Czech Centre for Phenogenomics

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

IMG:
From gene function to understanding gene mutation

functional genomics based on mouse and rat models

Radislav Sedláček

Czech Centre for Phenogenomics

IMG
basic facts

Director: Václav Hořejší

- construction costs – over 35 mil. €
- opened in January 2007
- modern animal facility (for 30.000 mice)
- conference hall for 300 people
- total - cca 350 people including 140 PhD and MSc students

Czech Centre for Phenogenomics

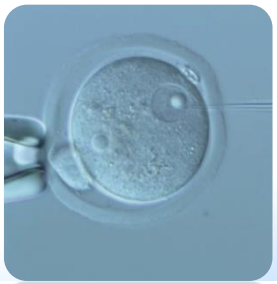
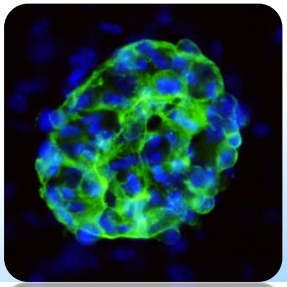
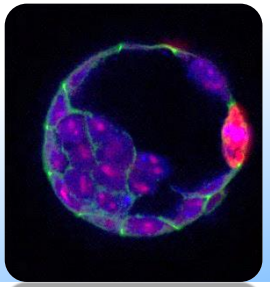
BIOCEV
basic facts

- project: IMG & 6 partners incl. Charles University, Prague
- 400 researchers & 200 students
- 55 research teams in 5 programmes
- integration into European Research Area
- estimated total cost: 120 mil. €
- completion: May 2015
- 5 state-of-the-art core facilities/infrastructures
  - Czech center for Phenogenomics

Czech Centre for Phenogenomics

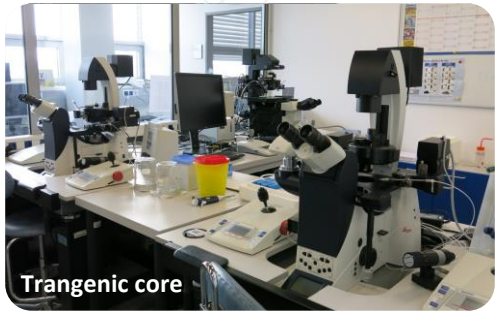
Czech Centre for Phenogenomics (CCP)
basic facts

- unique in the Czech Republic, large national infrastructure
- 7200 m2, 24 mil. €, 30 000 cages
- one of 5 in Europe, and of 12 in the world
- International centre, open acces

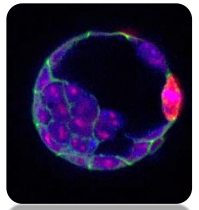

Czech Centre for Phenogenomics (CCP) activities


production


Trangenic core


Cryo-archiving unit

- new genetically modified (transgenic) models
- examination of the role of infomative mutation in all main physiological systems
- standardized primary (410 parameters) and secondary phenotyping
- cryo-archiving and distribution of transgenic models



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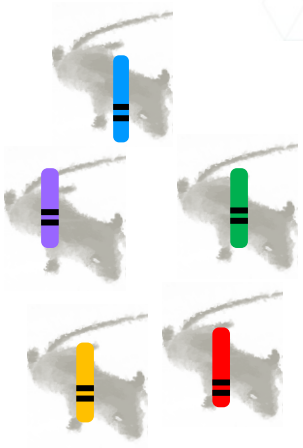
Phenogenomics:

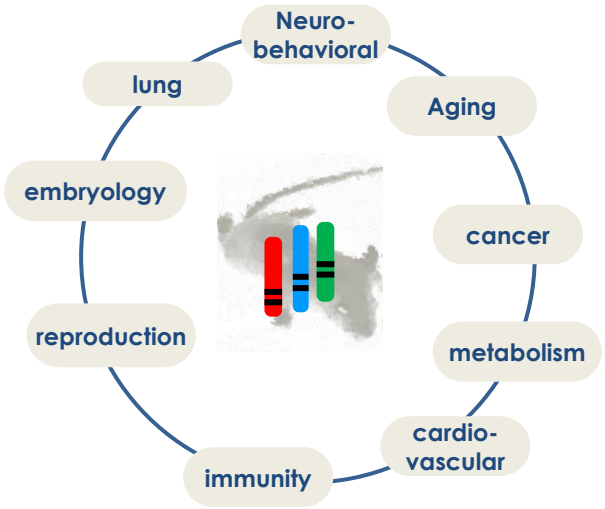
Systematic phenotypic analysis of animal models for annotation of gene function

genes to knock-out
(informative mutation)



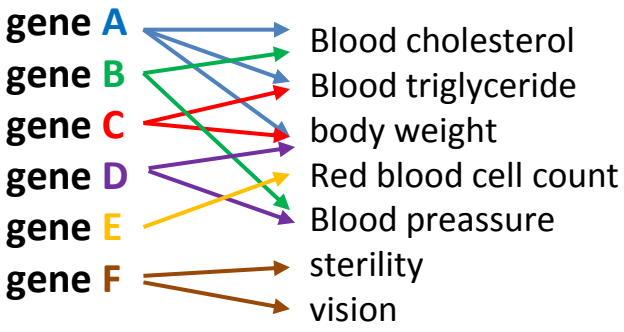
potential relevance to human diseases

comprehensive & large-scale phenotyping



more then 400 standardized parameters

Phenotype:
deciphering functions of individual genes



Encyclopaedic database on gene function

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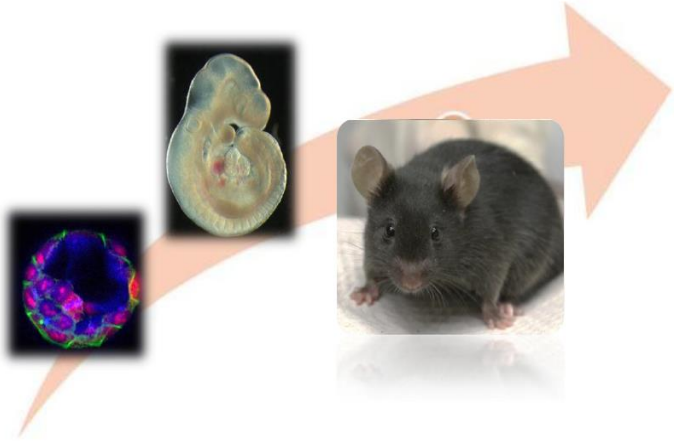
research projects



Systemic standardized phenotyping

(functions of individual genes)

