

# Heterologous Expression of Plant Cell Wall Genes from Grasses to Prove Biochemical Functions



U.S. DEPARTMENT OF  
**ENERGY**

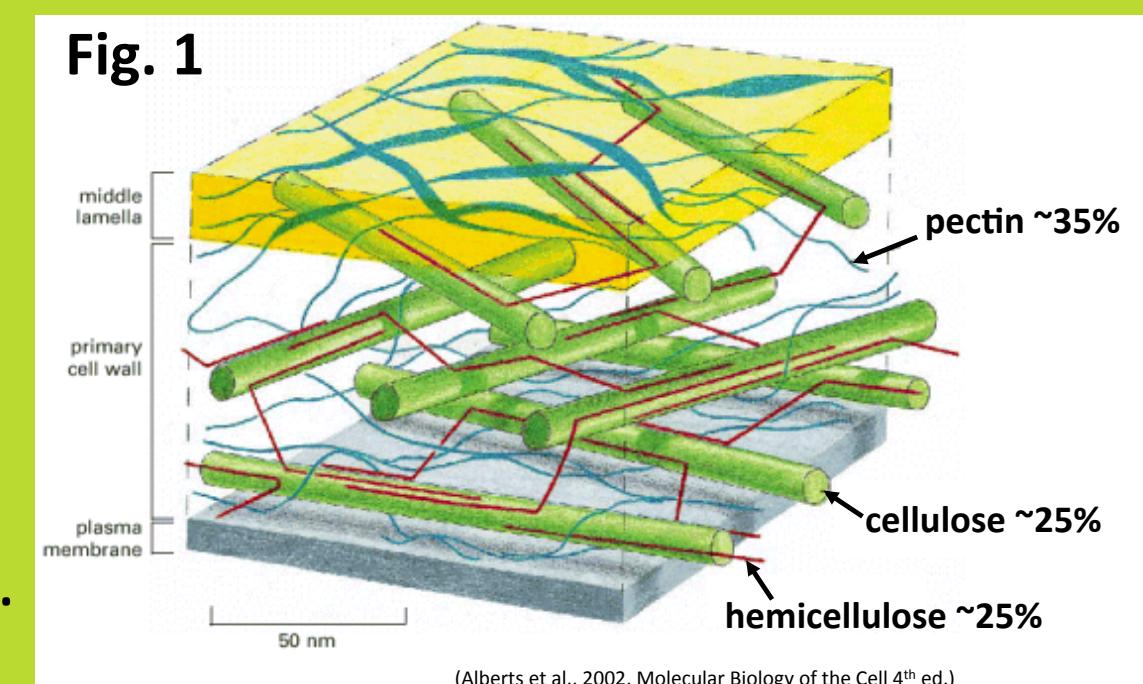
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## Introduction

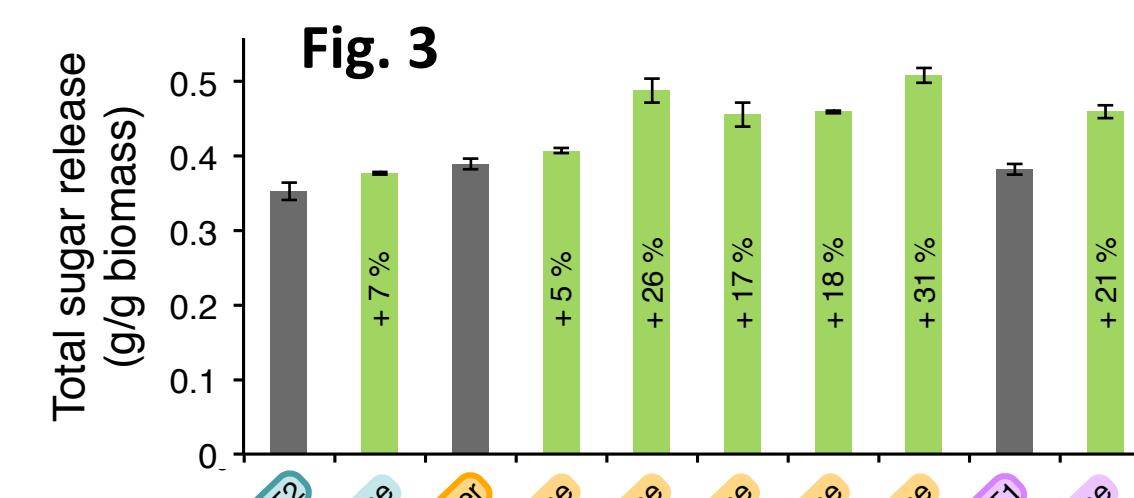
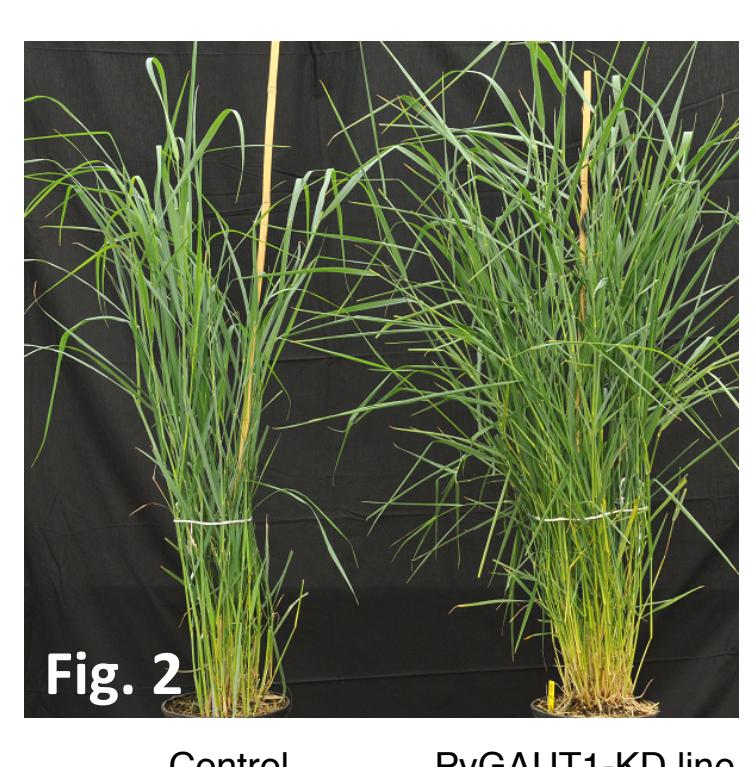
All plant cells are surrounded by an extracellular matrix known as the plant cell wall, which has a complicated and dynamic structure that is composed of different polysaccharides and proteins. Together, the structural components of the cell wall and their interactions have important functions in cell growth, shape, and differentiation. The major components of the plant cell wall include the polysaccharides cellulose and the non-cellulosic polysaccharides known as hemicellulose and pectin (Fig. 1).



