

# Activation tagging in aspen using an inducible two component Ac/Ds-enhancer element system



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# Poplar genome sequencing project

- Black cottonwood; also known as western balsam poplar or California poplar (*Populus trichocarpa*)
- Involved institutions
  - DOE Joint Genome Institute (JGI)
  - DOE Oak Ridge National Laboratory
  - Genome Canada
  - Umea Plant Science Centre
  - Ghent University
- Released September 2004, after two years of sequencing


*Populus trichocarpa* v1.0

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With a genome of just over 500 million letters of genetic code, *Populus trichocarpa* was sequenced eight times over to attain the highest quality standards. Poplar was chosen as the first tree DNA sequence decoded because of its relatively compact genetic complement, some 50 times smaller than the genome of pine, making the poplar an ideal model system for trees.

The poplar genome, divided into 19 chromosomes, is four times larger than the genome of the first plant sequenced four years ago, *Arabidopsis thaliana*.

Thus far, researchers have revealed poplar's genome to be about one-third heterochromatin, that is, regions of chromosomes thought to be genetically inactive, which should provide shortcuts to important regulatory features.

**Genome Project Notes**

The *Populus* genome assembly 1.0 is a preliminary release as part of the ongoing *Populus* genome project. A final draft sequence will be released in early 2005. The current assembly includes approximately 7.5X in small insert end-sequence coverage. Additional mapping and sequencing is ongoing.

Our goal is to make the genome sequence of Poplar widely and rapidly available to the scientific community. We endorse the principles for the distribution and use of large scale sequencing data adopted by the larger genome sequencing community and urge users of this data to follow them. It is our intention to publish the work of this project in a timely fashion and we welcome collaborative interaction on the project and analyses as appropriate.

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[http://genome.jgi-psf.org/Poptr1\\_1/Poptr1\\_1.home.html](http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html)

# Poplar genome sequencing project

- Approximately 7.5x coverage
- 485 Mio Bp on 19 chromosomes
- No of genes:  
*Arabidopsis*: 25.498 in about 11,000 gene families (Bennetzen 2001)  
 Human: 19.599 + 2.188 proposed ones
- *Populus*: annotation v1.1  
 JamboreeModels includes a total of 45,555 gene models
- Gene exons 41,908
- Function of the putative genes ?
- Mutants are needed


*Populus trichocarpa* v1.0

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<http://genome.jgi-psf.org/Poptr1/Poptr1.home.html>

# Poplar mutants ?

- Only few poplar mutants are known
- Classical mutagenesis (EMS, X-ray) strategy is not effective in trees
- Molecular strategies
  - ✓ Downregulation (antisense, RNAi)
  - ✓ Knock-out mutagenesis (T-DNA)
  - ✓ Knock-in mutagenesis (Activation tagging)

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# Insertion vehicle

## T-DNA

- Very useful for easy-to-transform plant species
- Complex integration pattern and chromosomal rearrangements near the insertion site
- Large number of independent transgenic lines needed

## Transposable elements (TE)

- Few transgenic lines are needed to use transgene as launching vehicle for TEs
- TEs can be remobilized to confirm the phenotype

# Gene tagging

1. **T-DNA tagged lines**
2. **Ac transposition**

# T-DNA tagged lines

- Most transgenic lines obtained were without any obvious phenotypic variation (diploid species)
  - + insertions in non-coding regions
  - + recessive alleles

- Loss-of-function: Few phenotypic variants observed (dominant alleles, null-alleles?)

(Fladung, MGG 1999, Kumar and Fladung, Planta 2001)

- Activation tagging of a GA2-oxidase gene in poplar  
(Busov et al., Plant Physiol. 2003)

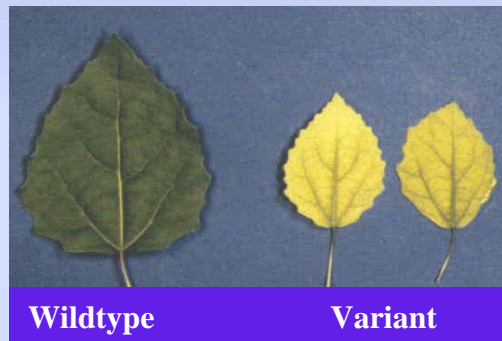
# Variants obtained so far

Out of 97 independent transgenic aspen 6 phenotypic variants were obtained:

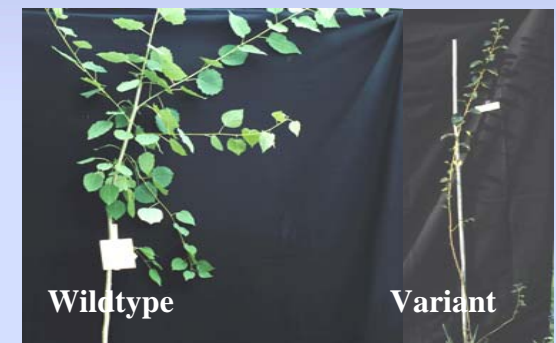
**W+2-3: „Glossy“**



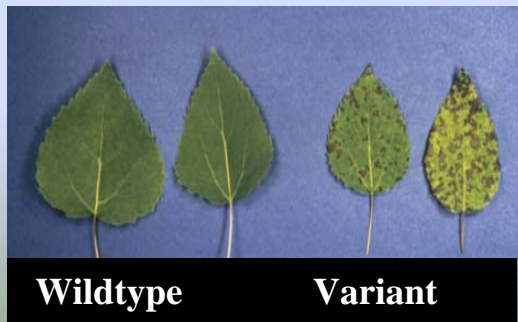
**W+23-5: „Pale green“**



**B2-2: „Lash“**



**E28-5: „Necrotic spots“**   **E28-22: „Lanceolate leaf“**



**E2-5: „Altered mycorrhiza“**

# Summary T-DNA analyses

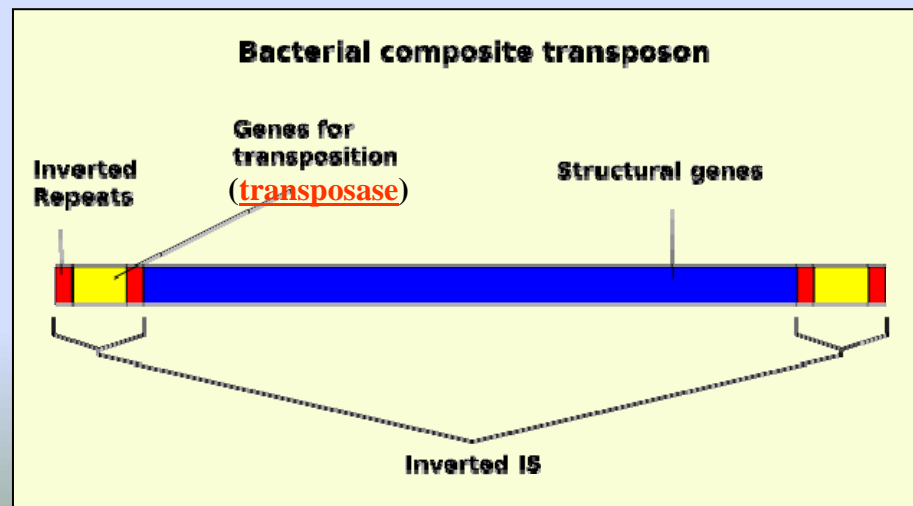
- About 10% of T-DNA flanking regions from obtained lines showed homologies to known genes
- Most of the transgenic lines don't reveal any phenotype
- T-DNA flanking genomic regions of three poplar variants are similar to sequences of T-DNA tagged *Arabidopsis* lines (GABI-KAT)