410 parameters

new technologies of genetic manipulation

aging, epigenetics, embryogenesis

Genetically modified mouse models to study human diseases:

Reproduction

mouse strains with chromosomal substitutions (consomic strains),

epigenetics, genetically controlled sterility, gene duplication

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research projects

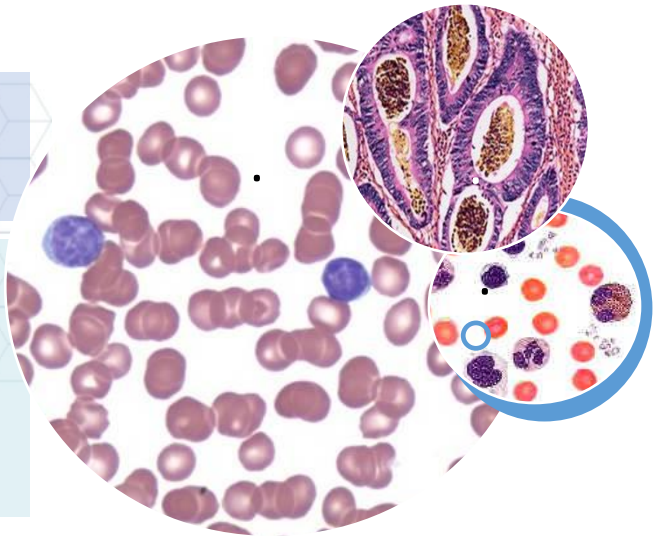
cancer:

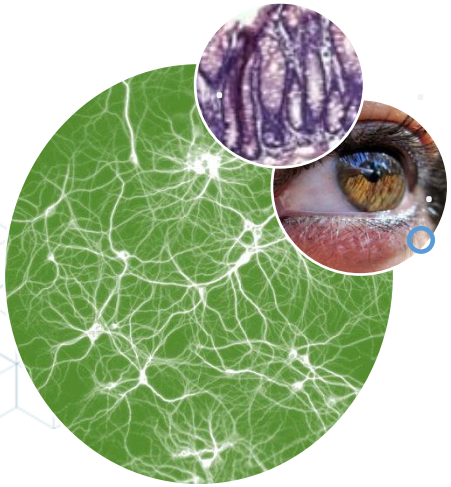
colorectal carcinoma

Inflammatory bowel diseases

Patho-physiology of gut epitelia

Metabolic and cardiovascular diseases





Hematologic disordes:

chronic lymphatic leukaemia, non-Hodgkin lymphoma

remodeling of chromatin structures

Neurobehavioral studies, auditory and visual functions

genetic disorders of eye, hearing impairment

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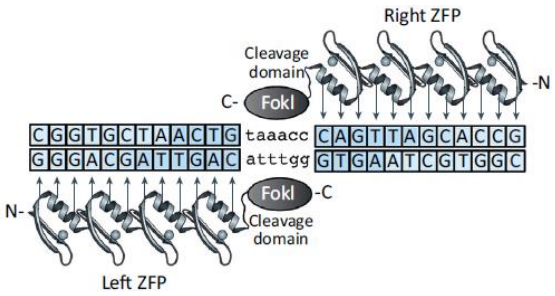
programmable nucleases

-mediated gene modifications

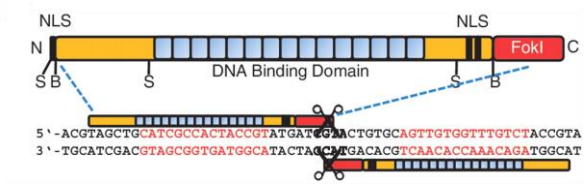
Zinc Finger Nucleases

- Cys2-His2 zinc finger domain
- Artificial arrays of 3-6 Zinc Fingers (9 – 18 bp)
- C-terminal fusion with endonuclease (FokI) – ZFN



Transcription Activator-Like Effectors nucleases (TALENs)

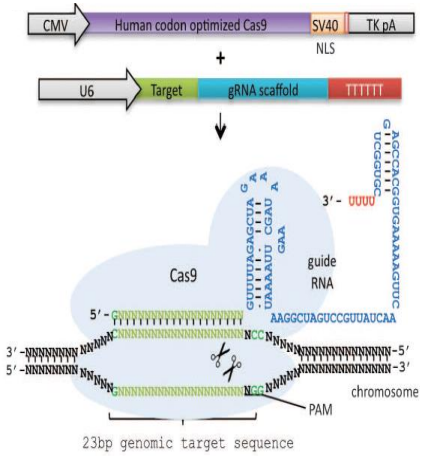
- Central Repeat Domain (CRD) responsible for DNA binding
- CRD consisting of 34aa highly homologous repeat modules
- DNA specifity determined by aminoacids 12 and 13 of each repeat
  – repeat variable diresidues (RVDs)



Modular assembly allows efficient and low-cost generation of TALEN vectors

CRISPR/Cas9 system

- interspaced short palindromic repeats (CRISPR) systems
- CRISPR RNAs (crRNAs) in complex with CRISPR-associated (Cas) proteins



Mali et al., Science 2013

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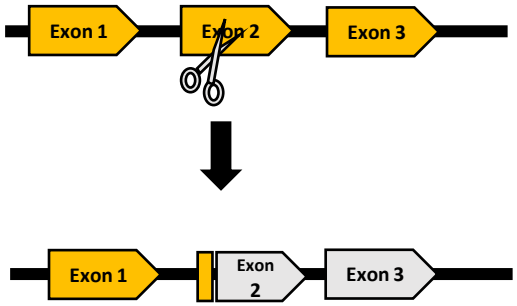
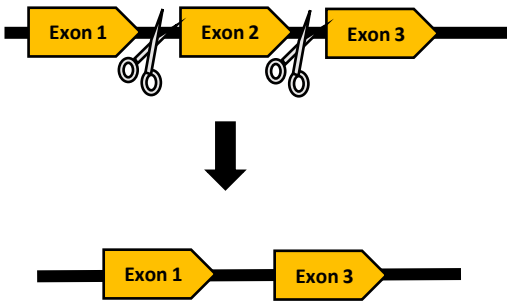
possibilities using programmable nucleases

