

Programovatelné nukleázy: nejefektivnější nástroje pro editaci genomu ve službách biomedicíny

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České Centrum Fenogenomiky
BIOCEV

Proč programovatelné nukleázy

RNA-Guided Nucleases

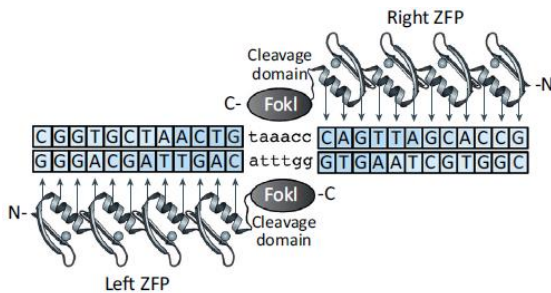
Nukleázy řízené RNA

- **vyjimečně výkonné nástroje využitelné k poznání jak pracuje genom**
- **Schopnost zacílit a upravovat specifické DNA sequence v celé šíři**
- **Možnost využití v lékařství**
- **a v dalších oborech ...**

programovatelné nukleázy

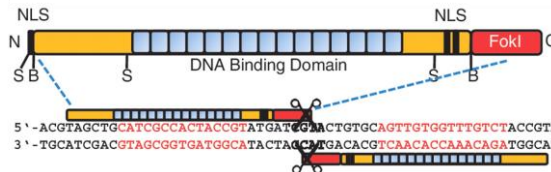
Zinc Finger Nucleases

- Cys2-His2 zinc-finger doména
- Umělé seřazení 3-6 Zinc-fingers (9–18 bp)
- C-terminální fúze s endonukleázou FokI



Transcription Activator-Like Effectors nucleases (TALENs)

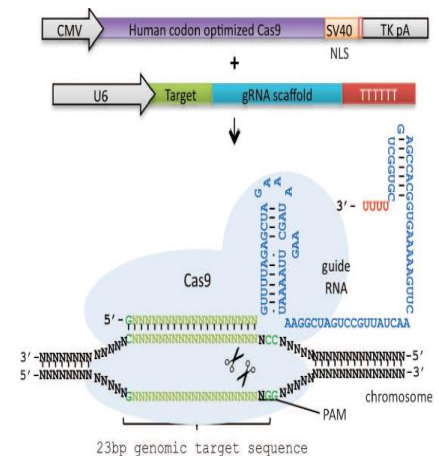
- Central Repeat Domain (CRD) odpovědná za vazbu na DNA
- CRD obsahuje moduly s vysoce homologní repeticemi o 34 aminokyselin
- Specifita vazby na DNA je determinována aminokyselinami 12 and 13 v každé repetici – repeat variable diresidues (RVDs)



Modulární kompletace dovoluje efektivní a ekonomickou tvorbu TALENových vektorů

CRISPR/Cas9 system

- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
- CRISPR RNAs (crRNAs) vytváří komplex s CRISPR-asociovaným proteinem (Cas)



Mali et al., Science 2013

Vědecká revoluce



CRISPR, THE DISRUPTOR

BY HEIDI LEDFORD

A powerful gene-editing technology is the biggest game changer to hit biology since PCR. But with its huge potential come pressing concerns.

Three years ago, Bruce Conklin came across a method that made him change the course of his lab. Conklin, a geneticist at the Gladstone Institutes in San Francisco, California, had been trying to work out how variations in DNA affect various human diseases, but his tools were cumbersome. When he worked with cells from patients, it was hard to know which sequences were important for disease and which were just background noise. And engineering a mutation into cells was expensive and laborious work. "It was a student's entire thesis to change one gene," he says.

Then, in 2012, he read about a newly published technique¹ called CRISPR that would allow researchers to quickly change the DNA of nearly any organism — including humans. Soon after, Conklin abandoned his previous approach to modelling disease and adopted this new one. His lab

is now feverishly altering genes associated with various heart conditions. "CRISPR is turning everything on its head," he says.

The sentiment is widely shared: CRISPR is causing a major upheaval in biomedical research. Unlike other gene-editing methods, it is cheap, quick and easy to use, and it has swept through labs around the world as a result. Researchers hope to use it to adjust human genes to eliminate diseases, create harderier plants, wipe out pathogens and much more besides. "I've seen two huge developments since I've been in science: CRISPR and PCR," says John Schimenti, a geneticist at Cornell University in Ithaca, New York. Like PCR, the gene-amplification method that revolutionized genetic engineering after its invention in 1985, "CRISPR is impacting the life sciences in so many ways," he says.

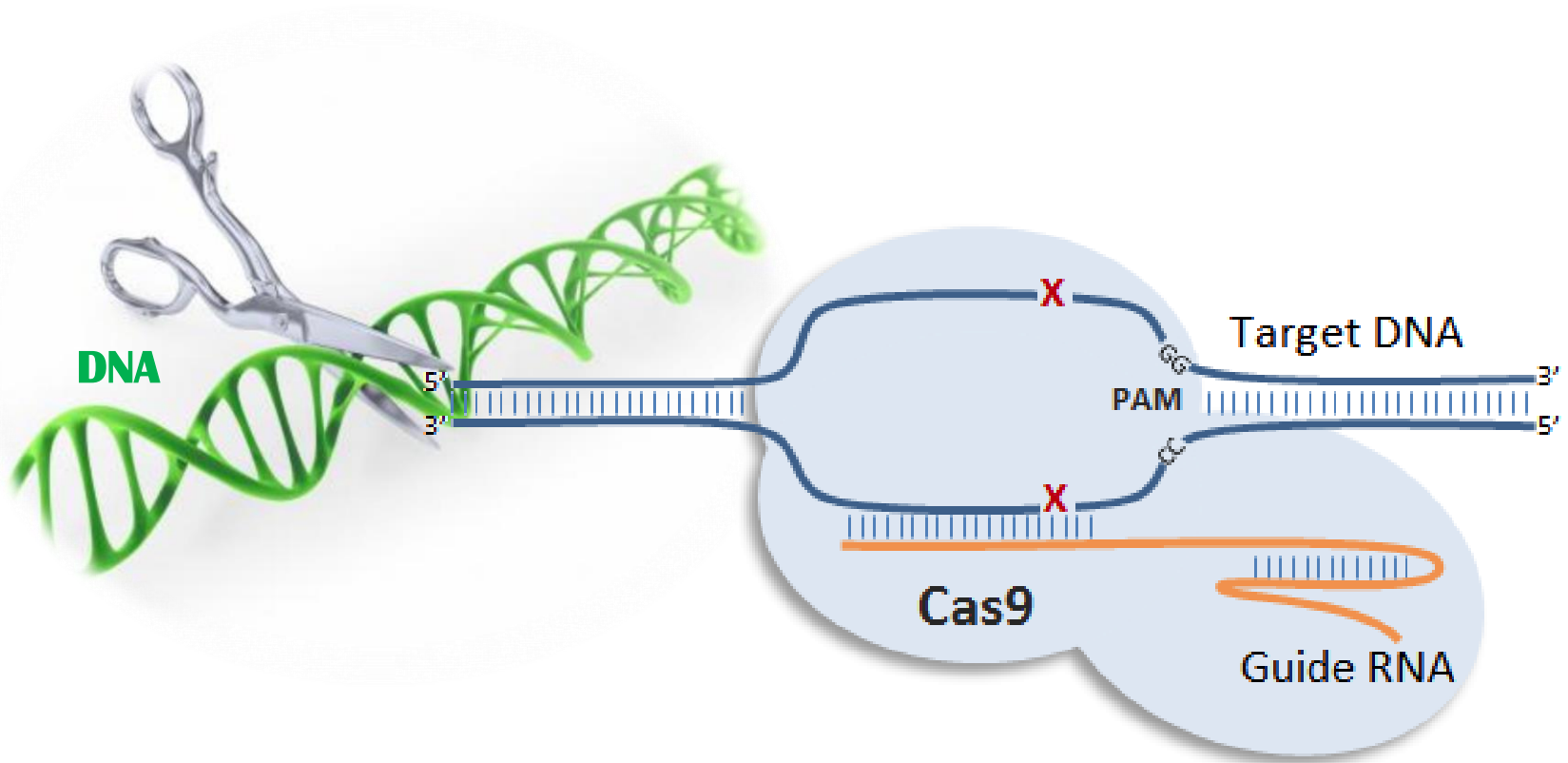
But although CRISPR has much to offer, some scientists are worried

