CG920 Genomics

Lesson 12

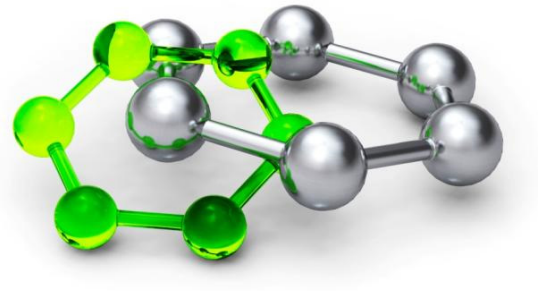
Practical Applications

Jan Hejátko

Functional Genomics and Proteomics of Plants,
CEITEC - Central European Institute of Technology
And
National Centre for Bimolecular Research,
Faculty of Science,

Masaryk University, Brno
hejatko@sci.muni.cz, www.ceitec.eu

MUNI
SCI



Literature

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.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., and Liu, D.R. (2017). Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. Nature **551,** 464-471.

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.

Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M., and Doudna, J.A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science **360,** 436-439.

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.

Li, X., Qian, X., Wang, B., Xia, Y., Zheng, Y., Du, L., Xu, D., Xing, D., DePinho, R.A., and Lu, Z. (2020). Programmable base editing of mutated TERT promoter inhibits brain tumour growth. Nat Cell Biol **22,** 282-288.

Outline

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

Outline

- Medicine
  - Molecular Diagnosis

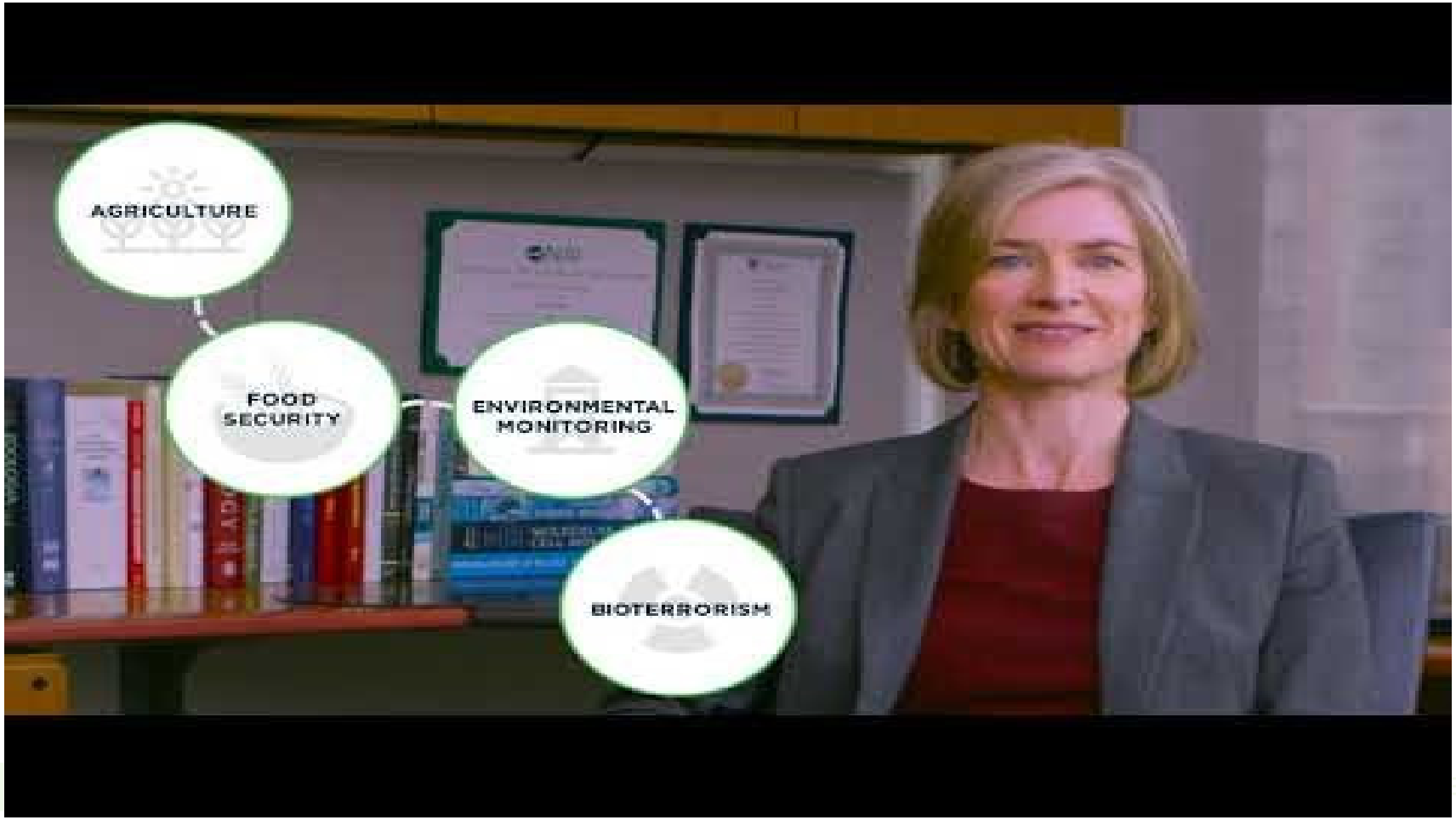
Molecular Diagnosis

- around 10,000 disorders in humans resulting from a single mutation
  - cystic fibrosis
  - sickle cell disease
  - muscular dystrophy
  - beta thalassemia
  - ….
- Early molecular diagnosis
  - mutations or infections
    - PCR
    - DNA (chip) hybridization
    - Cas-based

Molecular Diagnosis

- Mammoth Biosciences
  - Co-founded by Jenifer Doudna

https://youtu.be/IPe4IdgKGdQ



Outline

- Medicine
  - Molecular Diagnosis
  - Personalized Medicine

Personalized Medicine

- uses knowledge of the genome for:
  - prediction of health risks
  - diagnosis
  - selection of the most appropriate type of treatment
  - minimizing the side effects of treatment
  - prevention

Personalized Medicine

What is Personalized Medicine?

Current Practice	Personalized Medicine
	
One size fits all	The right treatment for the right person at the right time
Trial and error	

PMC Personalized Medicine Coalition

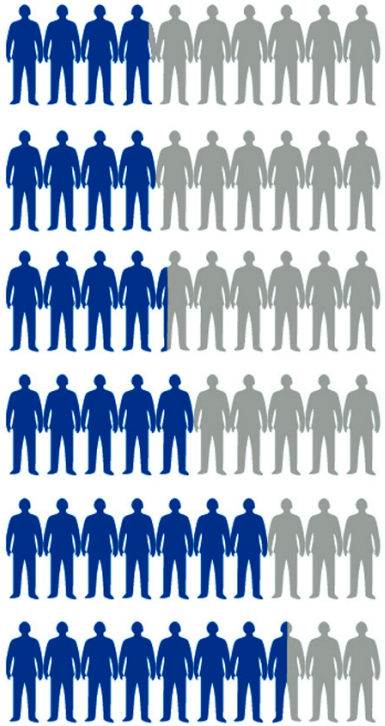
http://www.personalizedmedicinecoalition.org/

CEITEC

Personalized Medicine

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

ANTI-DEPRESSANTS (SSRIs)	38%
ASTHMA DRUGS	40%
DIABETES DRUGS	43%
ARTHRITIS DRUGS	50%
ALZHEIMER'S DRUGS	70%
CANCER DRUGS	75%



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.

- 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?"