**Homogalacturonan (HG)** is the most abundant pectic polysaccharides composed of unbranched 1,4-linked  $\alpha$ -D-galactosyluronic acids (GalA) polymers, making up around 60% of the pectin components in the plant primary cell wall (1). **Xylan** is the most abundant hemicellulosic polysaccharides after cellulose. Xylan polymer is composed of linear backbones of 1,4-linked  $\beta$ -D-xylosyl residues that are in grasses substituted with side chains of L-Ara, D-Xyl and 4-O-Me-  $\alpha$ -D-GlcA to form glucoronoarabinoxylan (2).

## Background

Understanding the enzymatic activities of the enzymes encoded by plant cell wall genes is an important step in understanding the cell wall structure, which in turn is critical to enable the modification and improvement of plant growth and response to the environment and to develop improved renewable biomaterial from plants.



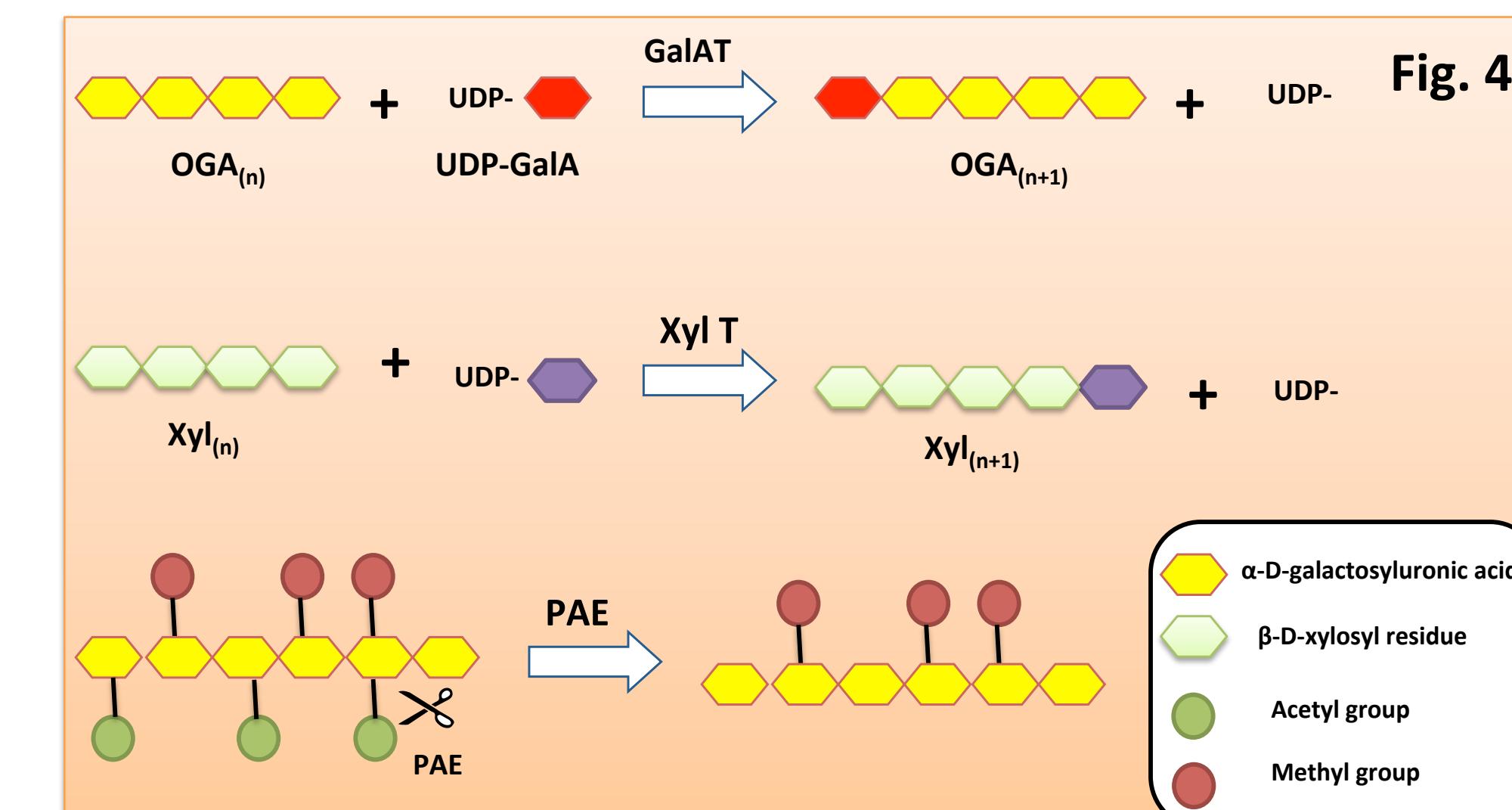
In our lab ongoing research (3), downregulation of switchgrass gene GAUT1 and IRX10L, as well as over-expression of foxtail millet PAE1 and PAE2 in switchgrass has shown increase in plant biomass (Fig. 2) and greater glucose conversion (Fig. 3).

The biochemical functions of orthologs of these genes from other plant species have been demonstrated (Fig. 4).

