Knockout of *BT1* Orthologs by CRISPR-Cas9 Approach to Improve Nitrogen Use Efficiency in Wheat (*Triticum aestivum* L.)

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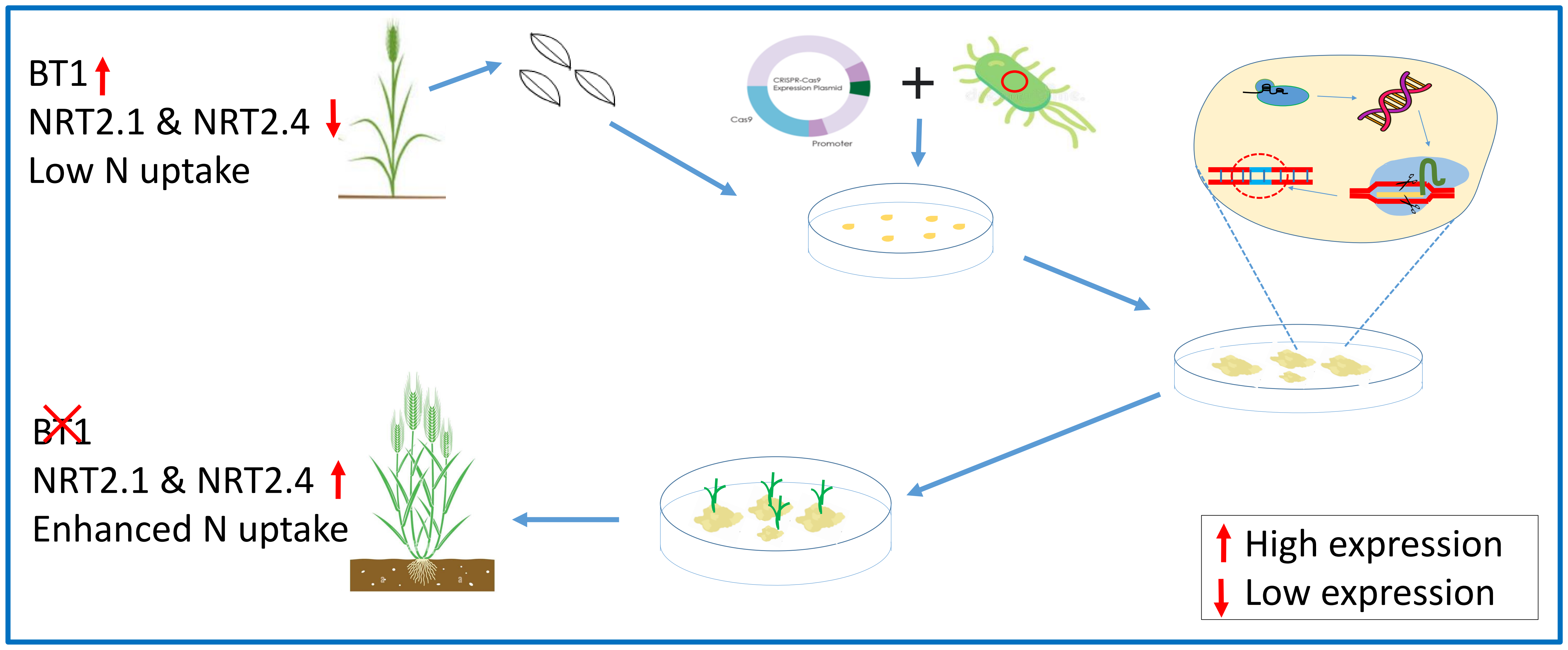

