ZNANSTVENI SASTANAK

GENOMSKE TEHNOLOGIJE

prehrambeno biotehnološki fakultet
Sveučilište u Zagrebu

Genomika CHO stanica
- dosezi i izazovi u farmaceutskoj biotehnologiji -

CHO cells genomics

- trends and challanges in pharma biotechnology -

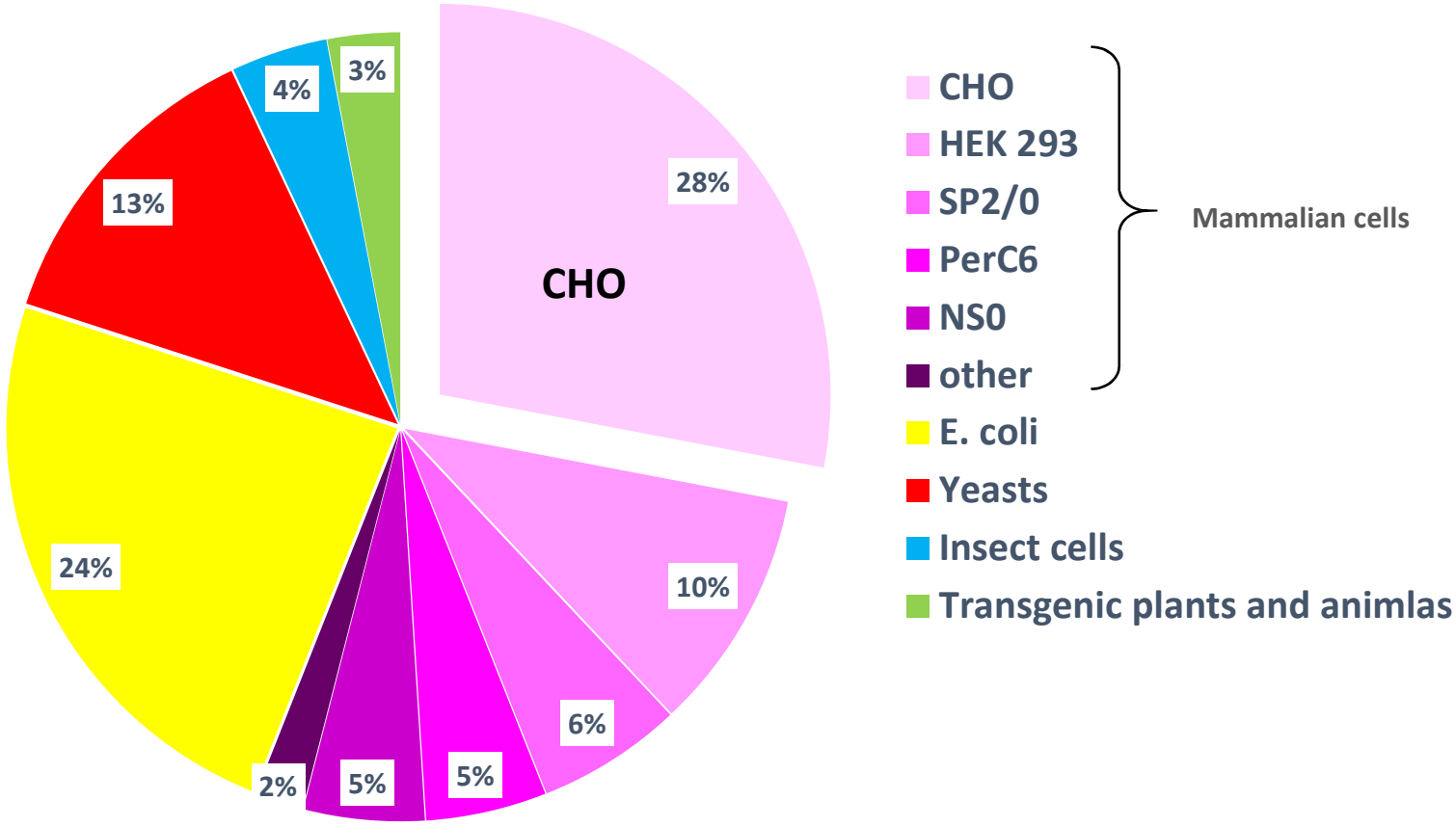
Igor Slivac

Chinese hamster ovary (CHO) cells

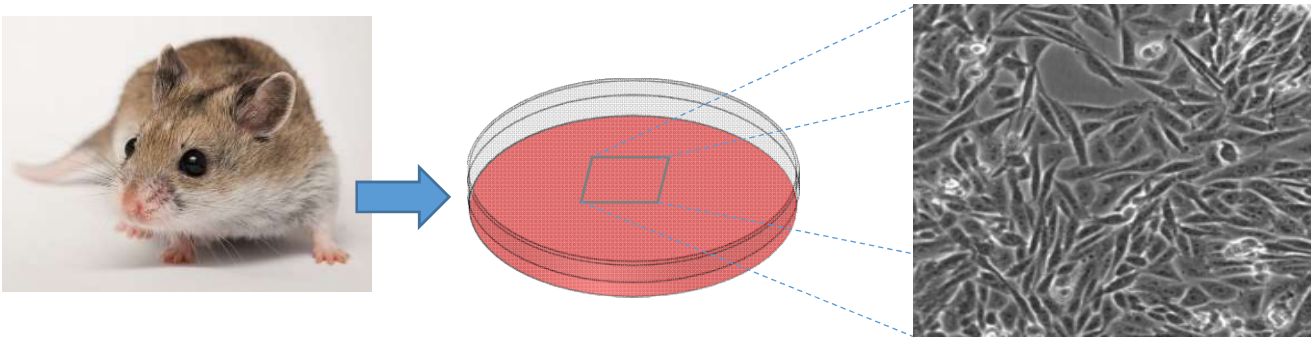
T. Puck (1957) from *Boston Cancer Research Foundation*

Chines hamster (*Cricetulus griseus*)

Expression systems for therapeutic protein production



