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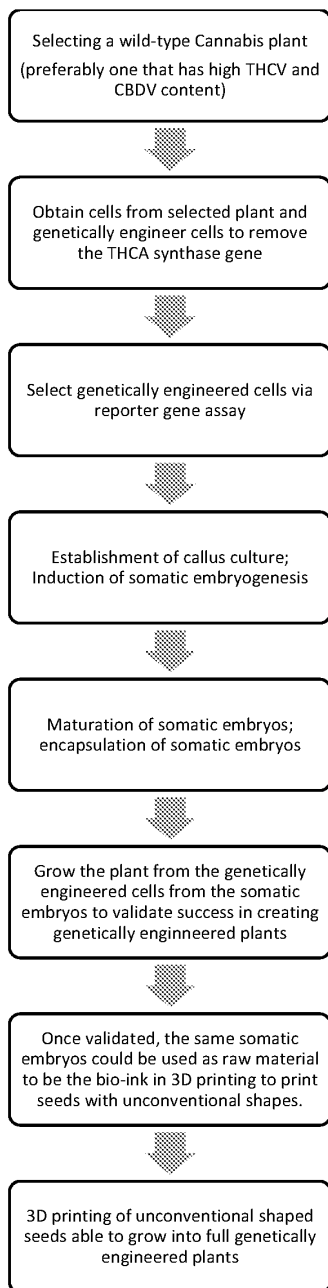
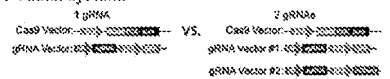


Figure 1

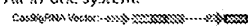
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1) Choose a knockout strategy

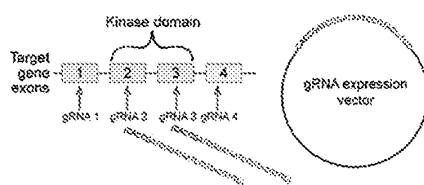
Two-vector system:



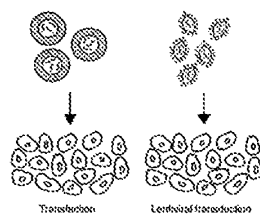
All-in-one system:



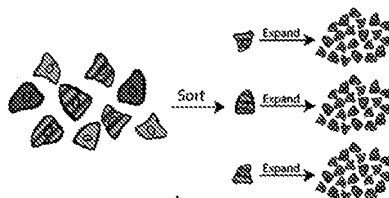
2) Select gRNAs and generate vectors



3) Introduce CRISPR plasmids



4) Isolate and expand single-cell clones



5) Verify clonal knockouts

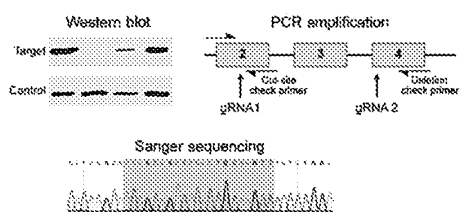


Figure 2

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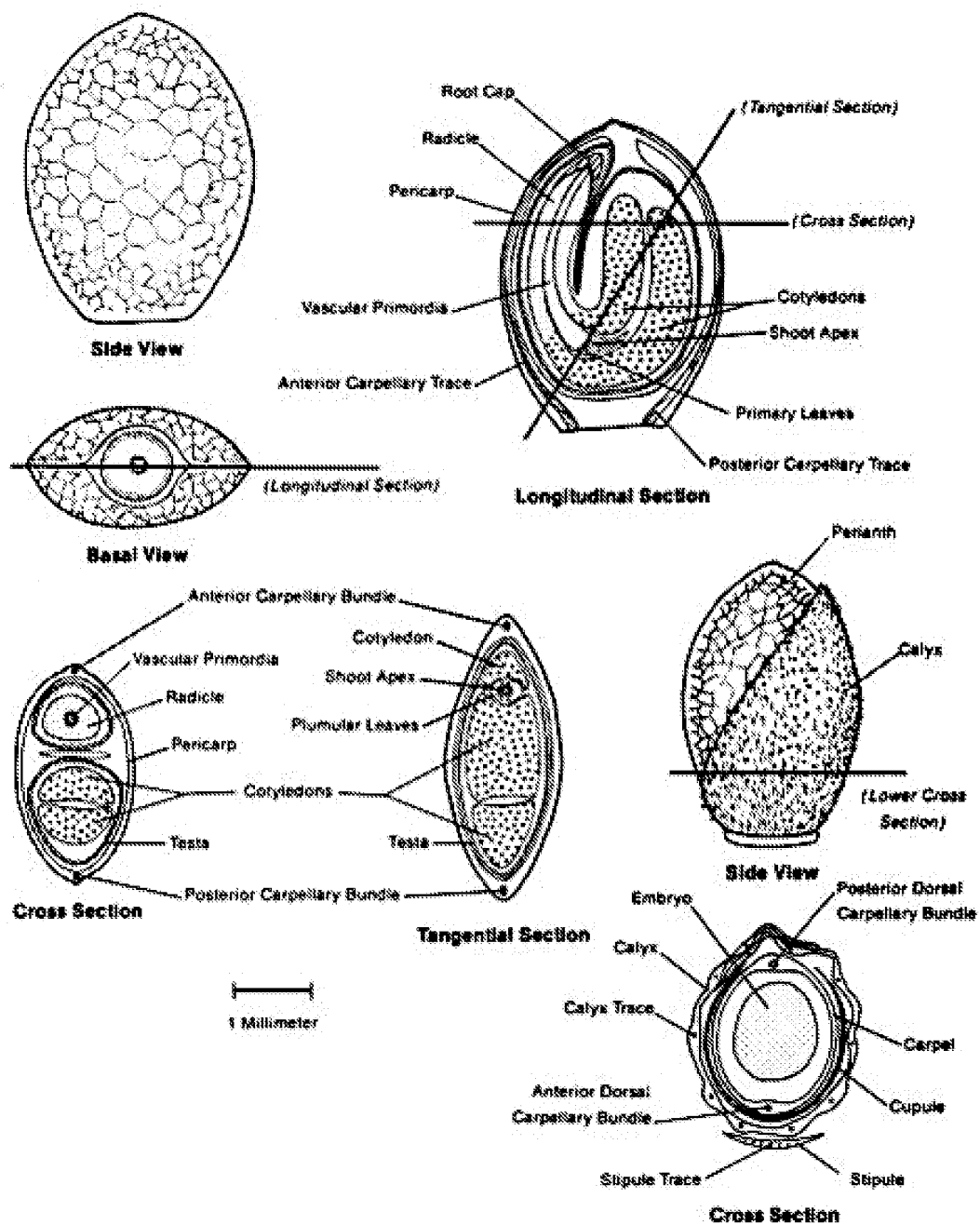


Figure 3

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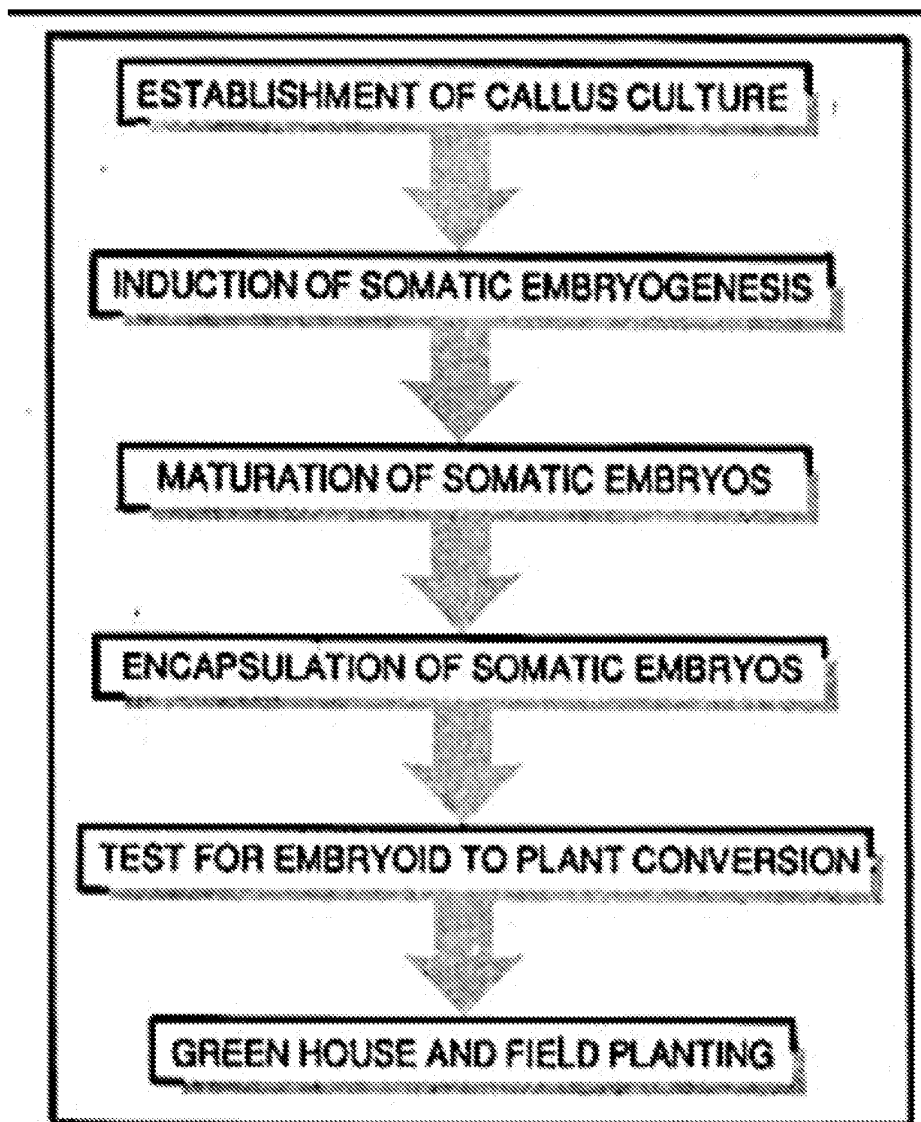


