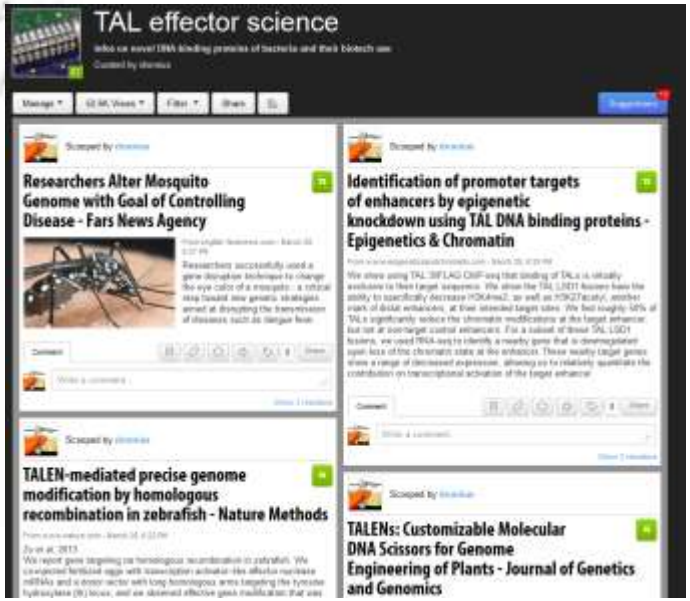
THE SAINSBURY LABORATORY
UNIVERSITY OF CAMBRIDGE

Targeted variation of genomes using TAL effectors

Sebastian Schornack

http://www.scoop.it/t/tal-effector-science



Read:

Schornack et al (2013) Annu Rev Phytopathol. 51:18.1–18.24
Vojtas (2013) Annu. Rev. Plant Biol. 64:327–50
Boch Bonas (2010) Annu Rev Phytopathol. 48:419-36

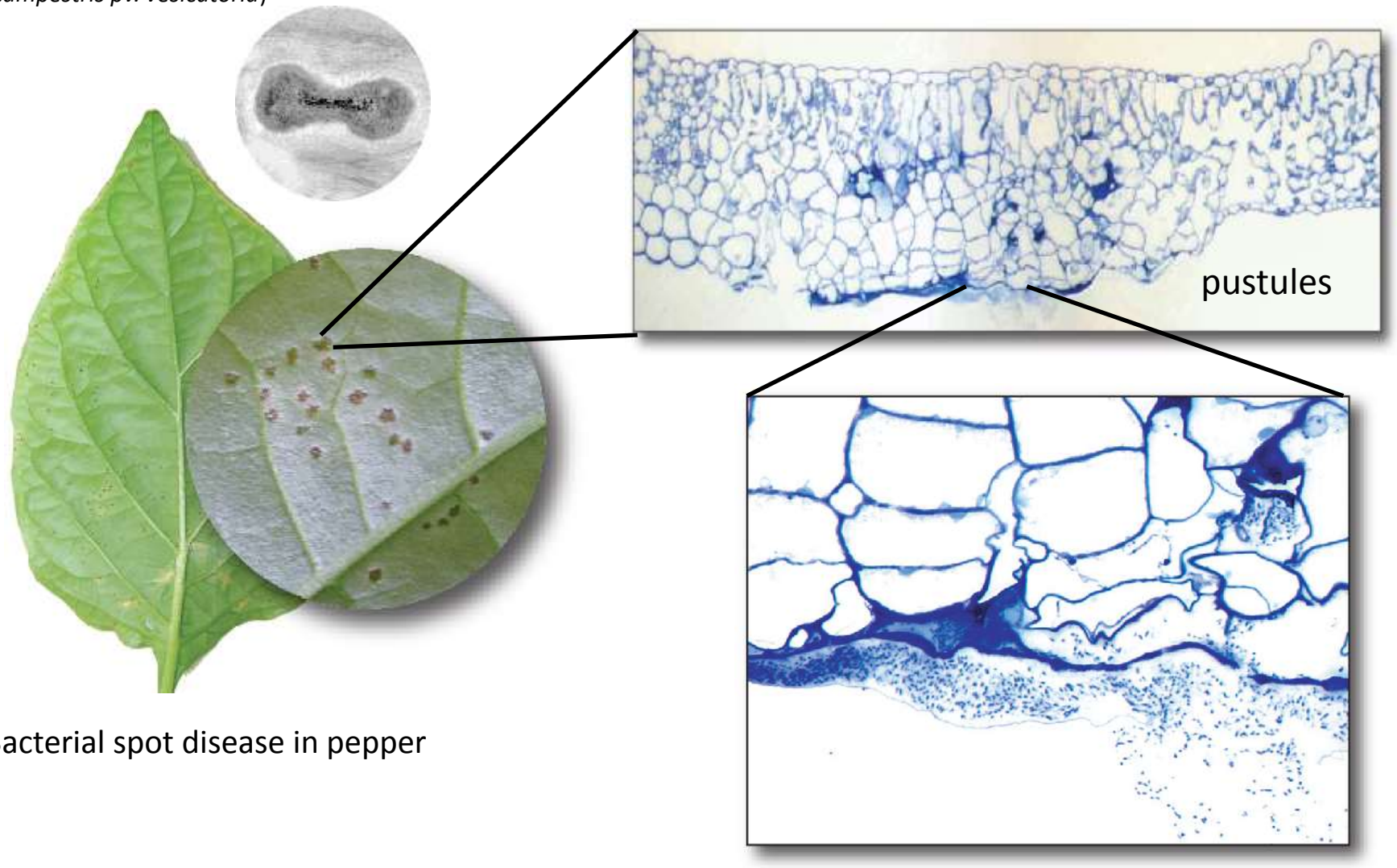
Nottingham
20.05.2013

Humans modify the genomes of animals and plants

By application of mutagens (chemicals, irradiation) and selective breeding

Xanthomonas bacteria reprogram plant cells to grow

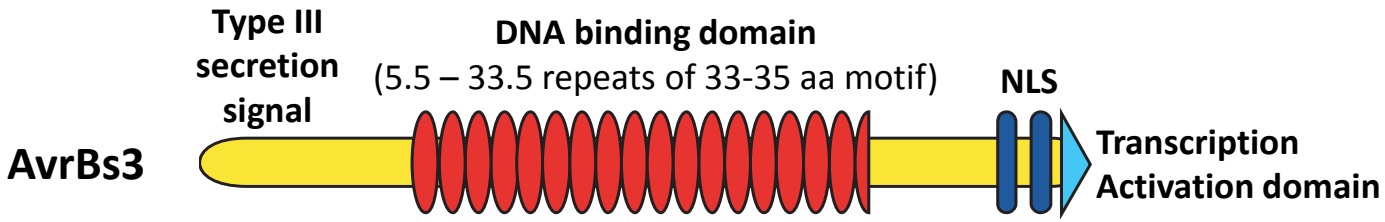
Xanthomonas euvesicatoria
(*X. campestris pv. vesicatoria*)

