Genomic DNA Chip:
Genome-wide profiling in Cancer

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Abstract

All cancers are caused by abnormalities in DNA sequence. Throughout life, the DNA in human cells is exposed to mutagens and suffers mistakes in replication, resulting in progressive, subtle changes in the DNA sequence in each cell. Since the development of conventional and molecular cytogenetic methods to the analysis of chromosomal aberrations in cancers, more than 1,800 recurring chromosomal breakpoints have been identified. These breakpoints and regions of nonrandom copy number changes typically point to the location of genes involved in cancer initiation and progression.

With the introduction of molecular cytogenetic methodologies based on fluorescence in situ hybridization (FISH), namely, comparative genomic hybridization (CGH) and multicolor FISH (m-FISH) in carcinomas become susceptible to analysis. Conventional CGH has been widely applied for the detection of genomic imbalances in tumor cells, and used normal metaphase chromosomes as targets for the mapping of copy number changes. However, this limits the mapping of such imbalances to the resolution limit of metaphase chromosomes (usually 10 to 20 Mb). Efforts to increase this resolution have led to the "new" concept of genomic DNA chip (1 to 2 Mb), whereby the chromosomal target is replaced with cloned DNA immobilized on such as glass slides. The resulting resolution then depends on the size of the immobilized DNA fragments.

We have completed the first draft of its Korean Genome Project. The project

proceeded by end sequencing inserts from a library of 96,768 bacterial artificial chromosomes (BACs) containing genomic DNA fragments from Korean ethnicity. The sequenced BAC ends were then compared to the Human Genome Project's publicly available sequence database and aligned according to known cancer gene sequences.

These BAC clones were biotinylated by nick translation, hybridized to cytogenetic preparations of metaphase cells, and detected with fluorescein-conjugated avidin. Only locations of unique or low-copy portions of the clone are identified, because high-copy interspersed repetitive sequences in the probe were suppressed by the addition of unlabelled Cot1 DNA. Banding patterns were produced using DAPI. By this means, every BAC fragment has been matched to its appropriate chromosomal location. We have placed 86 (156 BAC clones) cytogenetically defined landmarks to help with the characterization of known cancer genes.

Microarray techniques would be applied in CGH by replacement of metaphase chromosome to arrayed BAC confirming in oncogene and tumor suppressor gene: and an array BAC clones from the collection is used to perform a genome-wide scan for segmental aneuploidy by array-CGH. Therefore, the genomic DNA chip (arrayed BAC) will be undoubtedly provide accurate diagnosis of deletions, duplication, insertions and rearrangements of genomic material related to various human phenotypes, including neoplasias. And our tumor markers based on genetic abnormalities of cancer would be identified and contribute to the screening of the stage of cancers and/or hereditary diseases

CV

2001 년 현재: ㈜마크로젠 연구위원

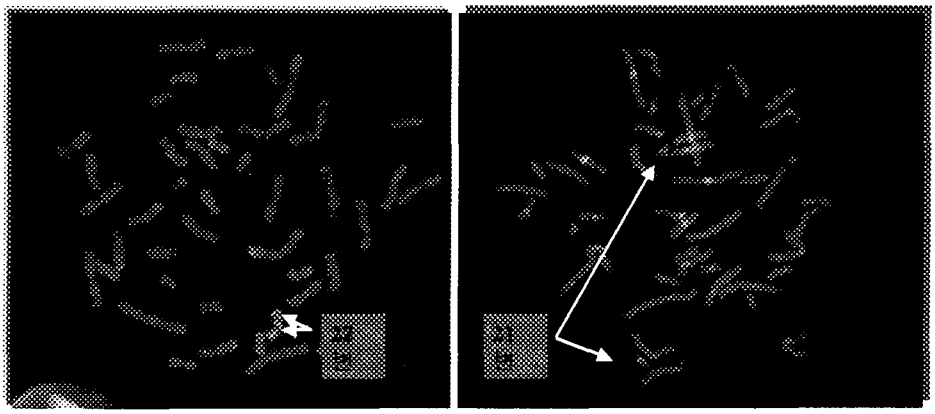
2000 년-2001 년: 서울대학교 의과대학 유전자이식연구소 선임연구원

1998 년: 서울대학교 농업생명과학대학 동물자원과학과 농학박사

1994 년-1998 년: 한국생명과학연구소 창단맴버 및 책임연구원

1991 년-1994 년: 마리아 산부인과 불임크리닉 선임연구원

Genomic DNA Chip : Genome-wide profiling in Cancer



이 종 호

㈜ 마 크 로 젠 : BAC응용사업부

Fluoresence in situ hybridization

1994

Probe

F I S H

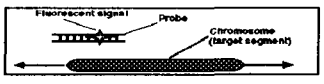
Preparation

Slide

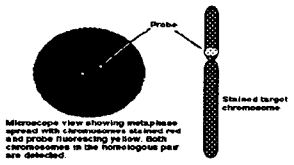
Conventional chr. prep.

Probe

1. Total genomic DNA
2. Known gene probe
3. Cosmid /PAC/BAC/YAC
4. Micro-FISH probe



Denaturation



Microscope view showing metaphase spread with chromosomes stained red and probe fluorescing yellow. Both chromosomes in the homologous pair are detected.

Hybridization

Detection

Confocal microscopy(LSM)

using digitized images of FITC and propidium iodide(PI)

Comparative Genomic Hybridization

1997

CGH

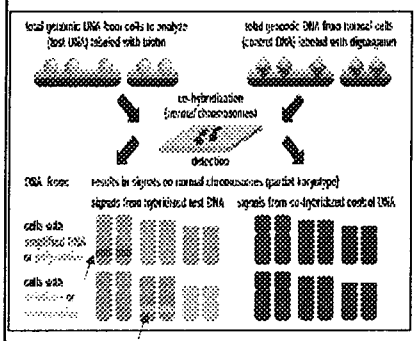
Slide

(FISH)

Preparation

Probe (Total Genomic DNA)

1. Reference DNA- Red
2. Test DNA - Green



Denaturation

Hybridization

Detection

Confocal microscopy(LSM)

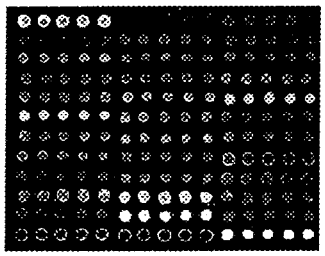
using digitized images of FITC and propidium iodide(PI)

Genomic DNA chip

2000

Slide

Genomic DNA chip



(FISH)

Preparation

Probe (Total Genomic DNA)

1. Reference DNA- Red
2. Test DNA - Green

Denaturation

Hybridization

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Confocal microscopy(LSM)

using digitized images of FITC and propidium iodide(PI)

FISH

Clinical Cytogenetic
Preimplantation Genetic Diagnosis (PGD)
Cancer Research
Gene Analysis

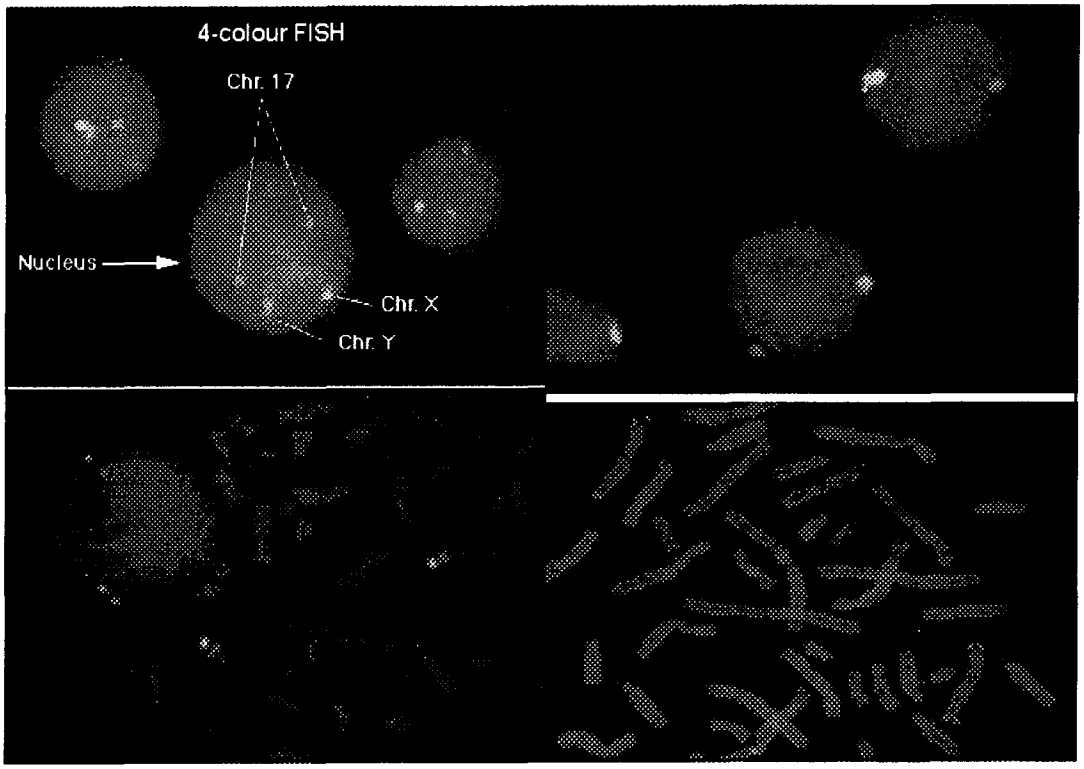
A major limitation is that FISH cannot be used if a suitable probes is not available.