BT1 ↑
NRT2.1 & NRT2.4 ↓
Low N uptake

~~BT1~~
NRT2.1 & NRT2.4 ↑
Enhanced N uptake

↑ High expression
↓ Low expression

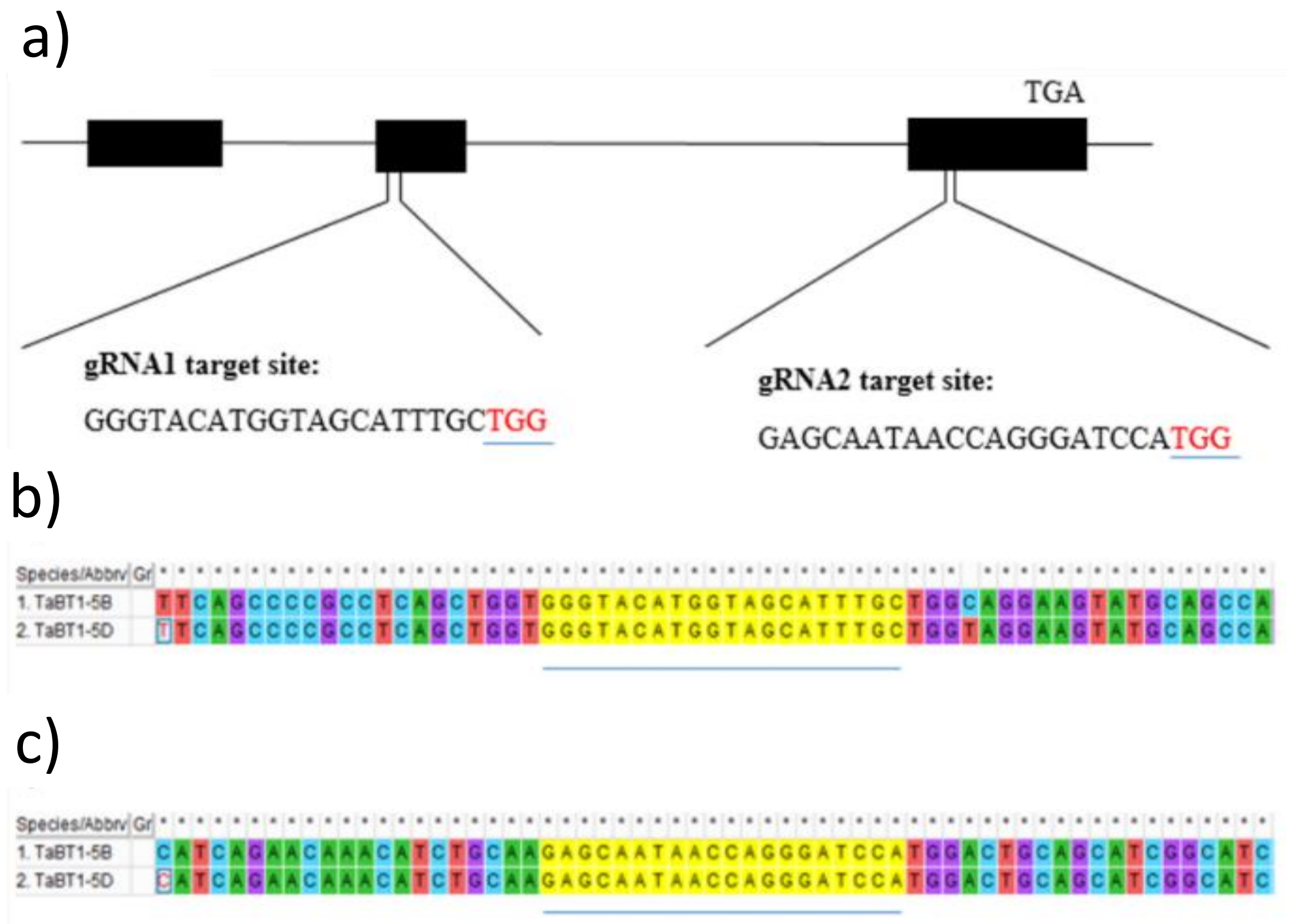
Introduction

Nitrogen (N) is a major determinant of wheat crop yield and quality. But semi-dwarf wheat cultivars absorb only 30-40% of the applied N. Recently, it has been identified that *BT1* is one of the central gene affecting NUE and knockout of *BT1* increases the N uptake in rice and *Arabidopsis* mutant plants. In this study orthologs of *BT1* were identified in wheat genome and knocked-out by CRISPR/Cas9 approach.