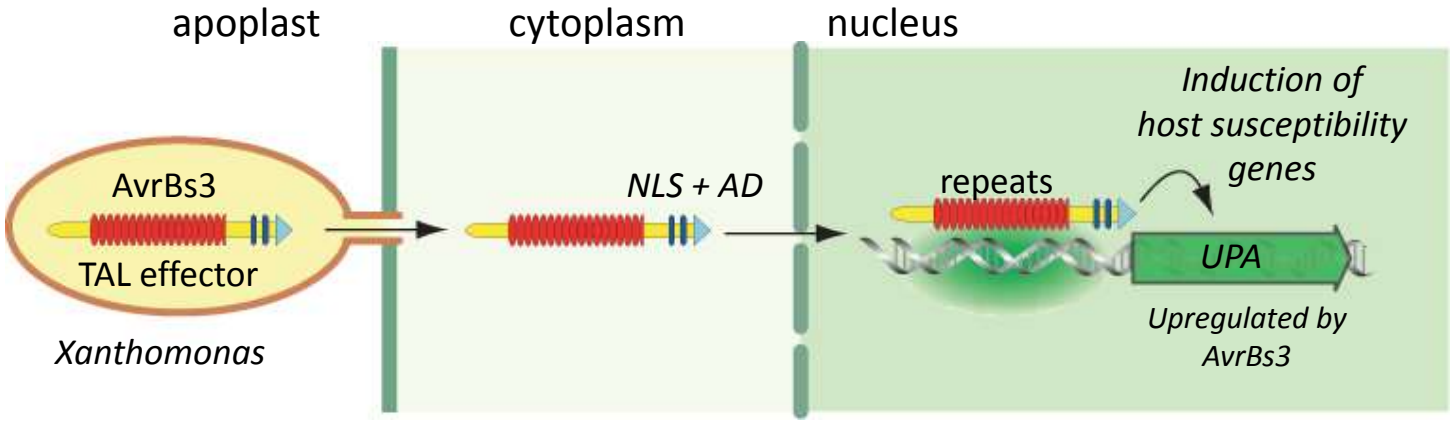


Bacterial spot disease in pepper

With G. Hause and T. Lahaye

TAL effectors = Transcription Activator-Like effectors



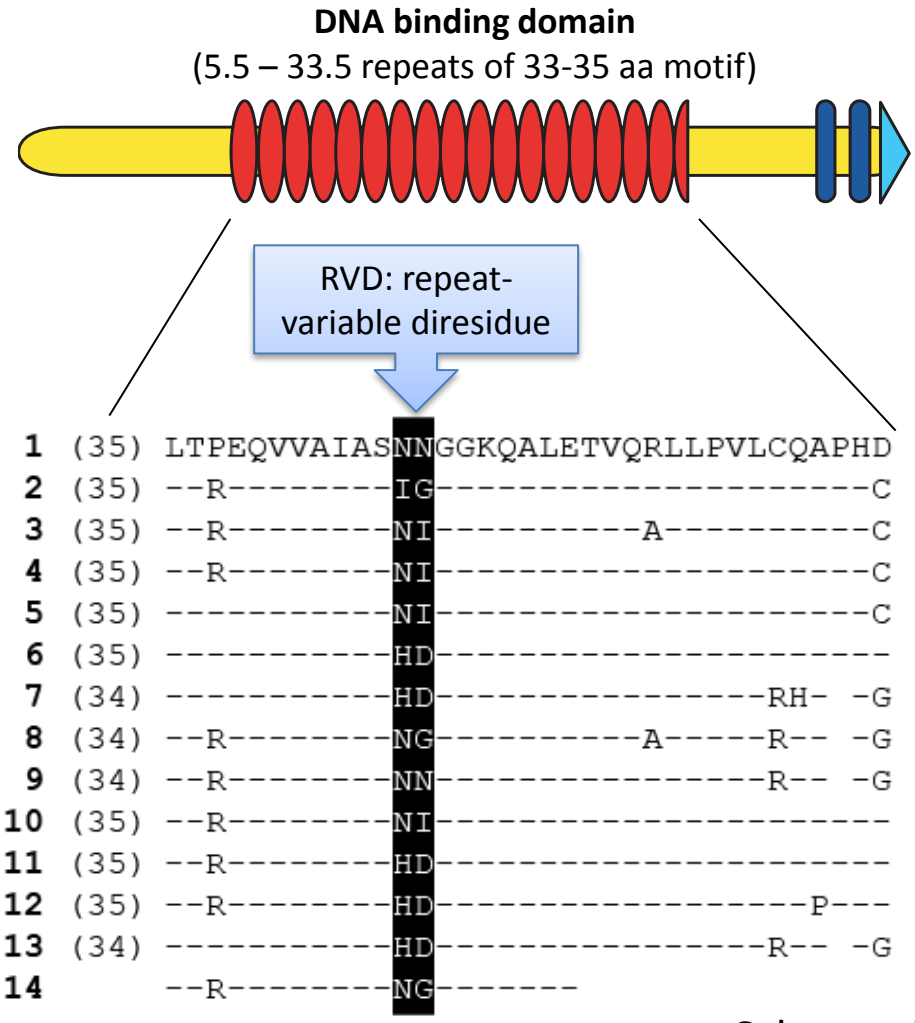


Bind promoter sequences in the host plant

Activate the expression of plant genes that aid bacterial infection.

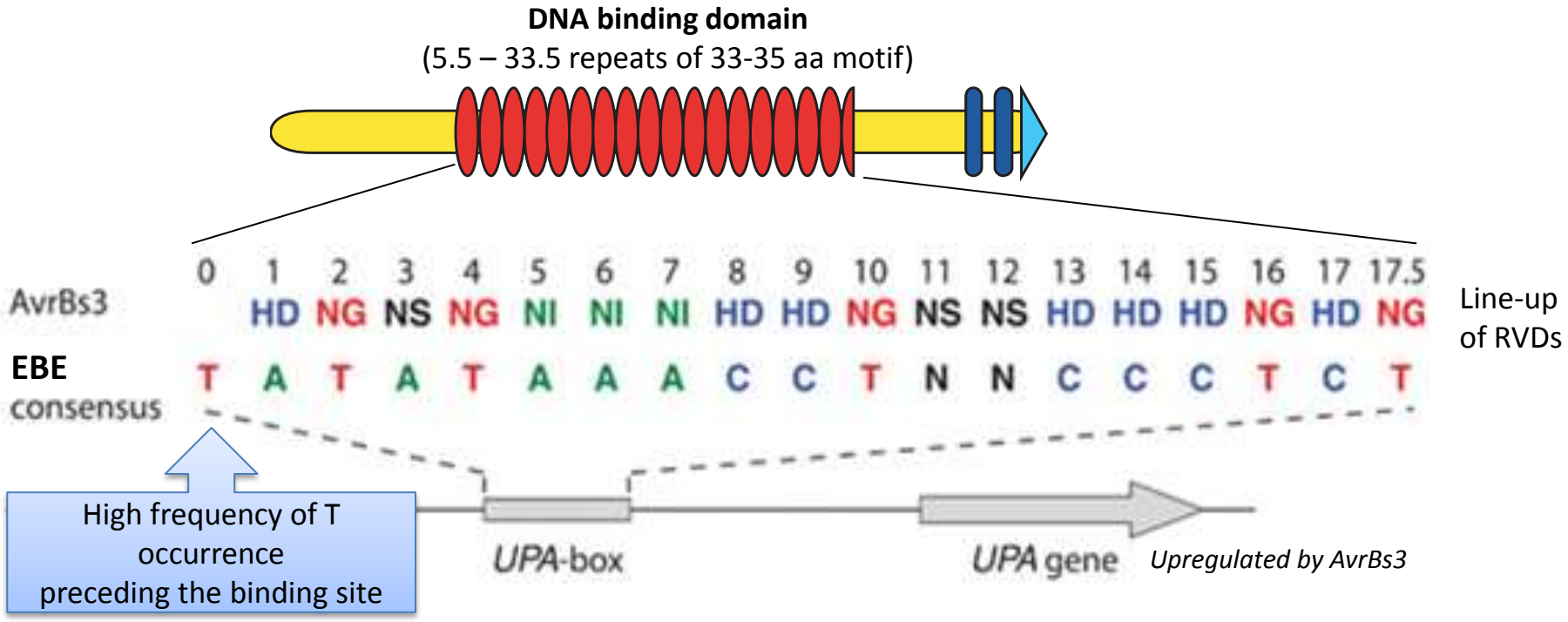
Marois et al (2002), Szurek et al (2001), Plant J., Kay et al, (2007), Science

