Kazan (Volga region) Federal University
Department of Morphology and General Pathology

Lecture 2

Topic: Molecular-genetic methods in clinical practice

R.V.Deev
M.O.Mavlikeev

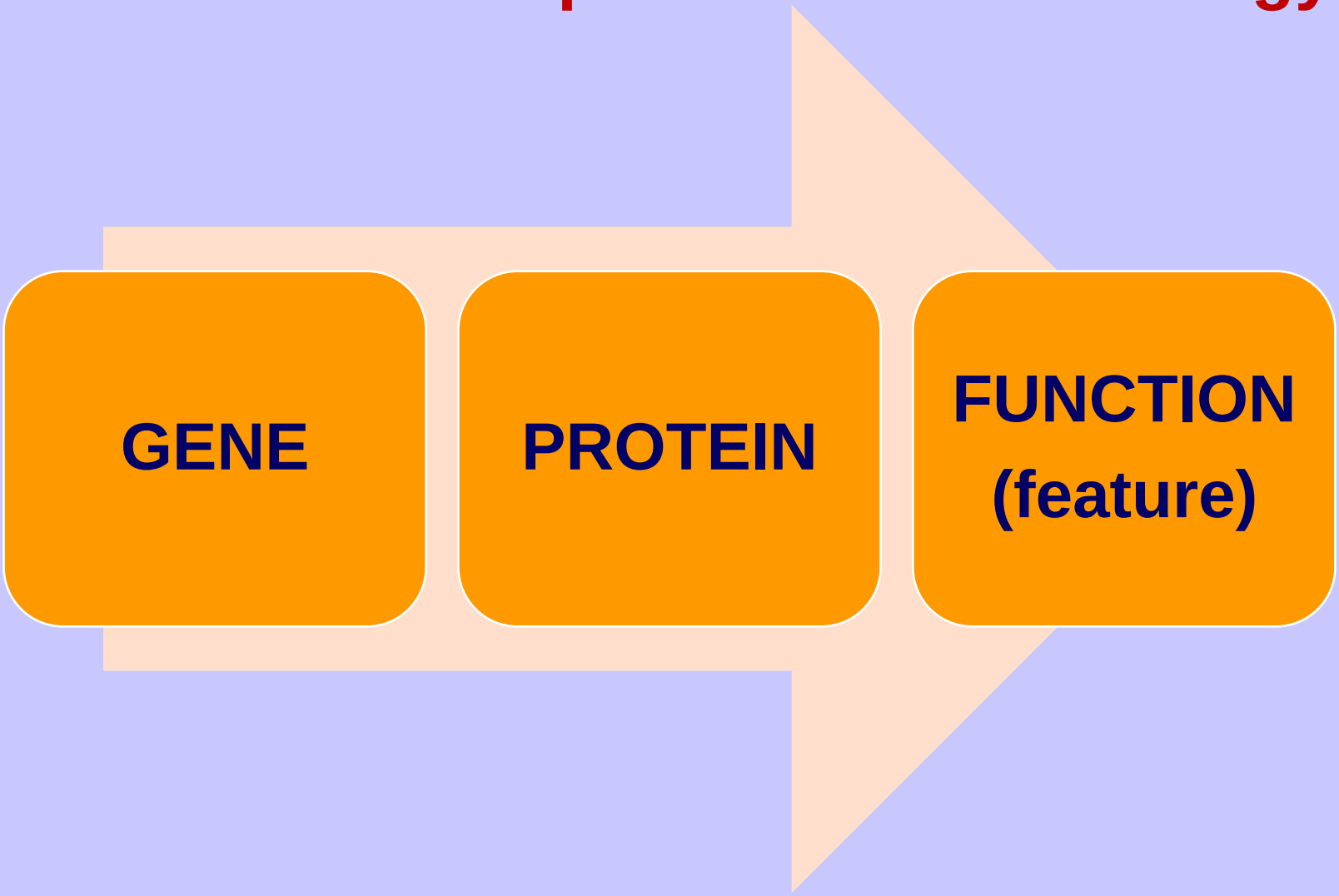
Kazan, 2015

Plan

- 1. Site of molecular genetic diagnostics in modern clinical practice.
- 2. Karyotyping
- 3. FISH-diagnostics
- 4. Polymerase chain reaction
- 5. Sequencing

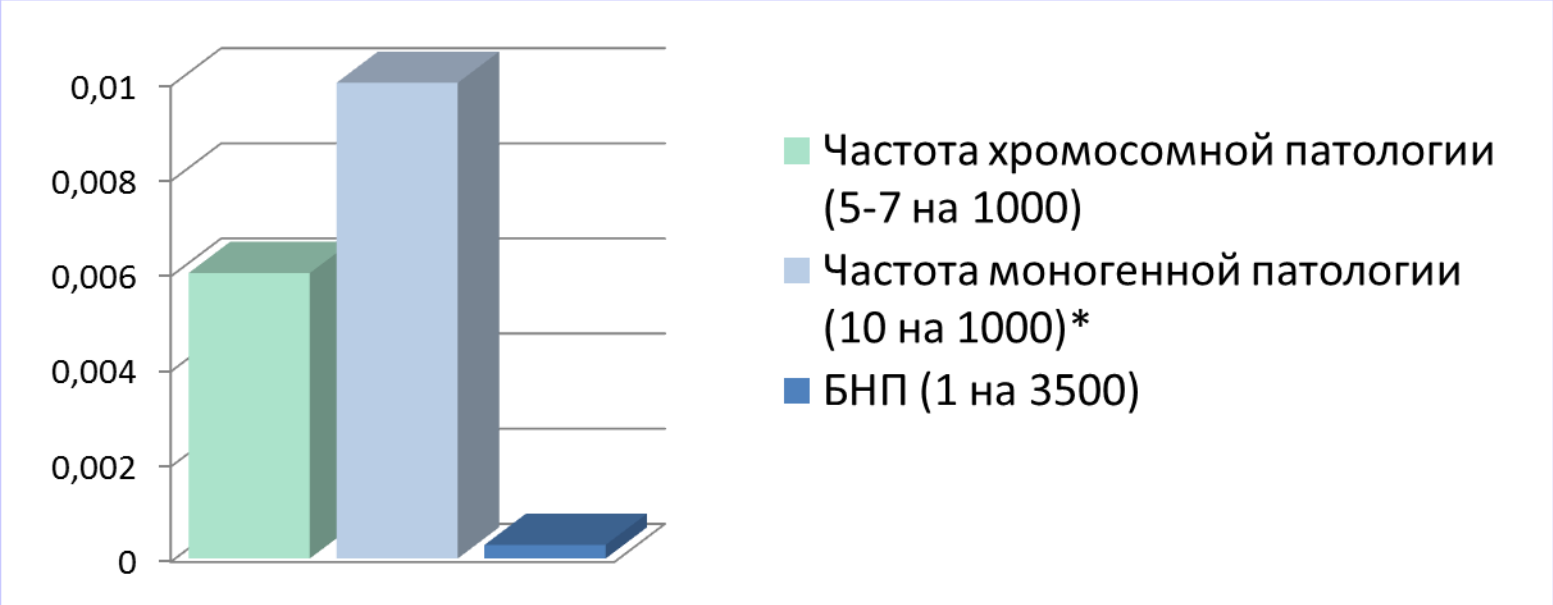
В лекции использованы слайды:
- А.С. Пушкина,
- С.Н. Бардакова,
- Е.А. Померанцевой,
- К.Г. Шевченко

The basic concept in molecular biology



Место наследственной и врождённой патологии в структуре заболеваемости населения

→ Частота врождённых аномалий - 3-4 на 100 новорождённых.



Каждый ЗДОРОВЫЙ человек является НОСИТЕЛЕМ 5-7 патологических мутаций

Частота моногенных заболеваний – 1/100

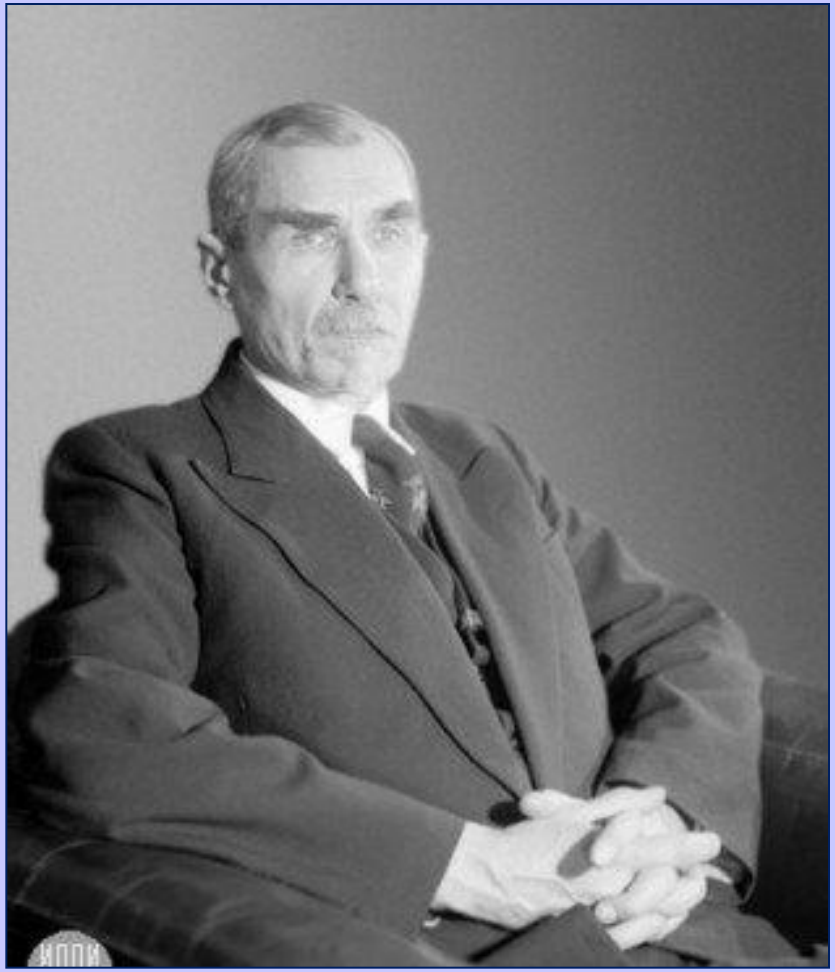
Частота синдрома Дауна – 1/700

*Community genetics services: report of a WHO consultation on community genetics in low- and middle-income countries. World Health Organization. 2011. ISBN 978 92 4 150114 9

Karyotype

- **Karyotype -** a set of attributes (number, size, shape etc.) **of a complete set of chromosomes of cells** of this particular species (species karyotype), of the organism (individual karyotype) or cell line (clone).
- Sometimes karyotype is called a visual representation of the full chromosome set (karyogram).

