



Comparative Genomic Hybridization (CGH) & Copy Number Variation (CNV)



Technical Note

Array CGH using DNA from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Samples



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Introduction

Identification of chromosomal aberrations in groups of related tumor samples using array-based comparative genomic hybridization (CGH) analysis has become an established procedure for identification of relevant genetic changes in abnormal cells¹. Formalin-fixed paraffin-embedded (FFPE) samples form an extensive archive of potential information about the relationship between focal somatic aberrations and cancer. For many years, however, this data has been largely inaccessible. Here, we describe an effective and simple method for isolating high-quality gDNA from FFPE samples for use in CGH analysis to identify focal aberrations in archived tumor tissue research samples.

The DNA isolation protocol has been carefully developed to reduce the cross-linking of DNA which takes place during formalin fixation. Additionally, because of the inconvenience of handling organic solvents normally used in phenol and chloroform based methods, this protocol utilizes a column-based DNA purification method.

It is important to note that even when this protocol is followed carefully, the resulting DNA may not produce CGH-quality DNA from all research samples. However, useful array CGH data can be obtained from most FFPE tissue samples archived in the past 15 - 20 years, if the DNA is purified using this method.

The method was created by Bauke Ylstra, Coordinator Micro Array Facility at VU University Medical Center, Amsterdam (www.vumc.com/afdelingen/microarrays). Data shown in this Technical Note was generated at this facility.

gDNA Isolation Method

Day 1

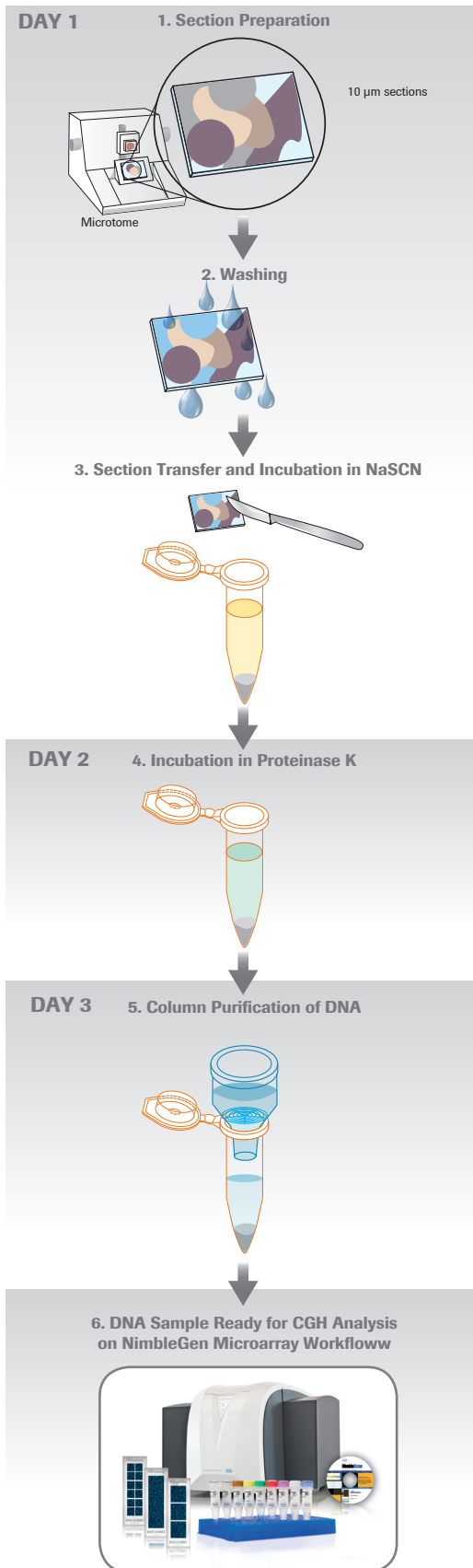
- 1. Section Preparation:** To obtain enough tissue and to ensure that the quality of the sample is satisfactory, several sections from each paraffin block are necessary. It is recommended to prepare two 3 μm sections and up to three 10 μm sections. Use the 10 μm sections, which should be located between the two 3 μm sections, for gDNA preparation. Use the 3 μm sections for hematoxylin and eosin (H&E) staining and histological analysis only. After microtome sectioning, mount both the 10 μm and 3 μm sections on glass slides using 0.1% BSA. Stain the 3 μm sections using standard H&E staining protocol. At the end of the staining procedure, mount (with Depex or Permount), and coverslip the 3 μm section slides. Confirm the presence of tumor cells by microscopic examination of these sections. Best results are obtained from research samples in which at least 70% of the visible cells in the section are tumor cells. Samples with fewer than 70% tumor cells can be included at the experimenter's discretion.
- 2. Washing:** Deparaffinize the 10 μm sections using three xylene washes (7 minutes each), two methanol washes (7 minutes each), two absolute ethanol washes (7 minutes each), one 96% ethanol wash (60 seconds), one 70% ethanol wash (60 seconds), and one quick tap water rinse (30 seconds). Incubate in hematoxylin for 1 - 2 minutes and rinse under running tap water for 5 - 10 minutes to permit the blue color to develop. After this brief staining procedure, use microscopic examination to identify necrotic tissue which can then be removed and discarded. This works best when the sections are still slightly wet.
- 3. Section Transfer and Incubation in NaSCN:** Carefully transfer sections from the glass slides to a 1.5 mL tube and add 0.5 - 1.0 mL 1 M NaSCN; 0.5 mL is sufficient for 1 cm^2 of tissue. Mix by inverting the tube several times and incubate overnight at 38 - 40 $^{\circ}\text{C}$.