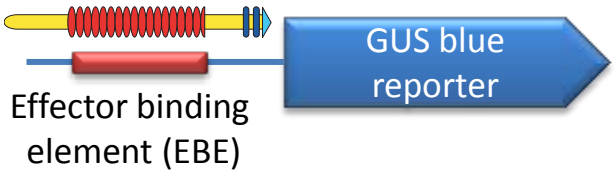
TAL effectors - hypervariable 12th and 13th amino acids

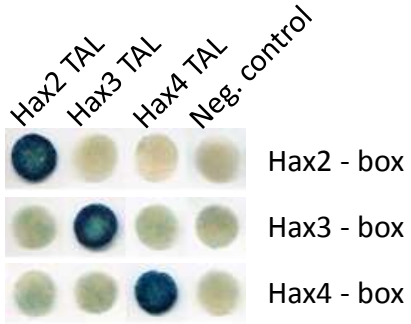


Schornack et al (2008) New Phytologist

TAL effectors – linear RVD-DNA base relationship







Boch et al. (2009) Science

TAL effectors – The DNA binding code

	HD	NG	NI	NS	NN	N*	HG	H*	IG
A	19	7	**55**	**20**	**7**	1	0	0	0
C	**69**	12	5	10	2	**7**	**2**	0	0
G	0	0	0	4	**7**	0	0	0	0
T	5	**50**	0	3	1	1	**2**	**1**	**1**

