Original Article

Reuse of Bacterial Artificial Clones Microarrays by Stripping with Sodium Hydroxide

Matchuporn Sukprasert MD[1], Chonthicha Satirapod MD[1], Worawan Lupthalug MD[1], Wicharn Choktanasiri MD[1], Kungpu Xu PhD[2]

[1] The Reproductive Endocrinology and Infertility unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[2] Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine and Infertility, Weill Cornell Medical College of Cornell University, New York, United State

Background: Array comparative genomic hybridization [CGH] was established as the method of choice for fast and accurate detection of numerical chromosomal abnormalities. However, the main limitation is due to the price of the array themselves. The present study tried to demonstrate a method for stripping DNA-DNA duplexes in bacterial artificial clone [BAC] array by using sodium hydroxide (NaOH)/stress corrosion cracking [SCC]. Additionally, after stripping, the microarray can be reused for further experiments.

Objective: To compare the accuracy in detected chromosomal aberrations between new and stripped slide by using NaOH/SCC as an array-stripping agent.

Materials and Methods: An experimental study was performed in an academic medical center. Two human known cell lines from fibroblast cell were used with whole genome amplification and microarray step according to the BlueGnome 24Sure protocol. Data processing was analyzed with Bluefuse software analysis. All detected copy number changes were compared to known aberrations listed in public databases. A 4 mM NaOH/0.5x SCC at 63°C was used as an array-stripping agent. The main outcome measure was the percentage agreement of chromosome interpretation between new and stripped microarray slide.

Results: The present study demonstrated that the stripped slides with NaOH/SCC at appropriate temperature (63°C) have almost identical hybridization pattern and 100% agreement of chromosome interpretation to the new slides.

Conclusion: The authors found that stripping BAC microarray by mild alkali treatment (4 mM NaOH/0.5x SCC) at appropriate temperature (63°C) do not compromise the results. It has been validated and showed 100% agreement of chromosome interpretation. The reused microarray slide is a cost-effective method to develop microarray experiment.

Keywords: Microarray, Stripping, Sodium hydroxide

J Med Assoc Thai 2018; 101 (5): 667-74
Website: http://www.jmatonline.com

Array comparative genomic hybridization [CGH] was introduced in the late nineties[1,2] and established as the method of choice for fast and accurate detection of numerical chromosomal abnormalities. It detects submicroscopic chromosomal imbalances and allows reliable detection of chromosomal deletions and duplications with higher resolution than the karyotype analysis. In addition, array CGH is far superior to the alternative methods for detection of genomic abnormalities that are clearly below the resolution of karyotype detection. Furthermore, it allows for massive DNA analysis in parallel with unprecedented speed.

Correspondence to:

Sukprasert M. Department of Obstetrics and Gynecology, Faculty of Medicine, Ramathibodi Hospital, 270 Rama 6 Road, Ratchathewi, Bangkok 10400, Thailand.
Phone: +66-2-2011412, **Fax**: +66-2-2011416
Email: matchuporn_m@yahoo.com

Bacterial artificial chromosome [BAC] array-CGH is a powerful method to identify DNA copy number changes on a genome-wide scale, it also has proven to be a specific, sensitive, and reliable technique, with considerable advantages compared to other methods used for the analysis of DNA copy number changes[3,4]. BACs vary in length from 150 to 200 Kb[5]. Microarray are fabricated by high-speed precision robotics on glass or nylon substrates, for which labeled probes are used to determine complementary binding allowing massively parallel gene expression and gene discovery studies[6-8]. Glass slides are more popular than nylon membranes for microarray fabrication, since nylon membranes have several problems, such as high autofluorescence and fragility. DNA microarray is a collection of microscopic DNA spots attached to a solid surface. In the process of creating microarray

How to cite this article: Sukprasert M, Satirapod C, Lupthalug W, Choktanasiri W, Xu K. Reuse of bacterial artificial clones microarrays by stripping with sodium hydroxide. J Med Assoc Thai 2018;101:667-74.

slides, the surface chemistry of the glass substrate is a major determinant of the stability of DNA attachment throughout the hybridization and washing steps. The most popular substrates for spotting DNA are polylysine and aminosilane-coated glass slides. The interaction between the positively charged amino groups of the silane molecule and the negatively charged phosphate groups on the DNA backbone helps the nucleic acids to be immobilized stably on the slide surface. This method increases the binding capacity. The attachment can be enhanced by treatment with heat or ultraviolet cross-link[9-12].