**Arabidopsis GAUT1** is an HG biosynthetic  $\alpha$ -1,4-galacturonosyl transferase that catalyzes the transfer of GalA from uridine diphosphate- GalA (UDP-GalA) onto endogenous and exogenous acceptors of HG (4,5).

**Arabidopsis IRX10L** is a xylan synthase that catalyzes the transfer of xylosyl residues from UDP-Xyl onto endogenous and exogenous acceptors of xylan (6).

**Populus PAE1** is a pectin acetyl esterase that catalyzes the removal of acetyl groups from the pectin polymers (7).

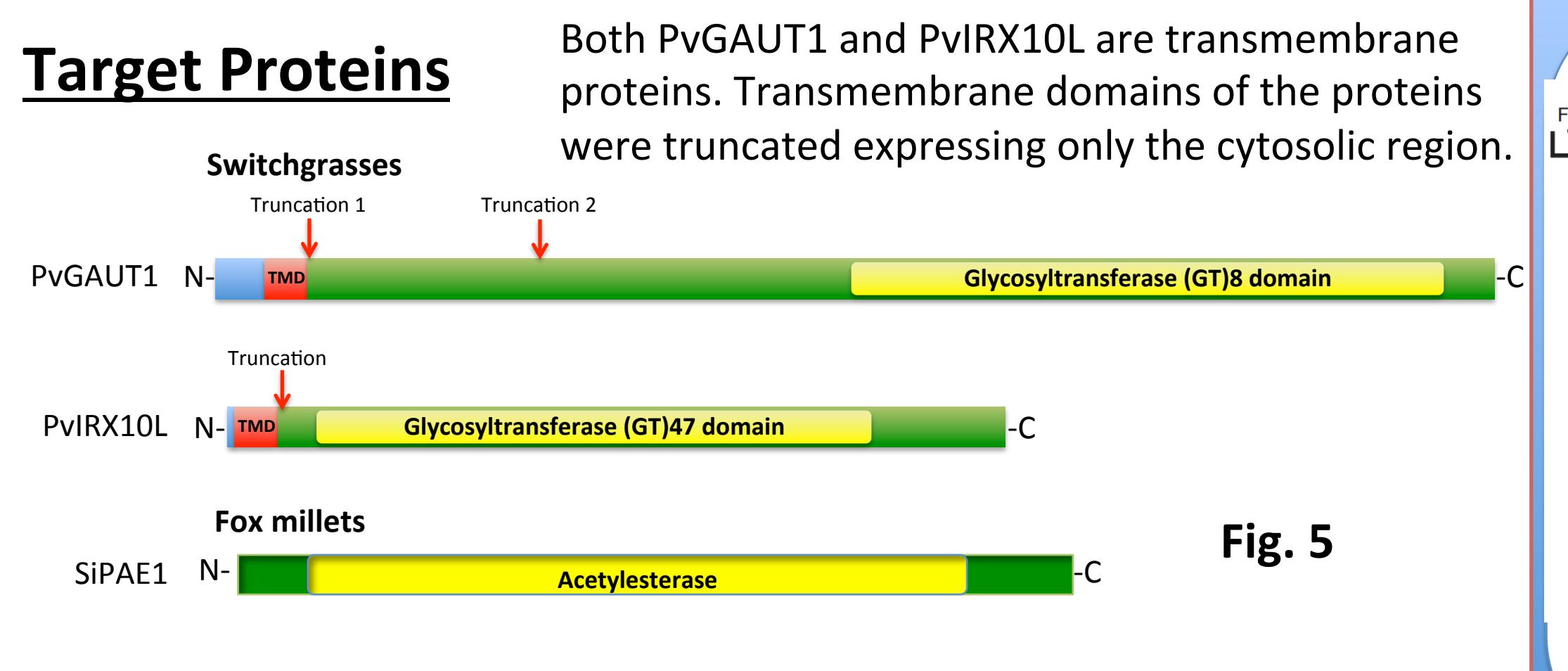


In the work presented in this poster, we aim to demonstrate the activities of the proteins encoded by the switchgrasses genes through heterologous expression in the mammalian Human Embryonic Kidney (HEK) 293 cells and *E. coli* to better understand the function of these genes.

