



DNA Fingerprinting

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'I don't want this to happen to anyone else'
Cancer-free woman underwent double mastectomy because of lab mix-up

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Lab Error Found

French lab admits they put the wrong number on Floyd Landis' B-sample

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News Front Page Last Updated: Saturday, 19 February, 2005, 10:00 GMT

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Lab error 'led to shattered life'

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Lab Mistakes

Each year, medical lab mistakes occur more than a million times in the U.S., and several thousand of these lab errors result in someone's death.

Although the healthcare system in America is sometimes described as "the best in the world," that's no comfort to a patient whose condition has worsened because of a lab mistake, or to the family members of someone who died due to a lab error.

Many Types of Lab Errors

Having medical tests performed is a very common element of a patient's diagnosis and treatment. Physicians must identify, confirm, and document what is ailing a patient. Lab errors occur when medical testing, images or procedures are mishandled. Examples of lab mistakes include:

- ▶ Images such as magnetic resonance imaging (MRI) and computed tomography (CT) are taken incorrectly
- ▶ A patient's test sample or paperwork is mixed up with that of another patient
- ▶ Specimens or samples are taken incorrectly
- ▶ Images or test samples are mishandled or lost
- ▶ Results are misinterpreted
- ▶ Patient identification errors are made

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Agenda

- Case presentation
- Molecular identity testing
- Risk management

Case Presentation

- 64 year old man with prostate biopsy
 - Carcinoma identified

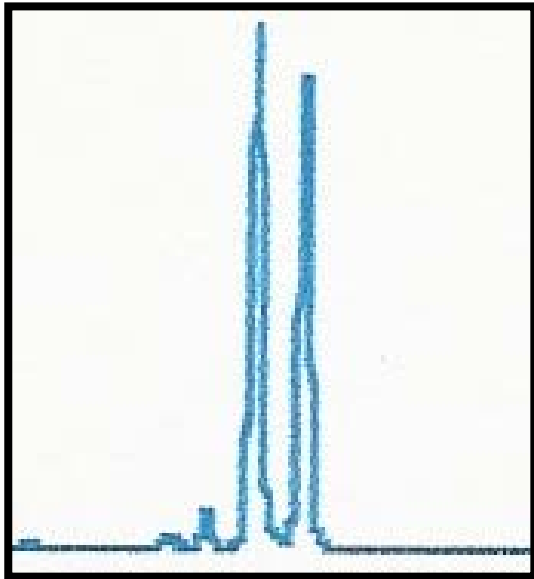
Case Presentation

- Prostatectomy
 - Benign

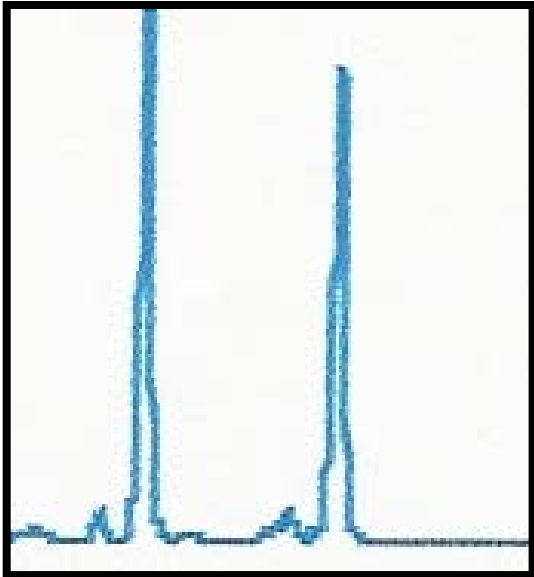
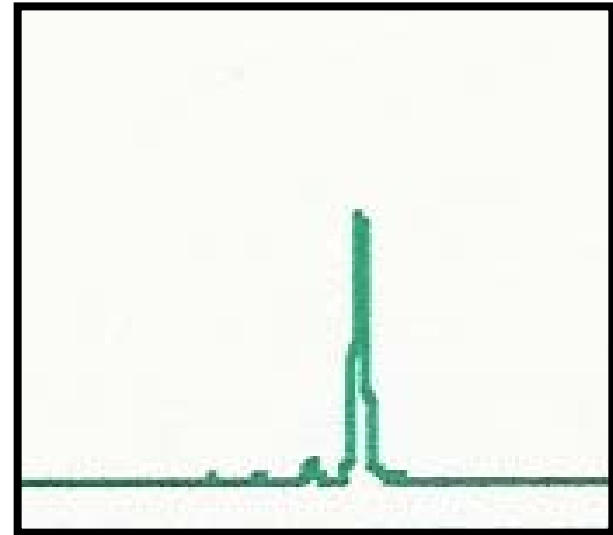
Case Presentation

Question:

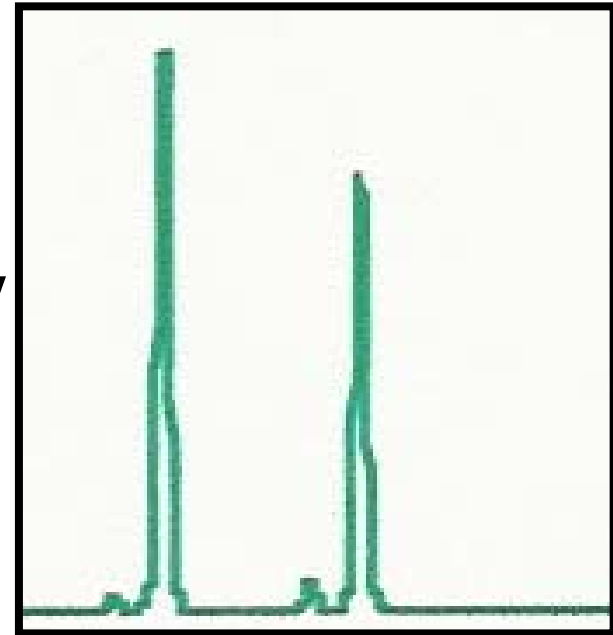
Did the sample with cancer come from
this patient?



**Prostate
Biopsy**



**Prostatectomy
Specimen**



Molecular Approaches to Identification of Tissue Contamination in Surgical Pathology Sections

Maria J. Worsham,* Sandra R. Wolman,[†] and Richard J. Zarbo*

From the Department of Pathology, Henry Ford Hospital, Detroit, Michigan; and the Uniformed Services University of the Health Sciences,[†] F. Edward Hebert School of Medicine, Bethesda, Maryland*

review at the time of case sign-out. However, frequency rose to 2.9% (range, 0–8.8%) when slid

A Microdissection and Molecular Genotyping Assay to Confirm the Identity of Tissue Floaters in Paraffin-Embedded Tissue Blocks

Jennifer L. Hunt, MD; Patricia Swalsky, BS; E. Sasatomi, MD, PhD; Laura Niehouse, BS; Anke Bakker, BS; Sydney D. Finkelstein, MD

Human Pathology (2007) 38, 378–382

Diagnosis of ectopic tissue versus contamination by genetic fingerprinting in a routine surgical pathology specimen[☆]

Marcello Venditti MD^a, Robert W. Hay MSc^e, Andrzej Kulaga MD, FRCPC^{b,e}, Douglas James Demetrick PhD, MD, FRCPC, FCAP^{b,c,d,e,*}

Molecular DNA Identity Testing

- Polymorphisms
- Testing
- Other applications

DNA Polymorphisms

- Define identity
 - Association with phenotype
- Define risk for disease
 - APO-E genotype
 - Alzheimer's disease
 - Cardiovascular disease
- Complex relationships

DNA Polymorphism Inheritance

- Polymorphisms are inherited
- 1 every 1000 basepairs
- Different types

Types of Polymorphism

- Single nucleotide polymorphisms (SNPs)
- Short tandem repeats (STRs)
 - Microsatellites
- Variable nucleotide tandem repeats (VNTRs)

DNA Polymorphisms

- Short tandem repeats

- 2 to 7 basepairs in length

- Dinucleotide, Trinucleotide, Tetranucleotide...

