

Table S6. Construction of plant expression vector and specific nucleotide sequences used.

1. Construction of plant expression vector

(1) The pUC57 plasmid was chosen as intermediate clone vector, and was digested with XmaI to produce linear vector. *CAMV35S* promoter, *Ubi1* promoter and *NOS* terminator were amplified by PCR using *pCAMBIA* plasmid and *pBI121* plasmid, respectively. Adjacent fragments were added 15bp overlapping ends by PCR amplification. Gibson Assembly® Master Mix – Assembly (E2611) (NEW ENGLAND Biolabs, Beijing) was used to assemble the targeted nucleotides to produce *pUC57-Pub1-Tnos* and *pUC57-Pcamv35s-Tnos*.

(2) Sequence of selected genes (*DzCYP97B1*, *DzCYP90G6*, *DzCYP94N8*, *DzCYP72A12-4*) were amplified by PCR and 15 bp overlapping ends were added to both ends of these genes, respectively. Linear vectors of *pUC57-Pub1-Tnos* or *pUC57-Pcamv35s-Tnos* were prepared by PCR amplification method too. The same strategy as above was used to produce *pUC57-Pub1-DzCYP90B71-Tnos* plasmid, *pUC57-Pcamv35s-DzCYP90G6-Tnos* plasmid, *pUC57-Pcamv35s-DzCYP90N8-Tnos* plasmid and *pUC57-Pcamv35s-DzCYP72A12-4-Tnos* plasmid.

(3) Linear pCAMBIA plasmid was produced by SbfI digestion and nucleotide purification. *Pub1-DzCYP90B71-Tnos* fragment was amplified with 15 bp overlapping ends added. Gibson assembly was used to produce *pCAMBIA-Pub1-DzCYP90B71-Tnos*, named *pCAMBIA-DzCYP90B71*. In the meantime, two restriction endonuclease recognition sites (SbfI & NruI) were added flanking the cassette facilitating multi-cassettes assembly. The *pCAMBIA-DzCYP72A12-4* construct was created in the same way.

(4) Linear *pCAMBIA-DzCYP90B71* was produced by digestion of NruI, and assembled with *Pcamv35s-DzCYP90G6-Tnos* cassette using Gibson Assembly method the same as above to produce *pCAMBIA-Pub1-DzCYP90B71-Tnos-Pcamv35s-DzCYP90G6-Tnos*, named *pCAMBIA -DzCYP90B71/DzCYP90G6*.

(5) Linear *pCAMBIA -DzCYP90B71/DzCYP90G6* was created by digestion of PmeI, and assembled respectively with *Pcamv35s-DzCYP90N8-Tnos* cassette and *Pcamv35s-DzCYP72A12-4-Tnos* cassette to produce *pCAMBIA-DzCYP90B71/DzCYP90G6/DzCYP90N8* and *pCAMBIA-DzCYP90B71/DzCYP90G6/DzCYP72A12-4*.

2. Sequences used in expression vector construction.

>CAMV 35S promoter

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>Ubi1 promoter (*Zea Mays*)