Karyotype



1878 - 1942

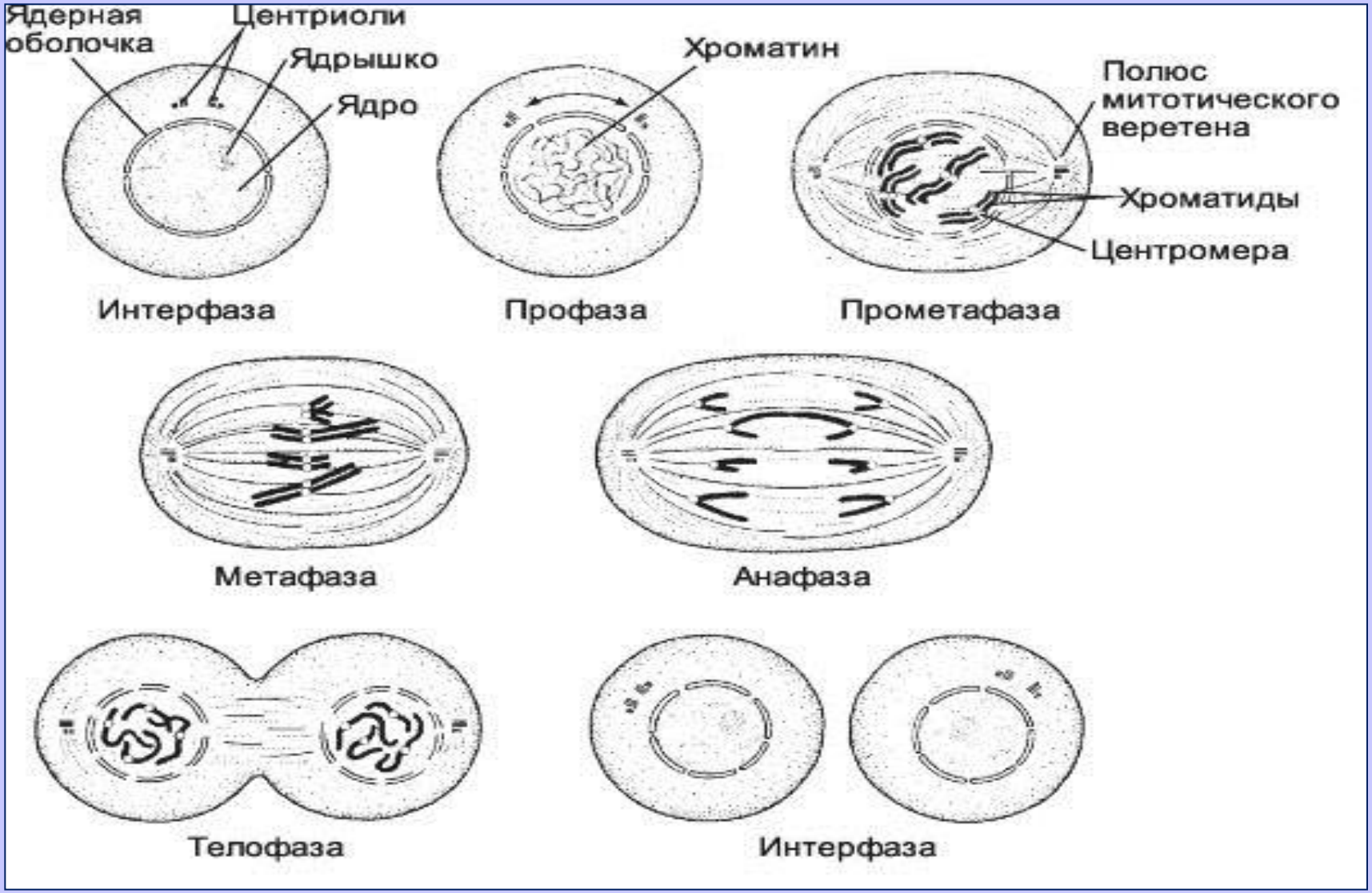
The term "karyotype" was introduced in 1924 by the Soviet cytologist Gregory A. Levitsky

Кариотип — статья из Большой советской энциклопедии (3-е издание)

Basic methods

- classical cytogenetic analysis (karyotyping);
- fluorescence in situ hybridization (FISH);
- chromogenic in situ hybridization (CISH);
- Polymerase chain reaction (PCR);
- Southern blotting;
- analysis of the primary sequence of DNA (sequencing);
- microchipping.

Karyotyping

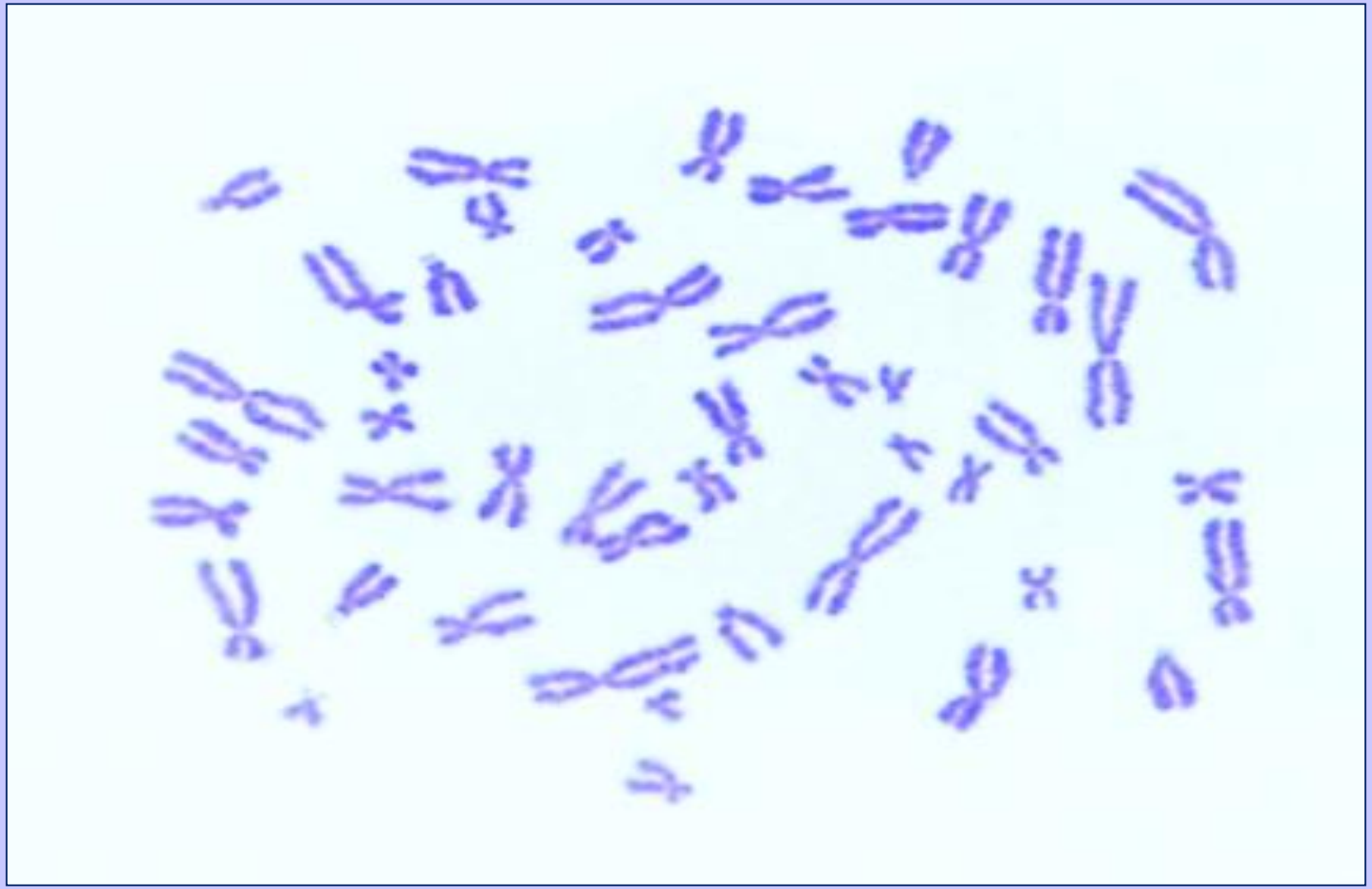


Улумбеков Г.Э. Гистология, эмбриология, цитология, 2007

The procedure for determination of the karyotype

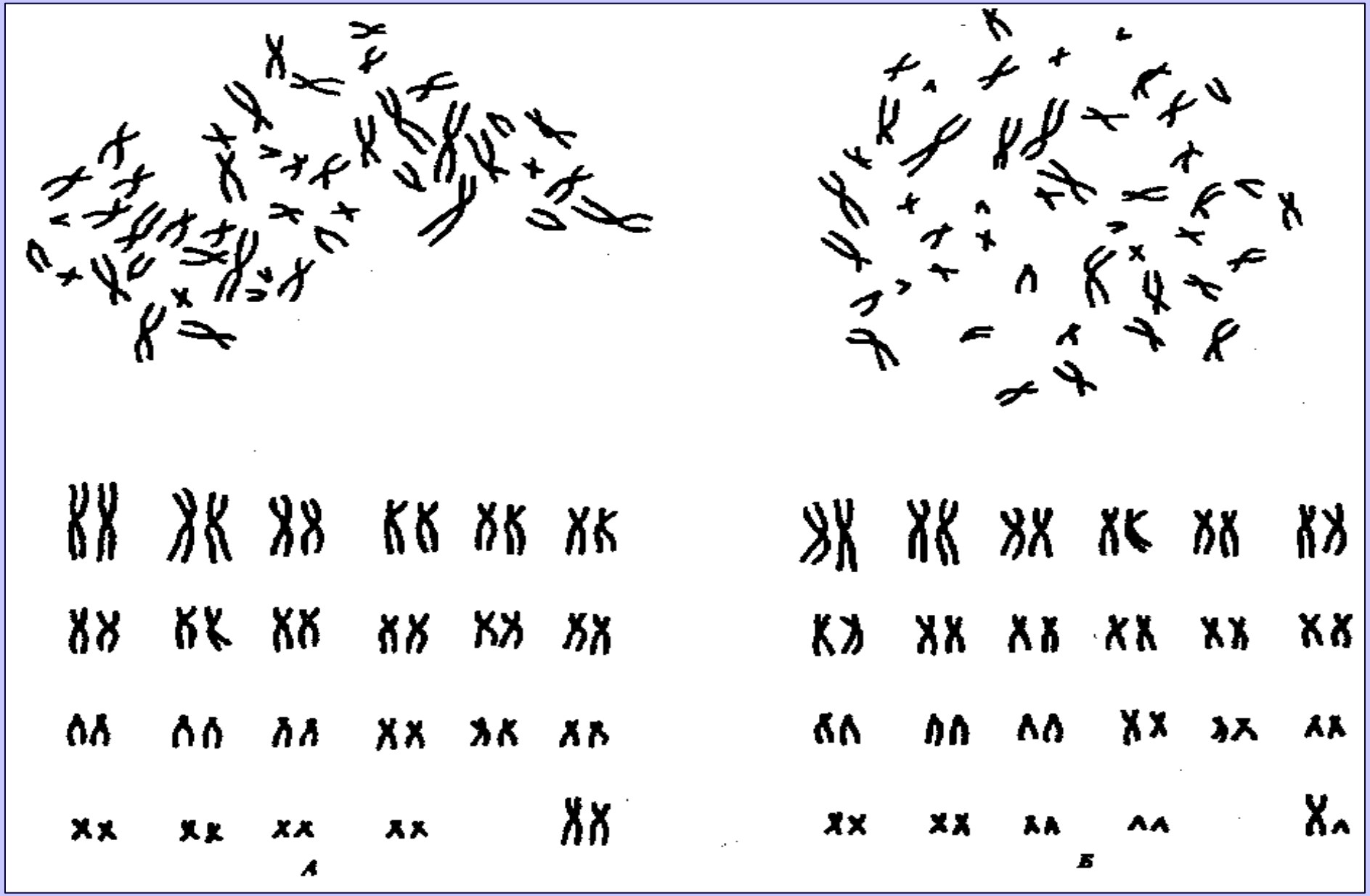
Бочков Н. П. Клиническая генетика, 2011

Non-detailed karyotype
(staining by Romanovsky-Giemsa)

