Laboratory of Radiation Biology
Joint Institute for Nuclear Research

Reconstruction of karyotypes of normal and tumor human cell lines using Multicolor Fluorescence *in situ* Hybridization (mFISH) approach

Pham Thi Duyen[1,2], Elena Nasonova[1]

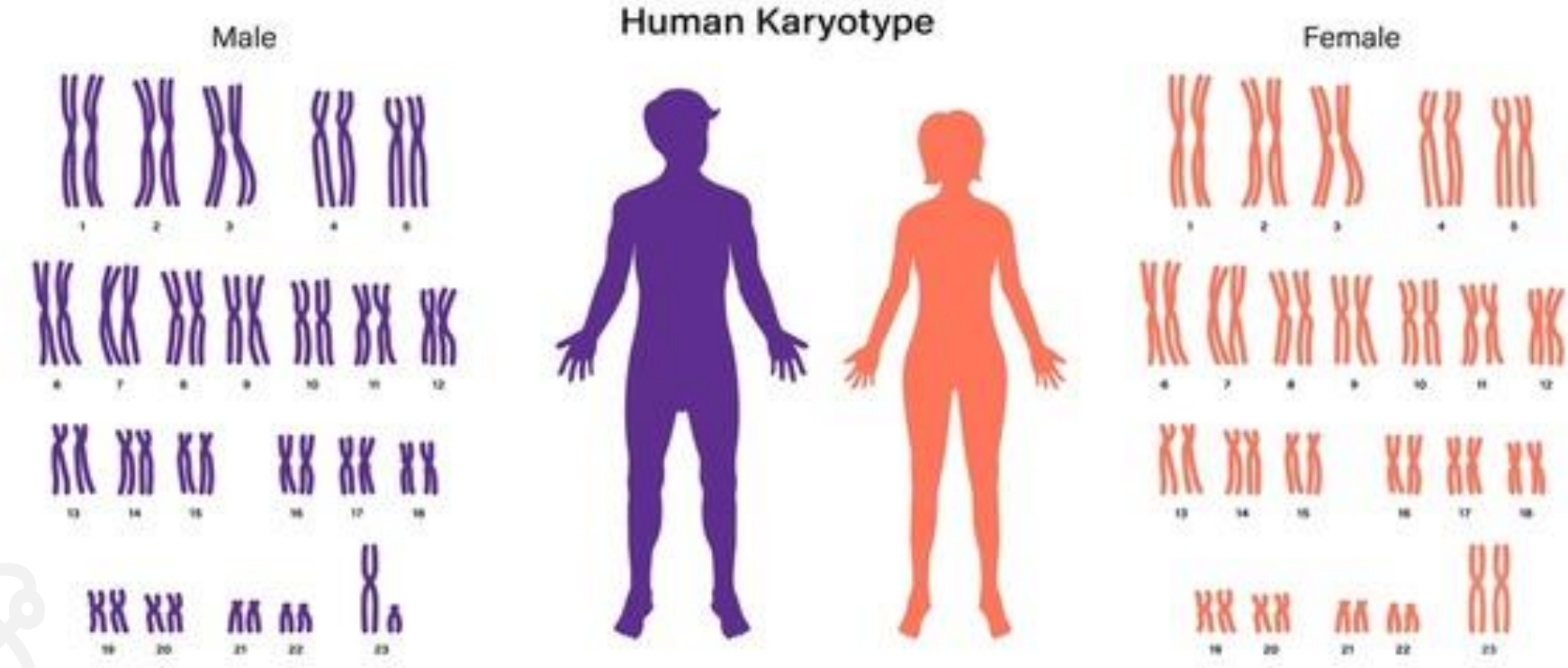
[1]Laboratory of Radiation Biology, Joint Institute for Nuclear Research, Dubna, Russia

[2]Vietnam National University, Hanoi, Vietnam

E-mail: phamduyen@jinr.ru

AYSS - 2025

Table of contents



What is a karyotype?

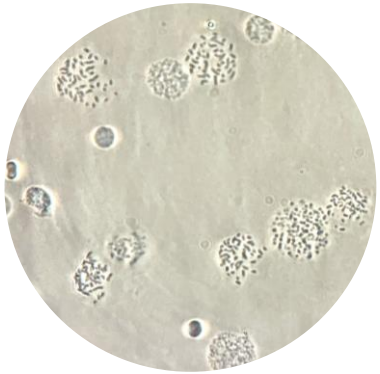
A karyotype: A chart of chromosomes are taken from a cell sample and chromosomes match up in pairs

The most general description of the number, morphology, and size of all chromosomes in the nucleus, representing the basic genetic information of species.

