

Recently, several studies of cancers by CGH using tissue microdissection and DOP-PCR have been published.^{16–18} These studies found that DOP-PCR was effective in obtaining CGH profiles, but they have not shown that DOP-PCR product from microdissected tissue gives the same CGH profile as standard nick translation. We made formalin-fixed, paraffin-embedded thin sections of cell pellet from U251NCI cells, microdissected small areas from them, and amplified DNA for CGH. We have previously verified CGH aberrations in nick-translated DNA from nine glioblastoma cell lines including U251NCI, using FISH and flow cytometry.¹⁰ We find that DOP-PCR CGH from this tissue can produce faithful CGH profiles if ≥ 50 pg of DNA is used.

The method of tissue staining had an influence on the CGH results. MG-stained tissue was an excellent material for producing faithful CGH profiles, irrespective of the amount of microdissected tissue. On the other hand, HE-stained tissue can produce false CGH results. Thus, for an experiment using DOP-PCR, hematoxylin stains should be avoided when preparing tissues as starting material, because hematoxylin interferes unpredictably with the reaction.¹⁹ Our result suggests that the efficacy of DOP-PCR from HE-stained tissue depends on the proportion of sample tissue to volume of the lysate: more tissue gave poorer results, even though the size of the DOP-PCR product from a larger HE-stained piece was bigger than those from a MG-stained piece and from a smaller HE-stained piece (Figure 3, lanes 7–11). This

suggests that higher concentrations of hematoxylin dye in the sample degrades the quality of DOP-PCR results. Diluting the crude DNA extract (tissue lysate) might help produce successful DOP-PCR experiments, but at the same time lowering DNA concentrations might produce invalid CGH profiles. In our controls for DOP-PCR CGH using SF210 and U251NCI, 50 pg of DNA produces accurate CGH profiles. Even though increasing magnesium during the second phase of DOP-PCR helped improve CGH results, practical difficulties in measuring hematoxylin concentration for each sample makes this solution unworkable.

As noted above, CGH from DNA extracted from fresh cells has been verified,¹⁰ and CGH of microdissected pieces of paraffin-embedded cell pellet provides faithful cytogenetic data under appropriate conditions (Figures 4 and 5). These data encouraged us to study cytogenetic characteristics from multiple regions within a single tissue sample using tissue microdissection and DOP-PCR CGH. When a single microdissected MG-stained section of oligo-astrocytoma was used, there were no chromosomal aberrations in a histologically normal region, but there were losses on 1p and 19q in a neoplastic region. These losses are commonly found in tumors of this type,^{20,21} and we have previously confirmed that CGH profiles at these sites from paraffin-embedded tumor sections where histologically normal tissue was removed are compatible with FISH and loss of heterozygosity studies.²¹ In cases of infiltrative tumors such as oligo-astrocytoma, normal tissue is often intermixed in a surgically excised tumor sample. This might mask chromosomal aberrations in tumor cells. However, because microdissection allows one to pick regions of relatively pure tumor, DOP-PCR CGH on microdissected samples may reveal the true cytogenetic characteristics of tumor cells. Furthermore, since retrospective investigations often require paraffin-embedded tissue, this technique might provide important information when accurate analysis of clinical data is needed. We have investigated grade II diffuse astrocytomas using microdissection and DOP-PCR in paraffin-embedded tissue and found CNAs in more than 80% of cases, although CGH using frozen tumor samples detected CNAs in only 40% of the cases (Hirose Y, Aldape K, Chang S, Larson D, Lamborn K, Berger MS, and Feuerstein BG, submitted data; and Mohapatra G, and Feuerstein BG, unpublished data). This discrepancy could be due to intermixed normal tissue within surgically resected samples. We compared CGH profiles from the central region of tumor with those from infiltrated region. Tissue microdissection together with DOP-PCR enabled us to find greater numbers of CNAs, and the clarity of existing CNAs was improved in regions of pure tumor (submitted). It is possible that cytogenetic analysis will provide important criteria for classifying astrocytomas, and that excluding intermixed normal tissue to obtain accurate information will increase the sensitivity of analysis.