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>DzCYP94N8

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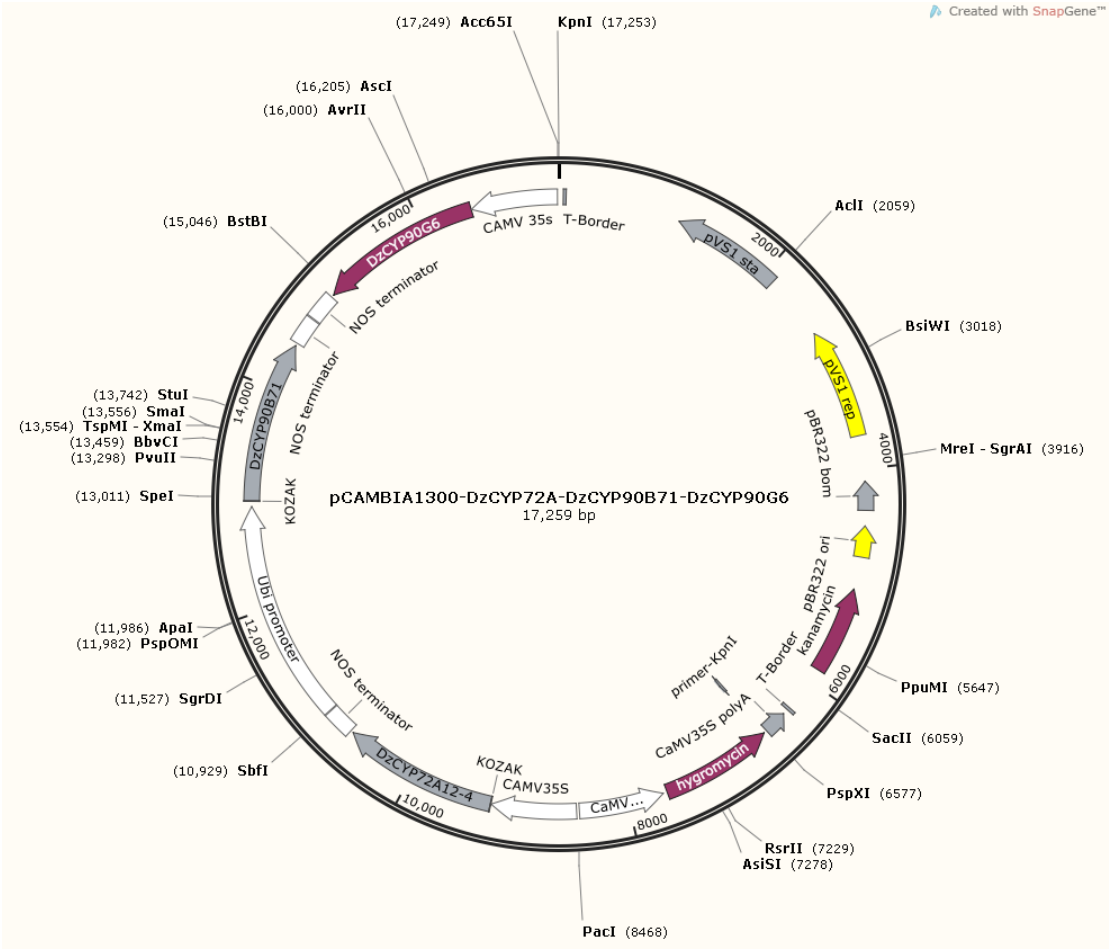


