



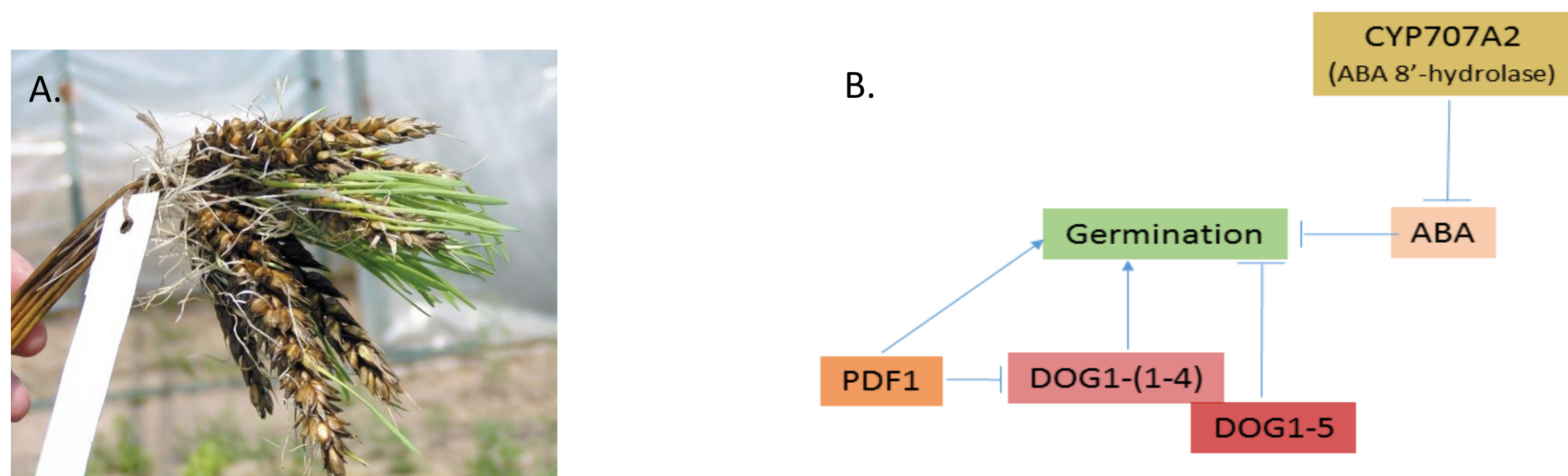
# TALEN design and assembly for gene editing of the seed dormancy – specific loci in Triticale

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## Introduction

Seed dormancy is an innate seed property and important component of plant fitness determined by genetic factors with substantial environmental influence. The pathway leading to pre-harvest sprouting (PHS) is represented by the seeds that go directly from development-maturation to germination on the mother plant. Pre-harvest sprouting causes substantial yield and end-quality losses, but the mechanism regulating PHS remains unclear.



