

# CG020 Genomika

## Přednáška 12

### Praktické aplikace genomiky

Jan Hejátko

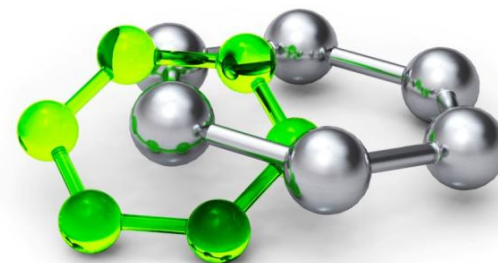
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**M U N I**  
**S C I**



# Literatura

- Literární zdroje ke kapitole 12:

**Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X., Streithorst, J.A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C.Y., Guevara, H., Wadford, D.A., Chen, J.S., and Chiu, C.Y. (2020).** CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol* **38**, 870-874.

**Dietel, M., and Sers, C. (2006).** Personalized medicine and development of targeted therapies: The upcoming challenge for diagnostic molecular pathology. A review. *Virchows Arch* **448**, 744-755.

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**Goh, K.I., Cusick, M.E., Valle, D., Childs, B., Vidal, M., and Barabasi, A.L. (2007).** The human disease network. *Proc Natl Acad Sci U S A* **104**, 8685-8690.

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**Koblan, L.W., Erdos, M.R., Wilson, C., Cabral, W.A., Levy, J.M., Xiong, Z.M., Tavarez, U.L., Davison, L.M., Gete, Y.G., Mao, X., Newby, G.A., Doherty, S.P., Narisu, N., Sheng, Q., Krilow, C., Lin, C.Y., Gordon, L.B., Cao, K., Collins, F.S., Brown, J.D., and Liu, D.R. (2021).** In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. *Nature*.

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# Osnova

- Lékařství
  - Molekulární diagnostika
  - Individualizovaná medicína
  - Genová terapie
- Biotechnologie
- Geneticky Modifikované Organismy
  - Transgenóza
  - Editování genomu
- Modelové organismy
- Principy PCR

# Osnova

- Medicine
  - Molecular Diagnosis

# Molekulární Diagnostika

- Cca 10,000 onemocnění u člověka je podmíněno mutací v jediném genu
  - cystická fibróza
  - srpkovitá anémie
  - svalová dystrofie
  - $\beta$ -talasémie
  - ....
- Časná molekulární diagnostika
  - mutace nebo infekce
    - PCR
    - Hybridizace na DNA čipu hybridization
    - Cas-based

# Molekulární Diagnostika

- Mammoth Biosciences

- Spoluzakladatelka Jenifer Doudna

<https://youtu.be/IPe4IdgKGdQ>



# Outline

- Medicine
  - Molecular Diagnosis
  - Personalized Medicine

# Individualizovaná Medicína

- Využívá znalost **genomu** pro:
  - Předpověď zdravotních rizik
  - **Diagnositku**
  - Výběr nejvhodnějšího typu léčby
  - **minimalizuje nežádoucí efekty léčby**
  - **prevence**



# Individualizovaná Medicína

What is Personalized Medicine?

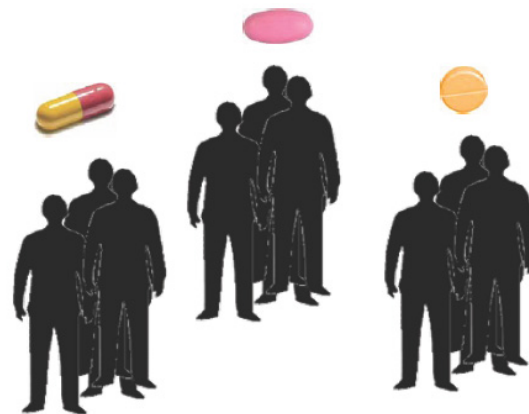
Current Practice



One size fits all

Trial and error

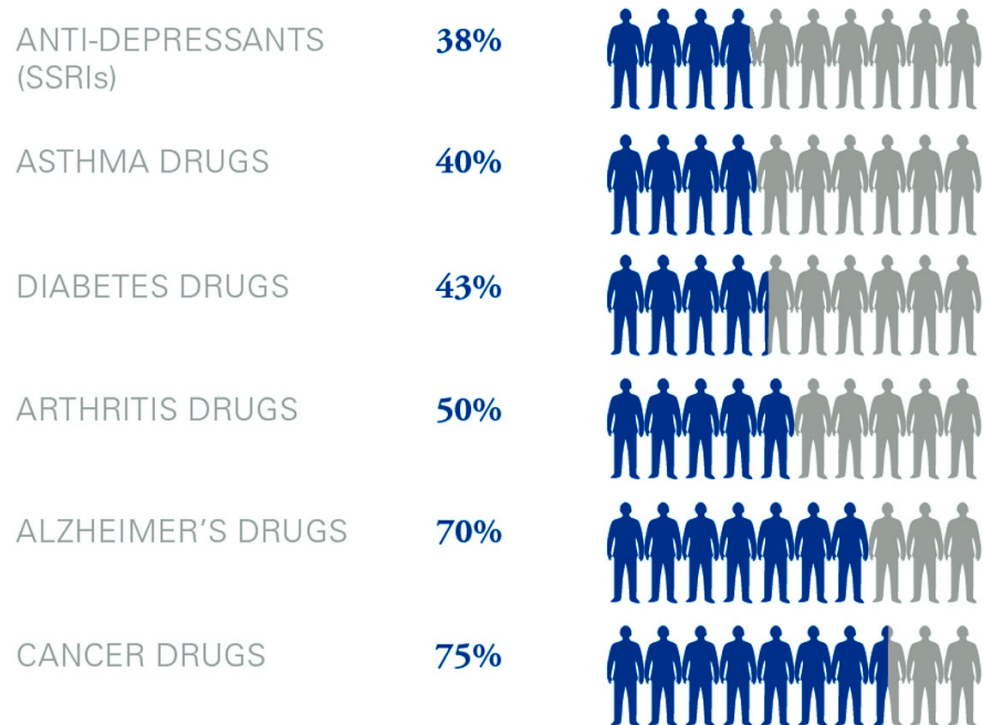
Personalized Medicine



The **right treatment** for  
the **right person** at the  
**right time**

# Individualizovaná Medicína

## PERCENTAGE OF THE PATIENT POPULATION FOR WHICH A PARTICULAR DRUG IS INEFFECTIVE, ON AVERAGE



- Just in hospitals: about 6.7% of patients (2.2 million) experience serious adverse drug reactions



Serious adverse drug reactions in even smaller percentages of treated populations have led to the withdrawal of several drugs from the market

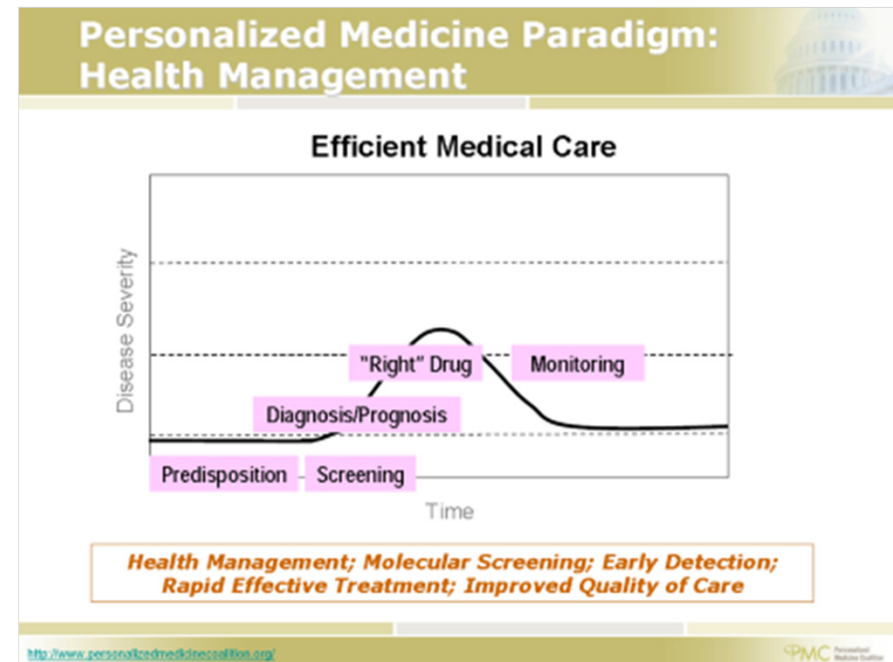
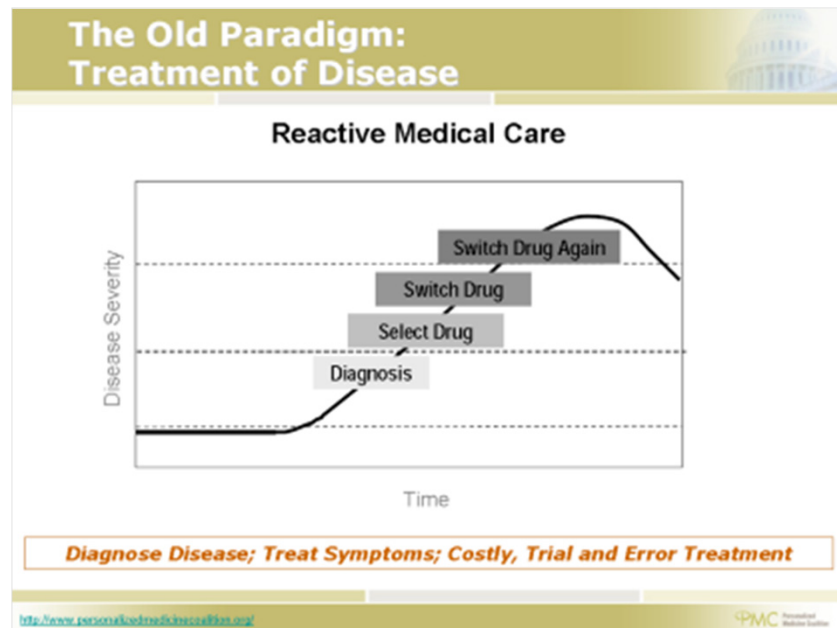
Zelnorm      Vioxx      Cylert

“Are good drugs going to the wrong people?”