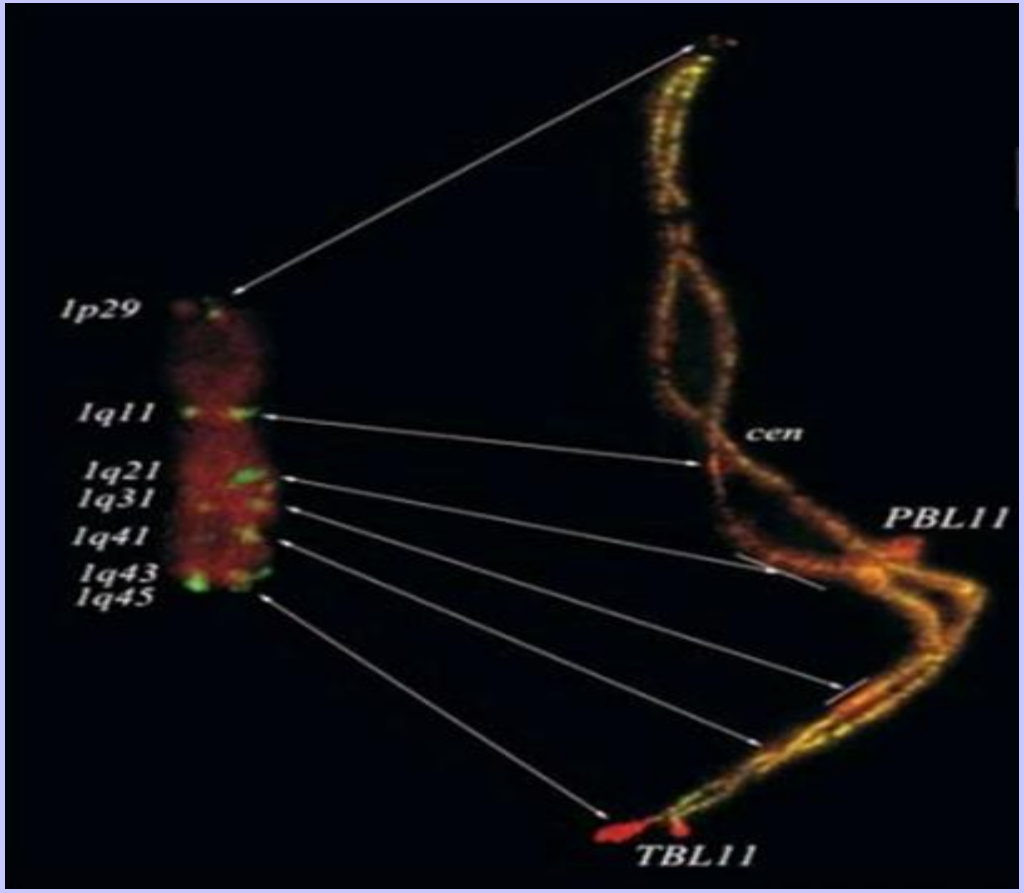


http://131.229.88.77/microscopy/home.html

Normal karyotype



Types of staining

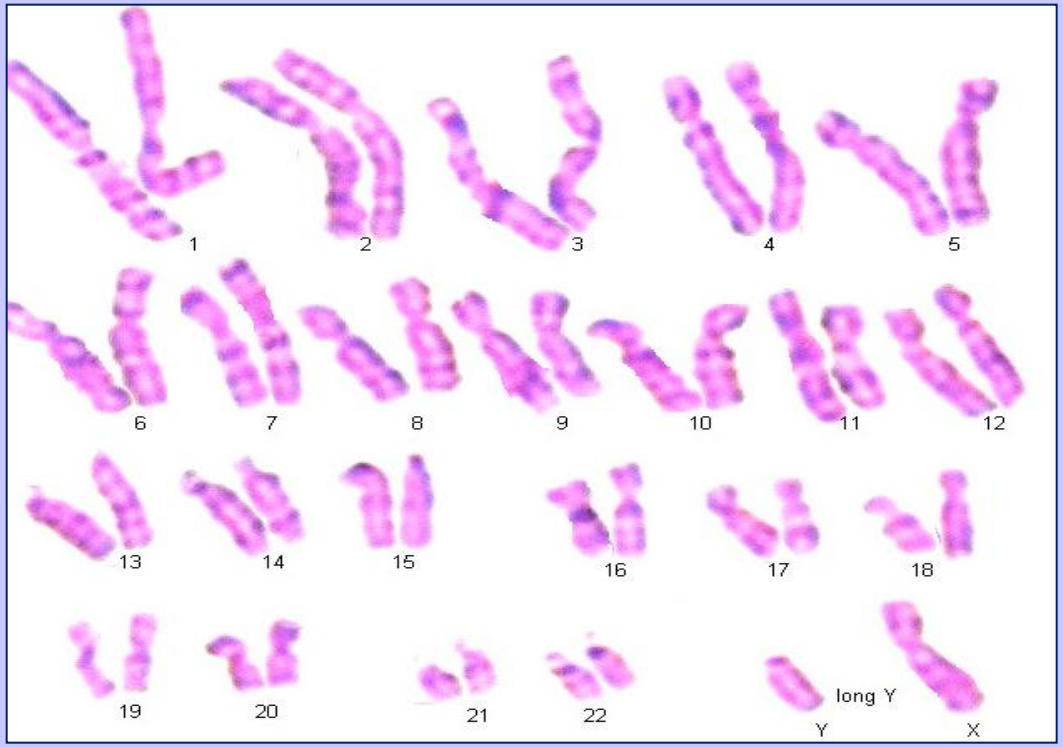


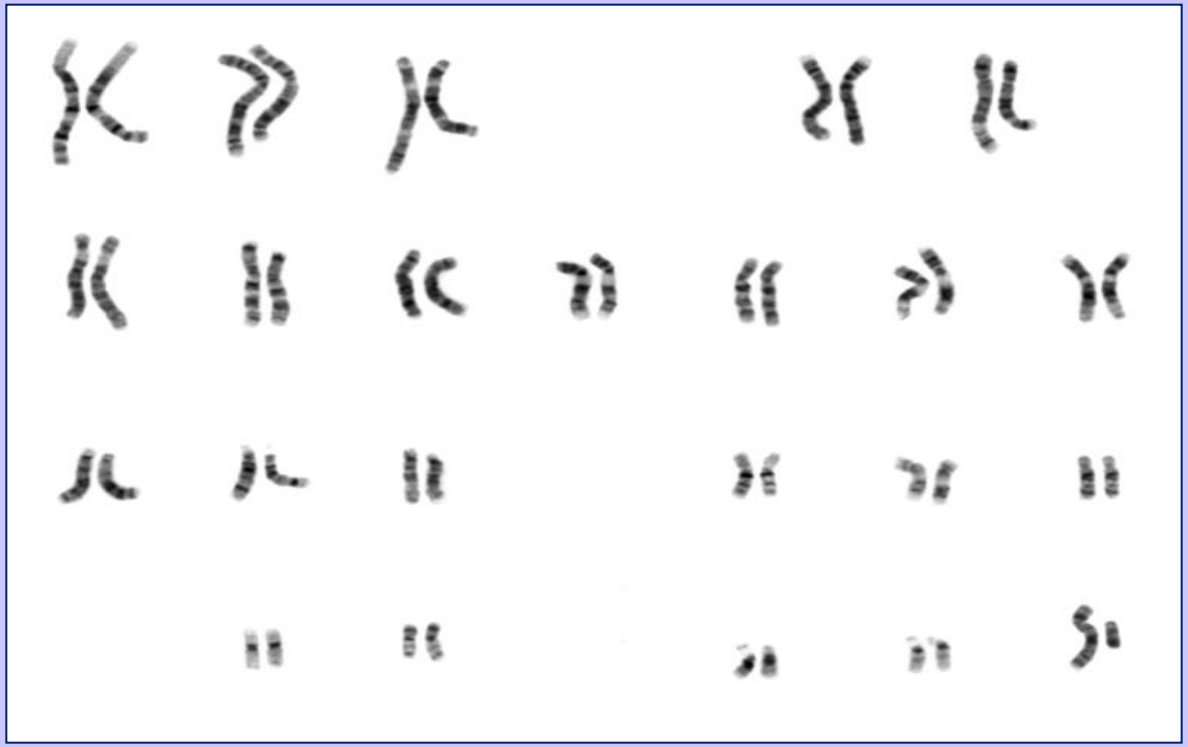
Q-banding

http://www.authorstream.com/Presentation/chhabra61-439811-chromosome-banding-techniques/

- Q-staining - staining by Kaspersson with acrikhin-ipritis with the investigation under a fluorescence microscope. Most often used for the study of Y-chromosome (rapid determination of genetic sex, identification of translocations between the X- and Y-chromosomes, or between a Y-chromosome and autosomes, screening mosaicism involving Y-chromosome)

Types of staining





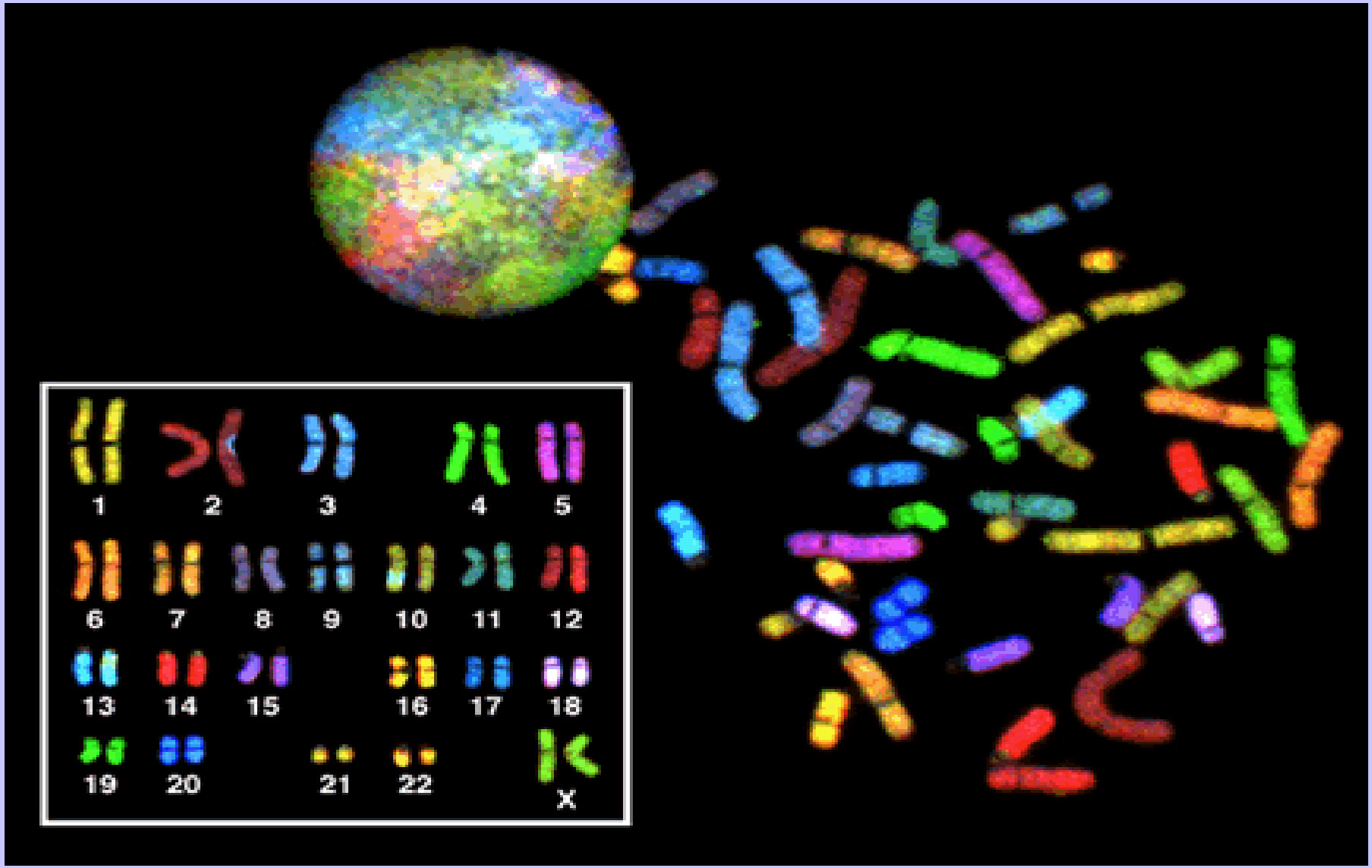
http://students.iitk.ac.in/projects/brain_karyotyping

G-staining - modified staining by Romanovsky - Giemsa. The sensitivity is higher than that of Q-staining, therefore it is used as a standard method for cytogenetic analysis. It is used for the detection of small aberration and marker chromosomes (segmented differently than normal homologous chromosomes)

Types of staining

- **R-staining** - using **acridine orange** and similar dyes, to stain G-staining-insensitive regions of chromosomes. It is used to identify the components of homologous G- or Q-negative regions of sister chromatids or homologous chromosomes.
- **C-staining** - used for the **analysis of centromeric regions** of chromosomes containing the constitutive heterochromatin and variable distal part of the Y-chromosome.
- **T-staining** - used for the **analysis of telomeric regions** of chromosomes.

Spectral karyotype (SKY - spectral karyotype)



Aberrations

Chromosomal aberrations (chromosome mutations or chromosomal aberrations) - the type of mutations that alter the structure of chromosomes.

- deletions (loss of chromosome region);
- inversions (changing the order of the genes on chromosome region reverse);
- duplications (repeat segment of a chromosome);
- translocations (transfer of the region to another chromosome);
- dicentric and ring chromosomes;
- isochromosome (carrying two identical shoulder).

- intrachromosomal (inversions, deletions, duplications, ring chromosomes);
- interchromosomal (duplications, translocations, dicentric chromosome).