ILLUSTRATIONS BY REBASTIEN THIBAUT

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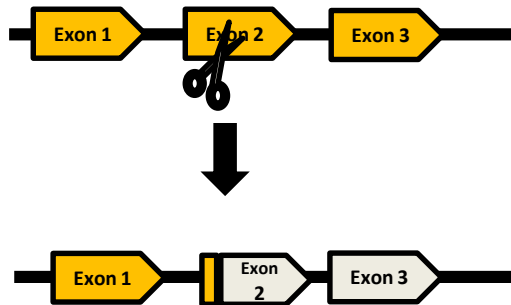
Jak to (CRISPR) funguje?



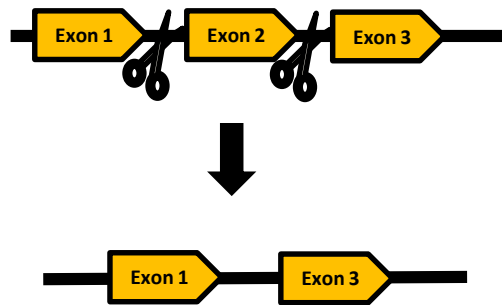
použití programovatelných nukleáz

základní možnosti

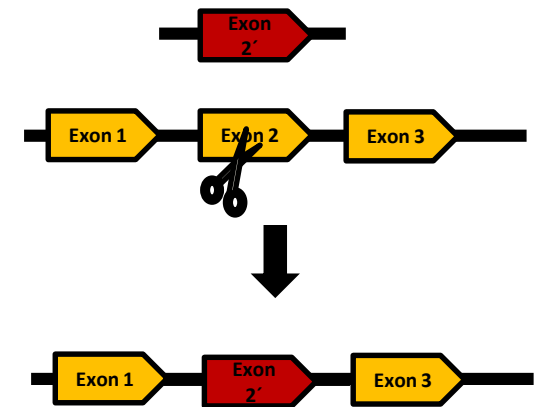
tvorba tzv. indel mutací



Excize/vyříznutí
Specifické DNA



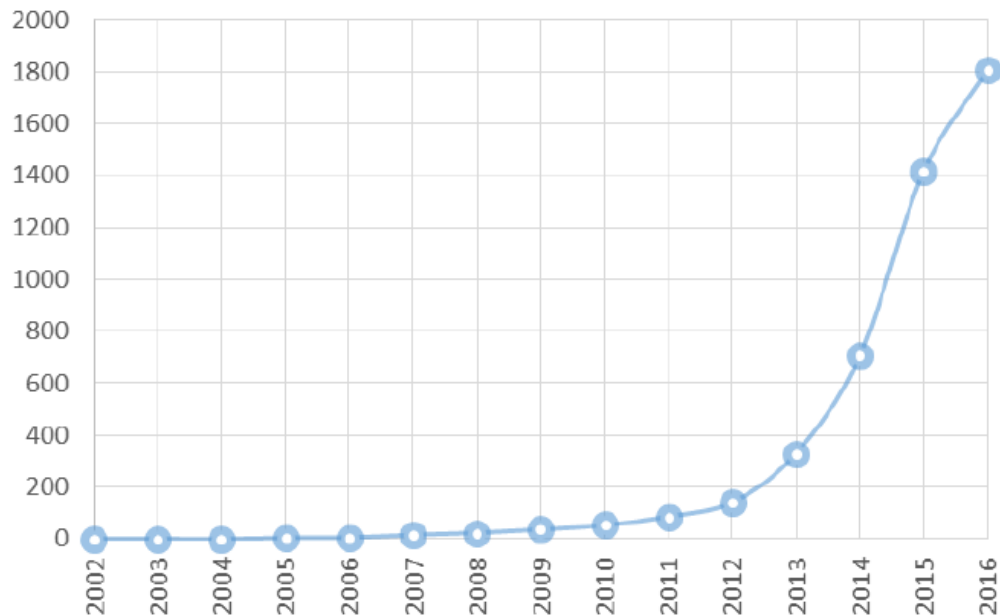
místně-specifická integrace
homologní rekombinace