A Single copy sequence : cDNA

Cloned genomic DNA : YAC, BAC, PAC, Cosmid

Chromosome marker : Repetitive sequence

Chromosome painting : FACS, Microdissection



Comparative Genomic Hybridization

1997

CGH

(FISH)

Slide

Preparation

Probe (Total Genomic DNA)

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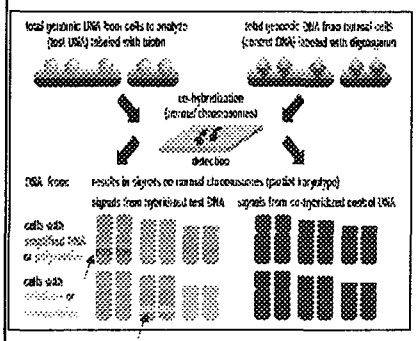

Denaturation

Hybridization

Detection

Confocal microscopy(LSM)

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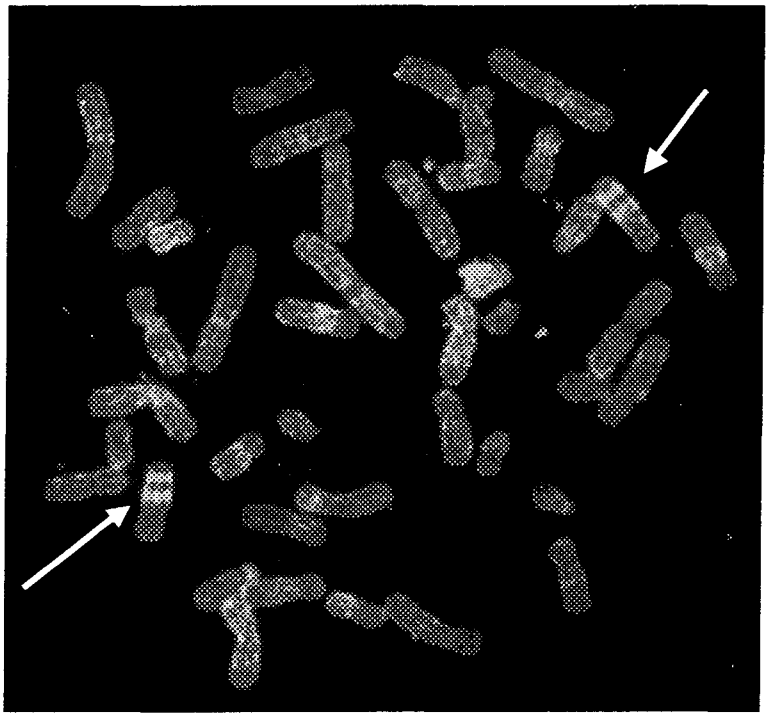
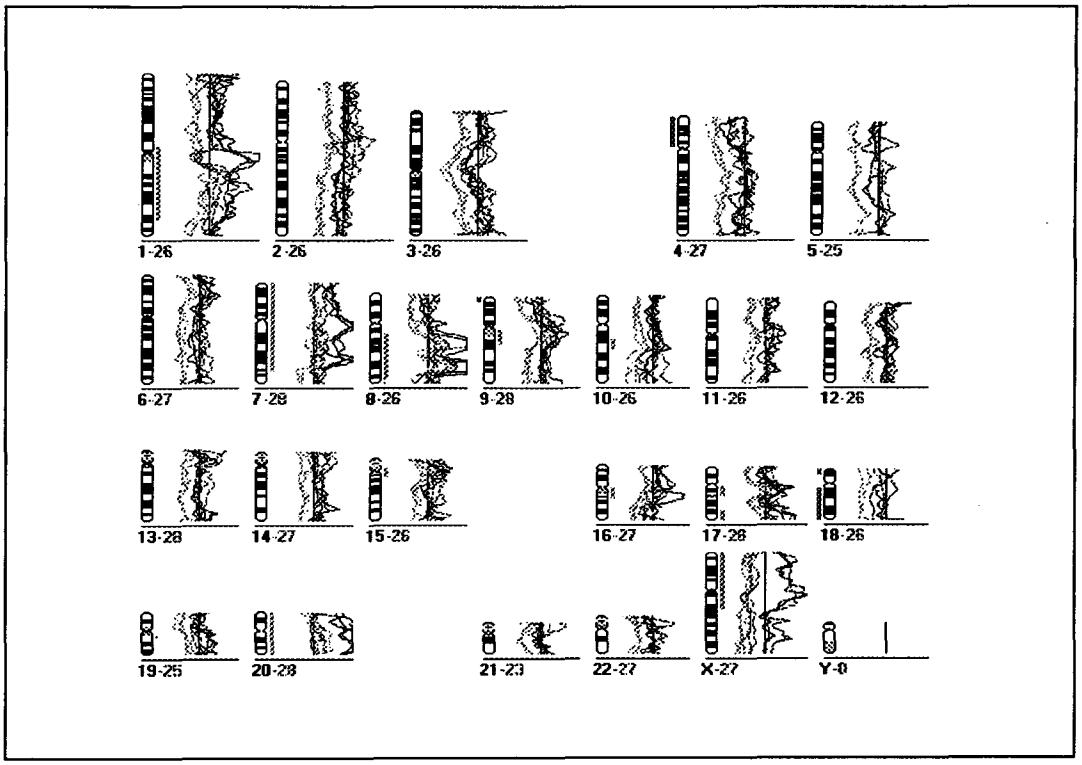




Chromosomal CGH

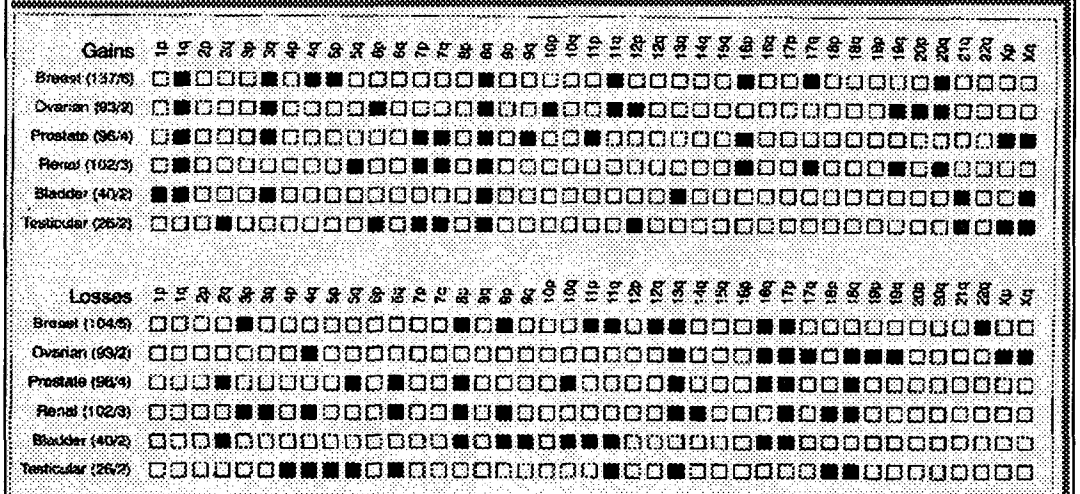
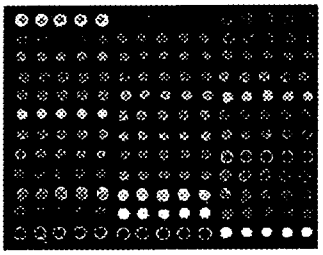


FIGURE 3. An overview of the most-common gains and losses reported in the published comparative genomic hybridization studies of selected genital and urological tumors (the references to the original studies can be obtained from http://nhgri.nih.gov/DIR/LCG/CGH). The number of tumors analyzed and the number of studies evaluated for each tumor type are indicated in parentheses. The three most-common changes to a particular tumor type (■), and the next most-common regions of involvement (■) are shown. Approximately ten of the most-common changes are indicated for each tumor type. The criterion for selecting the most-common changes was not only the frequency, but the systematic presence of the change in independent studies. Distinct patterns of losses and gains are seen for each tumor type, whereas some genetic changes are common to several tumor types.

TIG (1997)

Genomic DNA chip (FISH) 2000

Slide
Genomic DNA chip



Preparation

Probe (Total Genomic DNA)
1. Reference DNA- Red
2. Test DNA - Green

Denaturation

Hybridization

Detection

Confocal microscopy(LSM)
using digitized images of FITC and propidium iodide(PI)

FISH 와 Chip ?

\+ **BAC 과 HGP ?**

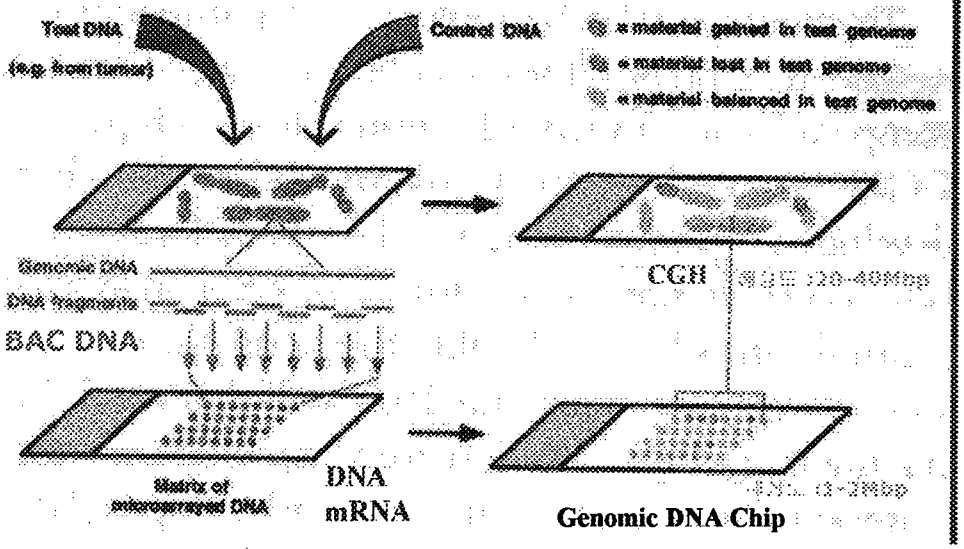
Genomic DNA Chip : G Chip : **BAC Chip** : Array CGH : matrix CGH

↓ ↑

Cancer 와 Genomic instability ?