Stripping is the method of stripping hybridized target oligonucleotides from a microarray while substantially leaving intact probe oligonucleotides on the microarray. After stripping, the microarray can be re-used for further experiments. In the microarray technology, several chemistries have been proposed for oligonucleotide detachment to glass surface such as formamide, sodium hydroxide (NaOH), sodium dodecyl sulfate, and dimethylurea. Even the enzyme RNase have been used for stripping microarrays on glass slide[13-18]. Most of these protocols for stripping microarrays on glass were developed in combination with specific surface chemistry and different coating for covalently immobilizing pre-synthesized DNA in a deposition process[19].

Sodium hydroxide is actually used to extract DNA from cells[20-22]. DNA made-up from the two strands are only held together with hydrogen bonding. The hydrogen bonding network can be very fragile. Heat, strong ionizing radiation, strong acid such as hydrofluoric acid, or a strong base can disrupt this bond and cause the DNA strands to separate. Several study used alkaline lysis extraction method for DNA. The benefits of an alkaline lysis extraction include minimal reagent cost, simple method, and less extraction time[23,24].

The first array stripping methods were developed from RNA hybridized onto DNA arrays. It is easy to start with RNA-DNA, stripping off protocol, because of the inherent difference between RNA-DNA and DNA-DNA duplexes. DNA-DNA duplexes are more tightly attached than RNA-DNA duplexes. That was the reason that the previous study used RNA-DNA attachment. The oligo-array was the most common array that the authors used because the short nucleic acid is easily stripped off while a long nucleic acid appropriate reagents and temperature for stripping off arrays such as BAC are difficult to find. The present study demonstrated the method for stripping DNA-DNA duplexes in BAC array by using NaOH/stress corrosion cracking [SCC] as an array-stripping agent.

Materials and Methods

DNA microarrays

Microarrays were purchased from BlueGnome (Cambridge, UK). The 24Sure V3 subarray contained BACs clones, from the Roswell Park (RP-nomenclature) human genome collection, spotted in duplicate. BAC clones on the array had been specifically selected for performance using data from about 1,000 dye swap hybridizations. Clones were selected to be at approximately 1 Mb interval. The effective resolution of 24Sure arrays was optimally 10 Mb (1 Mb genome coverage, 10 Mb effective resolution). BAC clones were polymerase chain reaction [PCR]-amplified and immobilized on codelink coated glass microarray slides (24Sure V3 Pack protocol, available in www.cytochip.com). The authors used the same print batch of slides in the present experiment.

Preparation of samples

Two human fibroblast cell line were obtained from the Coriell Cell Repository (Camden, NJ). The karyotype of cell line was 46, XY cell line (PCS-201-010) and 46, XX cell line (CCL-110). Cells were cultured in 1x Minimum Essential Medium (Gibco, Carlsbad, CA, USA) with 15% Fetal Bovine Serum (Invitrogen Corp., Carlsbad, CA, USA), 1% Non-Essential Amino Acid and 1% Penicillin-Streptomycin-Glutamine (Gibco, Carlsbad, CA, USA) at 37°C and 5% CO_2. Single cells were isolated following treatment with 0.05% trypsin/EDTA (Gibco, Carlsbad, CA, USA) to detach the adherent fibroblast cultures as recommended. Single cells were then picked up in 2.5 µl 1x PBS using pipette under a dissecting microscope and place in the bottom of a 0.2 ml PCR tube (Eppendorf, Hamburg, Germany). One µl of media was removed to serve as negative controls for each WGA method. Single cell was lysed, and genomic DNA was amplified using the SurePlex DNA Amplification System (BlueGnome, Cambridge, UK) according to the manufacturer's instructions. SurePlex was a WGA procedure based on random fragmentation of genomic DNA and subsequent amplification by PCR utilizing flanking universal priming sites. SurePlex could produce DNA of a standard concentration that could be used for a downstream application. The total WGA product was run on an agarose gel (in 1x TBE buffer) for check amplification efficiency.

SureRef reference male and female DNA was

amplified genomic DNA that was recommended for use as a reference sample that would be labelled and co-hybridized against an amplified single cell sample as part of the 24Sure protocol.

Microarray labeling, hybridization and washing

WGA products were processed according to the BlueGnome 24Sure protocol (available at www.cytochip.com). For array CGH, the test and reference DNA were co-hybridized to the 24Sure V3 (BlueGnome, Cambridge, UK). The reference DNA, both male and female, were derived from SureRef (BlueGnome, Cambridge, UK).