Day 2

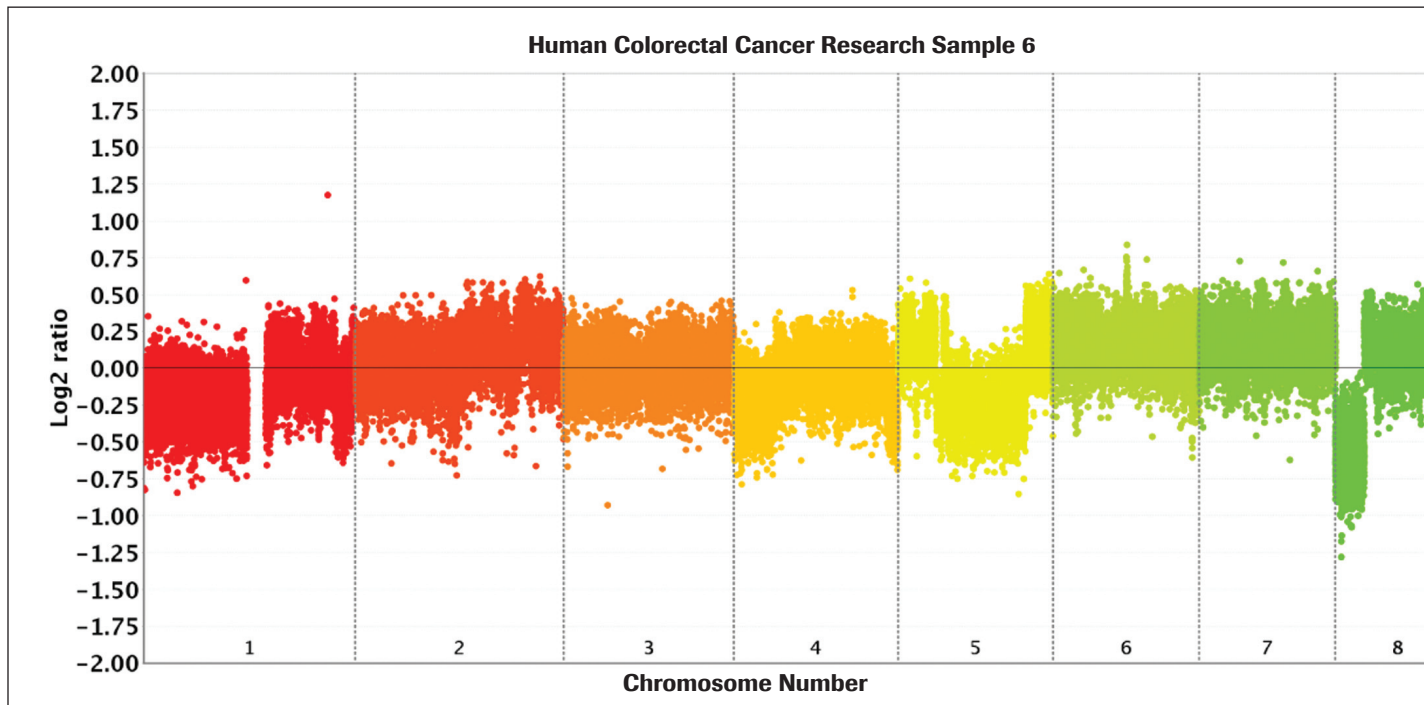
- 4. Incubation in Proteinase K:** Centrifuge sample tubes at full speed in a mini-centrifuge for 60 minutes. The tissue may not always form a pellet. Carefully remove and discard the NaSCN solution. Centrifuge again at maximum speed for 1 minutes, and again remove and discard the remaining NaSCN. Add 160 μL Buffer ATL (QIAamp DNA Micro kit) and 40 μL 20 mg/mL Proteinase K to each tube. Vortex each tube for 15 sec. Place tubes in a 55 $^{\circ}\text{C}$ heat block and incubate overnight. Mix tubes by vortexing as often as possible during this incubation, returning them promptly to the heat block after mixing. At the end of the digestion, the tissue lysate should be clear.

