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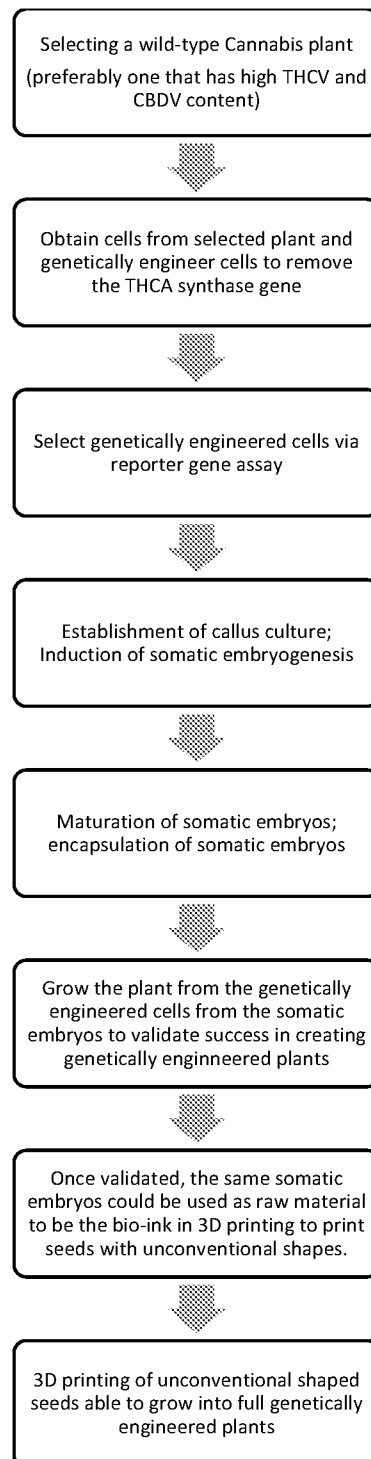
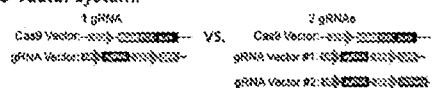


Figure 1

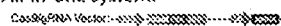
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1) Choose a knockout strategy

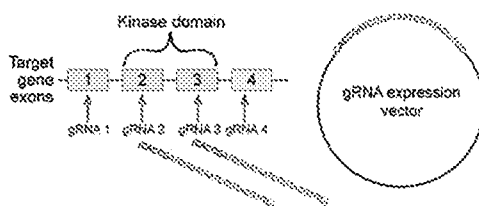
Two-vector system:



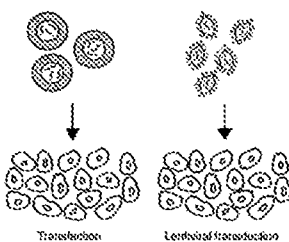
All-in-one system:



