

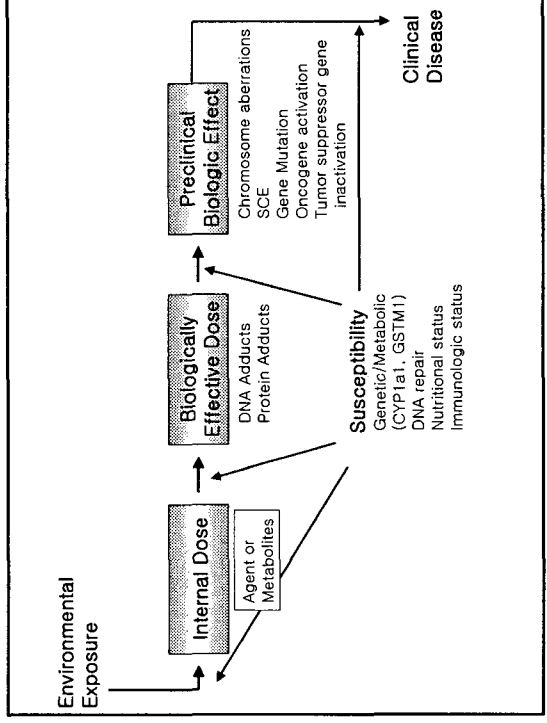
# 유전체연구를 위한 시료의 보관과 실험실적 환경

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## 순서

- ◆ 생체지표
- ◆ 시료의 채취와 보관
- ◆ DNA 분석
- ◆ 기타시료
- ◆ 자료관리
- ◆ 실험실환경
- ◆ 비용

# Molecular Biomarkers



### Examples of Markers of Internal Dose

Biomarker	Source of Exposure	Biologic Sample	Measurement
Cotinine	Cigarette smoke	Serum, urine, saliva	Higher levels in smokers compared to nonsmokers
Vitamine levels	Diet	Serum	Serum levels of Vitamines A, E, C, and D linked with risk for various cancers
Selenium	Diet	Hair, toe nails	Levels of selenium linked with risk for lung cancer
Levels of benzene And benzene Metabolites	Cigarette smoke	Urine, breath concentrations	Higher levels of benzene and metabolites in smokers compared to nonsmokers
Aflatoxin	Contaminated food	Urine	Higher levels of aflatoxin in urine of exposed compared to nonexposed

### Examples of Markers of Biologically Effective Dose

Biomarker	Source of Exposure	Biologic Sample	Measurement
DNA and protein adducts	Cigarette smoke	Blood	More common in smokers than nonsmokers
8-OH-dG	Cigarette smoke	Blood, urine	Higher levels in smokers than nonsmokers

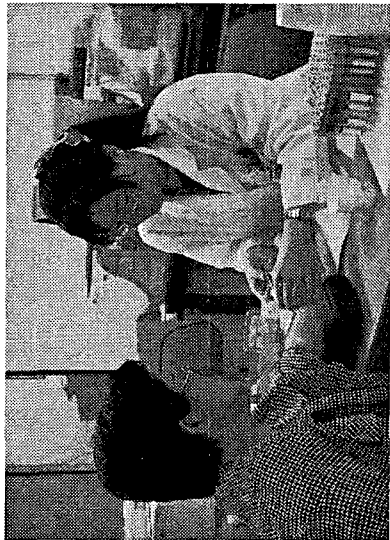
### Early Biologic Effect or Response

Compound analyzed	Exposure source	Biologic Sample	Population
Single strand breaks	Styrene	WBC	Workers
Sister chromatid exchange	Various industrial exposures, radiation, air pollution	WBC	Workers, residents
Micronuclei	Organic solvents, heavy metals, cigarette smoke, oral mucosa beta1 quid	WBC, mucosa	Workers
Chromosomal aberrations	Various industrial exposures, radiation, air pollution	WBC	Workers, residents
HPRT mutation	Chemotherapeutic agents, radiation	WBC	Patients, workers
GPA mutation	Chemotherapeutic agents, radiation	RBC	Patients, Japanese atom bomb survivors
Mutation in tumor suppressor genes	AFB1	Tumor tissue	Patients
Oncogene activation	PAH, cigarette smoke	Serum	Workers, cancer patients