Rezulin      Baycol      Lotronex\*

Source of data: Brian B. Spear, Margo Heath-Chiozzi, Jeffery Huff, “Clinical Trends in Molecular Medicine,” Volume 7, Issue 5, 1 May 2001, Pages 201-204.

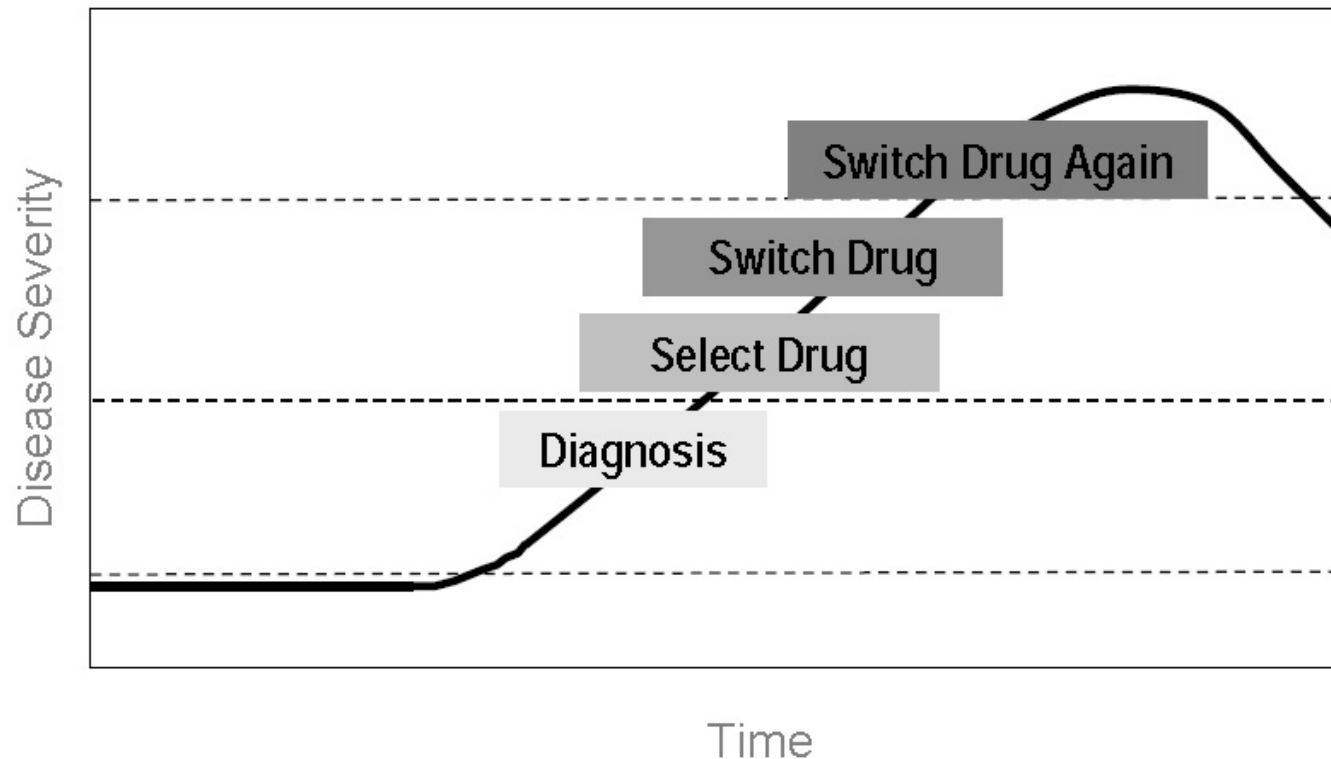
# Individualizovaná Medicína



# The Old Paradigm: Treatment of Disease



## Reactive Medical Care

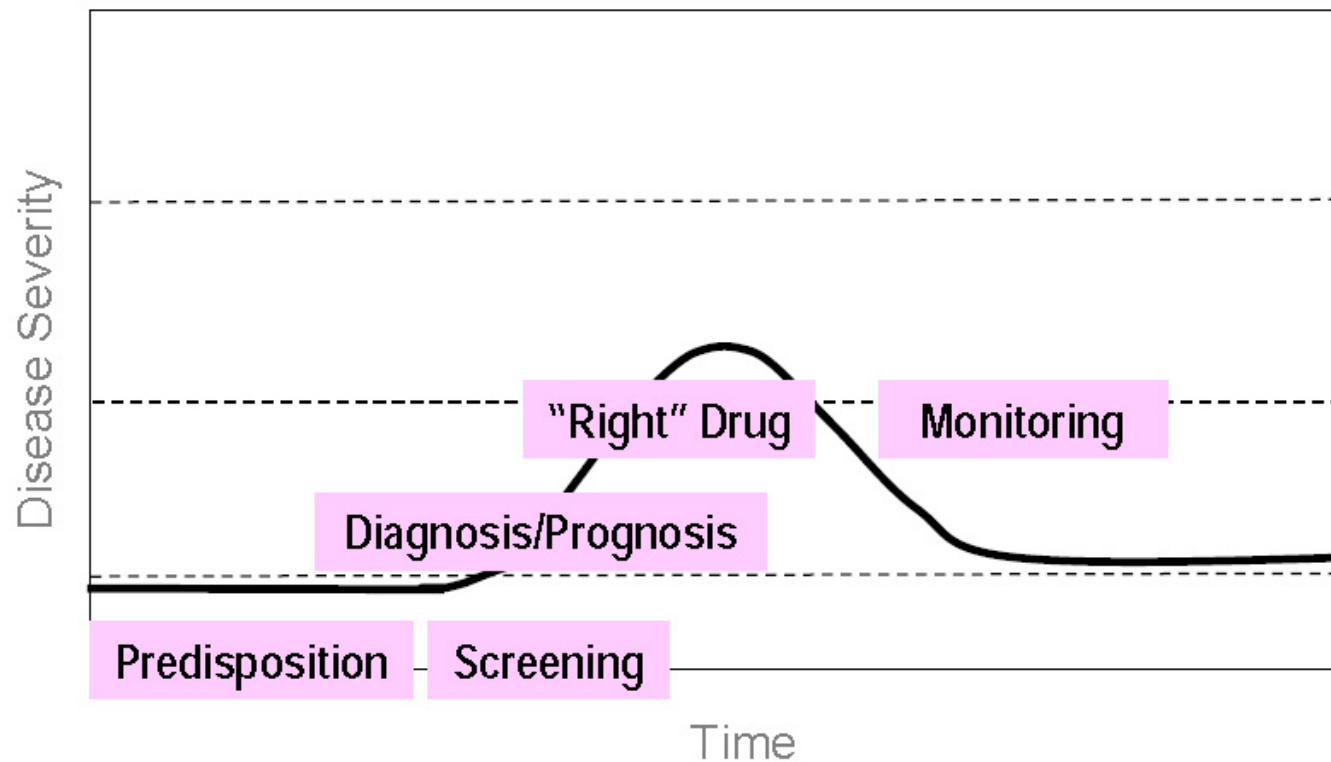


***Diagnose Disease; Treat Symptoms; Costly, Trial and Error Treatment***

# Personalized Medicine Paradigm: Health Management



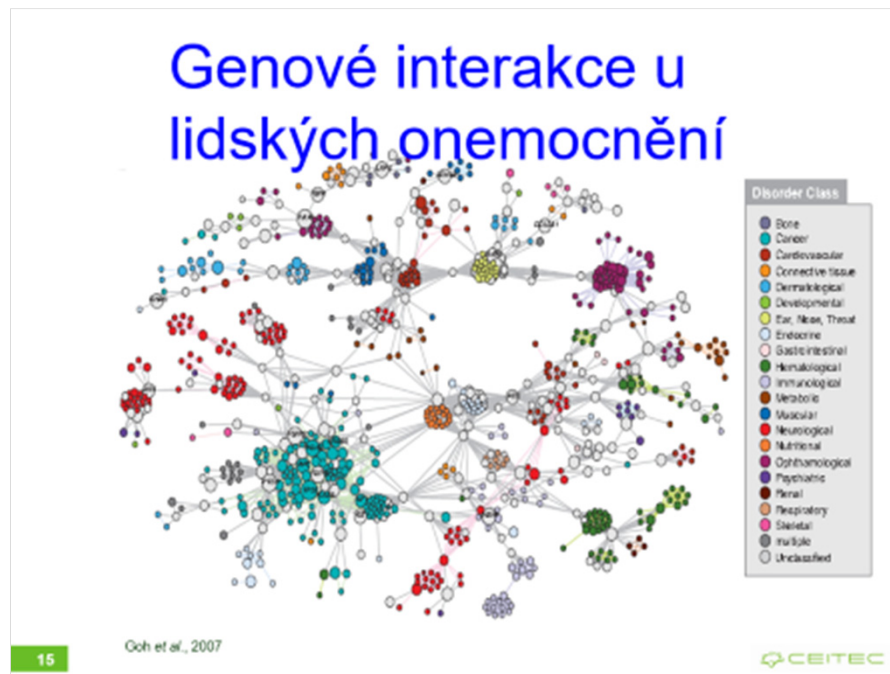
## Efficient Medical Care



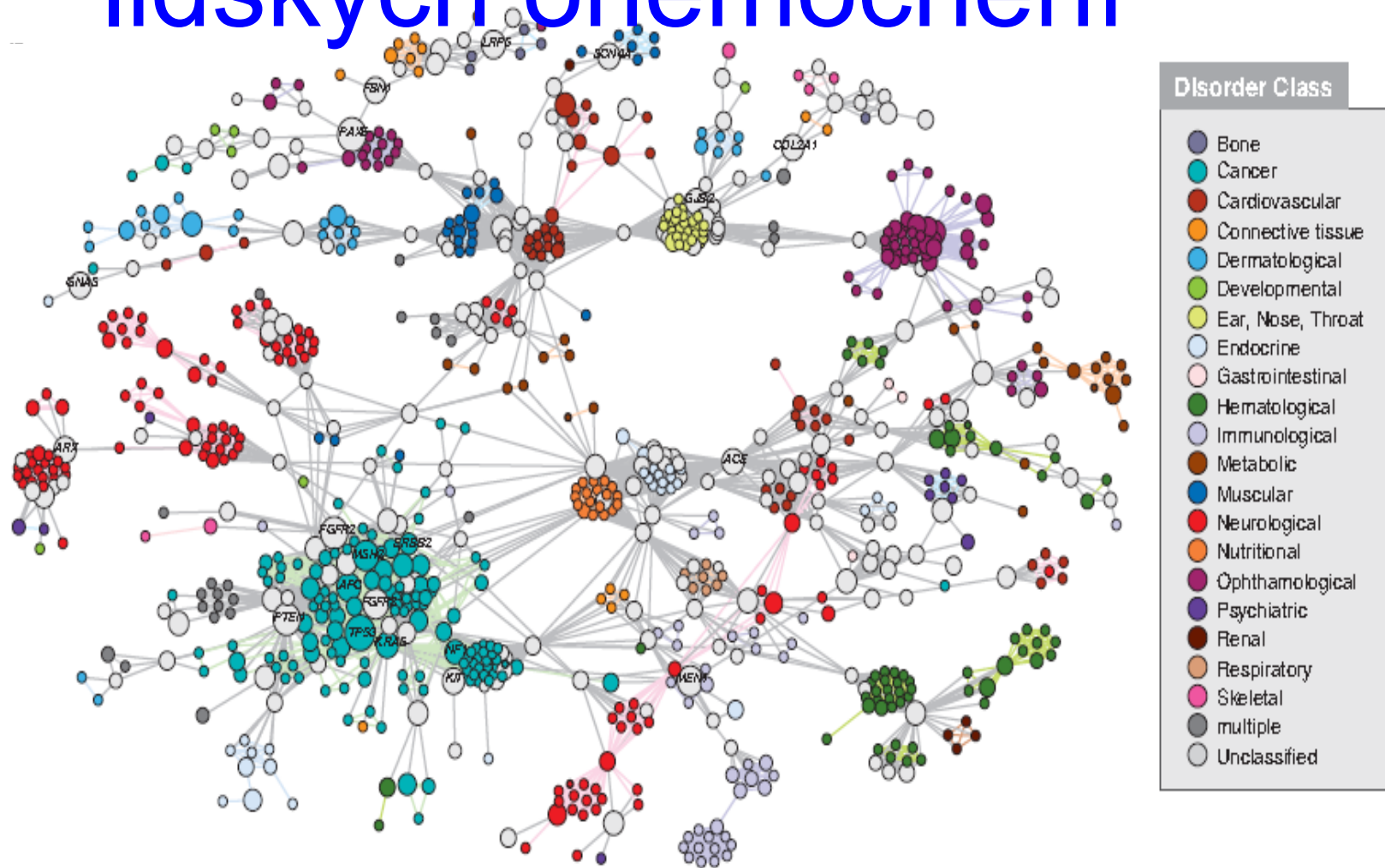
***Health Management; Molecular Screening; Early Detection;  
Rapid Effective Treatment; Improved Quality of Care***

# Individualizovaná Medicína

- Problém:
  - Mnohofaktoriální podmíněnost většiny lidských onemocnění