Reasons for CHO cell success in biotechnology



Small number of chromosome

- Human 46; Mouse 48 (Lab strains 40); Chinese hamster 22

Frequent, easily quantified chromosomal abberation

- At the time (cold war, nuclear weapon testing) CHO cells were used as standard for irradiation research

Large number of genetic variants available

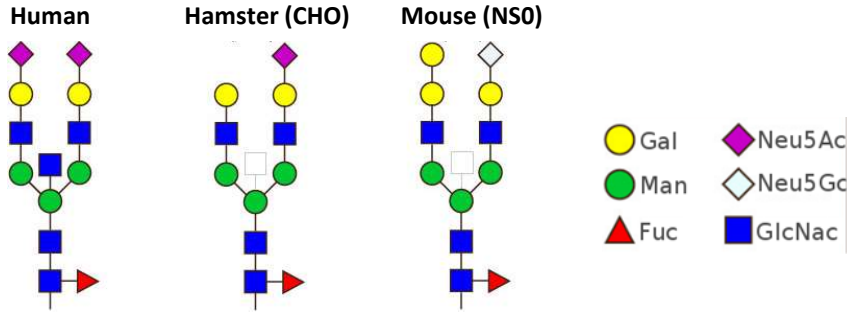
- Modified by irradiation or viral transduction
- Mutants for amino-acid (gs-) or nucleotide synthesis (dhfr-)

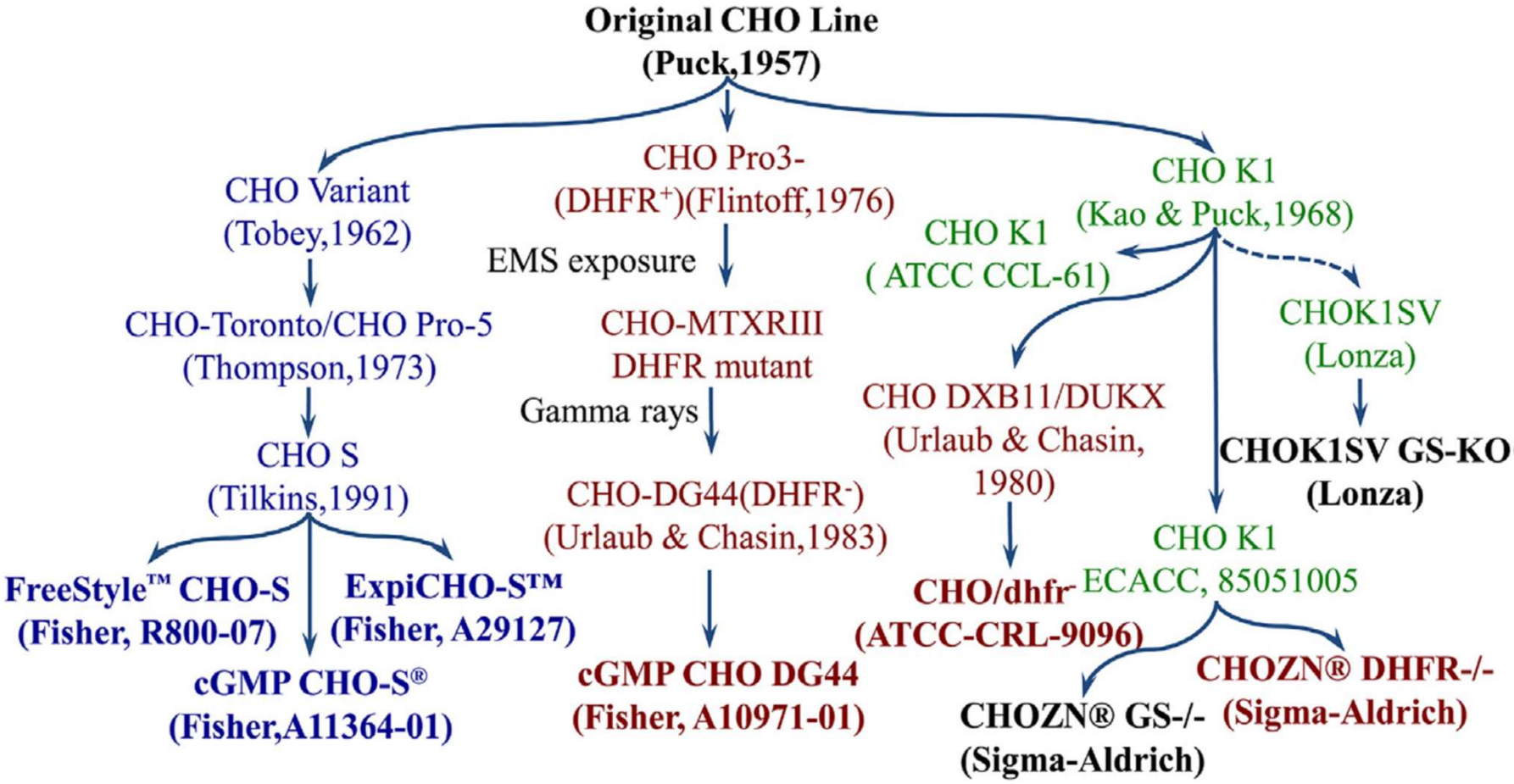
Lacks significant number of viral receptors