Day 3

- 5. Column Purification:** Vortex each tube briefly and centrifuge them at maximum speed for 15 seconds. Place the tube in a 98 $^{\circ}\text{C}$ heat block and incubate for 10 min. Centrifuge again at maximum speed for 1 minutes. Add 200 μL Buffer AL (QIAamp DNA Micro kit) and vortex for 15 seconds. Add 200 μL ethanol and vortex for 15 seconds. Incubate tubes at room temperature for 5 minutes. Centrifuge again at maximum speed for 1 minute and transfer the supernatant from each tube to a QIAamp MinElute column (QIAamp DNA Micro kit). Centrifuge columns at maximum speed for 1 minute. Discard flow-through and place columns in clean collection tubes. Add 500 μL Buffer AW1 (QIAamp DNA Micro kit) to each column and centrifuge at maximum speed for 1 min. Discard the flow-through. Add 500 μL Buffer AW2 (QIAamp DNA Micro kit) to each column, centrifuge columns at maximum speed for 1 minute, and discard the flow-through. Centrifuge the columns for 3 minutes at maximum speed to dry the membrane. Transfer the columns to properly marked collection tubes and add 20-30 μL Buffer AE (QIAamp DNA Micro kit) to each column. Incubate at room temperature for 5 minutes. Centrifuge the columns at maximum speed for 3 minutes. Discard the columns and store DNA in the collection tubes at the proper temperature (4 $^{\circ}\text{C}$ for short term, -20 $^{\circ}\text{C}$ for long term).
- 6.** The gDNA samples are now ready for CGH analysis on the NimbleGen microarray workflow.



◀ **Figure 1: Summary for gDNA isolation method from FFPE samples.** The three-day process includes histological examination and two overnight incubations.

