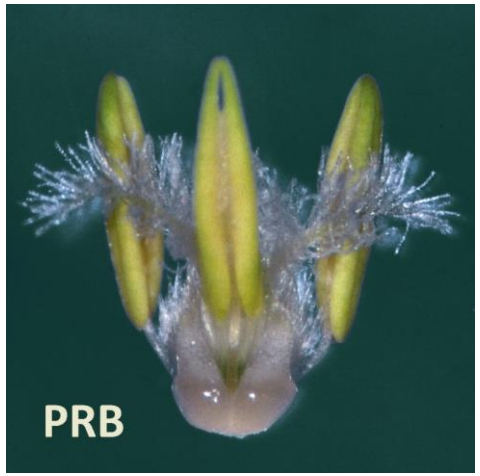
Site-directed mutagenesis of maize elite germplasm through pollination by *cas9*/gRNA-transgenic, haploidy-inducing lines



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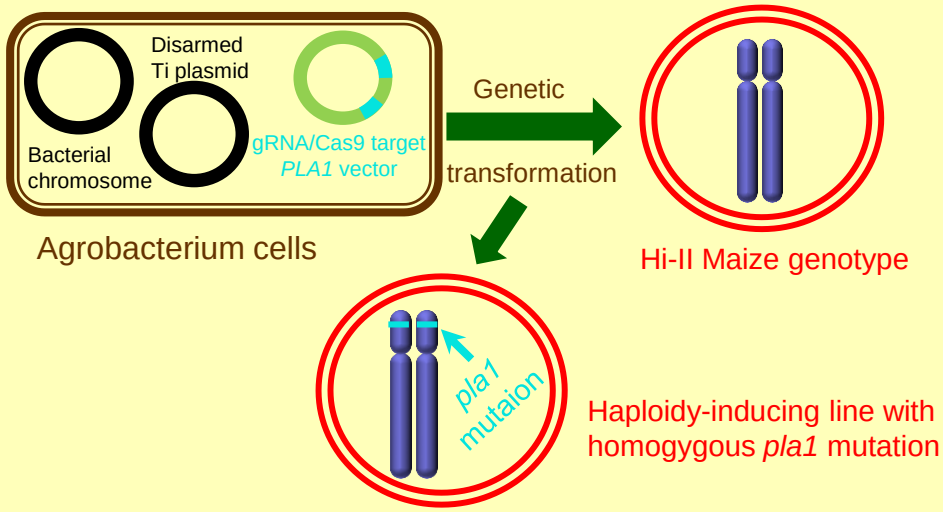
Introduction and concept

- RNA-guided Cas9 endonuclease provides a precise and powerful tool for site-directed genome modification in plant breeding.
- Aim of the study: induce site-directed modification in elite maize genotypes without integration of gRNA/Cas9-encoding DNA

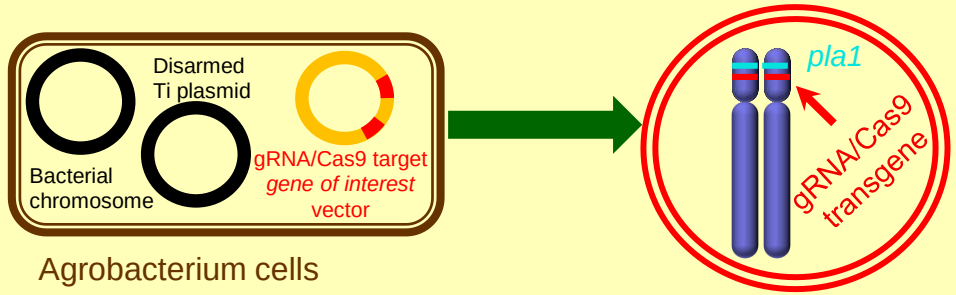
  how:
  - Generation of haploidy-inducing lines in maize plants via site-directed mutagenesis of *PHOSPHOLIPASE A1 (PLA1/MTL/NLD)*
  - Transform the generated haploidy-inducing lines with *cas9* and gRNA specified for a gene-of-interest
  - Cross-pollinate elite genotype(s) with haploidy-inducing, gRNA/*cas9*-transgenic line to generate progeny with site-specific alteration
- Loss-of-function of *PLA1* is sufficient to confer haploid-inducing ability to maize plants.
- After crossing, during early embryonic development, the haploidy inducer's genome is progressively degenerated and eliminated.
- A haploid embryo with site-directed mutation in the target gene of choice is generated.
- The genome of haploid plants will be chemically diploidized and then carries the site-specific mutation in homozygous condition.