Microdeletion and Aneuploidy

Genomic DNA Chip ?



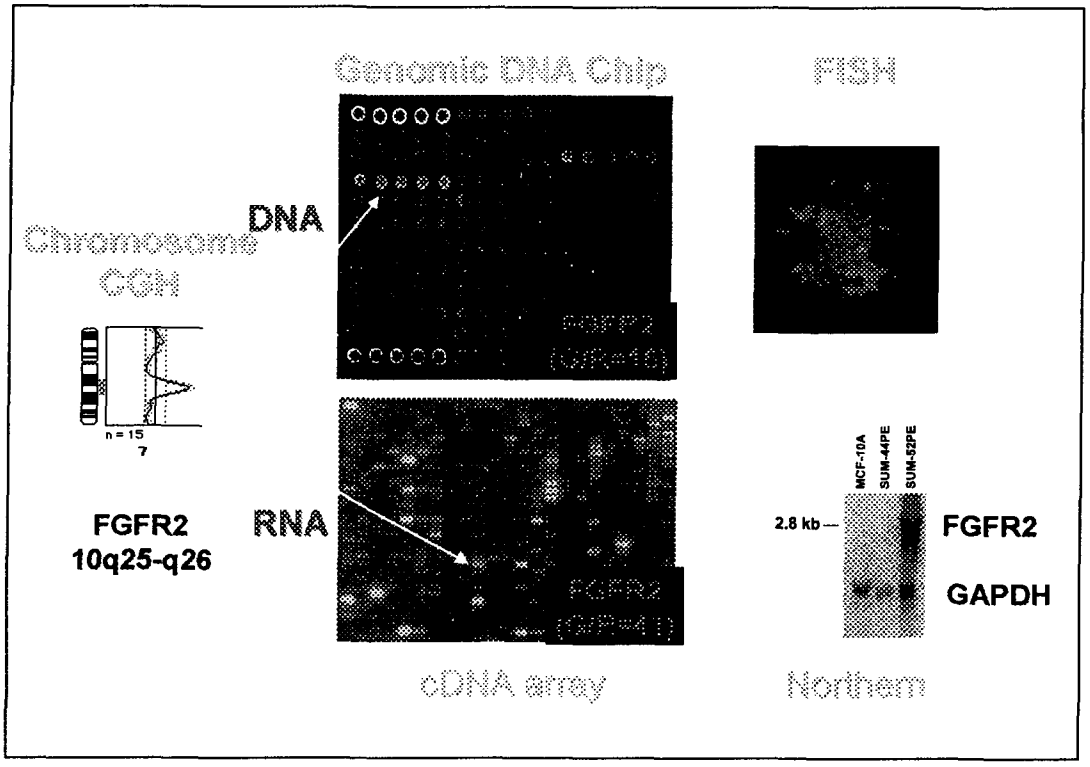
Genomic DNA Chip 와 cDNA Chip 차이점?

Genomic microarrays : **Detect the presence or absence of a gene.**

DNA → Target → **Screening** → Results

Expression Microarrays : **Determines expression levels of genes.**

mRNA → Random → Results → **Bioinformatics**



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↓

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Microdeletion and Aneuploidy

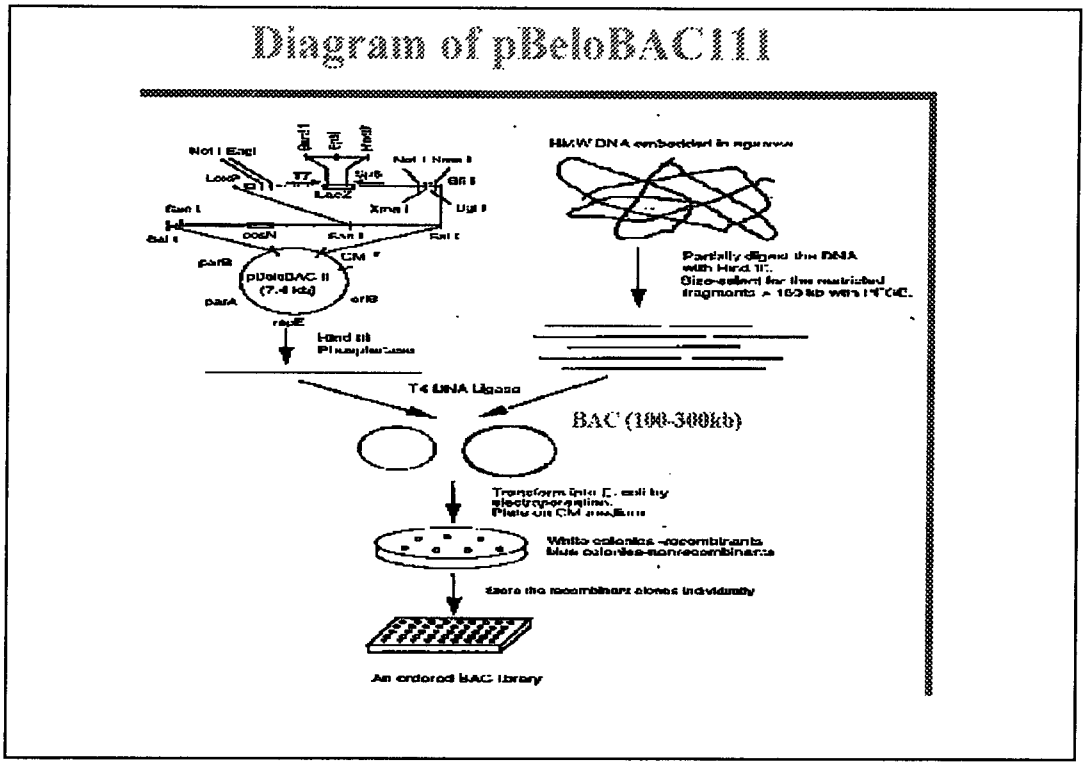
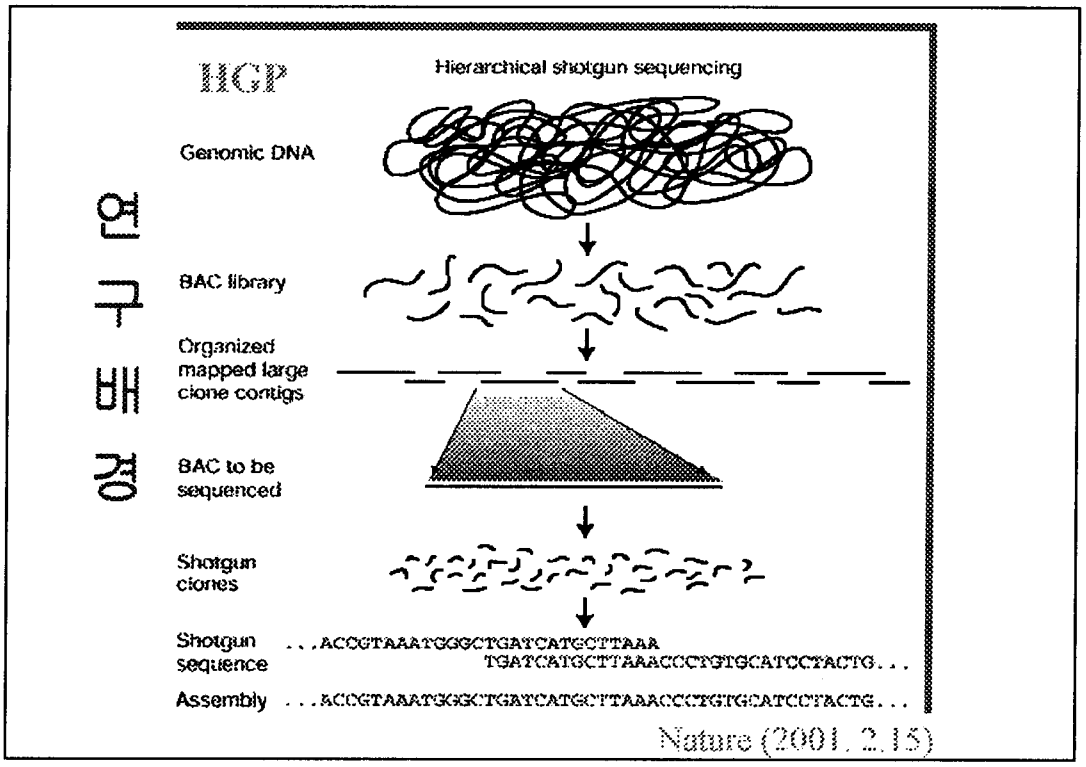


Diagram of pBeloBAC11
BAC (100-300kb)


HGP
Hierarchical shotgun sequencing
Genomic DNA
BAC library
Organized mapped large clone contigs
BAC to be sequenced
Shotgun clones
Shotgun sequence
...ACCGTAAATGGGCTGATCATGCTTAAA
TGATCATGCTTAAACCCTGTGCATCCTACTG...
Assembly
...ACCGTAAATGGGCTGATCATGCTTAAACCCTGTGCATCCTACTG...
Nature (2001. 2.15)