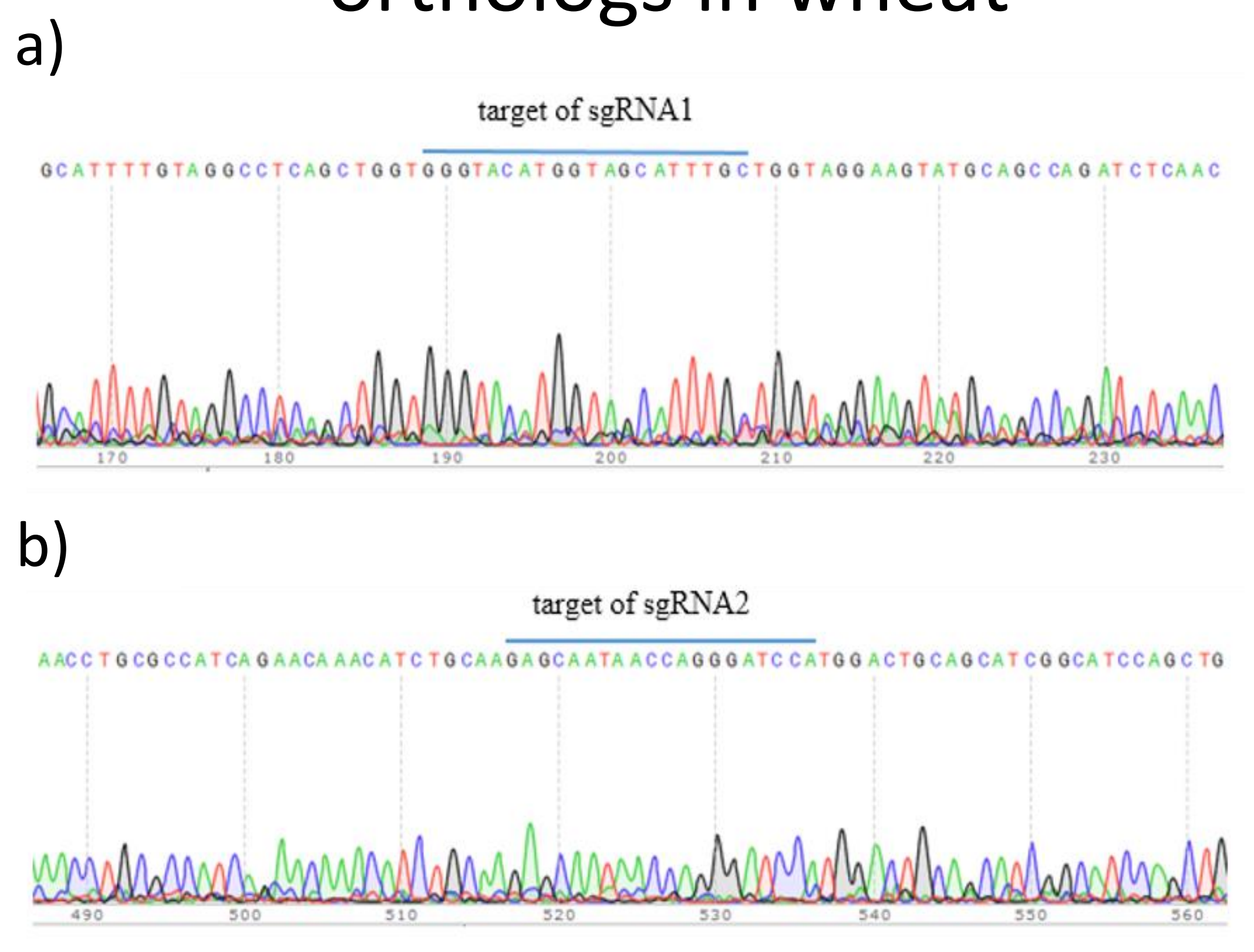
Material and Methods

- ✓ Identification of *BT1* orthologs in the wheat genome.
- ✓ Designing of sgRNAs.
- ✓ Validation of target regions in wheat cv. “Akbar-2019” by Sanger sequencing
- ✓ Cloning of sgRNAs in *pJA32* vector.
- ✓ *Agrobacterium* mediated transformation.