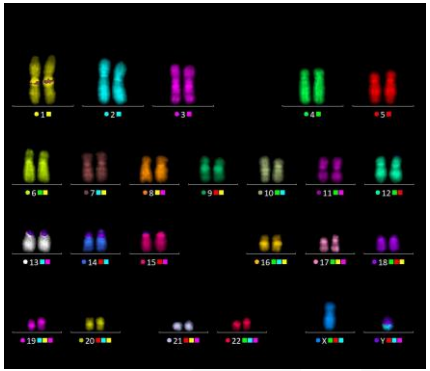
Normal Human Karyotype: Autosomes (22 pairs) and Sex chromosomes(XX, XY)

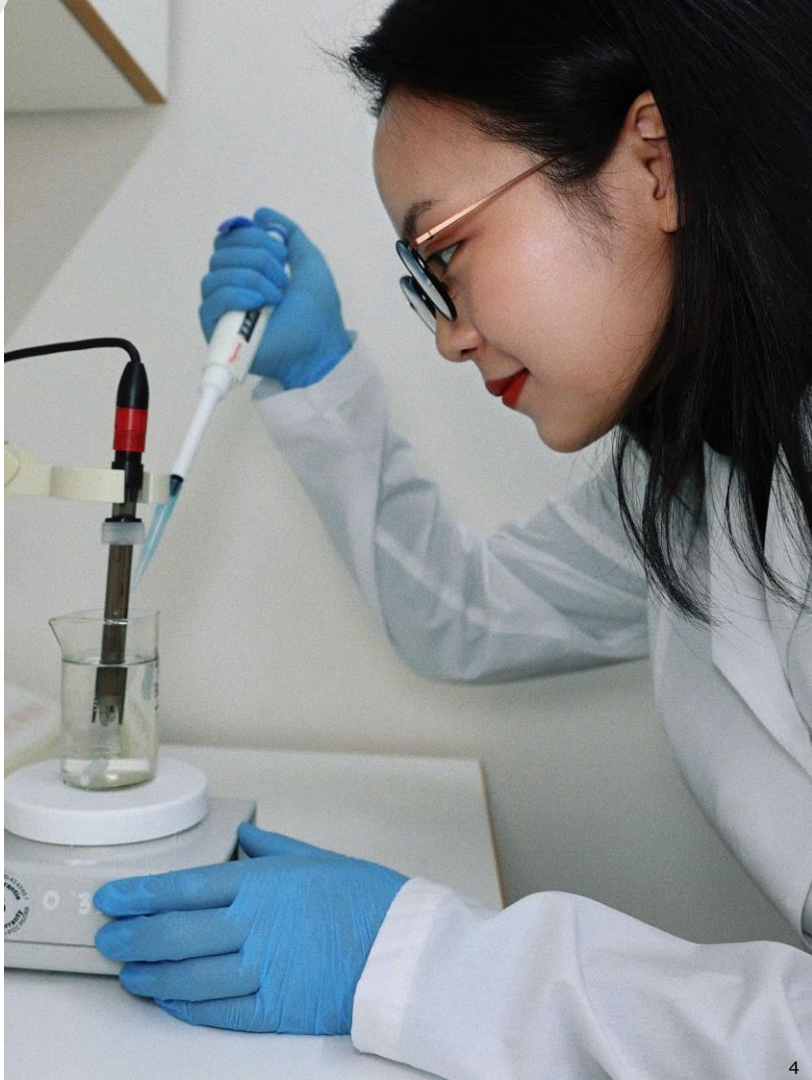
Purpose of research

Study the chromosome complement and genetic stability of cultured normal and tumor human cell lines (NDHF, Cal51, U87) of different origins using the modern and advanced method of molecular cytogenetics Multicolor Fluorescence *in situ* Hybridization (mFISH).