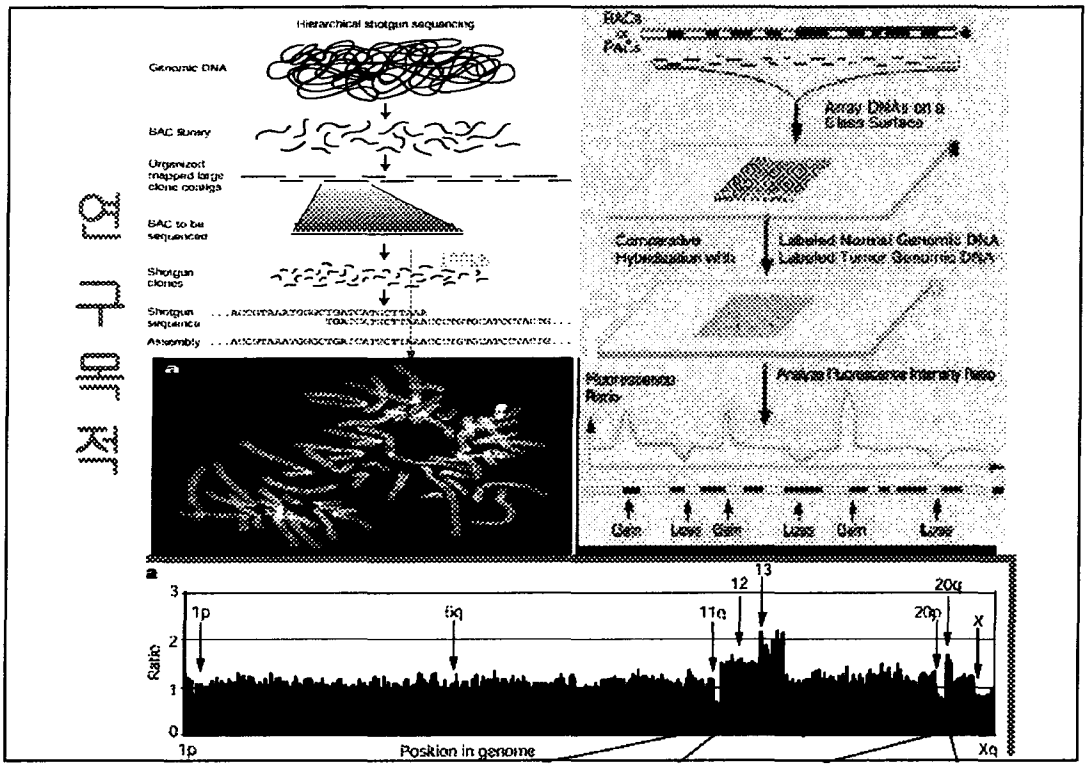
FISH 와 Chip ?

\+ BAC 과 HGP ?

Genomic DNA Chip : G Chip : **BAC Chip** : Array CGH : matrix CGH

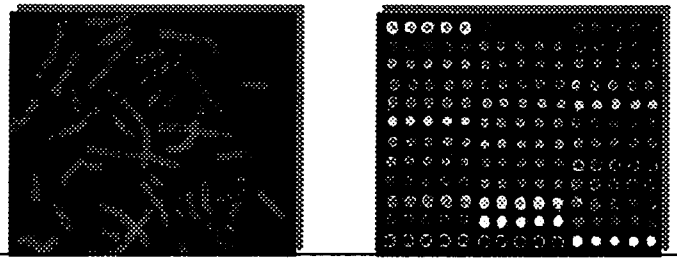
Cancer 와 Genomic instability ?

Microdeletion and Aneuploidy



Genomic DNA Chip using BACs
장 점

- This BACs will be of value to the cancer-research and cytogenetics community for analyzing chromosomal rearragements by FISH or by DNA array analyses



FISH 와 Chip ?

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↓↑

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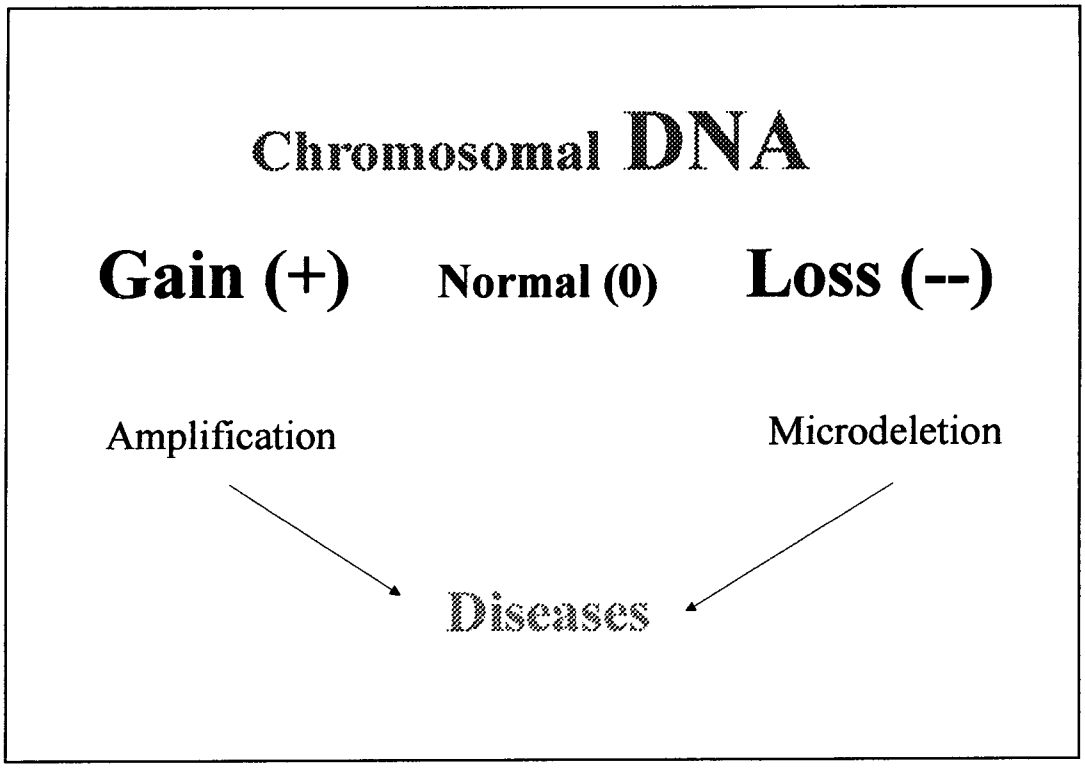


DNA copy number (gene dosage) variations

Cancer	Disease
↓	↓
Oncogenes – 100	Down, Prader Will,
Tumour-suppressor genes -30	Angelman, Cri du Chat syndrome
Genomic instability	**Gain or loss of one copy**

1) DNA sequence alteration
2) Aneuploidy
3) Gross chromosomal rearrangements
4) Gene amplification and deletion

Is there evidence for a causal relationship between
Checkpoint failure, Genomic instability, and Cancer ?

When DNA replication escape control !

↓

Spontaneous DNA Damage

↓

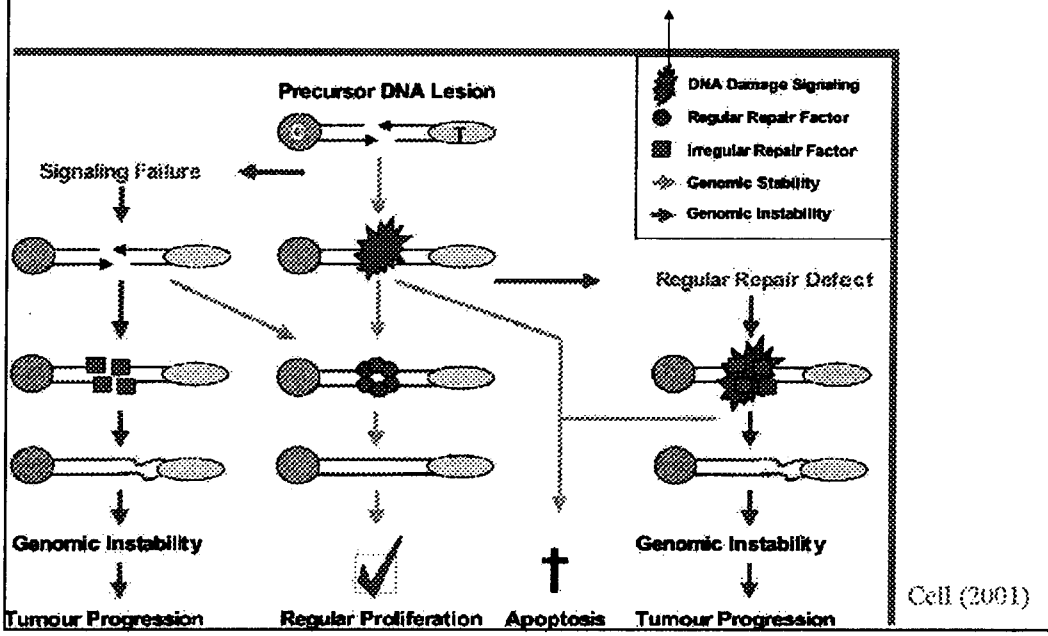
Genome instability

↓

Cancer

Cancer and Genome Instability

S phase-specific DNA damage sensor : Rfc5p, MEC1, DUN1, TEL1, 등



FISH 와 Chip ?