The labelling step, genomic DNA was labeled by random priming using 1 µl of Cy3-dCTP or Cy5-dCTP (BlueGnome, Cambridge, UK), 1 µl Klenow enzyme and 5 µl dCTP per sample. For hybridization, labeling mixes were combined and co-precipitated with Cot-1 DNA. Cot-1 DNA was used for blocking repetitive sequences in genomic DNA thereby preventing non-specific binding of labelled products to target sequences. Then, it was evaporated under centrifuge at 75°C for one hour and each pellet resuspended in 21 µl of prewarmed hybridization buffer at 75°C ensuring that the pellet was completely dissolved. 18 µl was spread onto the array slide and covered with a 22x22 mm coverslip. The slide was put in a hybridization box that presoaked with 2x SCC/50% formamide and incubated at 47°C for 16 hours. After hybridization, the slides were agitating in 2x SCC/0.05% Tween 20 to remove the cover slips and then washed with 2x SCC/0.05% Tween 20 at room temperature for 10 minutes then washed in 1x SCC, at room temperature for 10 minutes and then 0.1x SCC, at 60°C for five minutes. The last washing was in 0.1x SCC, at room temperature for one minute, and spin with 170 g for three minutes.

Processing image

Array image acquisition: After hybridization and washing, all microarray slides were scanned using the Agilent Surescan (Santa Clara, CA, USA) with the Cy3 channel/photomultiplier tube [PMT] setting at 450 and the Cy5 channel setting at 550. The scanner resolution was set at 10 µm.

Data processing: The image files were analyzed with Bluefuse software analysis (BlueGnome, Cambridge, UK). All detected copy number changes were compared to known aberrations listed in publicly available databases, such as ENSEMBL (Ensembl: http://www.ensembl.org), DECIPHER (http://dicipher.sanger.ac.uk), and the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/) using NCBI36/ng 18 University of California, Santa Cruz [UCSC] assembly.

Interpretation of the data: The principle was based on the co-hybridization of DNA sample and control, which were labelled with two different fluorochromes (Cy3 and Cy5) on the microarray. The region of copy number gain and loss for the BAC array-CGH were identified by creating sample specific thresholds. The clones with log2 ratios above or below a control samples threshold value were considered as gains or loss, respectively. All analyses were done on log2 ratios. The DNA copy number aberrations were measured by detecting intensity difference.

After scanning, the microarray slides were stored in a dark box with low humidity until the stripping procedure was applied.

Stripping microarrays

The stripping was initiated when the used micro-array slides were rinsed thoroughly with distilled water, pH 7.0 at 25°C for 60 second. Then, prewarm 75 ml of stripping buffer (4 mM NaOH/0.5x SCC) was put in the water bath at 63°C for 10 minutes. Then, the slide was immersed in 75 ml of prewarm stripping buffer in cooping jar in the water bath at 63°C for 10 minutes. This step was temperature critical. It needed to be monitored carefully because the arrays retained significantly signals at temperature lower than 60°C, and their coating became damaged at temperature higher than 64°C. Afterwards, the array was transferred to freshly prepared stripping solution (4 mM NaOH/0.5x SCC) and stirred at room temperature for two minutes with magnetic stirrer. Next, the slides were washed at room temperature for 15 seconds in distilled water and then washed in 70% ethanol for 15 seconds, and finally washed in 100% ethanol for 15 seconds. The last step, spin with 170 g for three minutes. To verify that stripping had been successful, the same area of each microarray slide was scanned with the same scanner before the rehybridization process was applied. The stripped slides were stored in a dark and desiccated environment until use.

Rehybridization steps

After the NaOH/SCC treated protocol, the bound cDNA appeared to be washed off completely as shown in Figure 2. For the rehybridization step, the stripped slides were hybridized, as they were initially, with the same labelled cDNA mixture. For the labelling,

hybridization and washing step were performed using the same protocol from BlueGnome (Cambridge, UK).

Statistical analysis

The data were analyzed using computer program STATA version 12.0 software (Stata Corp., College Station, TX, USA). Data with normal distribution were presented in mean and standard deviation. The comparison of percentage included clone among new, washed, and rehybridization slides were performed by means of multi-level models. The multi-level models were statistical models of parameters that varied at more than one level. They can also be used to analyze repeated measure data and deal with data in which the times of the measurements vary from subject to subject. The percentage agreement of chromosome interpretation between first and second round hybridization was performed by one-way analysis variance (one-way ANOVA). One-way analysis variance is a way used to compare the quality of three or more means at one time by using variances. A *p*-value of less than 0.05 was considered as statistically significant.