nucleotide frequency

NI = A

HD = C

HG = G

NG = T /mC

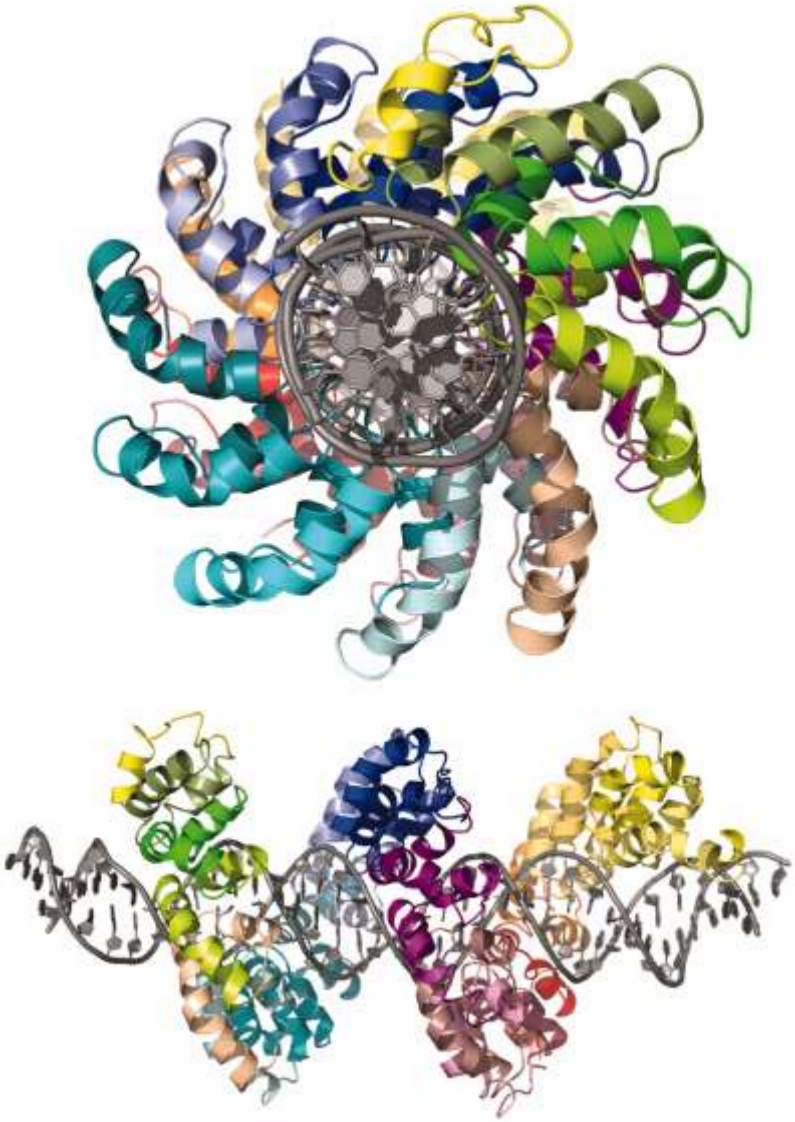
NN = G/A

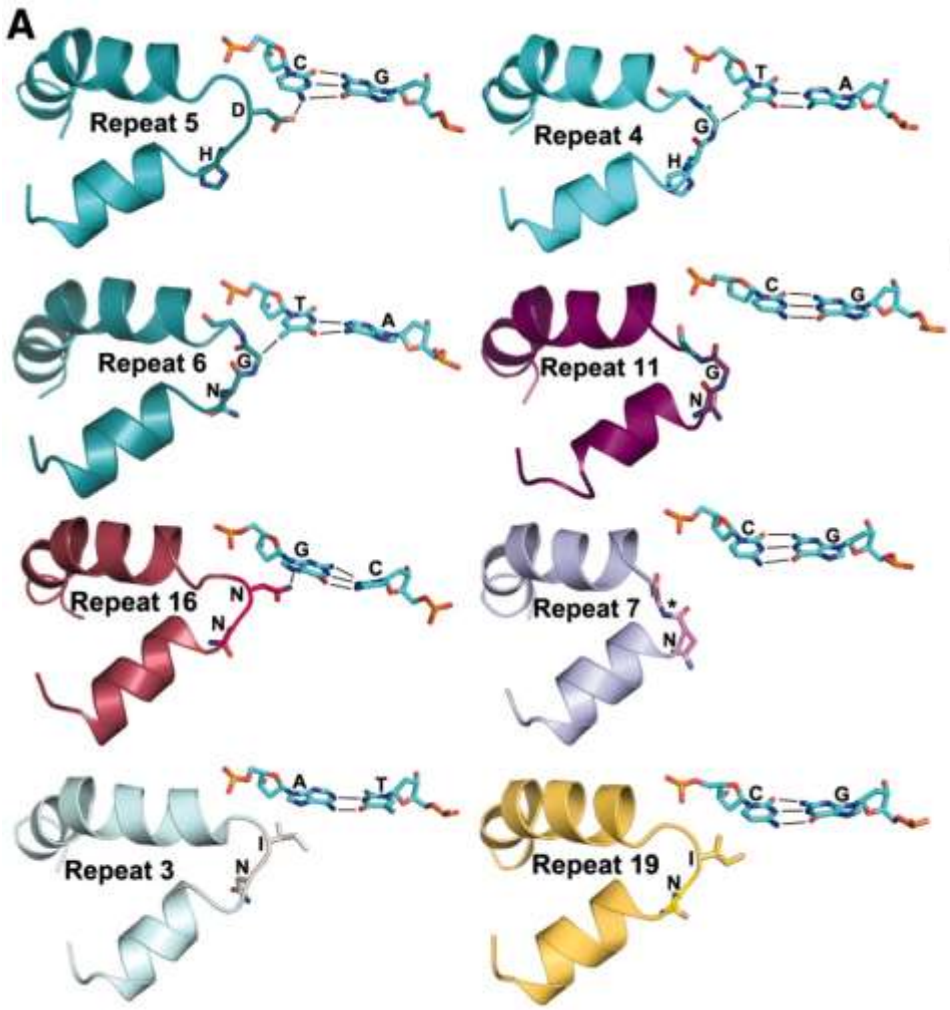
NS = A/C/G/T

N* = C/mC

Boch et al, (2009) Science, Moscou and Bogdanove (2009) Science

Repeats pair 1-on-1 with bases of the +DNA strand





Mak et al. (2012) Science

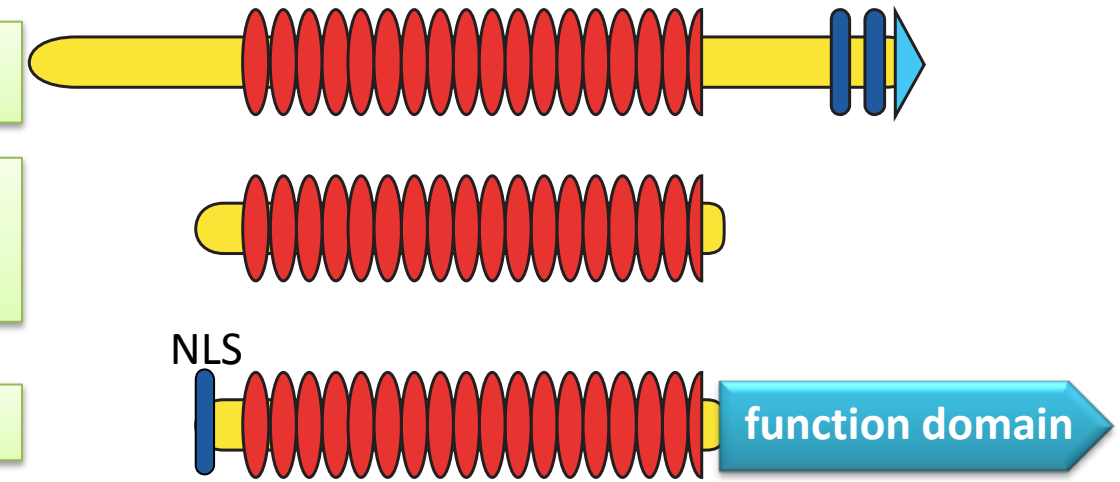
Customising the tool

DNA binding to
dsDNA, met-DNA, DNA/RNA hybrid

Xanthomonas TAL effector

Truncate and customise DNA binding domain

add features



- **Endonucleases**
- Cre, Flp, Ser-Recombinases
- De/Methylases
- Transcription activation
- Transcription repression

How to build/get a custom TAL effector?

1. Identify optimal DNA regions in a given target DNA sequence

TALeffectors.com

TAL effector nucleotide targeter - https://boglab.plp.iastate.edu/

Cellectis search tool: http://talen-hit.cellectis-bioresearch.com/search

http://baolab.bme.gatech.edu/Research/BioinformaticTools/assembleTALSequences.html

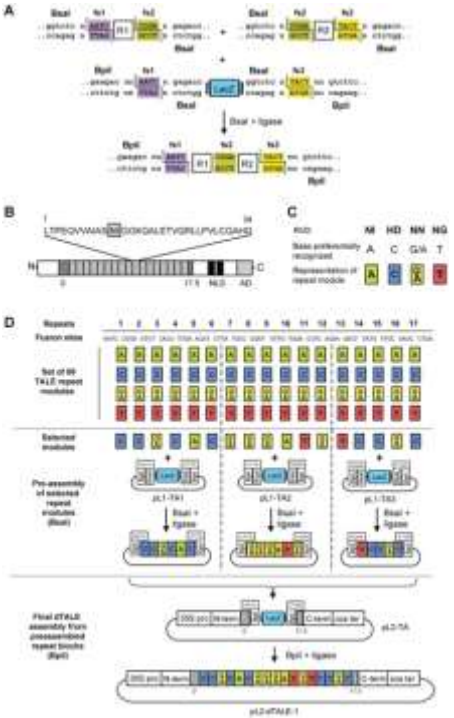
http://www.talendesign.org/mojohand_main.php

2a. Build it from single repeats using available modular cloning kits [Golden Gate cloning / Cut-ligation cloning]

addgene
A better way to share plasmids

- Bogdanove/Voytas Kit
- Joung Kit
- Zhang Toolbox

- Lahaye Kit
- Boch Kit

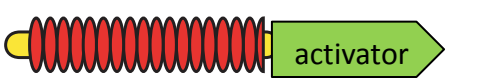


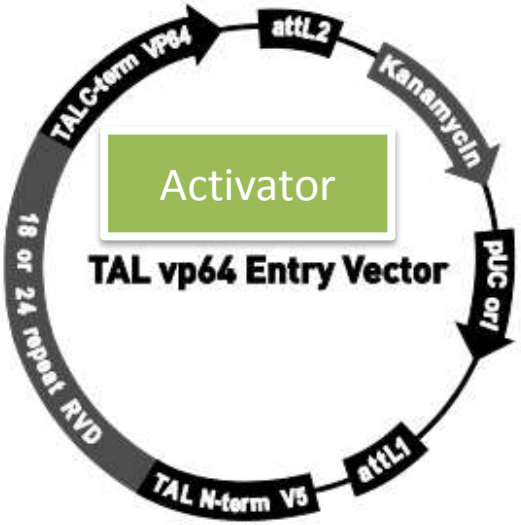
Weber et al (2011), PLOS One

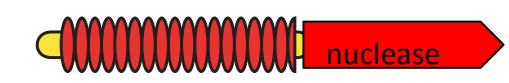
How to build/get a custom TAL effector?