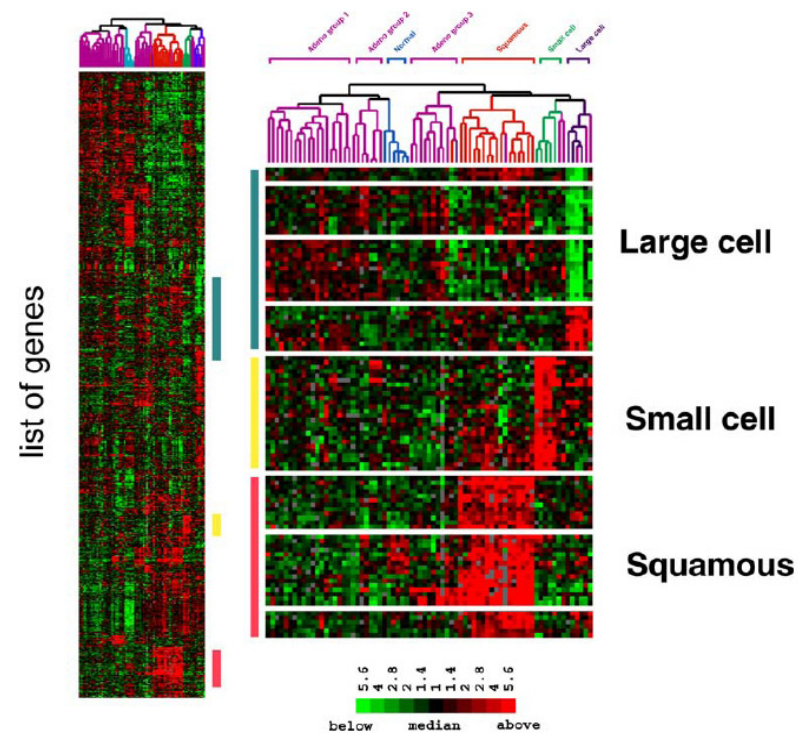


# Genové interakce u lidských onemocnění



# Individualizovaná Medicína

- Řešení problému:
  - **Systémová biologie** – využívá např. **genové klastrování** k identifikaci genů asociovaných s pozorovaným jevem (nemocí, poruchou, ..)





# Individualizovaná Medicína

- Řešení problému:
  - biomarkery
  - testy

**Table:** Selected Personalized Medicine Drugs, Treatments and Diagnostics as of September 2011 \*

*Indications in quotes and otherwise unattributed, are cited from the therapeutic or diagnostic product label.*

*Therapeutic product labels contain pharmacogenomic information as:*

Information only

Recommended

Required

Unhighlighted products have no pharmacogenomic information, recommendations or requirements in the label.