Results

The present study demonstrated that it is possible to strip and reuse commercial BAC microarray slides under a very mild alkali treatment (4 mM NaOH/0.5x SCC) with appropriate temperature (63°C). It was confirmed by visual examination in the same area of the feature as shown in Figure 1-3 (clone: RP11-226A18, RP11-30H9, RP11-468G5, RP11-213H22, RP-11-58G13, RP11-444B24, RP-231P15, RP11-162K11, RP11-392K14, RP11-320C15, RP5-960D23, RP11-263G22, RP11-501G7, RP11-27J5, RP11-115N5). The stripped slides had almost identical hybridization pattern and signal intensities as the new slides. The stripped slides had only slightly increased background intensities, whereas, reused arrays had similar intensities as the new slides. After a mild alkali treatment and the stripping protocol completed, the bound cDNA appeared to be washed completely off as shown in Figure 2. While the same stripping reagent and protocol was used for stripping the slide at 64°C, the clone and surface of the slide was damaged as shown in Figure 4. On the other hand, stripping at 60°C, the clone was retained as shown in Figure 5.

The percentage of included clone was assessed by BlueFuse software analysis (BlueGnome, Cambridge, UK). Before washing, the mean ± SD percentage included clone was 72.88±21.53. After washing with 4 mM NaOH/0.5x SCC at 63°C, mean ± SD percentage

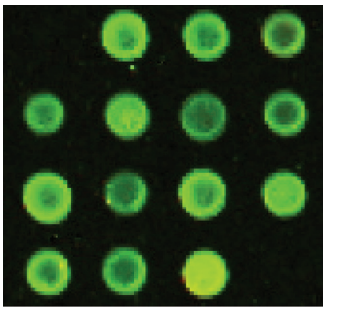

Figure 1. The first hybridization slide.

Figure 2. The stripped slide, used 4 mM NaOH/0.5x SCC at 63°C as an array-stripping agent.

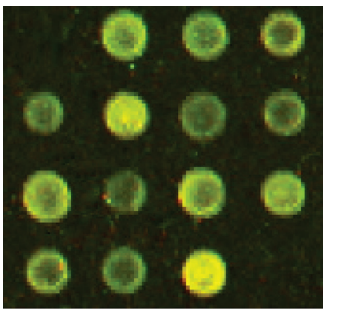

Figure 3. The rehybridization slide.

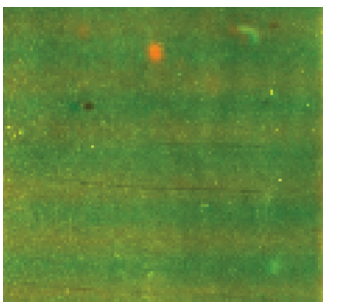

Figure 4. The stripped slide, used 4 mM NaOH/0.5x SCC at 64°C as an array-stripping agent (the surface damaged slide).

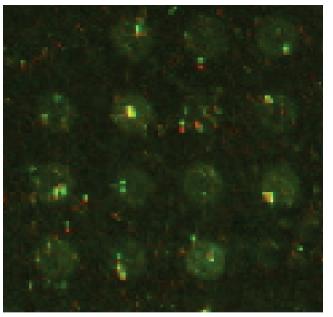

Figure 5. The stripped slide, used 4 mM NaOH/0.5x SCC at 60°C as an array-stripping agent (the clone retention slide).

included clone were decreased to 55.17±23.18. After rehybridization, mean ± SD percentage included clone were slightly increased to 55.27±26.37. The stripped slide had significantly lower percentage included clone than the new slide (p<0.05), and the rehybridization slide also had significantly lower percentage included clone than the new slide (p<0.05). However, there was no significant difference of percentage included clone between the stripped slide and rehybridization slide (p = 0.9721) (Table 1).

The median percentage included clone before washing was 53.96±10.30 then after the slides were washed with 4 mM NaOH/0.5x SCC at 60°C, the median percentage included clone was decreased to 29.75±14.93, while the median percentage included clone was decreased to 33.25±11.05 after rehybridization. However, there was no significant difference of percentage included clone before washing, after washing, and on rehybridization slides (p = 0.0856, 0.4228, respectively).