# Genomics in Poplar

1. T-DNA tagged lines
2. **Ac transposition**

# With the autonomous maize Ac transposon as a tag

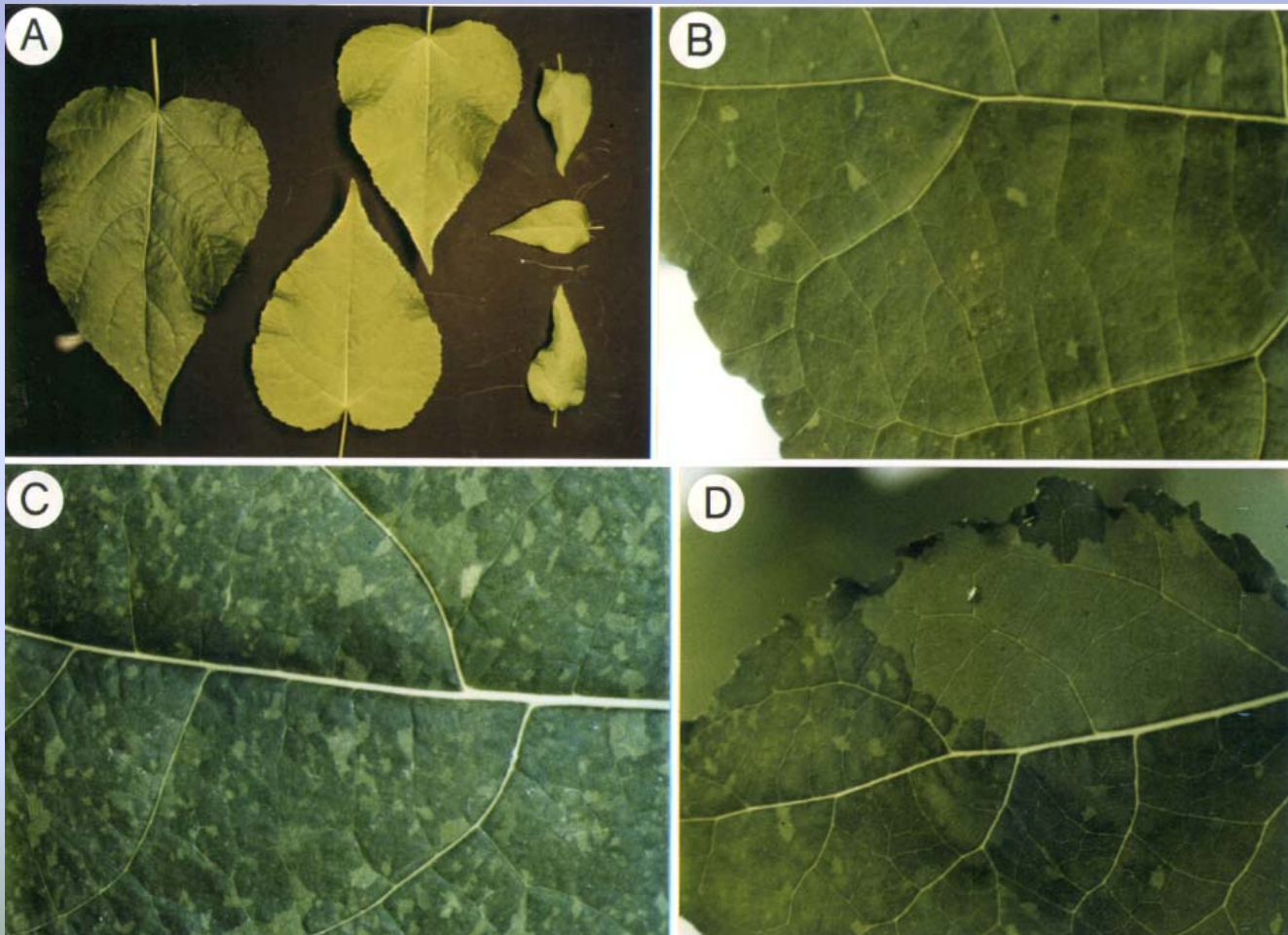
- Transposons are also called "jumping genes"
- Discovered by Barbara McClintock in 1940ies
- Examples of mobile genetic elements