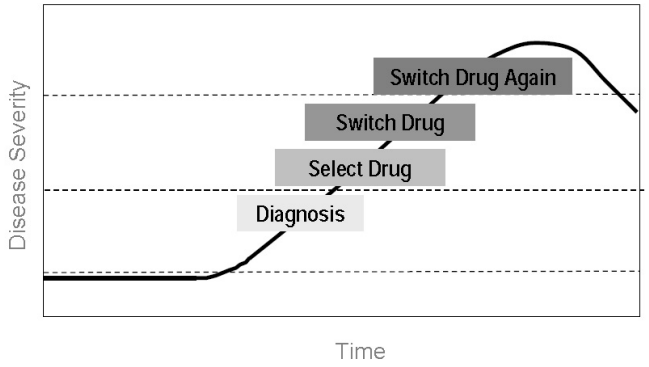
Rezulin Baycol Lotronex*

CEITEC

Personalized Medicine

The Old Paradigm: Treatment of Disease

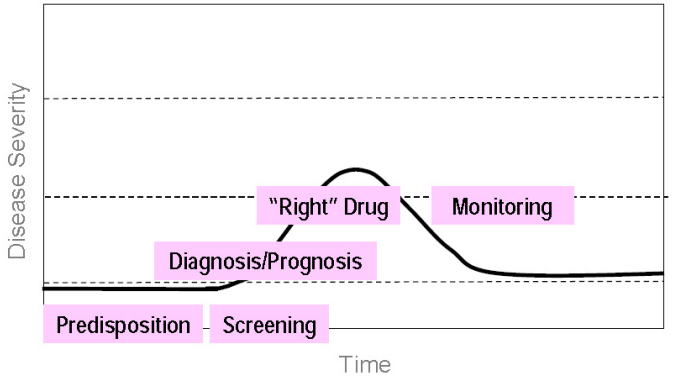
Reactive Medical Care



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

Personalized Medicine Paradigm: Health Management

Efficient Medical Care

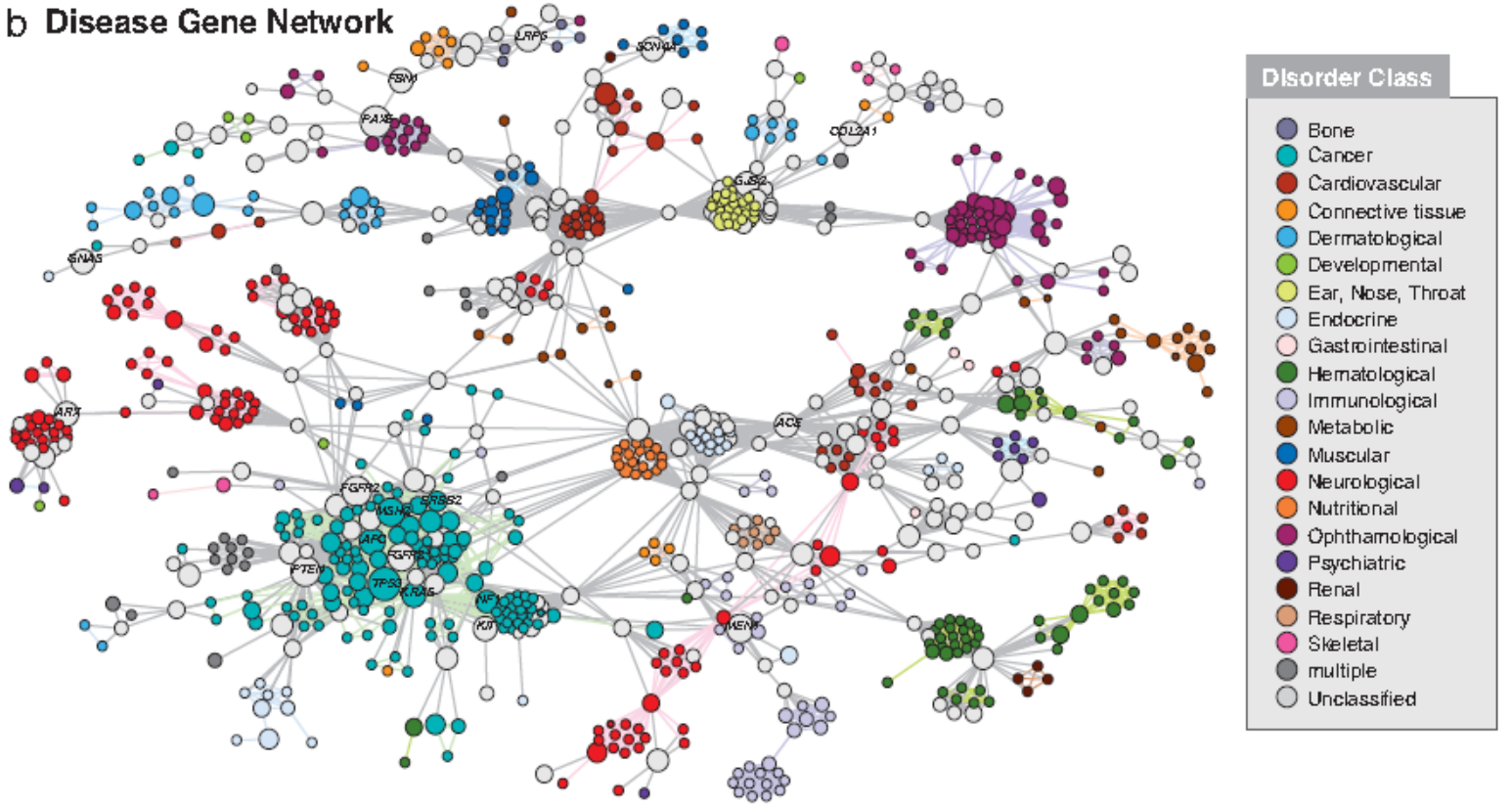


Health Management; Molecular Screening; Early Detection; Rapid Effective Treatment; Improved Quality of Care

http://www.personalizedmedicinecoalition.org/

Personalized Medicine

- Problem:
  - multigene conditionality of most human diseases

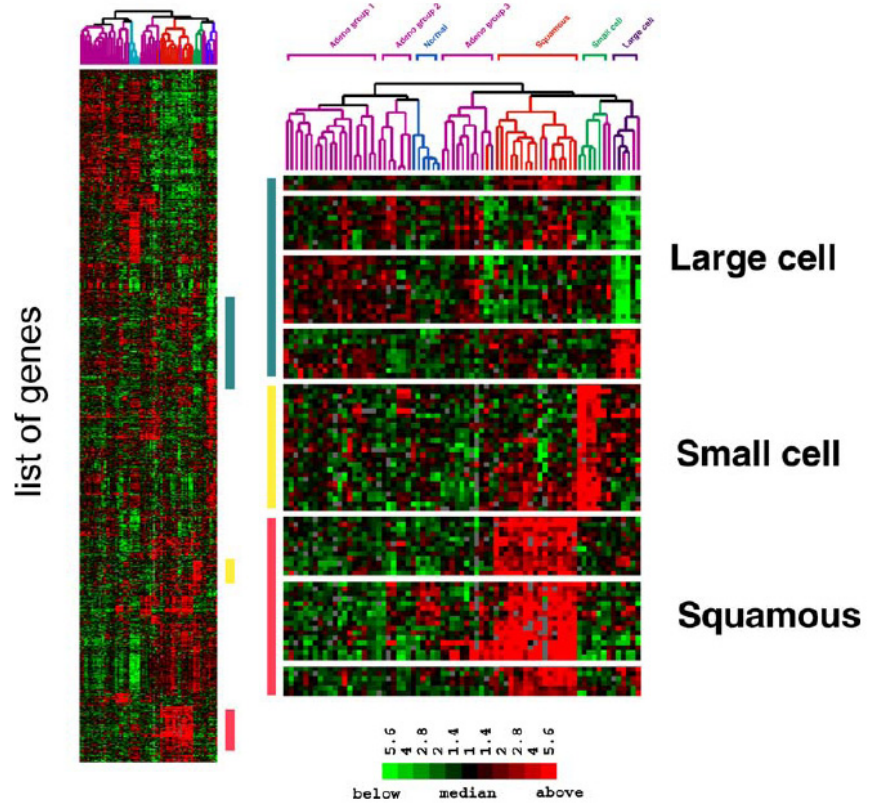


Goh *et al.*, 2007

CEITEC

Personalized Medicine

- Problem solving
  - **systems biology** - uses e.g. gene clustering to identify genes involved in the observed phenomenon



Dietel and Sers, 2006

Personalized Medicine

- Problem solving
  - biomarkers
  - tests

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	**Anesthesia adjunct**: "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*	**Arthritis**: "*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)*	**Bipolar disorder**: "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) Nolvaldex® (tamoxifen)	Estrogen Receptor (ER)	**Breast cancer**: Exemestane is indicated for adjuvant treatment of post-menopausal 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®	**Breast cancer**: 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®	**Breast cancer**: Assesses risk of distant metastasis in a 70-gene expression profile.
Chemotherapy	Oncotype DX® 16-gene signature	**Breast cancer**: 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	**Breast cancer**: The test predicts "time to event" for metastasis of breast cancer, following surgery or biopsy.
Faslodex® (fulvestrant)	Hormone Receptor *(HR)*	**Breast cancer**: 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	**Breast cancer**: "…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*	**Breast cancer**: Guides surveillance and preventive treatment based on susceptibility risk for breast and ovarian cancer.
Nolvadex® (tamoxifen)	Breast Cancer Index℠ *(HOXB13, IL17BR)*	**Breast cancer**: Calculates a combined risk analysis for recurrence after tamoxifen treatment for ER-positive, node-negative breast cancer.

The Case for Personalized Medicine, 3rd edition

Personalized Medicine

- Other problems
  - Ethical Issues
    - the condition is genetic testing or knowledge of the genome - easily abused
    - risk: insufficient data security
    - in some countries, employers or insurance companies do not have access to such data
  - High Costs
    - medicine could be divided into first-class and low-class services
    - globalization gap could grow even larger - poor countries would not be able to afford this
  - Privacy
    - crucial and complex issue
    - what information about oneself can/should be considered private?