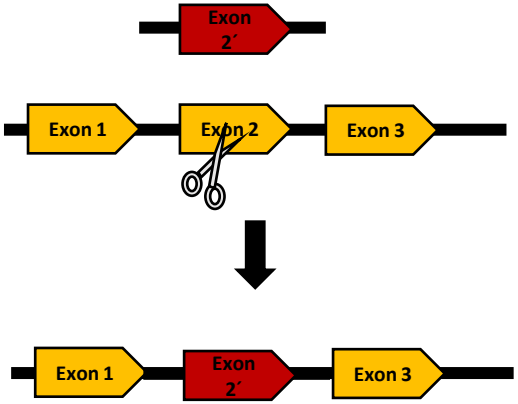
Generation of indel mutation => KO mouse



Excision of DNA fragment



Site-specific integration of DNA fragment



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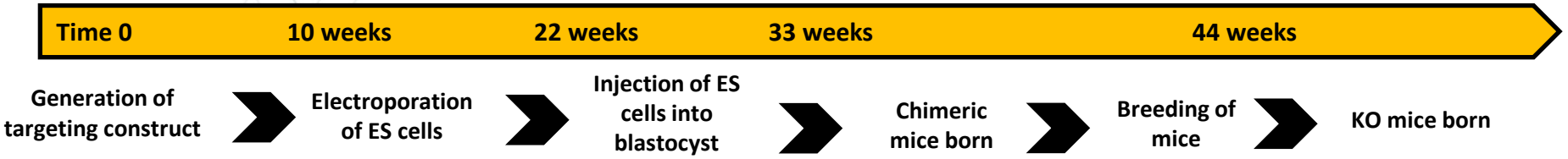
KO mouse generation by homologous recombination in ES cells vs TALEN technology



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programmable nucleases

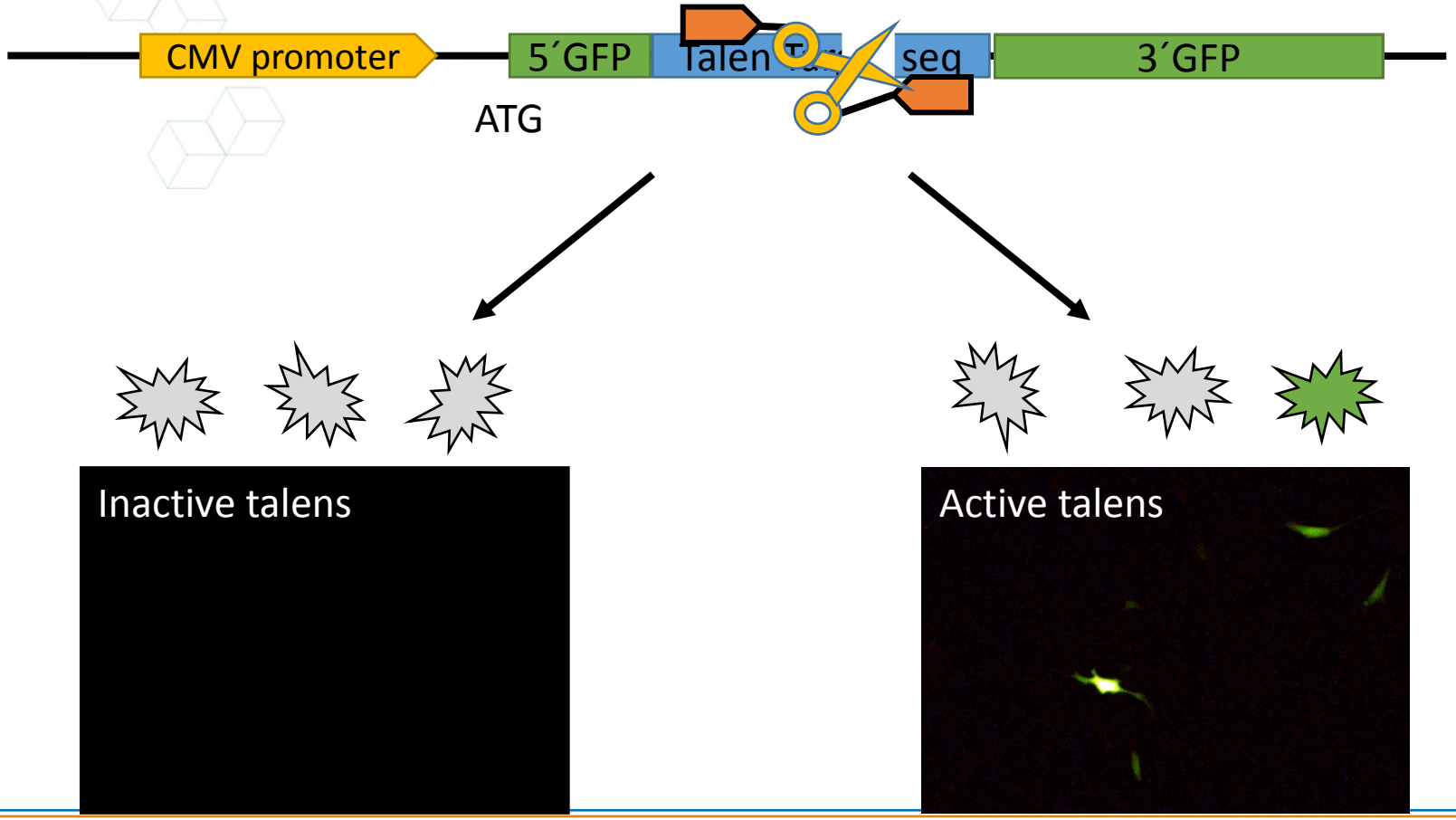
Transcription Activator-Like Effectors (TALEN) technology