- Balanced (without addition or loss of genetic material in the formation, carriers are usually phenotypically normal)
- Unbalanced (change the dose ratio of genes and, their carriage is associated with significant abnormalities)

Cytogenetics in Oncology

NCBI Resources How To

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed Search

Advanced

Abstract Send to:

Hum Genet. 1988 Nov;80(3):235-46.

Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes.

Cremer T[1], Lichter P, Borden J, Ward DC, Manuelidis L.

Author information

Abstract

Chromosome aberrations in two glioma cell lines were analyzed using biotinylated DNA library probes that specifically decorate chromosomes 1, 4, 7, 18 and 22 from pter to qter. Numerical changes, deletions and rearrangements of these chromosomes were readily visualized in metaphase spreads, as well as in early prophase and interphase nuclei. Complete chromosomes, deleted chromosomes and segments of translocated chromosomes were rapidly delineated in very complex karyotypes. Simultaneous hybridizations with additional subregional probes were used to further define aberrant chromosomes. Digital image analysis was used to quantitate the total complement of specific chromosomal DNAs in individual metaphase and interphase cells of each cell line. In spite of the fact that both glioma lines have been passaged in vitro for many years, an under-representation of chromosome 22 and an over-representation of chromosome 7 (specifically 7p) were observed. These observations agree with previous studies on gliomas. In addition, sequences of chromosome 4 were also found to be under-represented, especially in TC 593. These analyses indicate the power of these methods for pinpointing chromosome segments that are altered in specific types of tumors.

Fluorescence in situ Hybridization (FISH)

Hybridization in situ - method for the determination of specific nucleic acid sequences directly in the cytological or histological preparations.

Fluorescence in situ Hybridization (FISH)

MOLECULAR HYBRIDIZATION OF RADIOACTIVE DNA TO THE DNA OF CYTOLOGICAL PREPARATIONS

By Mary Lou Pardue and Joseph G. Gall

KLINE BIOLOGY TOWER, YALE UNIVERSITY

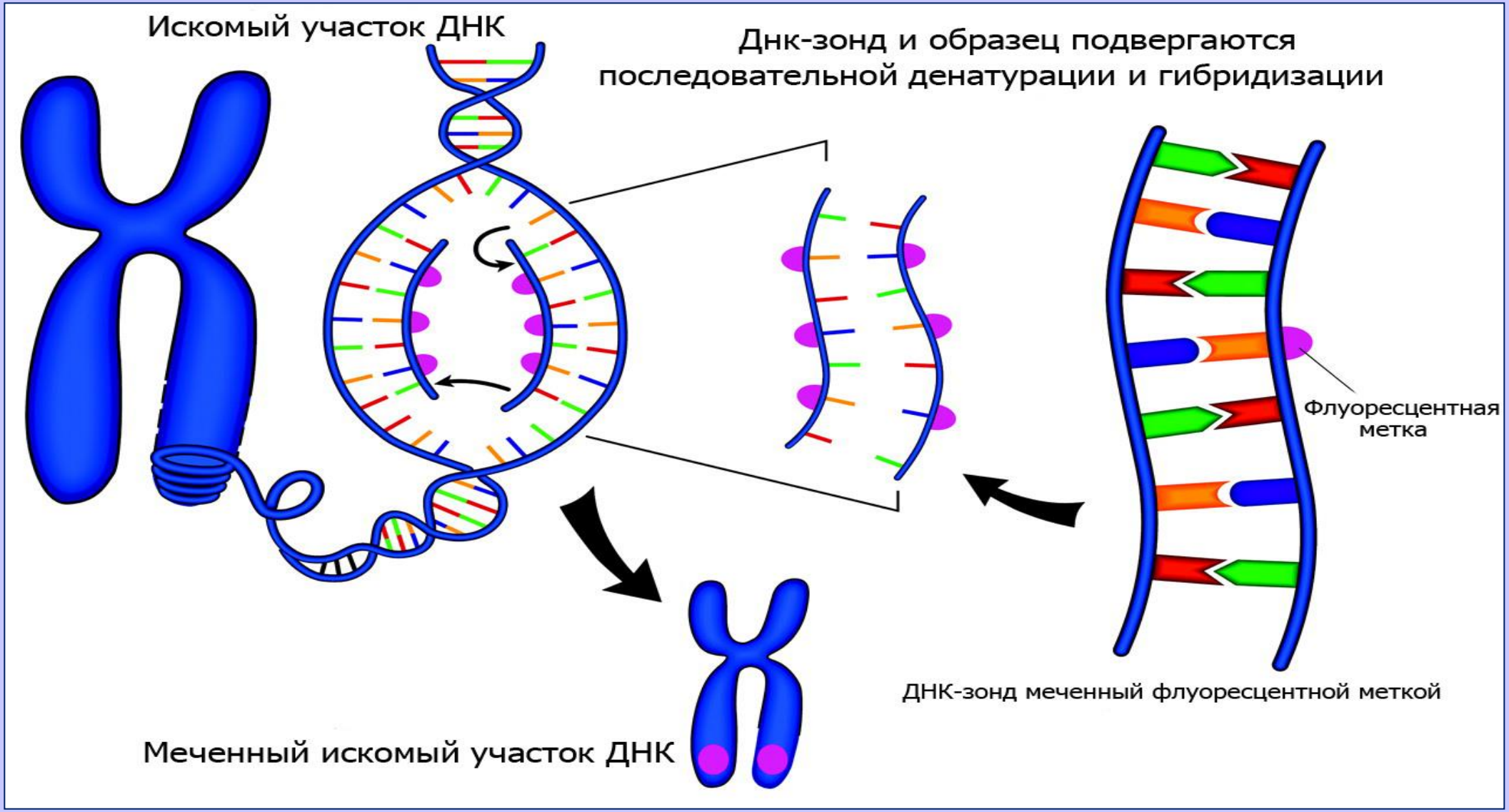
Communicated by Norman H. Giles, August 13, 1969

Abstract.—A method is presented for detecting the cellular location of specific DNA fractions. The technique involves the hybridization of a radioactive test DNA in solution to the stationary DNA of a cytological preparation. Sites of DNA binding are then detected by autoradiography. Experiments with DNA of the toad *Xenopus* are described.

The technique of DNA-DNA hybridization has been applied to a variety of genetic problems since its introduction by Schildkraut, Marmur, and Doty.[1] Hybridization of purified DNA has been used to investigate homologies between the DNA of phage and the DNA of the host bacterium,[2] to study genetic relationships among higher organisms,[3, 4] and to examine the relation of particular DNA fractions to the rest of the genome.[5] Reannealing kinetics have been used as a measure of genome complexity.[6, 7] Recently, substitutions and deletions in

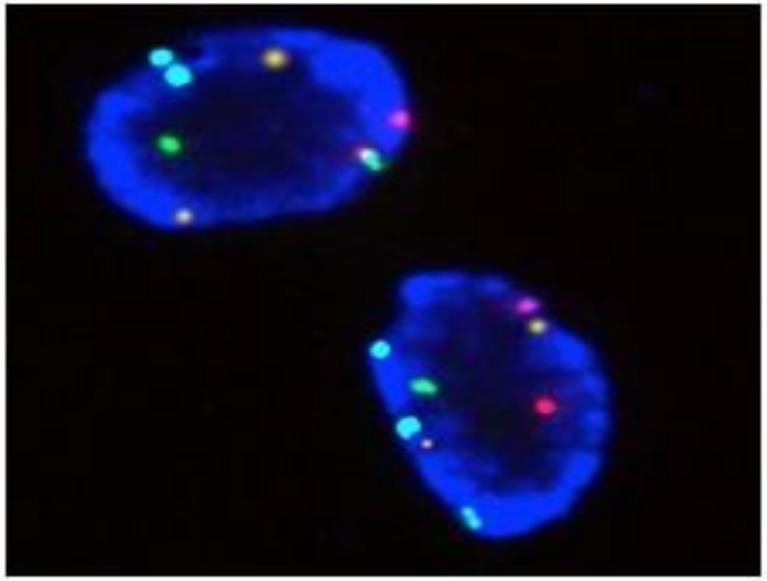
For the first time in situ hybridization has been described by Gall J. G., Pardue M. L. in 1969 using ^{32}P-labeled probe.

The procedure of the method

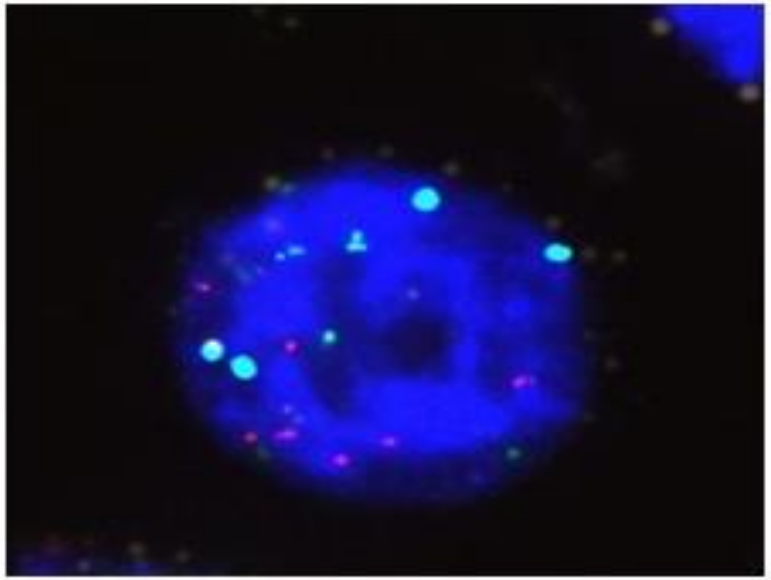


http://www.ajnr.org/content/28/3/406/F2.expansion.html

Микроскопическая картина нормальной и опухолевой клетки уротелия (UroVysion)