Outline

- Medicine
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy

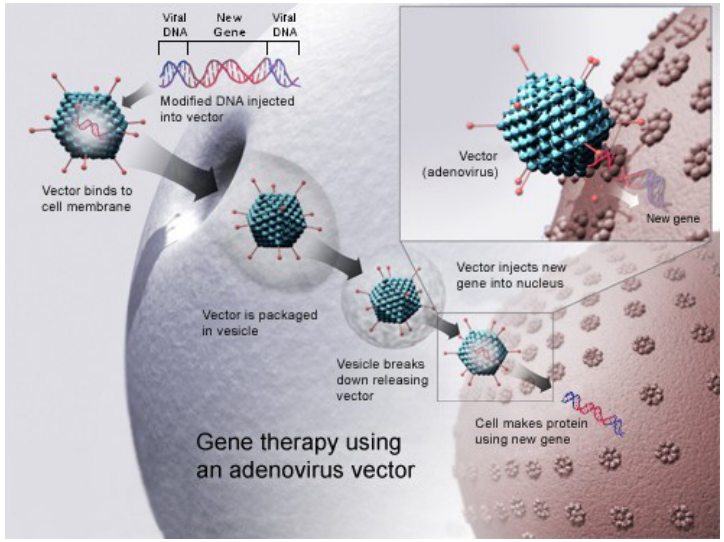
Gene Therapy

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

- 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



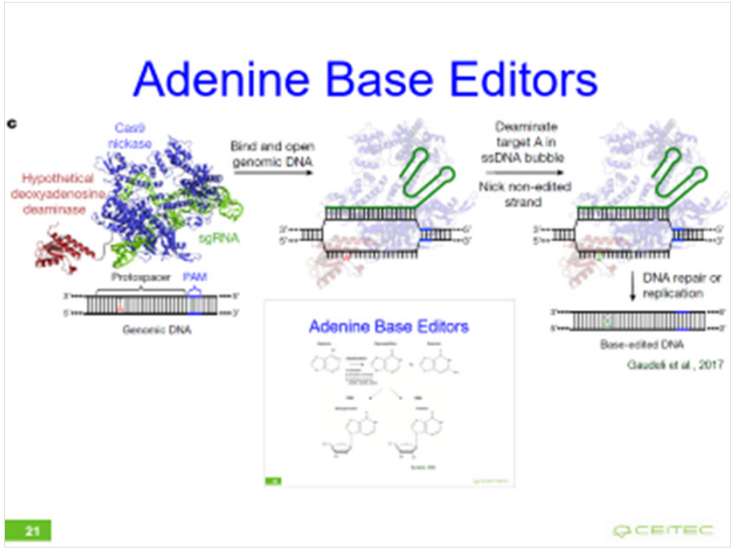
CEITEC

Gene Therapy

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

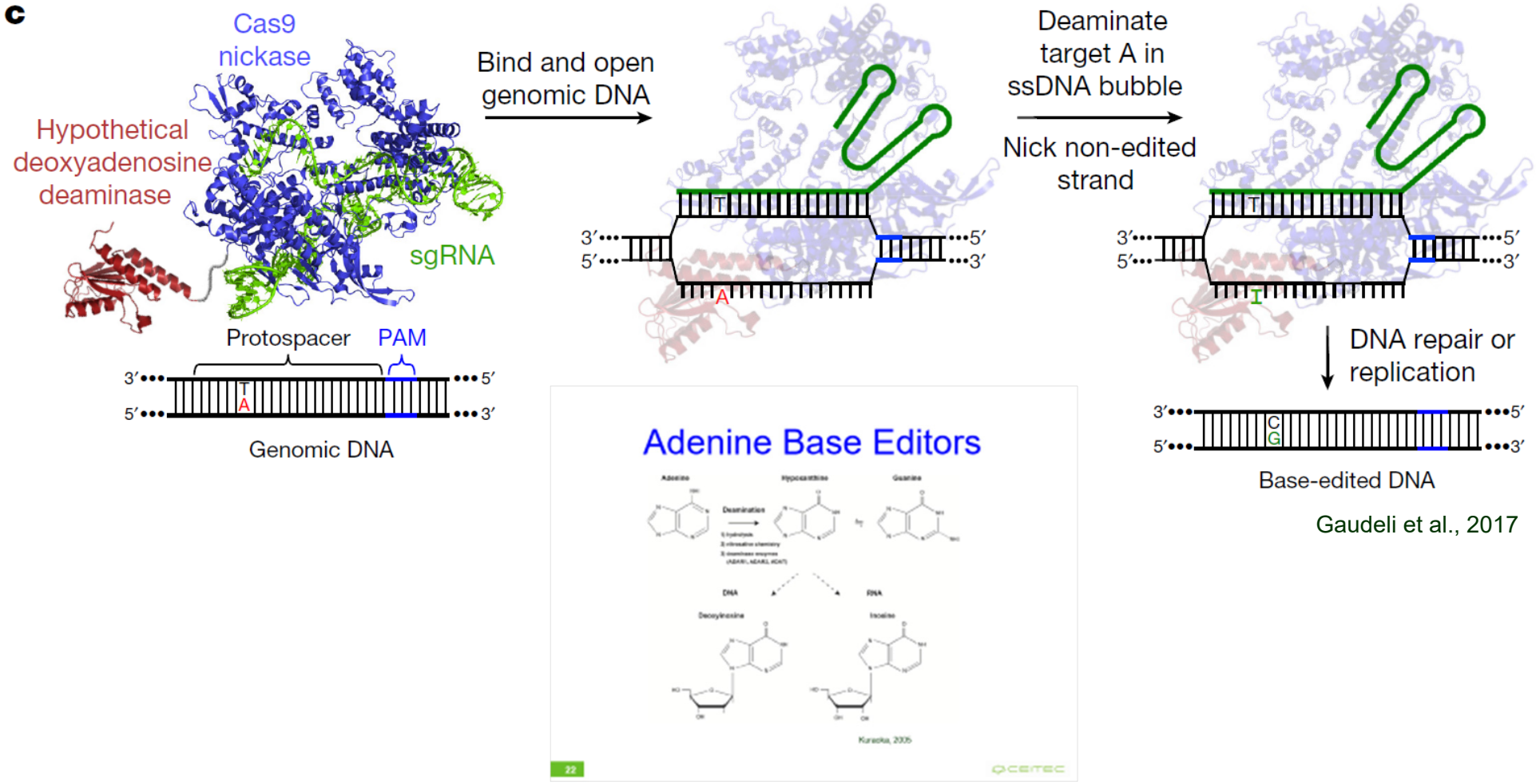
- Options:
  - replace the mutated gene
  - repair the mutation
  - deliver DNA encoding a therapeutic protein
  - antisense therapy
- In the future useful for treating e.g. hereditary diseases
- Types:
  - somatic gene therapy
  - gene therapy of germ cells

Gene Therapy

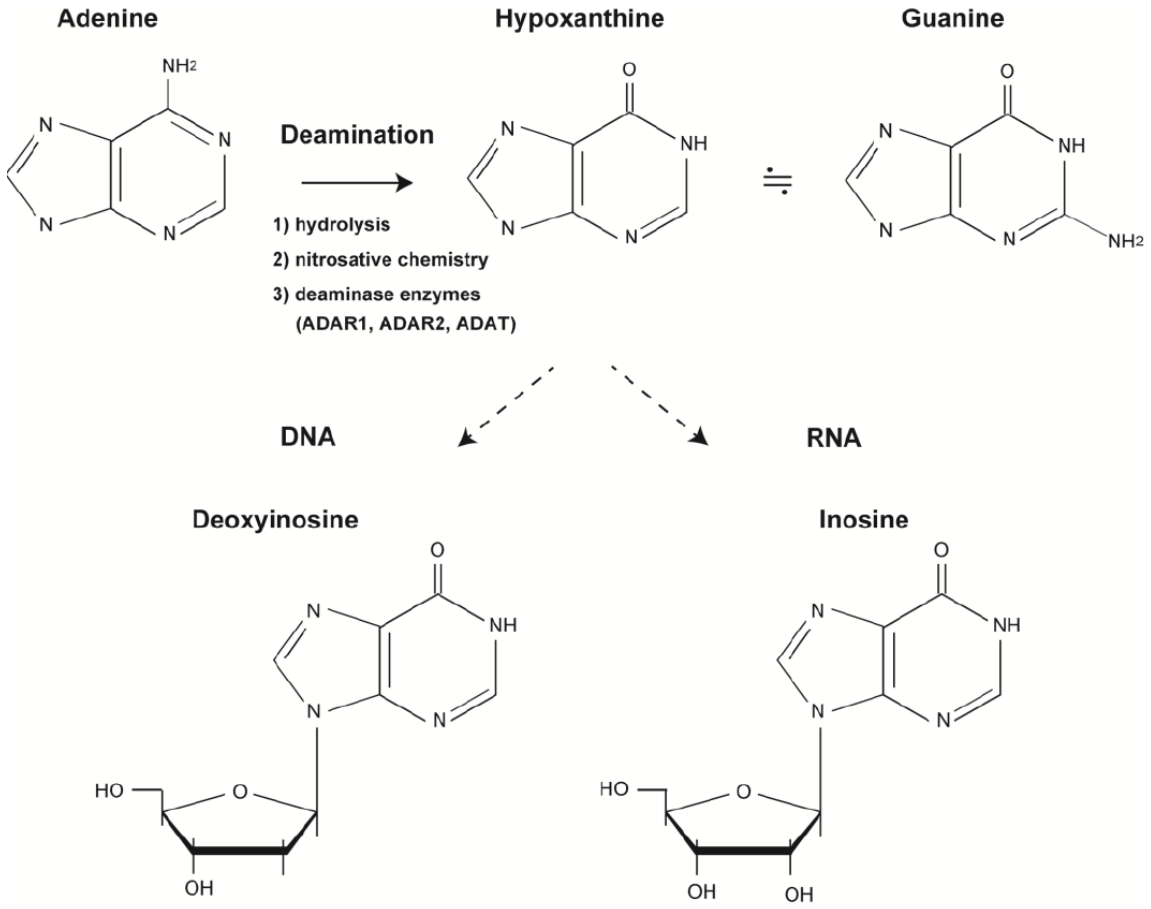


- Hutchinson–Gilford progeria syndrome
  - C•G-to-T•A mutace (c.1824 C>T; p.G608G) v genu pro laminin (*LMNA*)
  - Defekt v sestřihu RNA vede k tvorbě toxického proteoinu progerinu
  - Věk dožití cca 14 let
  - In vivo oprava pomocí ABEs potvrzena u myší a lidských fibroblastů (Koblan et al., 2021)