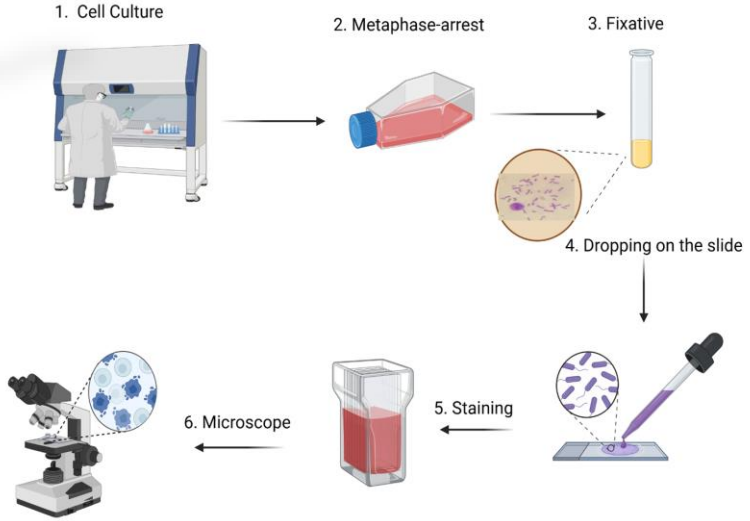


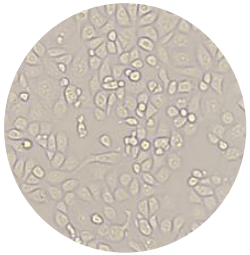
mFISH



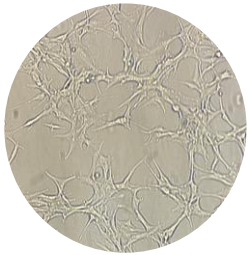


Materials and Methods—Metaphase method

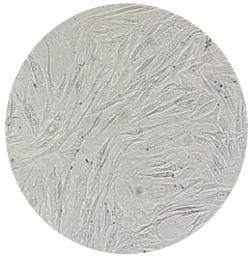




Cal51 cells



U87 cells

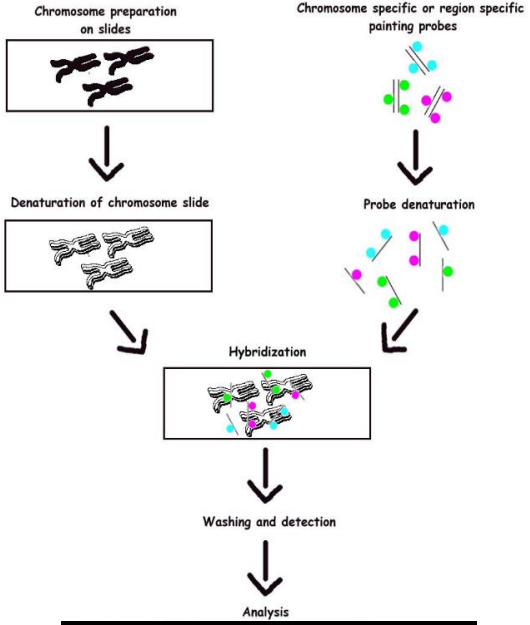


NDHF cells

Optimization of standard protocol for different cell lines culture

Cell line	Medium	FCS	AB *	Colcemide	Trypsin	0.075 M KCl	Fixative 3:1
Human breast carcinoma (Cal 51)	DMEM (low glucose)	15%	+	1 hour 10µL/mL	0.25% + EDTA 3 min	10 mins	X2
Human glioblastoma (U87)	DMEM (high glucose)	10%	+	1 hour 10µL/mL	0.05% + EDTA 5 min	8 mins	X2
Normal dermal human Fibroblasts (NDHF)	MEM	10%	+	2 hour 15µL/mL	0.25% + EDTA 3 min	6 mins	X2

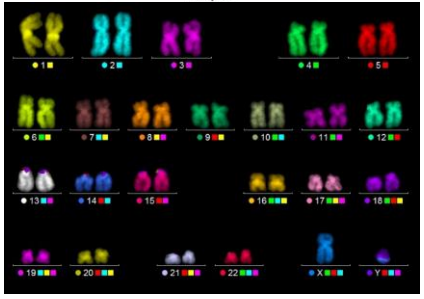
Metaphase analysis:
Multicolor Fluorescence *in situ* Hybridization – mFISH



The most advanced method of molecular cytogenetics - allows the identification of each pair of human chromosomes (22, X, Y).

- Based on reassociation of DNA single strands with specific DNA probes labeled with 5 different fluorochromes:
  FITC SpO TR Cy5 DEAC and **DAPI**-counterstaining
- Their unique combinations allows the simultaneous detection of all 24 different human chromosomes ➡ Unique color signature for each chromosome.
- Images are captured at fluorescence microscope using a specific filter set and analyzed by computer program ISIS (MetaSystems, Germany).

mFISH enables analysis of all chromosome aberrations, including complex aberrations and symmetric exchanges (translocations), on one slide.