ATCG

- Repeated a variable number of times

ATCG ATCG ATCG ATCG ATCG ATCG

DNA Polymorphisms

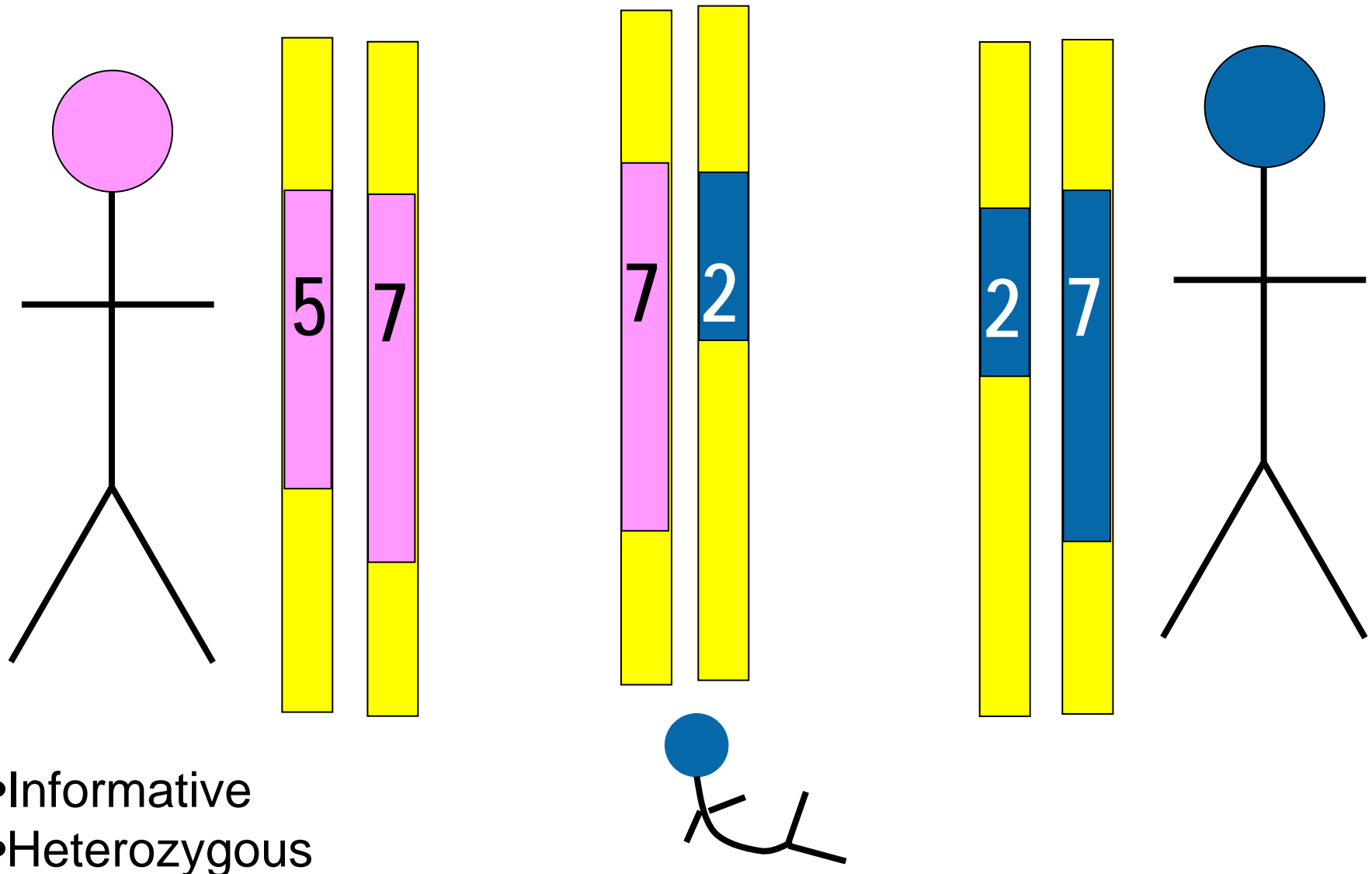
Allele 1



Allele 2

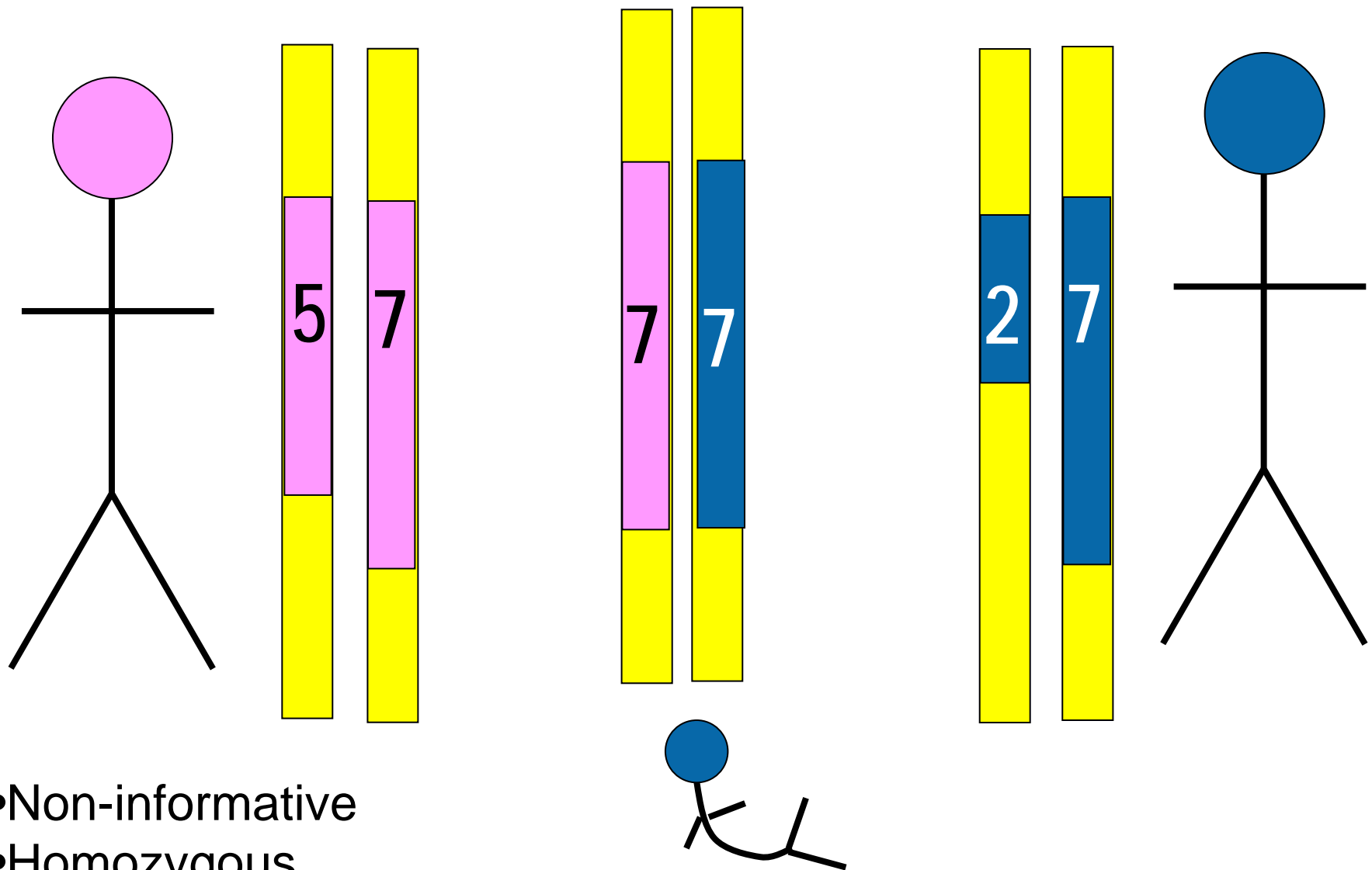


DNA Polymorphisms



- Informative
- Heterozygous