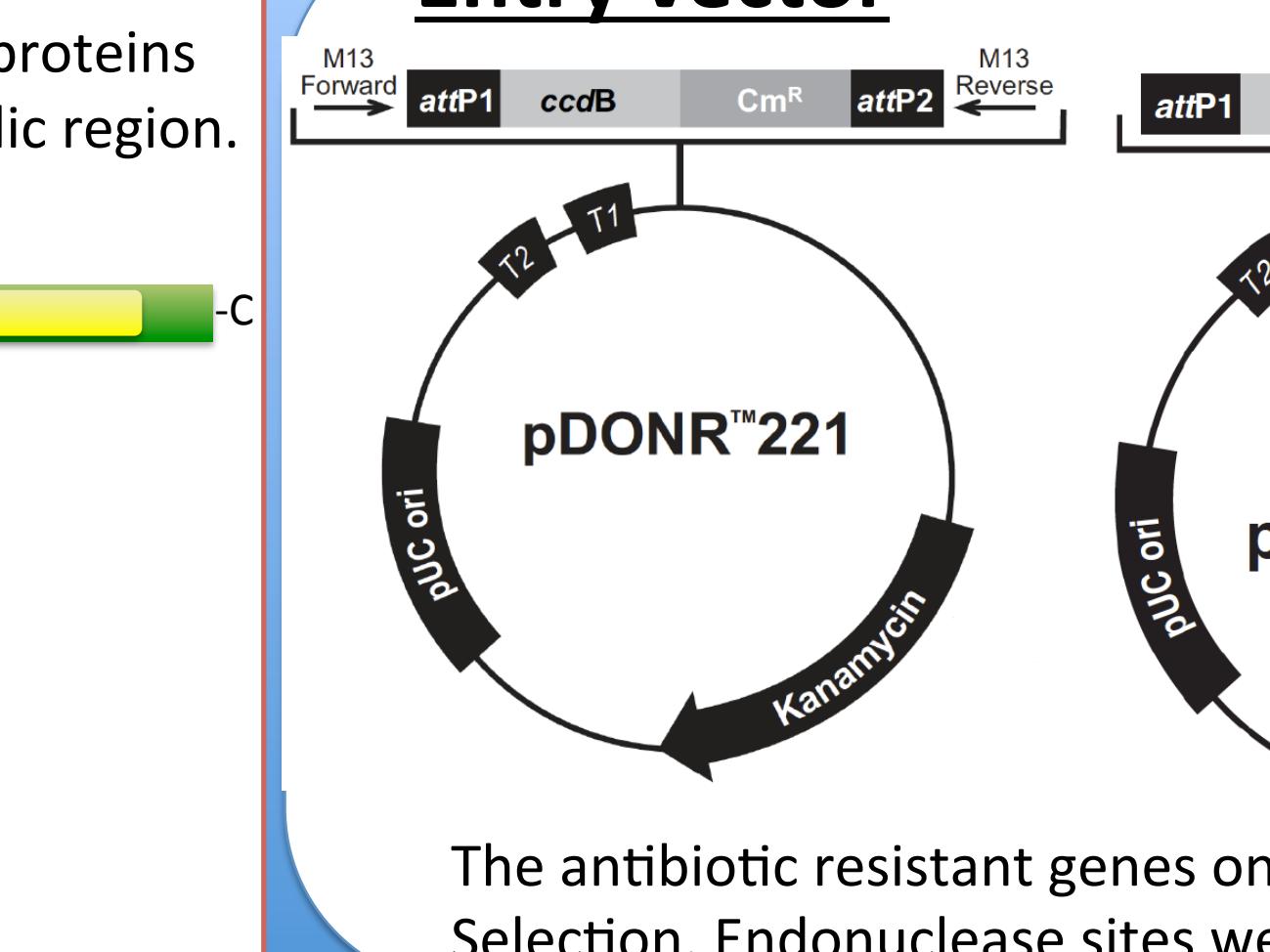
## Materials

**cDNA**  
Switchgrass cDNA: ST1 plant leave cultivar (PvGAUT1 truncation 1&2; PvIRX10L)  
Fox millet cDNA: YUGUI plant leave cultivar (SiPAE1; SiPAE2)

