References:


Till et al. 2003. Large-scale discovery of induced point mutations with high-throughput TILLING. Genome Research 13(3): 524-30

Heidi Scholze and Jens Boch, 2011. TAL effectors are remote controls for gene activation Current Opinion in Microbiology. Volume 14, Issue 1, February 2011, 47–53
Genetic analysis
It’s all about mutants and their phenotypes!

Forward genetics

• From mutant phenotype to gene, from gene to protein function

Reverse genetics

• From gene to mutant phenotype, to function
Reverse genetics

Because of the availability of oceans of genomic sequence information, you can learn about the biological function your favorite gene (FG) with Reverse genetics.

Knockout analysis:
1. Find a knockout mutant in FG
2. Analyze the mutant to see if there is any defects
3. Connect the defects with biological processes

Overexpression analysis/ectopic expression
1. Overexpress FG (endogenous promoter)
   Ectopic expression (CaMV 35S promoter)
2. Analyze the overexpresser to see if there are any defects/phenotypes
3. Connect the defects with biological processes
When to use reverse genetics?

1. Figure out function of YFG
2. Redundant genes
3. Essential genes
4. Assist forward genetics: second allele

Why forward genetics cannot find mutants carrying mutations in all genes?

1. Mutant missed from forward genetics screens
2. Subtle or not obvious phenotypes
3. Lethal mutations
4. Redundancy
Why do we still want to do forward genetics:

- Process more specific, it is less predictable in RG
- Suppressor or enhancer screens that may lead to new biology
Story on DAYSLEEPER

Background: DAYSLEEPER was found to bind to the promoter region of *Ku70*, which encodes a protein involved in DNA repair.

25 bp Kubox motif

25 bp Kubox motif
**DAYSLLEEPER** encodes a hAT-like transposase (Autonomous DNA type transposon encodes a transposase that enables them to mobilize to a new chromosomal position in the host genome by a cut-and-paste mechanism).

In Arabidopsis, there are 246 hAT-like elements
  - Active transposons: presence of 8bp duplication of the insertion site, and a short terminal inverted repeats (TIR)
  - Fossil elements are often transcriptionally silent

*Daysleeper* is a fossil element, but is being expressed
Q: DNA repair ------?????------ Transposon

How do you find out the function of DAYSLEEPER?

Q: What would a fossil transposable element mutant look like?
Q: How would you prove that the mutant phenotype is caused by mutation in *DAYSLEEPER*?

- co-segregation
- Transgene complementation
- Obtaining second allele
DAYSLEEPER overexpressers
Slower growth, delayed flowering, altered leaves, etc.

DAYSLEEPER is essential for plant development

Fossil elements are not always fossils, they have the potential to evolve functions that are essential for plant growth and development

Because of the availability of oceans of genomic information, you can study your favorite gene (FG)

Knockout analysis:
1. Find a knockout mutant in FG
2. Analyze the mutant to see if there is any defects
3. Connect the defect with biological processes

Overexpression analysis/ectopic expression
1. Overexpress FG (endogenous promoter)
   Ectopic expression (CaMV 35S promoter)
2. Analyze the overexpresser to see if there are any defects/phenotypes
3. Connect the defects with biological processes
Reverse genetics:
Gene Knockout Strategies

1. Homologous recombination (not for plants)
2. RNAi based (silencing, antisense, sense suppression, PTGS, etc.)
3. T-DNA or transposon based insertional mutagenesis
4. Deleteagene
5. TILLING
6. TAL effector-mediated DNA modifications (TALENS)
Homologous recombination (not for plants)
RNAi based methods

History:

Early 1990’s, phenomena first found by plant scientists: co-suppression

1998, in *C. elegans*, formally discover dsRNA as signal for RNA interference (Fire and Mello)

1999, small RNA species derived from mRNA detected (Baulcomb)