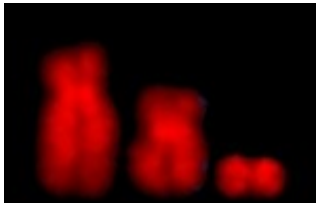


➡ **mFISH karyogram**

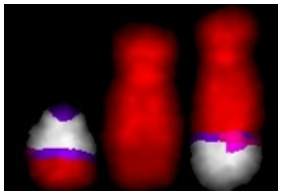
Classification & Analysis - mFISH

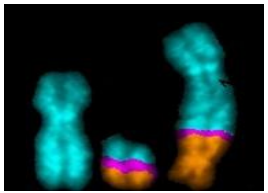
Simple aberrations:

- **One break** aberrations: *acentrics*

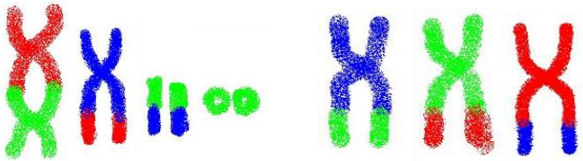


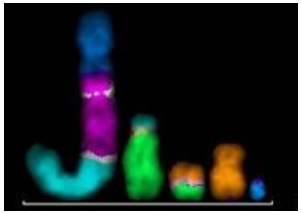
- **Two-break** aberrations: simple exchanges (*dicentrics, rings, translocations*)