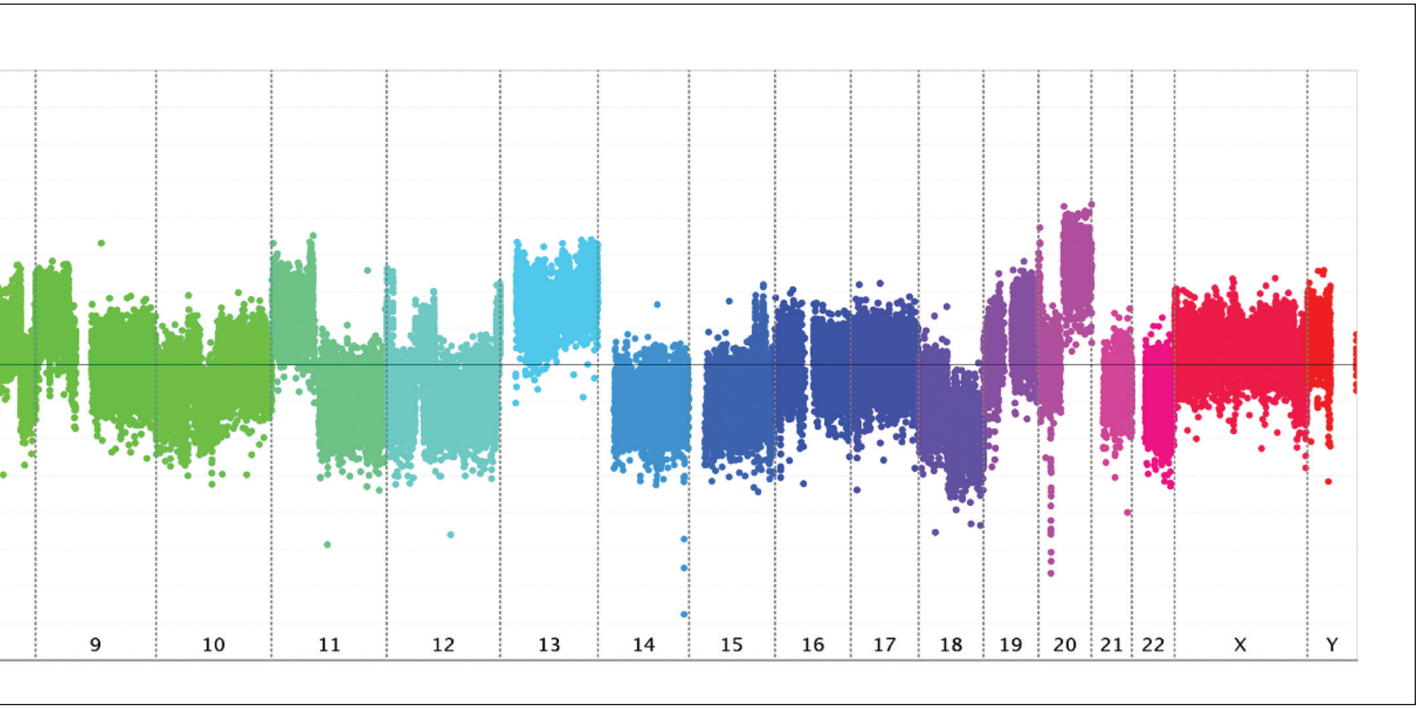


▲ **Figure 2: Whole-genome view of an FFPE colorectal cancer research sample, as hybridized to a NimbleGen Human CGH 12x135K Whole-Genome Tiling Array.** The Cy3 labeled Test DNA data and Cy5 labeled tissue matched reference DNA data was used to generate a \log_2 ratio (test/reference). The areas of signal above the “0” line indicate regions where DNA is duplicated, and the areas below the “0” correspond to deletion regions. Note focal aberrations on Chromosomes 6, 14, and 20.

Results

In this study, gDNA was isolated using the above method from eight FFPE human colorectal cancer tissues. One μg gDNA from each FFPE sample and reference sample was labeled using the NimbleGen Dual-Color DNA Labeling Kit and hybridized to a NimbleGen Human CGH 12x135K Whole-Genome Tiling Array using the standard protocol (NimbleGen Arrays User’s Guide: CGH Analysis v5.1). Arrays were scanned at $3\ \mu\text{m}$ resolution. Data was normalized and analyzed using the segMNT algorithm in NimbleScan v2.6 Software.

The mad.1dr (median absolute deviation, 1st derivative) score, a surrogate measure of noise in array CGH experiments was 0.11 ± 0.01 (SD, $N = 8$), which is equivalent to the average mad.1dr score observed in non-FFPE samples (0.12 ± 0.02 , $N = 30$). Figure 2 shows multiple focal aberrations commonly seen in DNA isolated from colorectal cancer research samples, such as amplification of an entire chromosome (Chromosome 13) as well as chromosome arms (Chromosome 20, q arm). In two research samples, several smaller (~ 1 Mb) common focal aberrations in overlapping regions were found (Figure 3), highlighting them as interesting candidate focal regions for further investigation.



▲ **Figure 3: Common focal aberrations in human colorectal research samples.**
 Chromosome 6 unaveraged segmented \log_2 -ratio data for two different FFPE samples.
 Note the similar somatic focal aberrations between different samples.