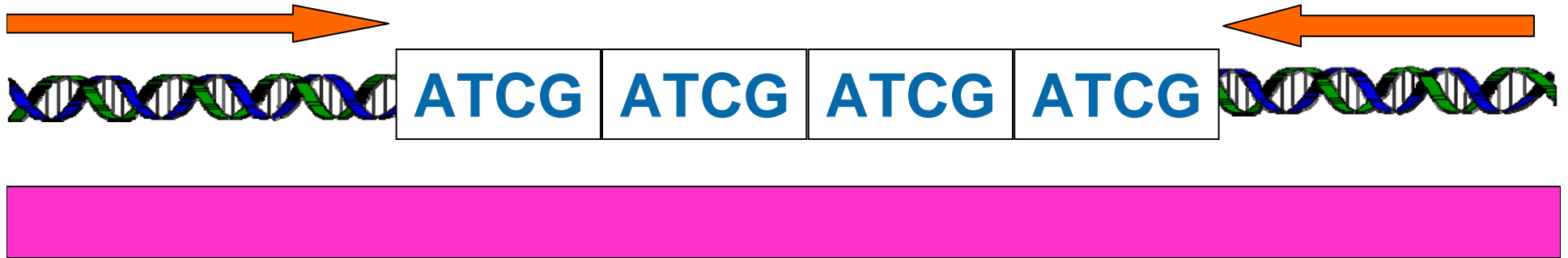
DNA Polymorphisms



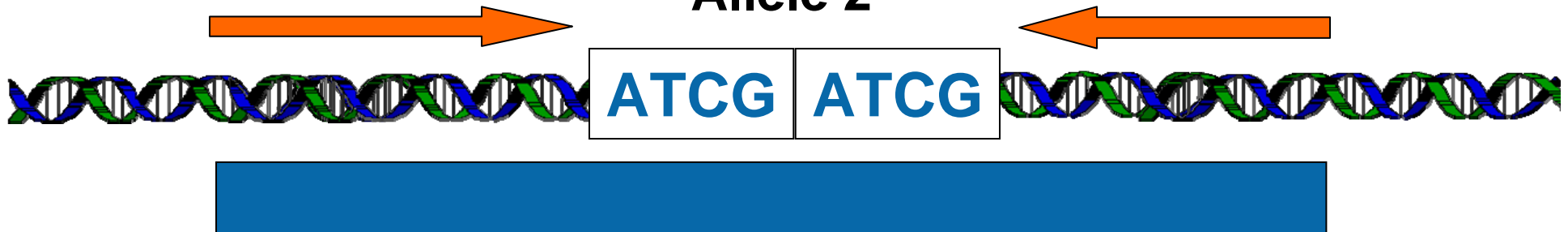
- Non-informative
- Homozygous

PCR Short Tandem Repeats

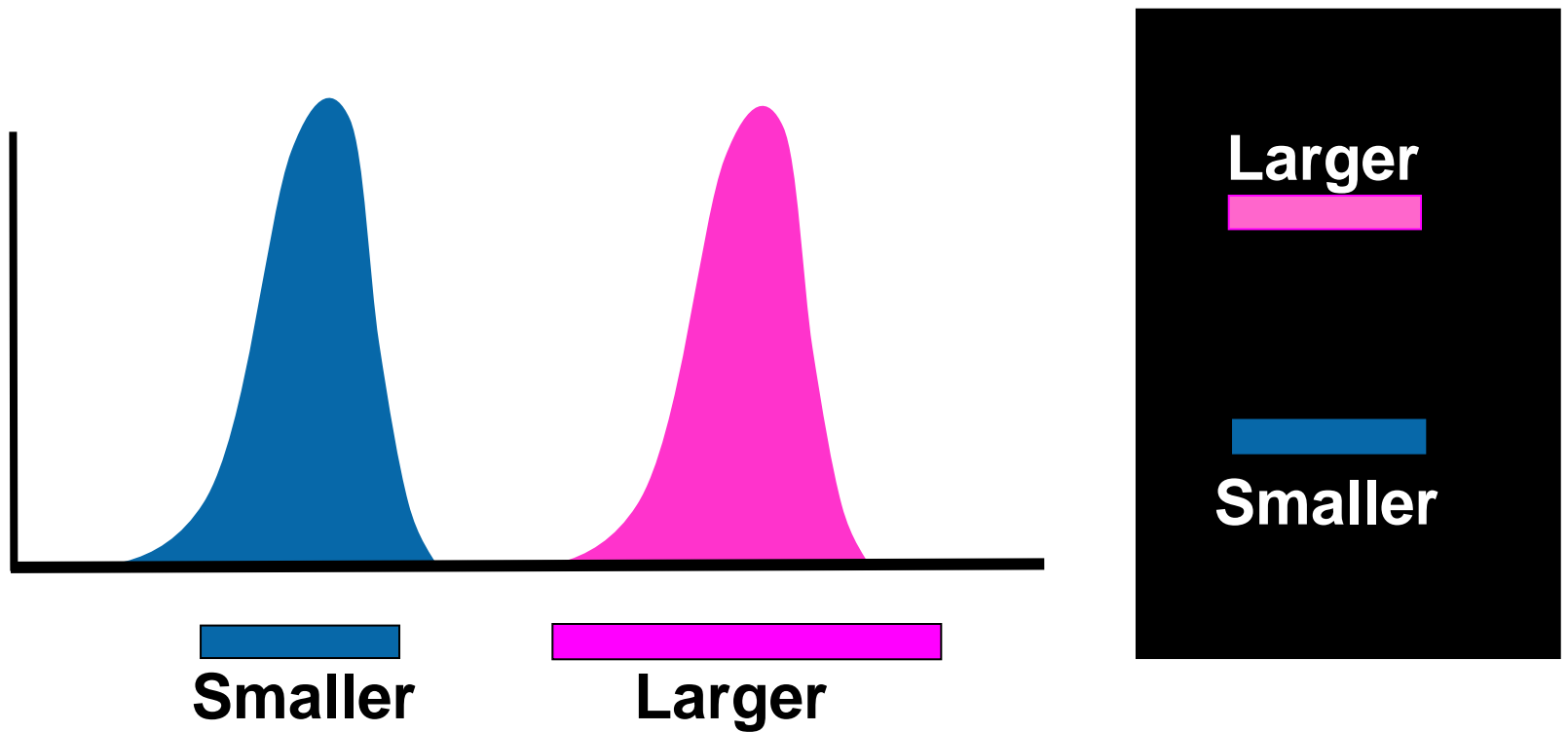
Allele 1



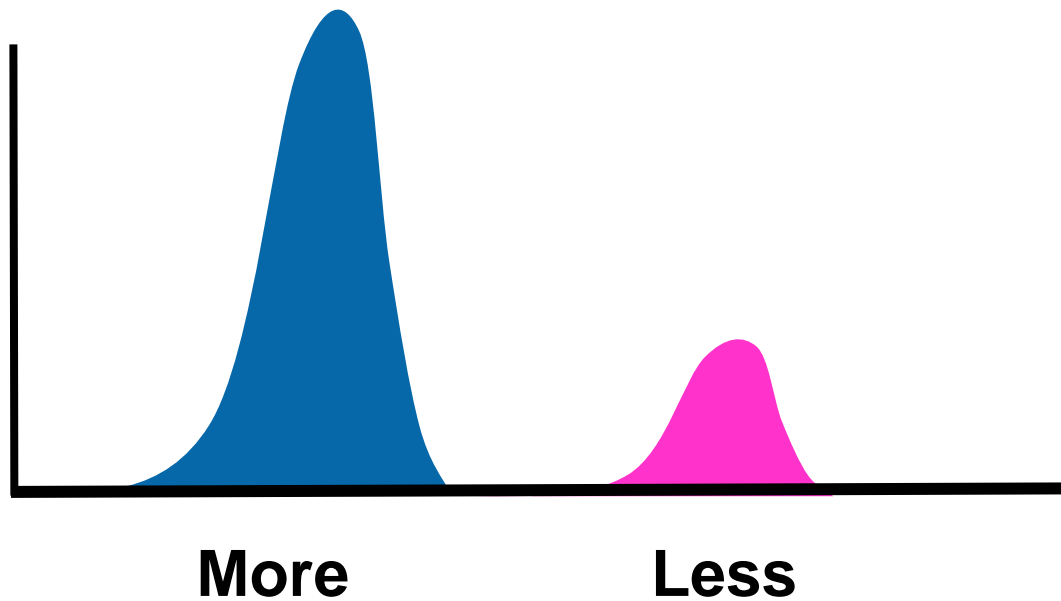
Allele 2