Adenine Base Editors



Adenine Base Editors



Kuraoka, 2005

Ethical Issues

- 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

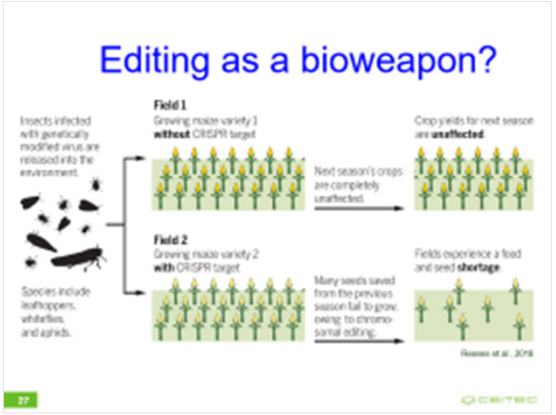
- Alliance for Regenerative Medicine
  - international group representing the cell and gene therapy sector
  - put out a “statement of principles” on genome editing endorsed by 13 of the most active companies in this field
  - changing heritable DNA in sperm, eggs or a new embryo — came true in November 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 commercialization. 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

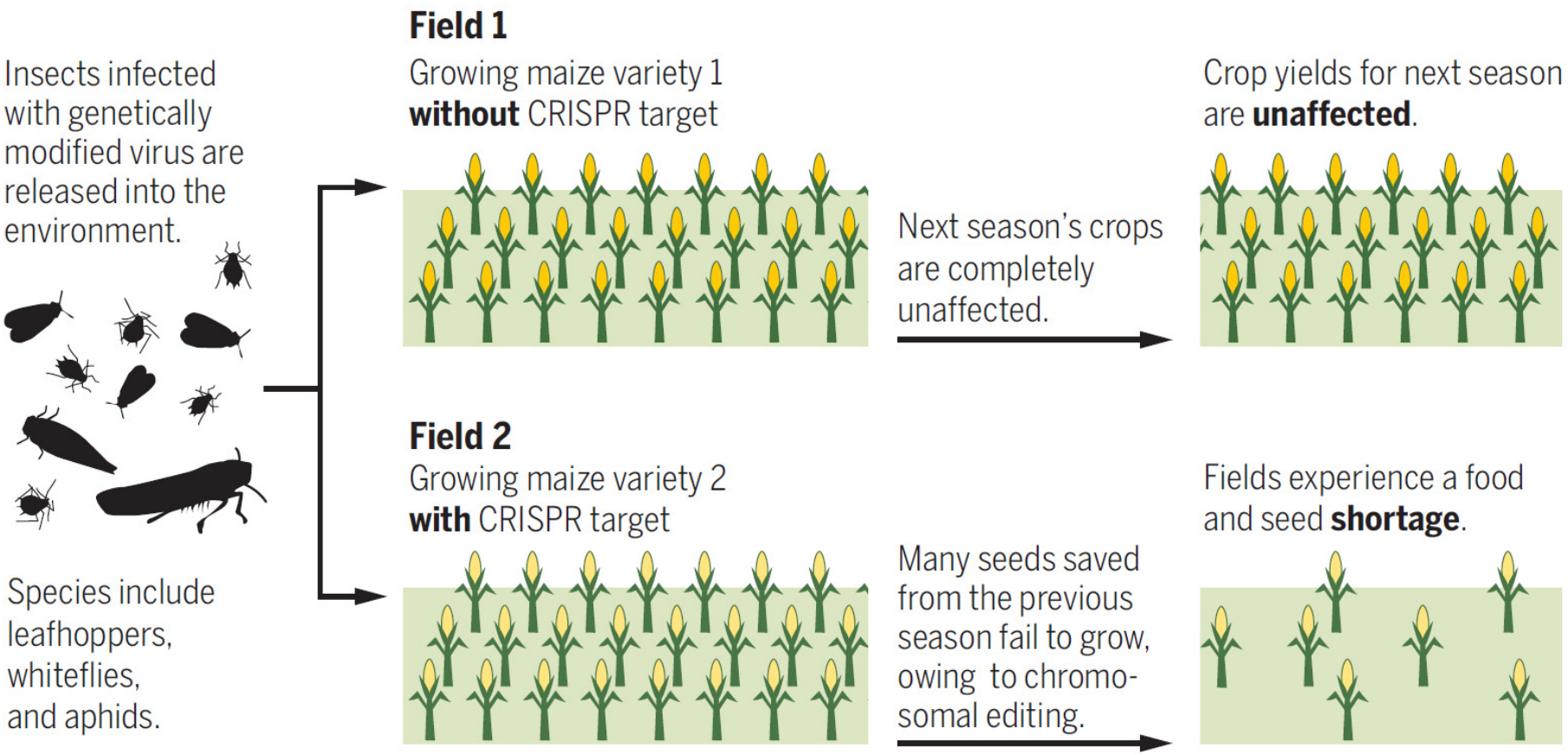
He Jiankui is Announcing Human Genome Editing

Ethical Issues

- Genome editing as a bioweapon?
  - ongoing research program funded by the U.S. Defense Advanced Research Projects Agency (DARPA)
  - aims to disperse infectious genetically modified viruses that have been engineered to edit crop chromosomes directly in fields
  - the means of delivery of these viral horizontal environmental genetic alteration agents (HEGAAs) into the environment should be insect-based dispersion
  - Part of scientific community does not find the program useful for the U.S. agriculture, but points to its possible misuse



Editing as a bioweapon?



Reeves et al., 2018

Outline

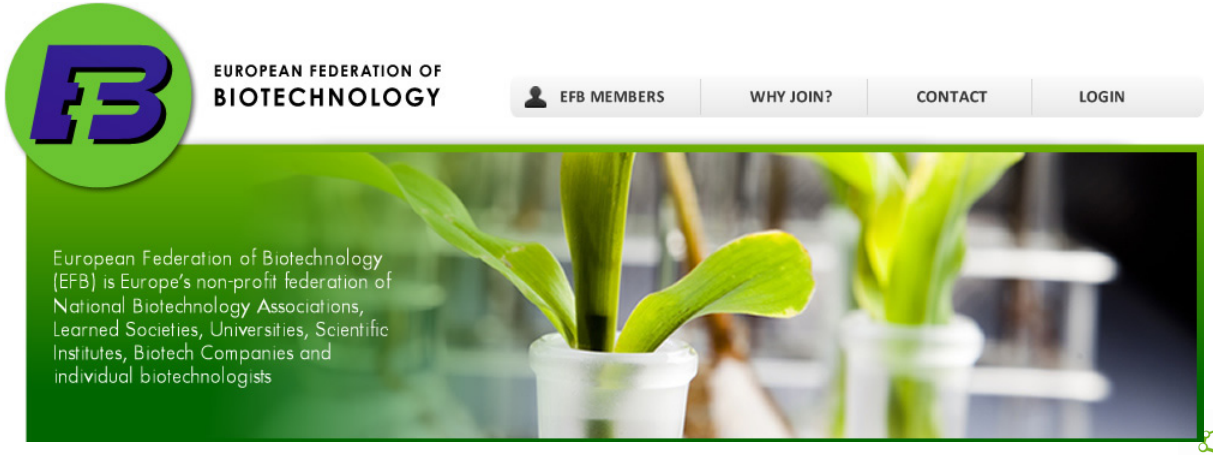
- Medicine
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- Biotechnology