Discussion

In recent years, mounting evidence for the contribution of somatic aberrations in the development and progression of a wide range of cancer has been facilitated by high-resolution CGH analysis. Archived FFPE samples provide a large source of potentially useful data, if properly processed. The low noise level in the CGH data from gDNA isolated using the method described in this Technical Note allows sensitive and reliable detection of focal aberrations in FFPE research samples. This simple method is ideal for large scale gDNA isolation, as it requires minimal hands-on time and does not involve caustic organic solvents. When used with high-resolution arrays, analysis of large numbers of tumor research samples will provide important insight into the genomic causes of malignancies, and thus shed light on direction for possible treatment.



References

1. Brosens, R, Haan, J, Carvalho, B, Rustenberg, F, Grabsch, H, Quirke, P, Engel, A, Cuesta, M, Maughan, N, Flens, M, Meijer, G, Ylstra, B. Candidate driver genes in focal chromosomal aberrations of stage II colon cancer. Accepted article, 16 March 2010, *J. Pathology*.

FFPE Sample Preparation - Ordering Information

Component	Manufacturer	Cat. No.
Microtome	Multiple Vendors	
Scalpel or needle	Multiple Vendors	
Pipettes	Multiple Vendors	
Centrifuge	Multiple Vendors	
Vortex	Multiple Vendors	
Coverglass	VWR	631-0146
Bovine Serum Albumin (BSA)	Roche	10 735 086 001
Uncoated Glass Slides	Thermo Scientific	8037/1
Xylene	VWR	28973.328
Depex Mounting Medium	VWR	361252B
Ethanol Absolute, 96%, 70%	Biosolve	05250601
Methanol	VWR	20847.320
Hematoxylin	Sigma-Aldrich	S7757
Eosin	Merck	1.00063
1M NaSCN	Sigma	467871-50G
1.5 mL Safe-Lock Tube	Eppendorf	03115836001
Proteinase K: 20 mg/mL (Recombinant, PCR Grade)	Roche	03 115 836 001
Spectrophotometer	Nanodrop	ND-1000
QIAamp DNA Micro Kit	Qiagen	56305

Roche NimbleGen Workflow - Ordering Information

Microarrays	 Delivery Cat. No.	 Service Cat. No.
NimbleGen Human CGH 2.1M Whole-Genome Tiling v2.0D Array	05 541 921 001	05 543 991 001
NimbleGen Human CGH 3x720K Whole-Genome Tiling v3.0 Array	05 520 797 001	05 520 860 001
NimbleGen Human CGH 3x720K Exon-Focused Array	05 542 073 001	05 544 122 001
NimbleGen Human CGH 12x135K Whole-Genome Tiling v3.0 Array	05 520 878 001	05 520 886 001
NimbleGen Human CNV 2.1M v1.0 Array	05 913 152 001	05 913 195 001
NimbleGen Human CNV 3x720K v1.0 Array	05 913 209 001	05 913 233 001
Reagents	Cat. No.	
NimbleGen Dual-Color DNA Labeling Kit	05 223 547 001	
NimbleGen Hybridization Kit	05 583 683 001	
NimbleGen Hybridization Kit, LS	05 583 934 001	
NimbleGen Wash Buffer Kit	05 584 507 001	
NimbleGen Array Processing Accessories	05 223 539 001	
NimbleGen Sample Tracking Control Kit	05 634 270 001	
Instruments	Cat. No.	
NimbleGen Hybridization System 4 (110V)	05 223 652 001	
NimbleGen Hybridization System 12 (110V)	05 223 679 001	
NimbleGen Hybridization System 4 (220V)	05 223 687 001	
NimbleGen Hybridization System 12 (220V)	05 223 695 001	
NimbleGen Microarray Dryer (110V)	05 223 636 001	
NimbleGen Microarray Dryer (220V)	05 223 644 001	
NimbleGen MS 200 Microarray Scanner	05 394 341 001	
Software	Cat. No.	
NimbleScan Software – Individual License	05 933 315 001	
NimbleScan Software – Site License	05 933 331 001	
SignalMap Software – Individual License	05 225 051 001	

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