- Resistant to viral infection/contamination

Posttranslational protein modifications → human-like

- Standard disulfide bonds
- Glycosylation pattern similar to human





CHO cell lineage

The generation of nutritional auxotrophs led to the development of a powerful gene amplification systems used to produce therapeutic proteins in CHO cells.

N. Xu et al. / Biochemical Engineering Journal 124 (2017) 122–129

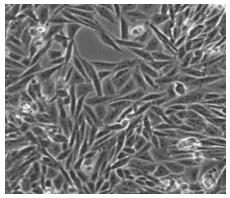
CHO cell line development for stable r-protein production

Cricetulus griseus

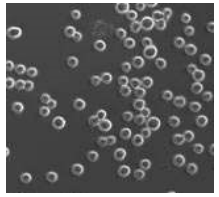
Cell isolation
(Puck, 1957)

Cell
immortalization

Production
CHO cell line
(1980s)



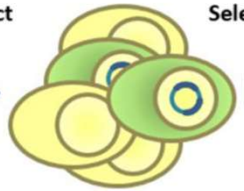
Adherent



Suspension

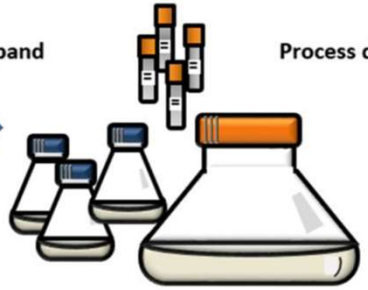
Transfect

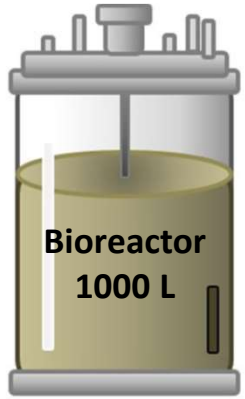
Select/clone



Screen/expand

Process development

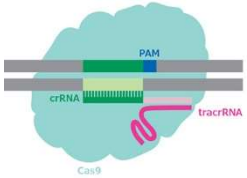