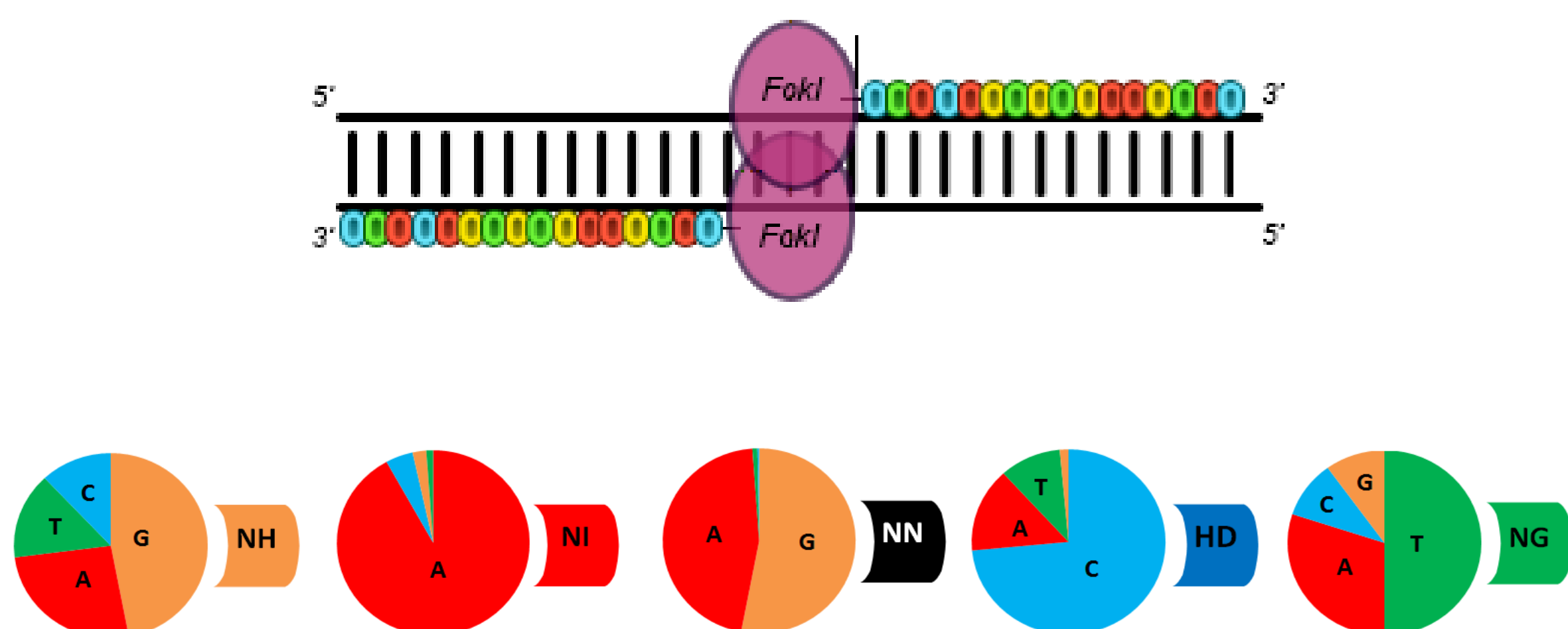
**Fig.1** A) Damage by PHS on a highly susceptible Triticale line. B) Sprouting time control pathways in Arabidopsis involve regulation of seed germination *via* hormones like ABA, but DOG1 independent pathway.

Seed germination is controlled by ABA 8'-hydroxylase (ABA8'OH) that regulates the level of abscisic acid (ABA). Four genes encoding ABA8'OH (*CYP707A1-A4*) were described in Arabidopsis (Kushiro et al. 2004) and two were reported for wheat TaABA8'OH (Chono et al. 2013). Negative regulator of seed dormancy PDF1 was detected as a loss of function mutant in Arabidopsis (Miatton, 2012). Selected for mutation PP2A is the wheat homolog of PDF1.

## TALEN gene editing

Transcription activator-like nucleases (TALEN) are customizable fusion proteins having a specific DNA binding domain and FokI endonucleases that induce double strand breaks (Fig.2).

TALEN enable targeted mutagenesis in a variety of organisms. The potential of TALEN technology for crop improvement was demonstrated (Li et al. 2012; Liang et al. 2013).



**Fig.2** Schematic representation of TALEN binding to the DNA. Pair of TALENs containing 16-20 individual modules, each one recognizes preferably a single base pair (shown in colours) and determines sequence specificity. FokI cleavage domains as dimer cleave the target sequence.

Although wheat and triticale are agriculturally important crops, only a few mutants with altered seed dormancy have been described (Warner *et al.* 2000), due to the difficulty in simultaneous mutation of 2, 3 loci. Therefore possibility of mutation by TALENs of all alleles of the targeted locus simultaneously is a particularly significant improvement in polyploids.

## Materials and methods

### TALEN design

- For TALEN design genomic sequence of the PP2A-A and TaABA8'OH1 was analysed and TALEN target sites were predicted for each gene of interest using TALE-NT web tool (Cermak et al., 2011) combined with own modifications. All target sites retained a T at the -1 position.

### Off-target prediction

- To assess potential off-target effects and increase the editing specificity of TALEN, genome-wide prediction of highly specific sequences in wheat and Brachypodium was performed.