PCR Short Tandem Repeats



Amount of PCR Product



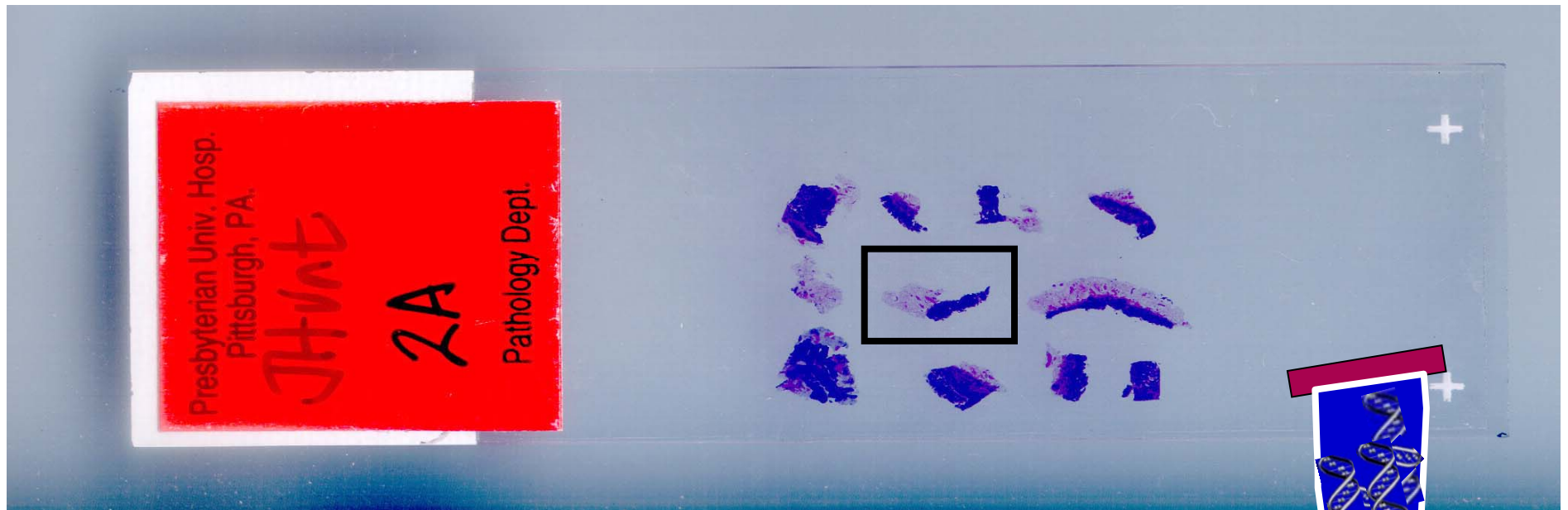
Lab DNA Fingerprinting

- Microdissect specimen
 - Floater, whole sections, biopsies, FNA cells
- Extract DNA
- Perform PCR
- Analyze PCR amplicon
- Compare samples