THERAPY	BIOMARKER/TEST	INDICATION
Mivacron® (mivacurium)	Cholinesterase gene	<b>Anesthesia adjunct:</b> "Mivacron is metabolized by plasma cholinesterase and should be used with great caution, if at all, in patients known to be or suspected of being homozygous for the atypical plasma cholinesterase gene."
Ansaid® (flurbiprofen)	CYP2C9	<b>Arthritis:</b> "In vitro studies have demonstrated that cytochrome P450 2C9 plays an important role in the metabolism of flurbiprofen to its major metabolite, 4'-hydroxy-flurbiprofen."
Depakote® (divalproex)	UCD (NAGS; CPS; ASS; OTC; ASL; ARG)	<b>Bipolar disorder:</b> "Hyperammonemic encephalopathy, sometimes fatal, has been reported following initiation of valproate therapy in patients with urea cycle disorders [UCDs]...particularly ornithine transcarbamylase deficiency [OTC]."
Aromasin® (exemestane) Arimidex® (anastrozole) Nolvadex® (tamoxifen)	Estrogen Receptor (ER)	<b>Breast cancer:</b> Exemestane is indicated for adjuvant treatment of postmenopausal women with ER-positive early breast cancer. Anastrozole is for treatment of breast cancer after surgery and for metastases in post-menopausal women. Tamoxifen is the standard therapy for estrogen receptor-positive early breast cancer in pre-menopausal women.
Chemotherapy	Mammostrat®	<b>Breast cancer:</b> Prognostic immunohistochemistry (IHC) test used for postmenopausal, node negative, estrogen receptor expressing breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy.
Chemotherapy	MammaPrint®	<b>Breast cancer:</b> Assesses risk of distant metastasis in a 70-gene expression profile.
Chemotherapy	OncoType DX® 16-gene signature	<b>Breast cancer:</b> A 16-gene signature (plus five reference genes) indicates whether a patient has a low, intermediate, or high risk of having a tumor return within 10 years. Low-risk patients may be treated successfully with hormone therapy alone. High-risk patients may require more aggressive treatment with chemotherapy.
Chemotherapy	CompanDx® 31-gene signature	<b>Breast cancer:</b> The test predicts "time to event" for metastasis of breast cancer, following surgery or biopsy.
Faslodex® (fulvestrant)	Hormone Receptor (HR)	<b>Breast cancer:</b> Fulvestrant is indicated for the treatment of hormone receptor positive metastatic breast cancer in post-menopausal women with disease progression following anti-estrogen therapy.
Herceptin® (trastuzumab) Tykerb® (lapatinib)	HER-2/neu receptor	<b>Breast cancer:</b> "...for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER-2 [Human Epidermal growth factor Receptor 2] protein and who have received one or more chemotherapy regimens for their metastatic disease." High levels of HER-2 expression have been associated with increased disease recurrence in breast cancer, but show a better response to trastuzumab.
Pharmaceutical and surgical prevention options and surveillance	BRCA 1/2	<b>Breast cancer:</b> Guides surveillance and preventive treatment based on susceptibility risk for breast and ovarian cancer.
Nolvadex® (tamoxifen)	Breast Cancer Index™ (HOXB13, IL17BR)	<b>Breast cancer:</b> Calculates a combined risk analysis for recurrence after tamoxifen treatment for ER-positive, node-negative breast cancer.

# Individualizovaná Medicína

- Další problémy
  - Etické otázky
    - Možnost zneužití znalosti genomu
    - riziko: nedostatečná ochrana dat
    - V některých zemích je uzákoněn omezený přístup pro určité typy zaměstnanců nebo pojišťovací společnosti
  - Vysoké náklady
    - Dělení medicíny na **first-class** and **low-class** služby
    - Zvětšování problému **globalizačního handicapu** – chudé země si nemohou takto pokročilý typ léčby dovolit
  - Soukromí
    - Zásadní a komplikovaná otázka
    - Jakou informaci lze považovat za soukromou?

# Outline

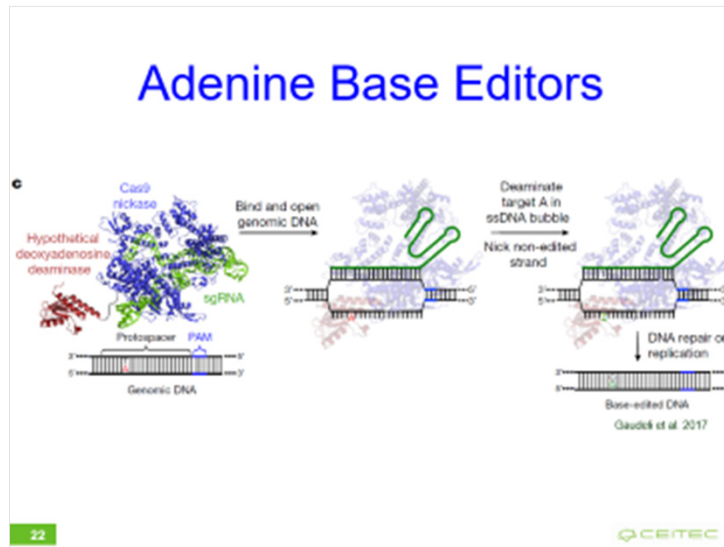
- Medicine
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy

# Gene Therapy

Procedure in which the DNA sequence is inserted into the patient genome to replace or supplement the original gene

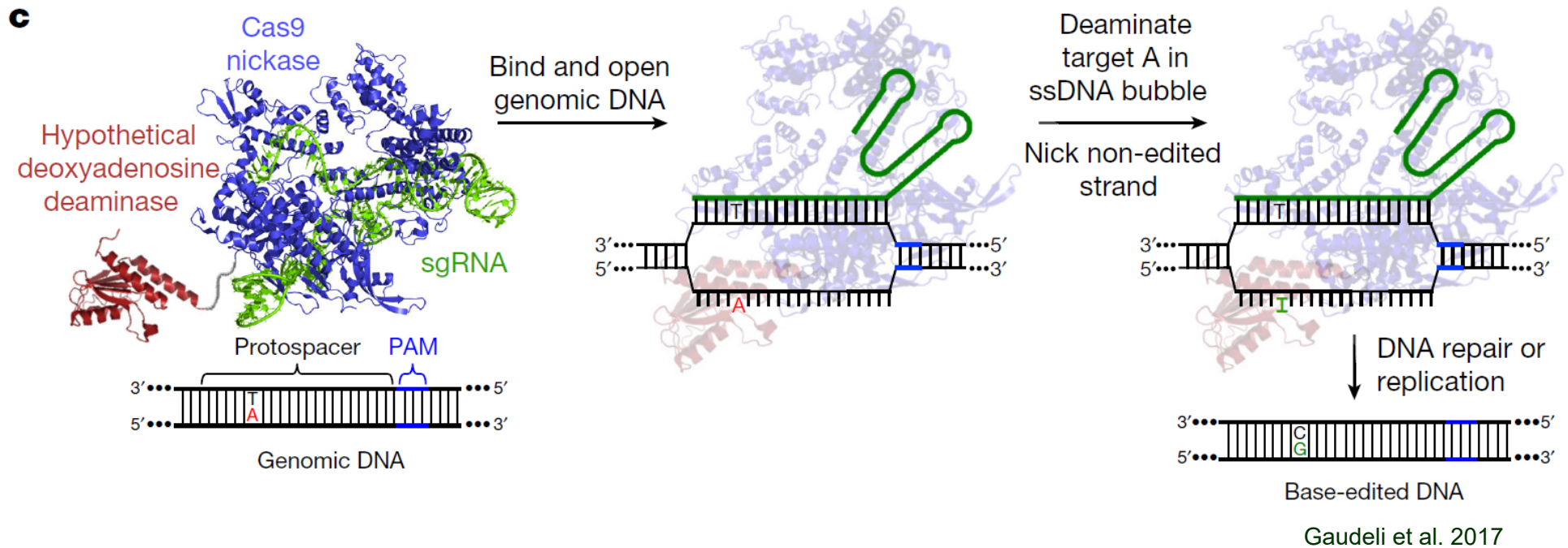
- Options:
  - replace the mutated gene
  - repair the mutation
  - deliver DNA encoding a therapeutic protein
  - antisense therapy
- In the future useful for treating e.g. hereditary diseases
- Types:
  - somatic gene therapy
  - gene therapy of germ cells

# Gene Therapy



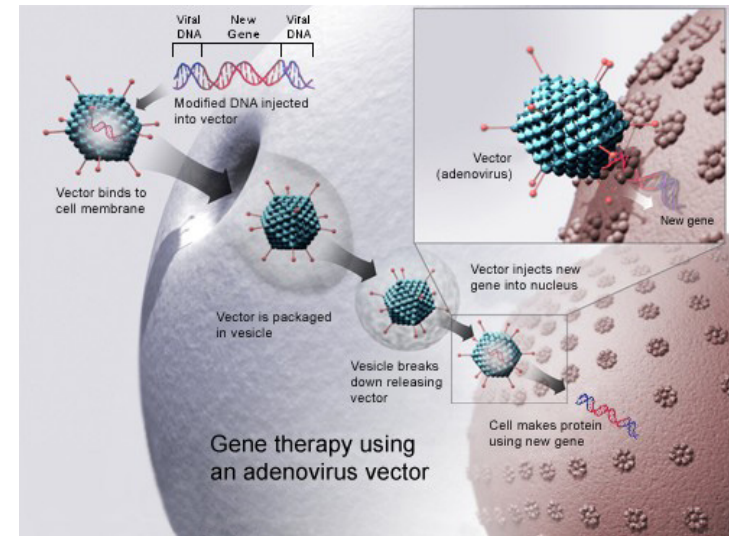
- Hutchinson–Gilford progeria syndrome
  - C•G-to-T•A mutace (c.1824 C>T; p.G608G) v genu pro laminin (*LMNA*)
  - Defekt v sestřihu RNA vede k tvorbě toxického proteoinu **progerinu**
  - Věk dožití cca 14 let
  - **In vivo oprava** pomocí ABEs potvrzena u myší a **lidských fibroblastů** (Koblan et al., 2021)