### TALEN assembly

- TALENs were assembled according to their corresponding repeat variable diresidues (RVDs) and were constructed by Golden Gate cloning (Addgene). The left and right TALEN units were cloned into yeast expression vector pZHY500 and introduced into Gateway entry vector pZHY013 and to pB7WG2D to form an expression cassette.

## Results

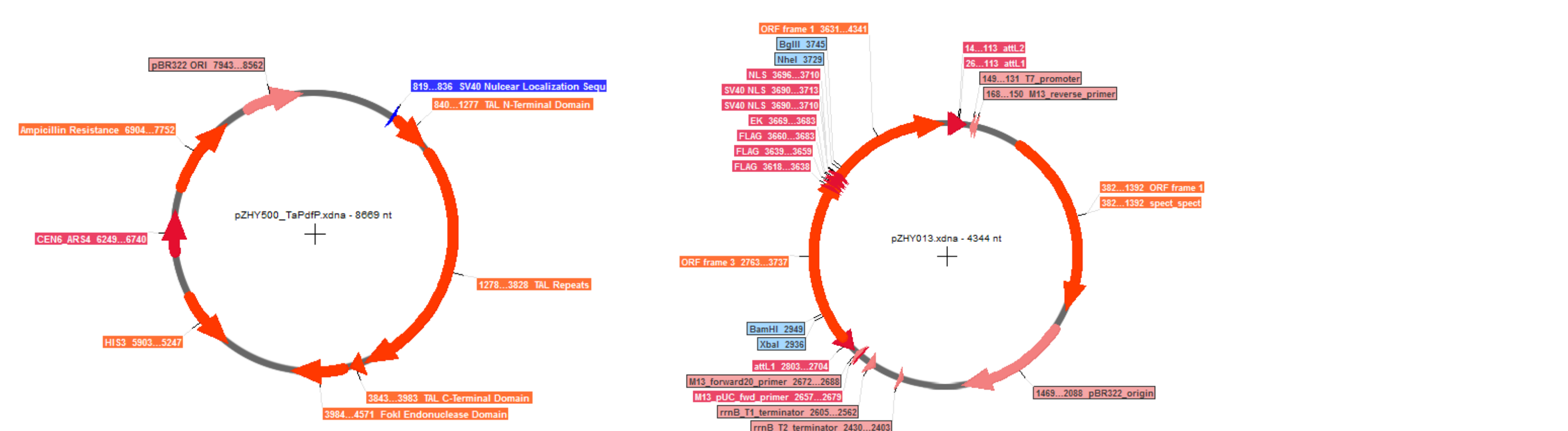
TALEN Triticum	RVD TAL-L HD NI NH NI NI NH NI HD NG NG NG HD NI HD NI HD NI NH max score: 4.34				RVD TAL-R HD NH HD NG NH NI NH NH NI NI NI NI NH NI NG NI HD NI HD NG NG max score: 4.19			
	Gene	Score	Targeted sequence	Genome targets (in Brachypodium)	Gene	Score	Targeted sequence	Genome targets (in Brachypodium)
	PP2A-A	4.34	CAGAAGACTTCCACTATTGCACGG	4 [7828358, 7828382] – score: 6.35	PP2A-A	4.19	CGCTGAGGAAGGAATCAGTTCTCT	4 [7907717, 7907741] – score: 11.41 4 [7828407, 7828431] – score: 14.93 1 [63402624, 63402648] – score: 16.35
	PP2A-B	4.34	CAGAAGACTTCCACTATTGCACGG	4 [7907668, 7907692] – score: 6.35	PP2A-B	4.19	CGCTGAGGAAAGGAATCAGTTCTCT	
	PP2A-D	4.34	CAGAAGACTTCCACTATTGCACGG	3 [17906547, 17906571] – score: 16.94	PP2A-D	4.19	CGCTGAGGAAGGAATCAGTTCTCT	