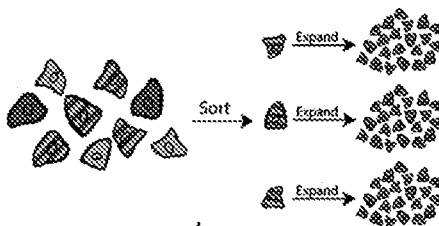
2) Select gRNAs and generate vectors



3) Introduce CRISPR plasmids



4) Isolate and expand single-cell clones



5) Verify clonal knockouts

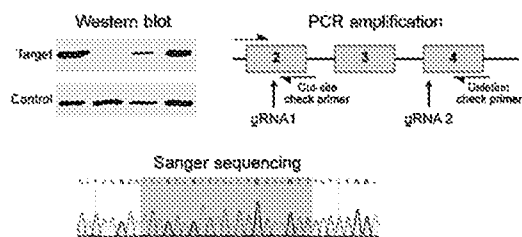


Figure 2

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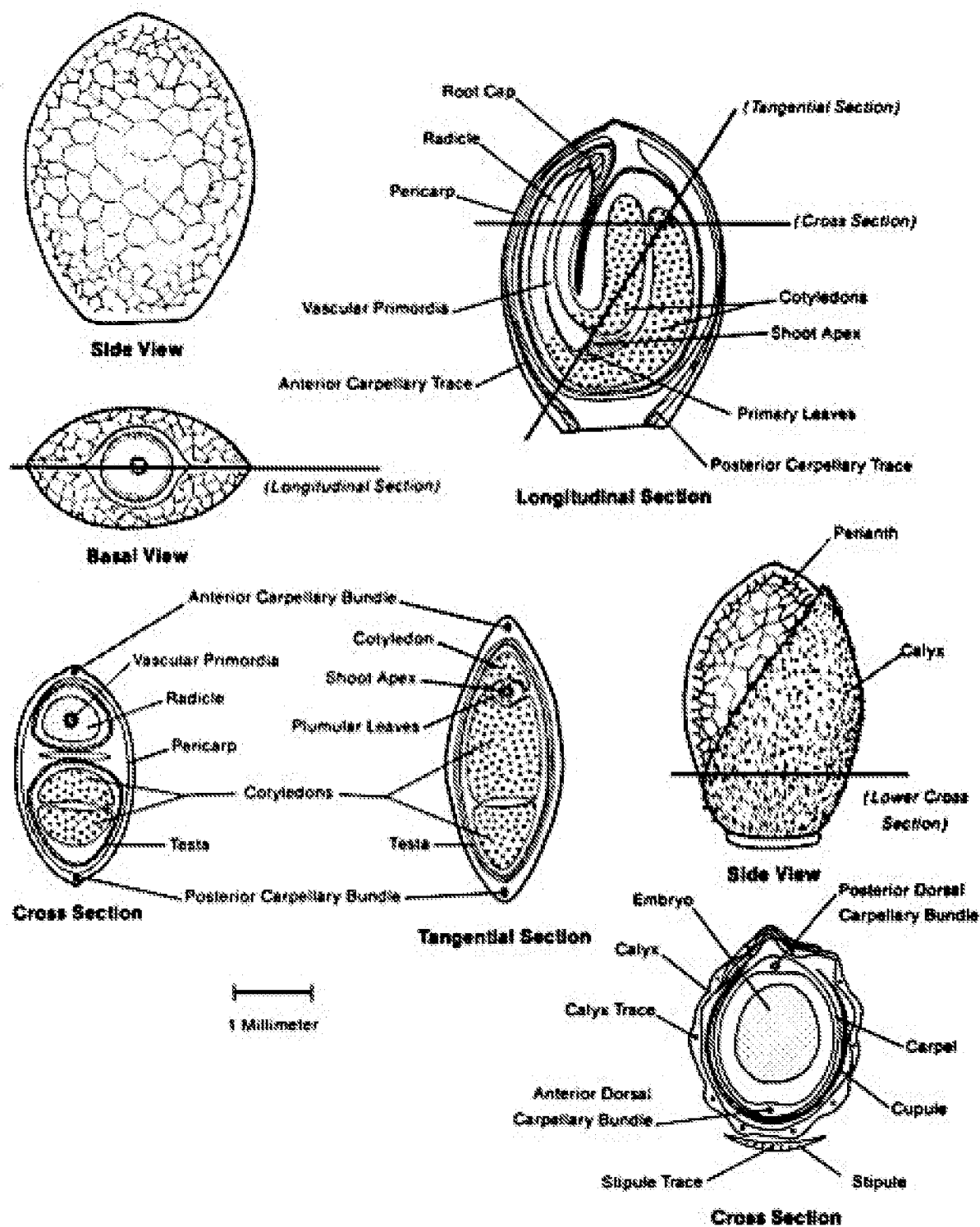