Норма



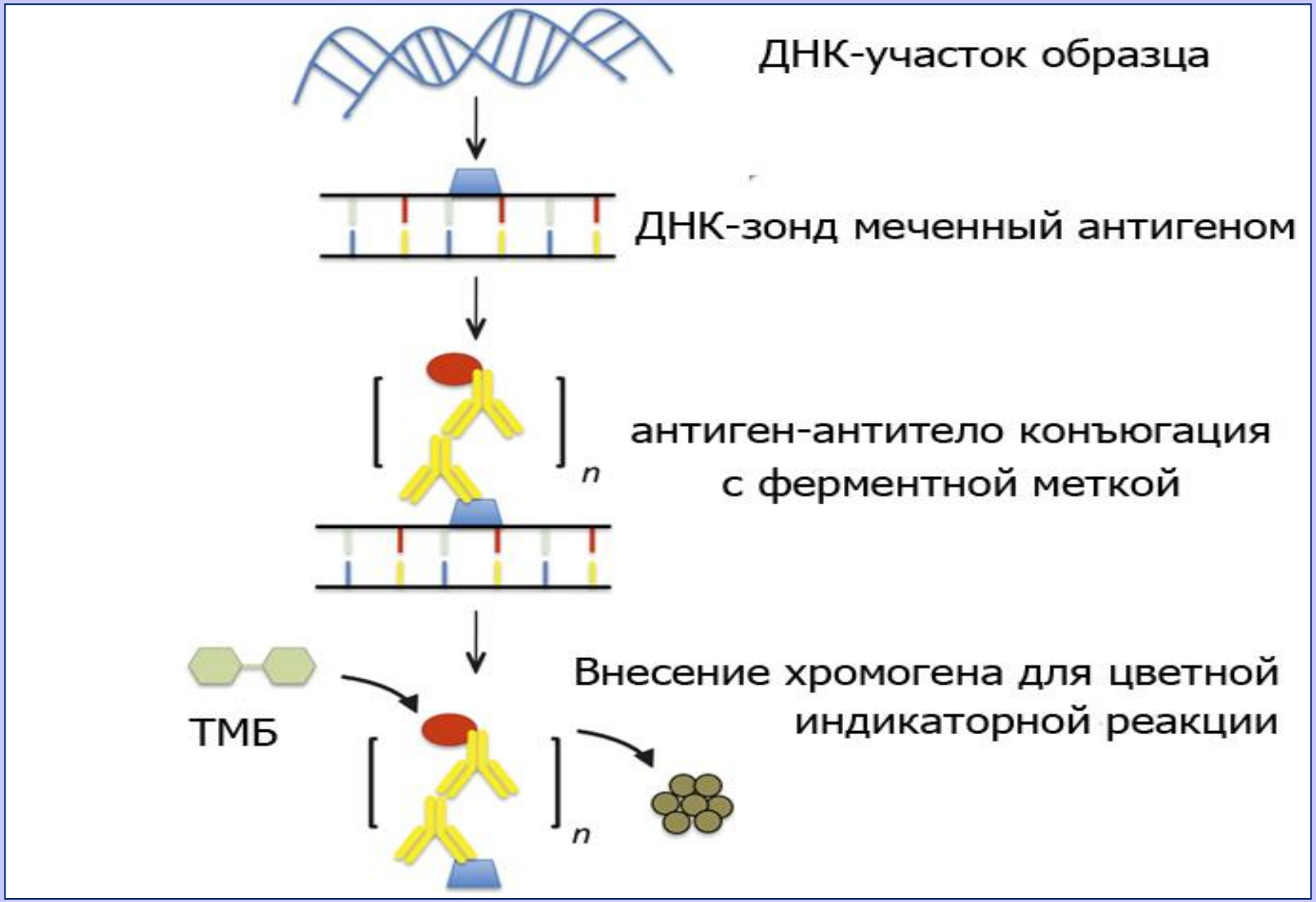
Патология

Пушкин А.С., 2012

FISH application

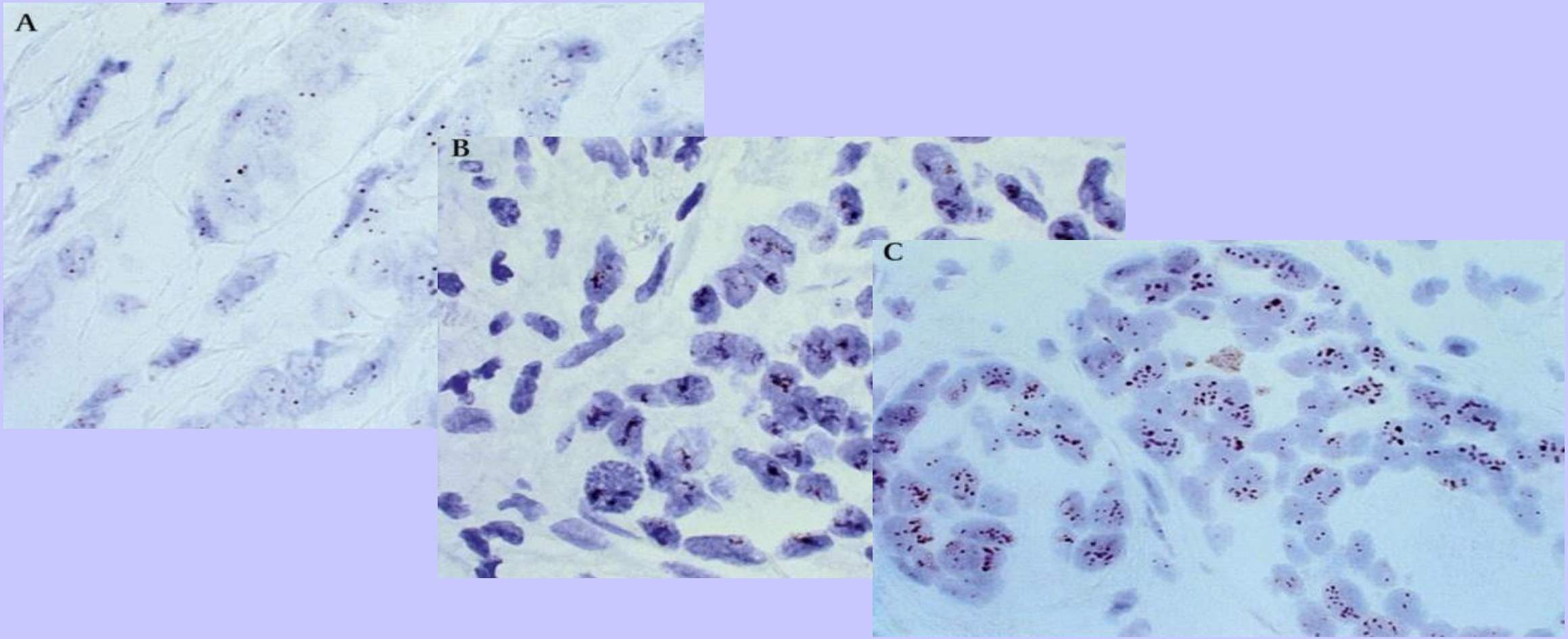
- diagnosis of solid tumors;
- Leukemia diagnosis
- preimplantation diagnosis;
- prenatal diagnosis of chromosomal abnormalities.

Chromogenic in situ hybridization (CISH)



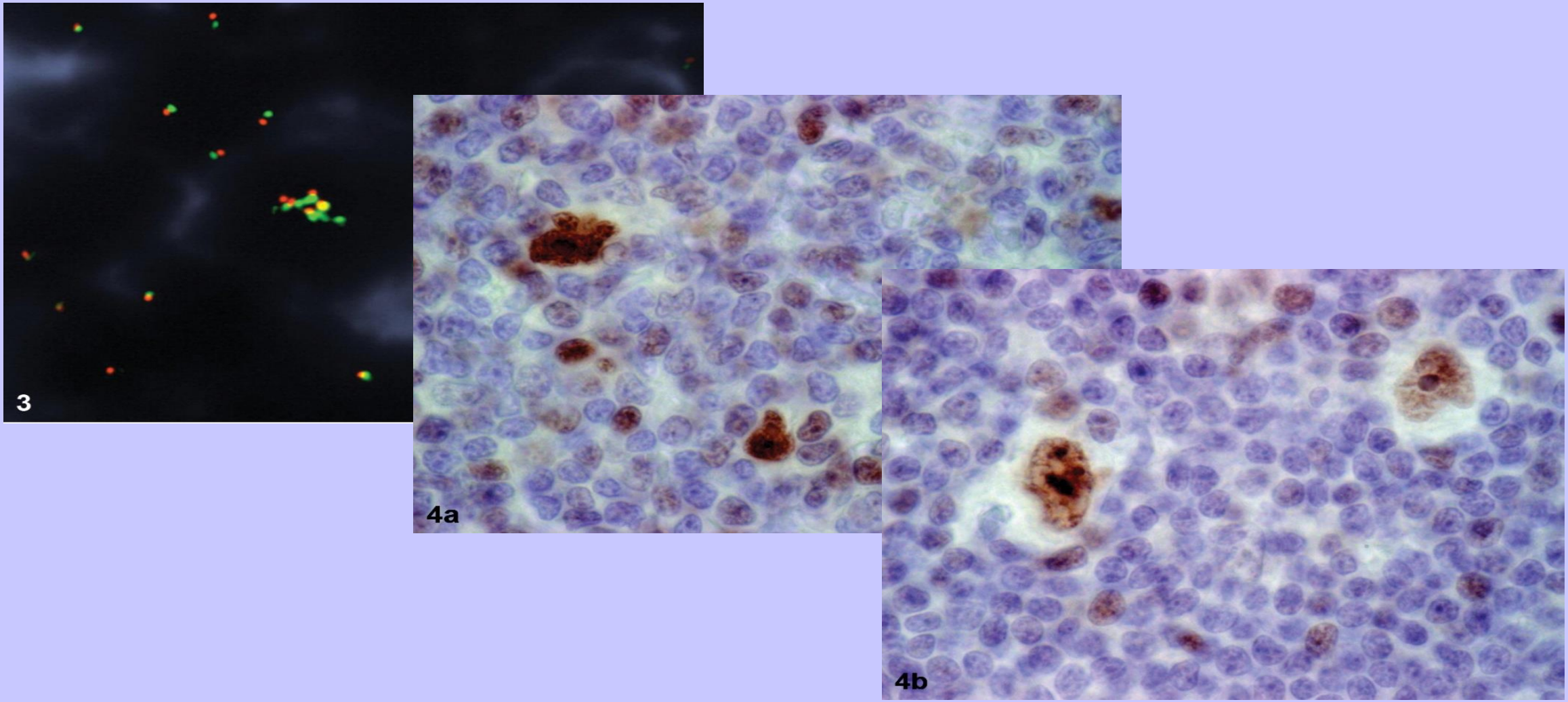
Aaron M. et al. Out of the darkness and into the light: bright field in situ hybridisation for delineation of ERBB2 (HER2) status in breast carcinoma, 2010

Chromogenic in situ hybridization (CISH)



Aaron M. et al. Out of the darkness and into the light: bright field in situ hybridisation for delineation of ERBB2 (HER2) status in breast carcinoma, 2010

FICTION (fluorescence immunophenotyping and interfase cytogenetics as a tool for investigation of neoplasms)



Alexei G. et al. (*2014*). Archives of Pathology & Laboratory Medicine

PCR diagnostics

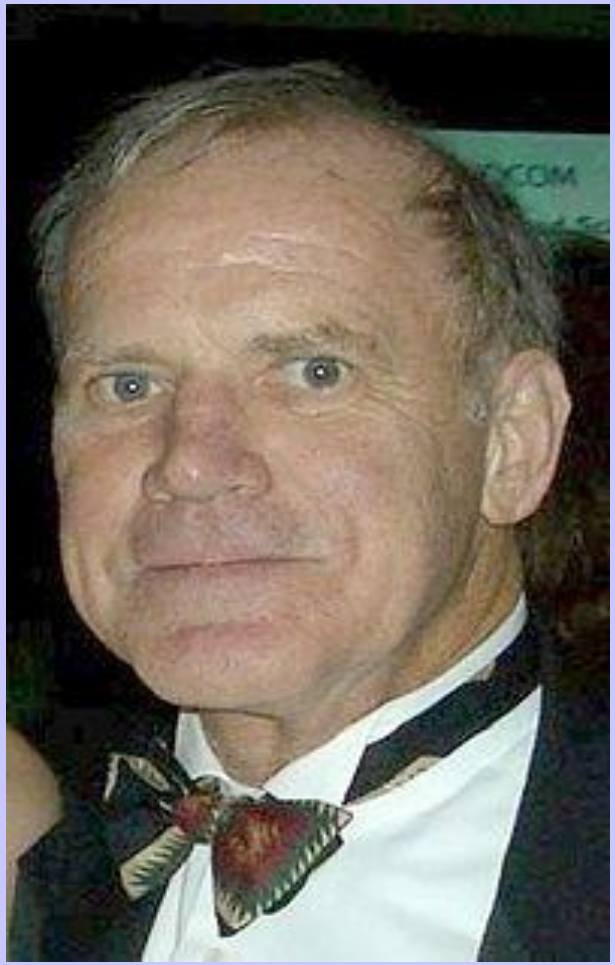
Polymerase chain reaction - an artificial process of multiple copying (amplification) of specific DNA sequences carried out in vitro.