2001, discovery of dsRNA processing enzyme Dicer

More components in RISC identified

2006, A. Fire and C. Mello won Nobel prize in medicine because of their discovery of dsRNA as mediator of RNAi
dsRNA-directed gene silencing mechanisms. Short dsRNA molecules can either be expressed by endogenous genes, invading viruses or by experimental means and are funneled into one of two different silencing mechanisms. The miRNA-dependent pathway, which mainly controls the translation of mRNAs, involves imperfect base pairing of the miRNA to its mRNA target, while siRNAs are perfectly complementary to their cognate mRNA species and induce their endonucleolytic cleavage and degradation. Although human miRNAs have been identified, their biological function in humans is currently unknown. Amplification of the RNAi signal by RDRP-dependent mechanisms, RNA-induced epigenetic control of gene expression as well as RNAi transfer between cells have been observed in some but not all species.
DNA constructs for RNA-mediated gene silencing.  

**a** | A DNA plasmid that can be propagated in *Escherichia coli* from which infectious potato virus X (PVX) RNA can be transcribed *in vitro*, using T7 polymerase. The PVX cassette contains sequence derived from the gene to be targeted.  

**b** | A transferred (T)-DNA plasmid that is propagated in *Agrobacterium*. When this plasmid-carrying *Agrobacterium* is inoculated onto a plant, it transfers the DNA between its left (LB) and right (RB) borders into the plant's cells. The region between the borders contains the viral sequences shown in part **a**, but in this vector, the T7 promoter has been replaced with the cauliflower mosaic virus promoter. This enables the transferred DNA to be transcribed by the plant's endogenous transcription machinery to generate infectious PVX (plus insert sequence) RNA. In amplicon transgene vectors, a selectable marker gene is also present between the left and right borders of this plasmid, enabling plants to be stably transformed with the transferred DNA.  

**c** | The tobacco rattle virus (TRV) virus-induced gene-silencing (VIGS) system. Two T-DNA plasmids that encode the TRV genome (one encoding TRV RNA1 and the other encoding TRV RNA2, which carries the inserted target sequence) are propagated separately in *Agrobacterium* and used to co-infect plant tissue.  

**d** | A typical T-DNA plasmid for the expression of hairpin RNAs (hpRNAs). This plasmid can be transiently introduced into plants by bombardment or stably introduced by agroinfiltration. A generic silencing precursor construct (pHANNIBAL) that enables hpRNA vectors to be easily constructed has different multiple cloning sites either side of the intron to enable the rapid insertion of target sequences in forward and reverse orientations. 35S, CaMV 35S promoter; CP, coat protein; M1,2,3, movement proteins 1, 2, 3; RdRP, RNA-dependent RNA polymerase; T7, T7 promoter; Term, transcription termination sequence.
Figure 3 | **Tobacco plant phenotypes after infection with a satellite-virus-induced silencing system.** Results show plant phenotypes four weeks after infection. Phenotypes caused by the silencing of the genes that encode **a** | cellulose synthase, **b** | transketolase and **c** | phytoene desaturase are shown. Images courtesy of M. Metzlaff, Ghent, Belgium. Reproduced with permission from Ref. 48 © (2002) Blackwell Publishing. **Please close this window to return to the main article.**

Note: some figures may render poorly in a web browser

Transketolase (TK) catalyzes the reversible transfer of a two-carbon ketol unit from xylulose 5-phosphate to an aldose receptor, such as ribose 5-phosphate, to form sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate. This enzyme, together with transaldolase, provides a link between the glycolytic and pentose-phosphate pathways.