### Sampling Strategy



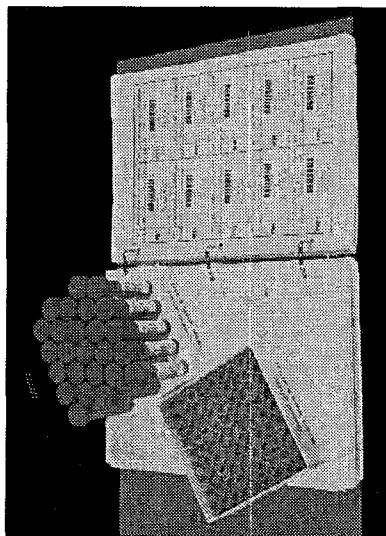
Sampling



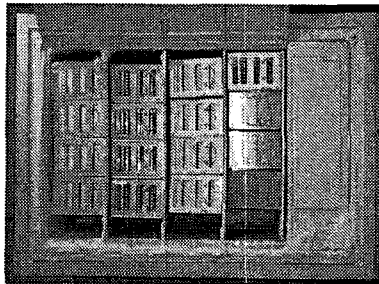
## Sample Storage

- ◆ 혈액은 barcode를 붙인 EDTA-vacutainer 에 채혈한 후 즉시 처리하는 것을 원칙으로 하나 여건에 따라 혈장, buffy coat, DNA 분리와 cell 시료 제작 시 (48시간 이내)까지 냉장 (+4℃) 보관한다.
- ◆ 시료의 향후 사용 목적에 따라 전혈 냉동용 1.5ml tube에 2개씩 분주하여 초저온 냉동고 (-80℃)에 보관하기도 하고, 전혈을 원심분리하여 혈장과 buffy coat를 분리하여 각각 1.5ml tube에 분주하여 초저온 냉동고 (-80℃)에 보관한다.

Sample & Barcode



Blood(-70℃)



## 시료 은행의 환경

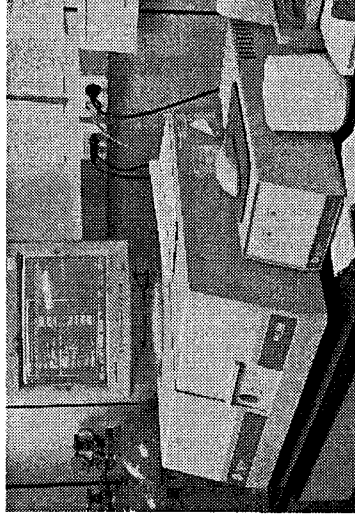
- ◆ 유전체 시료 보관은 안전한 곳에 다른 연구 시료 등과 공간을 분리하여 보관하며 시료 보관 용 장비는 alarm이 부착되어진 장비를 사용하여 정전시나 기기 오작동시 빠른 조치를 할 수 있도록 한다.
- ◆ 시료 보관 순서와 세심한 기술을 습득한 인원을 배치하여 bank를 유지하며 각 시료는 최소 두 군데 이상으로 분주하여 보관하고 보관된 시료는 일정한 시기마다 이상 유무를 확인한다.

## DNA analysis

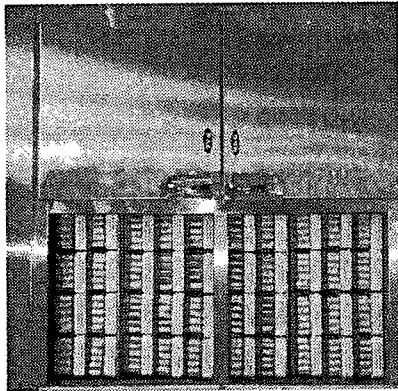
## Genomic DNA

- ◆ Genomic DNA는 채혈된 혈액 또는 냉동 보관된 혈액이나 buffy coat로부터 추출한다.
- ◆ 전통적인 phenol/chloroform 추출법은 경제적이긴 하나 노동력과 시간이 많이 드는 단점이 있어 medium-throughput DNA 추출에는 상품화되어진 DNA extraction kit을 사용한다. 또한 자동화된 DNA 추출기를 이용하기도 하는데 장비의 가격이 고가이긴 하나 DNA bank 구축 등의 high-throughput 연구에는 유용하다.
- ◆ 추출된 DNA는 UV spectrophotometer로 DNA의 순도 측정과 정량을 한 후 2개씩의 tube나 96-well plate에 분주하여 냉동고 (-20℃)에 보관한다.