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



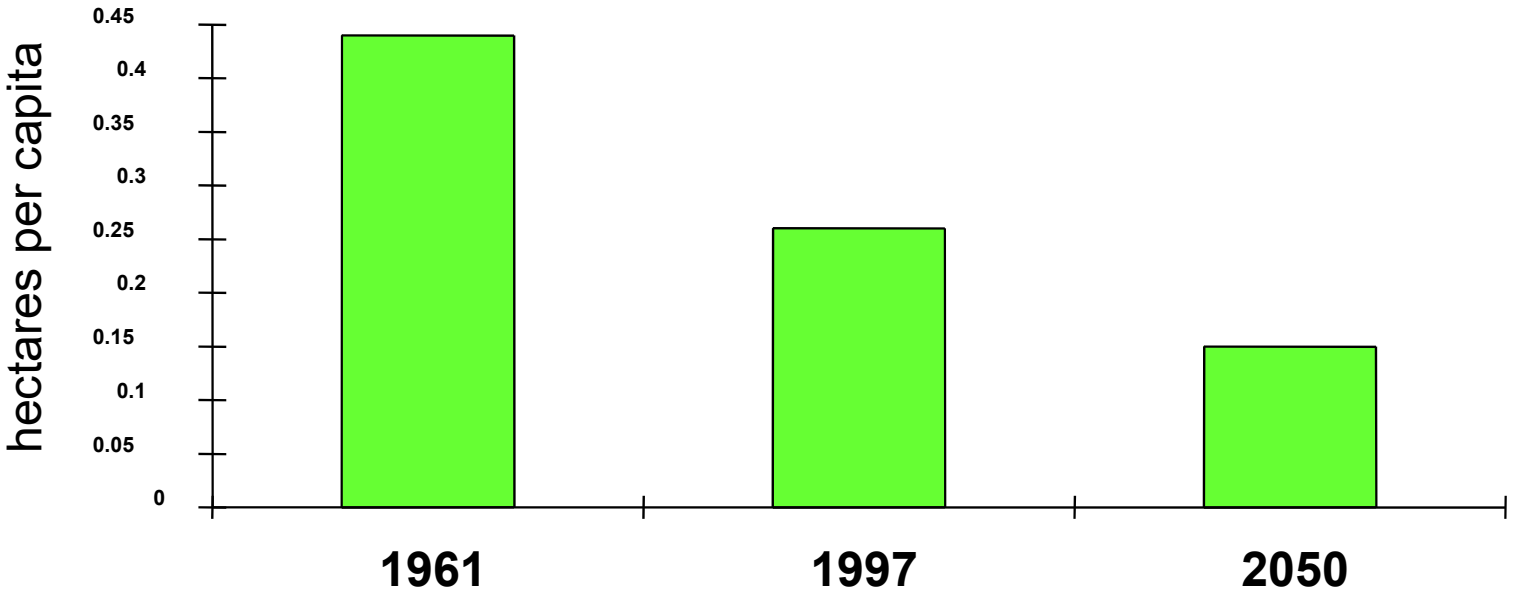
http://www.efb-central.org

CEITEC

Outline

- Medicine
  - Molecular Diagnosis
  - Personalized Medicine
  - Gene Therapy
- Biotechnology
- Genetically Modified Organisms
  - Transgenosis