application examples

From gene function to understanding gene mutation

Enrichment of TALEN-targeted cells

- **Reporter plasmid**
  - re-constitution of GFP activity

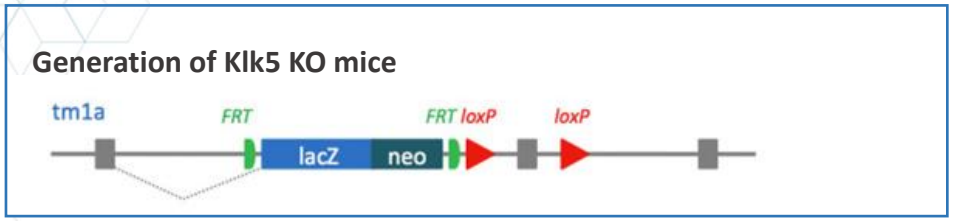


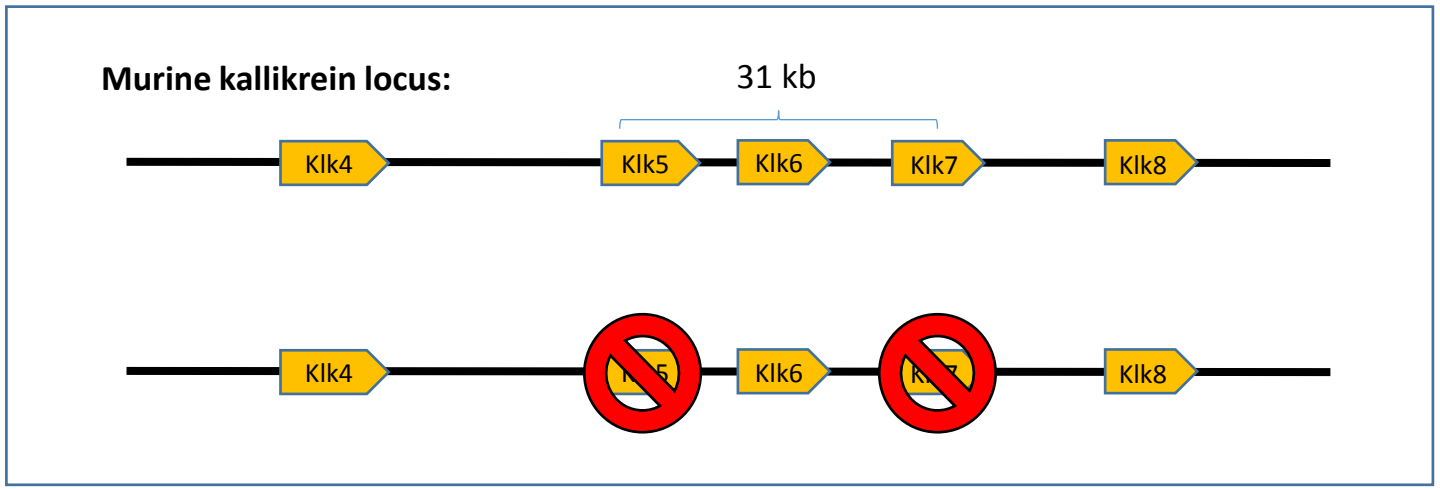
programmable nucleases

TALEN technology

deletion mutants/knockouts

Generation of Kallikrein 7 KO mouse & double KLK5/7 KO mouse





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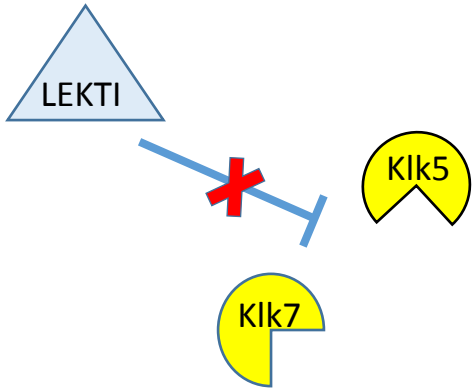
Generation of Kallikrein 7 KO mouse & double KLK5/7 KO mouse & triple Spink/KLK5/7 KO mouse

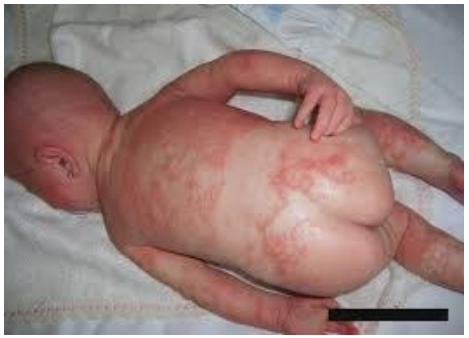


Netherton syndrome

Model:
Spink5 (LEKTI)-deficient mouse

Descargues P. et al., Nature Genetics, 2005

Patient



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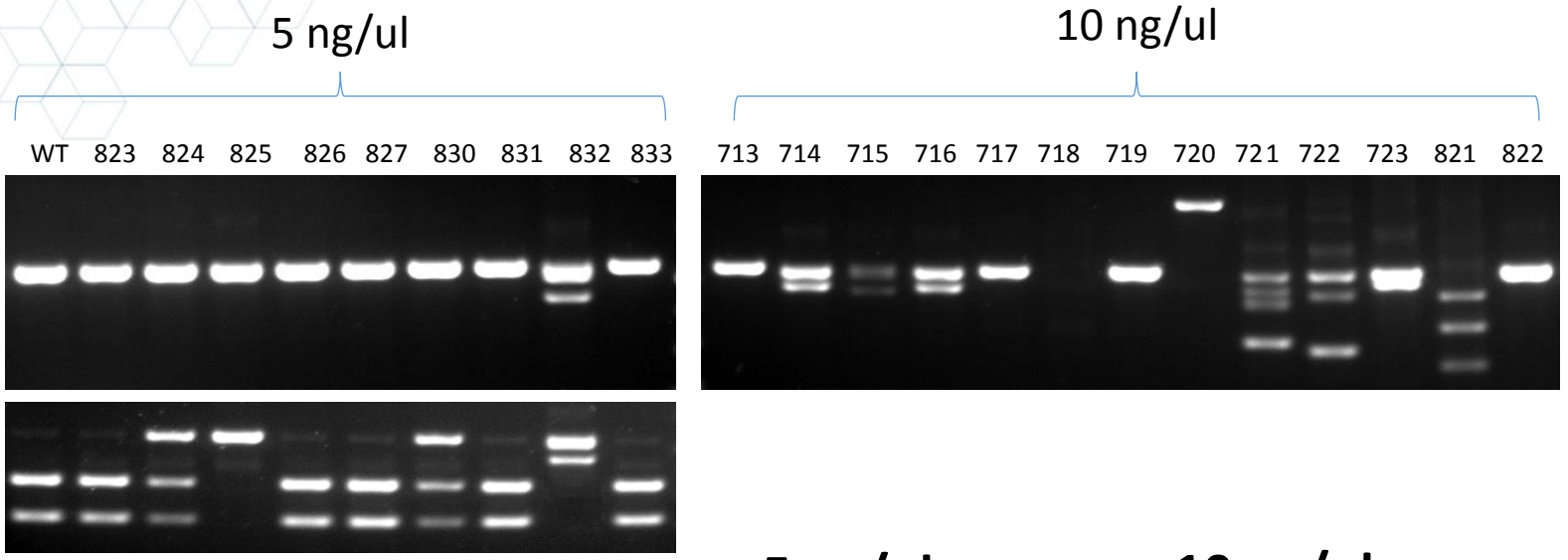
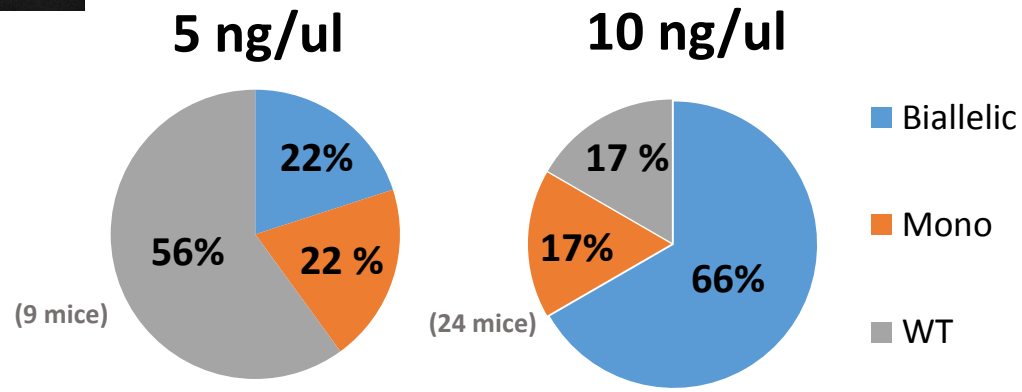
Generation of Klk7 KO mouse