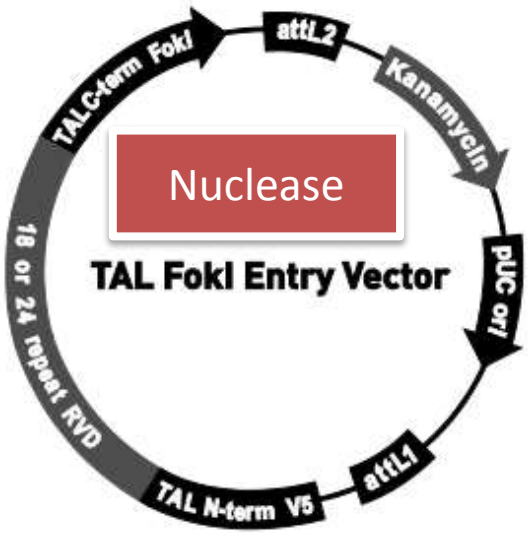
2b. Order it from a company

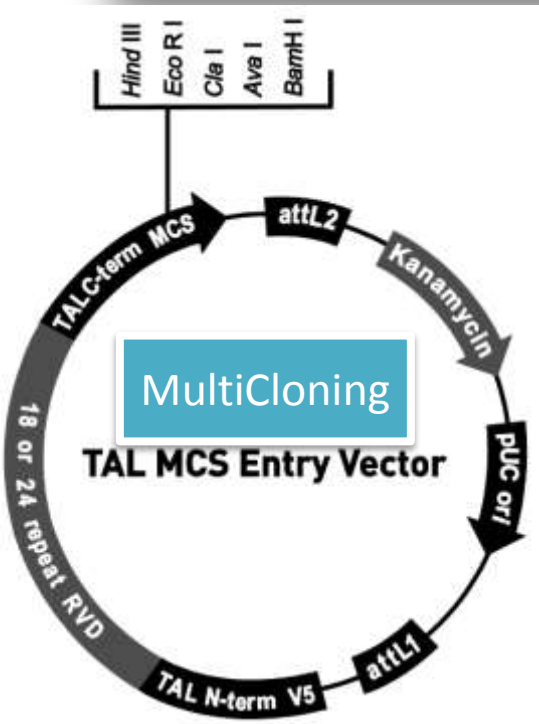
Life Technologies/GeneArt® TALs





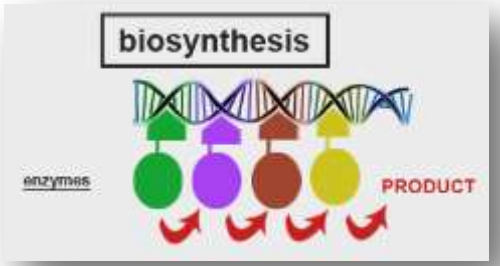






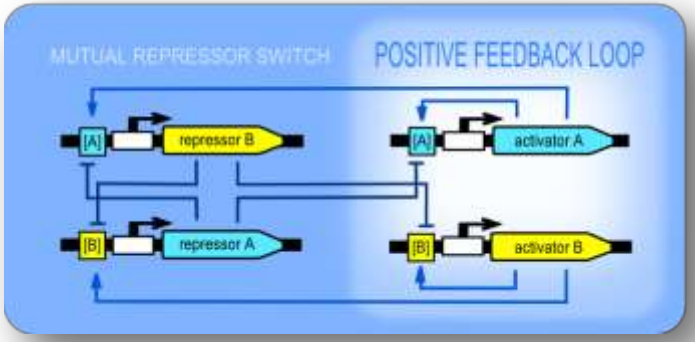
Applications

DNA binding domains to thether enzyme cascades (iGEM team Slovenia with ZFN)



http://2010.igem.org/Team:Slovenia/PROJECT/introduction

Synthetic signal switches/circuits

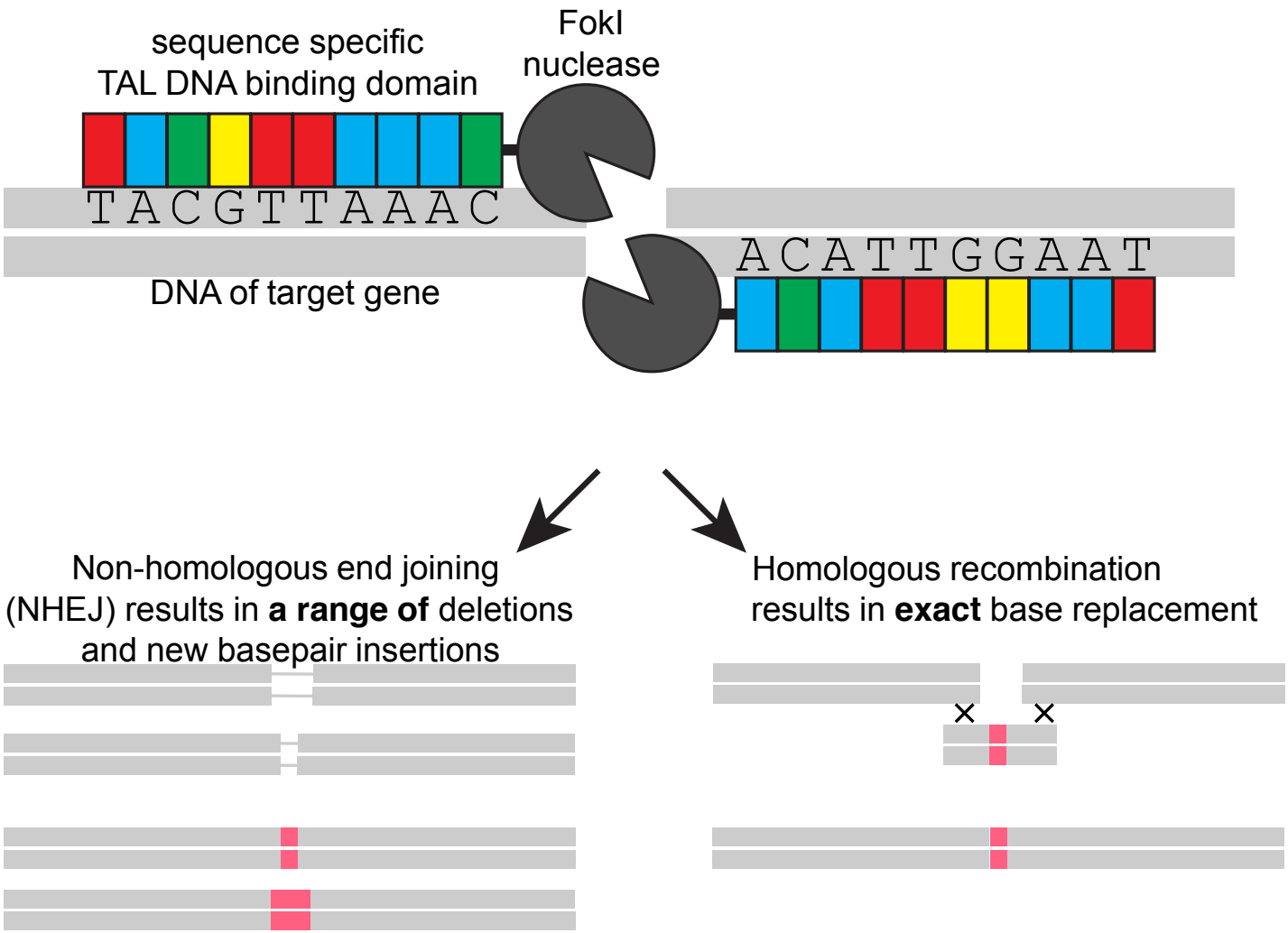


http://2012.igem.org/Team:Slovenia/TheSwitchPositiveFeedbackLoopSwitch

Subsampling of transcript pools

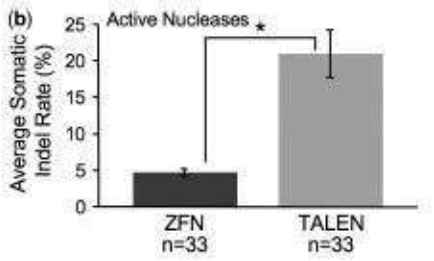
GTAACNNNNNNGTAAC
GTCACNNNNNNGTCAC
GTGACNNNNNNGTGAC
GTTACNNNNNNGTTAC

TAL effector nucleases / TALENs



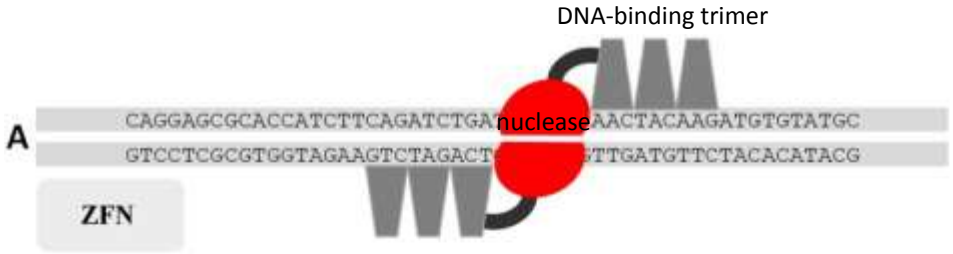
TAL effector nucleases are customisable and specific