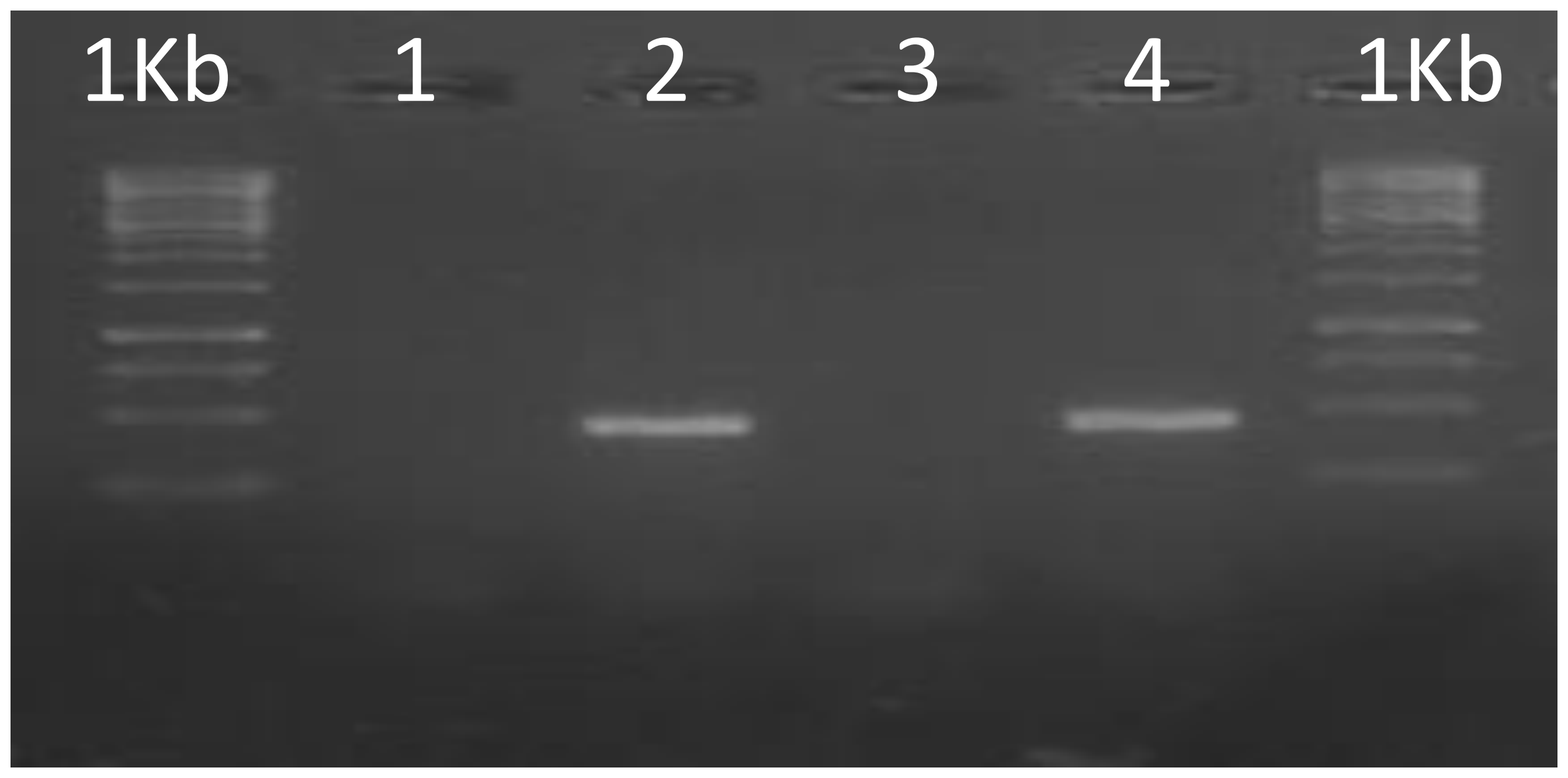
Results and Discussion



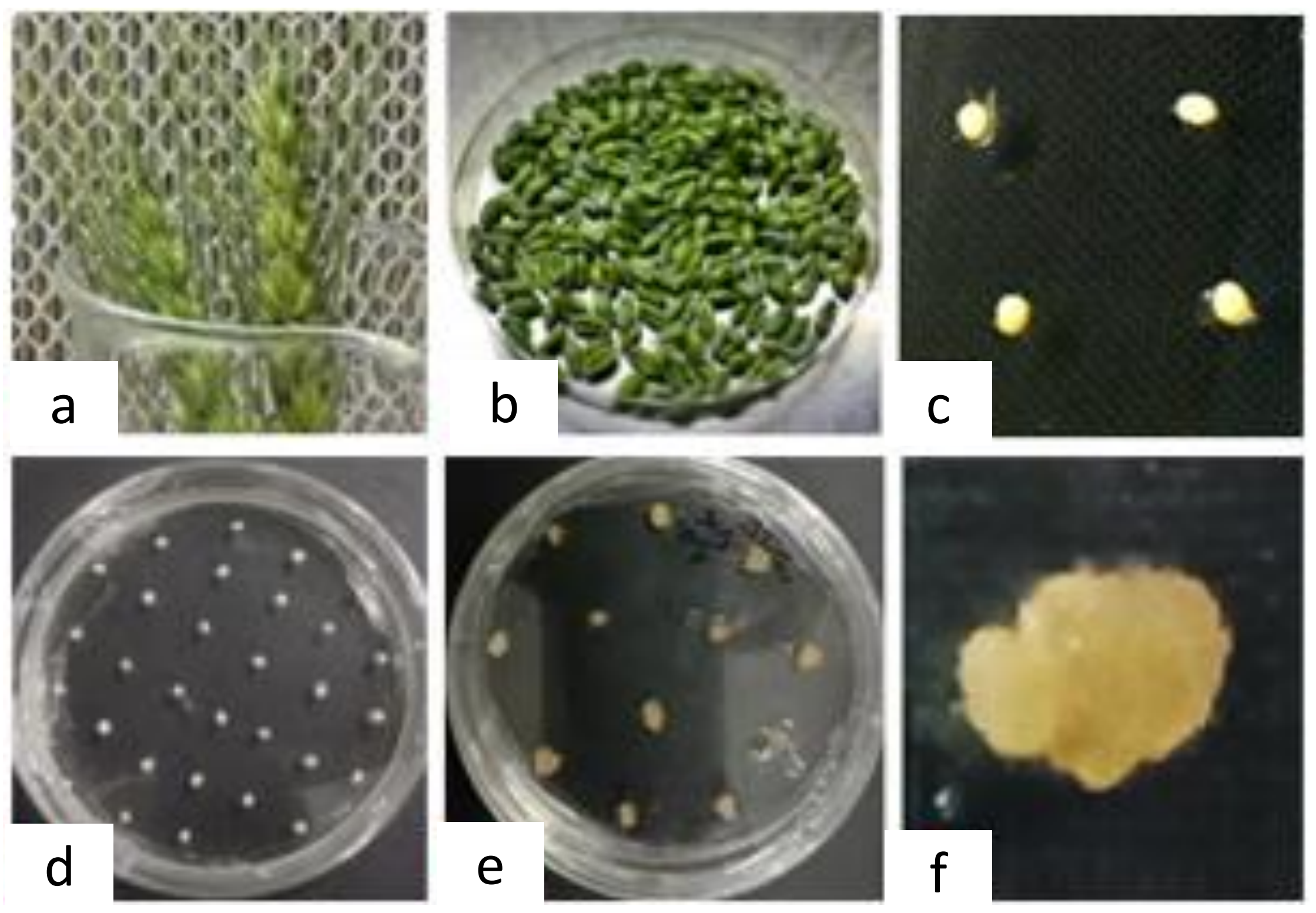
sgRNAs were designed in the conserved regions of *BT1* orthologs in wheat