Human Population vs Arable Land Availability



Source: UN Millennium Ecosystem Assessment

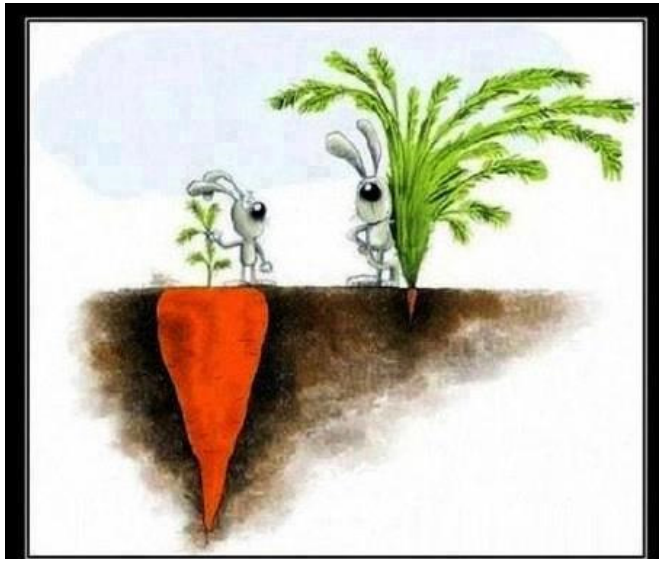
Nutrition Deficiency

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

CEITEC

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



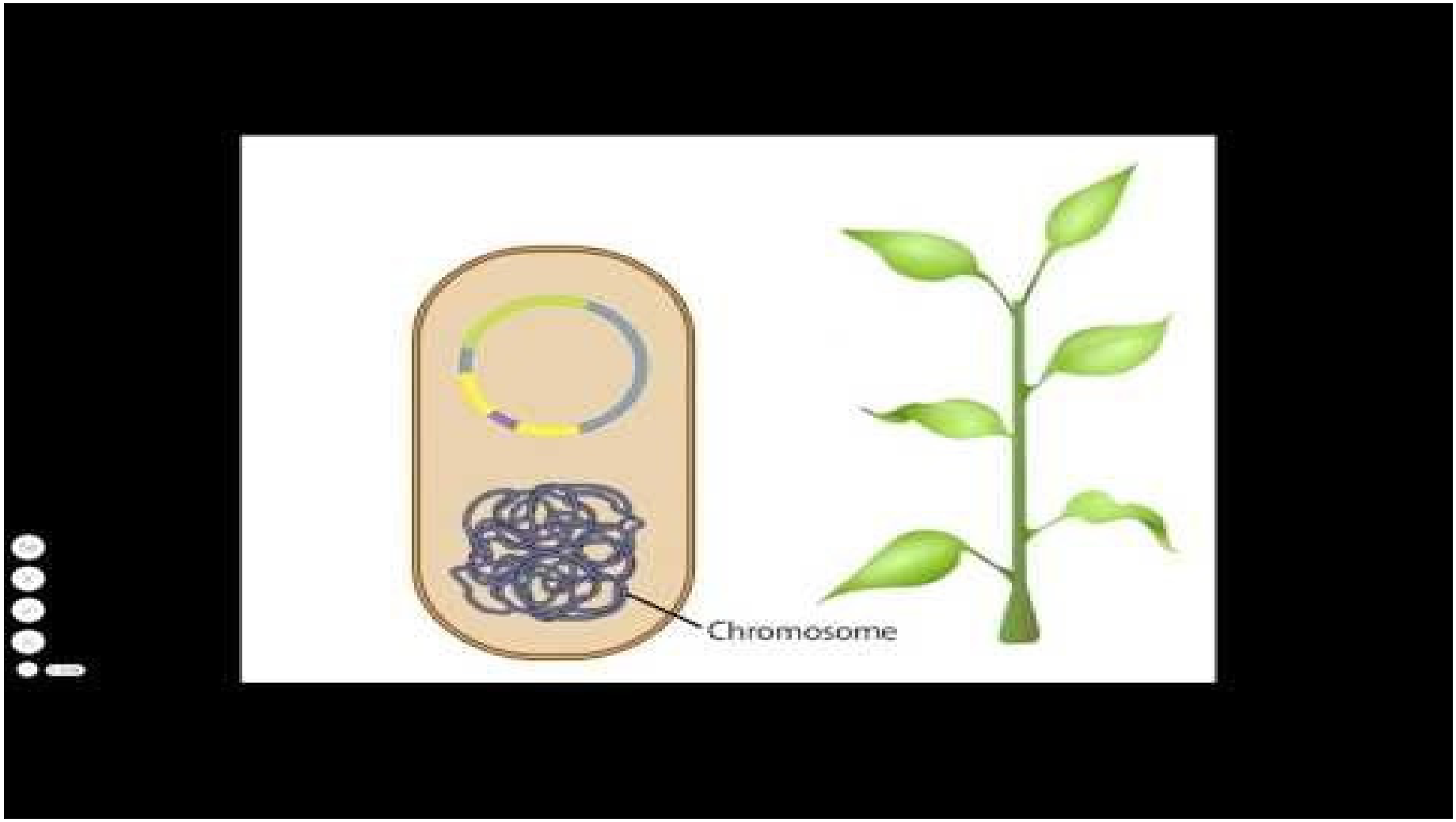
Success

is not always visible at a glance

Genetic Engineering

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

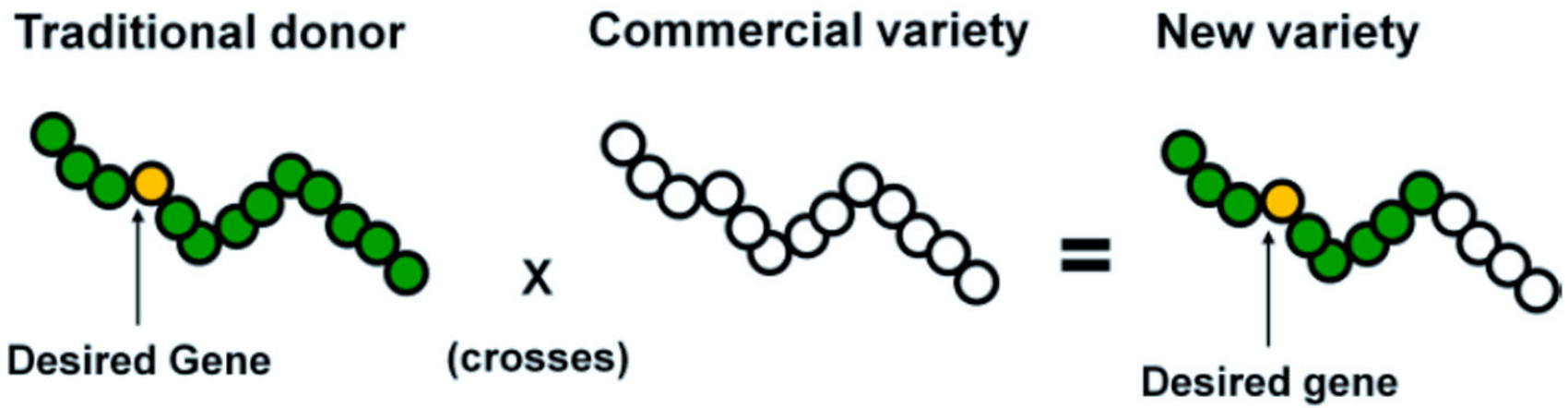
Plant Transgenosis



https://www.youtube.com/watch?v=yesNHd9h8k0

CEITEC

Breeding Vs. Genetic Engineering



Geneticaly 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/

Geneticaly Modified Plants

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

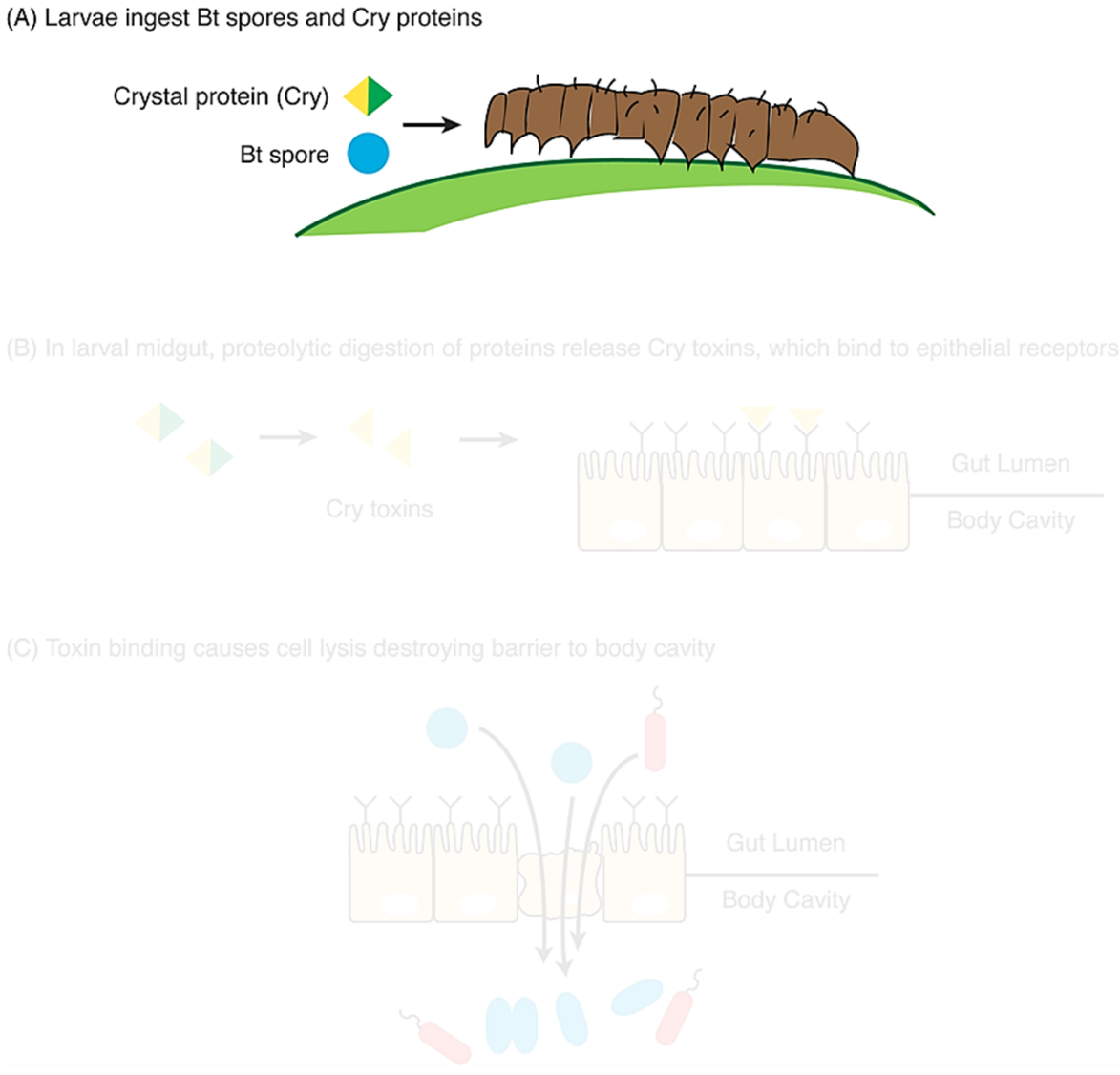
http://ipbo.vib-ugent.be/

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
Crystal protein (Cry)
Bt spore
(B) In larval midgut, proteolytic digestion of proteins release Cry toxins, which bind to epithelial receptors
Cry toxins
Gut Lumen
Body Cavity
(C) Toxin binding causes cell lysis destroying barrier to body cavity
Gut Lumen
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-enolpyrovylshikimate-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-Tollerant 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-Tollerant Plants

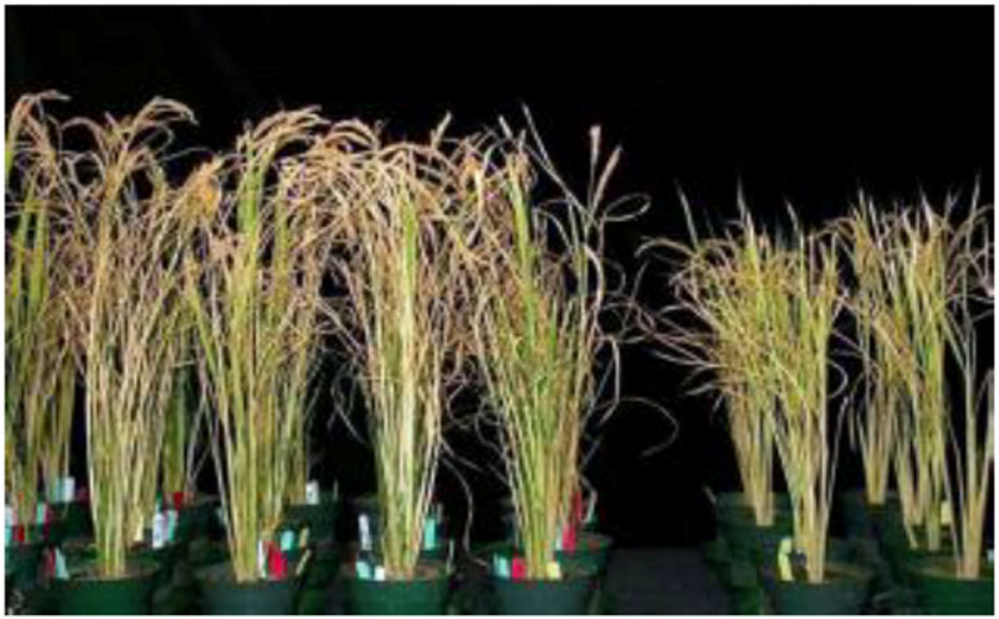
Drought in Ethiopia

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

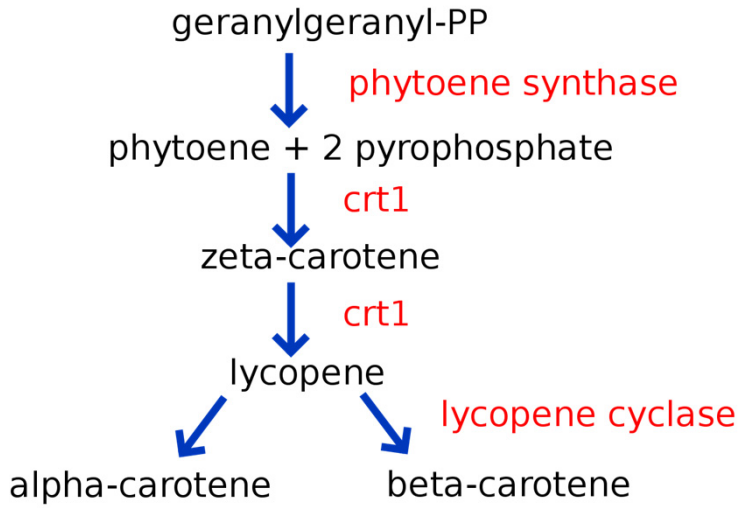
- use of nitrogen from fertilizers
  - rice with gene from barley - 3x higher nitrogen utilization under oxygen deficiency