Efficient targeting of Klk7 locus by TALE nucleases

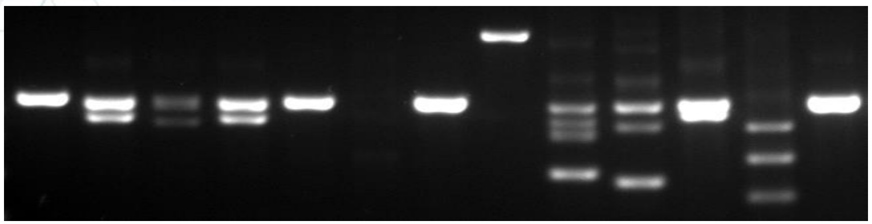




Generation of Klk7 KO mouse

Klk7 KO - Sequencing of indel mutations

PCR – Klk7 exon4



WT

AGTGGAGGCAGTCCAGCTTCCT**GAACACTGTGAACCC**CCAGGGACGTCATGTAC**CGTCTCTGGATGGGGCA**CCACAACCAGCCCAGACG

AGTGGAGGCAGTCCAGCTTCCT**GAACACTGTGAACCC**CCAGGG- - - - - - - -GTAC**CGTCTCTGGATGGGGCA**CCACAACCAGCCCAGACG -7 bp

AGTGGAGGCAGTCCAGCTTCCT**GAACACTGTGAACCC**CCAGGGACGTTACTCATGTAC**CGTCTCTGGATGGGGCA**CCACAACCAGCCCAGACG +4 bp

AGTGGAGGCAGTCCAGCTTCCT**GAACACTGTGAACCC**CCAGG- - -GTCATGTAC**CGTCTCTGGATGGGGCA**CCACAACCAGCCCAGACG -3 bp

AGTGGAGGCAGTCCAGCTTCCT**GAACACTGTGAACCC**CCAcGTCTGGGCTGGTTGtGgTGCCCCaTCCagAGACgtGACAACCAGCCCAGACG - 33 bp, + 37 bp

AGTGGAGG- -- - - - - - - - - TTCATGTAC**CGTCTCTGGATGGGGCA**CCACAACCAGCCCAGACG - 38 bp, + 1 bp

AGTGGAGGCAGTCCAGCTTCC**TGAACACTGTGAACCC**CCAGG- - - - - - - - -- -AA**CGTCTCTGGATGGGGCA**CCACAACCAGCCCAGACG - 12 bp, + 2 bp

P. Kasparek

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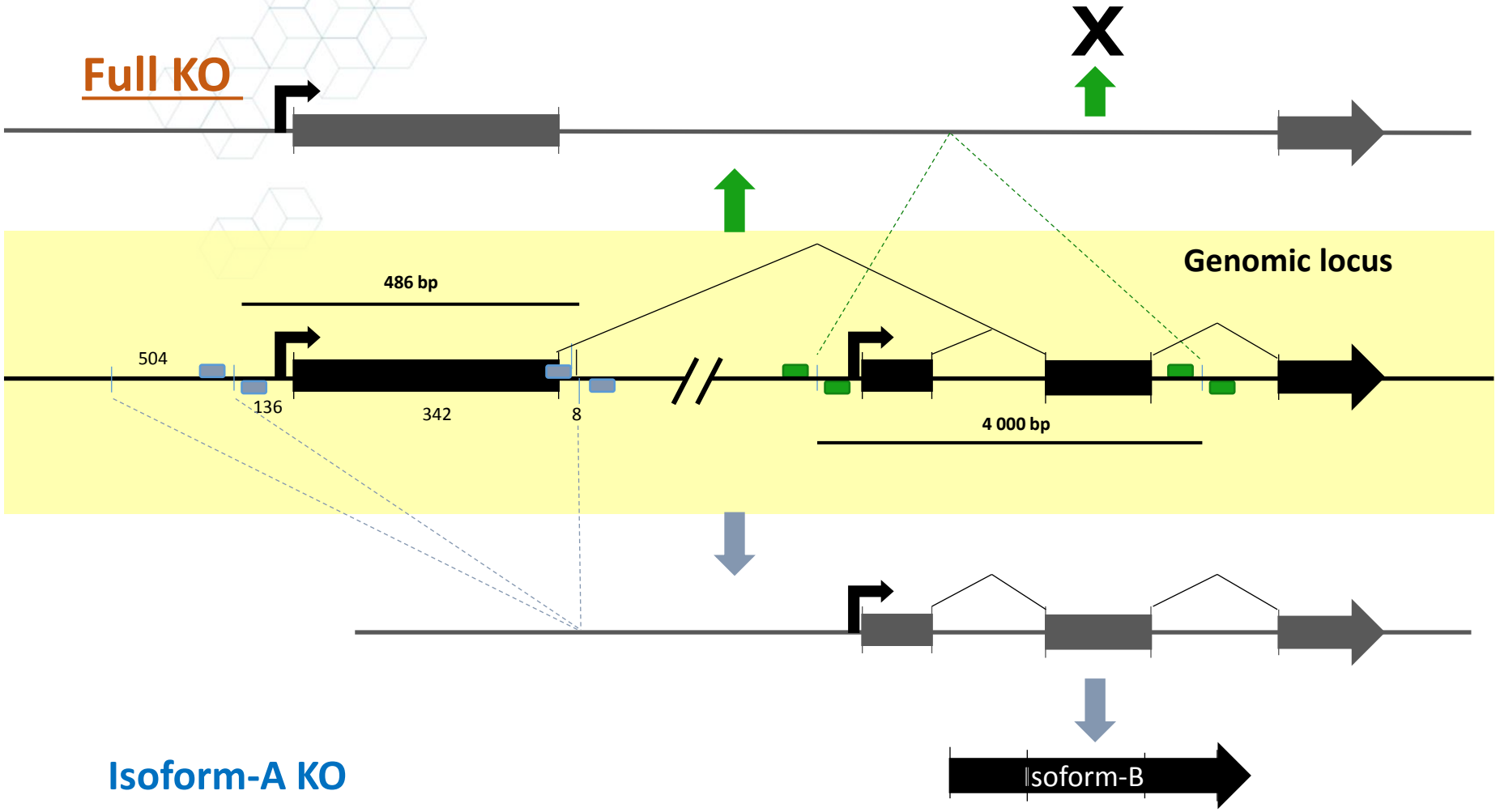
Isoform-specific KO of a microtubule associated protein:

full KO vs. isoform-A KO



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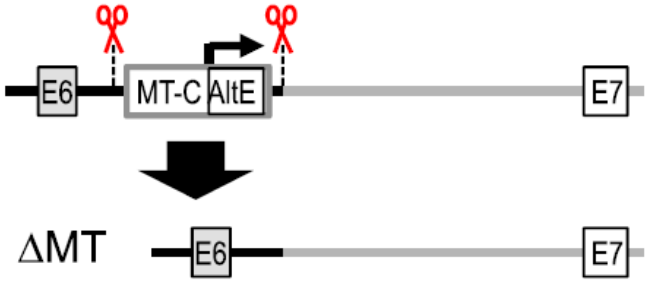
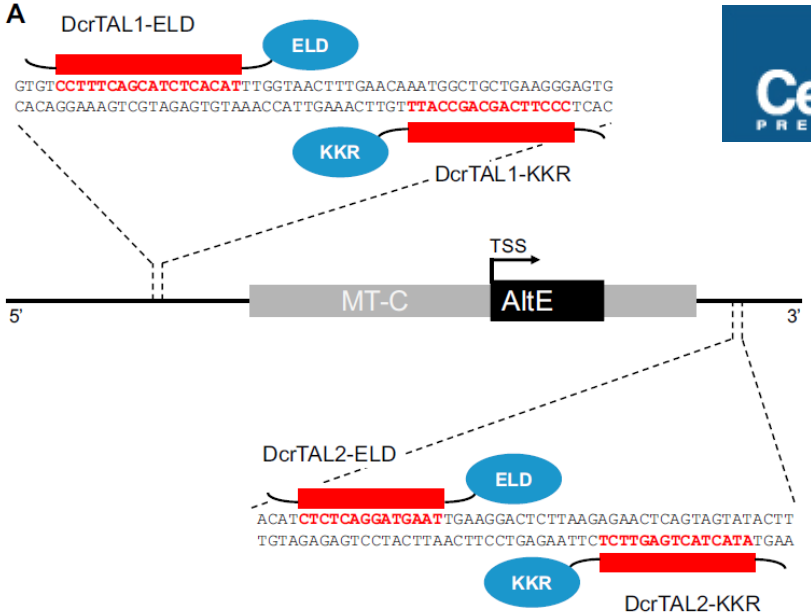
TALEN-mediated deletion of the MT-C element in Dicer intron 6

A Retrotransposon-Driven Dicer Isoform Directs Endogenous Small Interfering RNA Production in Mouse Oocytes

Matyas Flemr,[1] Radek Malik,[1] Vedran Franke,[2] Jana Nejepinska,[1] Radislav Sedlacek,[1] Kristian Vlahovicek,[2,3] and Petr Svoboda[1,*]
[1]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic
[2]Bioinformatics Group, Department of Molecular Biology, Division of Biology, Faculty of Science, University of Zagreb, Horvatovac 10000 Zagreb, Croatia
[3]Department of Informatics, University of Oslo, P.O. Box 1080 Blindern, NO-0316 Oslo, Norway
*Correspondence: svobodap@img.cas.cz
http://dx.doi.org/10.1016/j.cell.2013.10.001





B

animal	sex	genotype	allele	deletion position (m38/mm10)
28Y-06162	M	delMT$^{684/710}$	684	chr12:104,727,100-104,727,783
			710	chr12:104,727,089-104,727,798
28Y-06163	M	delMT$^{463\ /\ wt}$	463	chr12:104,727,100-104,727,562
28Y-06170	M	delMT$^{672\ /\ wt}$	672	chr12:104,727,110-104,727,781
28Y-06171	M	delMT$^{663\ /\ wt}$	663	chr12:104,727,114-104,727,776
28Y-06172	M	delMT$^{669\ /\ wt}$	669	chr12:104,727,109-104,727,777
28Y-06180	M	delMT$^{674\ /\ wt}$	674	chr12:104,727,106-104,727,779
28Y-06188	M	delMT$^{667\ /\ wt}$	667	chr12:104,727,110-104,727,776
28Y-06194	F	delMT$^{643\ /\ wt}$	643	chr12:104,727,104-104,727,746
28Y-06203	F	delMT$^{714\ /\ wt}$	714	chr12:104,727,086-104,727,799

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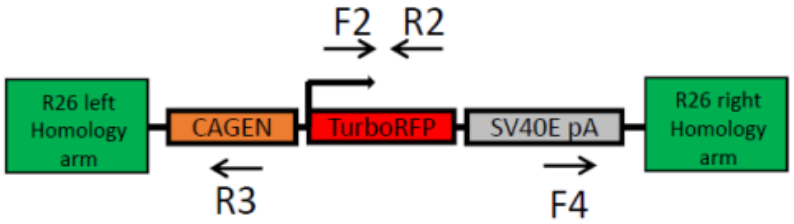
programmable nucleases