Počet vědeckých publikací zmiňující technologii CRISPR

vědecká REVOLUCE

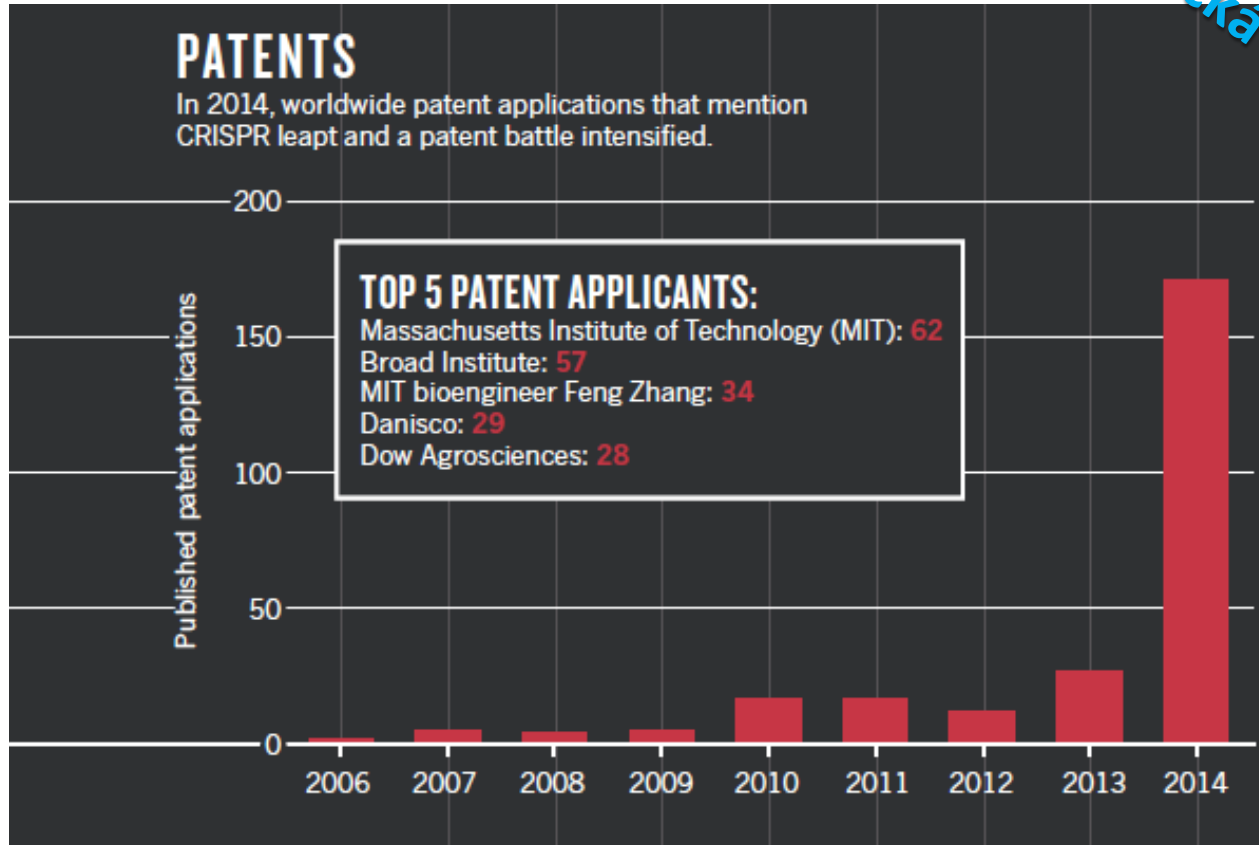
*obsahující slovo CRISPR (PubMed)



Vzestup technologie CRISPR

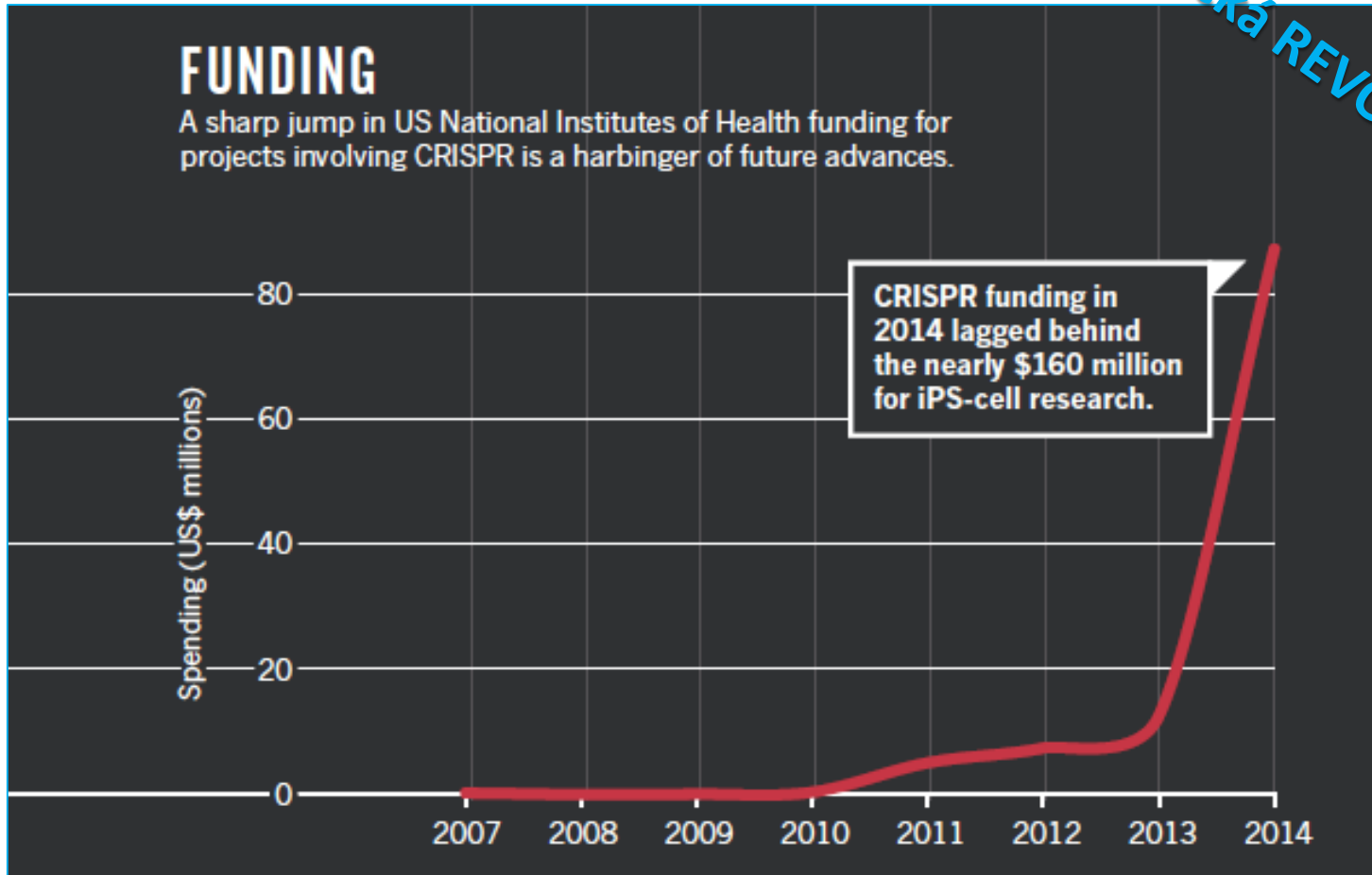
patenty

vědecká REVOLUCE



Vzestup technologie CRISPR

vědecká REVOLUCE



RNA-Guided Nucleases (RGN)