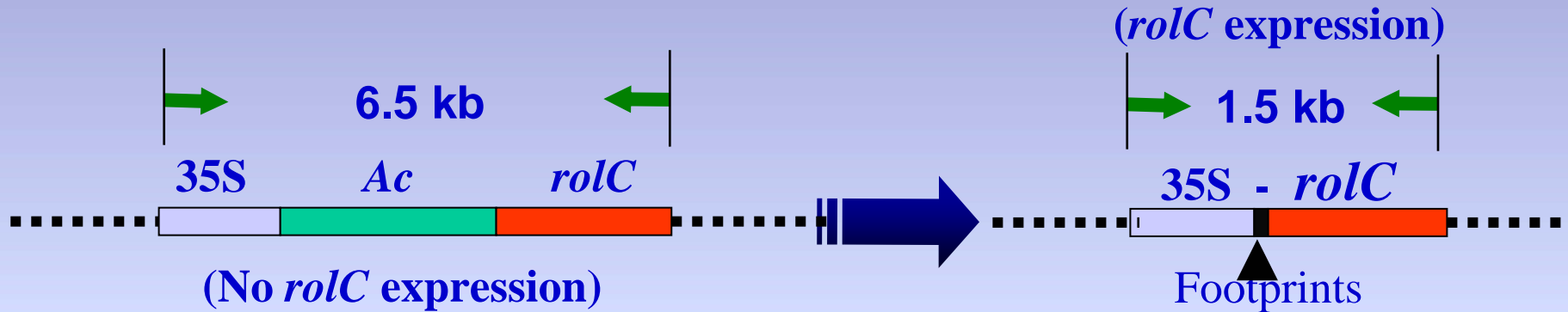
# Does *Ac* jump in *Populus*?

1. Transformation of three different aspen-*Populus* genotypes with the 35S-*Ac-roIC* and *rbcS-Ac-roIC* constructs (Fladung et al. 1997, Fladung and Ahuja 1997)
2. Transformation of haploid poplar (*Populus nigra* hybrid) with the 35S-*Ac-roIC* construct (Deutsch et al. 2004, Fladung et al. 2004)