# Adenine Base Editors



# Gene Therapy

- Methods
  - viral vectors
    - retroviruses
    - adenoviruses
    - herpes simplex virus
  - non-viral methods
    - injection of plasmid DNA into muscle
    - increased efficiency of DNA delivery
      - electroporation
      - sonoporation
      - „gene gun“ (biolistic)
      - magnetofection
  - genome editing



# Ethical Issues

- Regulace editace genomu v zemědělství a lidském zdraví
  - <https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/>
- International Commission on the Clinical Use of Human Germline Genome Editing
  - convened by the U.S. National Academy of Medicine (NAM), the U.S. National Academy of Sciences (NAS), and the Royal Society of the U.K. ...
  - ...to identify a number of scientific, medical, and ethical requirements that should be considered, and could inform the development of a potential pathway from research to clinical use — if society concludes that heritable human genome editing applications are acceptable
  - more details at <https://nationalacademies.org/gene-editing/international-commission/index.htm>



# Ethical Issues

- Alliance for Regenerative Medicine
  - international group representing the cell and gene therapy sector
  - put out a “statement of principles” on genome editing endorsed by 13 of the most active companies in this field
  - changing heritable DNA in sperm, eggs or a new embryo — came true in November 2018 when He Jiankui, a Chinese biophysicist, said that his lab had edited two baby girls to make them resistant to HIV infection. This mutation will be inherited by their descendants.
  - 31 clinical trials for gene edited therapies are in progress around the world, 20 of which are in oncology. None is yet close to commercialisation. The US has the largest number of trials (19) followed by China (10) and the UK (6)

FT, Clive Cookson, Science Editor August 27 2019



# Outline

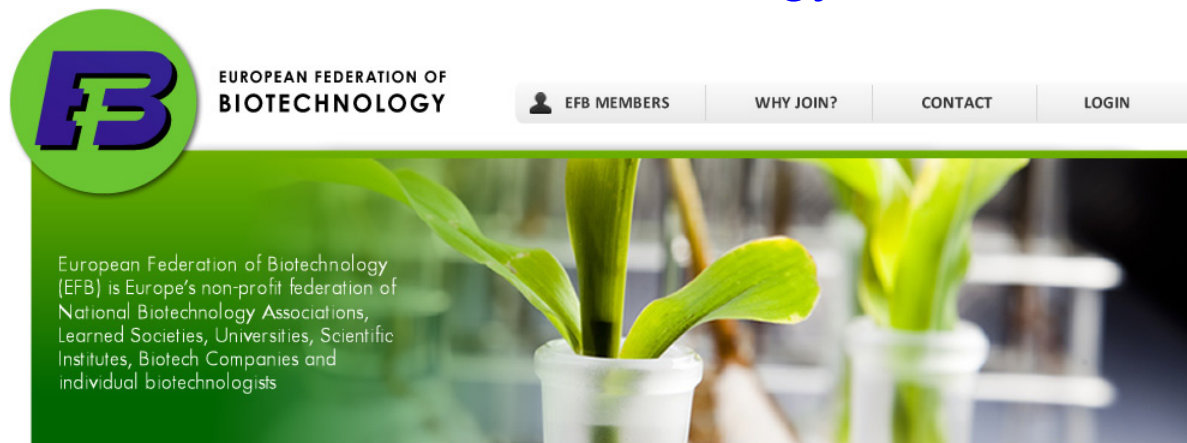
- Medicine
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- Biotechnology

# BIOTECHNOLOGY

- It uses **living organisms, cells or parts of cells (enzymes)** for research, leading to **new products and applications** in medicine, agriculture, food, environmental protection
- Also used in developing **better/sustainable production methods** for the **chemical industry** and **other industrial processes**
- An **interdisciplinary approach** requiring knowledge of chemistry, biology, physics, material sciences, engineering and informatics
- The **origin** of biotechnology can be traced **4,000 years back**, when the **Sumerians** (although not knowingly) used microbes for the production of **alcoholic beverages**.

# BIOTECHNOLOGY

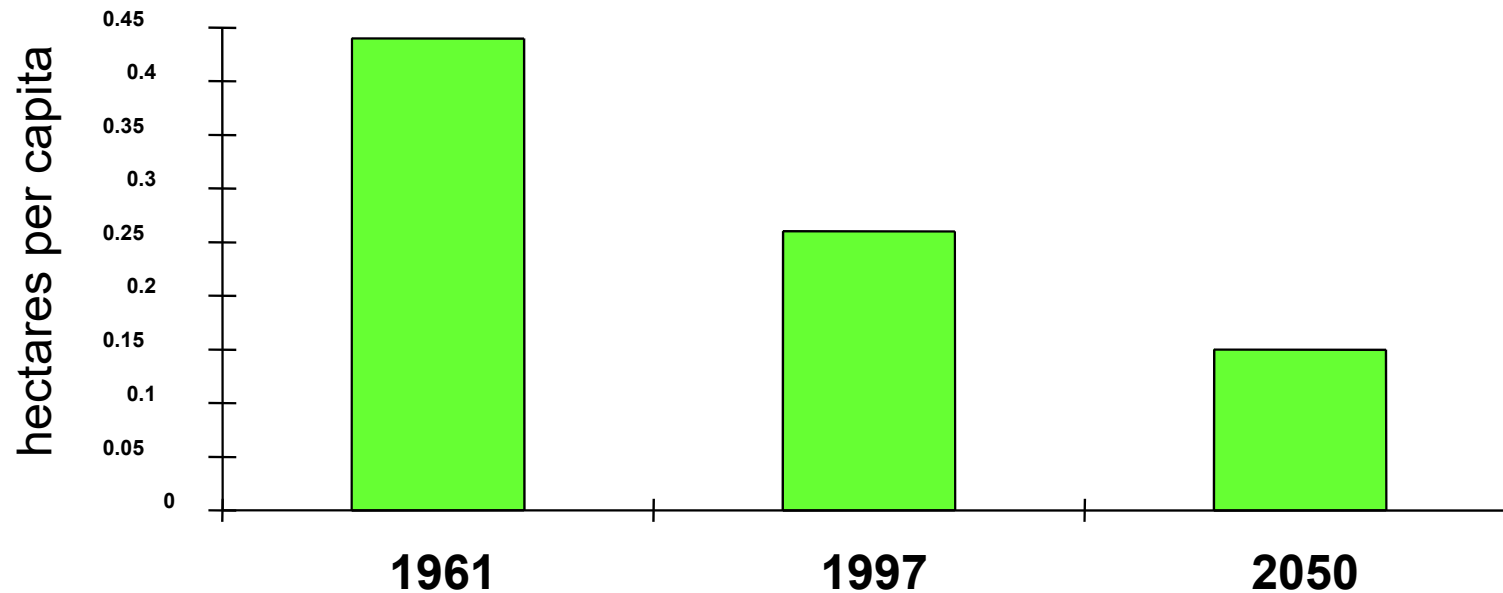
- Examples
  - effective utilization of **plant biomass** for **fuel production**
  - acquisition of starting material (**monomers**) for the **production of polymers** from living organisms instead of from fossil sources
  - **phytopharmaceuticals** – using plants in new vaccination methods such as expression of **antibodies** or **antigens** suitable for **immunization**
- **European Federation of Biotechnology**



# Outline

- Medicine
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- Biotechnology
- **Genetically Modified Organisms**
  - Transgenesis

# Human Population vs Arable Land Availability




Source: UN Millennium Ecosystem Assessment

# Nutrition Deficiency

The world-total deficiency in food production of ...

214 trillion additional calories is equal to:



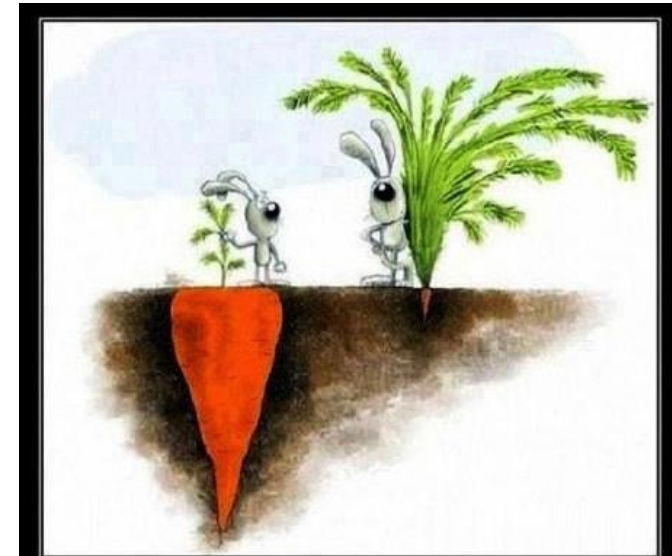
...as soon as in 2027?