TALEN technology

insertional mutants

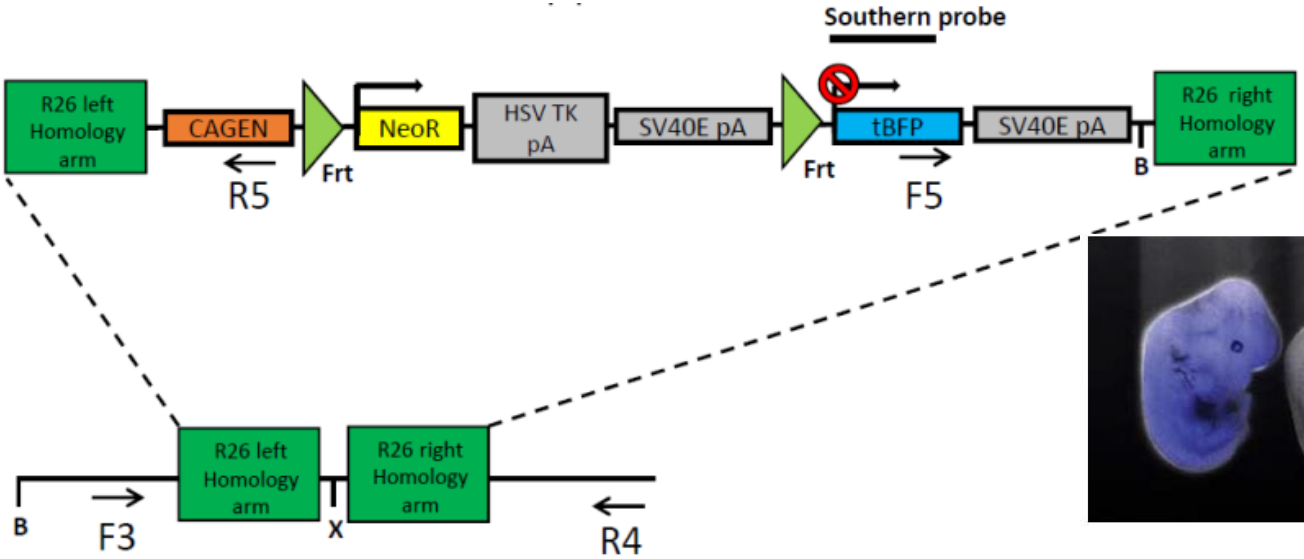
- point mutations
- reporter proteins/mouse
- fusion proteins

TALEN-mediated targeting into *ROSA* locus



turboRFP targeting vector

P. Kasparek



Blueflirt targeting vector

V. Korinek group (B. Fafílek)

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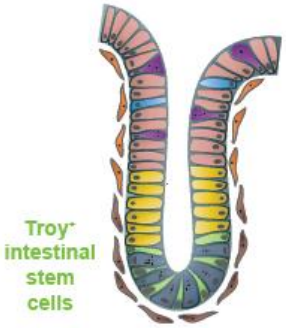
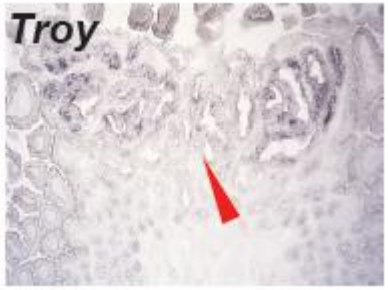
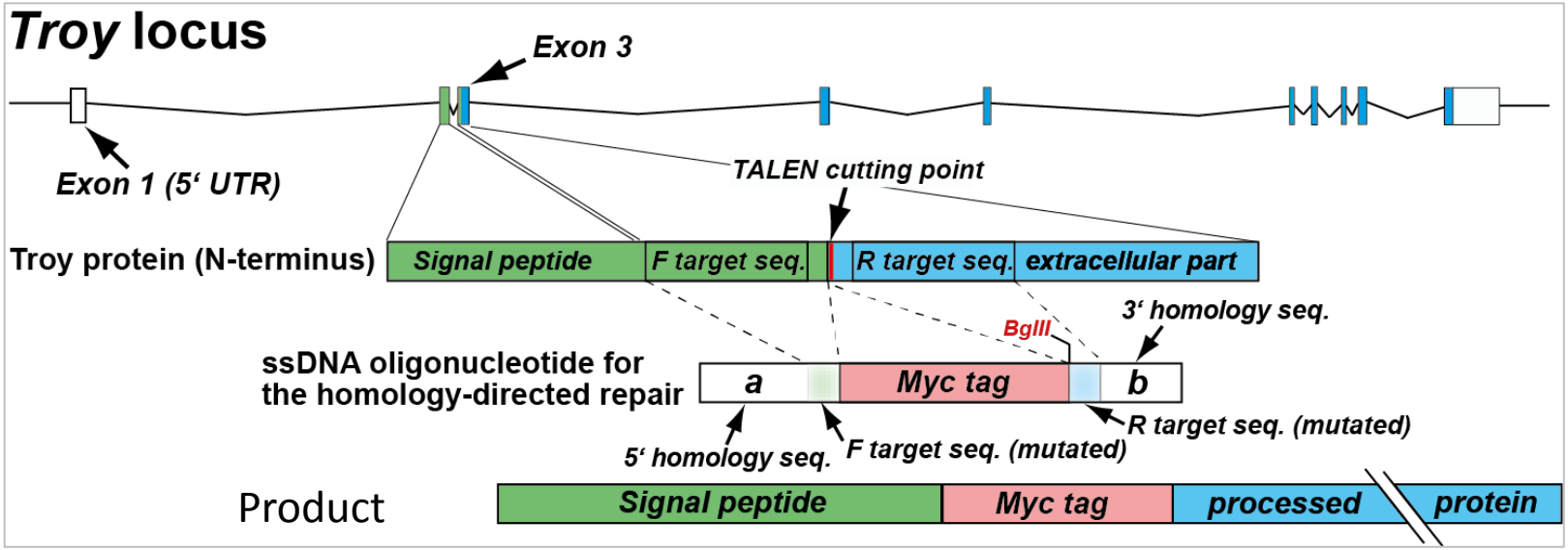
Reporter protein/mouse:

N-Myc TROY mouse







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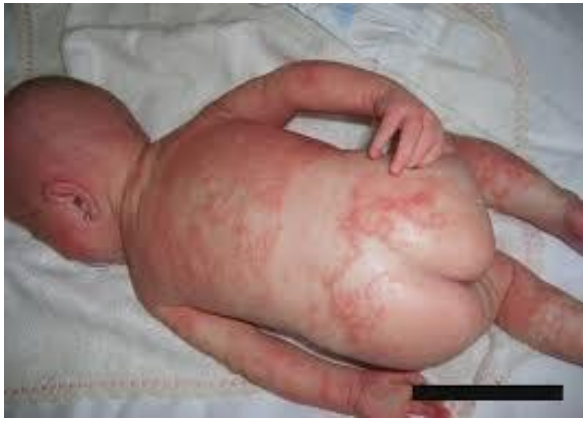
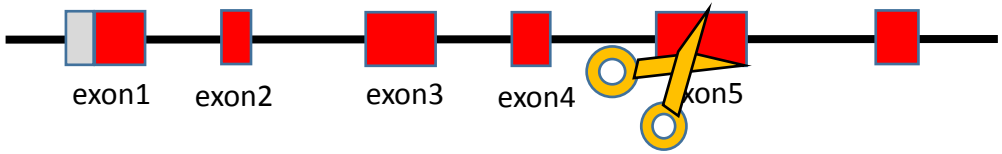
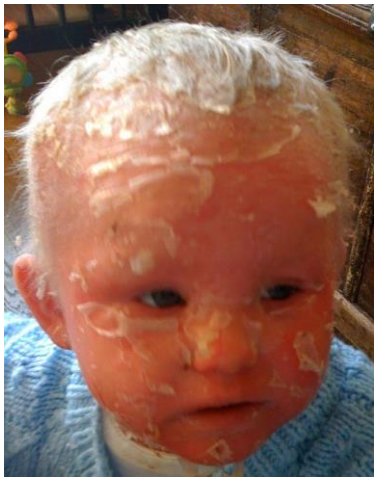
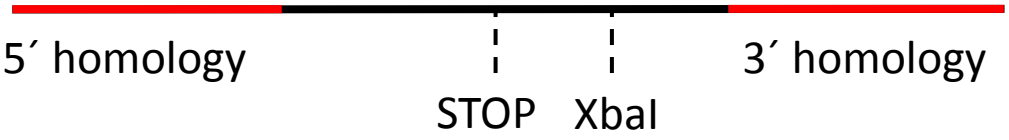
mouse model for Netherton syndrome

- **Autosomal resccesive skin disorder**
- **Point mutation in exon5 of Spink5 (-> STOP codon)**









P. Kasparek, unpublished

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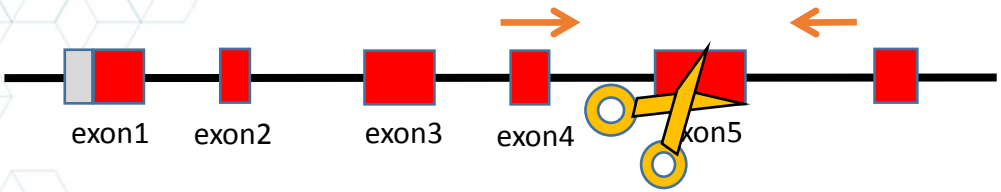
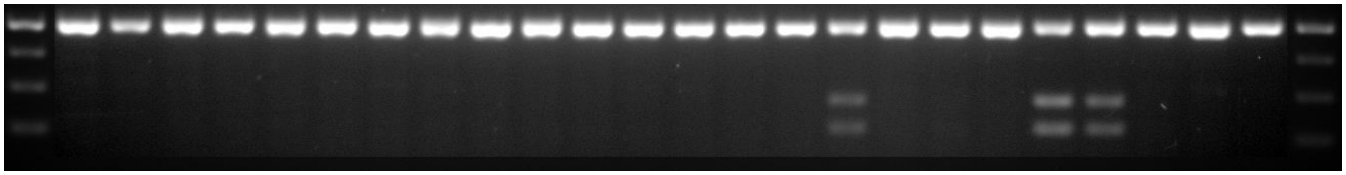
mouse model for Netherton syndrome



Spink5 PCR - XbaI



STOP XbaI

GGCACAGATGGGAAAACATACCGCAGTAGATGTGAACTGTGCTGATCTAGAATGCGTGAGTACCCTATAAAGCAGATATAGTGTTTATGCA

WT AGTTTGTGGCACAGATGGGAAAACATACCGCAGTAGATGTGAACTGTGTGCTGAGAATGCGTGAGTACCCTATAAAGCAGATATAGTGTTTATGCAAATC

AGTTTGTGGCACAGATGGGAAAACATACCGCAGTAGATGTGAACTGTGCTGATCTAGAATGCGTGAGGTACACCCTATAAAGCAGATATAGTGTTTATGCAAATC

STOP XbaI

P. Kasparek, unpublished

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mouse model for Netherton syndrome

AGTTTGTGGCACAGATGGGAAAACATACCGCAGTAGATGTGAACTGTGCTGATCTAGAATGCGTGAGGTACACCCTATAAAGCAGATATAGTGTTTATGCAAATC

STOP XbaI

WT

MKTATVPMLLTLAFYLTQDAAGEKGNQDPCMKFQAQMKNGTLTCPKGNNSSQSLNDIIFQSECILCKRALEQGAPTKIMNVKVLSRANRATDPAKLNCESFKQRRKDGDFICPSDTSSVCGTDGKTYRSRCELCAENAKSQNHVDVKSEGECGSSHLETDMCSDFRAY
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mut

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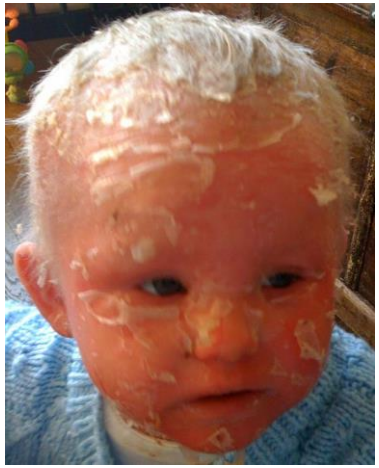

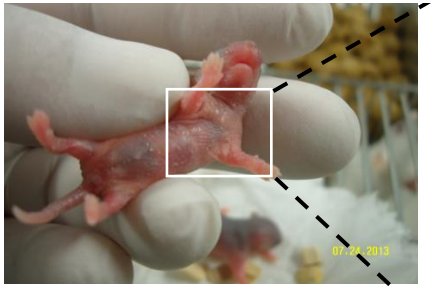

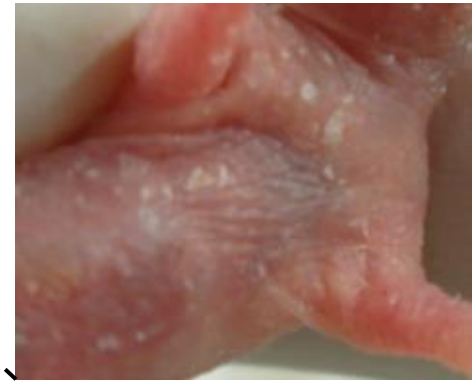

P. Kasparek, unpublished

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Thank you for your attention