Nukleázy řízené RNA

výhody

Jednoduchý design a tvorba

- jednoduchá pravidla pro plánování (programování) zacílení targeting
- rychlá procedura
- Podpora při plánování – několik softwarových nástrojů

Efektivní nástroje

- tvorba mutantů je rychlá
- ekonomická efektivnost (*)

RNA-Guided Nucleases (RGN)

Nukleázy řízené RNA

výhody

Dostupnost

- komponenty jsou komerčně dostupné nebo mohou být vytvořeny v laboratoři

Technologie se dále vyvíjí a zlepšují

- RGNs se nepoužívají jen pro „ničení“
- nabízí vynikající možnosti pro systémové přístupy
- modifikované RGN mohou být zapnuty a vypnuty (on/off)

RNA-Guided Nucleases (RGN)

Nukleázy řízené RNA

limitace

- **efektivita a specifita (?)**
 - off-targeting (?)
 - **Zvířecí modely**
 - **Buňky**
 - **lidé**
 - **mozaicismus**
 - **více pozměněných alel**
 - **různé mutace**
 - **potřeba klonování a sekvenování**

Globální vývoj: možnosti a limitace

CRISPR & právní otázky:

Patentová válka

- ❑ Dr. Jennifer Doudna, University of California, Berkeley, patentová aplikace na CRISPR-technologii – květen 2012, zároveň s Dr. Emmanuelle Charpentier.
- ❑ Dr. Feng Zhang, Broad Institute of Harvard and MIT patentová aplikace na CRISPR-technologii v 2013 „fast-track“ proces a obdržel oficiální patent v dubnu 2014.
- ❑ Řada dalších patentových přihlášek

CRISPR & právní otázky: Patentová válka

IN FOCUS NEWS

PATENTS

Court rules on CRISPR

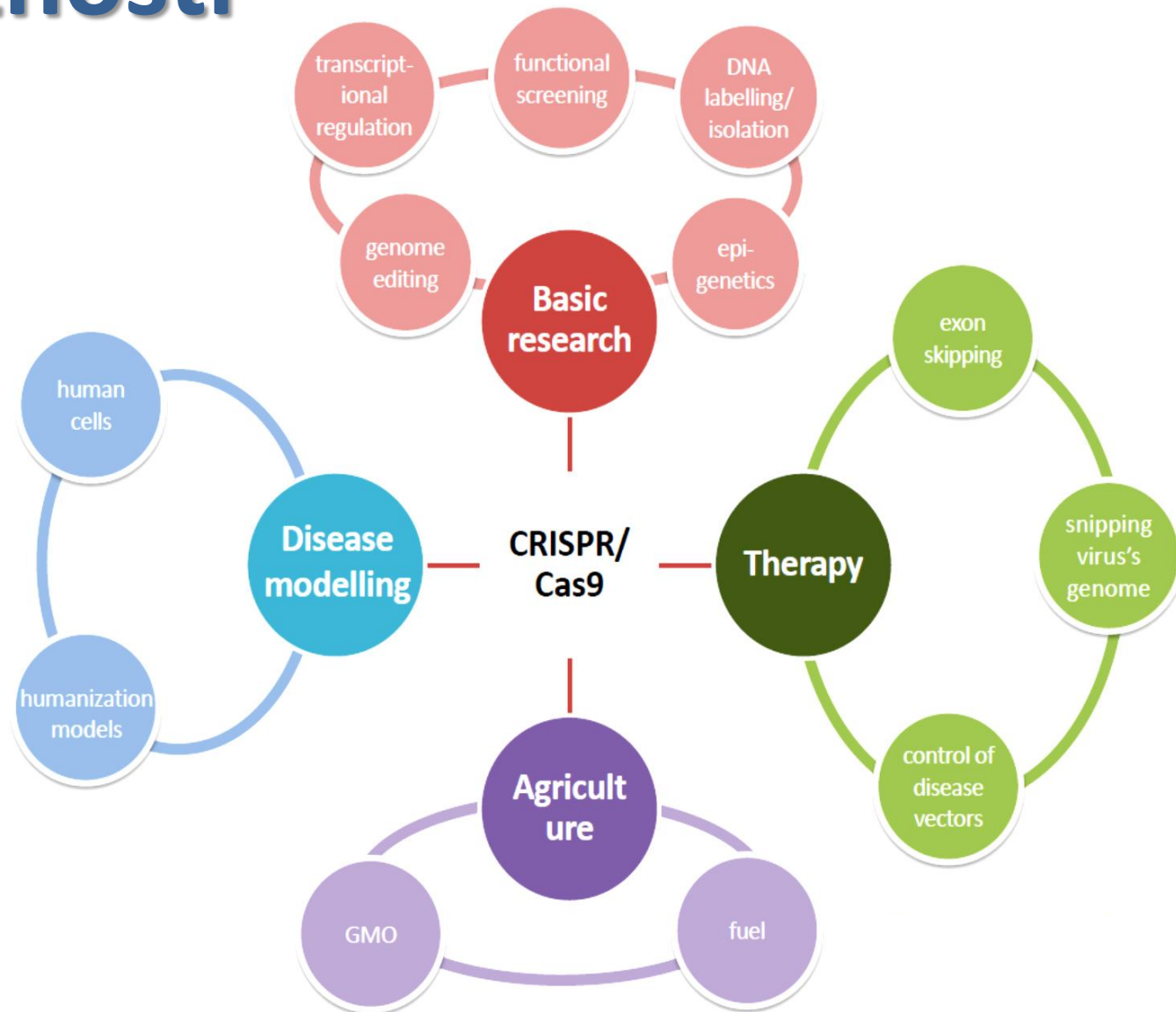
US Patent and Trademark Office sides with Broad Institute in genome-editing fight.