Microdissection

- By hand
 - Microscope
 - Implement
- Laser capture

Microdissection

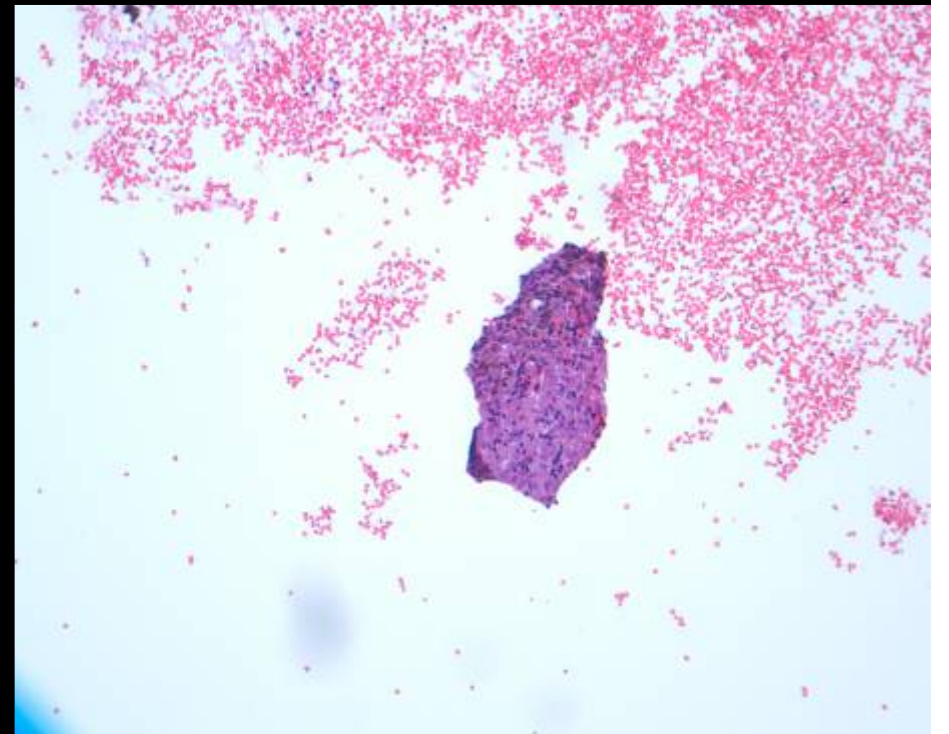
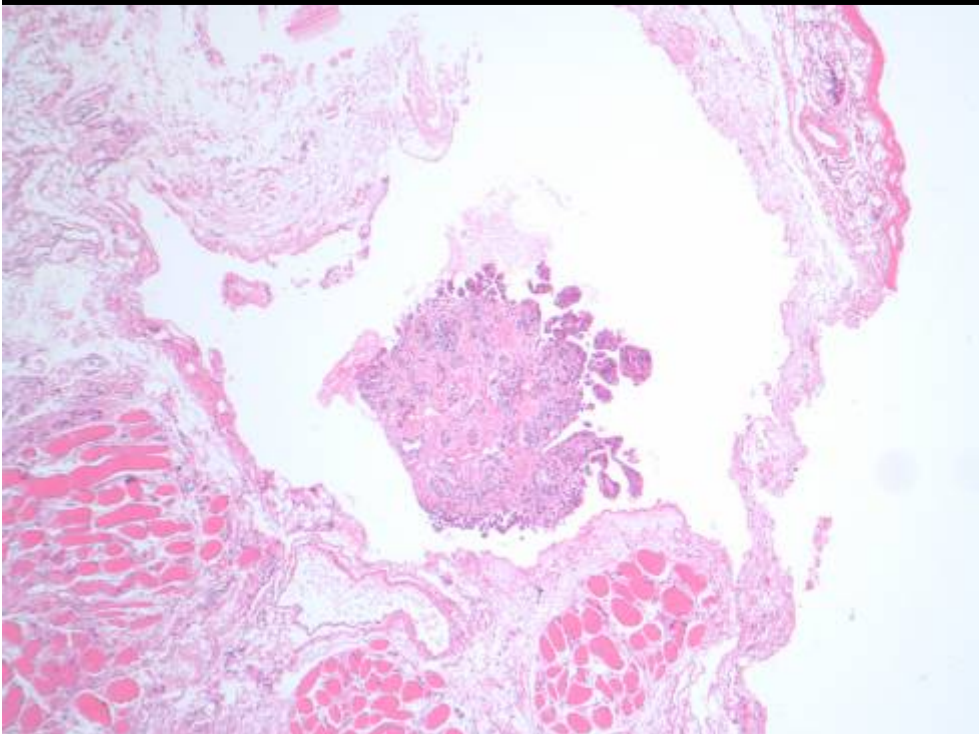
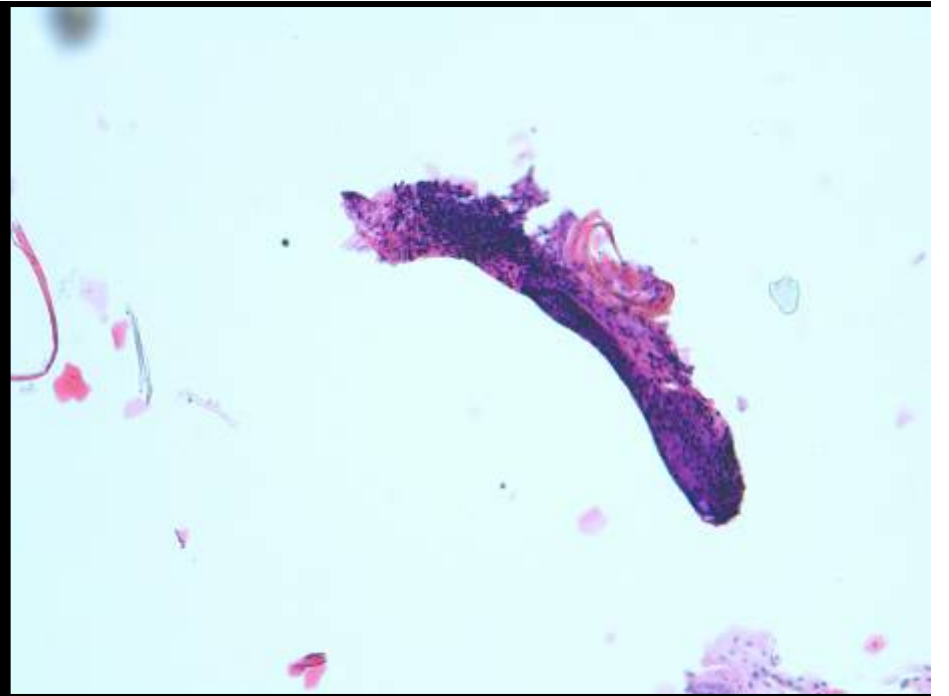
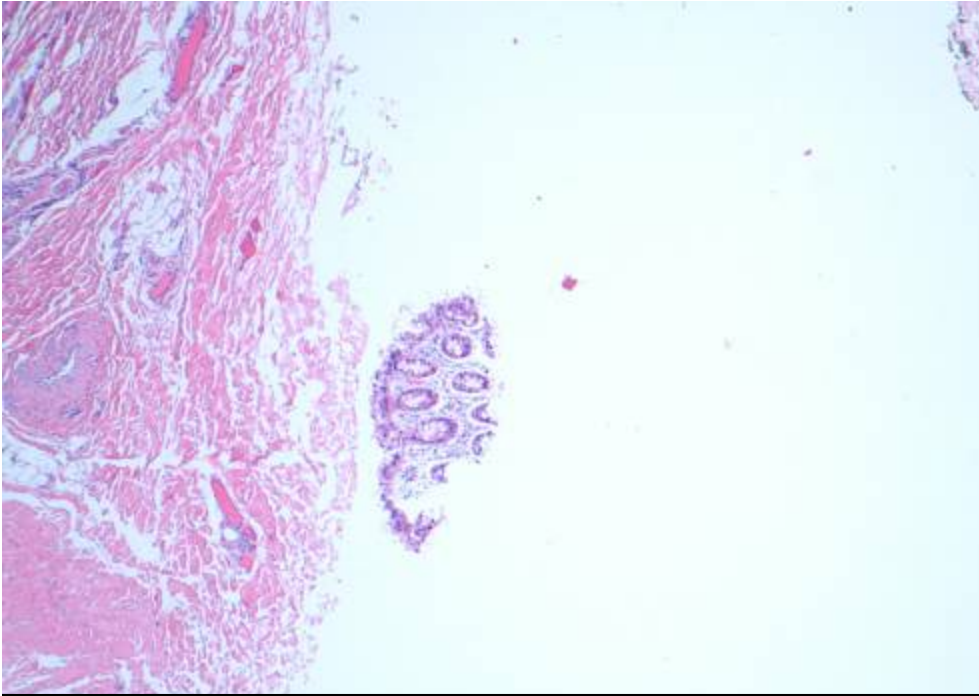