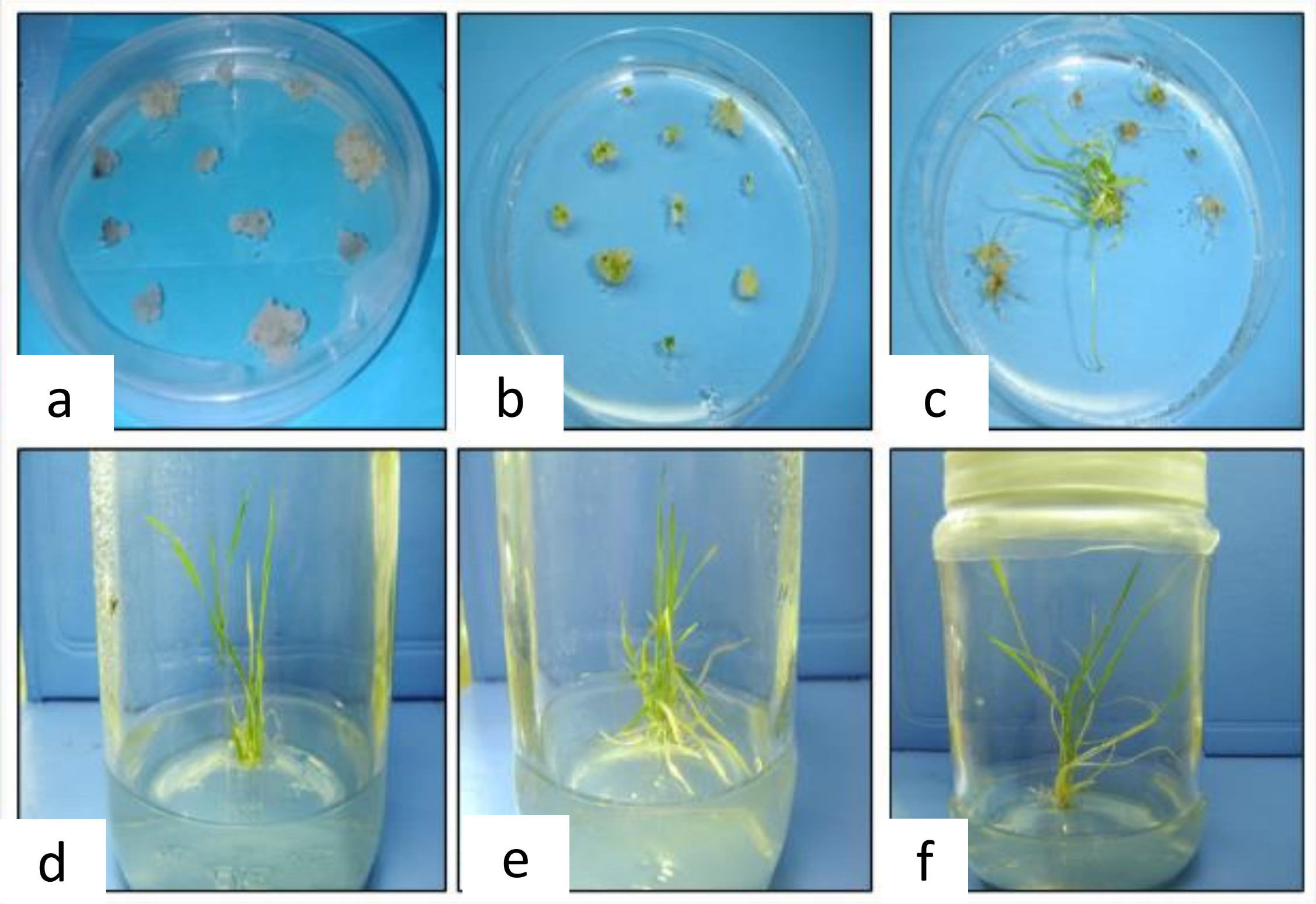
Validation of target regions by Sanger sequencing



PCR based Confirmation of *pJA32*-sgRNA constructs transformation in wheat cv. “Akbar-2019”



Agrobacterium mediated transformation in immature wheat embryos



Embryogenesis and regeneration

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