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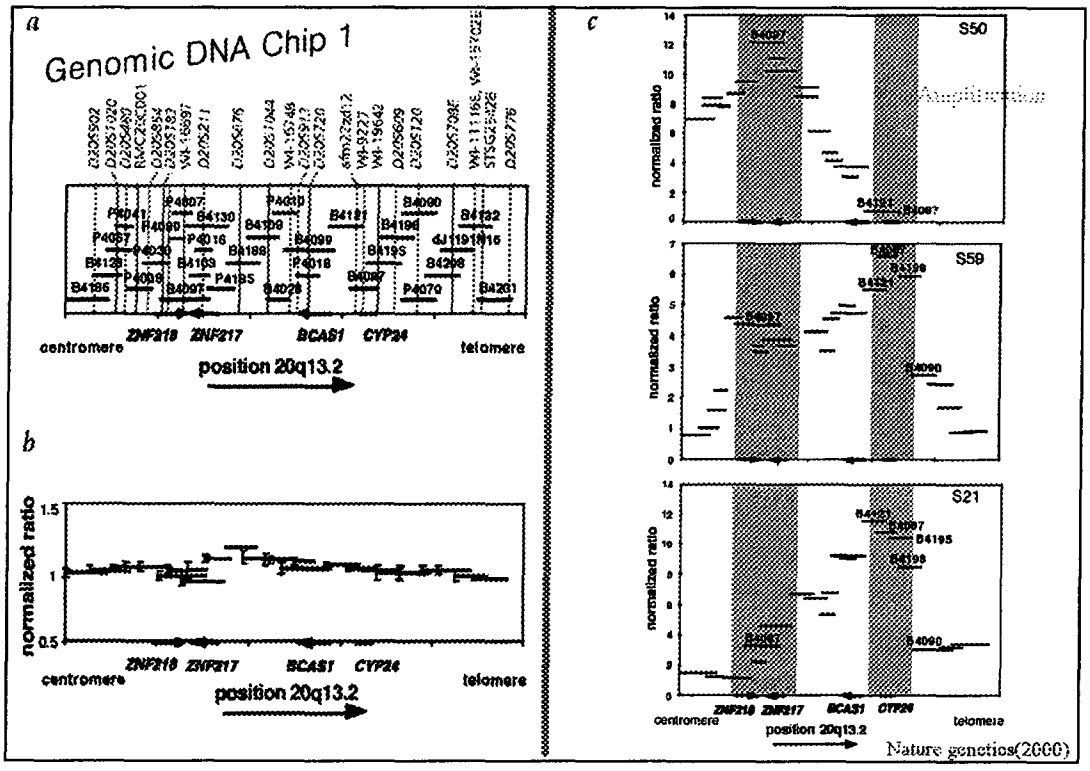
Genomic DNA Chip : G Chip : BAC Chip : Array CGH : matrix CGH

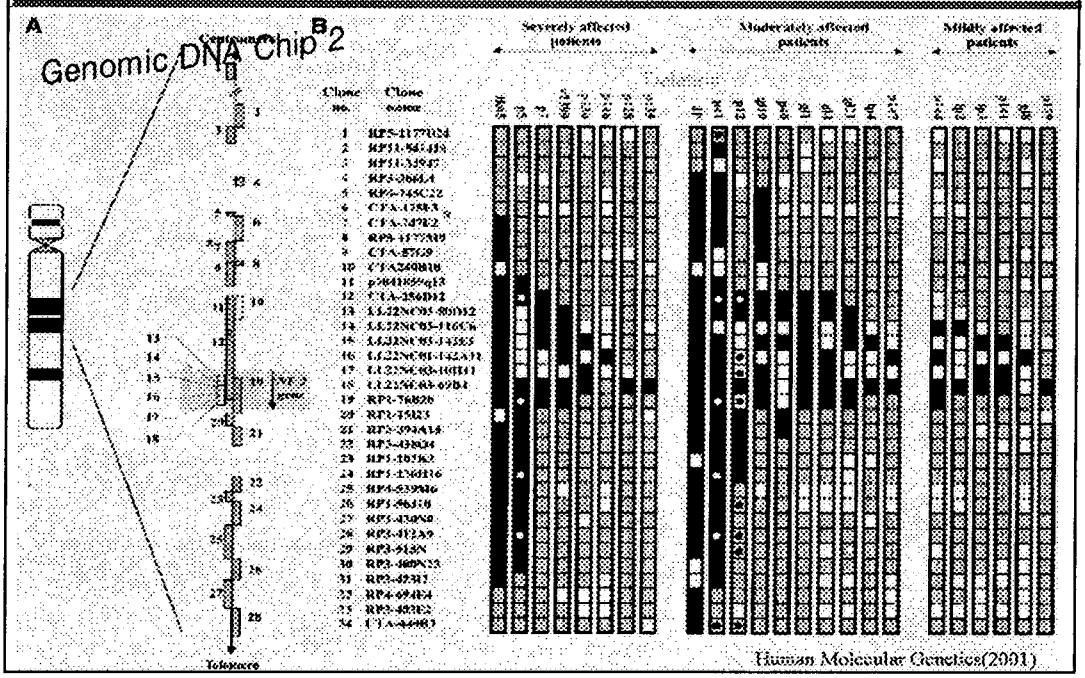
↑↓

Cancer 와 Genomic instability ?

Microdeletion and Aneuploidy



Gene progression by Genomic DNA Chip



Genomic DNA Chip 3

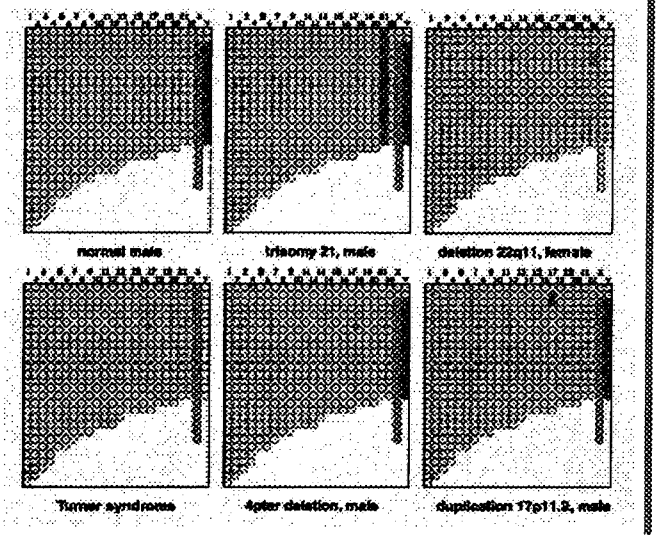
"BACking up the promises"

Control- G (female)

Test - R

XX : XR = Yellow

XY : XY = Green



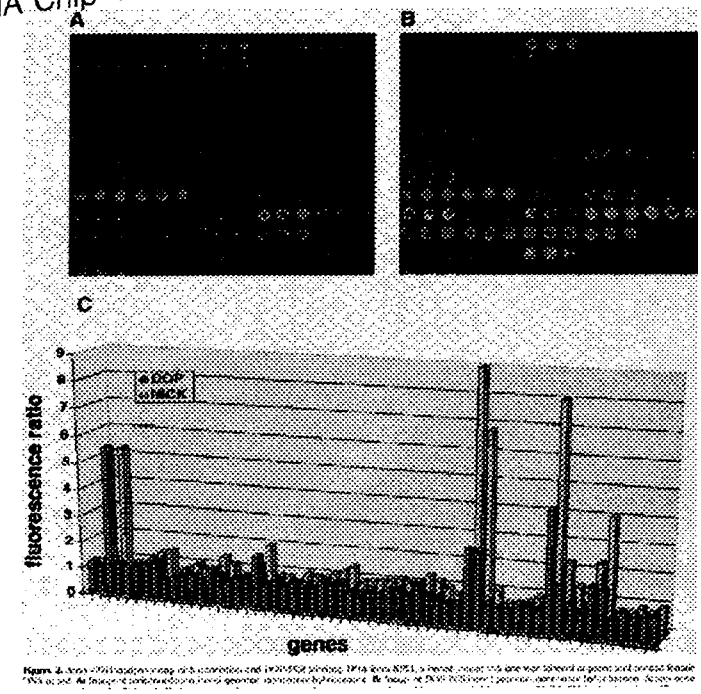
Without gain or loss

Balanced rearrangement
Inversion

Nature genetics(2001)

Genomic DNA Chip 4

Cancer gene chip



Am J Pathol 2001

MACROGEN

BAC-FISH map

Probe 化 : Human band specific chromosome
Microdeletion syndromes
Aneuploidy analysis
Oncology and pathology