BY HEIDI LEDFORD

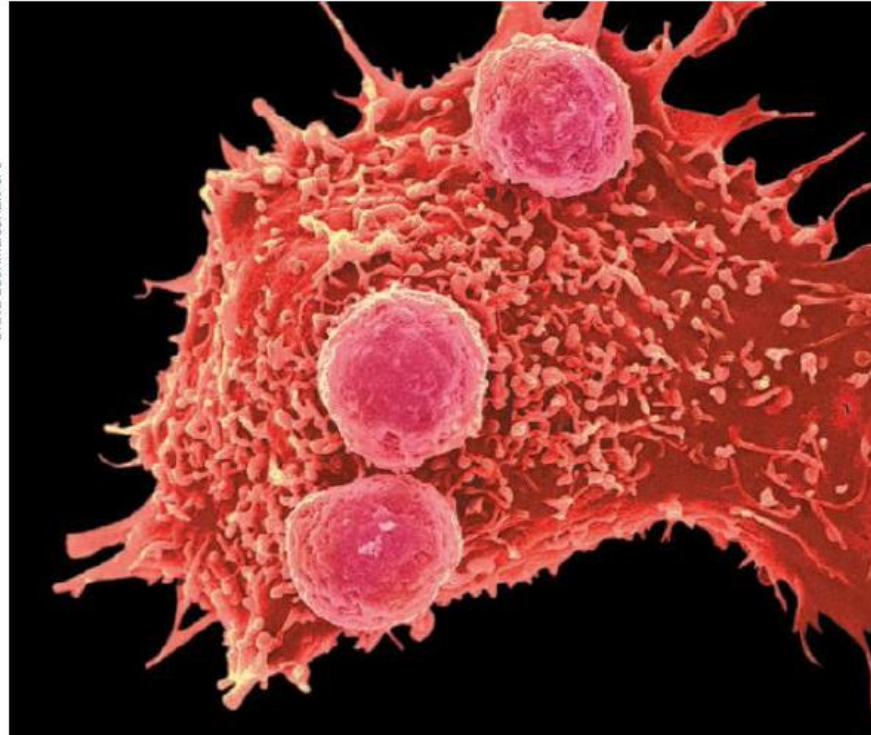
23 FEBRUARY 2017 | VOL 542 | NATURE | 401

Broad Institute (USA) vyhrává bitvu o CRISPR-patent

Možnosti



Nové možnosti v lékařství



Gene editing could improve the ability of immune cells (spherical) to attack cancer.

BIOTECHNOLOGY

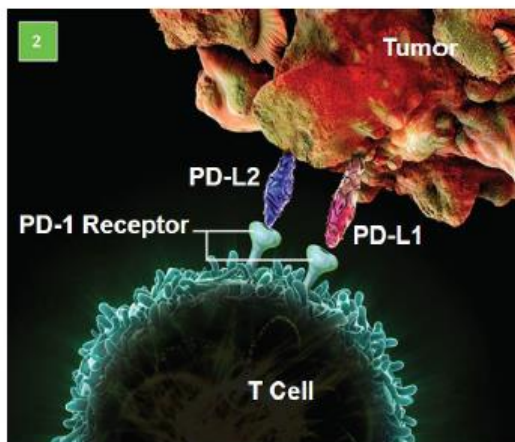
CRISPR gene editing tested in a person

Trial could spark biomedical duel between China and US.

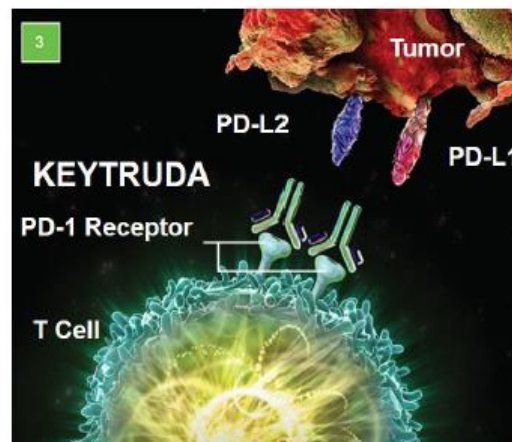
PD-1 léčba nádorového onemocnění

Keytruda(Pembrolizumab)

- humanizovaná terapeutická **protilátka** používaná k léčbě nádorového onemocnění
- Mechanismus účinku: váže se a blokuje PD1 receptor (programmed cell death)
- PD1 zamezeje, aby imunitní systém napadal tkáň vlastního těla
- Rakovinové buňky suprimují aktivitu T-buněk díky interakci s PD1 receptorem



Deaktivace T-buněk aktivovaná tumorem



Reaktivace T-buněk pomocí protilátek



Léčba pomocí PD-1 protilátek má množství negativních účinků

PD-1 léčba nádorového onemocnění

Article | [OPEN](#)

CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients



Shu Su, Bian Hu, Jie Shao, Bin Shen, Juan Du, Yinan Du, Jiankui Zhou, Lixia Yu, Lianru Zhang, Fangjun Chen, Huizi Sha, Lei Cheng, Fanyan Meng, Zhengyun Zou, Xingxu Huang  & Baorui Liu 

sběr CD4 T-buněk => *in vitro* CRISPR/Cas9 PD-1 genová editace

⇒ Upravené buňky jsou pak *in vitro* pomnoženy

=> Upravené pomnožené buňky jsou vpraveny zpět do pacienta

Genetické inženýrství v zárodečných buňkách u člověka



Intellia Therapeutics

Jennifer Doudna

- *Novartis*: New CRISPR/Cas9-based therapies using chimeric antigen receptor T cells (“CAR T cells”)
- *Regeneron Pharmaceuticals*: CRISPR/Cas-based therapeutic products primarily focused on gene editing in the liver



CRISPR Therapeutics

Emmanuelle Charpentier

- *Bayer HealthCare*: Create a joint venture, Casebia Therapeutics LLP (50% ownership)
- *Vertex*: Strategic research collaboration agreement
- *GlaxoSmithKline*



Editas Medicine

Feng Zhang

- *Juno Therapeutics*: Engineered T cells that have been genetically modified to recognize and kill other cells.
- *Adverum Biotechnologies*: Explore the delivery of genome editing medicines to treat up to five inherited retinal diseases
- *Cystic Fibrosis Foundation Therapeutics*: Modification of the cystic fibrosis transmembrane conductance regulator gene
- *Google*



source: <http://www.nanalyze.com>



Genetické inženýrství v medicíně

Závody ve vývoji nových terapií za použití Crispr-Cas9 technologie

5:00 PM EST SEP 22, 2016

By Amy Dockser Marcus and Joe Palazzolo

Some of the companies that are racing to develop therapies using the Crispr-Cas9 gene-editing system, without waiting to see who wins a patent dispute.

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BAYER AND CRISPR THERAPEUTICS

\$335 Million

Germany-based Bayer is investing \$335 million in a joint venture with Crispr Therapeutics. Together, they are pursuing treatments for blood disorders, blindness and heart disease in infants.

REGENERON PHARMACEUTICALS AND INTELLIA THERAPEUTICS

\$125 Million

Regeneron of Tarrytown, N.Y., is investing \$50 million into the startup and paying \$75 million to license Crispr to develop therapies via editing genes in the liver.