The image shows a woman in a blue patterned dress standing on a stage, presenting to an audience. The background features a large screen displaying the text and graphics described above.

<https://qz.com/africa/1064653/the-world-could-run-out-of-food-two-decades-earlier-than-thought/>

# Breeding

- organisms naturally vary due to mutations
- before the era of genetic engineering - question of chance
- breeding tools
  - selection and crossing
- modern breeder learned to change hereditary information – increase the mutants allele frequency
  - chemicals, radiation ...
- results are incidental/non-targeted



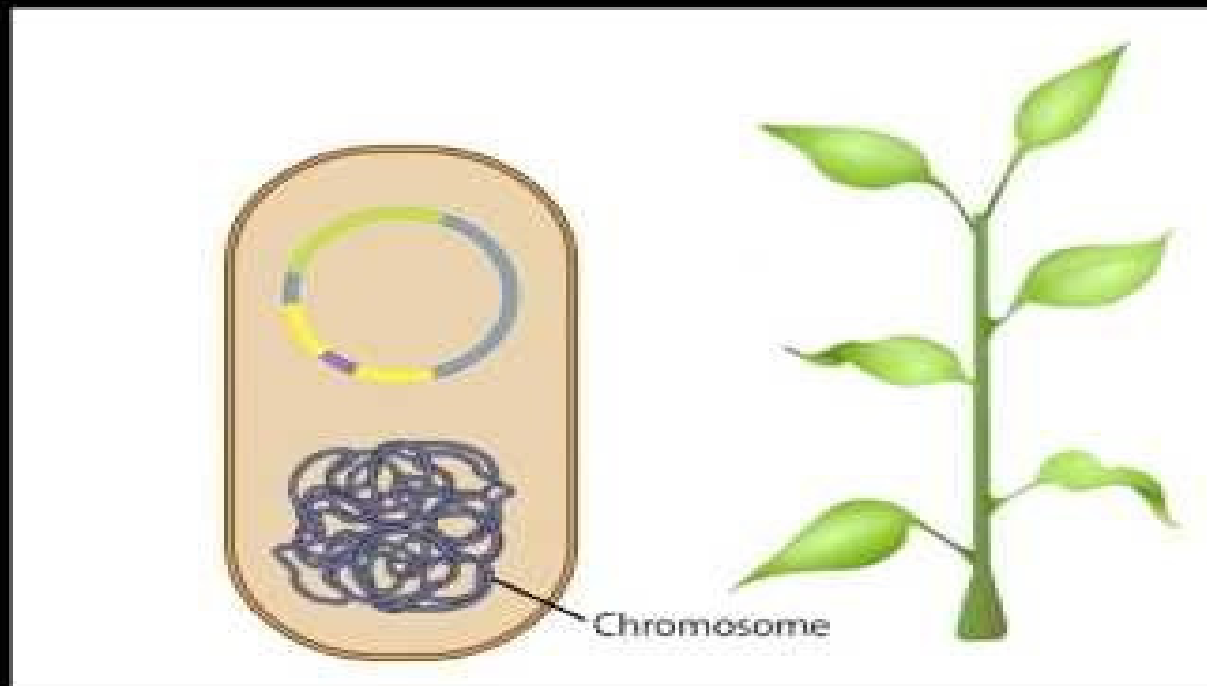
**Success**  
is not always visible at a glance



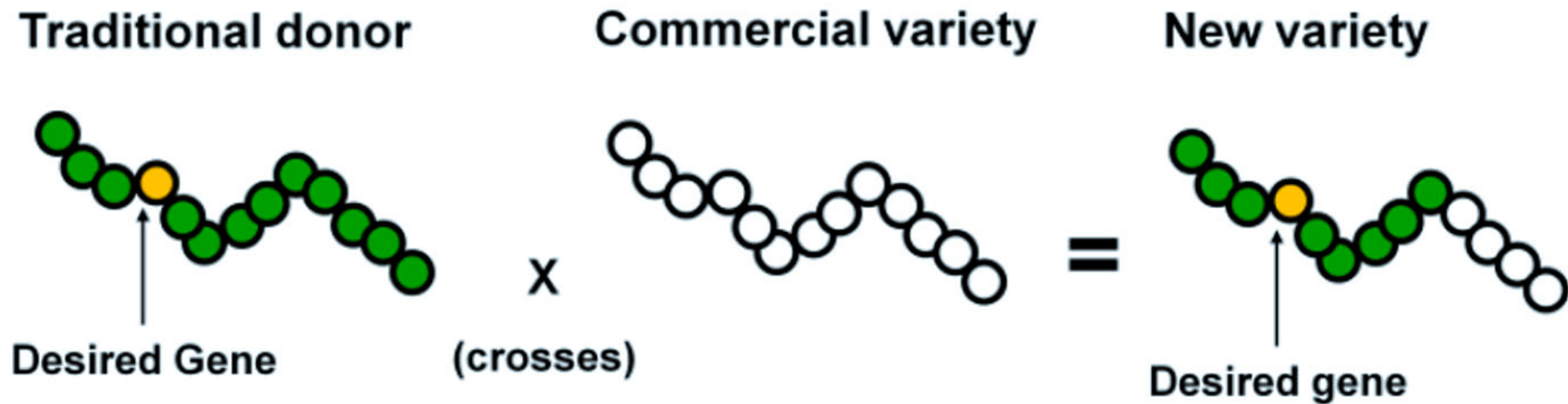
# Genetic Engineering

- Targeted modification ("targeted breeding")
  - ability to transfer genes = transgenesis
- the first practical application: production of human insulin in bacteria - 1978

# Plant Transgenesis



# Breeding Vs. Genetic Engineering



# Genetically Modified Organisms (GMOs)

- Organisms carrying modified genetic information – either own or foreign (from another organism), enabling targeted changes in the organism and its use for specific purposes
- GMOs
  - plants
  - bacteria
  - animals

<http://www.gmo-compass.org/>

# Genetically Modified Plants

- resistance to **pests**
- **herbicide** resistance
- resistance to **drought**
- resistance to **cold**
- resistance to **salinity**
- more efficient **nitrogen utilization**
- increasing **nutritional quality**



<http://ipbo.vib-ugent.be/>

# Bt Plants

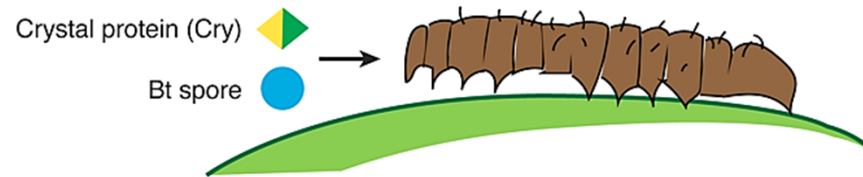
- resistance to **insect pests**
- corn, cotton, rice
- genes from *Bacillus thuringiensis* (**Bt**)
- Expression of crystalline delta-endotoxins - **Crystal (Cry)** proteins
- increasing **yields**, **reducing** the amount of **chemical sprays**



European corn borer damage and fungal infection in non-Bt (left) and Bt hybrids (right)

# Bt Plants

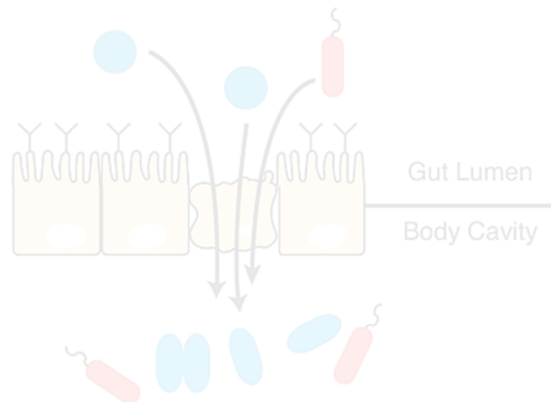
(A) Larvae ingest Bt spores and Cry proteins



(B) In larval midgut, proteolytic digestion of proteins release Cry toxins, which bind to epithelial receptors



(C) Toxin binding causes cell lysis destroying barrier to body cavity



# Ht Plants

- resistance to systemic herbicides
- glyphosate
  - interferes with the synthesis of aromatic amino acids; animals without the appropriate enzymatic apparatus = harmless
  - blocks the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in chloroplasts – affects green plants
  - ineffective for bacterial EPSPS - evolutionarily divergent
  - soya, maize, sugar beet, canola, cotton, alfalfa - added enzyme for tolerance
  - company Bayer (Monsanto), trade name Roundup



# Ht Plants

- resistance to systemic herbicides
- glufosinate (phosphinothricin)
  - prevents processing of ammonium - toxic
  - *Streptomyces hygroscopicus* synthesizes and transforms it: acetylation by the enzyme phosphinothricin acetyltransferase – coding gene isolated in 1987 - named *bar*
  - trade names: **Basta**, Liberty, Finale, Radical ...