## DNA 정량



DNA (-20°C)



### Specimens for DNA Banking

Specimen Type	Blood spots
DNA Yield	12-42 ng/ $\mu$ l (adults) 43-78 ng/ $\mu$ l (neonates) 1/4 -inch punch from 75 $\mu$ l volume yields about 12 $\mu$ l of blood
Advantages	Small sample size Ease of sample collection Ease of shipping (regular mail) Stability and low cost storage Offers a source for study of exogenous or endogenous compounds other than DNA Genotyping generally requires 10 ng/genotype, and with current technology as little as 2.5 ng per SNP so that scores to hundreds of genotypes could be obtained from one blood spot
Disadvantages	Low DNA yield : may not be suitable for whole-genome amplification Nonrenewable Smaller amplicons

### Specimens for DNA Banking

Specimen Type	Blood cells - Whole blood anticoagulated or blood clots - Buffy coat
DNA Yield	100-100 $\mu$ g/10 ml $\approx$ 200 $\mu$ g/ml
Advantages	Relatively low-cost storage (-80°C) Yield large quantities of high-quality genomic DNA Offers a source for study of exogenous or endogenous compounds other than DNA
Disadvantages	Invasive sample collection Shipping (special requirements) Nonrenewable

### Specimens for DNA Banking

Specimen Type	Transformed lymphocytes
DNA Yield	10 <sup>6</sup> Cells = 6 $\mu$ g 1-2 x 10 <sup>6</sup> Cells = 5-10 $\mu$ g
Advantages	Renewable source of DNA Yields large quantities of high-quality, genomic DNA
Disadvantages	Labor-intensive preparation High-cost storage (liquid nitrogen and periodic reculture) Does not offer a source for study of exogenous or endogenous compounds other than DNA or RNA

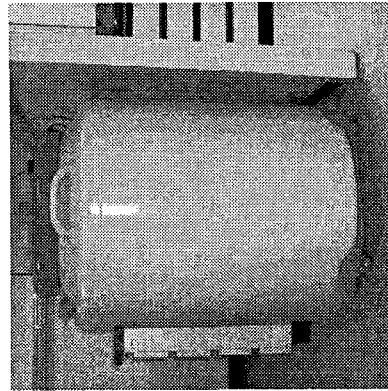
### Specimens for DNA Banking

Specimen Type	Buccal cells
DNA Yield	49.7 $\mu\text{g}$ mean; 0.2-134 $\mu\text{g}$ range (mouthwash, total DNA) 12-60 $\mu\text{g}$ range (mouthwash, total DNA) vs 16-30 $\mu\text{g}$ median; 1-290 $\mu\text{g}$ range (mouthwash, hDNA) 32 $\mu\text{g}$ median; 4-196 $\mu\text{g}$ range (mouthwash, hDNA) 1-1.6 $\mu\text{g}/2$ cytobrushes median; 6 ng-13 $\mu\text{g}$ range (hDNA) 1-2 $\mu\text{g}/2$ cytobrush (total DNA) 1-2 $\mu\text{g}/2$ swab (total DNA)
Advantages	Noninvasive collection Basic of sample collection (allows participant to collect and mail specimen) Genotyping generally requires 10 ng/genotype, and with current technology as little as 2.5 genotypes could theoretically be obtained from a buccal cell specimen
Disadvantages	Low DNA yield: not in general use for whole genome amplification Highly variable yield Does not offer a source for study of exogenous or endogenous compounds other than DNA or RNA Bacterial contamination must be addressed

### Lymphocyte Immortalization

- ◆ 각 개체의 genomic DNA를 필요시 다량으로 추출하기 위하여 lymphocytes를 stable cell line으로 제작한다.
- ◆ 채취된 혈액으로부터 Histopaque 1077 등을 이용하여 lymphocytes를 분리한 뒤 Epstein-Barr virus를 이용하여 immortalization 시킨다.
- ◆ 세포는 EB virus 감염여부를 확인한 후 액화 질소탱크 (-196°C)에 보관한다.

### Liquid Nitrogen Tank



### SNP의 분석

- Screening 방법**
- ◆ polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)
  - ◆ allele-specific PCR (AS-PCR)
  - ◆ TaqMan / Molecular beacons assay
  - ◆ dynamic allele-specific hybridization (DASH)
  - ◆ single base extension, pyrosequencing
  - ◆ oligonucleotide ligation assay (OLA)
  - ◆ matrix assisted laser desorption / ionization time-of-flight (MALDI-TOF)-mass spectrometry
- Scanning 방법**
- ◆ nucleotide sequencing
  - ◆ single strand conformation polymorphism (SSCP)
  - ◆ denaturing high performance liquid chromatography (DHPLC)
  - ◆ denaturing gradient gel electrophoresis (DGGE)
  - ◆ mismatch cleavage

## 기타 시료

## Serum

- ◆ 혈청은 혈액을 SST tube에 채혈한 후 상온에서 30분간 방치하여 혈액을 응고시킨 후 원심분리하여 혈청을 분리한 후 1.5ml tube에 2개씩 분주하여 초저온 냉동고 (-80℃)에 보관한다.