VERTEX AND CRISPR THERAPEUTICS

\$105 Million

The \$105 million deal included \$75 million in cash and a \$30 million equity investment to develop treatments for cystic fibrosis and sickle cell disease. Vertex has exclusive rights under the deal to license up to six new Crispr-Cas9 treatments from the collaboration.

FULCRUM THERAPEUTICS AND HORIZON DISCOVERY GROUP

\$55 Million

Fulcrum, which is developing therapies for Fragile X syndrome and a form of



Etické otázky

Etické otázky

Programovatelné nukleázy/CRISPR technologie

Junjiu Huang , Sun Yat-sen Univerzita, Guangzhou, China

- Popsal použití CRISPR–Cas9 technologie editovat genom lidských embryí
- Použití neživých embryo

>>> debata o etických otázkách editování genomu u lidských zárodečných buněk

Etické otázky

Programovatelné nukleázy/CRISPR technologie

First trial of CRISPR in people

Chinese team approved to test gene-edited cells in people with lung cancer.

476 | NATURE | VOL 535 | 28 JULY 2016

PD-1 léčba nádorového onemocnění

- Pacienti s metastatickou rakovinou plic, u nichž selhala chemoterapie i radioterapie
- Targeting PD-1 v T-buňkách k zesílení imunitní odpovědi proti tumorům
- fáze 1 (trial) - bezpečnost použití

Etické otázky

Programovatelné nukleázy/CRISPR technologie

BIOTECHNOLOGY

A prudent path forward for genomic engineering and germline gene modification

A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed

SCIENCE sciencemag.org

3 APRIL 2015 • VOL 348 ISSUE 6230

By David Baltimore,¹ Paul Berg,²
Michael Botchan,^{3,4} Dana Carroll,⁵
R. Alta Charo,⁶ George Church,⁷
Jacob E. Corn,⁴ George Q. Daley,^{8,9}
Jennifer A. Doudna,^{4,10*} Marsha Fenner,⁴
Henry T. Greely,¹¹ Martin Jinek,¹²
G. Steven Martin,¹³ Edward Penhoet,¹⁴
Jennifer Puck,¹⁵ Samuel H. Sternberg,¹⁶
Jonathan S. Weissman,^{4,17}
Keith R. Yamamoto^{4,18}

IGI Forum on Bioethics, Napa, California

“...we...discourage... germline genome modification for clinical application in humans, while... implications of such activity are discussed...”

...my ... nedoporučujeme... modifikace zárodečných buněk pro klinické aplikace u lidí ...

Etické otázky

Programovatelné nukleázy/CRISPR technologie



January 18, 2017

Proposals for NIH-funded human gene therapy clinical trials are discussed and reviewed for scientific, clinical, and ethical issues by the NIH's **Recombinant DNA Advisory Committee (RAC)**.

The RAC recently discussed (and did not find any objections to) the first clinical protocol to use CRISPR/Cas9-mediated gene editing.

RAC diskutoval (a nemá žádných námitek) k prvním klinickému protokolu pro použití CRISPR/Cas9-zprostředkované genové editace.

Etické otázky

Programovatelné nukleázy/CRISPR technologie

Sociální konsekvence

Nerovný přístup

“Prediction: my grandchildren will be embryo-screened, germline-edited. Won't 'change what it means to be human'. It'll be like vaccination.”

Dan MacArthur:

programovatelné nukleázy a geneticky modifikované organismy

Genome editing: scientific opportunities, public interests and policy options in the European Union



EASAC policy report 31

March 2017

ISBN: 978-3-8047-3727-3


This report can be found at
www.easac.eu

Science Advice for the Benefit of Europe

Programovatelné nukleázy v ČR a příklady

Czech Centre for Phenogenomics

IMPC: globální projekt



IMPC

Production of an
Encyclopaedia of Mammalian
Gene Function

International Mouse Phenotyping Consortium

Mark Moore, Ph.D.

<http://www.mousephenotype.org/>

Czech Centre for Phenogenomics

IMPC: global projekt

Group of Senior Officials on Global Research Infrastructures Progress Report 2015

Meeting of the G7 Science Ministers
8-9 October 2015

Annex Case Studies:

1. The International Mouse Phenotyping Consortium (IMPC) is comprised of 18 research institutions and 5 national funders, representing 12 countries from 4 continents and has been in operation since 2011. The mission of IMPC is to build the first comprehensive functional catalogue of a mammalian genome, which will give new insights into gene function and human disease. This bold goal will require the support, infrastructures and cooperation of multiple countries. The



G7 GERMANY 2015

Modely lidských nemocí

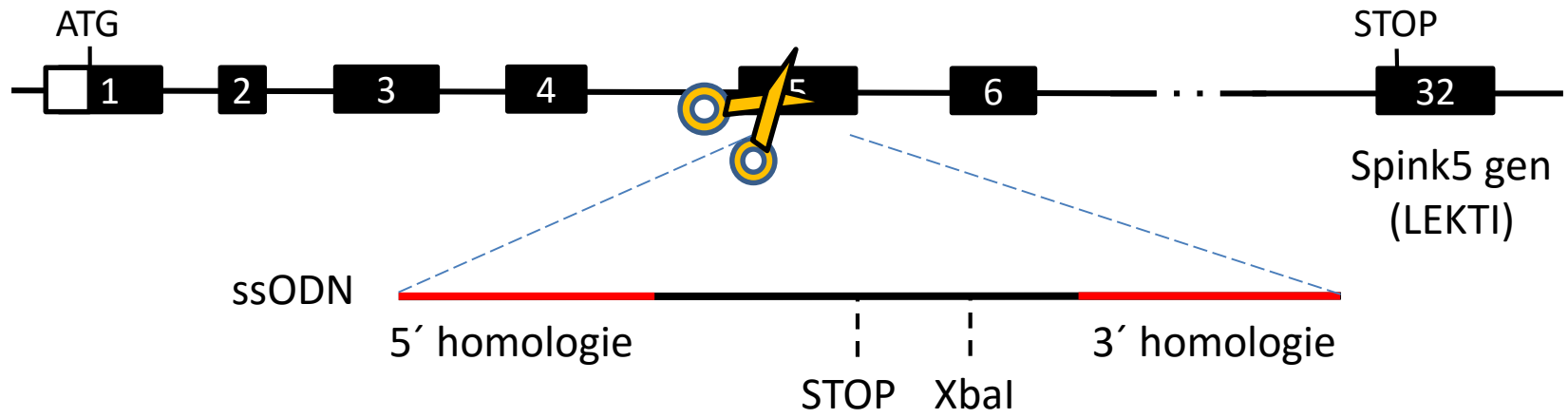
Nethertonův syndrom

- autozomálně-recesivně dědičná kožní porucha, která postihuje kůži, vlasy, imunitní systém (1:200 000)
- Chronický zánět epidermis, abnormální deskvamace, nefunkční epidermální bariera, časté alergické reakce
- abnormality vlasů a ochlupení
- bodová mutace v genu Spink 5