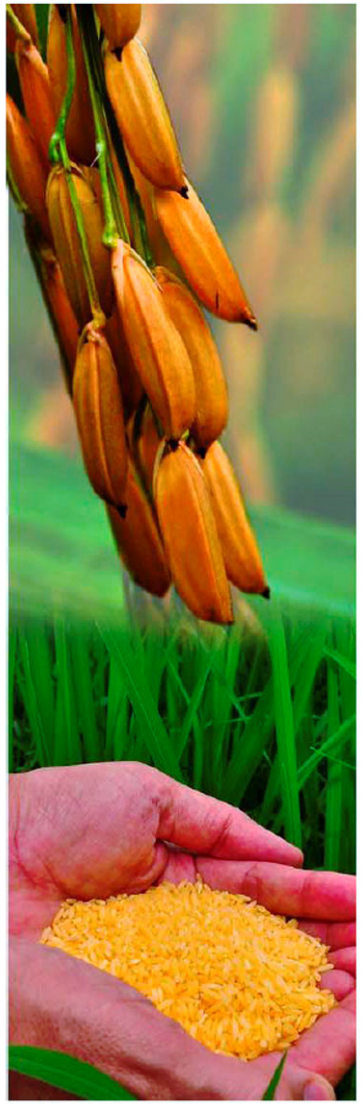
The effect of Nitrogen Use Efficiency (NUE) in rice growth with reduced N applications. Left: rice engineered

Improved Nutrition Value

- Golden rice
  - several genes from maize encoding enzymes for the biosynthesis of β-carotene (precursor of vitamin A)

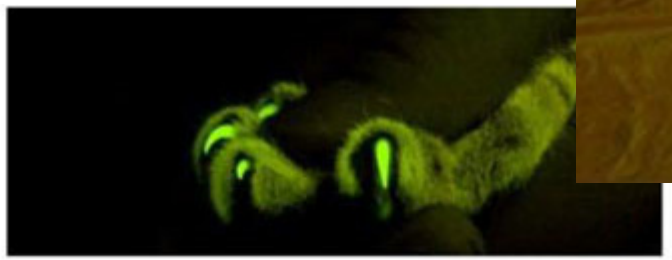


- Canola and Soybean
  - improved oil properties: stable, resistant to high temperatures, long storage