Before washing the slide with 4 mM NaOH/0.5x SCC at 64°C, the median percentage included clone was 86.52±1.44. After washing, median percentage included clone was decreased to 34.24±6.98. After rehybridization, median percentage included clone was increased to 40.32±18.30. The stripped and rehybridization slides were not significantly different in percentage included clone compared with the new slide (p = 0.0721, 0.1868, respectively). Additionally, no statistically significant difference between stripped and rehybridization slide was found (p = 0.5862) (Table 1).

Table 1. Mean and SD of percentage included clone among three different temperatures

Temperature	Mean ± SD of % included clone		
	Before washing	After washing	Rehybridization
60°C	53.96±10.30	29.75±14.93	33.25±11.05[b]
63°C	72.88±21.53	55.17±23.18[a]	55.27±26.37[b]
64°C	86.52±1.44	34.24±6.98	40.32±18.30

[a] p<0.05 percentage included clone before washing compare with after washing slides

[b] p<0.05 percentage included clone before washing compare with rehybridization slides

After washing with 4 mM NaOH/0.5x SCC at 63°C, the percentage agreement of chromosome interpretation between the new and rehybridization slide in the same array was 100%. After washing with the same protocol and solution at 60°C and 64°C, the authors found the percentage agreement of chromosome interpretation between the new and rehybridization slide was 93.48% and 86.96%, respectively (p = 0.3343).

The rehybridization array can detect genomic aberration ranging in size 0.5 to 24.3 Mb. The analysis found that no significant loss in precision in both sides duplication and deletions.

Discussion

When validating the data from the stripped and reused microarrays, several key factors need to be considered. First of all, eliminate all possible signals without affecting the array from the first hybridization and its surface defect must be minimized. Secondly, achieve paralleled intensities between second hybridization on stripped arrays and new arrays without dynamic range compression. Lastly, the accuracy and reproducibility of the reused microarray slides must be made.

Some studies have demonstrated that stripping of self-made deposit arrays on glass is possible, but depends on specific surface attachment chemistry used for the immobilization of DNA probes[10-12,16,25,26]. They describe 50% decrease of signal intensities with each round of consecutive stripping and probing[16]. Recently, it was reported that commercial in situ long oligonucleotide DNA microarrays could also be stripped and reprobed[18]. However, most of the published protocols typically used temperatures above 95°C, temperatures that damaged the surface of the array[10,16,27].

The authors have developed a method to strip glass-based BAC microarrays that used fluorescent DNA in the hybridization while leaving the DNA oligonucleotide probes intact and usable for a second experiment. The present study used mild alkali treatment (4 mM NaOH/0.5x SCC) for stripping slides. The authors used NaOH, based on a previously described study about evaluation of DNA probe removal from Nylon membrane. The largest amount of

genomic DNA was stripped from the membrane with NaOH[14]. Wu et al also described NaOH can break a long oligonucleotide DNA from the slide[28]. On the other hand, using high concentration alkali conditions may damage the surface of the slides. The exposure to mild alkali reagent is not enough to dissociate DNA-DNA duplexes. The most crucial factor is temperature. To the authors knowledge, most of the published microarray stripping protocols are derived from methods to strip Northern or Southern blots and utilize high-temperature alkaline incubations (>95°C) to remove bound probe[29]. For microarray slide, the authors cannot use the high temperature, because it will destroy the surface of the slide. Furthermore, arrays retain significantly signals at temperatures lower than 60°C. In the present study, when temperatures above 64°C were used, the clone and surface of the slide were damaged. Additionally, when temperatures below 60°C were used, the clone was not completely removed. The authors found 63°C is the most proper temperature for the procedure. The rehybridization slide had a slightly increase background because at the washing steps, some part of the chemical coating may get damaged.