# *Ro/C-* A visual excision marker



# Ac excises in diploid and haploid aspen

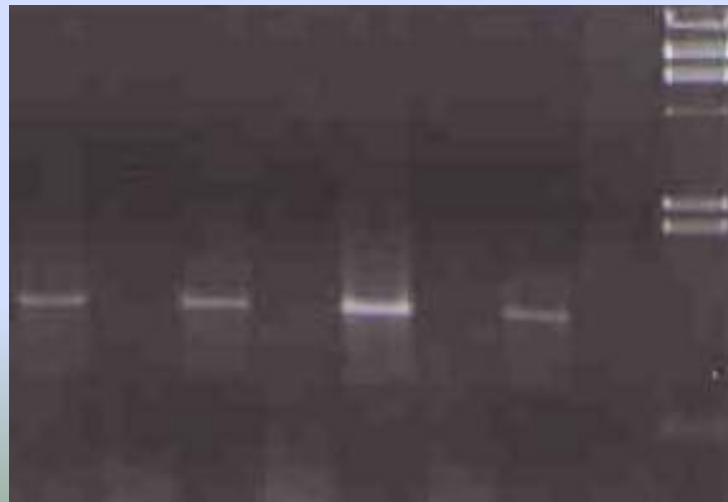


PCR

Leaf sectors

Ro/C C PG DG PG DG PG DG M

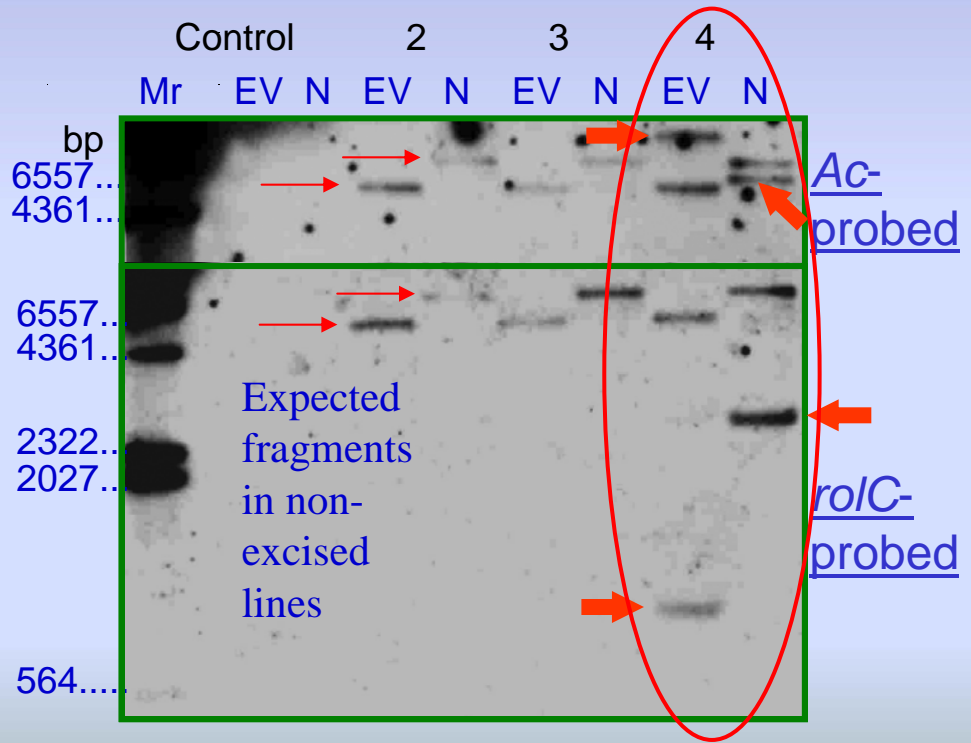
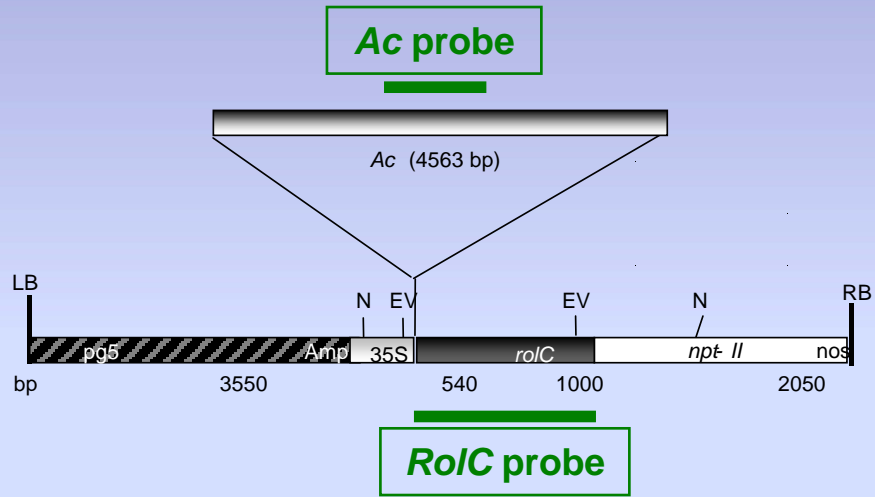
1.5 kb →



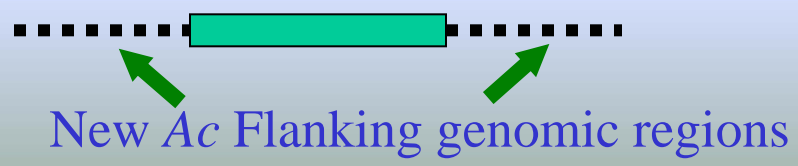
(Fladung et al. 1997, Kumar and Fladung 2003, Fladung et al. 2004)

And re-integration of Ac?

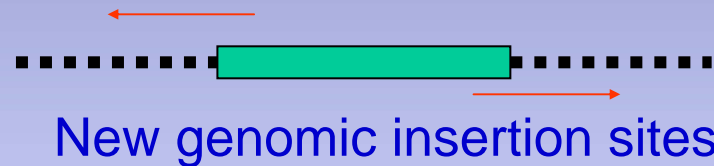
# Ac re-integrates in aspen



## Genomic integration of Ac



# Sequencing of new Ac flanking regions



Transgenic line	No. of patches analysed	Signifinant BLASTx Hits/Freq.
Esch5:35S- <i>Ac-rolC</i> #3	41	12 (29%)
Esch5:35S- <i>Ac-rolC</i> #10	24	6 (25%)
Esch5:35S- <i>Ac-rolC</i> #2	10	4 (40%)
<b>Total</b>	<b>75</b>	<b>22 (29%)</b>