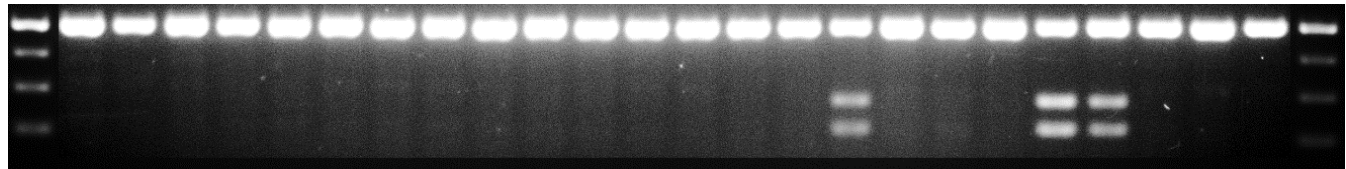


Modely lidských nemocí

Nethertonův syndrom Tvorba transgenního modelu



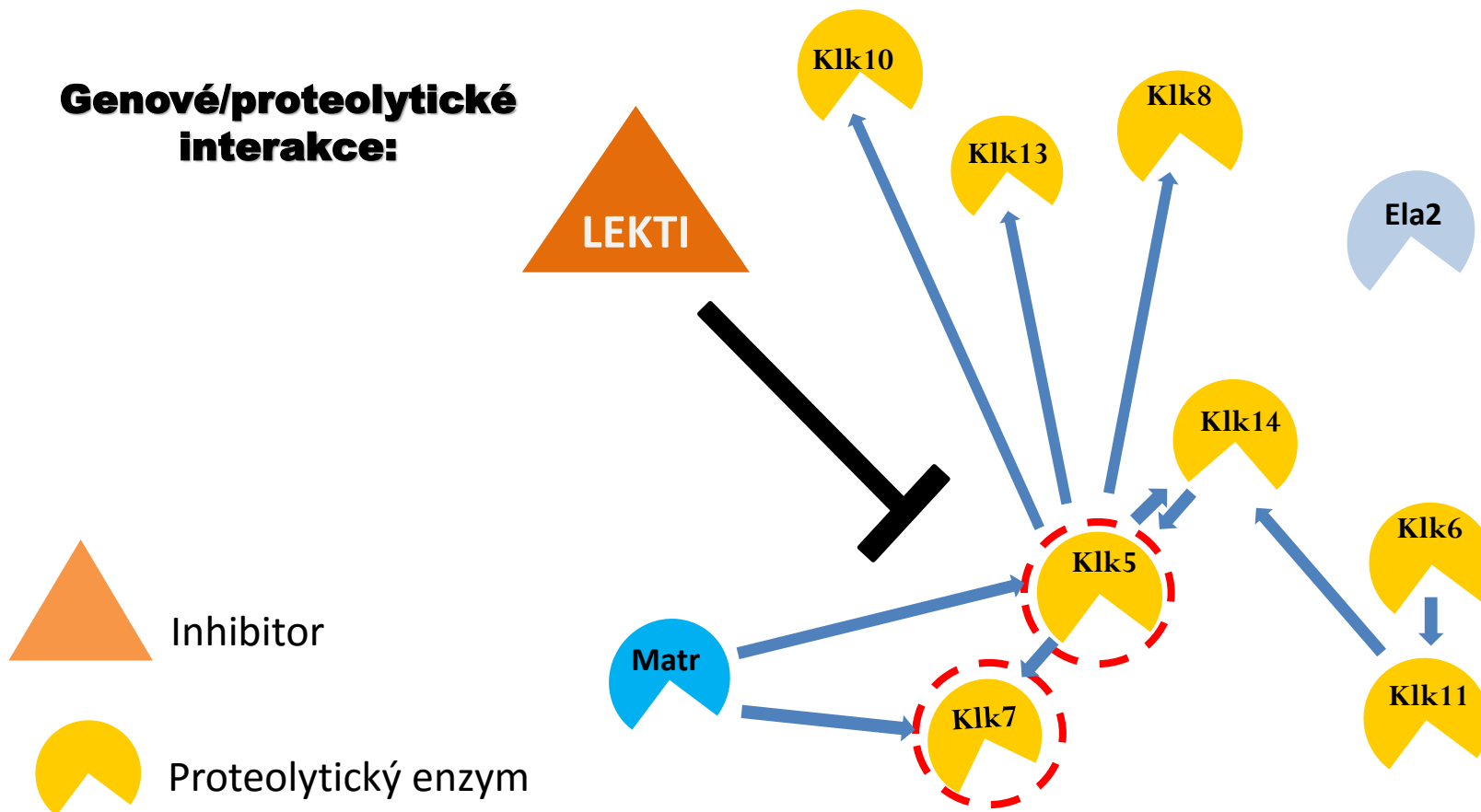
Spink5 PCR
- XbaI



Modely lidských nemocí

Nethertonův syndrom

Genové/proteolytické interakce:

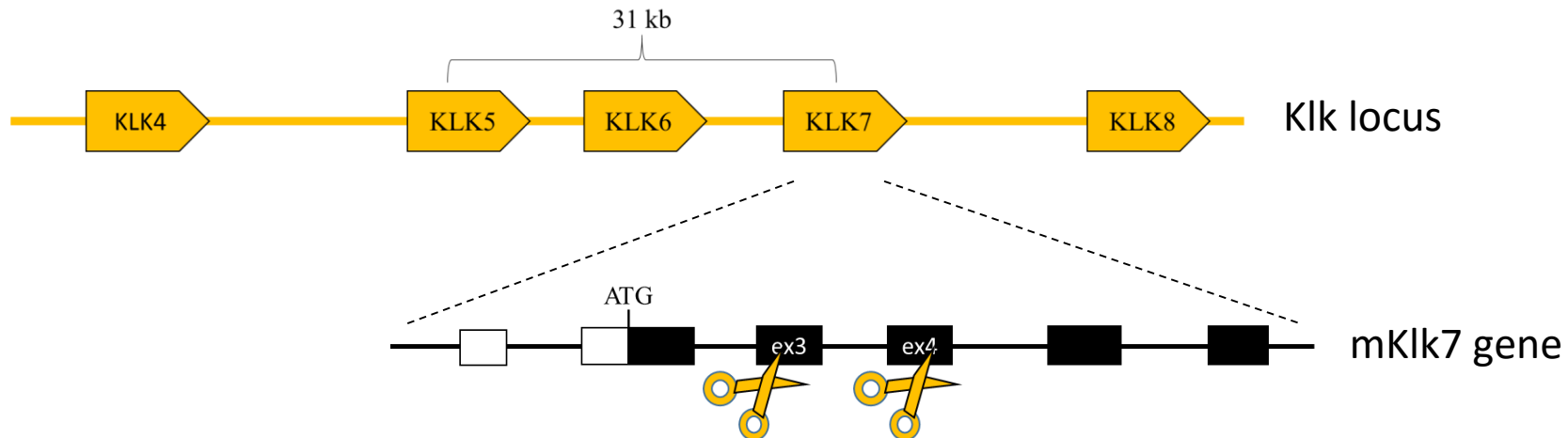


Kasperek P, et al., PLoS Genet. 2017 Jan 17;13(1)