phytoene desaturase: one of the enzymes in beta-carotene biosynthesis. Chlorophylls are derived from carotenoids pathway.
<table>
<thead>
<tr>
<th>Expression method</th>
<th>Introduction</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transient</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microprojectile bombardment</td>
<td>Rapid; valuable for single-cell assays; wide species range</td>
<td>Limited to cells on leaf surface; silencing does not persist</td>
<td></td>
</tr>
<tr>
<td><em>Agrobacterium</em> infiltration</td>
<td>Rapid; easy to apply; high throughput; low cost</td>
<td>Untested on most species</td>
<td></td>
</tr>
<tr>
<td>Viral-induced gene silencing (VIGS)</td>
<td>Rapid; easy to use; high-throughput vectors; can be applied to mature plants; good for species that are difficult or impossible to transform; useful in genetically intractable species</td>
<td>Host range limitations; might have restricted regions of silencing; depends on availability of infectious clones; viral symptoms might be superimposed on silencing phenotype; might have size restriction on insert</td>
<td></td>
</tr>
<tr>
<td><strong>Stable</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplicons</td>
<td>Extended host range; heritable; choice of tissues; no viral symptoms; tissue specificity controllable by promoter</td>
<td>Might have size restriction on insert; need efficient transformation technique</td>
<td></td>
</tr>
<tr>
<td>Hairpin transgenes</td>
<td>Not restricted by host range; heritable; controllable tissue specificity; range of degrees of silencing; high-throughput vectors</td>
<td>Need efficient transformation technique</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5 | Degrees of silencing produced by hairpin-RNA-encoding transgenes. The stable transformation of *Arabidopsis* plants with the same hairpin RNA (hpRNA) construct that is targeted against phytoene desaturase gives rise to lines that show a heritable photobleaching phenotype in: a | all tissues; b | sectors of tissue; or c | the cotyledons, but not the rest of the plant. Images courtesy of C.A.H. and P.M.W., CSIRO, Australia. Reproduced with permission from Ref. 63 © (2002) CSIRO Publishing.
Reverse genetics: Gene Knockout Strategies

1. Homologous recombination
2. RNAi based (silencing, antisense, sense suppression, PTGS, etc.)
3. T-DNA or transposon based insertional mutagenesis
4. Deleteagene
5. TILLING
Agrobacterium Ti plasmid-based transformation
<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Knock-out</strong></td>
<td>coding region or promoter</td>
<td>null</td>
</tr>
<tr>
<td><strong>Knock-down</strong></td>
<td>promoter or 3' UTR</td>
<td>reduced expression</td>
</tr>
<tr>
<td><strong>Knock-on</strong></td>
<td>promoter</td>
<td>increased expression</td>
</tr>
<tr>
<td><strong>Knock-about</strong></td>
<td>coding region</td>
<td>not null</td>
</tr>
<tr>
<td><strong>Knock-knock</strong></td>
<td>coding region or promoter</td>
<td>multiple KOs in one plant</td>
</tr>
<tr>
<td><strong>Knock-worst</strong></td>
<td>coding region or promoter</td>
<td>chromosomal rearrangement</td>
</tr>
</tbody>
</table>
Bigger genes have better chance being knocked out by T-DNA.
A

Vacuum infiltration
~15,000 plants

Kanamycin Selection
60,480 kan^r plants
grown on kanamycin plates

Pools of 9
6,720 pools
kan^r plants moved to soil
9 plants per pot
collected seed from entire pot

B

Primary PCR Screen
DNA from 30 "super pools"
5' + T-DNA L 30 reactions
3' + T-DNA L 30 reactions
5' + T-DNA R 30 reactions
3' + T-DNA R 30 reactions

Confirm positive results
DNA gel blot
DNA sequencing

Secondary PCR screen
DNA from 9 "pools of 225"

Tertiary PCR screen
germinate seed from twenty-five "pools of 9"
extract DNA & do PCR

Identify Individual Plant
sow ~50 seeds from one "pool of 9"
extract DNA from individual plants & do PCR

---

Pool 9 into one
Pool 25 into one
Pool 9 into one
Indexed T-DNA knockout lines

- Built using high throughput end-rescue for each T-DNA line in the population.

-Major sources: SALK Institute (SALK lines, USA) Syngenta Inc. (SAIL lines, USA) FLAG lines (French) GABI lines (German) SK lines (Canadian)
Moving forward in reverse: genetic technologies to enable genome-wide phenomic screens in Arabidopsis

Jose M. Alonso* and Joseph R. Ecker†

Abstract | Genome sequencing, in combination with various computational and empirical approaches to sequence annotation, has made possible the identification of more than 30,000 genes in Arabidopsis thaliana. Increasingly sophisticated genetic tools are being developed with the long-term goal of understanding how the coordinated activity of these genes gives rise to a complex organism. The combination of classical forward genetics with recently developed genome-wide, gene-indexed mutant collections is beginning to revolutionize the way in which gene functions are studied in plants. High-throughput screens using these mutant populations should provide a means to analyse plant gene functions — the phenome — on a genomic scale.

