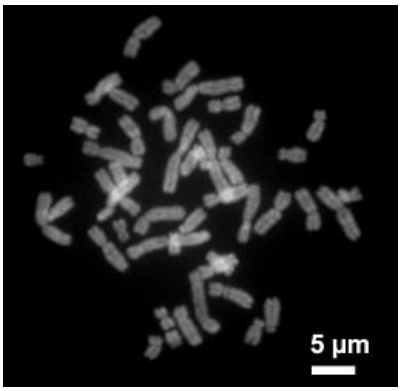
Human karyotype, methods of its examination

Human karyotype - set of 46 chromosomes (number – species specific characteristic)

- 22 homologous pairs of autosomes and 1 pair of sex chromosomes
- arranged and numbered according to the chromosome size and type (centromere position) and banding pattern into homologous pairs and seven chromosome groups
  - **Group A** (1-3) – large metacentric chromosomes (but chr. 2 – submetacentric)
  - **Group B** (4-5) – large submetacentric
  - **Group C** (6-12, X) – medium-sized submetacentric
  - **Group D** (13-15) – medium-sized acrocentric
  - **Group E** (16-18) – short submetacentric
  - **Group F** (19-20) – short metacentric
  - **Group G** (21-22, Y) – short acrocentric (but chr. Y – submetacentric)



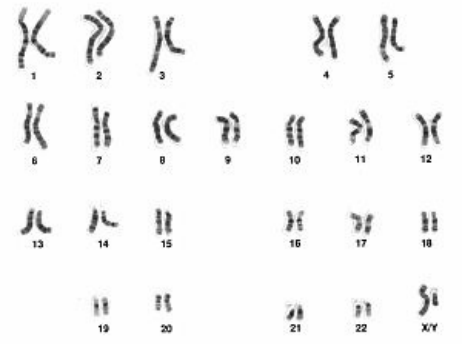
Chromosomes

Karyotype analysis

Karyotype analysis (***karyotyping***) – analysis of banded chromosomes in light microscope; chromosomes are visible only in condensed form during cell division – cell cultivation is necessary.

Samples: in general – nuclear and dividing cells (e.g. erytrocytes or neurons are excluded); in practise – routinely only some types of samples are used:

- **prenatally** – chorionic villi sampling (CVS), amniotic fluid, fetal blood (PUBS – percutaneus umbilical blood sampling), tissues of spontaneously aborted fetus;
- **postnatally** – peripheral blood (lymphocytes), bone marrow, fibroblasts.


Human male karyotype

Cultivation – incubator (37 ºC, culture medium, stimulation of growth), 72 hours (blood samples), longer – approx. 2 weeks (prenatal, tissue cultures).

Colchicine – stops the cell division, interacts with the spindle, cells are arested in metaphase (c-metaphase)
Cytogenetic processing: hypotonic saline, fixation, dropping of the cell suspension onto the slide, staining of preparations.

Types of staining

Conventional – only Giemsa, *monochromatic* chromosomes

Differential staining – **banding**

- **G-banding**: trypsin digestion + Giemsa, dark and light bands (dark bands – heterochromatin, gene-poor, usually repetitive sequences; light bands – euchromatin, gene-rich, unique sequences)
- **Q-banding**: historically first banding; quinacrine – non-stable fluorescent dye, the same banding pattern as G-bands
- **R-banding**: reverse banding pattern to G-bands; alkaline reagents and high temperature treatment

Selective staining

- **C-banding**: staining after denaturation in alkaline solutions; selectively highlights centromeres and another constitutive heterochromatin regions (namely in chromosomes 1, 9, 16 and Y)
- **Ag-NOR banding** (silver staining): selectively stains nucleolar organizing regions (NORs) located on the satellite stalks of the acrocentric chromosomes