- Preferential insertion of the *Ac* element in or near coding regions of the aspen genome (Kumar and Fladung 2003)



# Summary *Ac*-transformations

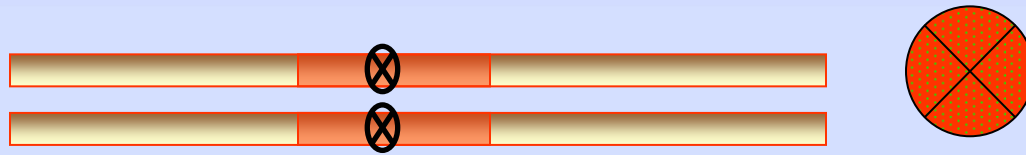
- Transformation of haploid and diploid poplar lines with the *Ac* transposon
- It was shown that *Ac* excises and re-integrates in the aspen genome
- *Ac* inserts frequently in or near coding regions
- A large number of poplar sequences were obtained and submitted to GABI database
- But no control of transposase expression  
    → *Ac*-transposition is autonomous

# New tagging approaches

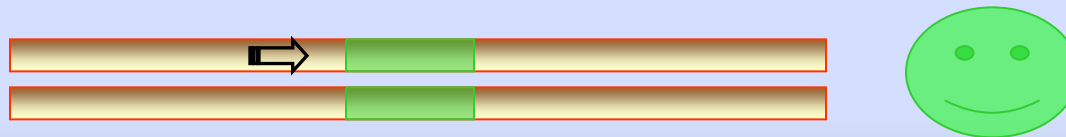
- (a) Independent from ploidy level
- (b) Control of transposition by using non-autonomous elements and inducible systems
- (c) Following induced transposition regeneration of plants from small cell clusters
- (d) Screen regenerants for variants

# Gene Tagging

Gene tagging is a gene isolation method using a tag that modifies gene expression

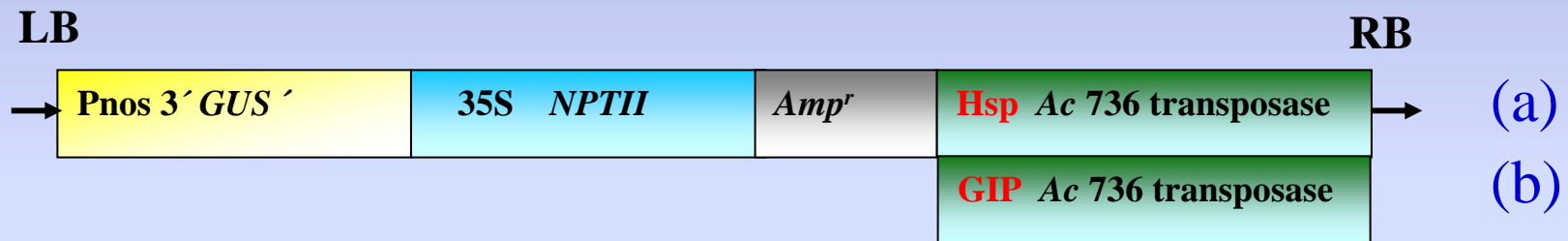


Knockout (Loss of function, most cases recessive, homozygous locus needed)