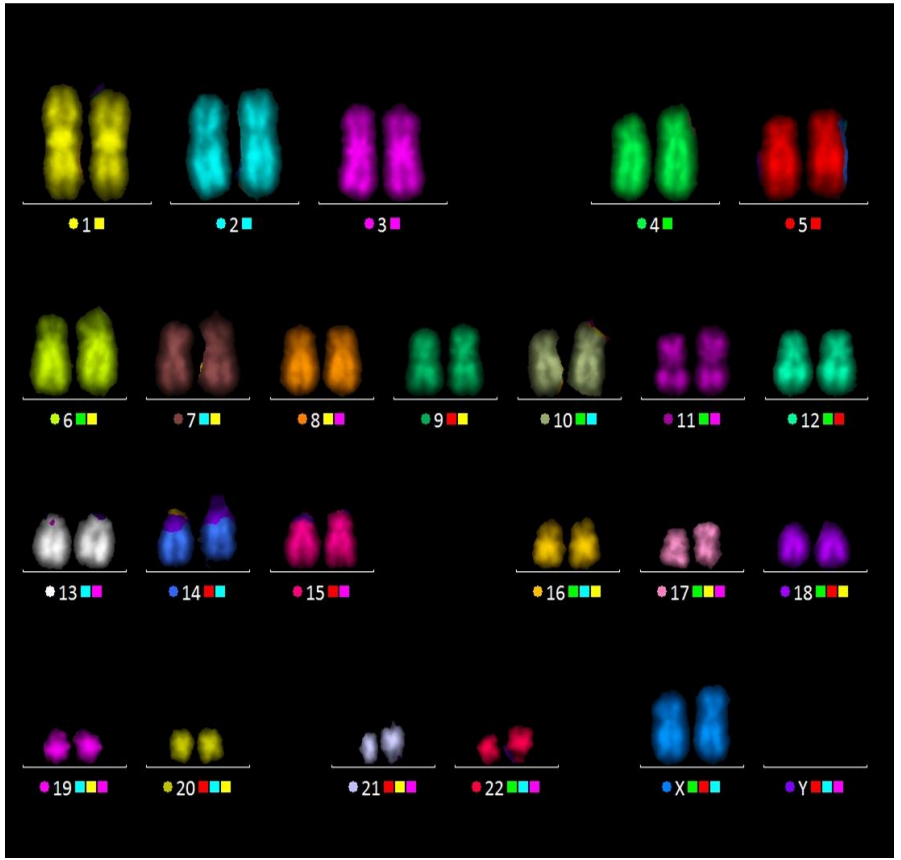




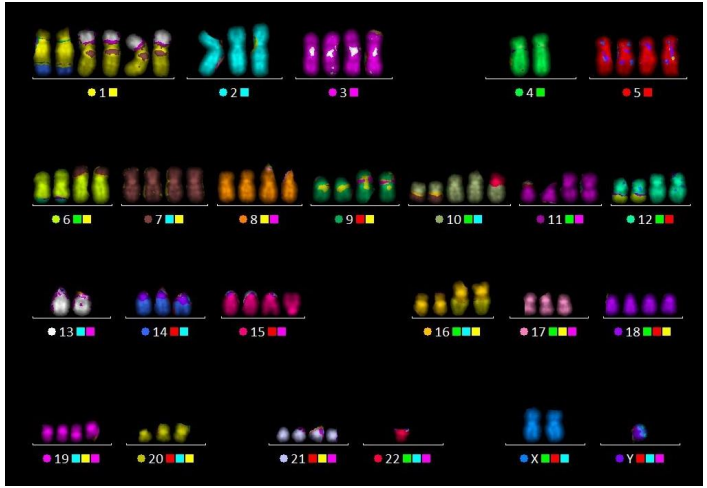
Complex aberrations: 3 or more breaks in 2 or more chromosomes.