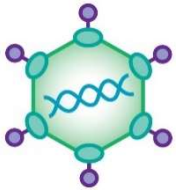


Transfection, random integration, selection, expansion

4-6 months

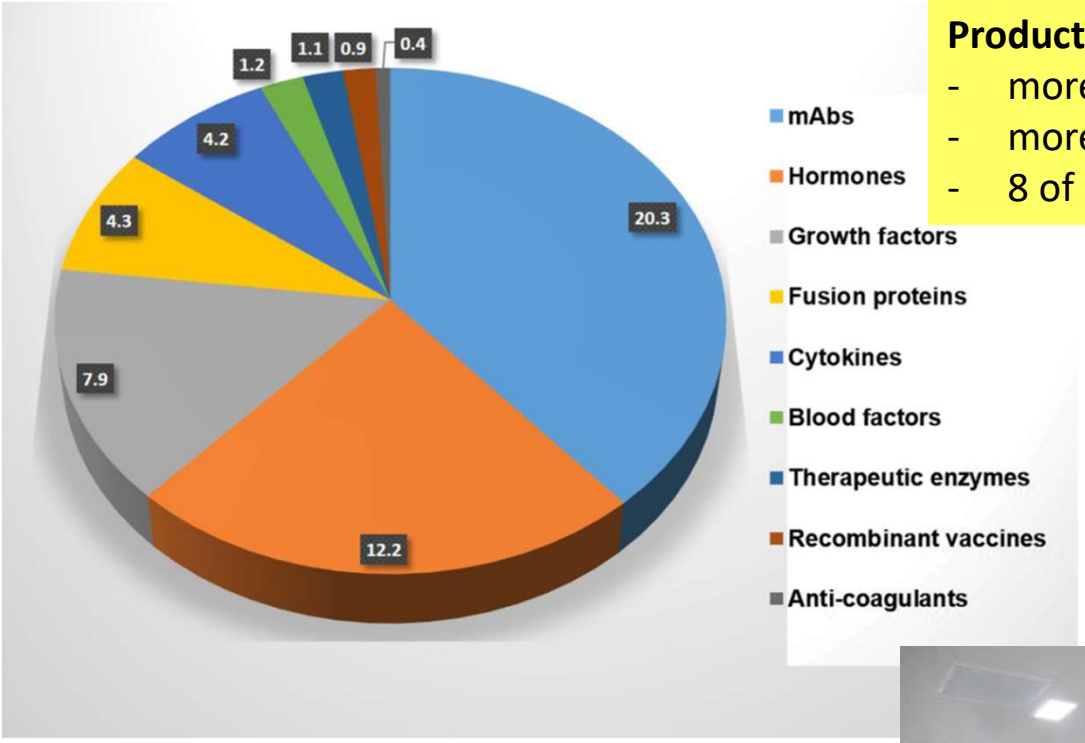
Site-specific integration and
Lentiviral tranduction





2 months

Mammalian cell culture technology (2016)



Products

- more than 100 biotherapeuticals (mAbs 20%)
- more than 400 in the pipeline
- 8 of 10 best-selling drugs

Platform technology

- Cells grow in suspension
- Cell doubling time 18-24h
- Protein free growth media
- r-protein production:
  IgG → standard 3 g/L
  titers up to 20 g/L reported

Lewis N.E. et al. (2013) Genomic landscapes of Chinese hamster ovary cell lines as revealed by the *Cricetulus griseus* draft genom. *Nat. Biotech.* 8 (**31**) 759–765.

Brinkrolf, K. et al. (2013) Chinese hamster genome sequenced from sorted chromosomes. *Nat. Biotechnol. 31*, 694–695

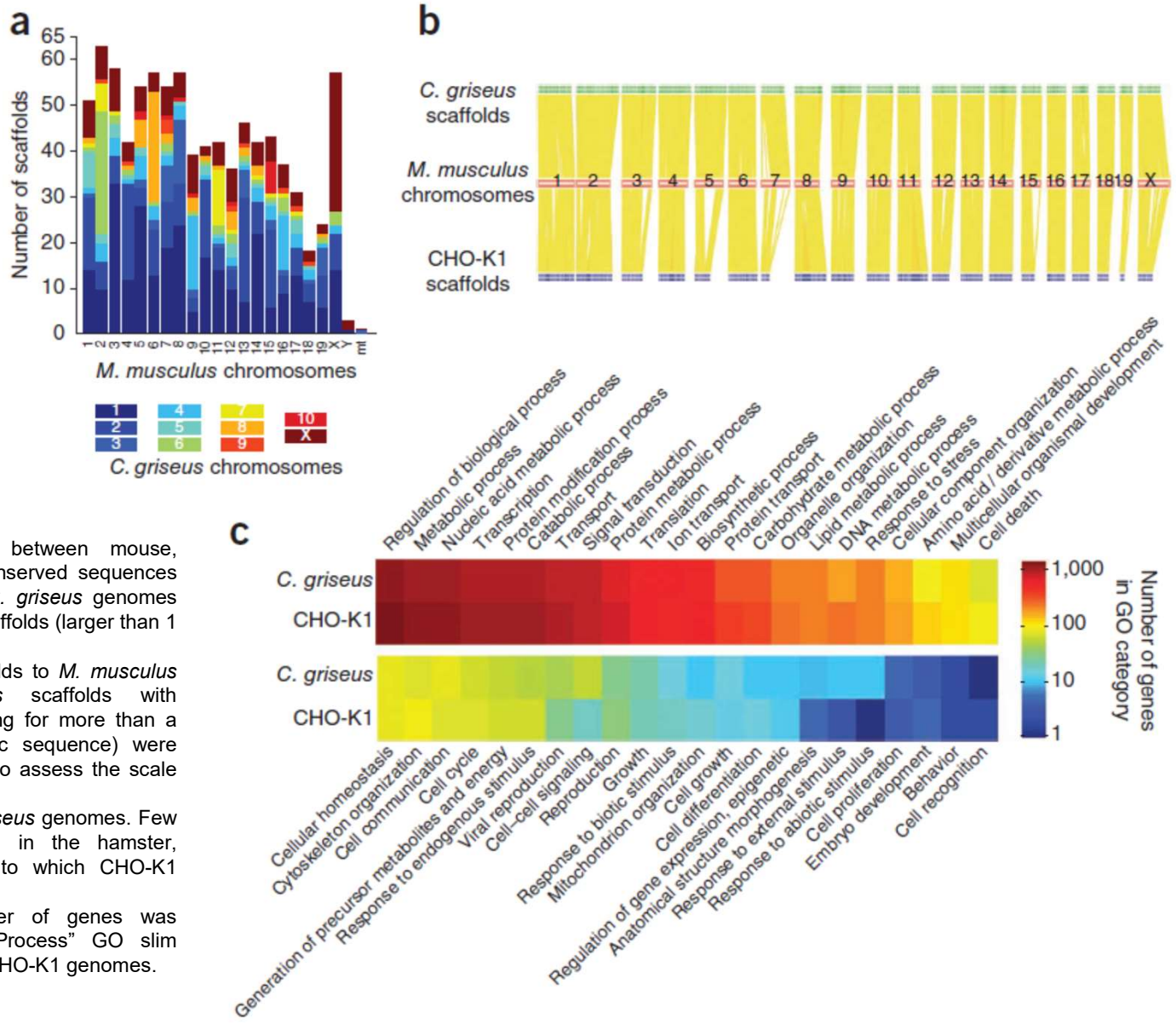
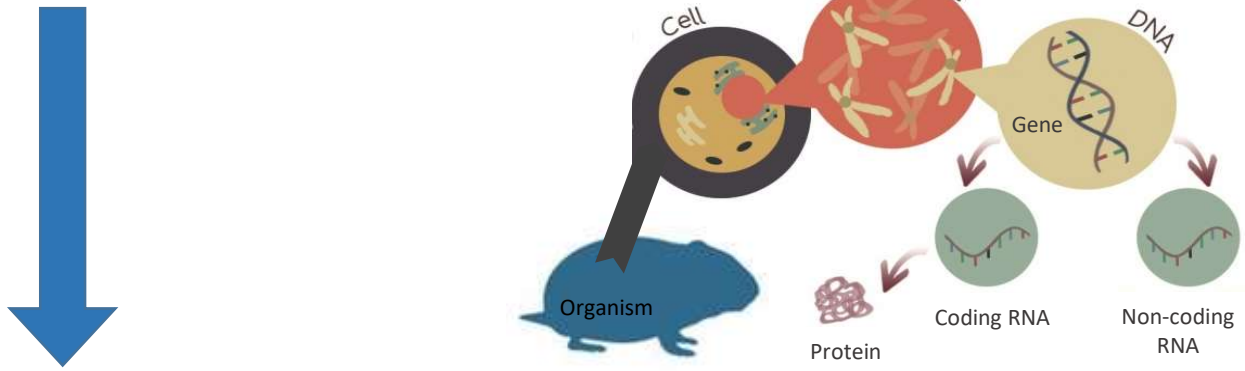