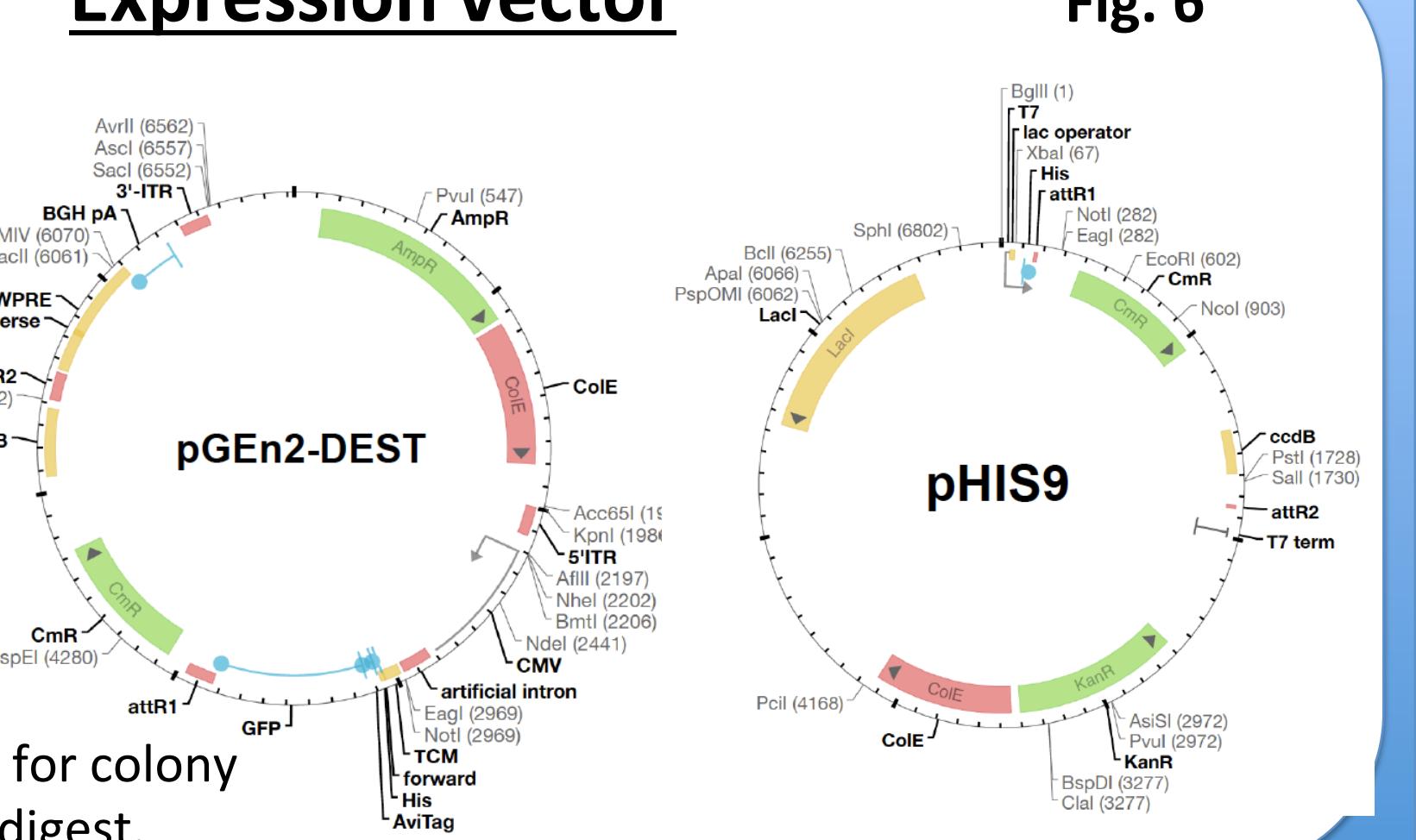
## Target Proteins



## Entry vector



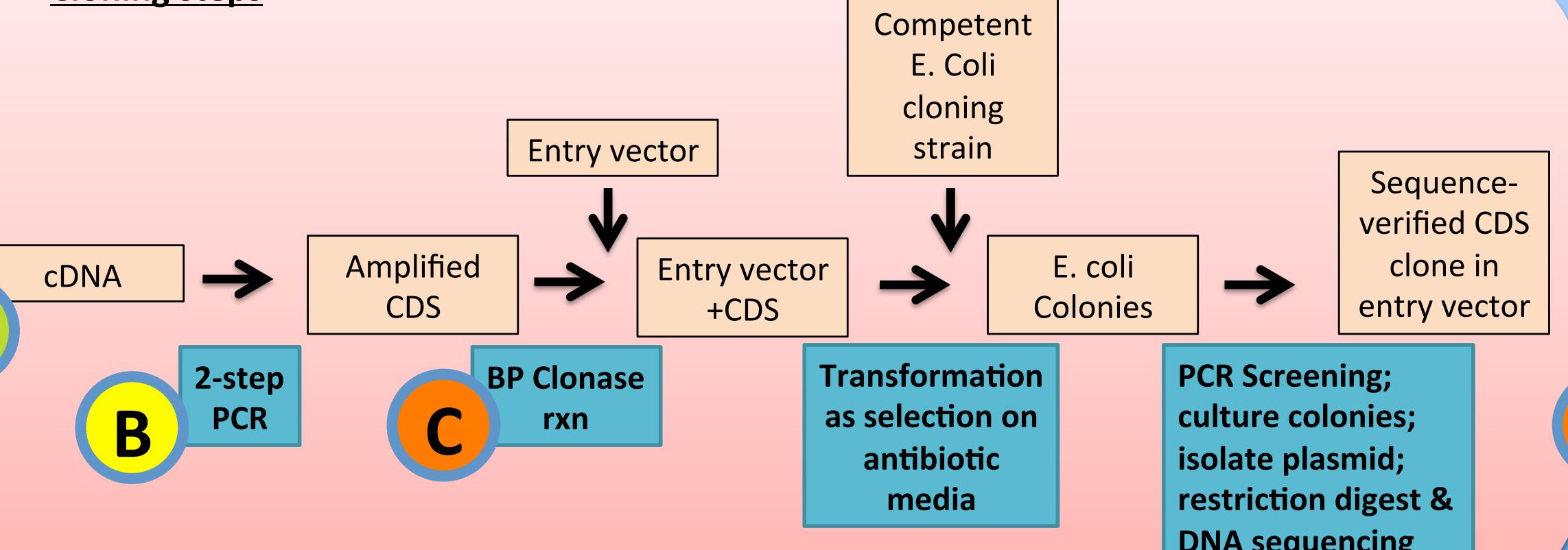