Zinc finger nucleases



Chen et al, 2013, Nucl.AcidsRes.

1-on-3
Customisation requires specificity tests

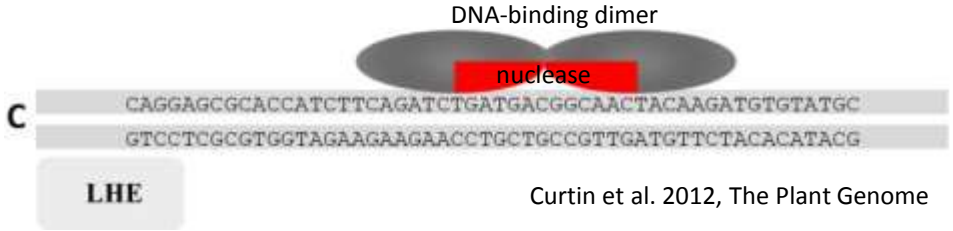


TAL effector nucleases

1-on-1
Perfectly customizable

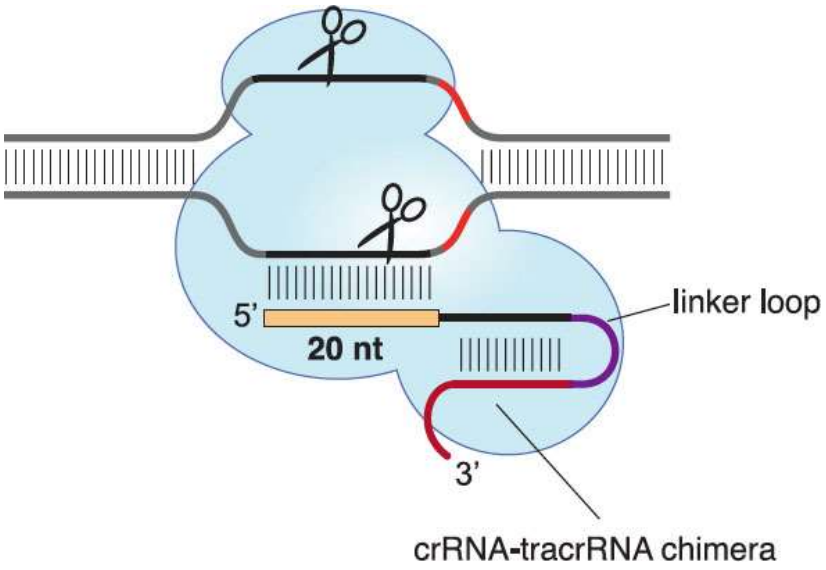
Meganucleases/ Homing endonucleases

1-on-motif
Complicated customization



Curtin et al. 2012, The Plant Genome

A new kid on the block: CRISPR/Cas9 nuclease



- fast, easily programmed , inexpensive
- Requires that the target sequence is followed by a 3'-NGG (the so-called "protospacer-adjacent motif ").
- limited to 12-bp off the RNA + GG PAM motif, so effectively ~14bp of specificity
- this enables unique targeting of ~40% of human genome

Advantage over ZFNs or TALENs → multiplexing. Simply add multiple guide RNAs.

Unknowns:
Specificity for single site?
Cas9 fused to other domains possible?
Efficiency?
RNA scaffold differences between systems?
Sequence requirements within the Protospacer adjacent motif

See also: www.addgene.org/CRISPR/guide/

Scientific applications in plants

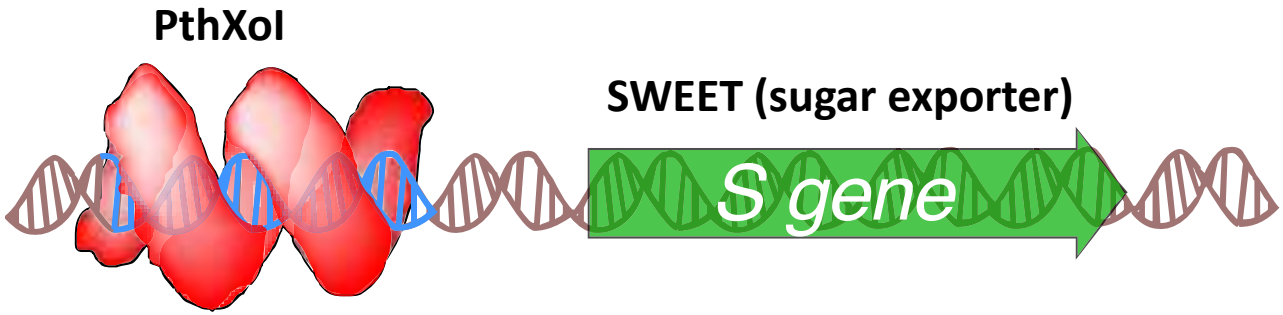
Successfully applied in
bacteria, fungi, algae, higher plants, insects, frogs, fish, mouse, rat, pigs, cows

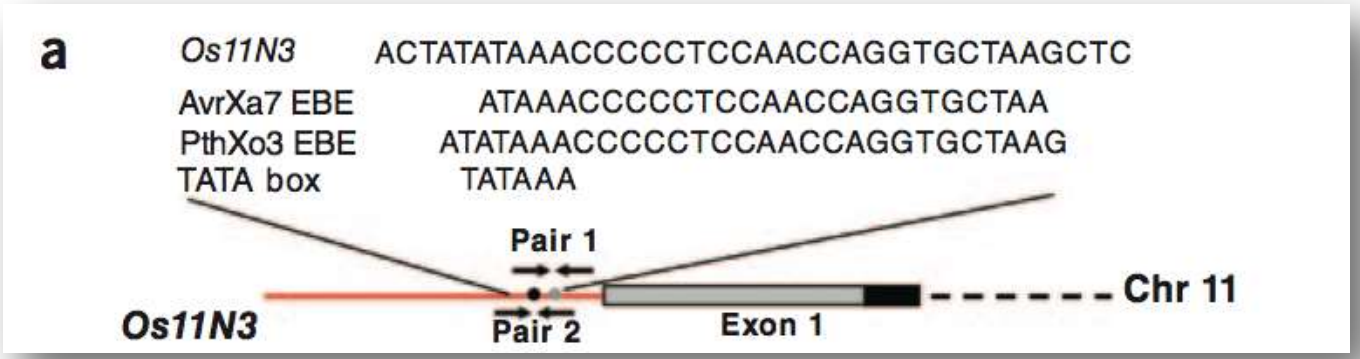
See also: http://eendb.zfgenetics.org/een.php