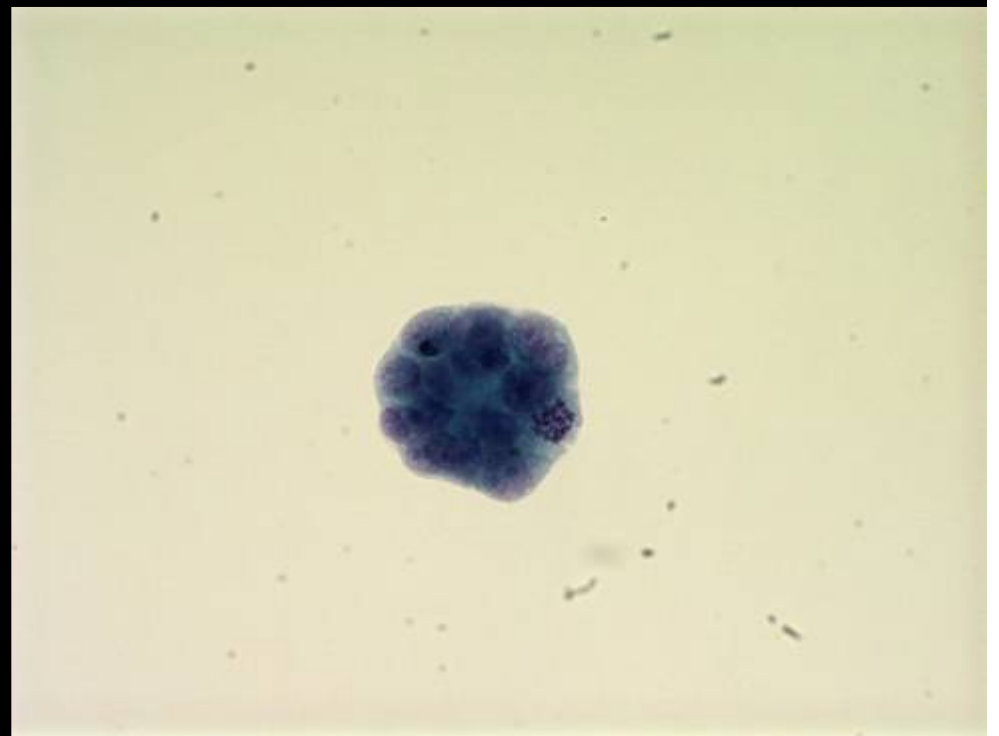
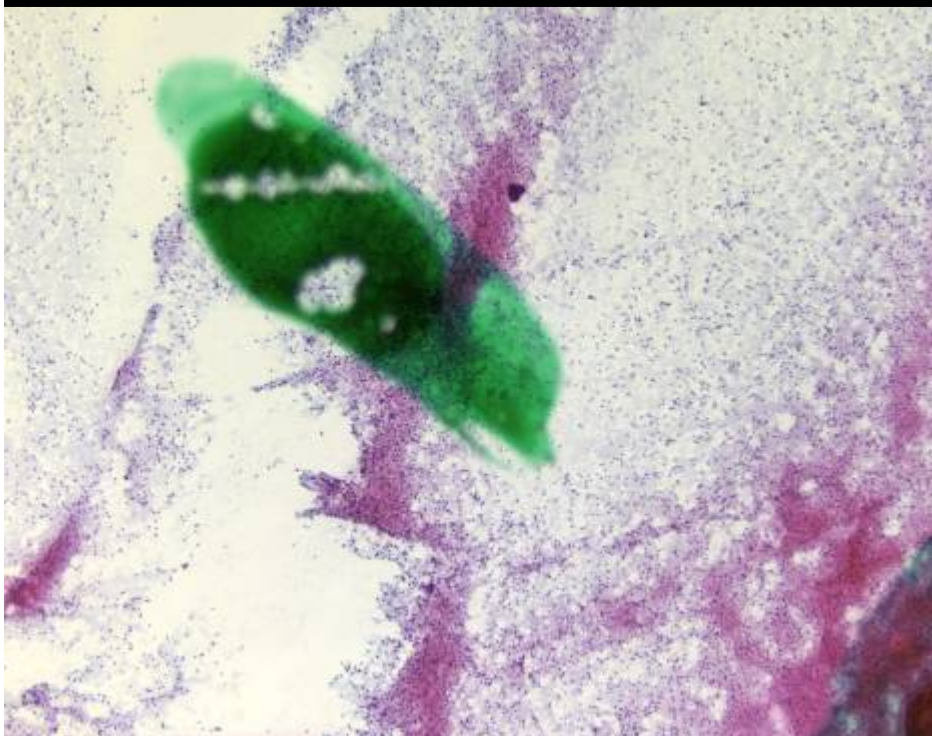
Implement