The percentage included clone after washing with mild alkali and a temperature of 63°C. It significantly decreased, This means that stripping reagent was appropriate to strip DNA-DNA duplexes. While percentage that included clone between stripped slide and rehybridization slide was not significantly different, such residual signals usually do not pose a problem for slide reuse because they are always in a tiny minority and easily identifiable. An interesting and unknown issue was that DNA probes were differentially affected by stripping process. There are two plausible explanations. One is the difference in the extent of labeling and the length of the DNA probes. Zhang et al[30] provided experimental evidence that the length of oligonucleotide probes positively influences the retention of probe on microarray slides. They showed that probes that were 44 nucleotides long adequately maintained their attachment after stripping, while the 20 to 40 nucleotides long probes tended to lose potential signal intensities faster[30]. The other was that certain probes were preferentially washed off a slide during stripping. The attachment of unmodified DNAs to microarrays is mediated by complicated interactions that are poorly characterized. During a typical array printing, a solution containing an oligonucleotide probe is spotted onto the surface of a microarray slide. The probe probably adheres to the slide through hydrogen bonds and through electrostatic attraction between the negatively charged phosphate groups in the DNA and the positively charged glass surface, leading to the formation of strong ionic interactions or a varying number of covalent bonds, respectively, between the surface and the DNA oligomers. Probes are then fixed to the slide by UV-cross linking. As crosslinking efficiency is never 100%, all DNA are not crosslinked to a slide.

To access microarray accuracy and reproducibility, the authors computed the percentage agreement of chromosome interpretation by one-way ANOVA. At the temperature 63°C for stripping slide, the result showed a 100% agreement of chromosome interpretation between the first-round and the second-round hybridization in the same arrays. The authors accepted only 100% agreement. It represented that the reuse slides gene expression profile was 100% accurate. While at the 60°C and 64°C, the percentages agreement of chromosome interpretation were 93.48% and 86.96%, respectively. Even if the percentage of agreement was high, the authors could not accept for diagnostic application, because the accuracy and reproducibility are critical issues. Reuse of stripped arrays with 4 mM NaOH/0.5x SCC at 63°C gave comparable results as compare to unused arrays with no significant loss in accuracy.

The merit of the present study was that the simply performed protocol and material can be found easily in any laboratory. The authors were also using the same print batch of microarray slides to make the data more reliable and decrease intrinsic factor variation. The authors evaluated probes that hybridized to identical targets.

The limitation of the present study was different batch of SureRef Male and Female samples that hybridized in the new and rehybridization slide, but it was produced from the reliable source.

Conclusion

The success of the stripping methods for BAC microarrays depends on several factors such as type of arrays, coating material, and hybridization conditions. To reduce the costs of microarray experiments, the authors developed a stripping protocol for BAC arrays. The recommended stripping BAC microarray by mild alkali treatment (4 mM NaOH/0.5x SCC) with appropriated temperature (63°C) do not compromise the results. It is easy to perform, inexpensive, and achieves accurate results. Stripping only once does not compromise reproducibility. It was validated and has shown 100% agreement of chromosome interpretation.

What is already known on this topic?

Array CGH is a powerful tool to identify 24 chromosomal abnormalities. It also increases the number of samples analyzed at one time. The process must create microarray slides with very high binding capacity between spotting DNA and aminosilane-coated glass slides. Microarray is an established method for fast and accurate detection of numerical chromosomal abnormalities. However, the main limitation is due to the high price of the array.

What this study adds?