Figure 2. Genome comparison between mouse, Chinese hamster and CHO-K1. Conserved sequences among the mouse, CHO-K1 and *C. griseus* genomes were determined by aligning their scaffolds (larger than 1 Mb) to the mouse genome.
(**a**) Assignment of *C. griseus* scaffolds to *M. musculus* chromosomes. The *C. griseus* scaffolds with chromosomal assignment (accounting for more than a quarter of the 2.4 Gb of genomic sequence) were compared to mouse chromosomes to assess the scale of chromosomal rearrangement.
(**b**) Alignment of CHO-K1 and *C. griseus* genomes. Few large DNA stretches are missing in the hamster, whereas there are more regions to which CHO-K1 scaffolds could not align.
(**c**) Gene annotation. The number of genes was determined for each “Biological Process” GO slim category in both the *C. griseus* and CHO-K1 genomes.

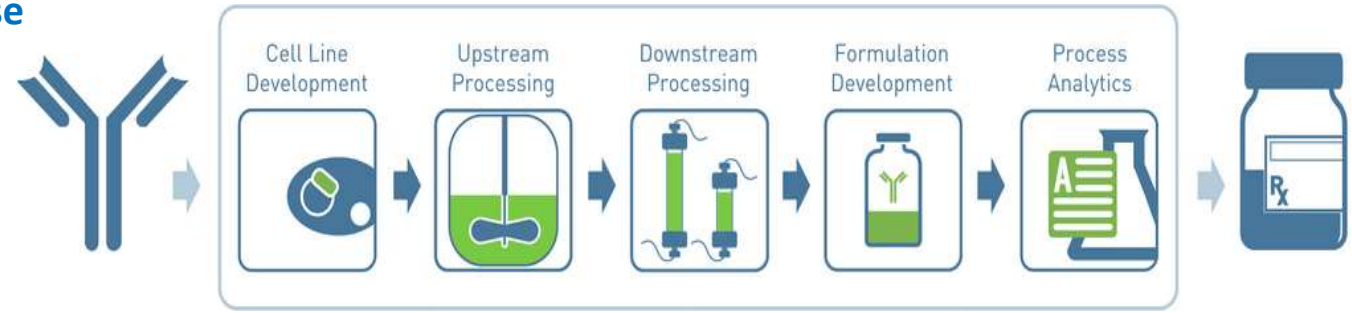
CHO genome:

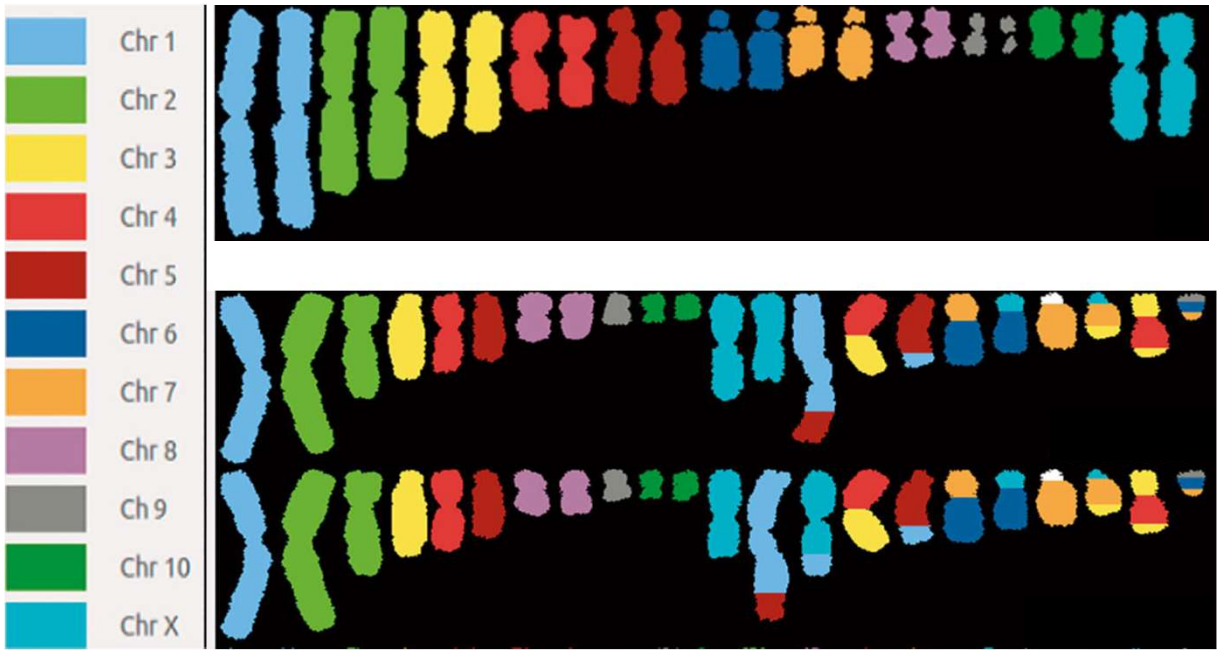
- ca. **2.35 Gb**, ca. **28k genes** and 10-13k expressed in every cell
- Contains genes for more than **100 cell types**
- Multiple levels of regulation: chromatin state, transcription, post-transcription, translation, post-translation, protein activity



Reflection in biotech therapeutic production:

- High genomic **variation** even within clonal population → subclones
- Gene expression **instability**
- **Lack** of production process predictability
- **Big effort** in GMP and QC documentation
- **Delay** in product commercialization
- Product **price increase**