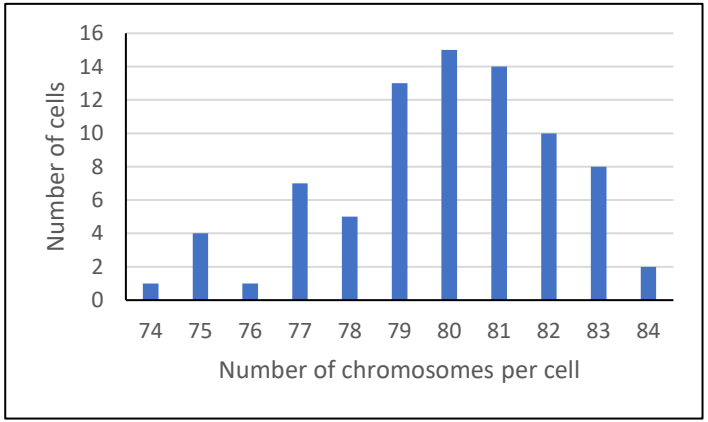




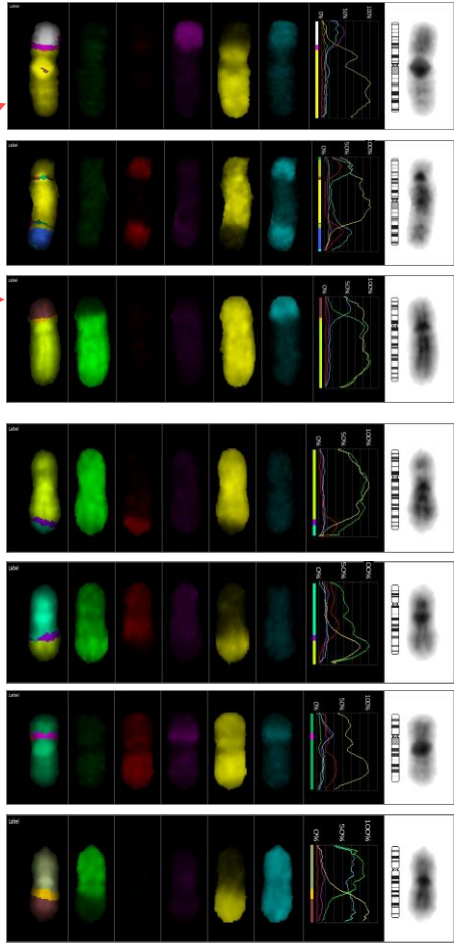


Karyogram of U87 glioblastoma cells



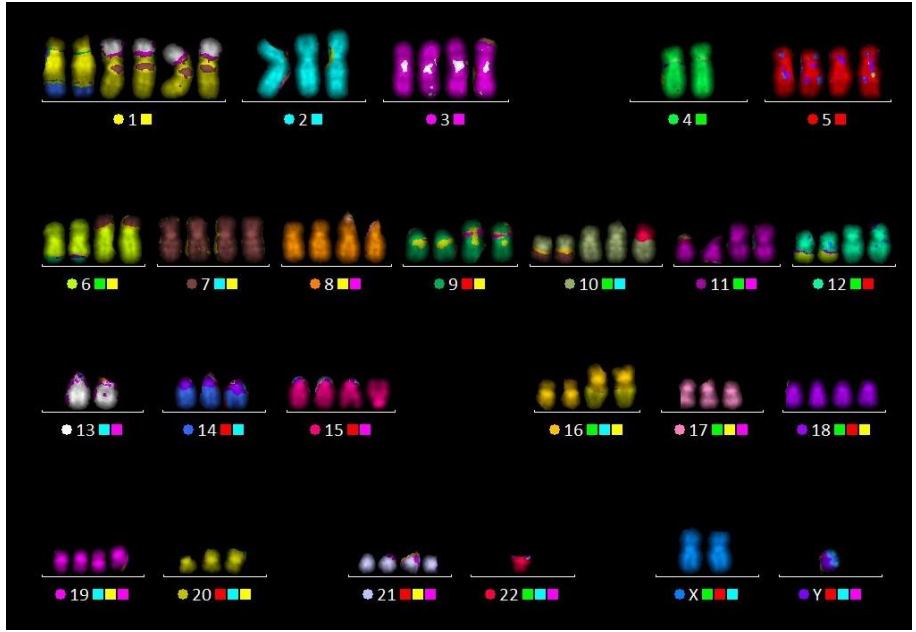


Number of analyzed cells - 80
Modal chromosome number – 80 (75-84)
Truncated chromosomes:
9’; 11’; 20’;
Translocated chromosomes:
tr 1’-13;
tr 20’-1-14;
tr 6’-7;
tr 6’-12
tr 12’-6
reciprocal translocation
tr 9’-13-9;
tr 10’-7;
tr 16’-1;
tr 22’-10.

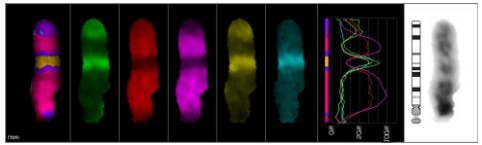


Additional spontaneous aberrations - Found in 75% of cells (truncated chromosomes (44), translocations (38), dicentric chromosomes (14), Robertsonian translocations (3) and complex aberrations (11))

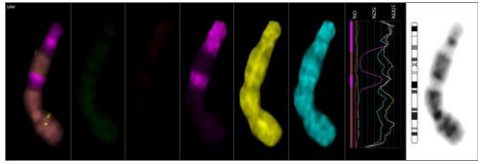
A. Representative karyotype.
B. tr(ins) 15'-16-15-18.
C. dic 7'-19-7-19' (add. mat. 7 and 19)
D. dic 1-16'-1-20' produced from two marker chromosomes tr 16'-1 and tr 20'-1-14
(The parts of marker chromosomes participating in dicentric are in red circles – right figure).



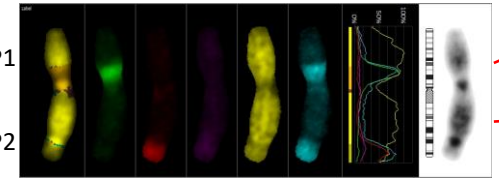
A.



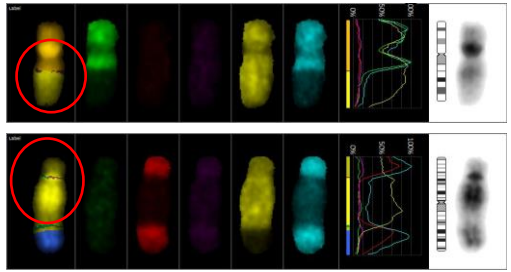
B.



C.