Figure 3

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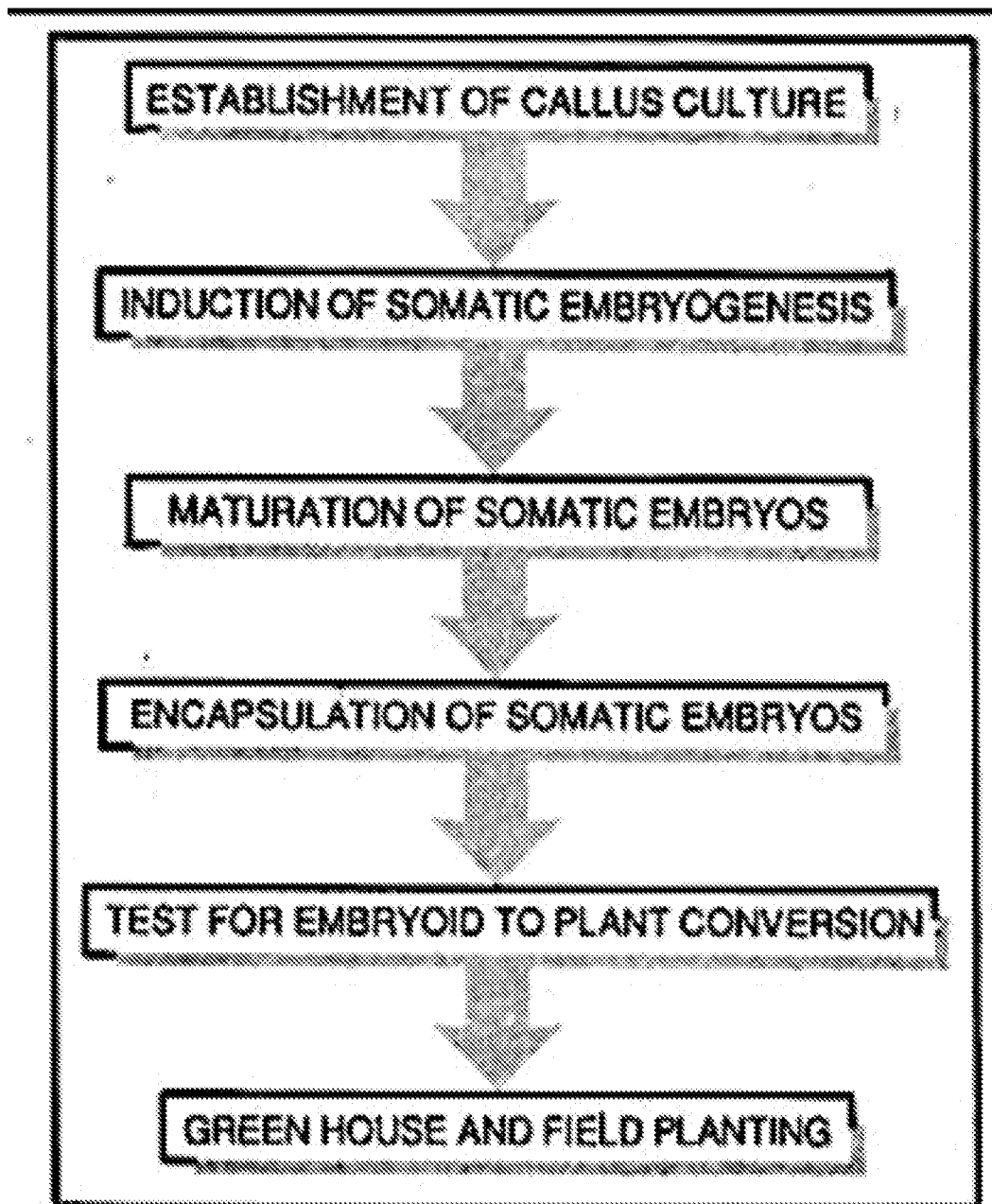


Figure 4

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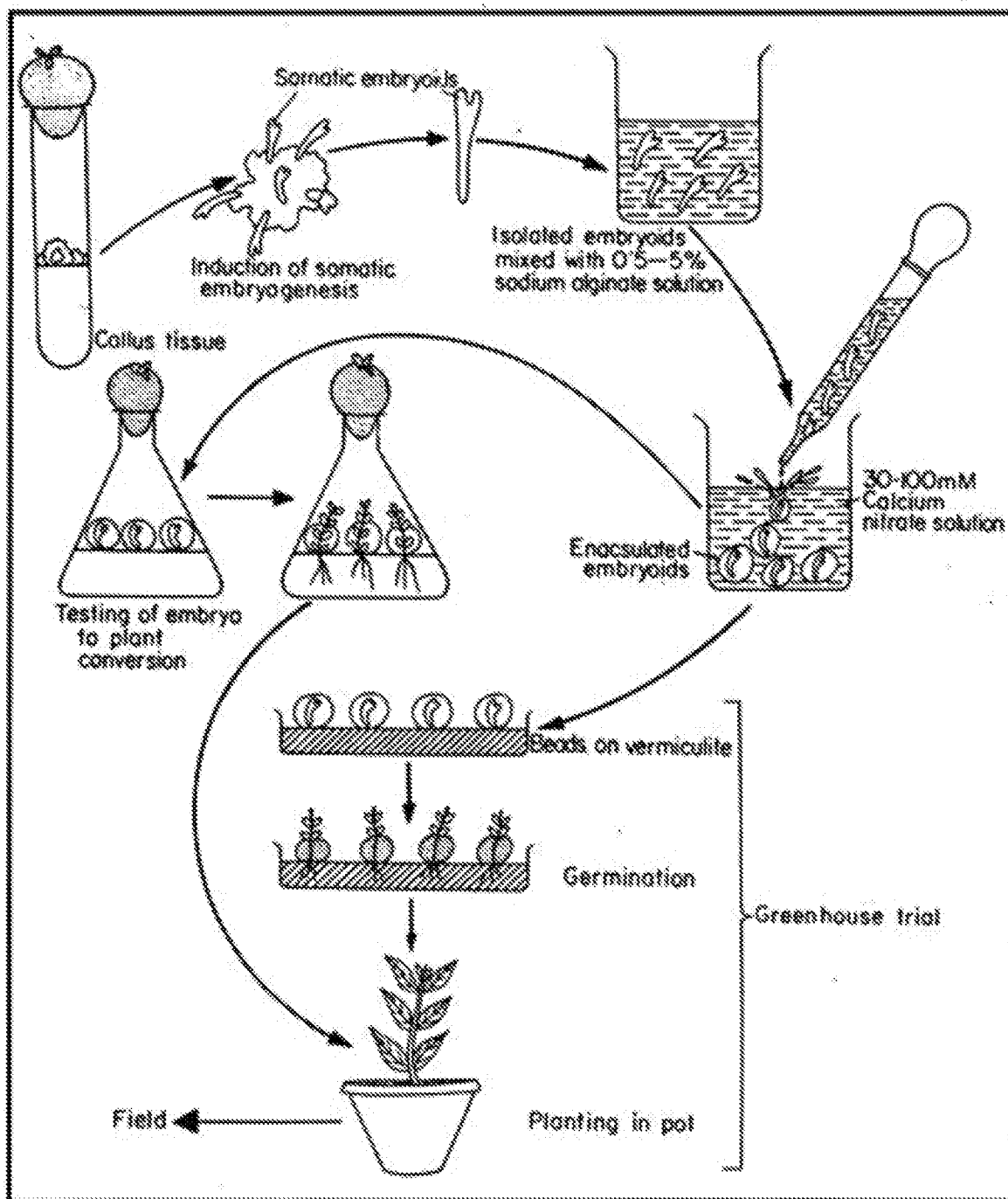