Introduction and concept

Step 1a: Generation of haploidy-inducing line in Hi-II background

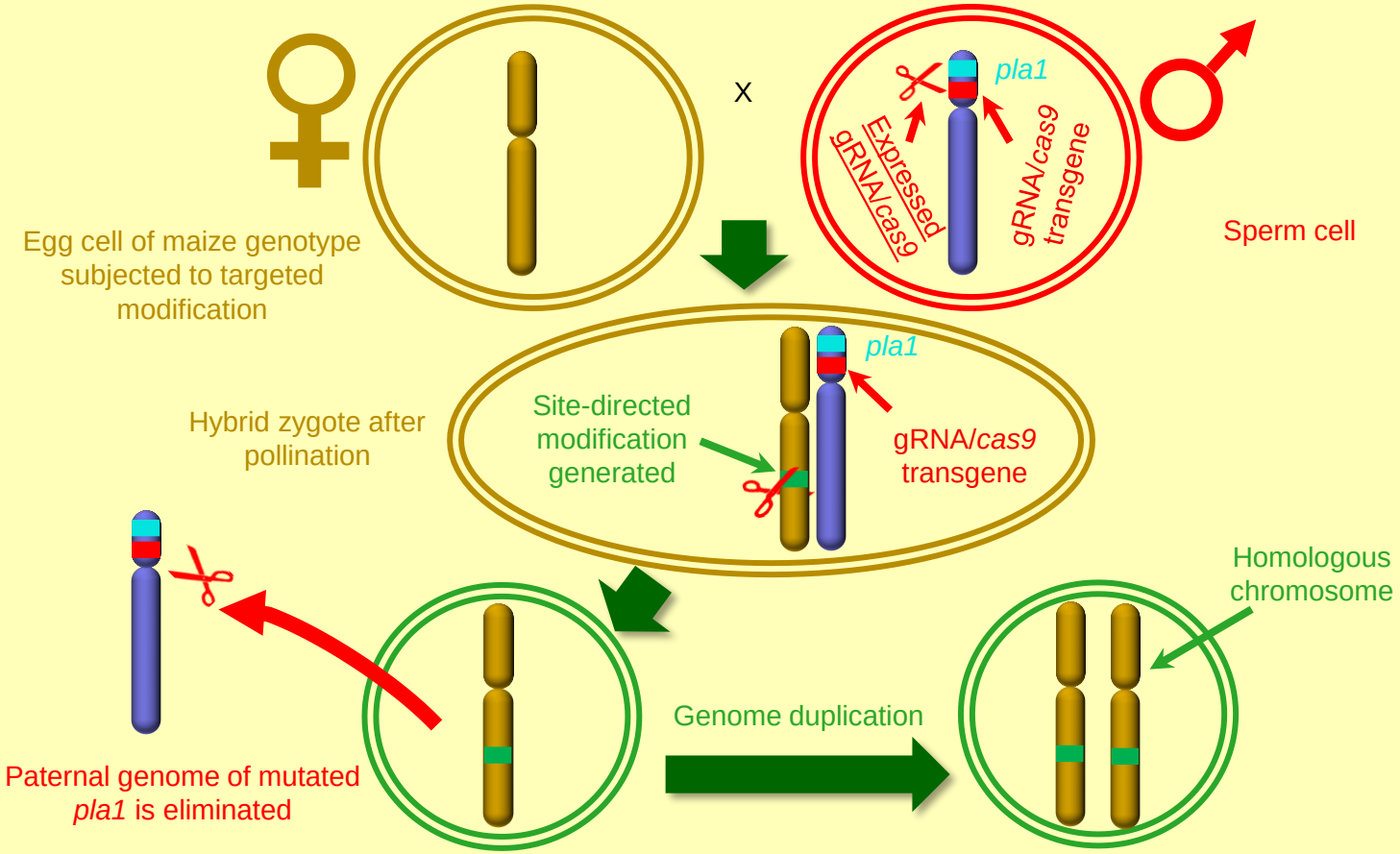


Step 1b: Transformation of haploidy-inducing line with gRNA/*cas9* specified for target gene of choice



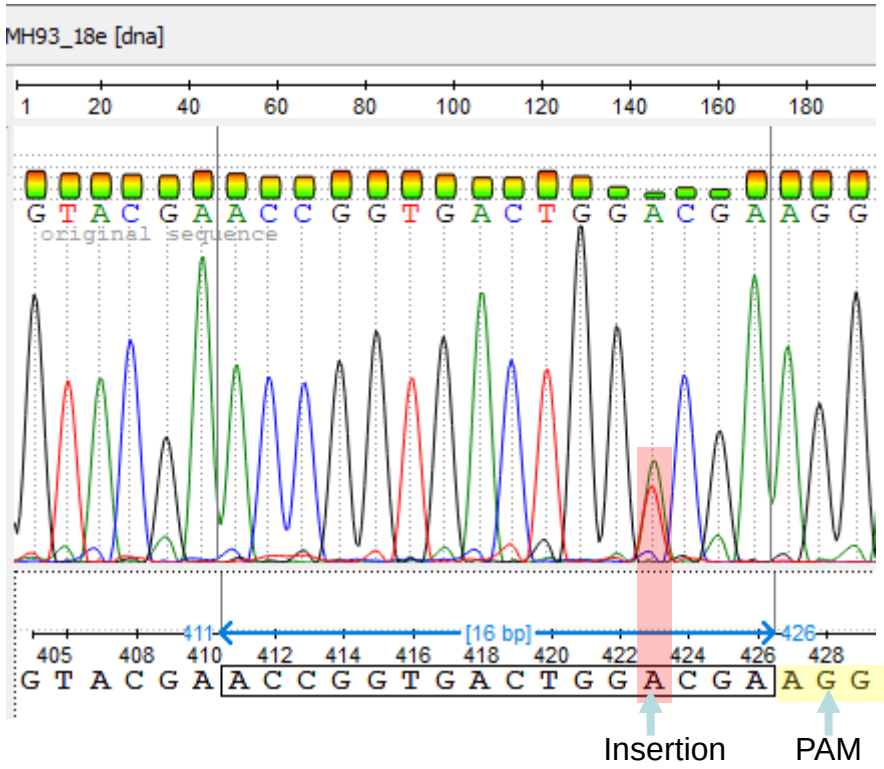
Introduction and concept

Step 2: Generating homozygous, site-specific alterations in elite maize genotypes



Results and summary

- Three target motifs in the *PLA1* gene were selected and corresponding gRNAs were cloned along with in cas9 into binary vectors.
- Hi-II genotype immature embryos were subjected to transformation with these vectors.
- Amplicons from one of the PLA1 target motifs were analyzed by Sanger-sequencing, whereby 22 of the 32 transgenic plants proved to carry targeted mutations.
- Currently, progeny of these primary mutants is screened for homozygosity of mutations and loss of transgene sequences by segregation.
- The current concept offers various advantages:
  - Reduced genotype-dependence.
  - The absence of any recombinant DNA in the modified genome,
  - Site-directed modifications can be achieved in a variety of elite maize lines using just one transgenic haploidy inducer line.



The DNA sequencing chromatograms from Sanger sequencing represent the mutation location. The arrow denotes a heterozygous mutated nucleotide.

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