Diagram of pCambia-DzCYP72A12-4/DzCYP90B71/90G6

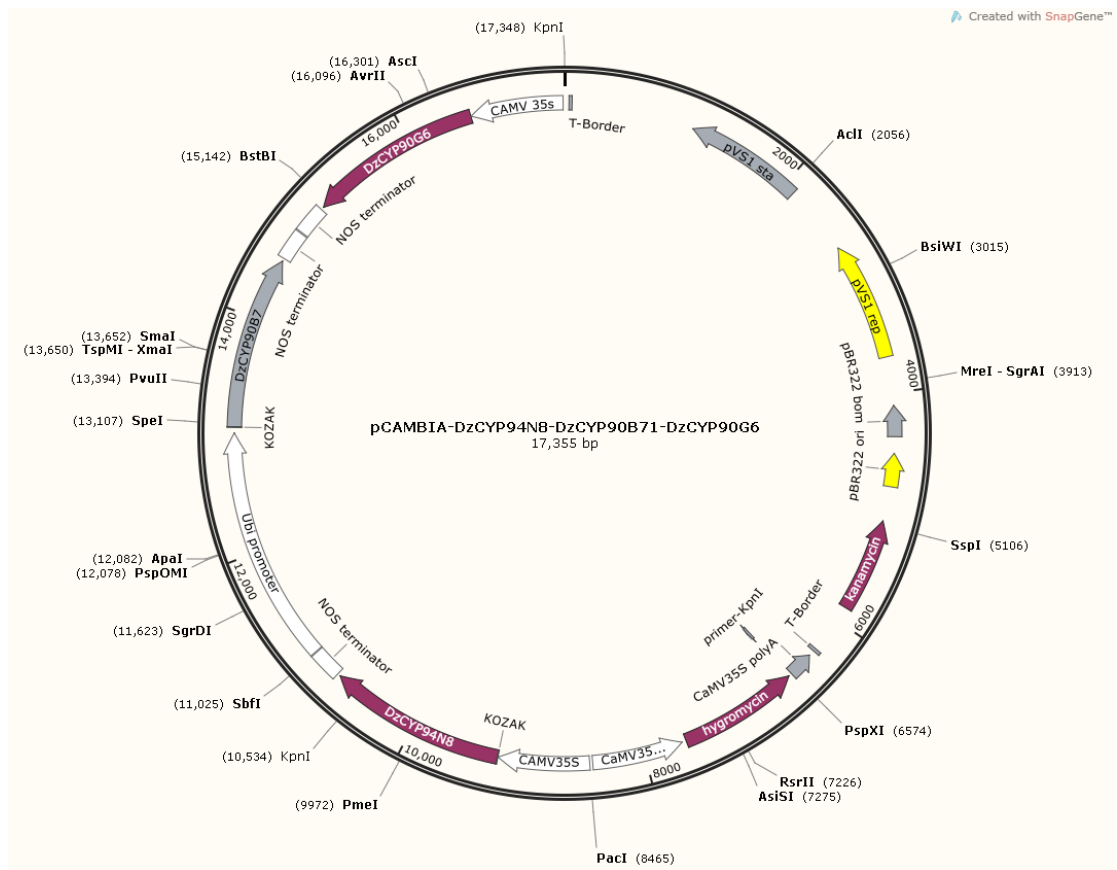


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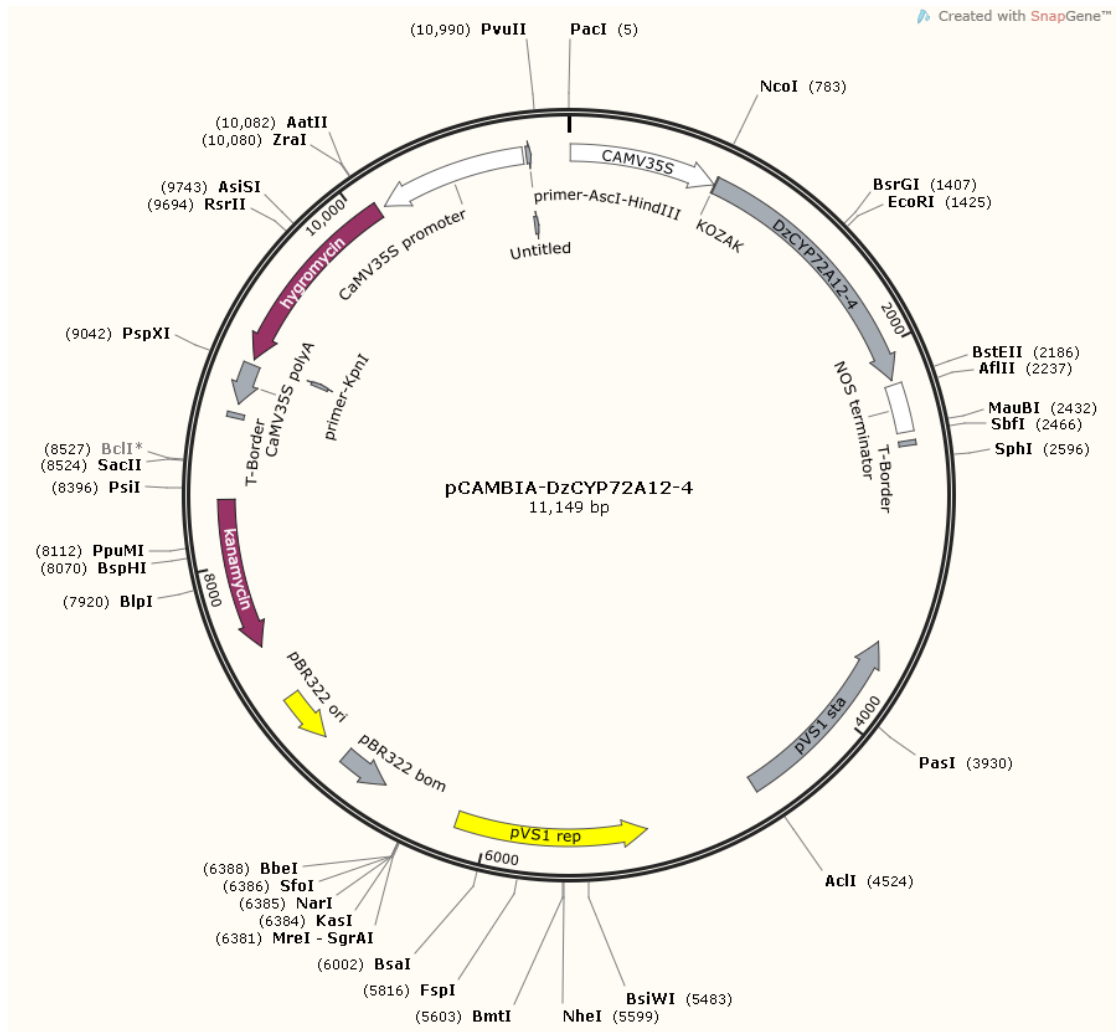