PCR diagnostics

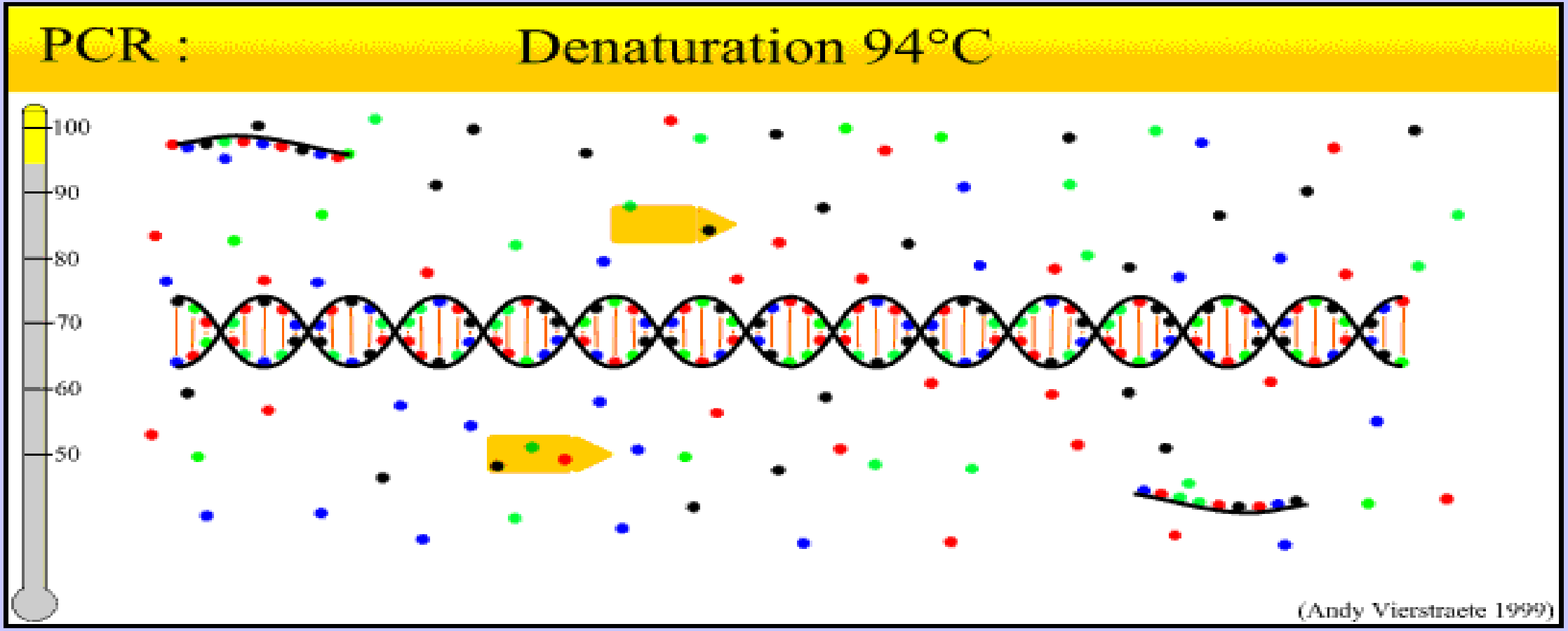
The principle of PCR was developed by Kary Mullis in 1983.

Opening PCR has become one of the most outstanding events in the field of molecular biology in recent decades.

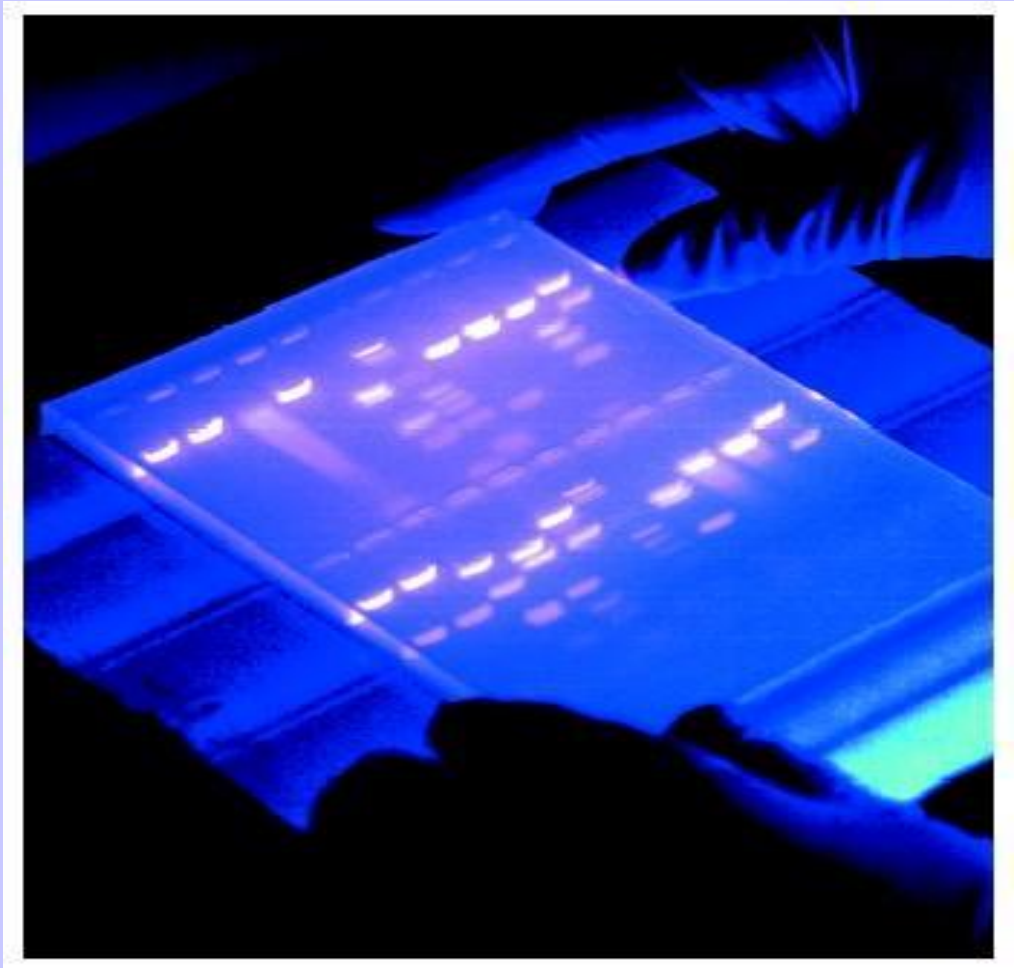
For the development of the method Kary Mullis was awarded the Nobel Prize in chemistry in 1993 .



Principle of the method



Detection



http://www.mehanfamily.com/4/electrophoresis-gel

Real-time PCR

http://premiereflooring.com/pages/8/real-time-pcr-machine-price-list

Modifications of PCR

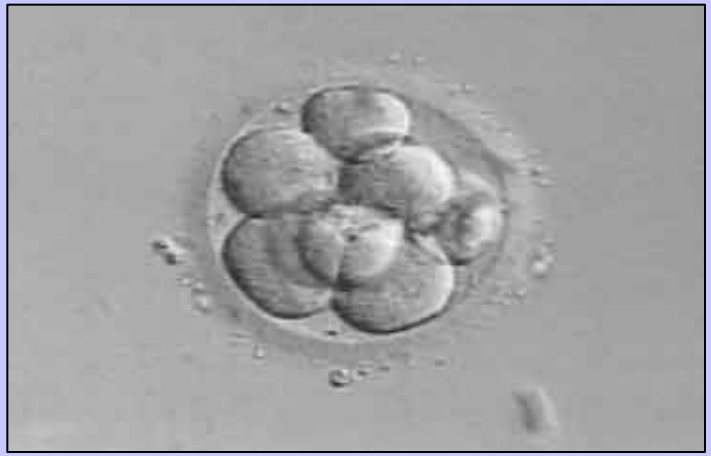
- nested-PCR (nested-polymerase chain reaction);
- RT-PCR (reverse transcription-polymerase chain reaction);
- Long-PCR;
- PCR in situ (PRIMS — polymerase reaction in situ);
- multiplex PCR (multi-polymerase chain reaction).

PGD
Preimplantation genodiagnostic

IVF procedure allows you to get multiple embryos, usually 5-15. The question - which of the embryos are better suited for implantation into the uterus. Morphological criteria help to assess the viability of embryos, but not their quality from the genetic point of view. It has been shown that embryos with an extra chromosome 21 (Down syndrome) or with mutations in the genes of the hematopoietic system (hemophilia, thalassemia, sickle cell anemia and others) according to morphological criteria are indistinguishable from healthy embryos.

PGD allows us to estimate the genetic characteristics of embryos before transfer to the uterus and select healthy embryos - with a normal number of chromosomes and without parental mutations.

From which of these embryos child with Down syndrome will be obtained?

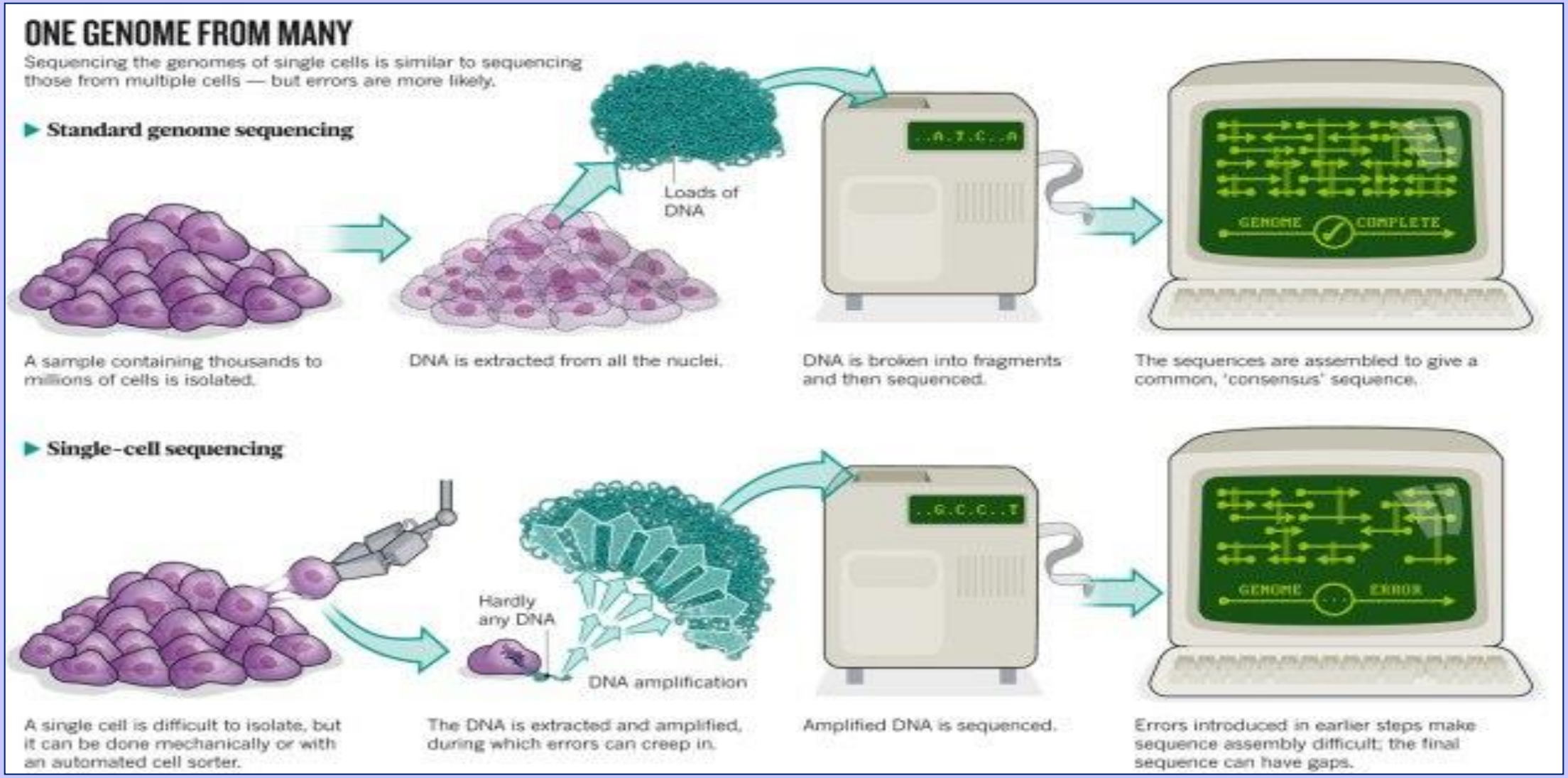


Sequencing

- DNA sequencing technology appeared due to the work of scientists Walter Gilbert (Maxam A, Gilbert W, 1977) and Frederick Sanger (Sanger F, Coulson A, 1975) in the 70-s of the last century turned our understanding of human biology.
- Continuous improvement of these technologies has led to the fact that the project for the sequencing of the human genome, "the Human Genome" (Human Genome Project, HGP), has been implemented for one year in 2001 after ten years of work (Watson J, 1990; Venter J, et al , 2001).
- It is expected that the genome sequencing (sequencing, reading the information from the DNA) will provide benefits to humanity in the understanding of human health and will advance to the individual treatment.