Chip化 : Genomic DNA Chip

Scanner of Genome-wide array

Genomic DNA Chip을 위한 5 단계 기술

1 단계 : BAC library

2 단계 : BAC end sequence

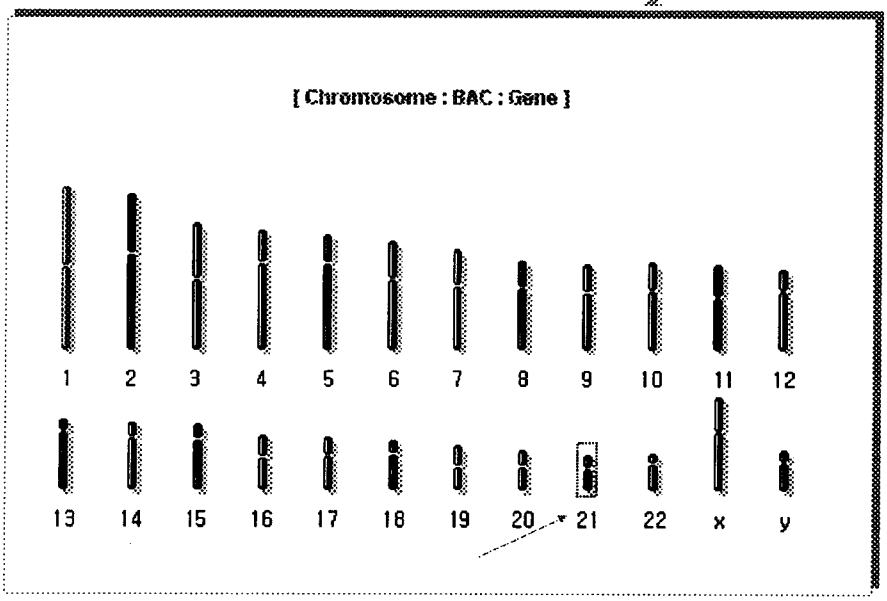
3 단계 : Bioinformatics

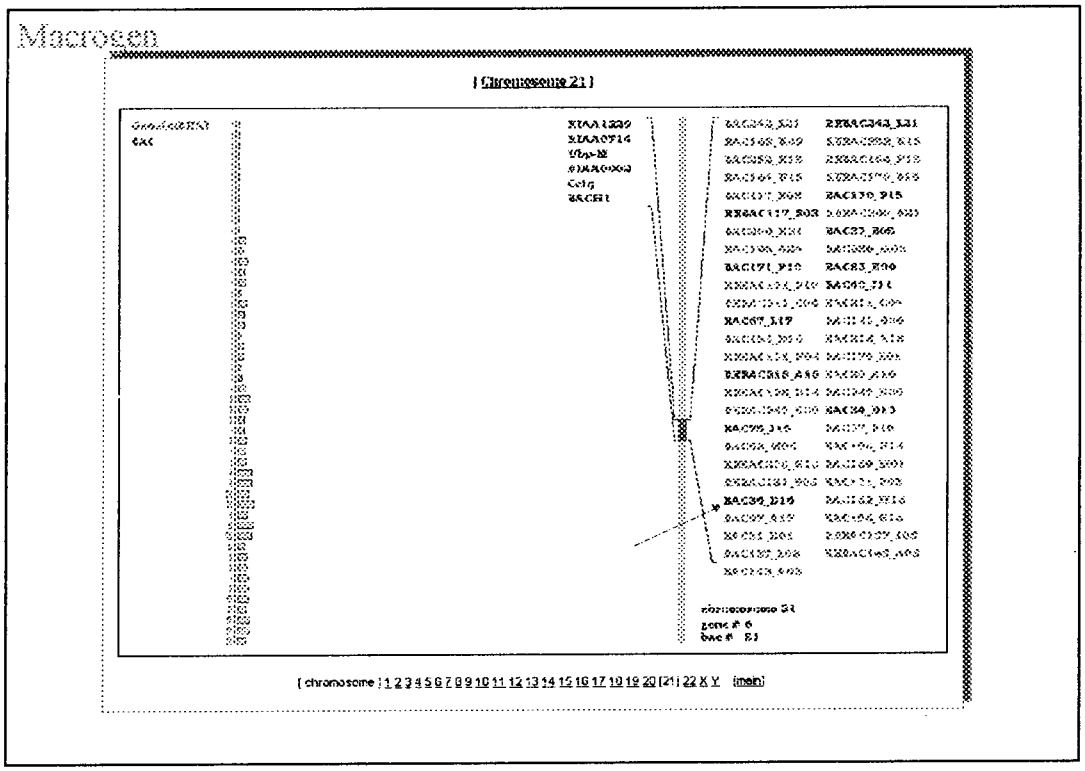
4 단계 : FISH

5 단계 : Chip 化

Macrogen

Korean BAC Map





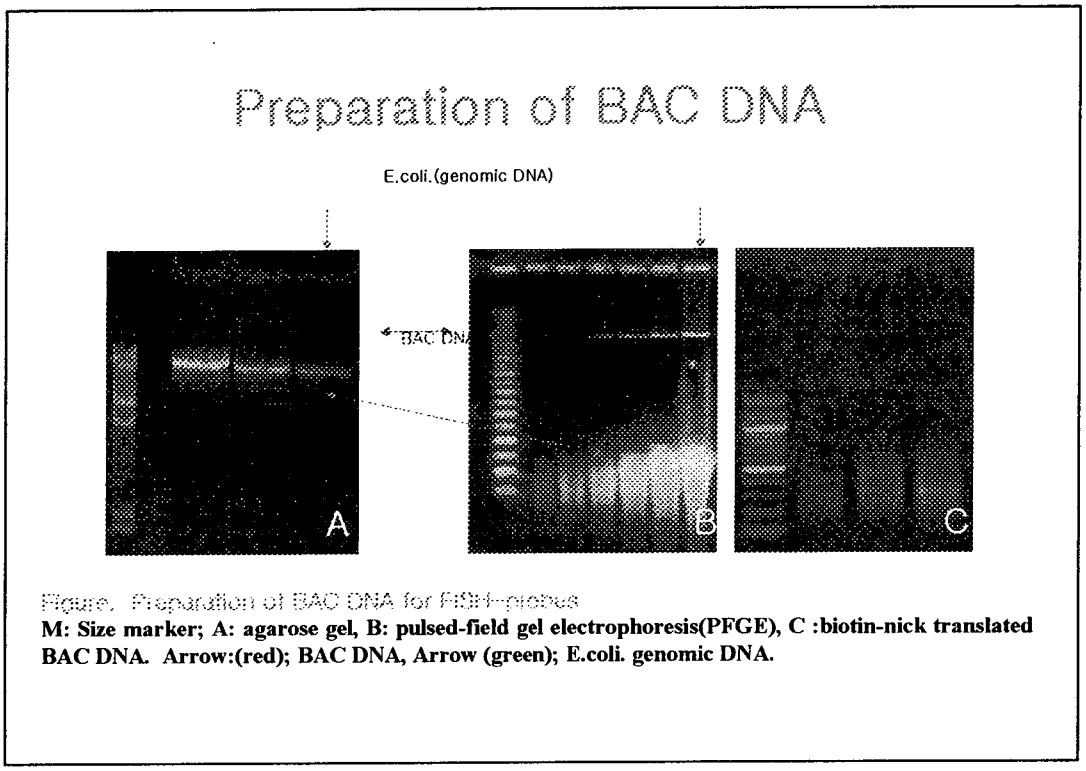
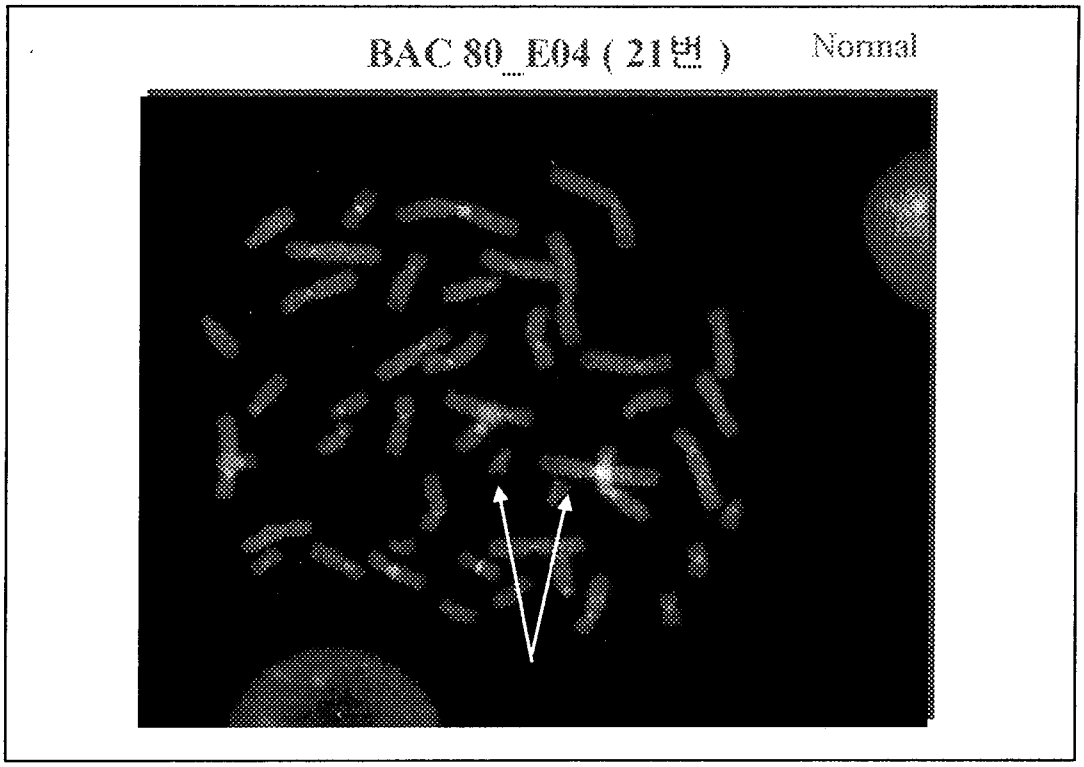
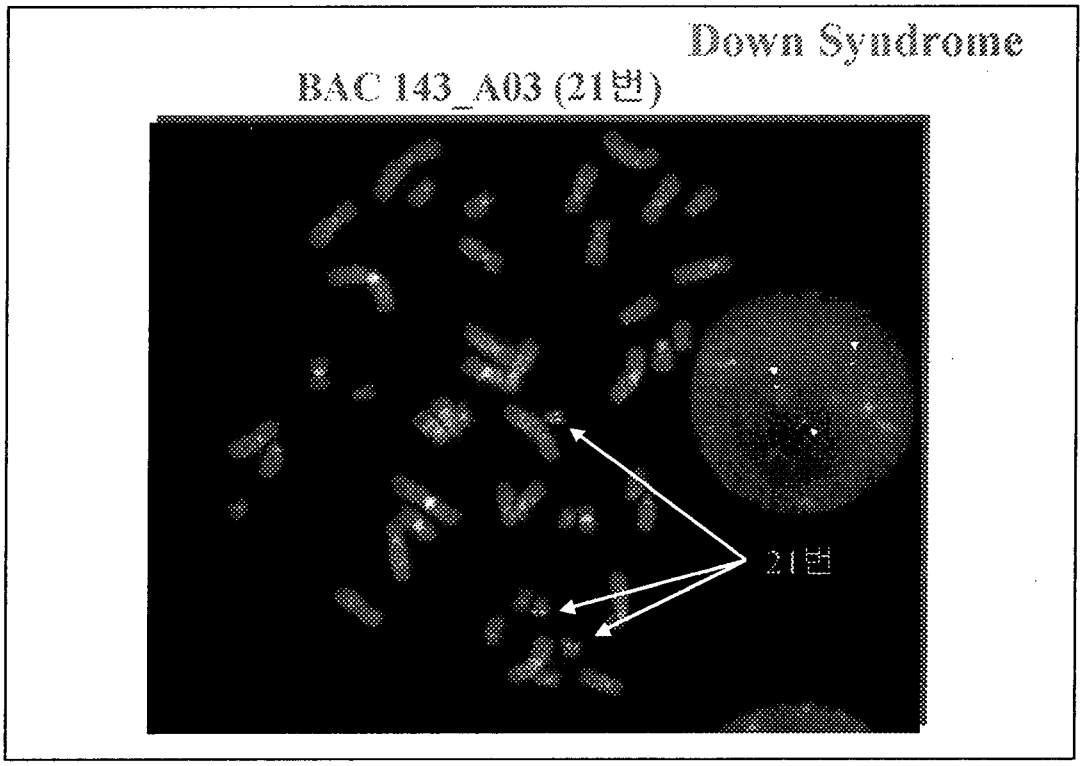