# Multiresistant Plants

- Bt resistance + herbicide
- multiresistant corn - the majority of total production in the USA
- example of multiresistant corn:
  - three Bt genes for resistance to air pests
  - three Bt genes for resistance against soil pests
  - two genes for herbicide resistance

# Disease-Tolerant Plants

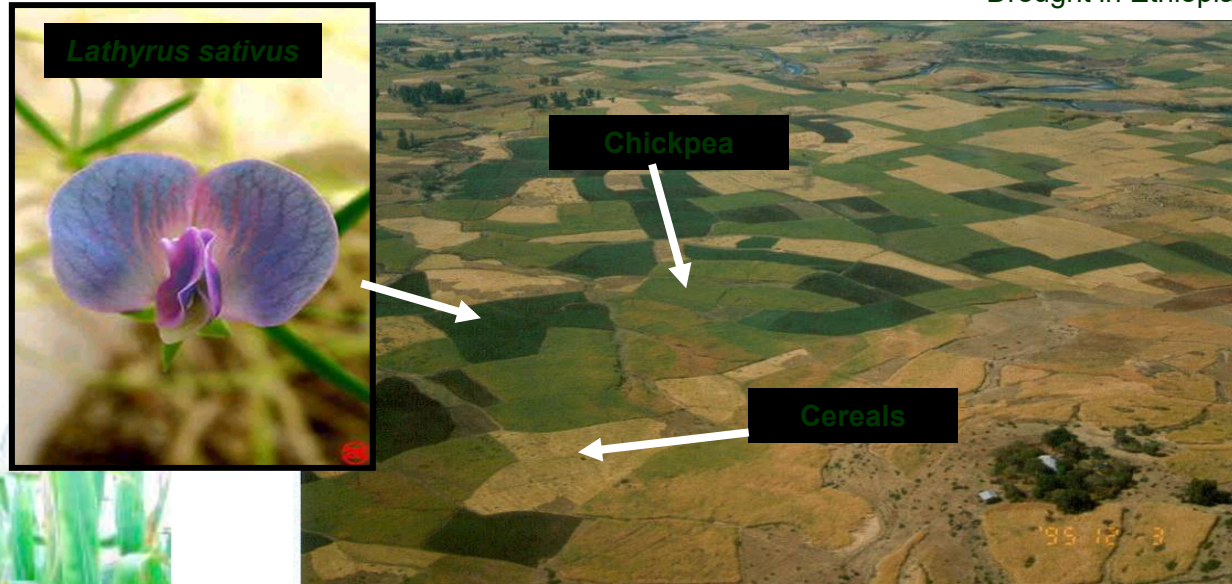
- **viruses** - no chemical agents available
- gene encoding **non-infectious viral envelope** protein - increases resistance to viral infection
  - **banana; papaya** - Hawaii, Southeast Asia
  - **cassava** - a basic food ingredient for more than **500 million people** + animal feed



Left: Papaya with Papaya ringspot disease  
Right: Biotech Papaya resistant

# Disease- and Stress-Tolerant Plants

Drought in Ethiopia



New drought-tolerant maize (right) needs less water.

- Chickpeas - more resistant to drought, but toxic
  - GMOs with inactivated toxin
- Corn resistant to drought

# Nitrogen Use Efficiency

- use of nitrogen from fertilizers
  - rice with gene from barley - 3x higher nitrogen utilization under oxygen deficiency

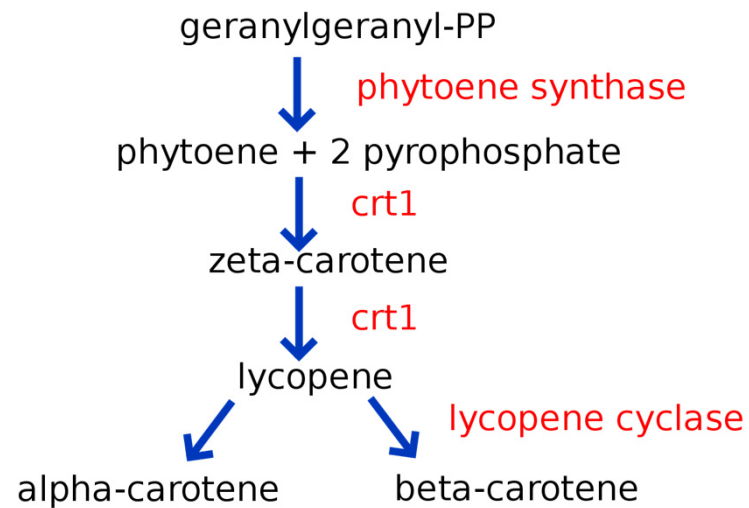


The effect of Nitrogen Use Efficiency (NUE) in rice growth with reduced N applications. Left: rice engineered

# Improved Nutrition Value

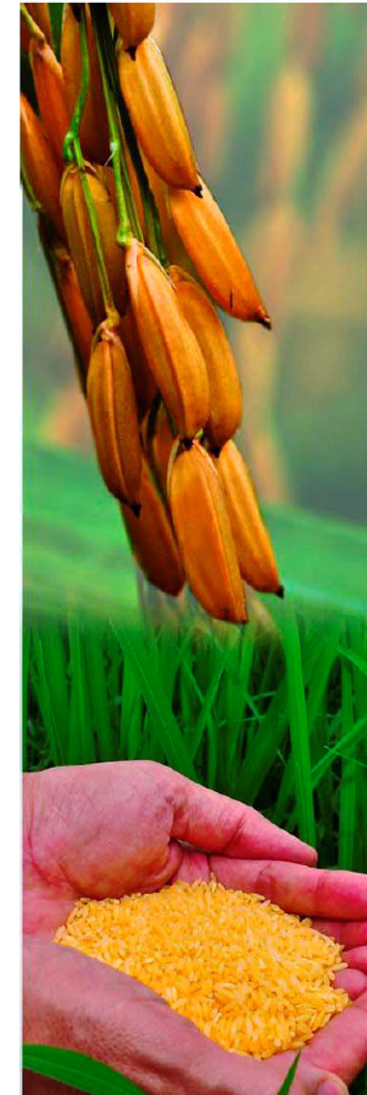
- Golden rice

- several genes from maize encoding enzymes for the biosynthesis of  $\beta$ -carotene (precursor of vitamin A)



- Canola and Soybean

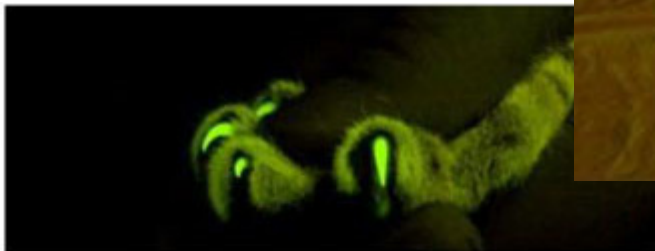
- improved oil properties: stable, resistant to high temperatures, long storage



Golden Rice  
LEI TEL

# GMO Animals

- Transgenic cats
  - lentiviruses are sensitive to restriction factors
    - specific restriction factor: rhesus macaque TRIMCyp + eGFP
  - uniform expression, no mosaicism and no silencing in F1 generation
  - lymphocytes of transgenic animals resistant to replication of FIV