Figure 4

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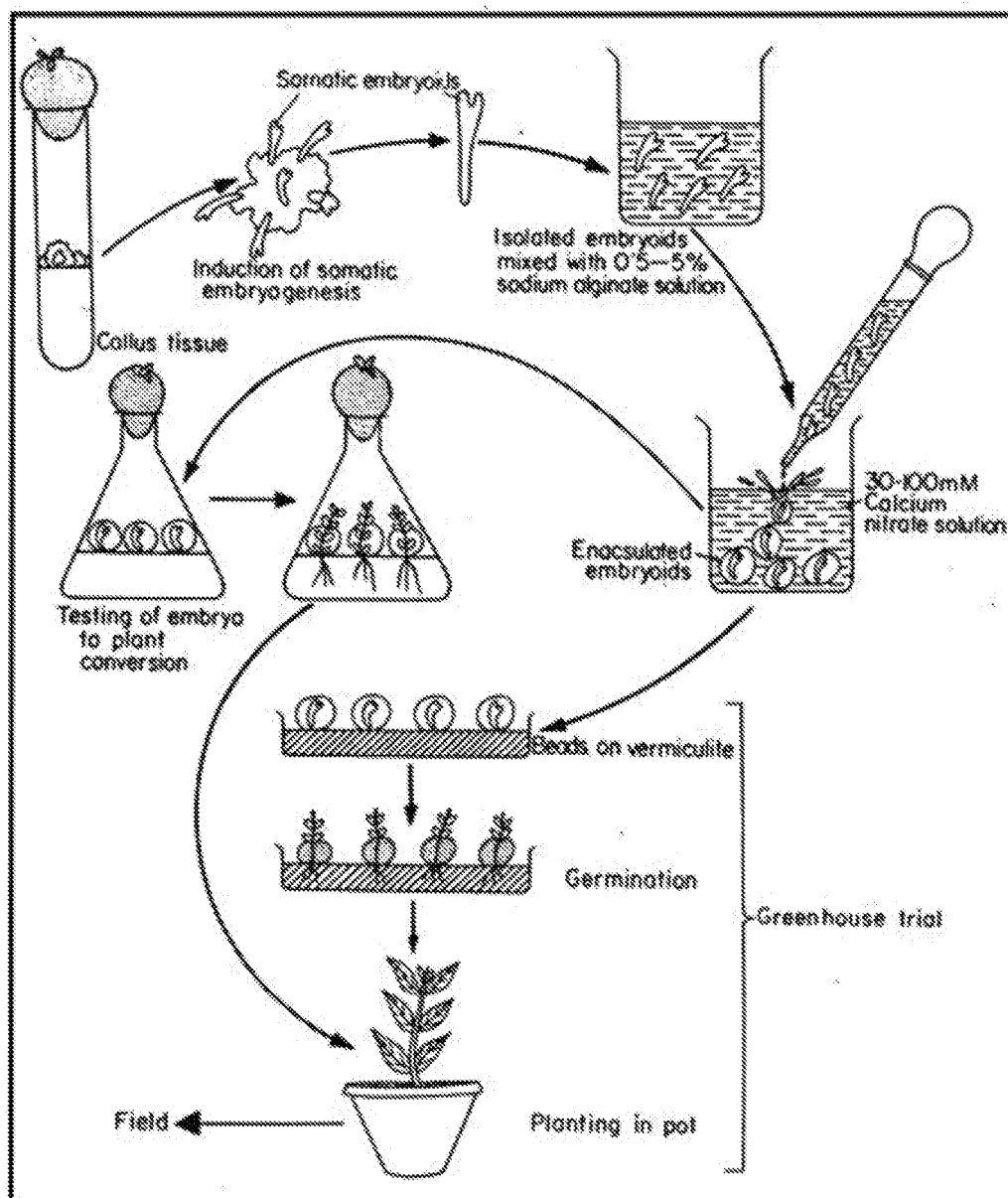