	Organism	Specific application
Activator	Arabidopsis	Transcriptional activation of endogenous KNAT1 and EGL3 genes with TALEs directed to their promoters
	Tobacco	Transcriptional activation of reporter gene
Repressor	Arabidopsis	RD29A promoters in reporter constructs and endogenous genes repressed with EAR repressor domain fusions
	Yeast	Repression of reporter genes
Nuclease	Arabidopsis	NHEJ-induced deletions with Fok1 fusions
	Tobacco	NHEJ based repair of inactive reporter gene
	Tobacco cells	NHEJ and HR based modification of ALS genes
	Brachypodium	NHEJ-induced modifications and large deletions with Fok1 fusions
	Rice	NHEJ based modification of a host susceptibility gene for Xanthomonas
	barley	NHEJ-induced deletions with Fok1 fusions
	Yeast	Repair of lacZ reporter gene constructs containing target sequences from various organisms

Schornack et al. (2013) Annual Rev Phytopathology

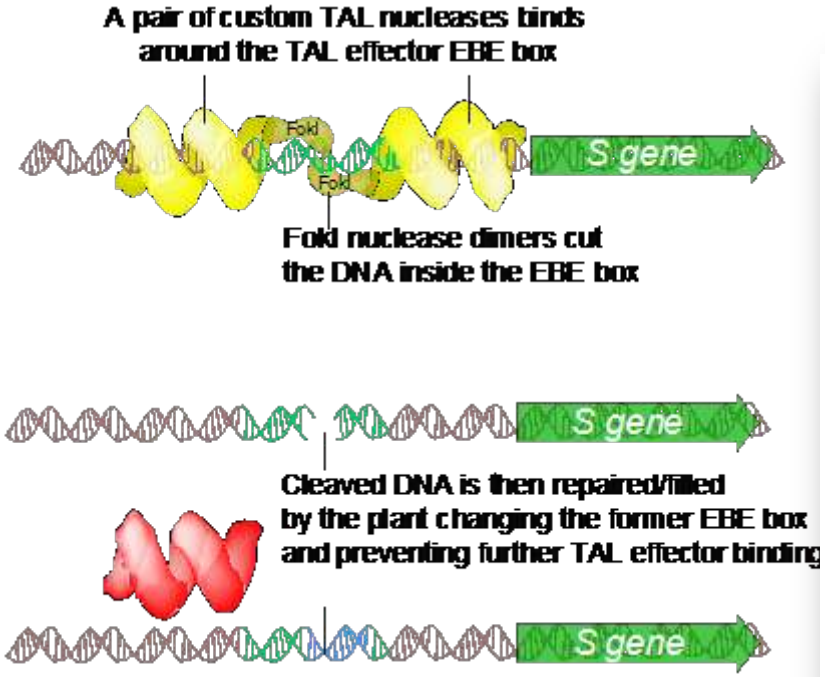
Generation of disease resistant rice by removing DNA

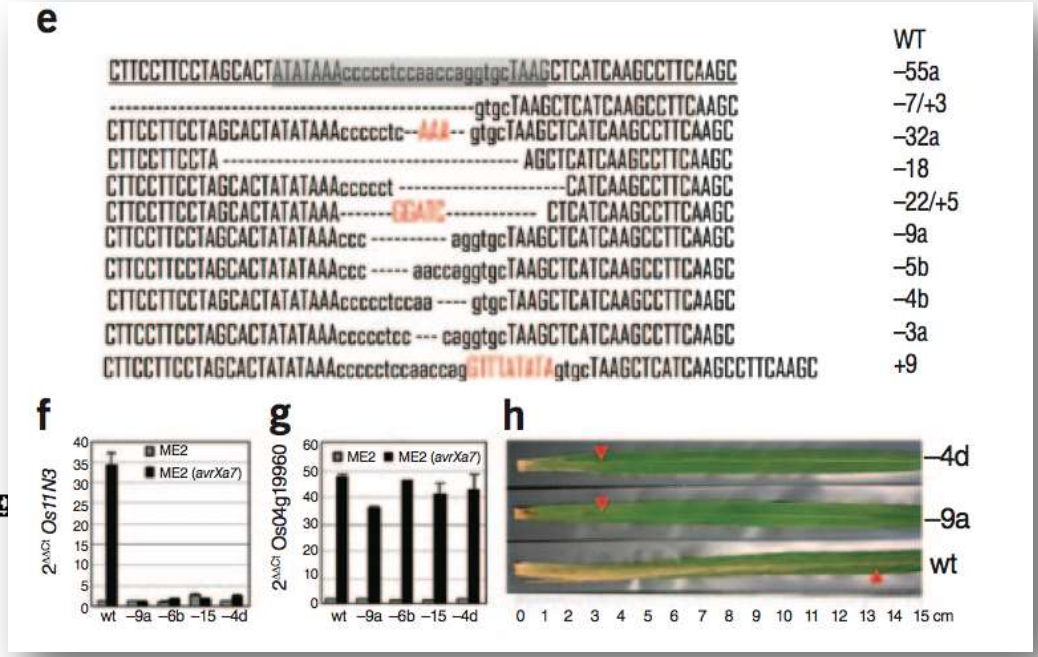




Li et al. (2012) Nature Biotechnology

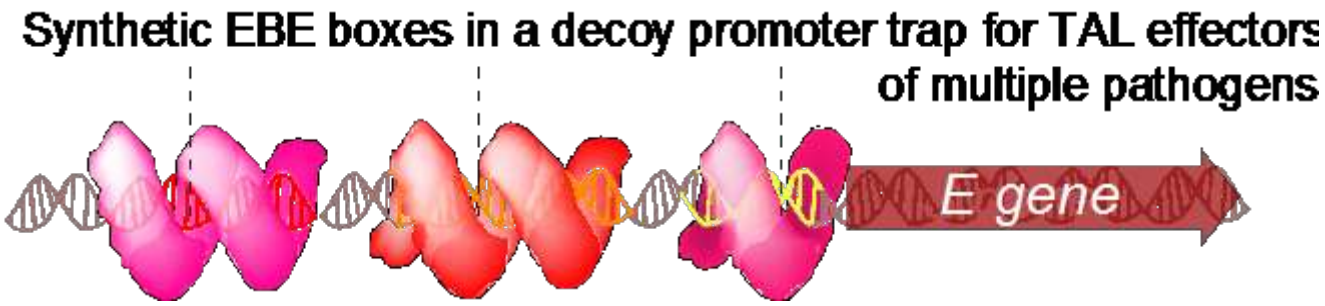
Generation of disease resistant rice by removing DNA