## Expression vector



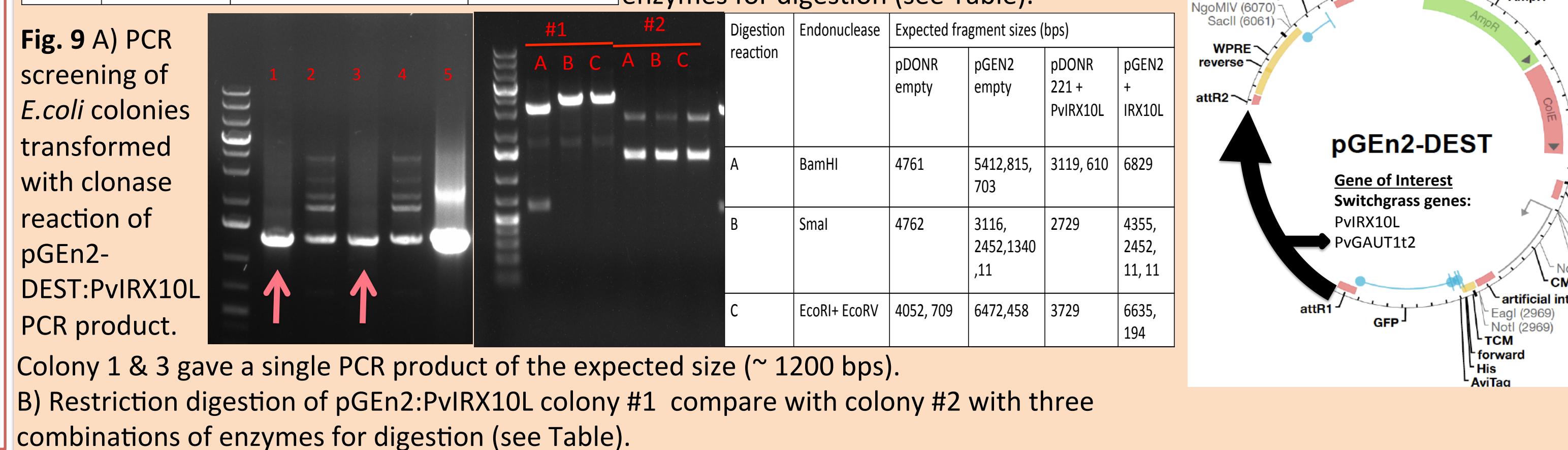
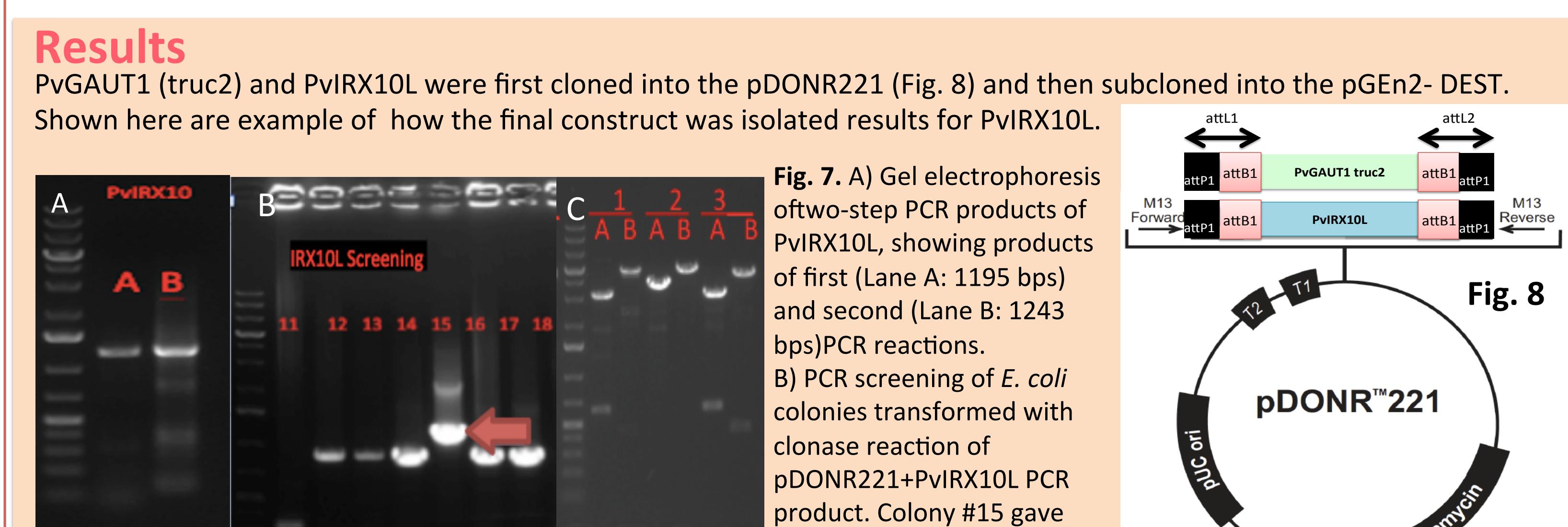
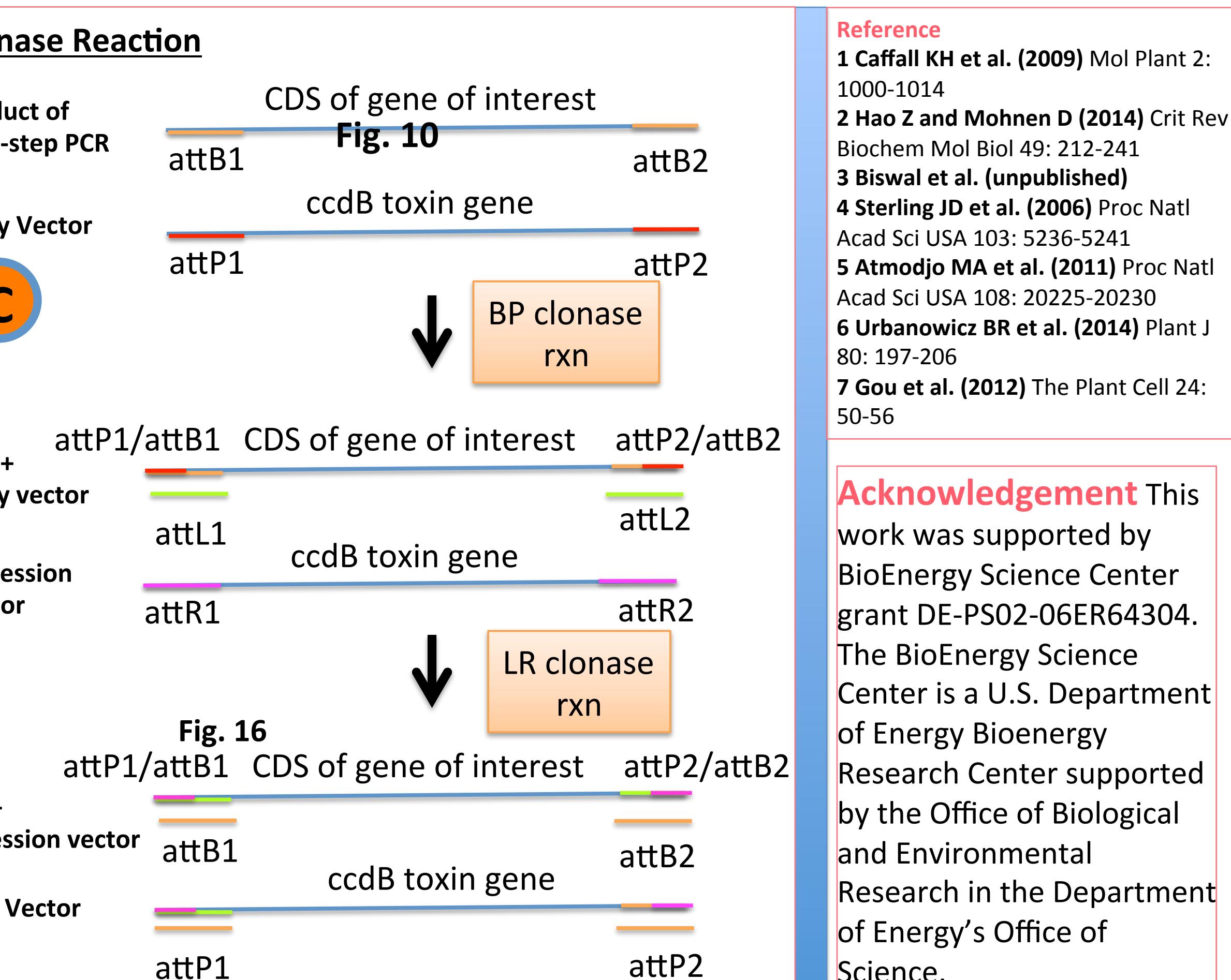