Figure 5

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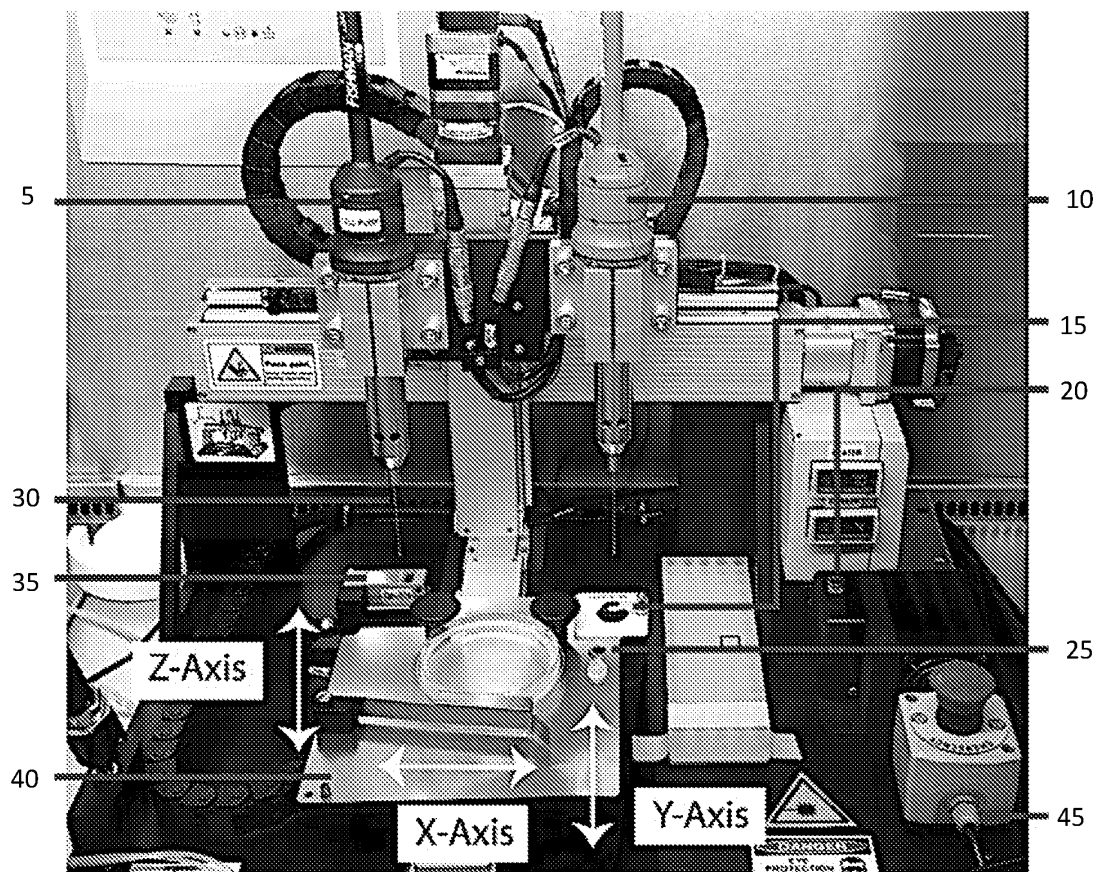