a Plate 1 of 521
b Screen 521 plates for resistance to compound X
c Screen 521 plates for resistance to compound Y

List of gene-indexed mutants that show resistance to compound X

<table>
<thead>
<tr>
<th>Plate</th>
<th>Position</th>
<th>Gene name</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A3</td>
<td>At1g10540</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>A4</td>
<td>At1g10540</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>F7</td>
<td>At1g36710</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>F8</td>
<td>At1g36710</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>D9</td>
<td>At2g20010</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>D10</td>
<td>At2g20010</td>
<td>2</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

List of gene-indexed mutants that show resistance to compound Y

<table>
<thead>
<tr>
<th>Plate</th>
<th>Position</th>
<th>Gene name</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D3</td>
<td>At1g20090</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>D4</td>
<td>At1g20090</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>A11</td>
<td>At1g49000</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>A12</td>
<td>At1g49000</td>
<td>2</td>
</tr>
<tr>
<td>23</td>
<td>H1</td>
<td>At3g00110</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>H2</td>
<td>At3g00110</td>
<td>2</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Reverse genetics: Gene Knockout Strategies

1. Homologous recombination (not for plants)
2. RNAi based (silencing, antisense, sense suppression, PTGS, etc.)
3. T-DNA or transposon based insertional mutagenesis
4. Deleteagene: useful for tandem repeats and small genes
5. TILLING: useful to get dominant or partial l-o-f mutations
- Bigger genes have better chance being knocked out by T-DNA.
- Tandem repeats cannot be knocked out by T-DNAs.
Deleteagene™

Fast Neutron Deletion Mutagenesis-based Reverse Genetics Approach for Plants
Fast neutron bombardment
- random deletions

HTP preferential PCR
- screen libraries of mutants to find target gene deletions
DELETION SCREEN RECONSTRUCTION

5 kb Deletion

Wild type (6.4 kb)

ga1-3 band (1.4 kb)

GA1 Locus

WT

6.4 kb

1.4 kb

ga1-3
DELETION LIBRARY CONSTRUCTION

- Treat wild type seeds with fast neutron
- Plant M1 seeds and grow up population
- Collect M2 seeds from individual plants
- Plant some seeds from each line
- Collect tissue and extract DNA
**ARABIDOPSIS MUTANT SCREEN**

20 mega pools (2592 lines per pool) → WT

9 super pools (288 lines per pool) → WT

8 pools (36 lines per pool) → WT

2 sub pools (18 lines per pool) → WT

Individual lines → WT

Deletion
MEGAPOOL ANALYSIS

MEGAPOOL ANALYSIS

SUPERPOOL ANALYSIS

SUPERPOOL ANALYSIS

PLANT ANALYSIS

PLANT ANALYSIS

POOL ANALYSIS

POOL ANALYSIS

1.7 kb Deletion
**ARABIDOPSIS MYB19**

*AtMyb19* Deletion Analysis

WT 39371 gcattctttta attcaattg - - - aacaacaaca tgatcatgaa 41090
Mut 39371 gcattcttt/ /a tgatcatgaa 41090
## DELETION vs. OTHERS

<table>
<thead>
<tr>
<th></th>
<th>Deletional Knockout</th>
<th>Insertional Knockout</th>
<th>RNAi Knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applicability in Crop Species</strong></td>
<td><strong>Wide</strong></td>
<td>Limited</td>
<td>Limited</td>
</tr>
<tr>
<td><strong>Cost &amp; Time</strong></td>
<td><strong>Inexpensive &amp; Fast</strong></td>
<td>Slow &amp; Expensive</td>
<td>Slow &amp; Expensive</td>
</tr>
<tr>
<td><strong>Gene Specificity</strong></td>
<td>Gene-/Tandem Gene-Specific</td>
<td>Gene-Specific</td>
<td>Family</td>
</tr>
<tr>
<td><strong>Tissue Specificity</strong></td>
<td>None</td>
<td>Limited</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Penetrance</strong></td>
<td><strong>High</strong></td>
<td>High</td>
<td>Unreliable</td>
</tr>
</tbody>
</table>

In Arabidopsis, Deleteagene is especially useful for knocking out small genes and tandem repeats.
TILLING: Targeting Induced Local Lesions In Genomes

Detection of point mutations in target genes within mutagenized or natural populations of plants by heteroduplex analysis.