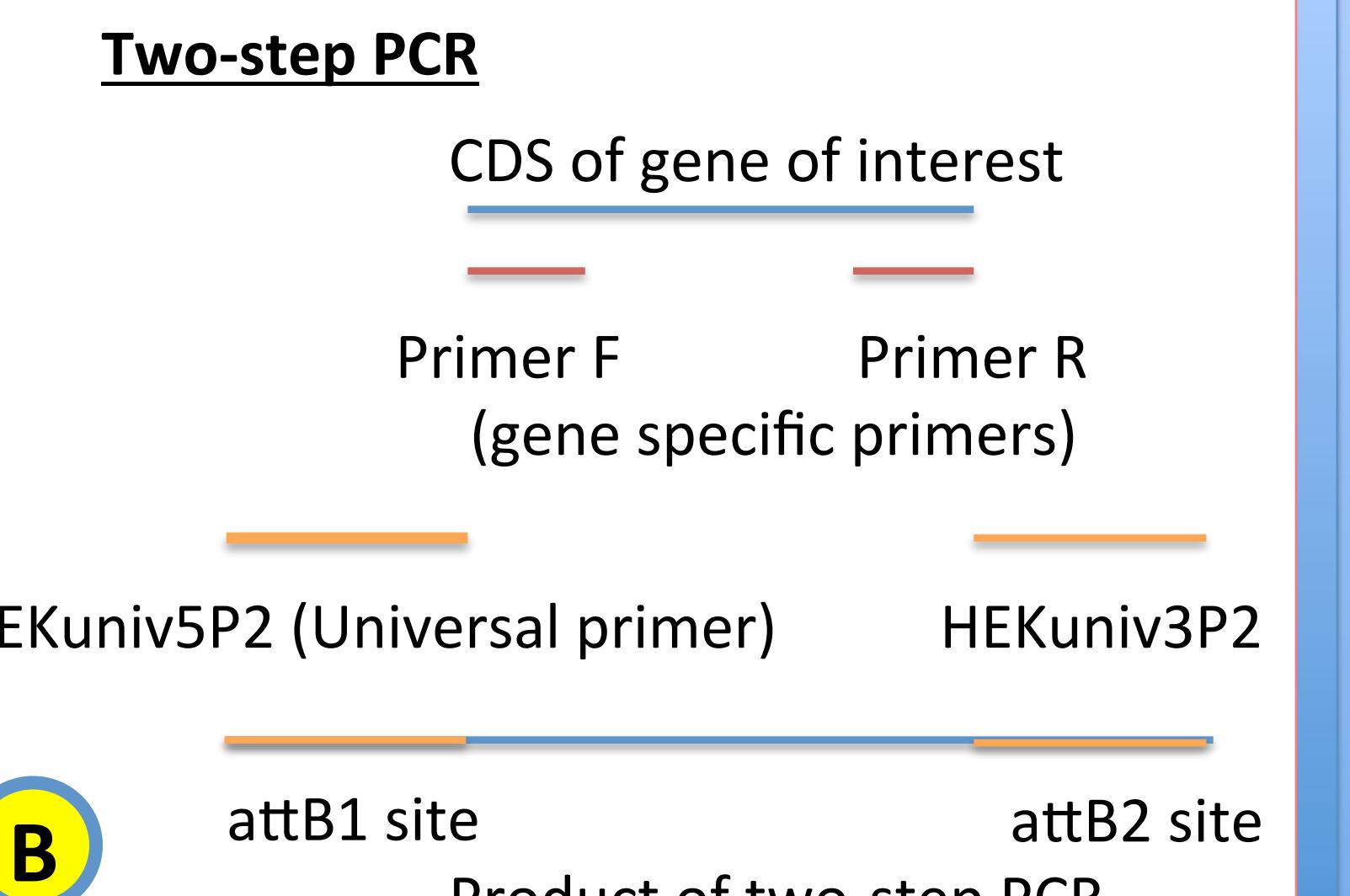
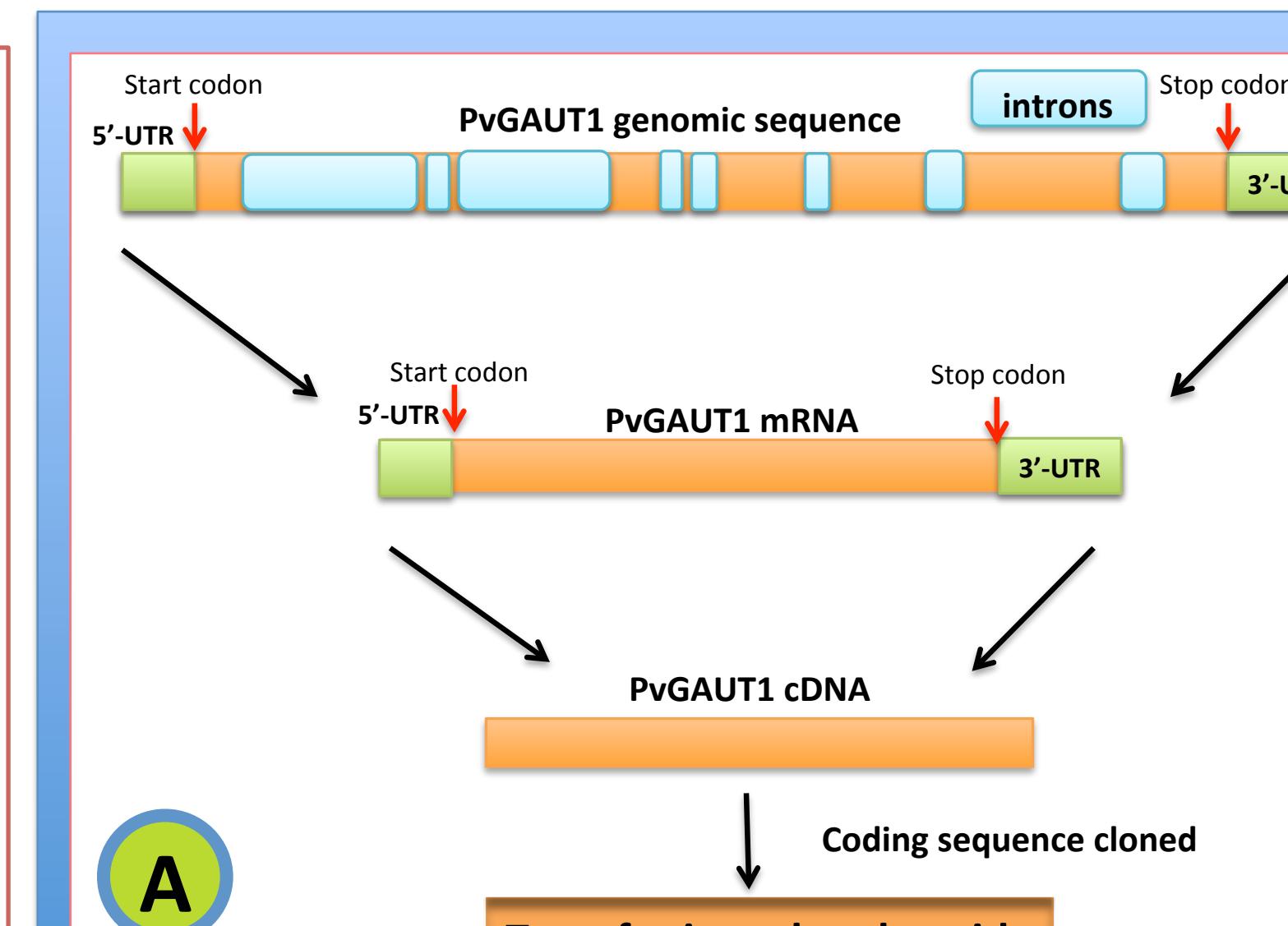
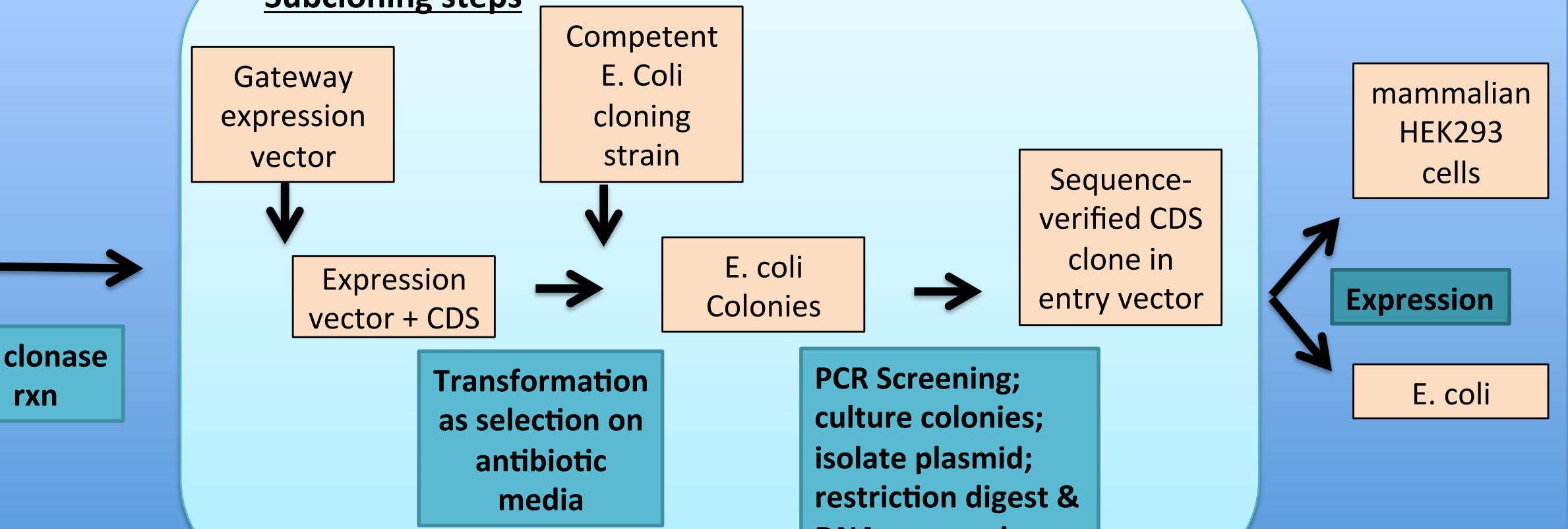
## Methods