Diagram of pCAMBIA-DzCYP72A12-4

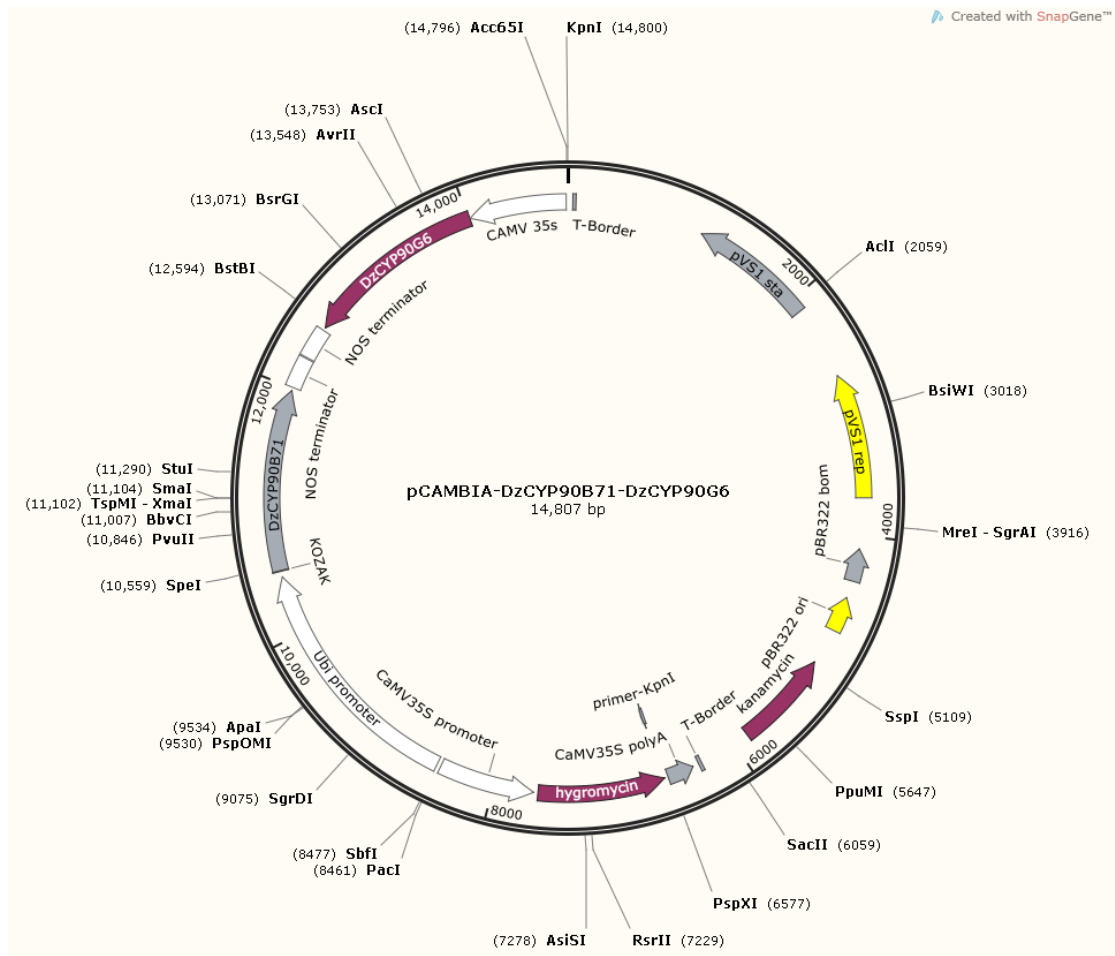


Diagram of pCambia- DzCYP90B71/90G6