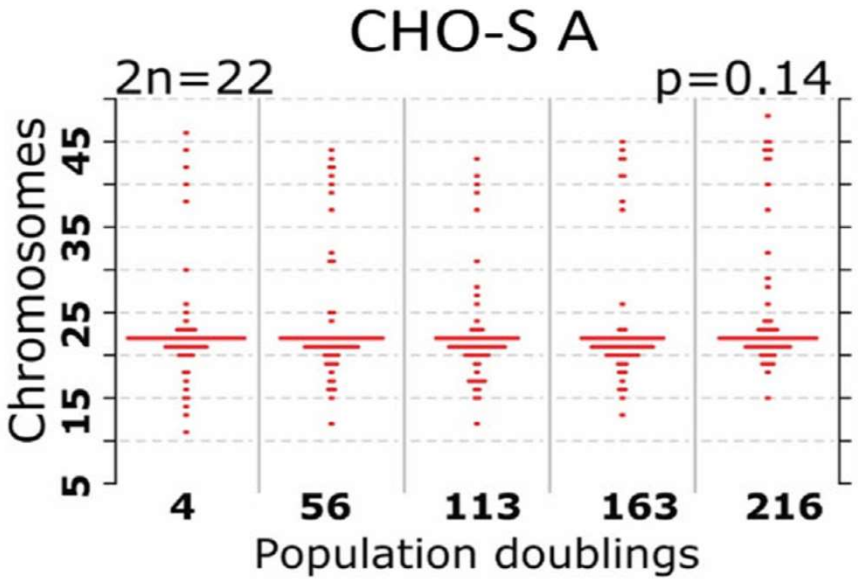




Chromosomes isolated from fibroblsats of female chinese hamster

CHO-S T0

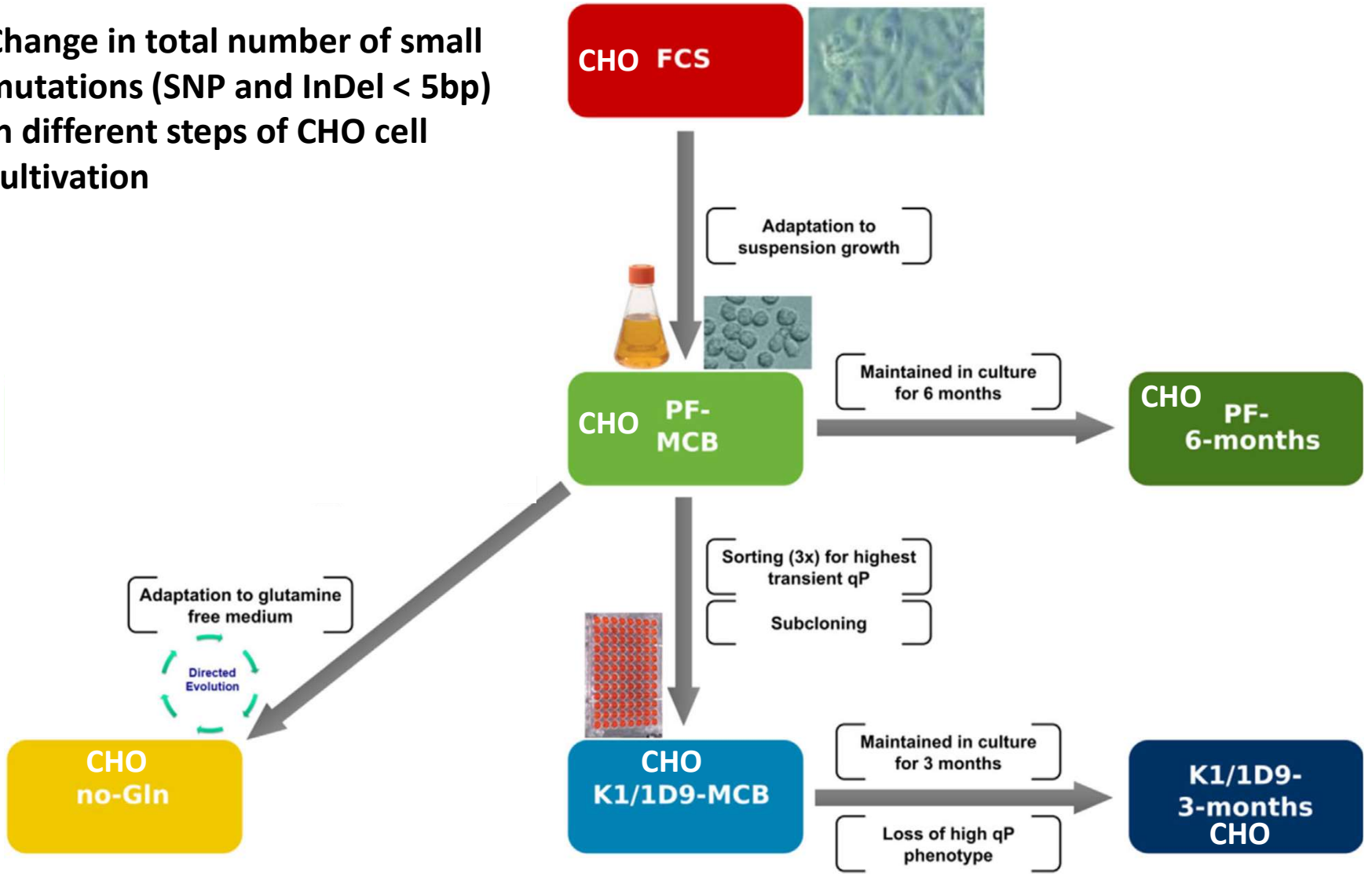
CHO-S T216



Chromosome number variation during CHO cells cultivation (216 cell doublings)

Veclar S. et al (2018) Karyotype variation of CHO host cell lines over timein culture characterized by chromosome counting and chromosome painting. *Biotech. Bioeng.*115:165–173