Developed by:
Steve Henikoff, Fred Hutchinson Cancer Inst.
Luca Comai, University of Washington
CAN-TILL

The Canadian TILLING Initiative

University of British Columbia
Vancouver, BC

George Haughn (Primary Investigator)
Erin Gilchrist (Research Associate)
Fariba Aboutorabi (Technician)
Arabidopsis EMS Mutagenesis

- Mutation frequency can be as high as
  - 500 mutations/genome or
  - 1 mutation/1000 bp/300 plants
  - 5% truncations, 50% missense, 45% silent

Greene, et al., 2003, Genetics 164: 731-740
PCR Amplification of Target Gene from Pooled Genomic DNA
CEL1 Cleavage

Denature

Resolve on Li-Cor Gel
LI-COR Scanning Results:

IR DYE 700

Normal | Mutant
--- | ---
1.0kb | 0.2 kb
0.8kb

IR DYE 800

Normal | Mutant
--- | ---
1.0kb | 0.8kb
0.2 kb
Colbert et al., 2001, Plant Physiol. 126:480-84
Advantages of TILLING as an Approach for Reverse Genetics

• Both natural and mutagenized populations in any organism can be screened. Mutagenized plants have a large number of randomly distributed mutations per plant genome.

• Plants heterozygous for a mutation can be detected (lethality not a problem).

• Both nonsense (knockout) and mis-sense mutations can be recovered.

• No transgenic manipulations required.
<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homologous recombination*</td>
<td>• Allows for exact replacement or modification of the targeted gene</td>
<td>• Very low efficiency</td>
</tr>
<tr>
<td></td>
<td>• Highly specific to the target gene (no off-target effects)</td>
<td>• Low throughput</td>
</tr>
<tr>
<td></td>
<td>• Results in stable mutations</td>
<td></td>
</tr>
<tr>
<td>Gene silencing†</td>
<td>• Possibility of generating allelic series, allowing the study of</td>
<td>• The degree of gene silencing is unpredictable</td>
</tr>
<tr>
<td></td>
<td>essential genes</td>
<td>• Risk of off-target effects</td>
</tr>
<tr>
<td></td>
<td>• Possibility of restricting the alterations to specific tissues or</td>
<td>• Instability of phenotypes</td>
</tr>
<tr>
<td></td>
<td>developmental stages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Study of gene families with high degree of functional redundancy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Can be adapted for high-throughput screens</td>
<td></td>
</tr>
<tr>
<td>Ectopic expression†</td>
<td>• Similar to gene silencing</td>
<td>• Similar to gene silencing</td>
</tr>
<tr>
<td></td>
<td>• Allows for the analysis of gain-of-function alleles</td>
<td>• Possibility of generating misleading neomorphs</td>
</tr>
<tr>
<td></td>
<td>• Can be adapted for high-throughput screens</td>
<td>• Its use is limited to transformable species</td>
</tr>
<tr>
<td>Zinc-finger nuclease s*</td>
<td>• Highly specific</td>
<td>• Low throughput</td>
</tr>
<tr>
<td></td>
<td>• Results in stable mutations</td>
<td>• Its use is limited to transformable species</td>
</tr>
<tr>
<td>TILLING†</td>
<td>• Allows the identification of loss-of-function alleles, hypomorphs and</td>
<td>• Based on random mutagenesis, so the desired mutation might never be found</td>
</tr>
<tr>
<td></td>
<td>gain-of-function alleles</td>
<td>• Low to medium throughput</td>
</tr>
<tr>
<td></td>
<td>• Can be used in non-transformable species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Results in stable mutations</td>
<td></td>
</tr>
<tr>
<td>Deleteagene*</td>
<td>• Allows the identification of two or more genes in close proximity</td>
<td>• Based on random mutagenesis, so the desired mutation might never be found</td>
</tr>
<tr>
<td></td>
<td>• Can be used in non-transformable species</td>
<td>• Limited to loss-of-function mutations</td>
</tr>
<tr>
<td></td>
<td>• Results in stable mutations</td>
<td>• Low to medium throughput</td>
</tr>
<tr>
<td>Insertional mutagenesis.§</td>
<td>• High throughput</td>
<td>• Based on random (T-DNA) or non-targeted (transposon) mutagenesis, so the</td>
</tr>
<tr>
<td></td>
<td>• Can be adapted for both loss-of-function and gain-of-function studies</td>
<td>desired mutation might never be found</td>
</tr>
<tr>
<td></td>
<td>• Results in stable mutations</td>
<td>• Cannot be used to study tandemly repeated genes (T-DNA mutagenesis)</td>
</tr>
<tr>
<td></td>
<td>• Few unwanted mutations</td>
<td>• Only limited information can be obtained for essential genes</td>
</tr>
</tbody>
</table>