"Агентство по инновациям и развитию", http://www.innoros.ru/publications/articles/13/sovremennye-metody-polnogenomnogo-sekvenirovaniya-rasshifrovki-dnk-v-diagno

Sequencing


ONE GENOME FROM MANY
Sequencing the genomes of single cells is similar to sequencing those from multiple cells — but errors are more likely.
Standard genome sequencing
Loads of DNA
..A.T.C..A
GENOME
COMPLETE
A sample containing thousands to millions of cells is isolated.
DNA is extracted from all the nuclei.
DNA is broken into fragments and then sequenced.
The sequences are assembled to give a common, 'consensus' sequence.
Single-cell sequencing
Hardly any DNA
DNA amplification
..G.C.C..T
GENOME
ERROR
A single cell is difficult to isolate, but it can be done mechanically or with an automated cell sorter.
The DNA is extracted and amplified, during which errors can creep in.
Amplified DNA is sequenced.
Errors introduced in earlier steps make sequence assembly difficult; the final sequence can have gaps.

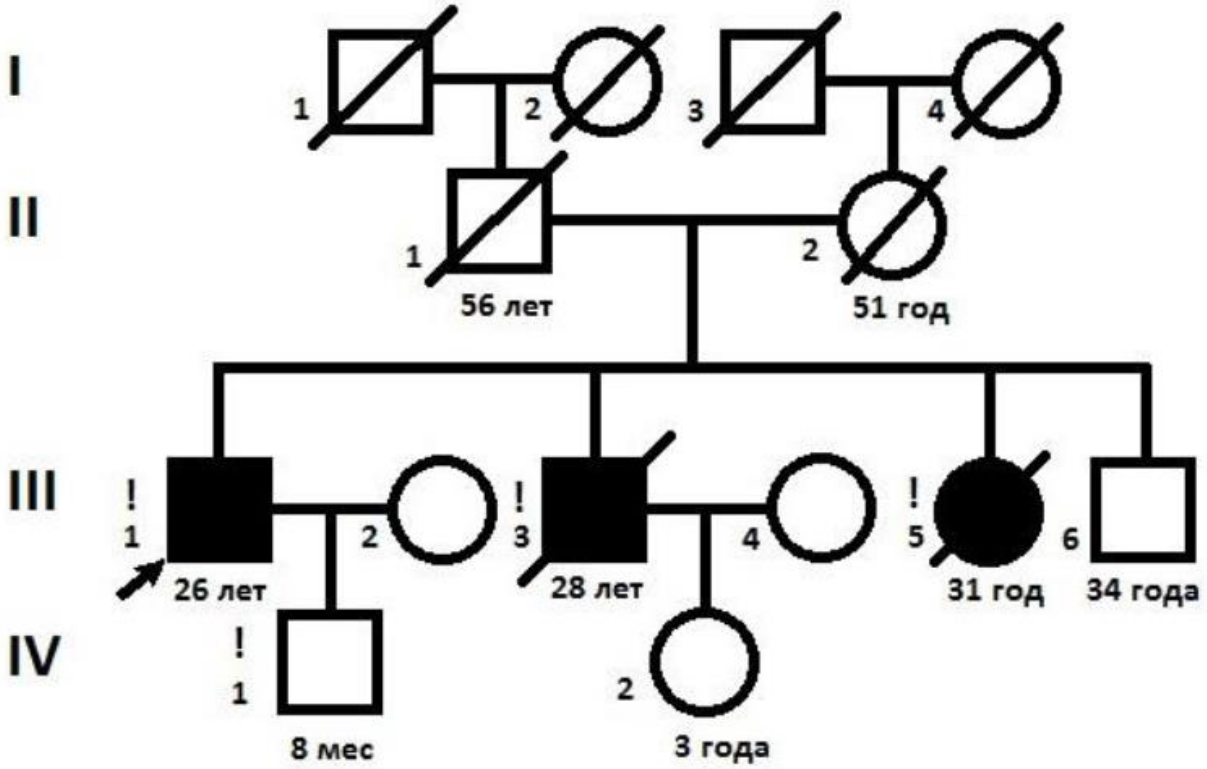
Application of sequencing

- Personal medicine
- Cancer and Genetic Diagnosis
- Other diseases and genetic diagnosis (Alzheimer's disease, infertility, and others.)
- Pharmacogenomics and whole genome sequencing

Sequencing in Clinical Oncology

- оценки лекарственной устойчивости в мониторинге лечения онкологических пациентов.
- Molecular preventing of hereditary diseases (breast cancer, ovarian cancer, nonpolyposis colon cancer etc.);
- differential diagnosis of neoplasias (diagnosis of gastrointestinal tumors by C-kit mutation, mutations in the EGFR gene in supratentorial brain tumors and others.);
- determination of mutations in oncogenes and tumor suppressor genes (p53, APC, PTEN etc.);
- determination of microsatellite instability in lung cancer and colon cancer to choose the tactics of treatment and prognosis;
- evaluation of drug resistance in the treatment monitoring of cancer patients.

Clinical case



Примечание:

II:1 - смерть в 56 лет, кардиопатология;

II:2 - смерть в 51 год, опухоль печени;

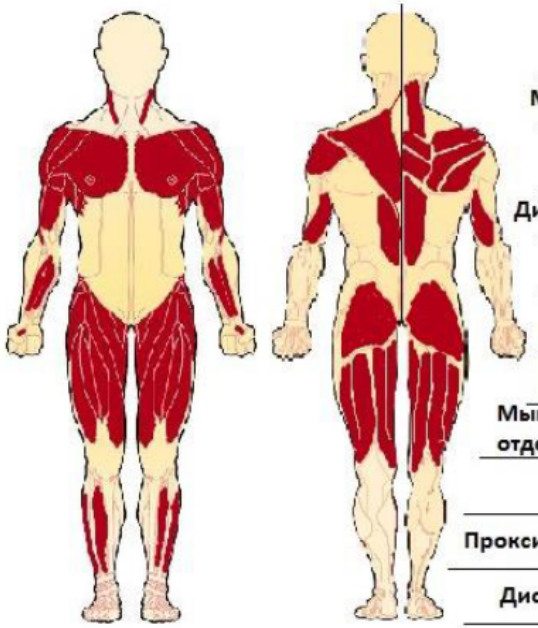
III:3 - смерть в 28 лет, спонтанный пневматоракс;

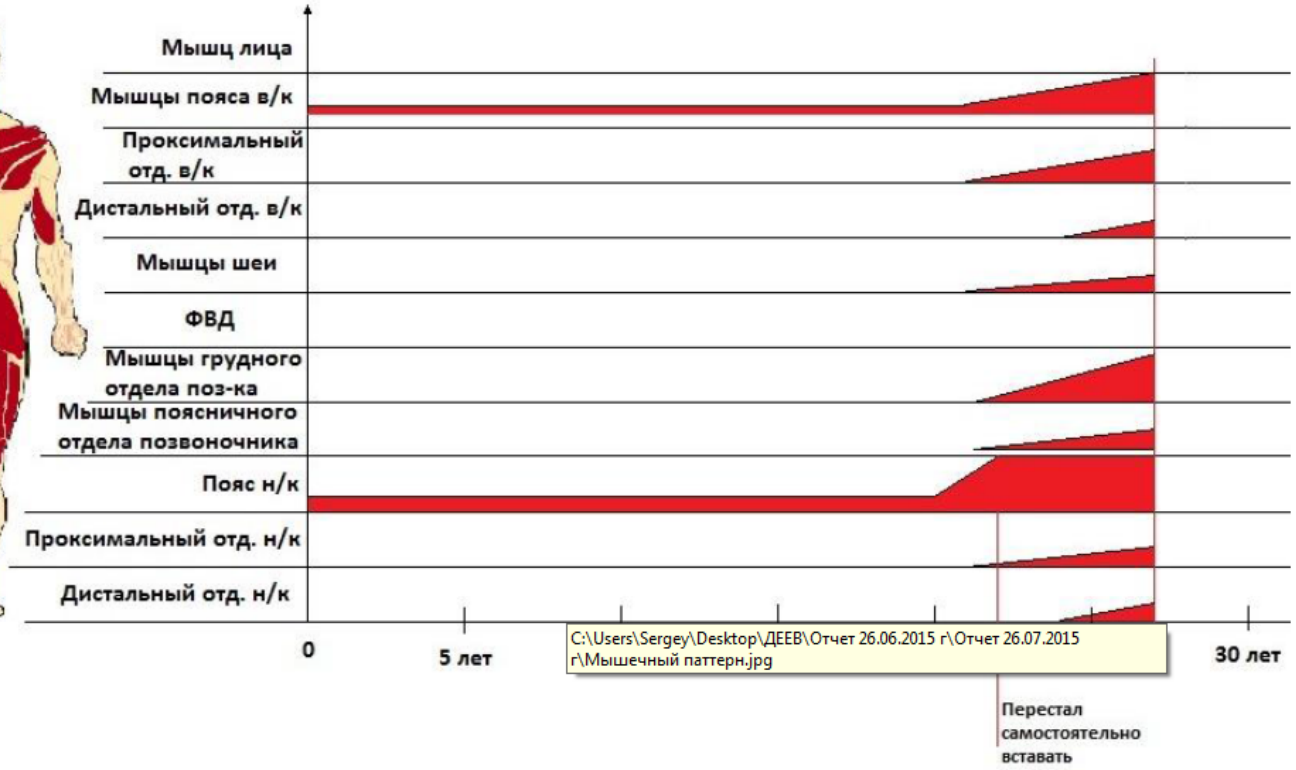
III:5 - смерть в 31 год, рестриктивная ДН-2-3 ст.

Clinical case

Жалобы

Анамнез заболевания





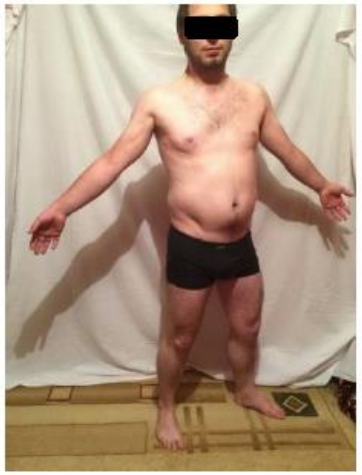
Clinical case

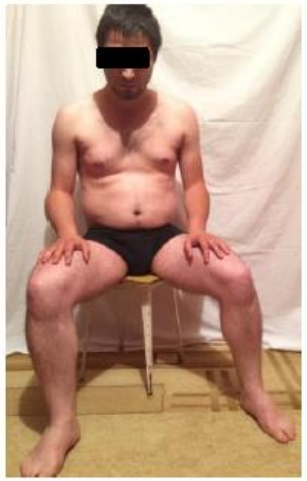
Неврологический статус

Объем активных движений

- **В верхних конечностях**: отведение в плечевых суставах 45◦, сгибание 30◦, разгибание 30◦;
- **В нижних конечностях**: сгибание в тазобедренных суставах сидя – рывковым движением кратковременно приподнимает бедро; лежа - самостоятельно невозможно, пассивное сгибание до 150◦ (норма); самостоятельное разгибание не возможно; приведение в тазобедренных суставах до 30◦.

- **Глубокие рефлексы** не вызываются.
- **Самостоятельная ходьба** до 30-50 метров.