Figure 5

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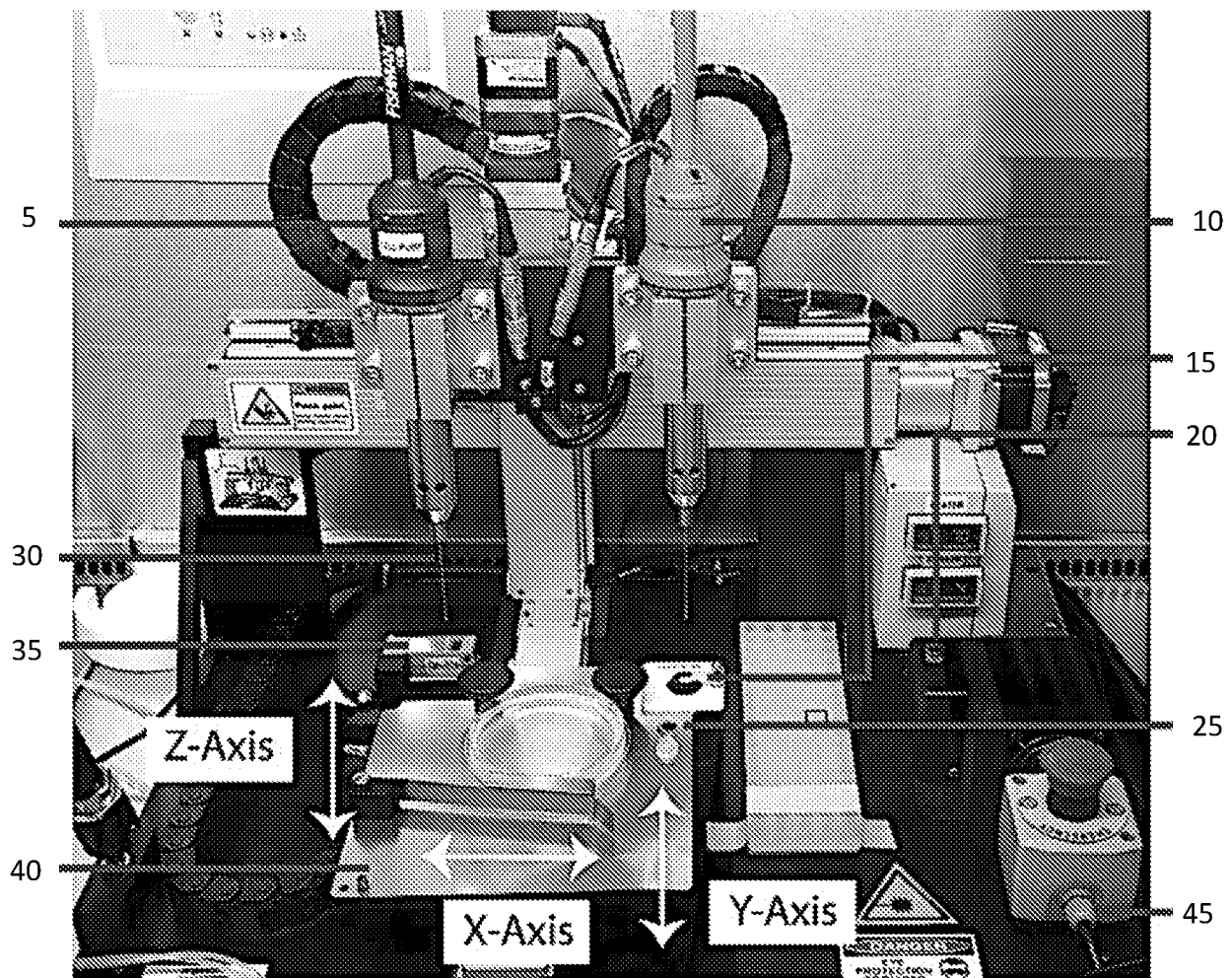