*Each of these approaches has so far been of limited practical use, with fewer than 10 successful examples reported. †Each of these approaches was used as a tool to study gene function in more than 50 publications. ‡There are more than 500 publications that used this technique. T-DNA, transferred DNA.
TAL effectors are remote controls for gene activation
Heidi Scholze and Jens Boch
Current Opinion in Microbiology
Volume 14, Issue 1, February 2011, Pages 47–53

Dr. Ulla Bonas
Institute of Biology, Dept. of Genetics
Martin- Luther- University
Halle- Wittenberg
Xanthomonas campestris pv. vesicatoria (Xcv)  
Bacterial spot of tomato and pepper
*Xcv* Type III secretion system (T3SS) secretes effectors to disturb host immunity.
Proposed model for the molecular mechanisms underlying virulence and avirulence activity of AvrBs3 from *X. campestris* pv. *vesicatoria*. Characteristic features of AvrBs3 are the central 17.5 nearly identical 34 amino acid repeats, two functional C-terminal nuclear localization signals (NLSs) and an acidic activation domain (AAD). Delivery of AvrBs3 into the host cell is mediated by the TTS system. In the plant cell, the NLSs bind to importin α, which together with importin β targets AvrBs3 to the plant cell nucleus. Direct or indirect (via a target protein X) interaction of AvrBs3 with the plant DNA leads to the modulation of the host's transcriptome and presumably results in hypertrophy, a disease symptom in susceptible plants. In resistant plants, specific plant defense responses are induced upon recognition of AvrBs3 by the R protein Bs3 (Bs, bacterial spot).
TAL effectors: Sequence-specific transcription factors
Transcription activator-like effectors (TALEs)

http://www.genome-engineering.org/taleffectors/?page_id=261
TAL nucleases (TALNs) promote genome editing. (a) TALNs are fusions between TAL effectors and the FokI endonuclease domain. A tailored TAL repeat domain controls DNA-binding specificity. (b) Two TALNs bind neighboring DNA boxes and FokI dimerization induces DNA cleavage in the spacer region between the boxes. DNA double-strand breaks can promote nonhomologous end-joining (NHEJ) or homologous DNA recombination (HDR) enabling targeted genome modifications like deletions or insertions.
Transcription Activator-Like Effector Genome Editing

1. I'd love to edit your favorite gene!
2. Design TALEN
3. Predict assembly
4. Assembly PCR
5. Transfection via Electroporation
6. Target Cell
7. Nucleus
8. TALEN
9. mDNA
10. Exogenous DNA Introduced
11. Homology Directed Repair
12. Non-Homologous End-Joining
13. Double Strand Break
14. YFG
15. Error Introduced

Exogenous DNA Introduced
References:


Till et al. 2003. Large-scale discovery of induced point mutations with high-throughput TILLING. Genome Research 13(3): 524-30

Heidi Scholze and Jens Boch, 2011. TAL effectors are remote controls for gene activation Current Opinion in Microbiology. Volume 14, Issue 1, February 2011, 47–53