Golden Rice

GMO Animals

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

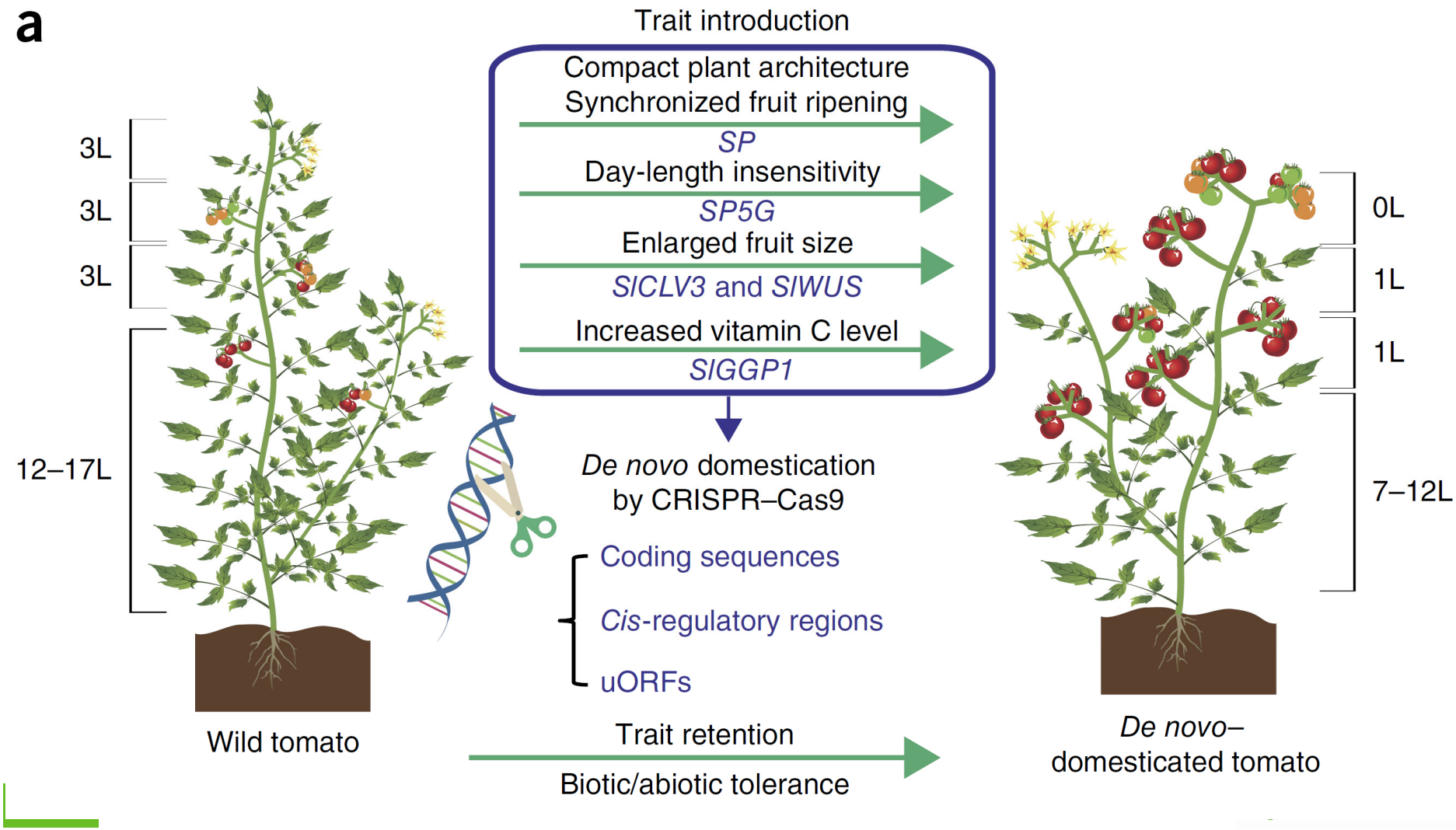


Wongsrikeao *et al*., 2011, Nature Methods

Outline

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

Gene Editing in Plant Domestication



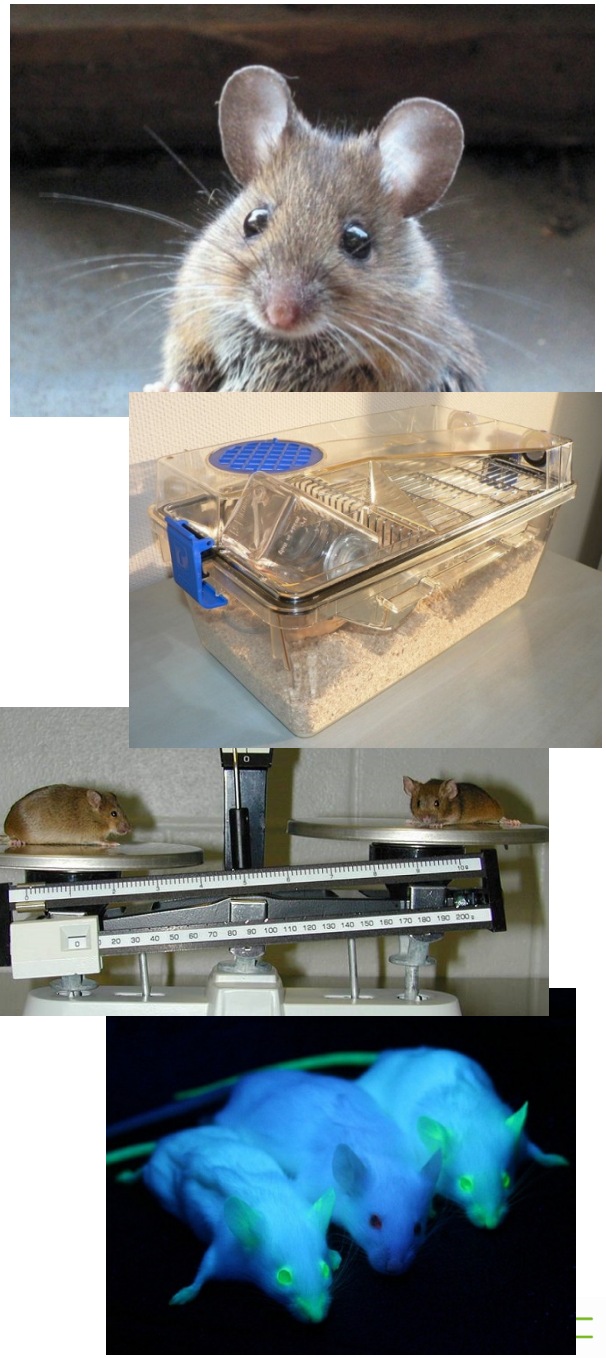
Outline

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

Mus musculus

house mouse

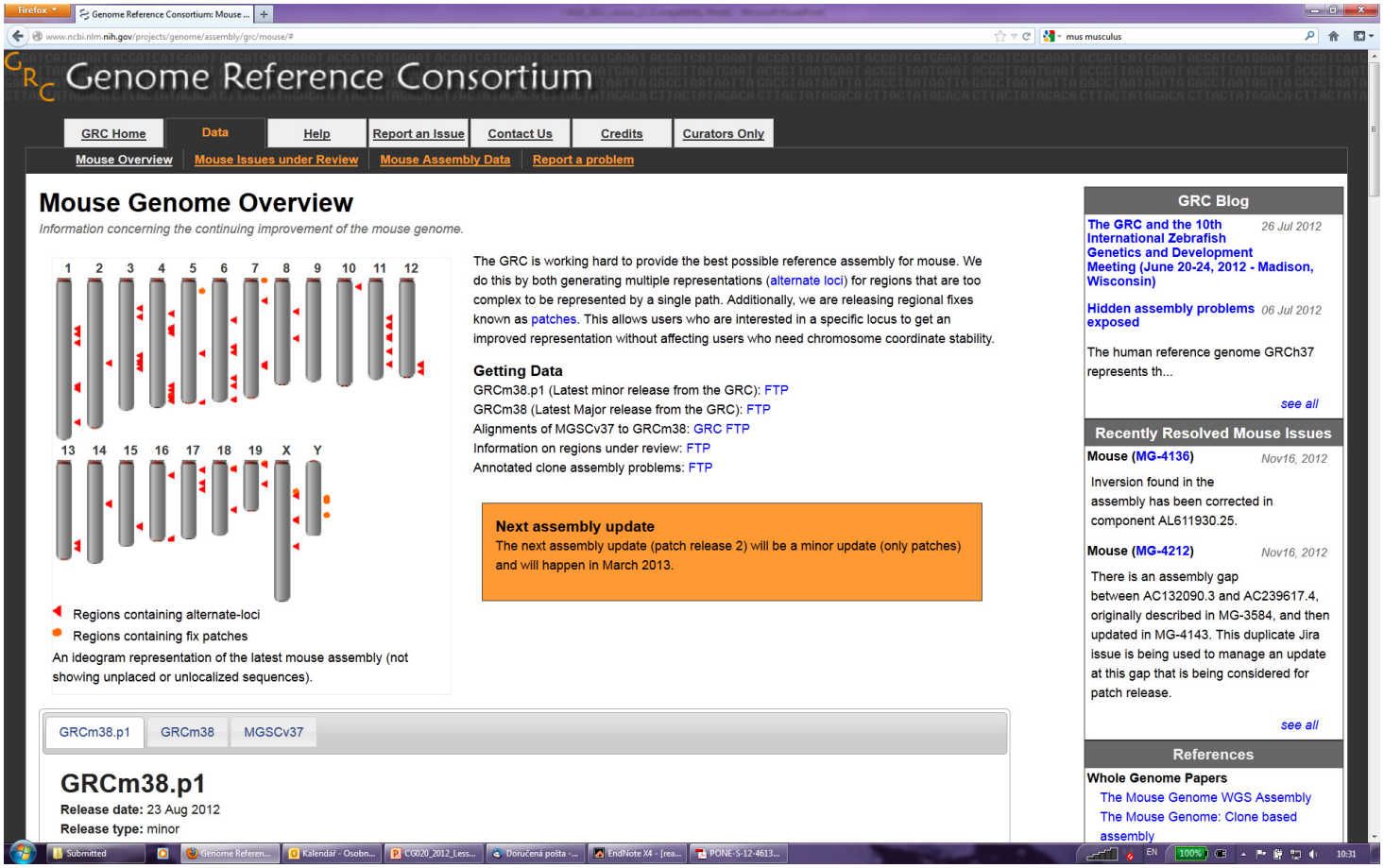
- Low requirements for area
- Relatively large number of offspring (3-14, 6-8 on average)
- Genome size is close to the size of human genome (about 3000 Mbp), the number of genes as well (about 24K)
- 20 chromosomes (19+1)
- Suitable for a wide range of physiological experiments (anatomical and physiological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



Mus musculus

house mouse

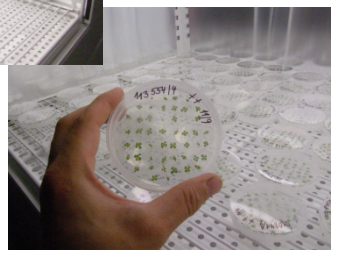
- Genome known since 2002 (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/mouse/)

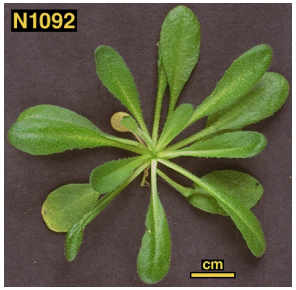


Arabidopsis thaliana

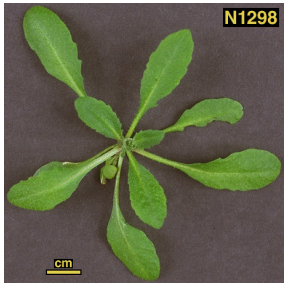
mouse-ear cress

- 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 od physiological experiments
- High natural variability (approximately 750 ecotypes (Nottingham Arabidopsis Seed Stock Centre))

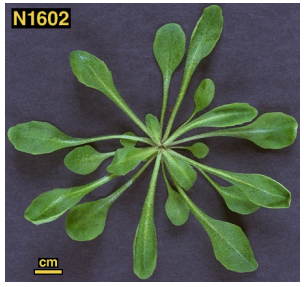




Columbia 0



Landsberg 0



Wassilewskija 0

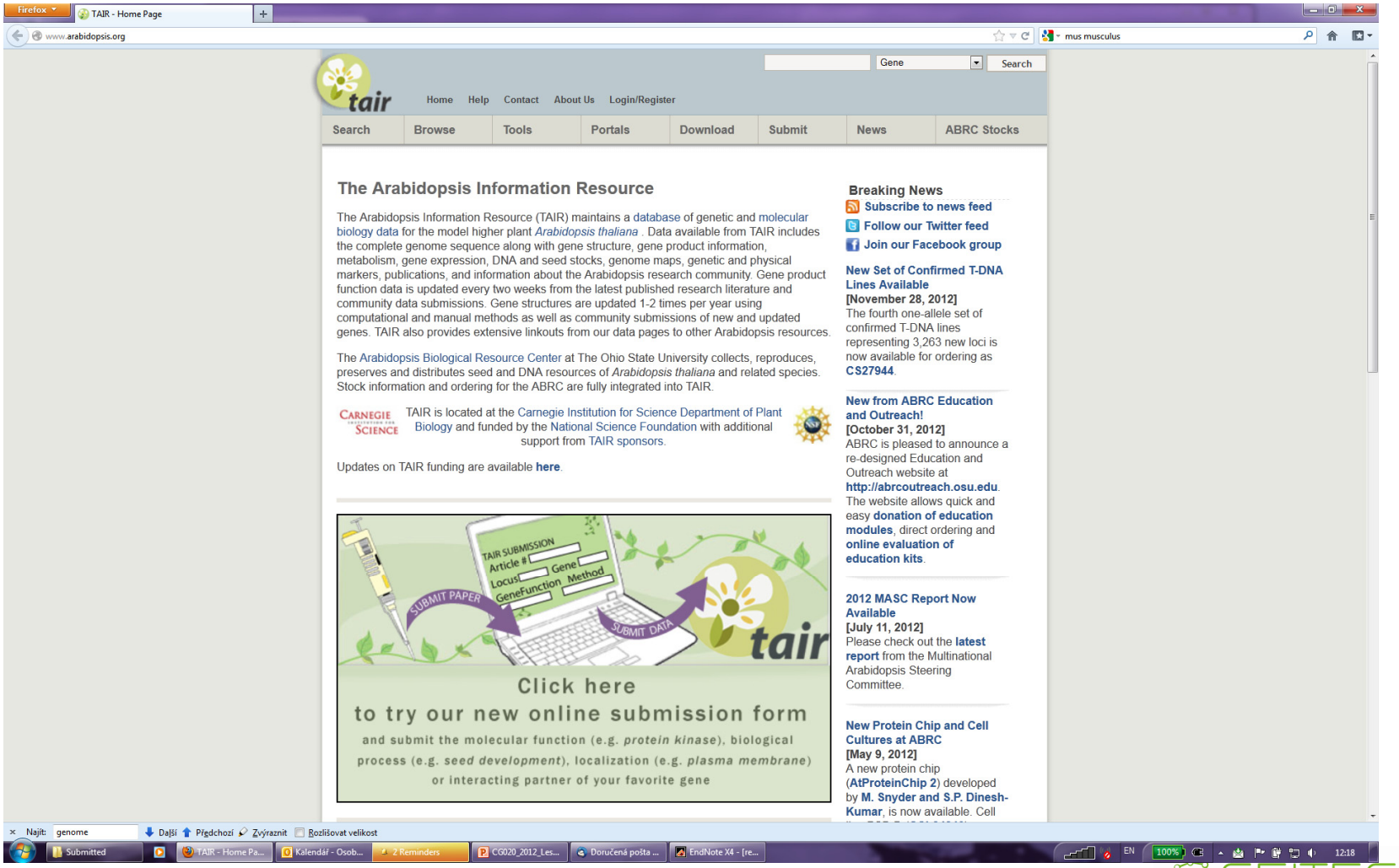
http://seeds.nottingham.ac.uk/

CEITEC

Arabidopsis thaliana

mouse-ear cress

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



Outline

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

Polymerase Chain Reaction

Key Concepts

- The techniques employing advance genetic and genomic approaches substantially improve our possibilities to reach desired effects in agriculture and medicine
- The programmable genome editing is promising principal changes in the cure of particularly inherited disorders and in the breeding of new varieties
- The rigorous control, but NOT complete ban is necessary

Discussion