Figure 6

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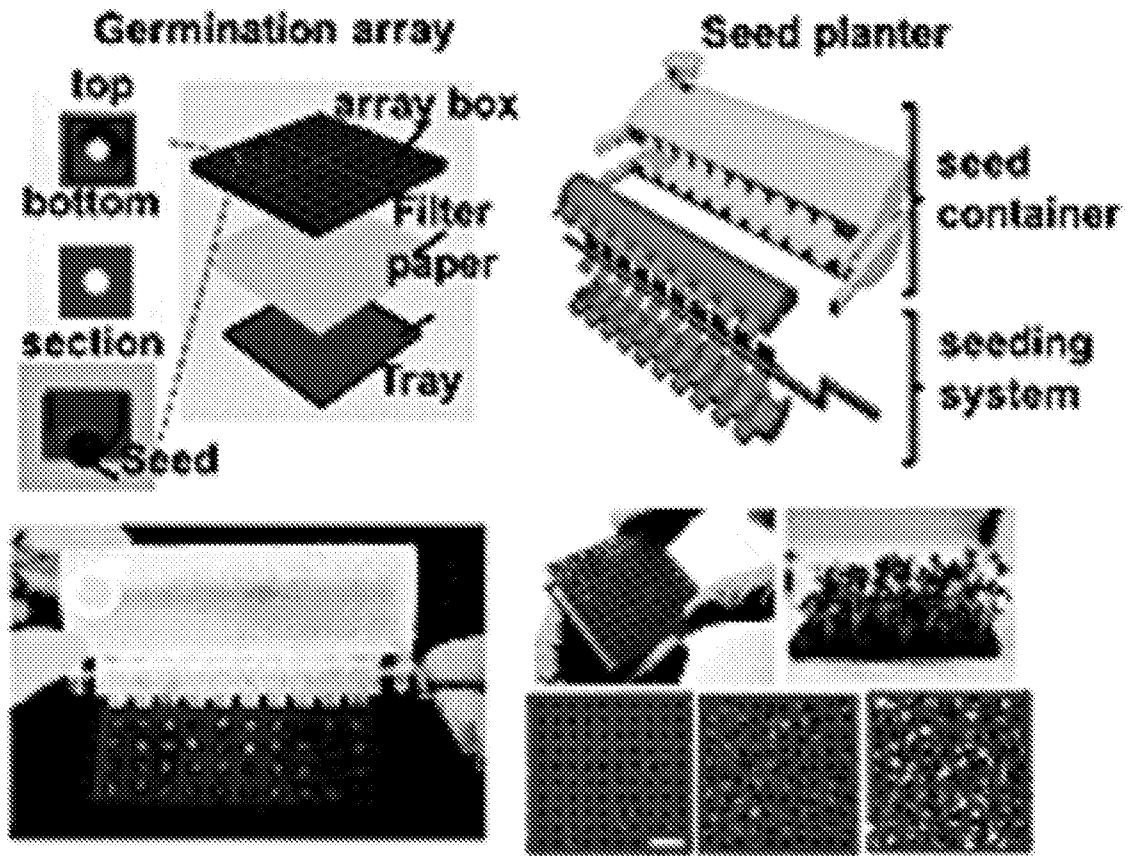