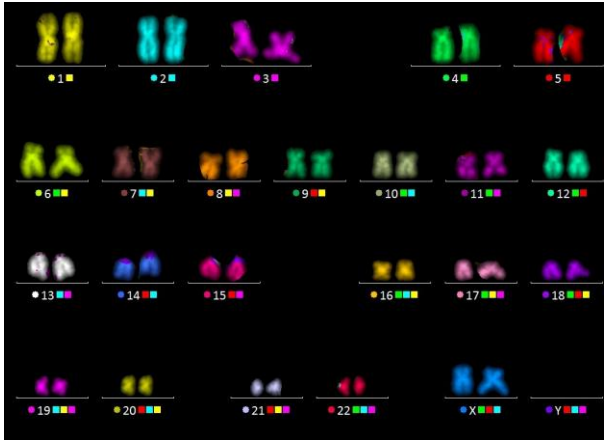


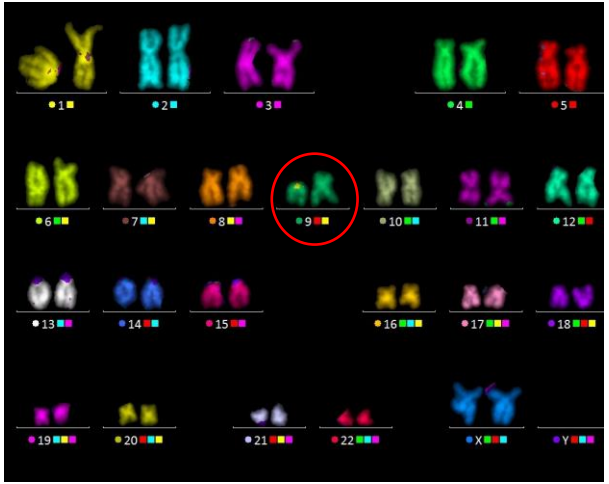
D.



Karyogram of U87 glioblastoma cells

Karyogram of the Cal 51 human breast carcinoma cells



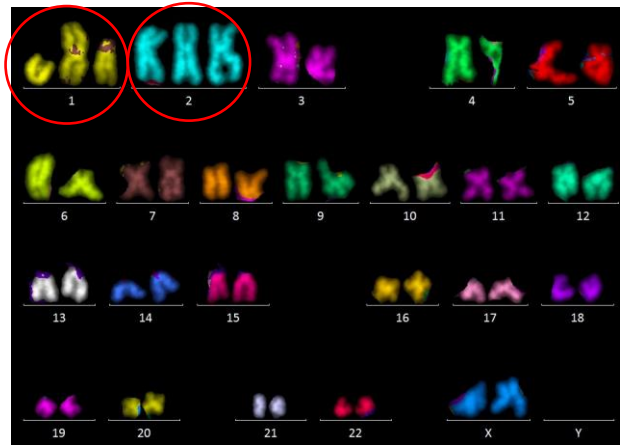


Normal diploid karyotype 22, XX
311 cells were analyzed, including:

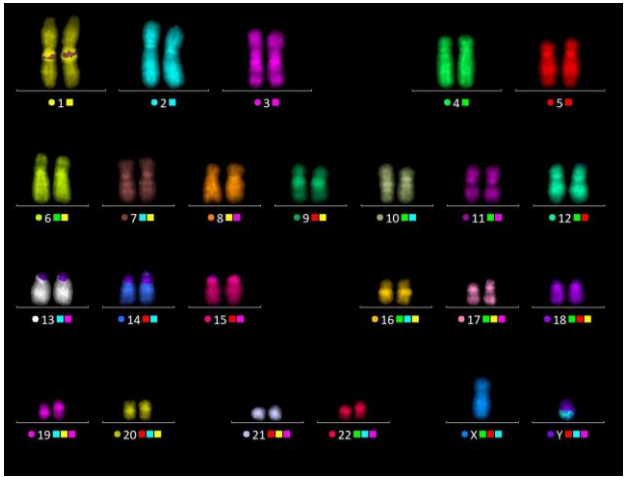
Numerical aberrations 9/311:
trisomy 2’ – 3/311;
trisomy 1’ (T1’) – 1/311;
trisomy 5’; 8’;9’;22’ - 4/311

Structural aberrations: 19/311
6,1% of aberrant cells
20 aberrations (6,5/100):
ace + T’ – 5/20 (1,6)
T’ – 8/20 (2,6)
trans – 7/20 (2,3)

Additional chromosome material
3/311



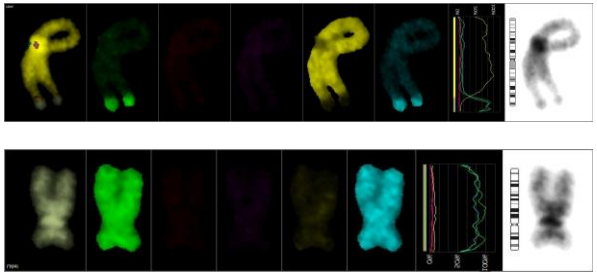
Karyogram of normal dermal human skin fibroblasts (NDHF)



Normal diploid karyotype 22, XY
106 cells were analyzed including:

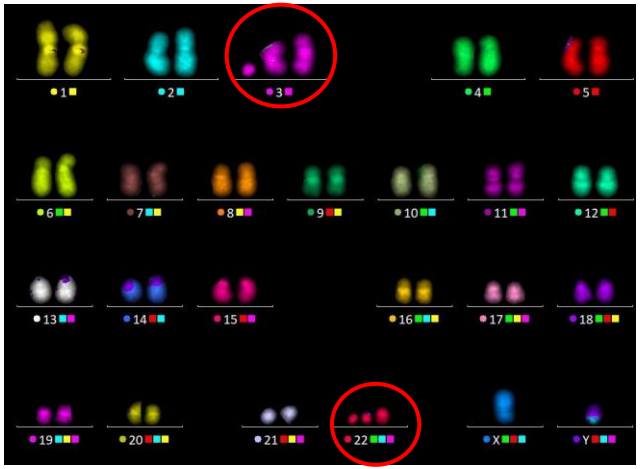
Numerical aberrations - 17/106

- trisomy 4’ (T4’) – 1/106;
- trisomy 5’ - 2/106
- Trisomy 6’ – 1/106
- trisomy 8’ (T 8’) – 5/106;
- trisomy 10’ – 2/106;
- trisomy 11’ – 1/106
- trisomy 12’/106 – 1/106
- Trisomy 15’/106 – 1/106
- trisomy 21’/106 – 1/106
- trisomy 22’/106 – 2/106



Structural aberrations - 6/106
3,7 % of aberrant cells
4,6/100 aberrations:

- ace 3 + T 3';
- T 4'; - add 4';
- ace 6 + T 6';
- tr 1'-10 + T 10';
- tr 15'-4 - add mat 4 and add 15';



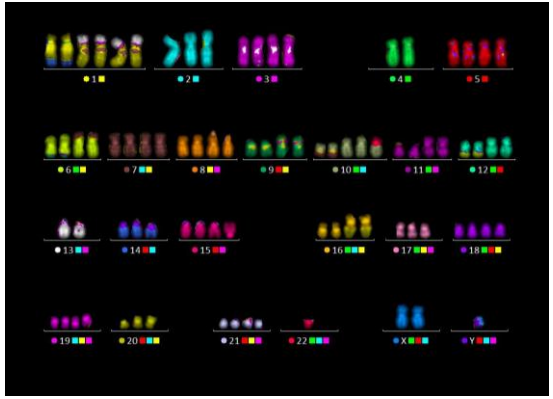
Summary

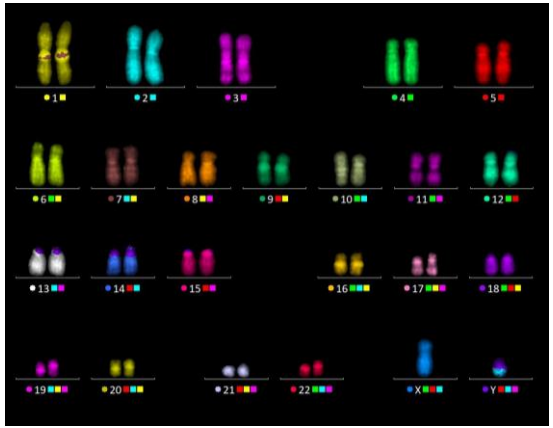
1. Long-term culture of mammalian cells leads to numerical and structural changes of chromosomes; therefore, the chromosome complement and genetic stability of cell lines should be controlled.

2. The NDHF has a normal human karyotype of 22, XY with a low level of spontaneous aberrations and numerical changes.

3. The Cal 51 breast carcinoma cell line has a normal human karyotype of 22, XX with a low level of spontaneous aberrations and numerical changes, which is extremely rare observeb in tumor cell lines.

4. The Human glioblastoma U87 line shows highly rearranged but stable hypotetraploid karyotype (modal number of chromosomes 80) with multiple translocated and truncated chromosomes and additional spontaneous aberrations.





Thank you for your attention

My deep gratitude is expressed to my supervisor, Dr. Elena Nasonova, for guiding me throughout the research process as well as contributing to the results of this research. Thanks to my colleagues Tatiana Hramko and Marina Krupnova in the Laboratory of Radiation Biology - JINR for supporting me in preparing the cells with the best quality for this research.

Thanks to the 2025 Grant number 24-701-03 (Order 1091, 27.12.2024) from the Association of Young Scientists and Specialists of JINR (AYSS) for funding and giving me more motivation to carry out the report.