Li et al. (2012) Nature Biotechnology

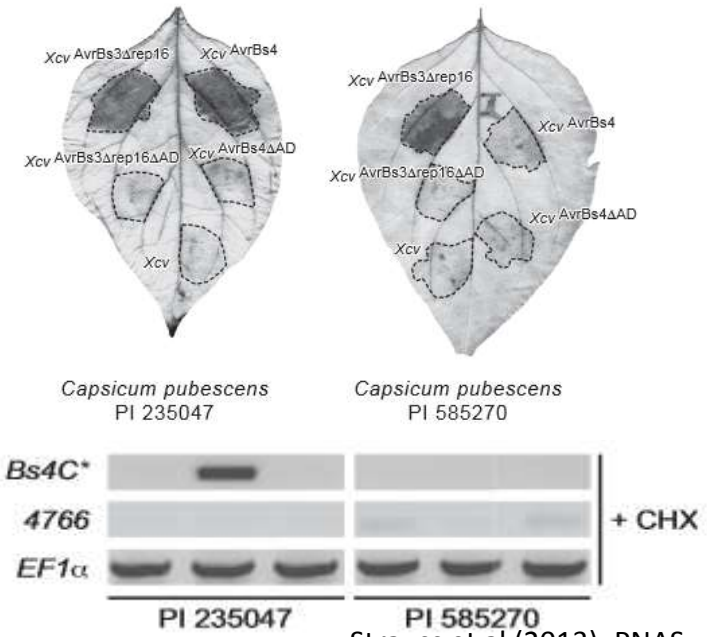
Plants with broad *Xanthomonas* resistance



Schornack et al. (2013) Annual Rev Phytopathology

We need more executors (E genes)

Rice *Xa27*
Pepper *Bs3*
Pepper *Bs4P*

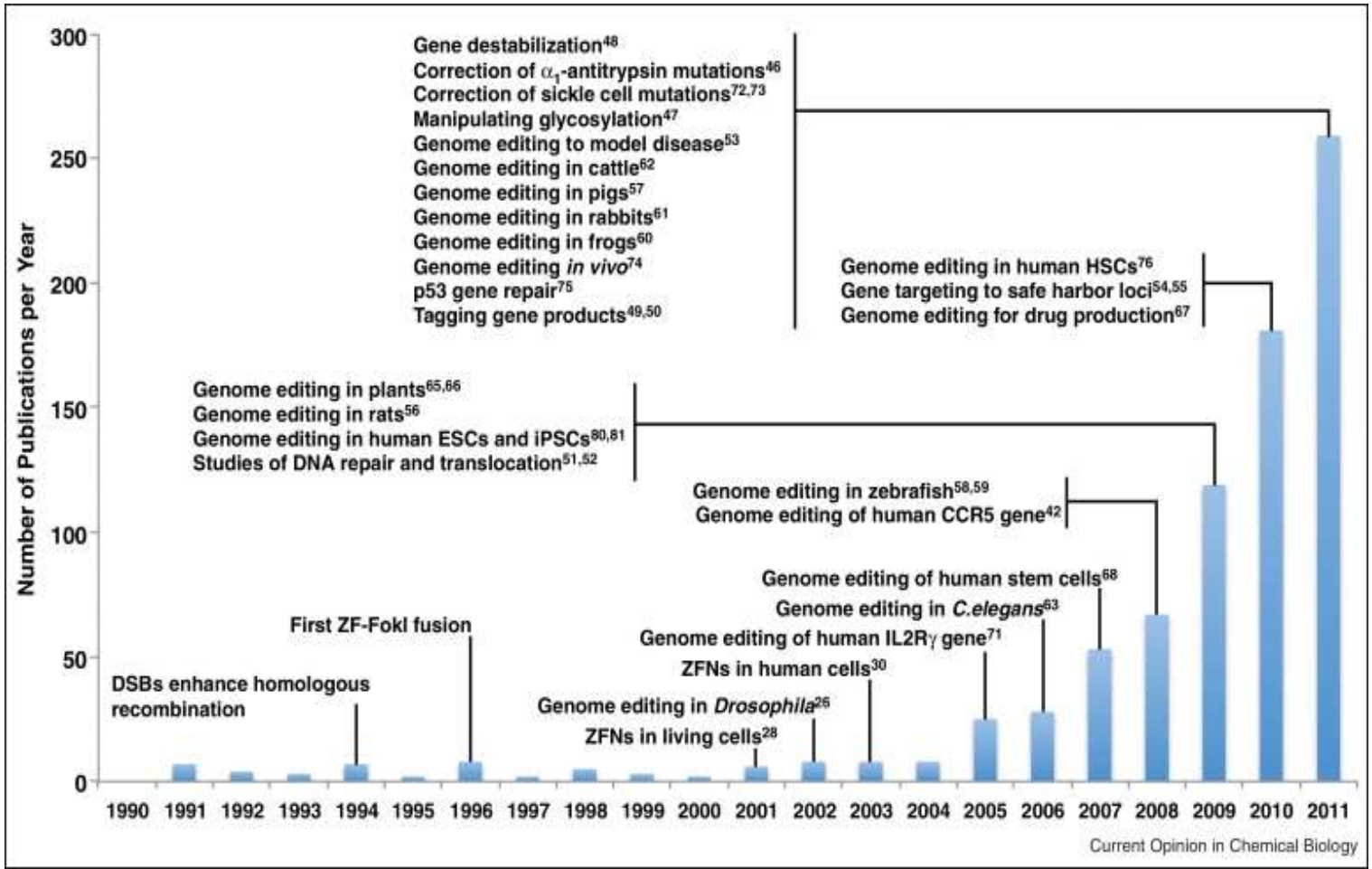


Strauss et al (2013), PNAS

Genome editing – an accepted next-gen biotechnology?

Genome editing – an accepted next-gen biotechnology?

Targeted variation:
Site-specific alteration of DNA-anchored information



Possibilities for targeted variation

Bind ds-DNA

Bind methylated DNA

Bind DNA-RNA hybrids (to the DNA strand)

- Transcriptional regulation
  - Activation
  - Repression
- DNA modification
- DNA cleavage – nucleases/recombinases
  - deletion
  - substitution
  - insertion
- ssRNA protection

Processes do not result in organisms with foreign DNA

TAL effector technology – How will it be regulated?

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

Position statement of the german ZKBS – June 2012

A **segment must contain at least 20 nucleotide pairs (NP) to lead to a recombinant nucleic acid**.

An intentional change of less than 20 NP cannot be distinguished with sufficent confidence from random occurrence of this sequence. Certain sequences of less than 20 NP […] are indistinguishable from the genetic changes caused by conventional mutagenesis or natural mutation (random occurrence) (Cao et al., 2011).

The mutations induced by mutagenesis methods are according to to § 3 no. 3b. Sentence 2 letter a GenTG (mutagenesis) **no genetic modifications**.

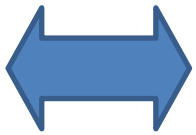
Netherlands Commission on Genetic Modification (COGEM)

COMMISSIE COGEM GENETISCHE MODIFICATIE

the issue of whether the modification process or the characteristics of the modification product should be the determining factor

modification <u>process</u>
Process based legislation
Europe

Product: GMO



characteristics of the modification <u>product</u>
Product based legislation
America, Canada, others

Product: Not GMO

Transcription factors do not involve genetic modification
If ...introduced into a cell in protein form, there will be no alterations to the DNA sequence in the hereditary material ...There would therefore seem to be no genetic modification involved. Under this line of reasoning these activities do not fall under the EU directives.

Some medicines on the European market have a similar effect and do not fall under the GMO regulations. An example is Vidaza (azacitidine), which blocks the synthesis of DNA and RNA.

the use of [sequence specific] nucleases in protein form is largely comparable to the use of chemical mutagens
the question is whether activities involving the use of nucleases in protein form should be covered by the GMO regulations, or not.

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Deutsche Forschungsgemeinschaft DFG

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