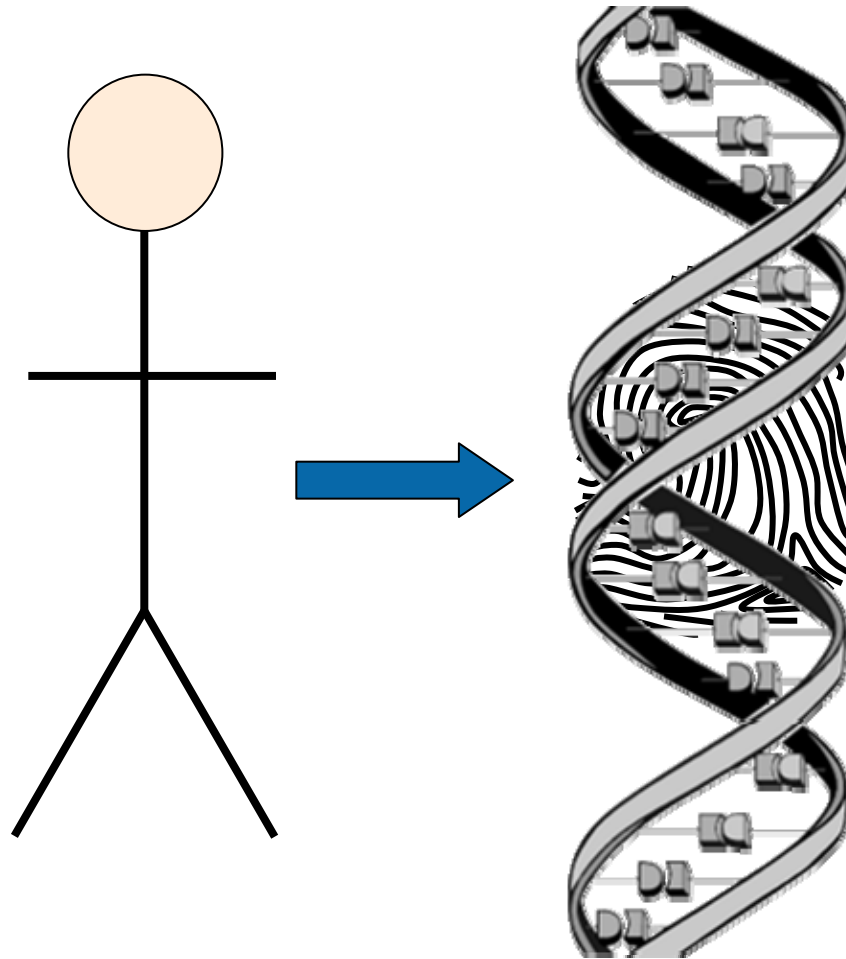
Microscope







Identity Testing



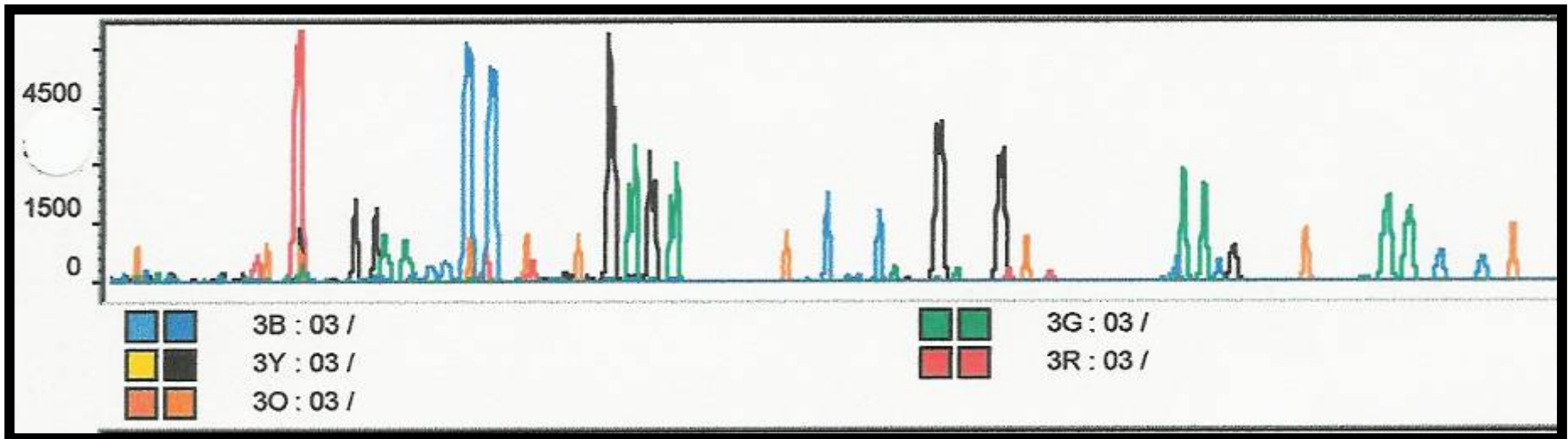
Identity testing

- Also known as...
 - DNA Profile
 - Genotype
 - DNA Fingerprint

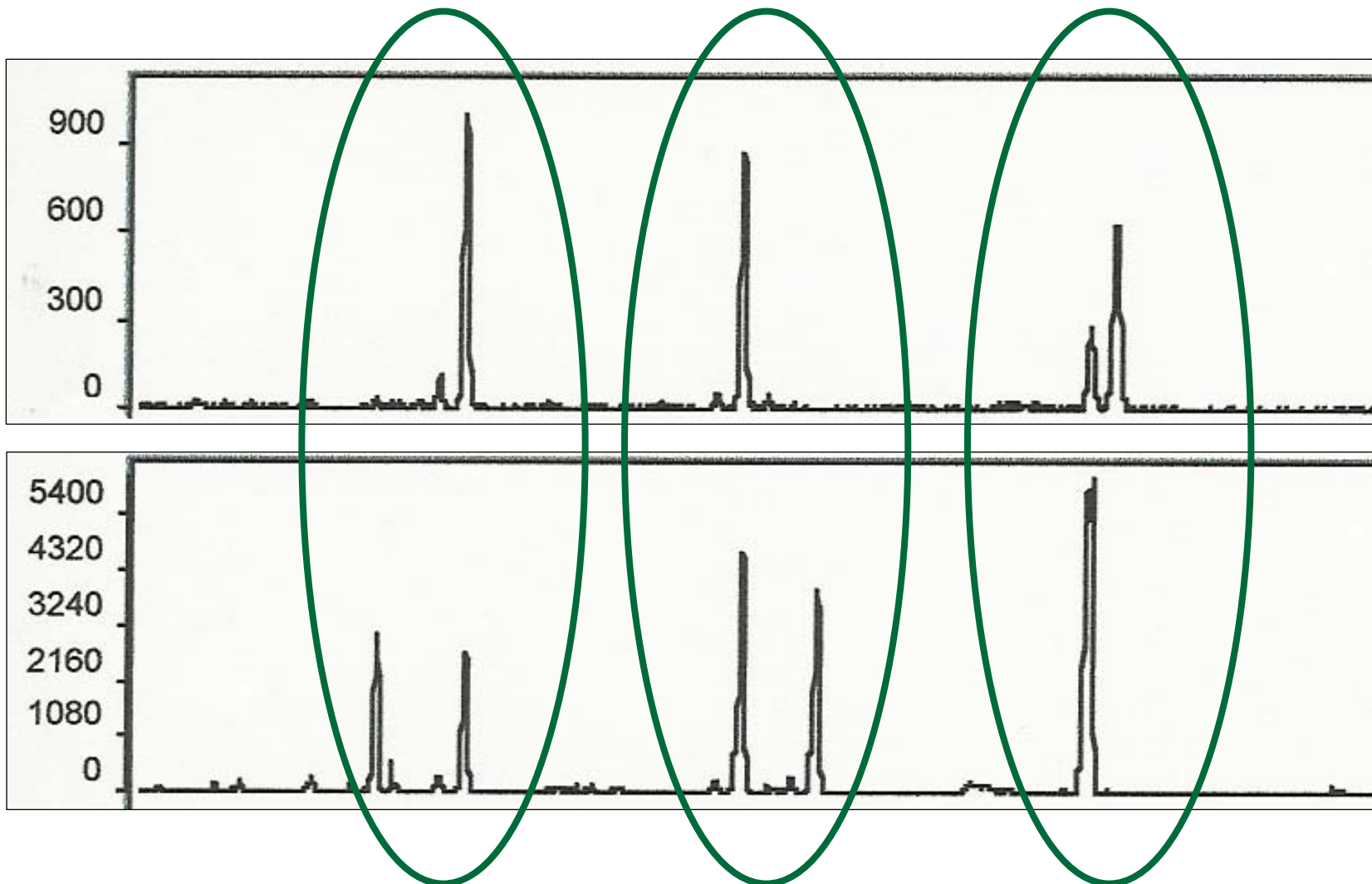
DNA Fingerprinting Kits

- Multiplex assays
 - Multiple primers all in one tube
 - Assess short tandem repeats
 - Assess (X, Y) status
- Capillary electrophoresis analysis

Multiplex PCR Product Analysis



Comparing Two Samples



Other Applications

- Floaters and contaminants
- Gestational trophoblastic disease
- Transplant
 - Tumors
 - Chimerism
- Paternity testing
- Forensic testing

Contaminants and Floaters

Contaminants present	.6 to 3% of slides
Malignant contaminants	6 to 13% of slides

Gephardt GN, Arch Pathol Lab Med. 120(11):1009, 1996

Contaminants and Floaters

Contaminant on slide only	59 to 73%
Contaminant in block	16 to 28%

Gephardt GN, Arch Pathol Lab Med. 120(11):1009, 1996

Contaminants and Floaters

Severe diagnostic difficulty	<ul style="list-style-type: none">• 0.1 to 0.4% of slides
Molecular resolution	<ul style="list-style-type: none">• 21 cases (100%)• 16 (76%) discordant

Hunt JL, Arch Pathol Lab Med. 127(2);213, 2003

Gephardt GN, Arch Pathol Lab Med. 120(11):1009, 1996

Impact

- Floaters & contaminants are common
- Many are malignant
- Some create diagnostic difficulties
- Can be resolved using molecular

When To Test

- Floaters
 - Malignant or change in diagnosis
- Question of specimen mix-up
- Benign resection & malignant biopsy

Guidelines

- Don't cut into blocks
 - Tissue needed!
- Talk to your clinicians
- Talk to risk management or legal
- Look around the lab
 - Potential sources
 - Unusual diagnoses



Thank
YOU

CAP Companion Meeting at USCAP 2008
Value Added Diagnostics: Emerging Tests in Clinical Practice
Specimen Mix-ups and Floater Identification

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DNA Fingerprinting and Identity Testing

DNA Fingerprinting

In the past, immunohistochemical stains were used with some variable success, to assess for the ABO blood group antigens or HLA antigens on tissue fragments that were suspected of being floaters [1, 2]. However, because biopsies can be small and can exhibit edge artifact, the staining patterns can be difficult to interpret. Recently molecular techniques have been detailed that can reliably differentiate tissues from different patients based upon the DNA fingerprinting [3-7].

In the DNA fingerprinting test, two samples are compared: a sample that is known to be from the patient (either a blood sample, or another confirmed tissue sample) and the specimen of. If the sample of interest is a floater or a carry-over contaminant, the fragments are microdissected from the glass slides. If the tissue is all suspected as being a mixed-up sample, less exact microdissection is needed. DNA is extracted from the tissue fragments and PCR is performed for a set of polymorphic markers.

The polymorphisms that are tested for in typical identify testing assays are called short tandem repeats. These are short sequences of DNA, ranging between 1 and 5 basepairs in length that are repeated for a variable number of times. The number of the repeats is what is polymorphic, since most people will harbor two alleles with different numbers of repeats. These are inherited, and thus the alleles can be traced back to the father's and mother's alleles. In the identity test, we usually use tetranucleotide repeats (4 basepair sequence that is repeated), but there are also mononucleotide, dinucleotide, trinucleotide, and even pentanucleotide repeats.