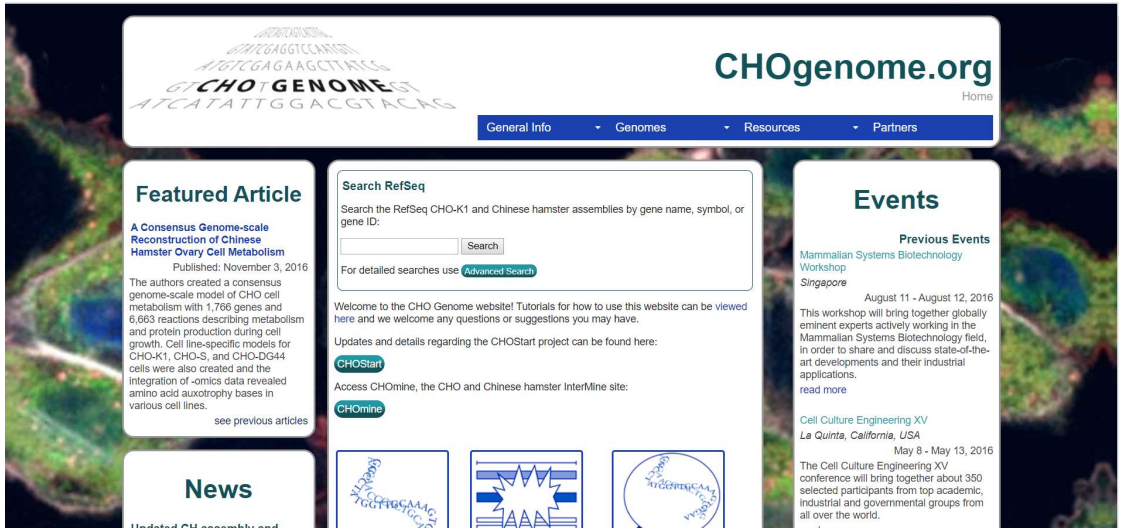
Change in total number of small mutations (SNP and InDel < 5bp) in different steps of CHO cell cultivation



Feichtinger J. et al (2016) Comprehensive Genome and Epigenome Characterization of CHO Cells in Response to Evolutionary Pressures and Over Time, *Biotechnol Bioeng* 2016 113(10):2241-53

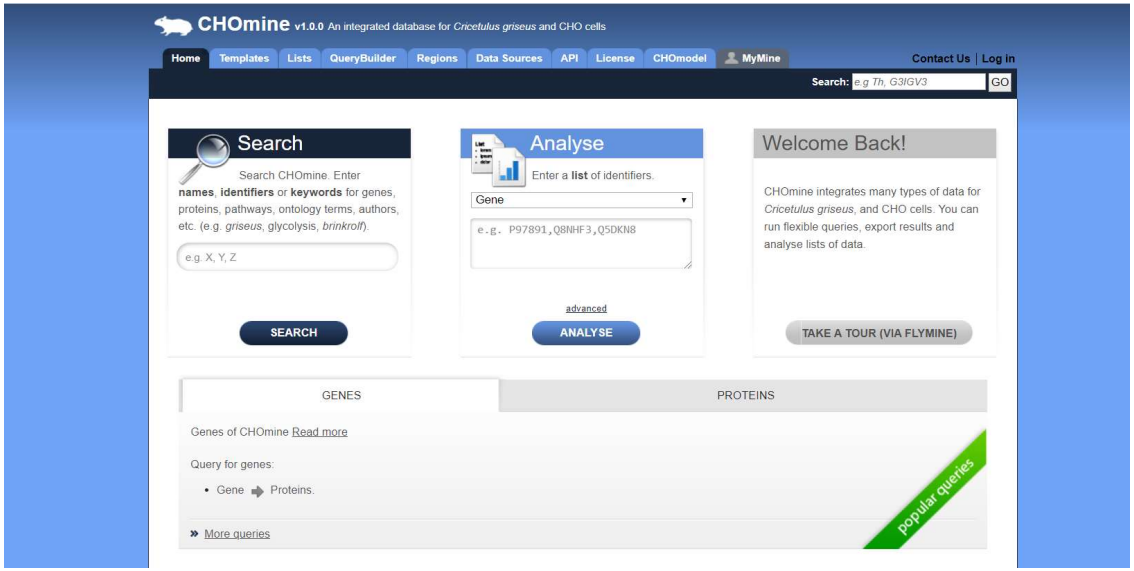
Data mining tools

https://chogenome.org



- The access point for all publicly available genome-wide data of chinese hamster and CHO cell lines
- CHOgenome-specific literature
- CHO-specific BLAST service

https://chomine.boku.ac.at



- A warehouse for CHO genome data with analysis tools
- Provides links to external websites and integrates recently published genome scale models
- Translates gene IDs and names
- Support cell line engineering approaches and CHO cell bioprocesses

CHO cells in Croatian biotech

PLIVA

Biosimilar production (rhEPO) in CHO cells

Parnham M.J. *et al.* (2007) Non-clinical safety studies on biosimilar recombinant human erythropoietin 100(2):73-83.

Pfizer

Hospira

Biosimilar production → filgrastim

Non-glycolyzed form in *E. coli*

CHO?

BIOtech

Student graduation theses

prehrambeno biotehnološki fakultet

Sveučilište u Zagrebu

Laboratory for cell culture technology and biotransformation

Current HRZZ rsearch project *Hydro-pep-cell* (2017) (prof. V. Gaurna Srček)

- growth media optimisation for improved (CHO) cell cultivation

Thank you for your attention!