Figure. Preparation of BAC DNA for FISH-probes

M: Size marker; A: agarose gel, B: pulsed-field gel electrophoresis(PFGE), C :biotin-nick translated BAC DNA. Arrow:(red); BAC DNA, Arrow (green); E.coli. genomic DNA.


BAC 80_E04 (21번)
Nonnal


Down Syndrome
BAC 143_A03 (21번)
21번

Incidence of common aneuploidies resulting in live births

Chromosome make-u	Incidence	Phenotype
Trisomy 21	1/770	Mental retardation
Trisomy 13	1/15,000	Mental/physical retardation
Trisomy 18	1/4000-1/8000	Mental/physical retardation
45, XO	1/10,000 females	Sexual immaturity
47, XXY	1/1000 males	Sexual immaturity
47, XXX	1/1000 females	Tall, thin, menstrual irregularity
47, XYY	1/1000 males	

Microdeletions

Microdeletions	Test Population	Incidence 1/
CATCH 22(22q)	Newborns w/symptoms	5,000
Cri du Chat (5p15)	Newborns w/symptoms	20,000
Wolf-Hirschhorn (4p)	Newborns w/symptoms	500,000
Prader-Willi (15q11-13)	Children w/symptoms	10,000
Williams Syndrome (7q11)	Children w/symptoms	10,000
Xp22.3 Variety of syndromes	Children w/symptoms	15,000
Xp21 deletions variety of syndromes	Children w/symptoms	15,000
Angelman (15q11-13)	Children w/symptoms	25,000
Smith-Magenis (17p11)	Children w/symptoms	25,000
Kallmann (Xp22.3)	Young adult w/symptoms	25,000
HNPP (17p12)	Children w/symptoms	50,000
Allagille syndrome (20p11)	Children w/symptoms	70,000

Cancer

Cancer Types	Amplification in Literature
Colon Adenocarcinoma	C-MYC
Retinoblastoma	N-MYC
Breast cancer	HER-2, C-MYC EGFR
Breast ductal cancer	HER-2, Amplicon 20q
Breast adenocarcinoma	HER-2, BCL-1/CYC-D, INT-2
Breast adenocarcinoma	EGFR
Femoral sarcoma	GLI, MDM2, CDK4/SAS
Leukemia (CML)	ABL
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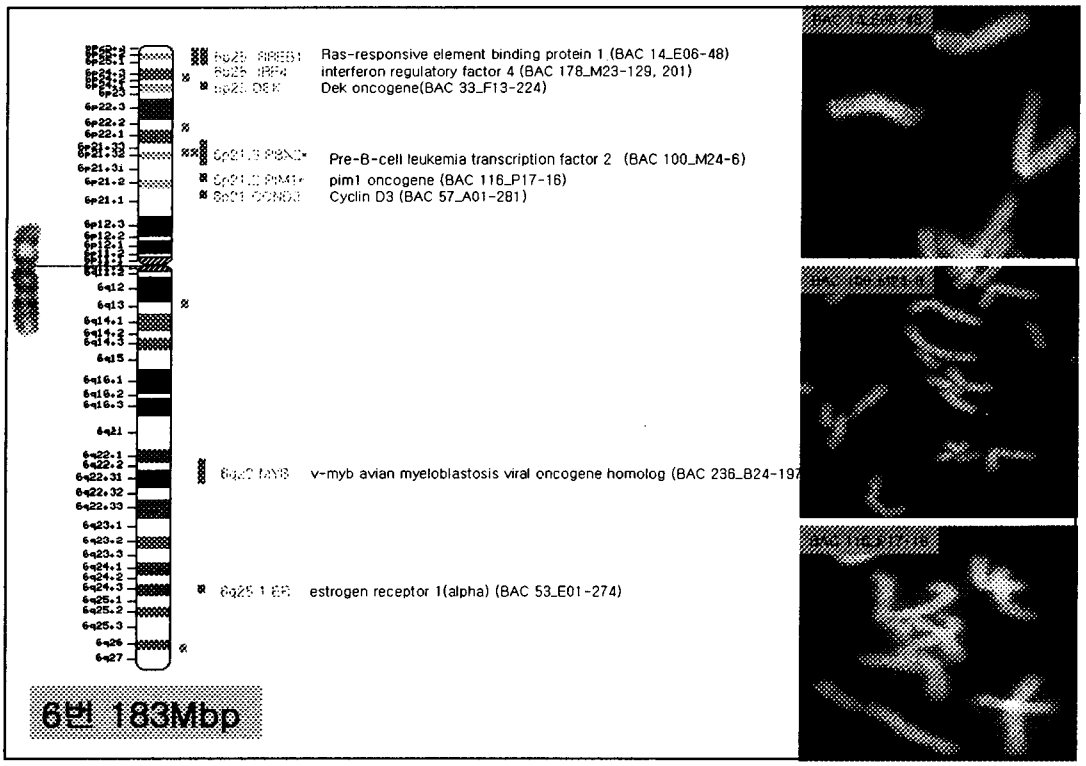
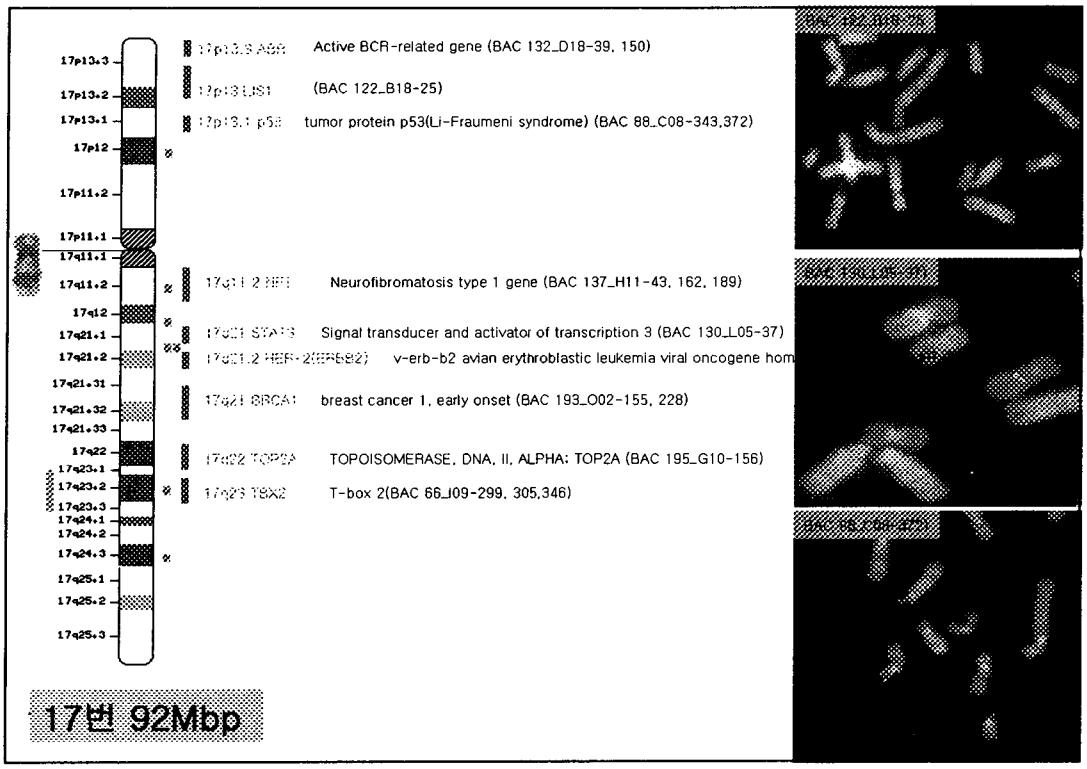
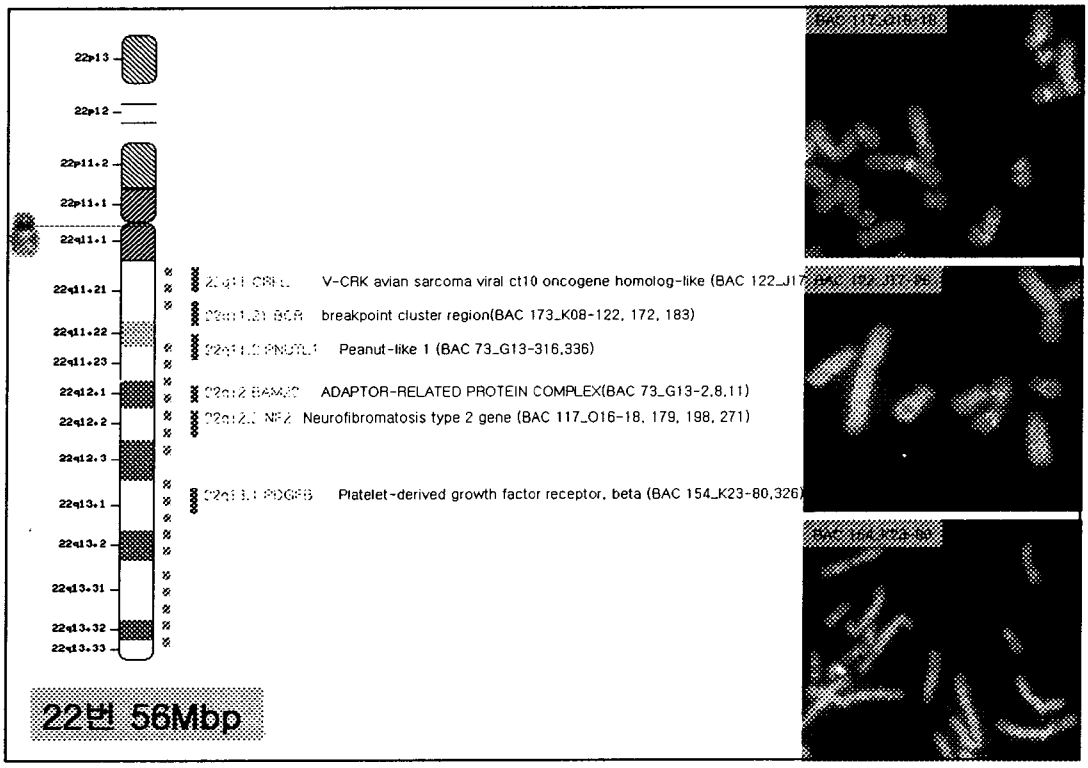
.	.
.	.
.	.