Modely lidských nemocí

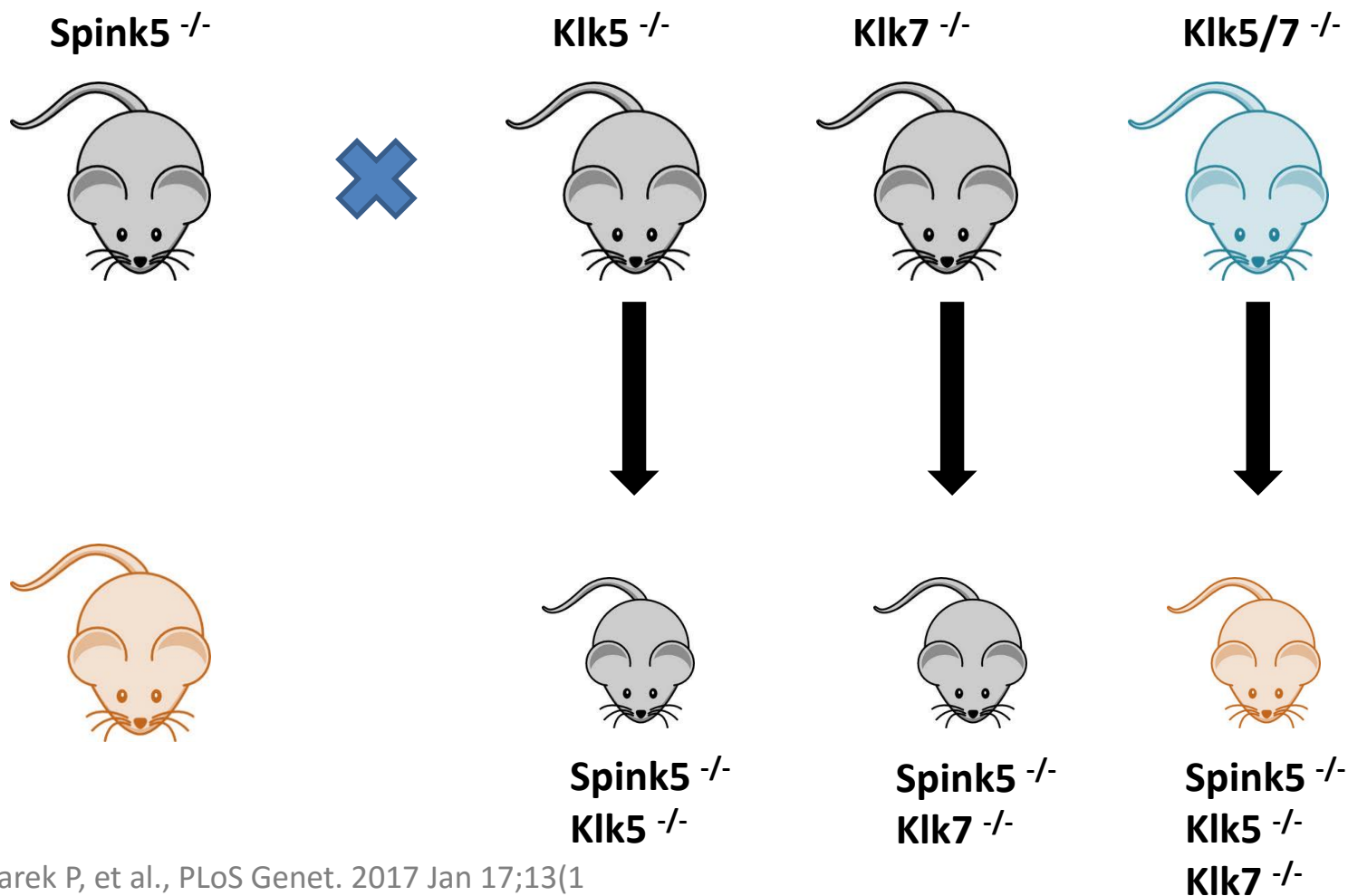
Nethertonův syndrom

tvorba transgenních modelů (Klk5/Klk7 DBKO)



Modely lidských nemocí

Nethertonův syndrom



Kasperek P, et al., PLoS Genet. 2017 Jan 17;13(1)



Our research is

enhancing the understanding of the genetic bases for human diseases

Phenotyping

The genomes of humans, mice and other species have been completely sequenced, yet the knowledge of genome sequences as such does not shed light on questions concerning the functions of these sequences. In order to describe biological functions of a gene, informative genetic modifications are introduced into the genes...

[Learn more →](#)

CCP: model generation services

Genetically modified mouse models have become a key tool in basic and biomedical research. The ability to engineer the mouse genome has greatly transformed biomedical research in the last decade.

[Learn more →](#)

Research

The research program is focused onto functional genomics using genetically engineered models and is closely connected to the infrastructure of Czech Centre for Phenogenomics that provides the project indispensable core facilities

[Learn more →](#)

Latest news

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Phenotyping course as part of 13th Transgenic Technology Meeting (TT2016) in Prague, Czech Republic

As part of 13th Transgenic Technology Meeting (TT2016) we are arranging 3-day workshop designed to ...

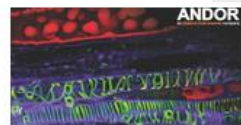
[Read more →](#)



Phenotyping course as part of 13th Transgenic Technology Meeting (TT2016) in Prague, Czech Republic

As part of 13th Transgenic Technology Meeting (TT2016) we are arranging 3-day course on mouse ...

[Read more →](#)



Phenotyping course as part of 13th Transgenic Technology Meeting (TT2016) in Prague, Czech Republic

We would like to draw your attention to this seminar by Sasa Pospesch from Andor ...

[Read more →](#)

Děkuji za pozornost