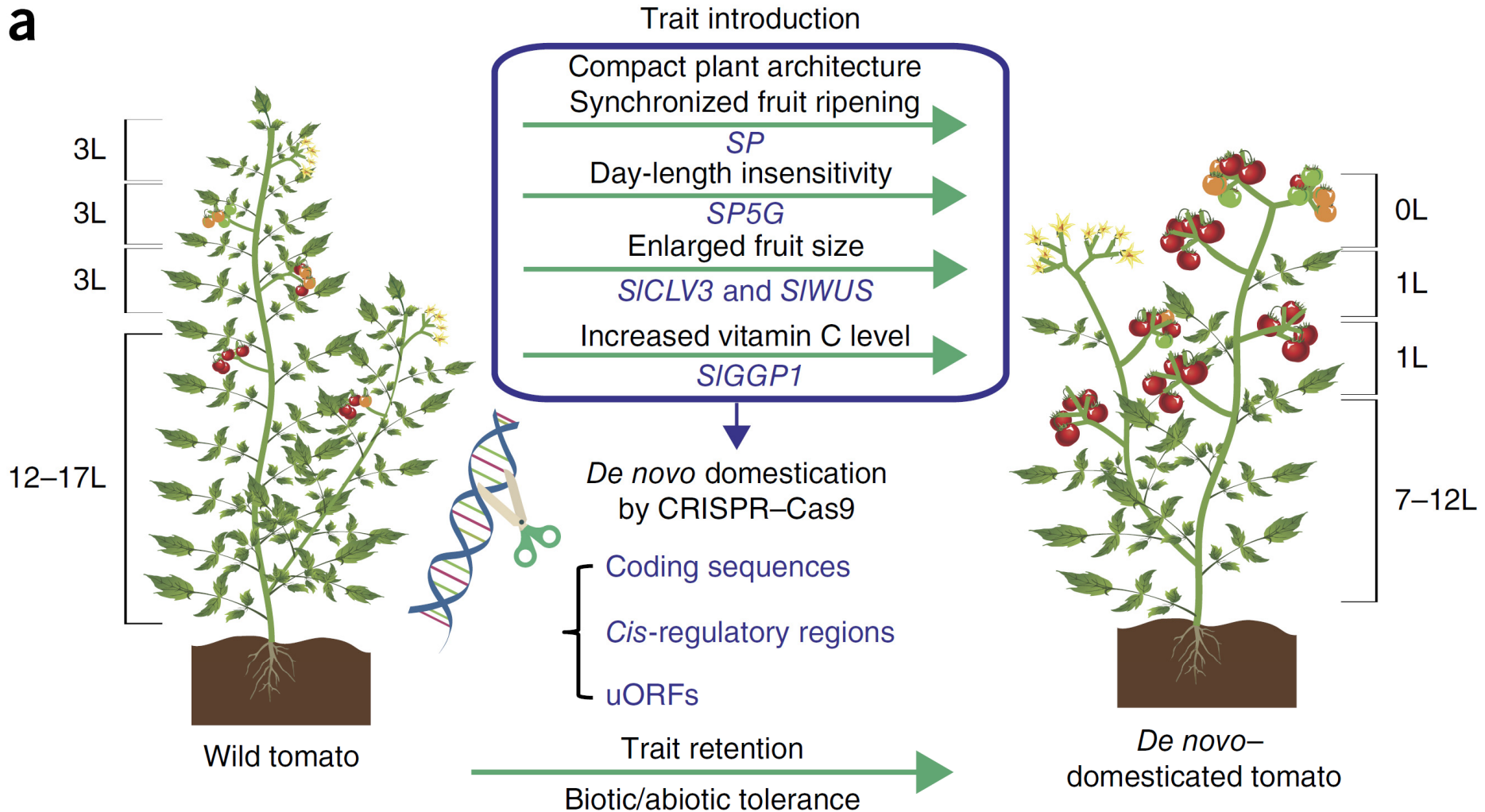


# Outline

- **Medicine**
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- **Biotechnology**
- **Genetically Modified Organisms**
  - Transgenesis
  - **Genome Editing**



# Gene Editing in Plant Domestication



# Outline

- **Medicine**
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- **Biotechnology**
- **Genetically Modified Organisms**
  - Transgenesis
  - Genome Editing
- **Model Organisms**

# *Mus musculus*

## house mouse

- Low requirements for area
- Relatively large number of offspring (3-14, 6-8 on average)
- Genome size is close to the size of human genome (about 3000 Mbp), the number of genes as well (about 24K)
- 20 chromosomes (19+1)
- Suitable for a wide range of physiological experiments (anatomical and physiological similarity to human)
- Possibility to obtain (quite easily) KO mutants and transgenic lines



# Mus musculus

## house mouse

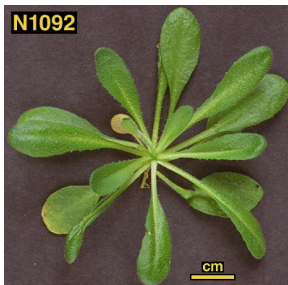
- Genome known since 2002  
(<http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/mouse/>)

The screenshot shows the 'Mouse Genome Overview' page from the Genome Reference Consortium. The page features a navigation bar with links like 'GRC Home', 'Data', 'Help', 'Report an Issue', 'Contact Us', 'Credits', and 'Curators Only'. Below the navigation, there are sub-links for 'Mouse Overview', 'Mouse Issues under Review', 'Mouse Assembly Data', and 'Report a problem'. The main content area is titled 'Mouse Genome Overview' and includes an ideogram of the mouse genome with chromosomes 1-19, X, and Y. Red triangles indicate regions with alternate loci, and orange triangles indicate regions with fix patches. A text box explains the GRC's goal to provide the best reference assembly by generating multiple representations for complex regions. It also provides links for getting data, including the latest minor release (GRCm38.p1) and major release (GRCm38). A prominent orange box highlights the 'Next assembly update' information: 'The next assembly update (patch release 2) will be a minor update (only patches) and will happen in March 2013.' On the right side, there is a 'GRC Blog' section with recent posts and a 'Recently Resolved Mouse Issues' section listing specific problems like MG-4136 and MG-4212. At the bottom, there are tabs for 'GRCm38.p1', 'GRCm38', and 'MGSCv37', and a 'References' section with links to whole genome papers.

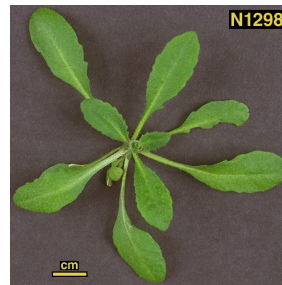
# *Arabidopsis thaliana*

mouse-ear cress

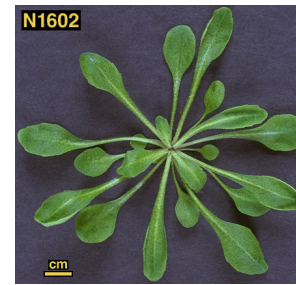
- Low requirements for cultivation area
- High number of seeds (20.000 per plant and more)
- Small and compact genome, (125 MBp, about 25.000 genes, average size 3 kb)
- 5 chromosomes
- Suitable for wide range of physiological experiments
- High natural variability (approximately 750 ecotypes (Nottingham Arabidopsis Seed Stock Centre))



Columbia 0

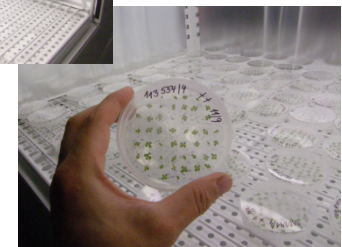


Landsberg 0



Wassilewskija 0

<http://seeds.nottingham.ac.uk/>



# Arabidopsis thaliana

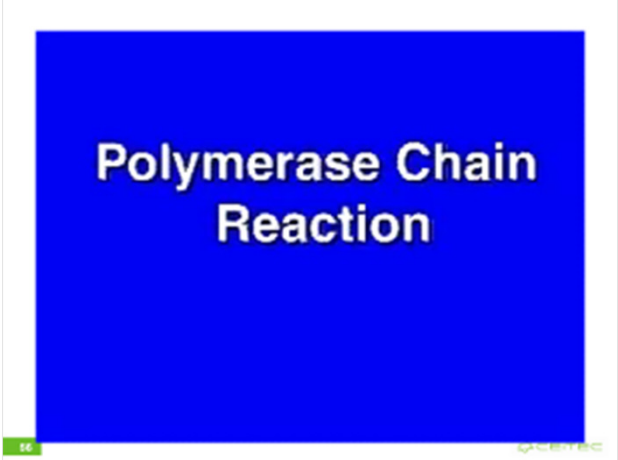
## mouse-ear cress

- Genome known since 2000 (<http://www.arabidopsis.org/>)

The screenshot shows the TAIR website homepage. The browser window title is "TAIR - Home Page" and the address bar shows "www.arabidopsis.org". The page has a navigation menu with links for Home, Help, Contact, About Us, and Login/Register. Below the menu is a search bar with a dropdown menu set to "Gene" and a "Search" button. The main content area is titled "The Arabidopsis Information Resource" and contains several paragraphs of text describing the database and its updates. To the right of the main text are several news articles, including "Breaking News" with links to subscribe to a news feed, follow on Twitter, and join a Facebook group. There is also a section for "New Set of Confirmed T-DNA Lines Available" dated November 28, 2012, and another for "New from ABRC Education and Outreach!" dated October 31, 2012. At the bottom of the page, there is a large banner for a new online submission form, which includes a form with fields for "TAIR SUBMISSION Article #", "Gene", "Locus", "GeneFunction", and "Method". The banner also includes a "SUBMIT PAPER" button and a "SUBMIT DATA" button. Below the form, there is a call to action: "Click here to try our new online submission form and submit the molecular function (e.g. protein kinase), biological process (e.g. seed development), localization (e.g. plasma membrane) or interacting partner of your favorite gene".

# Outline

- **Medicine**
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- **Biotechnology**
- **Genetically Modified Organisms**
  - Transgenesis
  - Genome Editing
- **Model Organisms**
- **Principles of PCR**



Polymerase Chain  
Reaction

# Polymerase Chain Reaction



# Klíčové koncepty

- Techniky využívající pokročilé přístupy zásadním, způsobem mění naše možnosti v medicíně i zemědělství
- Možnost programovatelné editace genomu slibuje zásadní obrat v léčbě zejména dědičně podmíněných chorob a ve šlechtění nových odrůd i ras
- Je nezbytná přísná kontrola s jasně nastavenými pravidly pro všechny, ale nikoliv úplný zákaz

# Diskuse