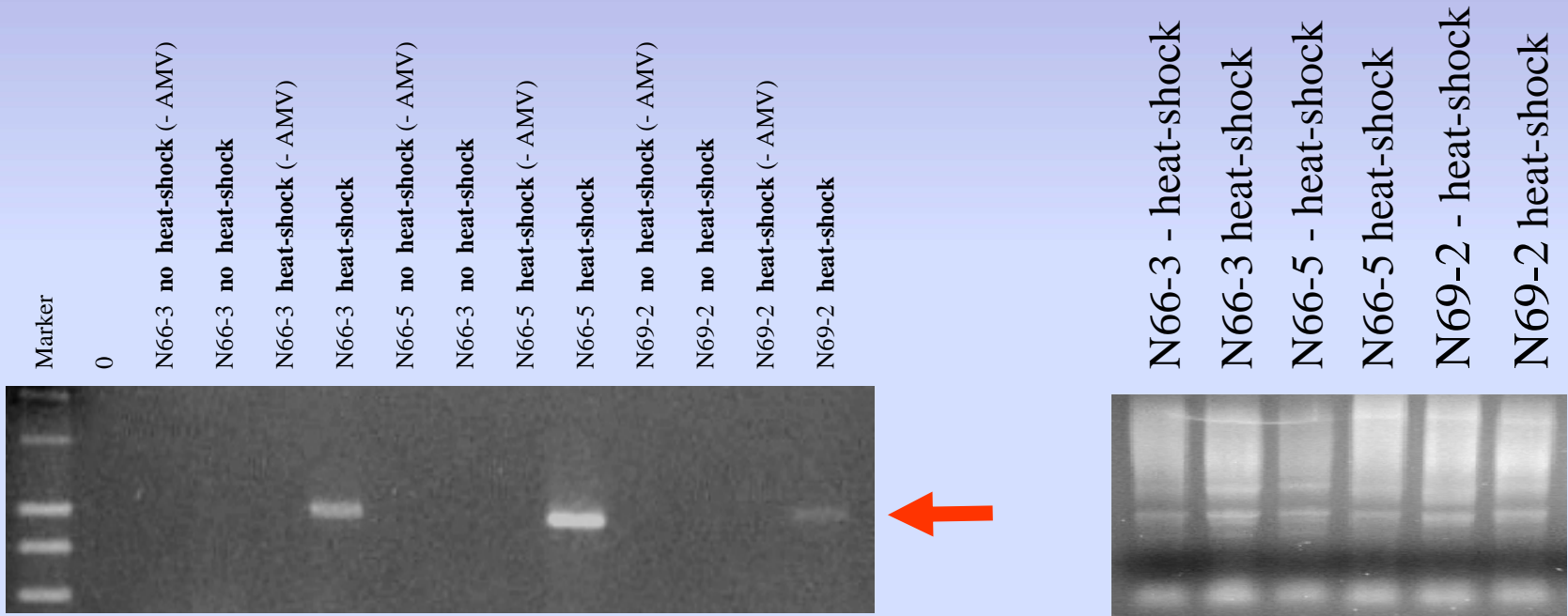
Knock in (Gain of function, dominant) = **Activation tagging**

# Inducible transposase expression



- (a) T-DNA containing heat shock promoter (Hsp) and *Ac 736* transposase (Balcells et al. 1994).
- (b) In the second construct the Hsp is replaced by a glucocorticoid-inducible promoter (GIP, Ouwerkerk et al. 2001)