## Urine and Tissue

- ◆ 소변은 소변컵을 이용하여 채취한 뒤 barcode가 붙어있는 15ml centrifuge tube에 분주하여 냉동고 (-20℃)에 보관한다.
- ◆ 조직은 연구방법에 맞게 전처리 후 cryo-vial에 넣어 초저온 냉동고 (-80℃)나 액화질소탱크 (-196℃)에 보관한다.

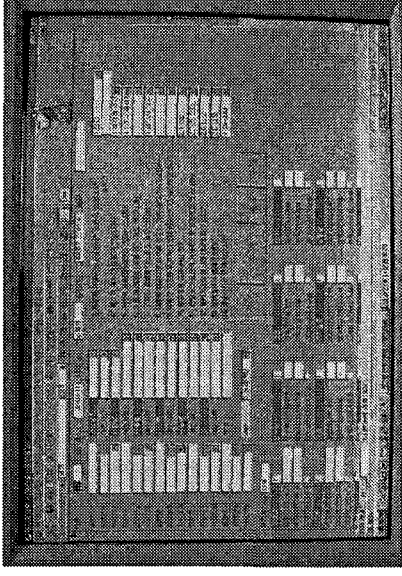
## Data Management



## 자료의 정리 및 보관

- ◆ 유전체 시료 제공자의 기본정보, 가족력, 생활 습관, 환경요인 및 임상정보는 Access 등의 프로그램 이용하여 데이터베이스화하여 보관한다.
- ◆ 데이터베이스용 컴퓨터는 정보 입력 인원만이 접근할 수 있도록 암호화하여 관리한다.
- ◆ 입력된 정보는 일정기간 단위로 백업을 하여 보관하며 입력 완료된 설문지와 동의서는 절멸을 하여 잠금장치가 되어 있는 보관함에 보관한다.

Data Management



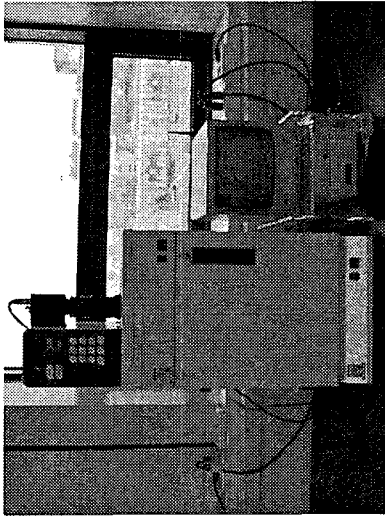
## Laboratory Environment

- ◆ Biomarker Lab
- ◆ Genomics Lab

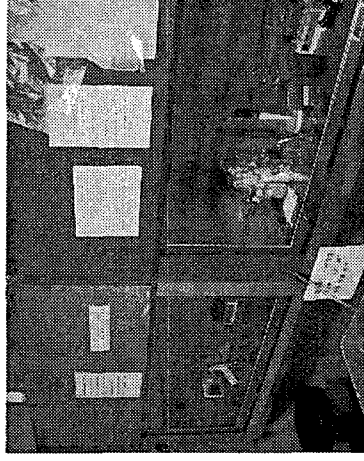
PCR



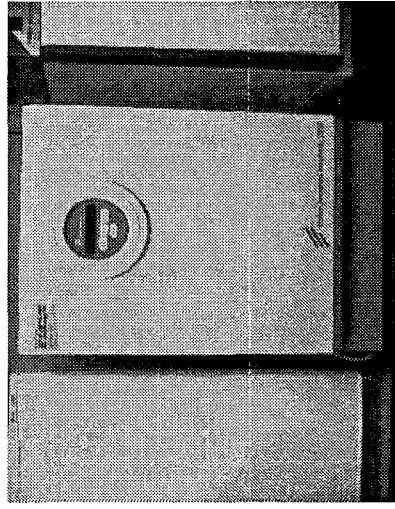
**Image Analyzer**



**Clean Bench**



**CO2 Incubator**



**Cost...**

- ◆ Minimal Genomic Lab
- ◆ Biomarker Lab