cDNA was used as the starting material to amplify the coding sequence of the targeted proteins. (Fig. 5) Gene specific primers were designed to include adaptors for Gateway cloning. PCR products were first transferred into entry vectors and then later subcloned into expression vectors (Fig. 6). The desire construct were selected by transforming into *E. coli* and screen the resulting colonies by using media with antibiotics and PCR. The plasmids were then verified by restriction digest and sequencing.

## Cloning steps



## Subcloning steps



Reference
1 Caffall KH et al. (2009) Mol Plant 2: 1000-1014
2 Hao Z and Mohnen D (2014) Crit Rev Biochem Mol Biol 49: 212-241
3 Biswal et al. (unpublished)
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5 Atmodjo MA et al. (2011) Proc Natl Acad Sci USA 108: 20225-20230
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## Summary & Future works

- Switchgrass PvGAUT1 truc2 and PvIRX10L have been successfully cloned into pDONR 221 and were sequence verified.
- Switchgrass PvGAUT1 and PvIRX10L were subcloned into pGEN2-DEST and sequence verified. The construct will be expressed in the mammalian HEK 293 cells.
- Foxtail millet SiPAE2 was cloned into pDONR 207 and sequence verified. It was also subcloned into pHIS9 and will be sequenced. The construct will be expressed in *E. coli*.
- PvGAUT1 truc1 was cloned into pDONR 221 and will be sequenced. Then the construct will be subcloned into pGen2-DEST.
- We will keep working on the cloning of Foxtail millet SiPAE1.