Figure 7

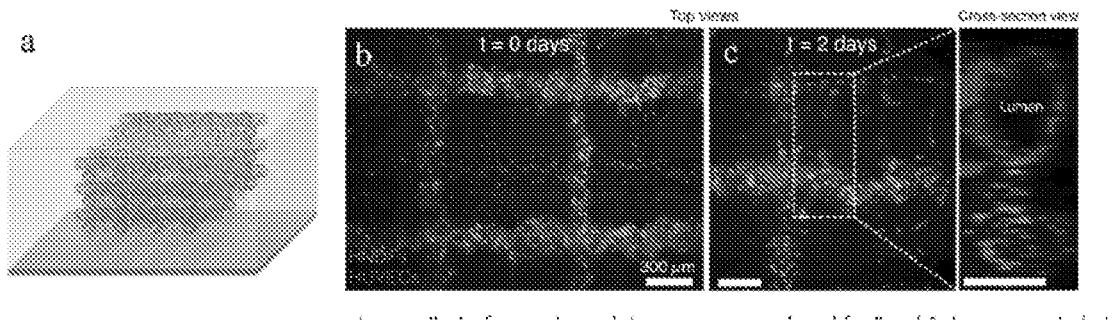


Figure 8

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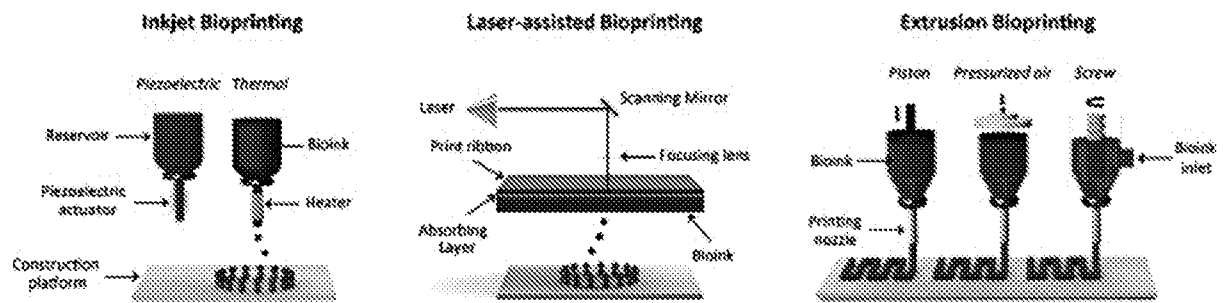


Figure 9

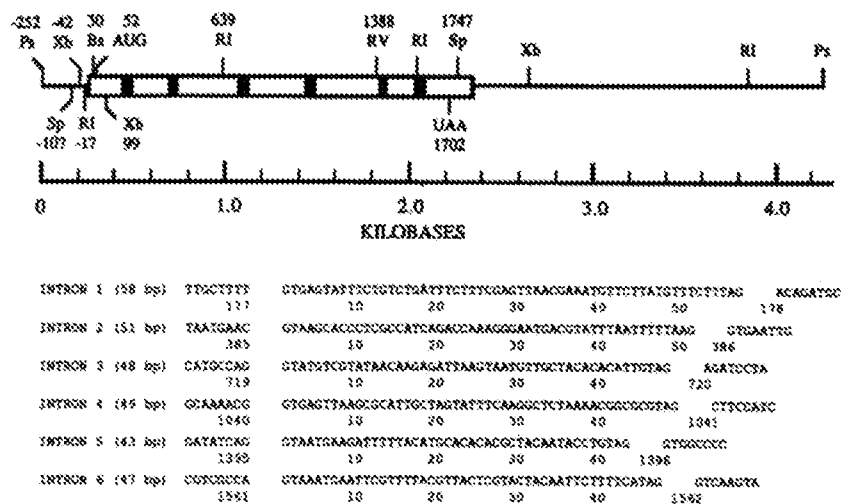
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 -184 -154 -130 -90
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 -64 -34 1 30
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 lys arg tyr ala leu val pro gly thr ile ala phe thr asp ala his ile glu val asn ile thr tyr ala glu tyr phe glu met ser
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 GTT CGG TTG GCA GAA GCT ATG AAA CGA TAT GGG CTG AAT ACA AAT CAC AGA ATC GTC GTA TCC AGT GAA AAC TCT CTT CAA TTC TTT ATG
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 pro val leu gly ala leu phe ile gly val ala val ala pro ala asn asp ile tyr asn glu arg glu leu leu asn ser met asn ile
 CCG GCG TTG GCG GCG TTA TTT ATC GGA GTT GCA GTT GCG CTC GCG AAC GAC ATT TAT AAT GAA CGT GAA TGG CTC AAC AGT ATG AAC ATT
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 ser glu pro thr val val phe val ser lys lys gly leu glu lys ile leu asn val glu lys lys leu pro ile ile glu lys ala ile
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 960 990 1020
 phe his leu pro gly ile arg glu gly tyr leu thr thr ser ala ile leu ile thr pro glu gly asp asp lys pro gly
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 ala val gly lys val val pro phe phe glu ala lys val asp leu asp thr gly lys thr leu gly val asn glu arg gly glu leu
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 1230 1260 1290
 gly asp ile ala tyr trp asp glu asp glu his phe phe ile val asp arg leu lys ser leu ile lys tyr lys gly tyr glu val ala
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 1320 1350 1380
 pro ala glu leu glu ser ile leu leu glu his pro asn ile phe asp ala gly val ala gly leu pro asp asp asp ala gly glu leu
 CCG GCT GAA TTG GAA TCG ATA TTT TTA CAA CAC CCG AAC ATC TTC GAC GCG GCG GTG GCA GGT CTT CCG GAT GAC GCG GGT GAA CTT
 1410 1440 1470
 pro ala ala val val val leu glu his gly lys thr met thr glu lys glu ile val asp tyr val ala ser glu val thr thr ala lys
 CCG GCG GCG GTT GTT GTT TTT GAG CAC GAA AAG ACG ATG ACG GAA AAA GAG ATC GCG GAT TAC GTC GCG AGT CAA GTA ACA ACC GCG AAA
 1500 1530 1560
 lys leu arg gly gly val val phe val asp glu val pro lys gly leu thr gly lys leu asp ala arg lys ile arg glu ile leu ile
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 1590 1620 1650
 lys ala lys lys gly gly lys ser lys leu ***
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 1680 1710 1740 1770
 TAATTGTGGTCACTGTACTATTTTACGATATTAATTAATTAAGTATAGGTAACTAAATA
 1800 1830