This study found a method of stripping hybridized target oligonucleotides from a microarray while substantially leaving intact the probe oligonucleotides on the microarray. After sodium hydroxide have been used for stripping microarrays on glass, this study demonstrated that it is possible to strip and reuse microarray slides. The stripped slides have almost identical hybridization pattern and signal intensities as new slides. There was no significant different of percentage included clone between the stripped slide and the rehybridization slide.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, et al. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 1997;20:399-407.
2. Shizuya H, Kouros-Mehr H. The development and applications of the bacterial artificial chromosome cloning system. Keio J Med 2001;50:26-30.
3. Cowell JK, Wang YD, Head K, Conroy J, McQuaid D, Nowak NJ. Identification and characterisation of constitutional chromosome abnormalities using arrays of bacterial artificial chromosomes. Br J Cancer 2004;90:860-5.
4. Cai WW, Mao JH, Chow CW, Damani S, Balmain A, Bradley A. Genome-wide detection of chromosomal imbalances in tumors using BAC microarrays. Nat Biotechnol 2002;20:393-6.
5. Ylstra B, van d, I, Carvalho B, Brakenhoff RH, Meijer GA. BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). Nucleic Acids Res 2006;34:445-50.
6. Ren H, Francis W, Boys A, Chueh AC, Wong N, La P, et al. BAC-based PCR fragment microarray: high-resolution detection of chromosomal deletion and duplication breakpoints. Hum Mutat 2005;25: 476-82.
7. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995;270:467-70.
8. Ramsay G. DNA chips: state-of-the art. Nat Biotechnol 1998;16:40-4.
9. Belosludtsev Y, Iverson B, Lemeshko S, Eggers R, Wiese R, Lee S, et al. DNA microarrays based on noncovalent oligonucleotide attachment and hybridization in two dimensions. Anal Biochem 2001;292:250-6.
10. Dolan PL, Wu Y, Ista LK, Metzenberg RL, Nelson MA, Lopez GP. Robust and efficient synthetic method for forming DNA microarrays. Nucleic Acids Res 2001;29:E107.
11. Strother T, Hamers RJ, Smith LM. Covalent attachment of oligodeoxyribonucleotides to amine-modified Si (001) surfaces. Nucleic Acids Res 2000;28:3535-41.
12. Chiu SK, Hsu M, Ku WC, Tu CY, Tseng YT, Lau WK, et al. Synergistic effects of epoxy- and amine-silanes on microarray DNA immobilization and hybridization. Biochem J 2003;374:625-32.
13. Hughes TR, Shoemaker DD. DNA microarrays for expression profiling. Curr Opin Chem Biol 2001; 5:21-5.
14. Noppinger K, Duncan G, Ferraro D, Watson S, Ban J. Evaluation of DNA probe removal from nylon membrane. Biotechniques 1992;13:572-5.
15. Joos B, Kuster H, Cone R. Covalent attachment of hybridizable oligonucleotides to glass supports. Anal Biochem 1997;247:96-101.
16. Consolandi C, Castiglioni B, Bordoni R, Busti E, Battaglia C, Bernardi LR, et al. Two efficient polymeric chemical platforms for oligonucleotide microarray preparation. Nucleosides Nucleotides Nucleic Acids 2002;21:561-80.
17. Rehman FN, Audeh M, Abrams ES, Hammond PW, Kenney M, Boles TC. Immobilization of acrylamide-modified oligonucleotides by co-polymerization. Nucleic Acids Res 1999;27: 649-55.
18. Hu Z, Troester M, Perou CM. High reproducibility using sodium hydroxide-stripped long oligonucleotide DNA microarrays. Biotechniques 2005; 38:121-4.
19. Hahnke K, Jacobsen M, Gruetzkau A, Gruen JR,

Koch M, Emoto M, et al. Striptease on glass: validation of an improved stripping procedure for in situ microarrays. J Biotechnol 2007;128: 1-13.
20. Osmundson TW, Eyre CA, Hayden KM, Dhillon J, Garbelotto MM. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples. Mol Ecol Resour 2013;13:66-74.
21. Oloyede OA. DNA extraction from chorionic villi for prenatal diagnosis of foetal haemoglobin genotype. West Afr J Med 2011;30:400-3.
22. Kouduka M, Suko T, Morono Y, Inagaki F, Ito K, Suzuki Y. A new DNA extraction method by controlled alkaline treatments from consolidated subsurface sediments. FEMS Microbiol Lett 2012; 326:47-54.
23. Hudlow WR, Buoncristiani MR. Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence. Forensic Sci Int Genet 2012;6:1-16.
24. Li X, Jin H, Wu Z, Rayner S, Yin J, Yu Y, et al. An automated process to extract plasmid DNA by alkaline lysis. Appl Microbiol Biotechnol 2007; 75:1217-23.
25. Benters R, Niemeyer CM, Drutschmann D, Blohm D, Wohrle D. DNA microarrays with PAMAM dendritic linker systems. Nucleic Acids Res 2002; 30:E10.
26. Fixe F, Dufva M, Telleman P, Christensen CB. Functionalization of poly(methyl methacrylate) (PMMA) as a substrate for DNA microarrays. Nucleic Acids Res 2004;32:e9.
27. Bao Z, Wenli M, Rong S, Ling L, Qiuye G, Wenling Z. Re-use of a stripped cDNA microarray. Br J Biomed Sci 2002;59:118-9.
28. Wu H, Bynum J, Stavchansky S, Bowman P. Reuse of cDNA microarrays hybridized with cRNA by stripping with RNase H. Biotechniques 2008;45: 573-5.
29. Li JK, Johnson TM, Parker BD. Multiple uses of liquepipets in Southern, northern, and western blots. Anal Biochem 1988;174:471-6.
30. Zhang X, Xu W, Tan J, Zeng Y. Stripping custom microRNA microarrays and the lessons learned about probe-slide interactions. Anal Biochem 2009;386:222-7.