TALEN Triticum	RVD TAL-L NH NG HD HD NI NH HD HD HD NI NH NG NG HD max score: 3.76				RVD TAL-R NH NH HD HD NH NH NI NI NH NH NG NI HD NG NG NH NI NI NH NI NH NH max score: 3.35			
	Gene	Score	Targeted sequence	Genome targets (in Brachypodium)	Gene	Score	Targeted sequence	Genome targets (in Brachypodium)
	TaABA8'OH1 -A	3.76	GTCCAGCCCCGAGGCCGCAAGTTC	3 [53531438, 53531462] – score: 12.85	TaABA8'OH1 -A	3.35	GGCCGGGAAGTAGCCTGAAGAGG	3 [53531483, 53531507] – score: 10.47
	TaABA8'OH1 -B	7.30	GTCCAGCCCCGAGGCCGCAAGTTC	2 [41572693, 41572717] – score: 14.93	TaABA8'OH1 -B	3.35	GGCCGGGAAGTAGCCTGAAGAGG	
	TaABA8'OH1 -D	7.30	GTCCAGCCCCGAGGCCGCAAGTTC		TaABA8'OH1 -D	3.35	GGCCGGGAAGTAGCCTGAAGAGG	

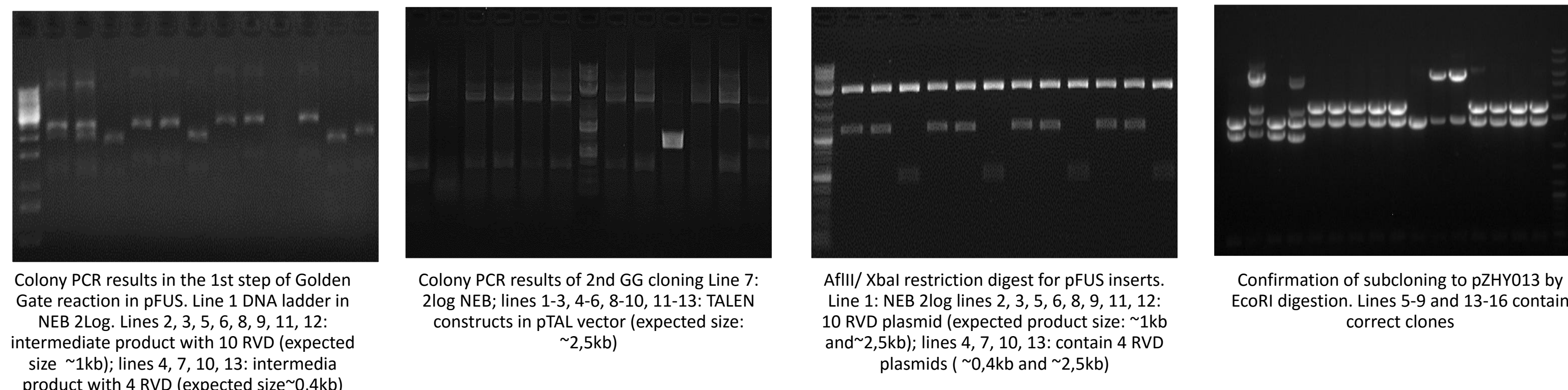
**Tab 1.** TALEN RVD sequences for left and right monomers designed for wheat PP2A gene and for TaABA8'OH from wheat. Genome targets in wheat and off-target sequences based on Brachypodium genome are listed.



**Fig.3** Schematic sequence of two wheat dormancy negative regulator genes PP2A-A (A) and TaABA8'OH1-A (B). The arrow indicates exon region predicted for TALEN mutation.



**Fig.4** The left and right TALEN units constructed by Golden Gate cloning were introduced into pZHY500 vectors and cloned into BamHI/XbaI and BglII/NheI sites of pZHY013 Gateway compatible intermediate vector. Gateway LR recombinations were performed to obtain an expression T-DNA cassette in pB7WG2D plant expression vector (VIB). Cloning steps were confirmed by colony PCR, restriction digest and sequencing.



## Conclusions

- TALEN mediated targeted genome modification becomes a powerful tool for genome editing in polyploids.
- Here we propose a selected TALEN architecture for knock-out of two negative regulators of seed dormancy in Triticale (PP2A and TaABA8'OH1), targeting all loci of these genes in the wheat genome.

## References

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