Nucleotide sequence of the *P. pyralis* luciferase cDNA and 5'-flanking sequence. The DNA sequence is listed 5' to 3', left to right, and is in the same sense as the luciferase mRNA sequence. Sequence from the *Pst*I site at -252 through residue +68 was determined solely from the analysis of genomic luciferase DNA; the remainder of the sequence was determined from the analysis of both luciferase cDNA and genomic DNA. The firefly luciferase cDNA, Luc23, extended from +69 through +1840 and was bounded at both termini by *Eco*RI linkers (5'-GGAATTCC-3', not shown). The complete sequence of both strands of the cDNA was determined. The transcriptional start site of the major luciferase mRNA is defined as residue +1 (see Fig. 4). The predicted amino acid sequence is shown directly above the only long open reading frame found within the nucleotide sequence. TATA boxes and the TATA-like sequence TATTTAA are underlined in the 5'-flanking region. The presumed polyadenylation signal, AATAAA, is underlined beginning at base 1813. The sequences and locations of the six luciferase gene introns are presented in Fig. 3.

Figure 10

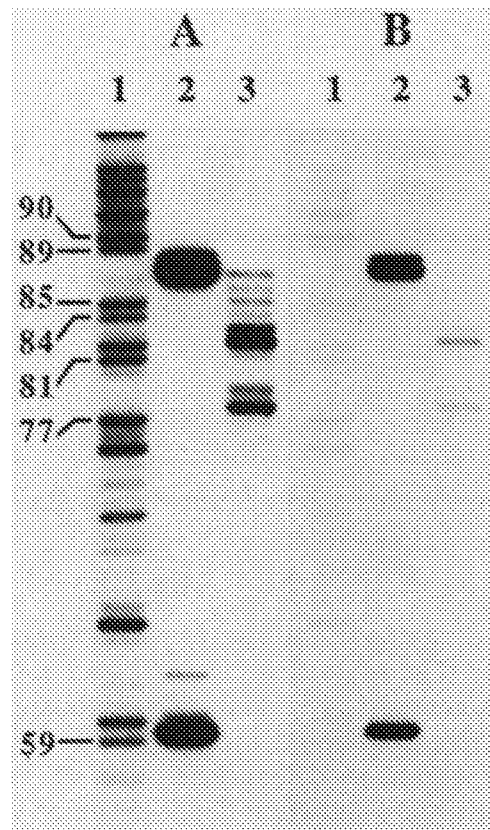
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Structure of *P. pyralis* luciferase gene. The map of the genomic *PstI* restriction fragment shows the luciferase gene exons as open boxes and introns as shaded boxes. Restriction sites are indicated with the following abbreviations: Ps, *PstI*; Sp, *SspI*; Xb, *XbaI*; RI, *EcoRI*; Bx, *BsmI*; RV, *EcoRV*. The coordinates of the sites correspond to their locations within the sequence shown in Fig. 1. This numbering does not include the introns. The nucleotide sequences of the six luciferase gene introns plus eight residues of each flanking exon are shown directly below the map of the luciferase gene. The coordinates of the terminal bases of the exons are indicated and correspond to their locations in the DNA sequence shown in Fig. 1. The sequences of introns 2, 3, 4, and 5 were determined by sequencing both strands of the DNA. Only one strand of the DNA was sequenced for introns 1 and 6.