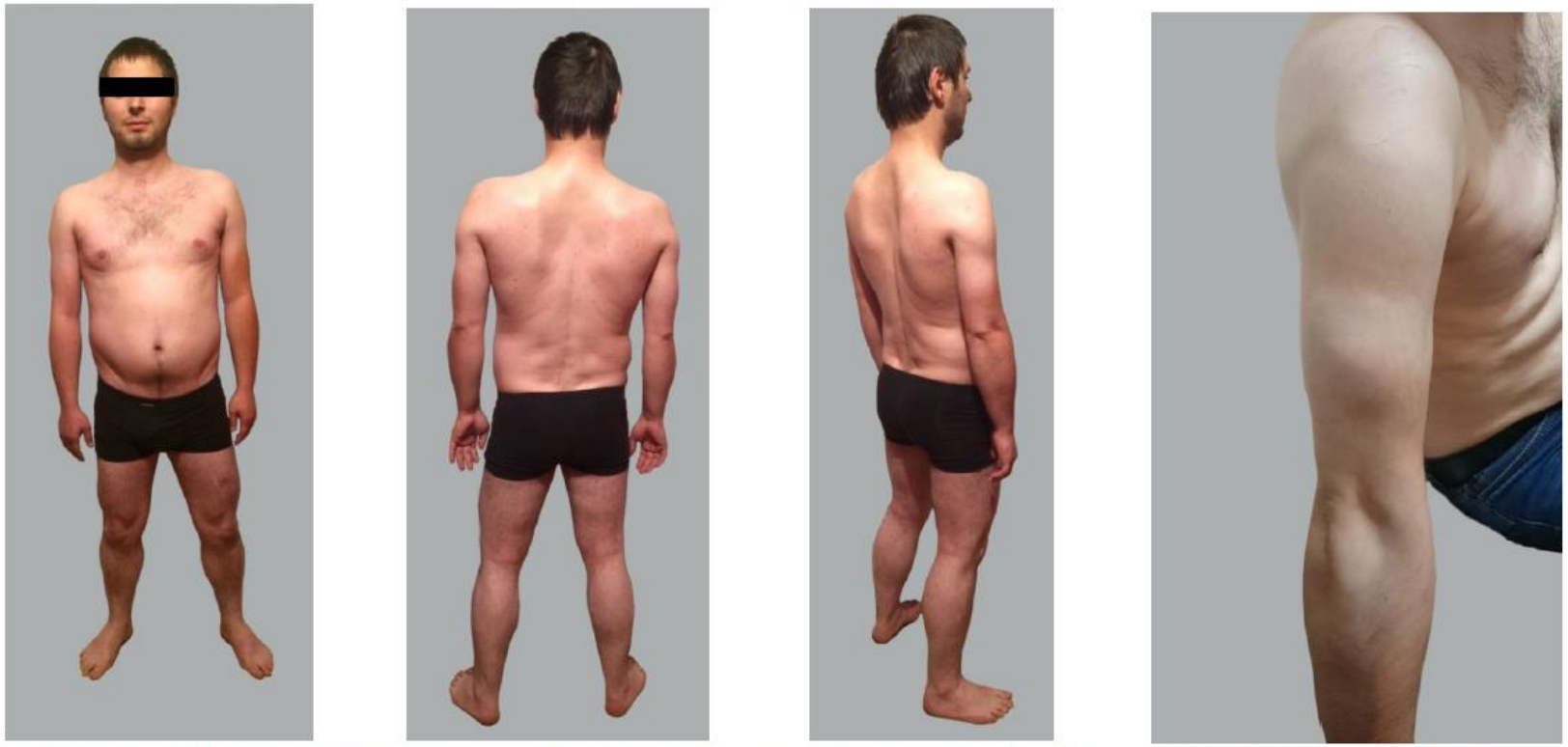




Clinical case

Неврологический статус

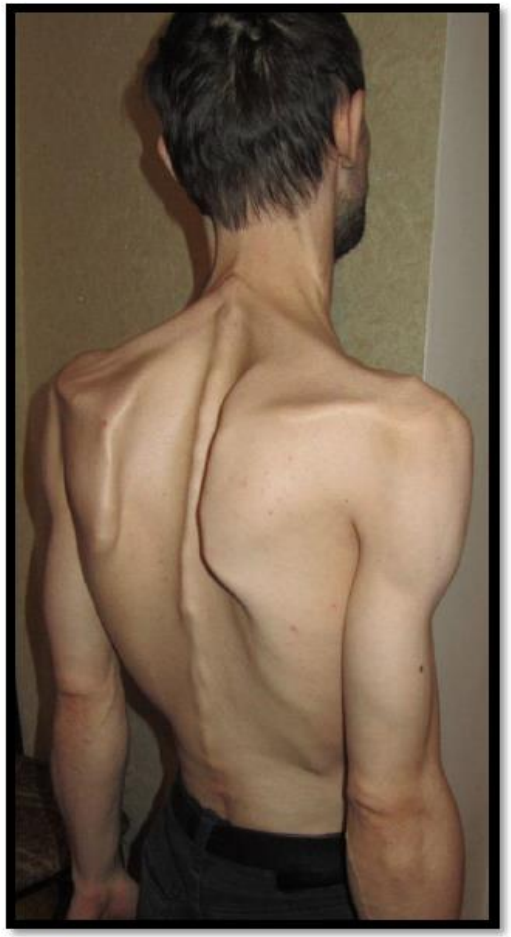
Амиотрофии



- **Выраженные атрофии** паравертебральных мышц грудного и поясничного отделов позвоночника.
- **Умеренные атрофии** в проксимальных отделах верхних конечностей (в большей степени бицепса плеча, в то время как дельтовидная мышца достаточного объема, а трицепс имеет гипертрофию латеральной головки).

Clinical case

Сибсы



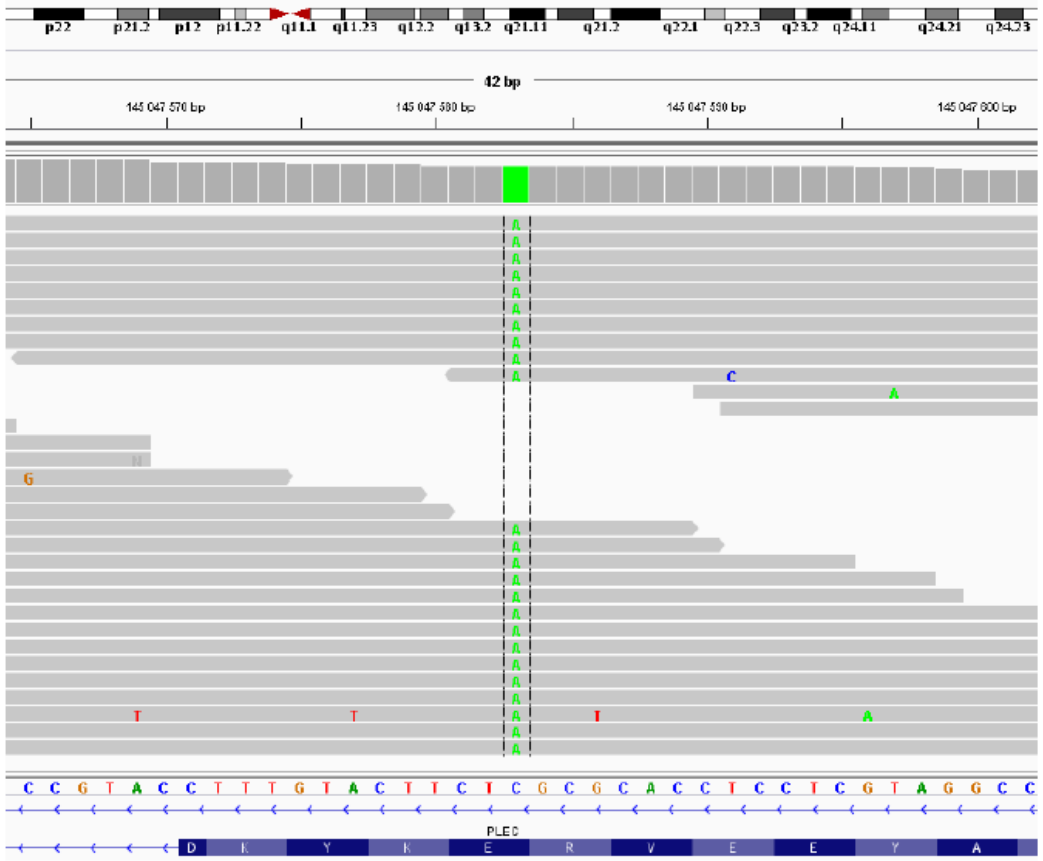
М. 29 лет, смерть от спонтанного пневмоторакса.

- А.31 год, смерть от прогрессирующей дыхательной недостаточности.

Clinical case

Генетический анализ

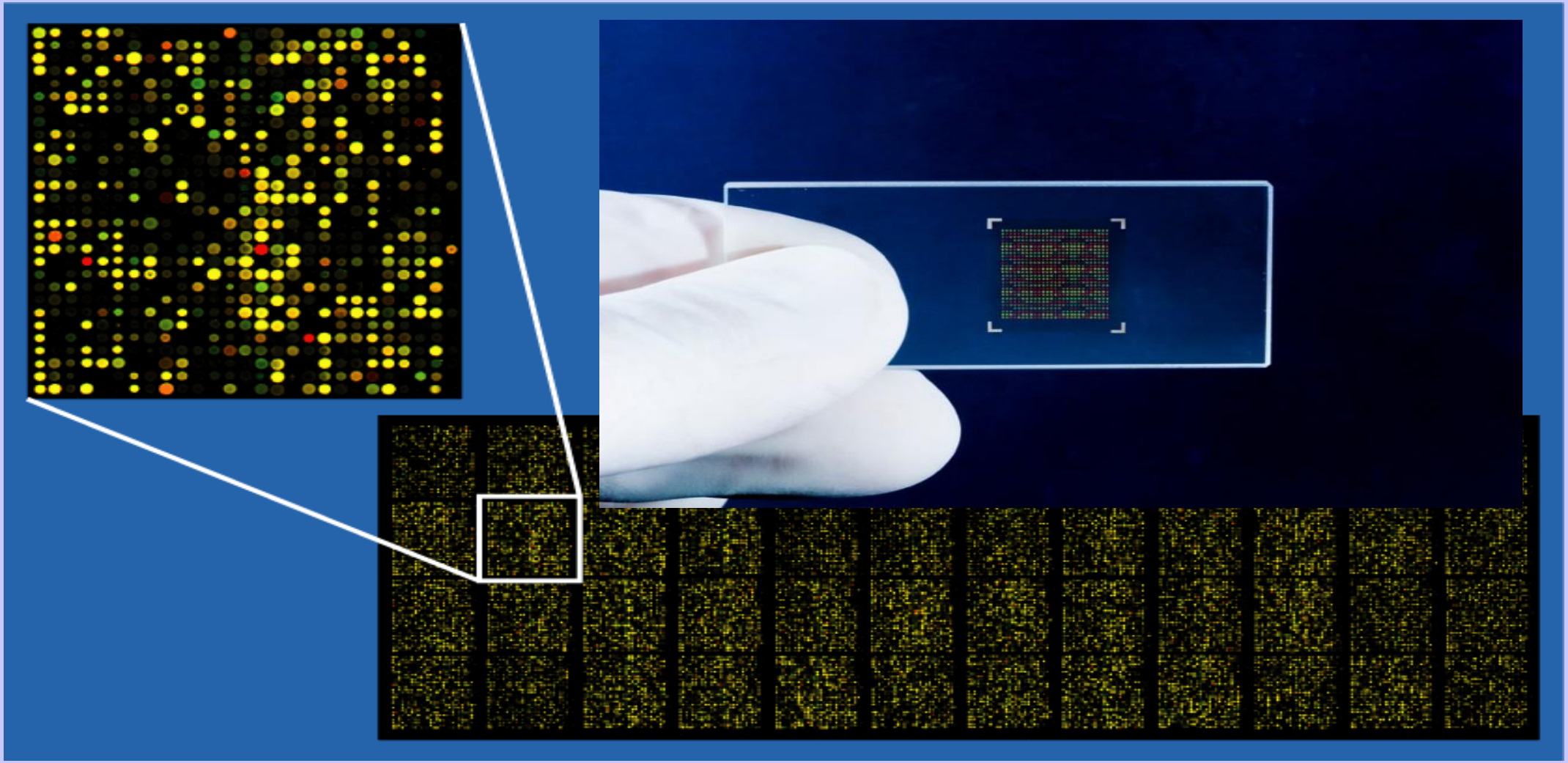
- С помощью NGS была обнаружена новая гомозиготная мутация chr8: 145047583C>A, Glu20ter в гене PLEC, изоформа 1f. (Биоинформационная обработка Ф. Коновалов, лаборатория Genetico, г. Москва (2014))
- Результат подтвержден референсным методом (секвенирование по Сэнгеру)



Microarray

- Biological microarrays - a set of DNA molecules (at least - proteins), orderly arranged on special media, so-called "platform".
- Platform can serve as a plate of glass, plastic, silica, or polymer membrane.
- Size diagnostic microchip surface may vary from a few square millimeters to 50 square centimeters.
- Each microarray can range from 30-50 up to several tens or even hundreds of thousands or microassay orderly deposited samples.

Microarray



http://sgugenetics.pbworks.com/w/page/38184737/Methods%2520of%2520Pathogen%2520identification%2520using%2520molecular%2520methods&sa

Application

Clinical diagnostics to determine:

- viruses and microorganisms;
- hormones;
- allergens;
- drugs;
- any bioactive substances in any small concentrations;
- in oncology;
- in criminology;
- for research in the field of ecology and biosafety.

Conclusions