Most laboratories use kits that are pre-formulated to contain a large set of known robust polymorphic markers [8]. In these kits, multiple PCR primers are multiplexed so that all of the primers are in one tube and can be run with one PCR reaction. This type of complex multiplexing is difficult and requires substantial quality control and validation. However, the interpretation of the resulting genotype, or fingerprint, is usually fairly straightforward. Because the markers are highly polymorph in the general population, it is highly likely that each unique patient will have a different DNA profile at these markers. If the genotypes match at multiple loci, it is statistically highly likely that they came from the same patient. If they do not match, however, the specimens definitely did not come from the same patient.

This type of assay can also be used to identify specimen floaters or carryover artifacts in paraffin blocks. In cases of identity testing in paraffin blocks, it is extremely useful to be able to test another sample from the patient that is known to contain genomic DNA (such as blood, or another biopsy sampling from another time) and to also test the tissue from which the putative floater is suspected of being derived. In busy anatomic pathology practices, it may be difficult to determine which case a floater arose from. But, when possible, this adds an additional confirmatory layer to molecular testing for carry-over artifact or floater contamination.

Specimen Mix-ups and Floater Identification

Jennifer L. Hunt, MD

Many different types of samples are amenable to this type of molecular testing. It can be performed on recut unstained slides, which are the most successful source of tissue [9]. Even single fragments on stained H&E slides, however, yield fairly good results with this assay. The smaller the fragment, the more likely it is to be difficult to amplify.

Other anatomic pathology applications of DNA fingerprinting

DNA fingerprinting is an extremely robust and powerful molecular technique. These assays have gained public awareness as they are commonly used in forensic pathology, for identification of remains and for linking suspects with crimes based upon the presence of body fluids or residual cells. Although the panel of polymorphic markers that are used in forensic testing varies from that used clinically [10], the techniques and interpretation are essentially identical.

The technique of DNA fingerprinting also has another very valuable application in the arena of transplant pathology. In bone marrow transplantation, the percentage of donor and recipient cells, either in peripheral blood or in the bone marrow sampling, can be ascertained in a semi-quantitative manner, using capillary electrophoresis [11]. When the recipient and the donor are related, as is often the case, multiple polymorphic markers may have to be tested in order to find informative alleles, as related individuals will be expected to share genotypes at more genetic loci than those who are not genetically related.

The DNA fingerprinting assay can also be useful in very rare cases in which a transplant patient develops a carcinoma that is suspected of being of donor origin [12, 13]. Genotyping of the tumor can prove that it originated from the recipient or the donor cells. The test can also be used to identify a complete molar pregnancy, based on the presence of paternal-only alleles [14, 15]

Specimen Mix-ups and Floater Identification

Jennifer L. Hunt, MD

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