Figure 11

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Mapping the 5' end of luciferase mRNA. The 5' end of the *P. pyralis* luciferase mRNA was located by primer extension and S1 nuclease protection as described in the text. Restriction fragments used in the mapping experiments were obtained from the genomic *Pst*I clone (pJD183) and were 5' end labeled with ^{32}P at base +87 after cleavage with *Nar*I. Lane 1, Marker fragments produced by cleavage of the 5' *Nar*I-to-*Pst*I fragment at A residues (34) (numbers on left are in bases). Lane 2, A 59-base primer extending from the *Nar*I site to the *Bsm*I site located at +30 was hybridized to *P. pyralis* lantern poly(A)⁺ RNA and extended with avian myeloblastosis virus reverse transcriptase. Lane 3, The 331-base *Nar*I-to-*Pst*I fragment was hybridized to total lantern RNA and digested with S1 nuclease. The samples were analyzed by electrophoresis in a 7 M urea-8% polyacrylamide sequencing gel followed by autoradiography. Panel B is a shorter exposure of the same gel as in panel A.

Figure 12

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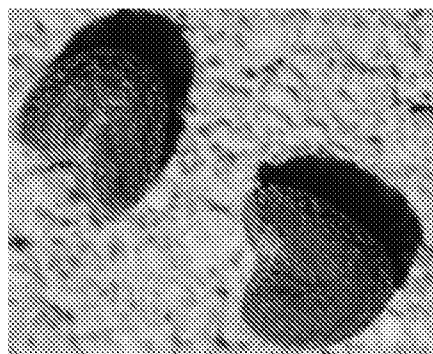


Figure 14(a)



Figure 14(b)

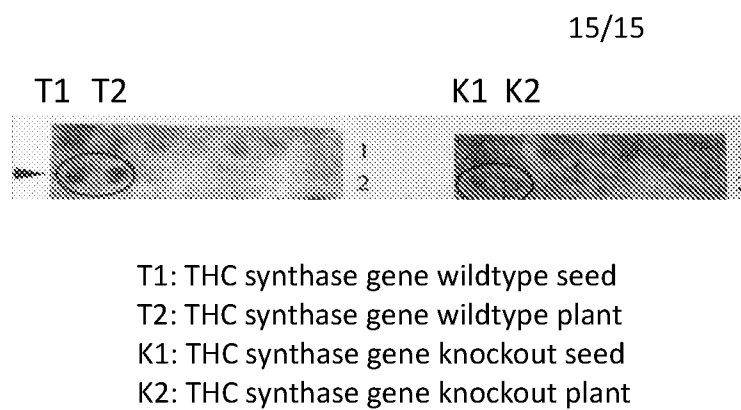


Figure 15(a)

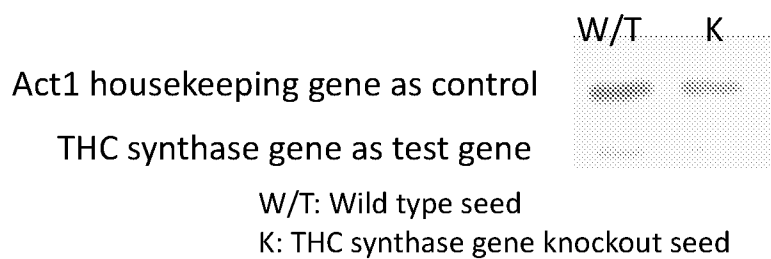


Figure 15(b)

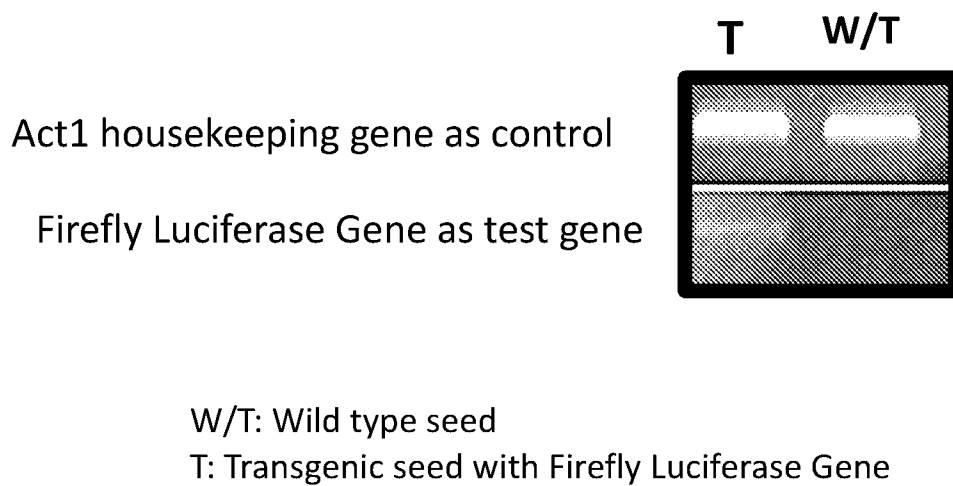


Figure 15(c)