Furthermore, microdissection might be useful to investigate genetic heterogeneity. To date, most previous CGH studies on gliomas used nick-translated DNA extracted from relatively large samples of frozen or paraffin-embedded tissues.^{22–26} Data from these studies could

detect a CNA only if it were present in a high proportion of cells. If cytogenetic subpopulations of cells existed within these large samples, these methods might have missed them. This would be unfortunate if such minor subpopulations represent higher-grade or drug-resistant populations. Thus, sampling small regions of tumor might reveal these subpopulations and lead to a more certain diagnosis. Studies of heterogeneity in gliomas are underway in our laboratories to investigate these possibilities using DOP-PCR CGH of microdissected samples.

An important limitation of CGH is its inability to assay chromosome aberrations of a single cell. However, its strength is its ability to screen the whole genome for CNAs. This study provides evidence that very small regions of tissue can be assayed by CGH. These methods may find important applications in evaluating small samples and in investigating the role of heterogeneity as a prognostic factor.

References

1. Telenius H, Carter NP, Bebb CE, Nordendkjöld M, Ponder BA, Tunnacliffe A: Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. *Genomics* 1992, 13:718–725
2. Speicher MR, du Manoir S, Schröck E, Holtgreve-Grez H, Schoell B, Lengauer C, Cremer T, Ried T: Molecular cytogenetic analysis of formalin-fixed, paraffin-embedded solid tumors by comparative genomic hybridization after universal DNA-amplification. *Hum Mol Genet* 1993, 2:1907–1914
3. Cheung VG, Nelson SF: Whole genome amplification using a degenerative oligonucleotide primer allows hundreds of genotypes to be performed on less than one nanogram of genomic DNA. *Proc Natl Acad Sci USA* 1996, 93:14676–14679
4. Kuukasjärvi T, Tanner M, Pennanen S, Karhu R, Visakorpi T, Isola J: Optimizing DOP-PCR for universal amplification of small DNA samples in comparative genomic hybridization. *Genes Chromosomes Cancer* 1997, 18:94–101
5. Klein CA, Schmidt-Kittler O, Schardt JA, Pantel K, Speicher MR, Riethmüller G: Comparative genomic hybridization, loss of heterozygosity, and DNA sequence analysis of single cells. *Proc Natl Acad Sci USA* 1999, 96:4494–4499
6. Huang Q, Schantz SP, Rao PH, Mo J, McCormick SA, Chaganti RSK: Improving degenerative oligonucleotide primed PCR-comparative genomic hybridization for analysis of DNA copy number changes in tumors. *Genes Chromosomes Cancer* 2000, 28:395–403
7. Kallioniemi A, Kallioniemi OP, Sudar S, Rutovitz D, Gray JW, Waldman FM, Pinkel D: Comparative genomic hybridization of molecular cytogenetic analysis of solid tumors. *Science* 1992, 258:818–821
8. Kallioniemi A, Kallioniemi OP, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Dray JW, Waldman FM: Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc Natl Acad Sci USA* 1994, 91:2156–2160
9. Mohapatra G, Kim DH, Feuerstein BG: Detection of multiple gains and losses of genetic material in ten glioma cell lines by comparative genomic hybridization. *Genes Chromosomes Cancer* 1995, 13:86–93
10. Mohapatra G, Moore DH, Kim DH, Grewal L, Hyun WC, Waldman FM, Pinkel D, Feuerstein BG: Analysis of brain tumor cell lines confirm a simple model of relationships among fluorescence in situ hybridization, DNA index, and comparative genomic hybridization. *Genes Chromosomes Cancer* 1997, 20:311–319
11. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual*, 1st ed. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1992, pp 9.16–9.19
12. Piper J, Rutovitz D, Sudar D, Kallioniemi A, Kallioniemi OP, Waldman FM, Gray JW, Pinkel D: Computer image analysis of comparative genomic hybridization. *Cytometry* 1995, 19:10–26
13. Faulkner SW, Leigh DA: Universal amplification of DNA isolated from small regions of paraffin-embedded, formalin-fixed tissue. *Biotechniques* 1998, 24:47–50
14. Sanchez-Cespedes, Cairns P, Jen J, Sidransky D: Degenerate oligonucleotide-primed PCR (DOP-PCR): evaluation of its reliability for screening of genomic alterations in neoplasia. *Biotechniques* 1998, 25:1036–1038
15. Wells D, Sherlock JK, Handyside AH, Delhanty JDA: Detailed chromosomal and molecular genetic analysis of single cells by whole genome amplification and comparative genomic hybridization. *Nucleic Acids Res* 1999, 27:1214–1218
16. Aubele M, Mattis A, Zitzelsberger H, Walch A, Kremer M, Hutzler P, Höfler H, Werner M: Intramural heterogeneity in breast carcinoma revealed by laser-microdissection and comparative genomic hybridization. *Cancer Genet Cytogenet* 1999, 110:94–102
17. Jung V, Romeike BFM, Henn W, Feiden W, Moringlane JRM, Zang KD, Urbschat S: Evidence of focal genetic microheterogeneity in glioblastoma multiforme by area-specific CGH on microdissected tumor cells. *J Neuropathol Exp Neurol* 1999, 58:993–999
18. Kim SH, Godfrey T, Jensen RH: Whole genome amplification and molecular genetic analysis of DNA from paraffin-embedded prostate adenocarcinoma tumor tissue. *J Urol* 1999, 162:1512–1518
19. Burton MP, Schneider BG, Brown R, Escamilla-Ponce N, Gully MC: Comparison of histologic stains for use of microdissected, paraffin-embedded tissues. *Biotechniques* 1998, 24:86–92
20. Bigner SH, Matthews MR, Rasheed BKA, Wiltshire RN, Friedman HS, Friedman AH, Stenzel TT, Dawes DM, McLendon RE, Bigner DD: Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. *Am J Pathol* 1999, 155:375–386
21. Smith JS, Alderete B, Minn Y, Borell TJ, Perry A, Mohapatra G, Hosek SM, Kimmel D, O'Fallon J, Yates A, Feuerstein BG, Burger PC, Scheithauer BW, Jenkins RB: Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. *Oncogene* 1999, 18:4144–4152
22. Weber RG, Sabel M, Reifenberger J, Sommer C, Oberstraß J, Reifenberger G, Kiessling M, Cremer T: Characterization of genomic alterations associated with glioma progression by comparative genomic hybridization. *Oncogene* 1996, 13:983–994
23. Schröck E, Blume C, Meffert MC, du Manoir S, Bersch W, Kiessling M, Lozanowa T, Thiel G, Witkoeski R, Ried T, Cremer T: Recurrent gain of chromosome arm 7q in low-grade astrocytic tumors studied by comparative genomic hybridization. *Genes Chromosomes Cancer* 1996, 15:199–205
24. Sallinen SL, Sallinen P, Haapasalo H, Kononen J, Karhu R, Helen P, Isola J: Accumulation of genetic change is associated with poor prognosis in grade II astrocytomas. *Am J Pathol* 1997, 151:1799–1807
25. Nishizaki T, Ozaki S, Harada K, Ito H, Arai H, Beppu T, Sasaki K: Investigation of genetic alterations associated with the grade of astrocytic tumor by comparative genomic hybridization. *Genes Chromosomes Cancer* 1998, 21:340–346
26. Mohapatra G, Bollen AW, Kim DH, Lamborn K, Moore DH, Prados MD, Feuerstein BG: Genetic analysis of glioblastoma multiforme provides evidence for subgroup within the grade. *Genes Chromosomes Cancer* 1998, 21:195–206