# Inducible transposase expression

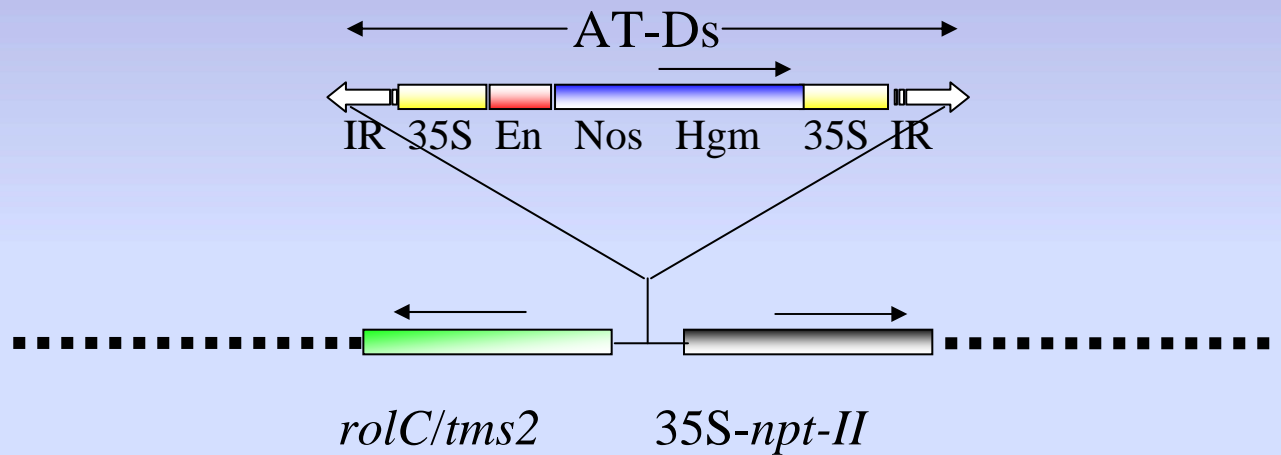


RT-PCR analysis for the transposase transcript

RNA quality test

# Activation construct

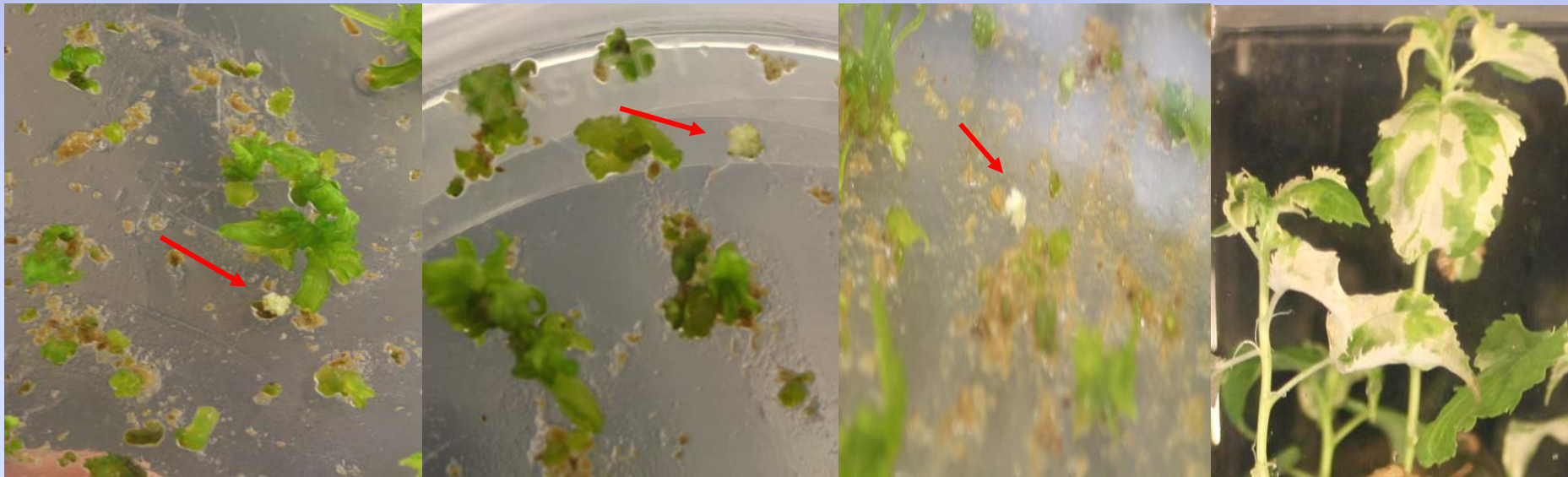
... containing the non-autonomous *Ds* element



- Activation constructs: AT-Ds contains two CaMV 35S promoters and four tandem repeats of enhancer fragments (En) of the 35S promoter, flanked by terminal inverted repeats (IR) (Suzuki et al. 2001). A similar one: Sundaresan et al. (1995)
- Either *rolC* or *tms2* gene is outside of AT-Ds, and the 35S promoter keeps these genes active under non-excised conditions.



# Putative tagged mutants



- Three putative chlorophyll-defective variant calli (arrows), following DEX treatment
- Chimeric plant
- At present a large tagging experiment with 8 lines is ongoing

# Conclusions

- Activation tagging via T-DNA and/or transposons is a useful tool to induce mutants in tree species
- The use of the transposon *Ac* is a strategy to efficiently produce stable transposon-based activation tags in *Populus* genome
- The *Ac* tagging principle suggested is applicable across other tree species because a small number of primary transgenic lines is required

# Many thanks to

## Lab and greenhouse

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**Many thanks for your  
attention !**