17p13.3
17p13.2
17p13.1
17p12
17p11.2
17p11.1
17q11.1
17q11.2
17q12
17q21.1
17q21.2
17q21.31
17q21.32
17q21.33
17q22
17q23.1
17q23.2
17q23.3
17q24.1
17q24.2
17q24.3
17q25.1
17q25.2
17q25.3
Active BCR-related gene (BAC 132_D18-39, 150)
(BAC 122_B18-25)
tumor protein p53(Li-Fraumeni syndrome) (BAC 88_C08-343,372)
Neurofibromatosis type 1 gene (BAC 137_H11-43, 162, 189)
STAT3 Signal transducer and activator of transcription 3 (BAC 130_L05-37)
v-erb-b2 avian erythroblastic leukemia viral oncogene hom
BRCA1 breast cancer 1, early onset (BAC 193_O02-155, 228)
TOPOISOMERASE, DNA, II, ALPHA; TOP2A (BAC 195_G10-156)
TBX2 T-box 2(BAC 66_I09-299, 305,346)
17번 92Mbp


22p13
22p12
22p11.2
22p11.1
22q11.1
22q11.21
22q11.22
22q11.23
22q12.1
22q12.2
22q12.3
22q13.1
22q13.2
22q13.31
22q13.32
22q13.33
V-CRK avian sarcoma viral ct10 oncogene homolog-like (BAC 122_J17
breakpoint cluster region(BAC 173_K08-122, 172, 183)
Peanut-like 1 (BAC 73_G13-316,336)
ADAPTOR-RELATED PROTEIN COMPLEX(BAC 73_G13-2,8,11)
Neurofibromatosis type 2 gene (BAC 117_O16-18, 179, 198, 271)
Platelet-derived growth factor receptor, beta (BAC 154_K23-80,326)
22번 56Mbp

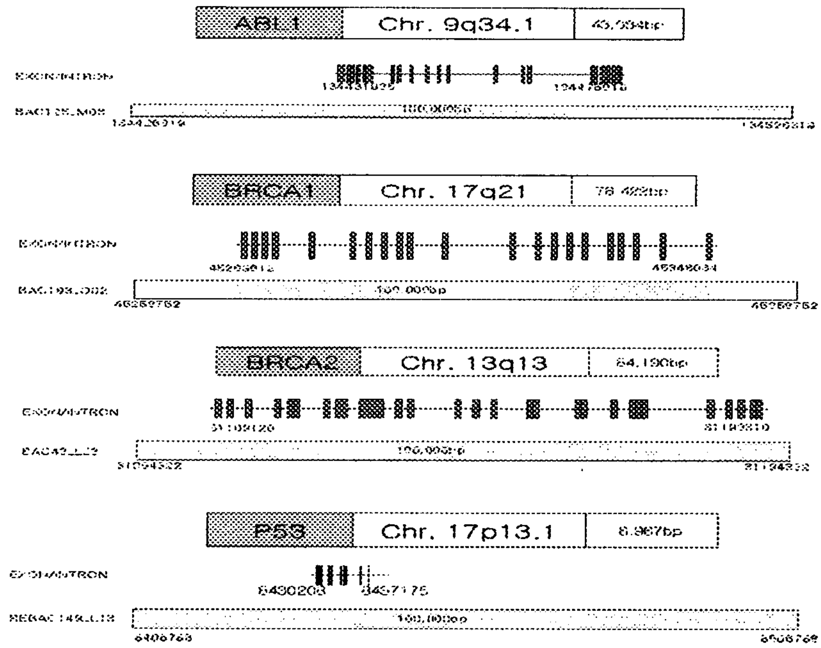


Figure. Schematic representation of cancer gene and BAC clones

List of cancer related gene

Symbol	Gene Name	CytoBand
ABL1	[illegible]	9q34.1
ABR	Active BCR-related gene	17p13.3
AKT2	[illegible]	19q13.1
ARP	Arginine-rich protein gene	3p21.1
BRCA1	breast cancer 1, early onset	17q21
BRCA2	breast cancer 2, early onset	13q13
C8G	complement component 8, gamma subunit	9q34.3
CCND1	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	11q13
CCND2	cyclin D2	12p13
CCND3	cyclin D3	6p21
COL1A2	collagen, type I, alpha 2	7q22.1
CRKL	[illegible]	22q11
CSF1	colony stimulating factor 1	1p21
CTSB	cathepsin	8p22
DEK	DEK oncogene	6p23
DPC4	MAD(mothers against decapentaplegic, Drosophila) homolog 4	18q21.1
EGFR	epidermal growth factor receptor	7p12
EGR2	early growth response 2	10q21.1
EMS1	EMS1 oncogene	11q13
EPOR	[illegible]	19p13.3
ER	[illegible]	6q25.1
EXT2	Multiple exostoses type 2 gene	11p13
FGF5	fibroblast growth factor 5	4q21
FGR	[illegible]	1p36
FLG	[illegible]	8p11.2
FRAT1	frequently rearranged in advanced T-cell lymphomas	10q23
HER-2(ERBB2)	[illegible]	17q21.2
HKR1	[illegible]	19qter
HOX11	homeo box 11	10q24
IGF1	insulin-like growth factor 1	12q24.1
IL2	[illegible]	4q27
IRF4	interferon regulatory factor 4	6p25
LMO2	LIM domain only 2	11p13
LTK	leukocyte tyrosine kinase	15q15.1
MEN1	Multiple endocrine neoplasia type [illegible]	11q13
MET	met proto-oncogene	7q31
MLL	[illegible]	11q23
MYB	[illegible]	6
NF2	neurofibromatosis type 2 gene	22q12
NOV	nephroblastoma overexpressed gene	8q24.1
ODC1	ornithine decarboxylase 1	2p25
p53	tumor protein p53(Li-Fraumeni syndrome)	17p13.1
PAEP	progestagen-associated endometrial protein	9q34
PBX2	pre-B-cell leukemia transcription factor 2	6p21.3
PDGFB	v-sis platelet-derived growth factor beta polypeptide oncogene homolog	22q13.1
PDGFRA	platelet-derived growth factor receptor, alpha	4q12
PIM1	pim-1 oncogene	6p21.2
PNUTL1	peanut-like 1	22q11.2
RAB3A	RAB3A, member RAS oncogene family	19p13.1
RARB	retinoic acid receptor, beta	3p24
RASSF1	Ras association domain	3p21.3
RET	RET proto-oncogene	10q11.2
RREB1	ras responsive element binding protein 1	6p25
SAS	sarcoma amplified sequence	12q13
SET	SET translocation (myeloid leukemia-associated)	9q34.1

Table. A cytogenetic resource of cancer-related genes and FISH-mapped clones followed BAC end sequence (Korean map).

Chr.	FISH mapped	Type of clones		Cancer related genes
		Marker	Cancer	
1		12	3	
2		5	1	
3		3	5	
4		3	2	
5		3	1	
6		5	2	
7		3	4	
8		3	3	
9		4	4	
10		10	4	
11		4	3	
12		4	4	
13		2	1	
14		5	1	
15		4	2	
16		3	1	
17		7	9	
18		2	4	
19		1	5	
20		5	3	
21		21	0	
22		25	6	
X		4	2	
Y		0	1	
total	249	163	86	

All clones are associated with BAC-end sequence; localized directly to cytogenetic bands by FISH (2001. 9. 1.).* End-sequence BACs and single-site FISHed

Genome-wide array (Korean)

The 'BAC chip' will soon be an off-the-shelf, relative cheap product