Figure 6

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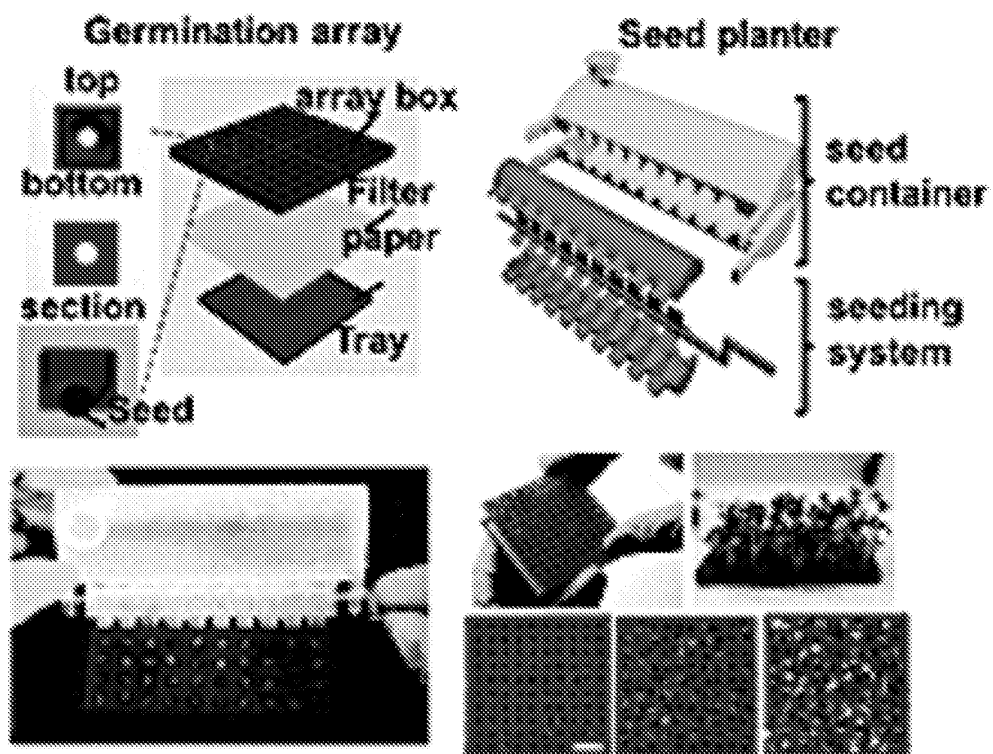


Figure 7

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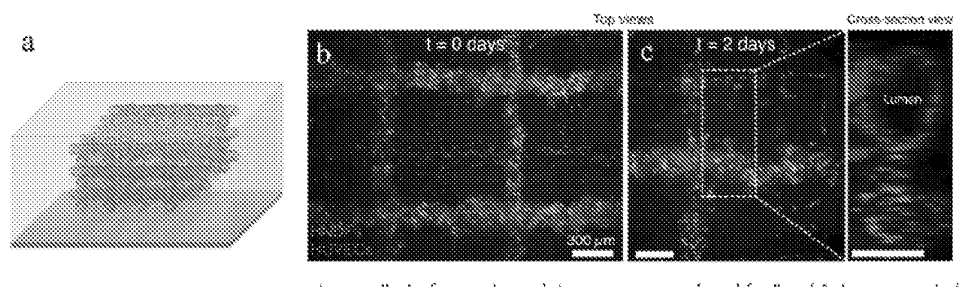


Figure 8

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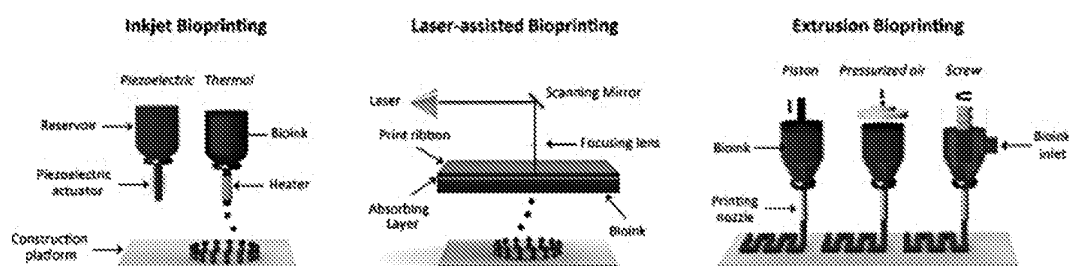


Figure 9

[illegible]

Figure 10

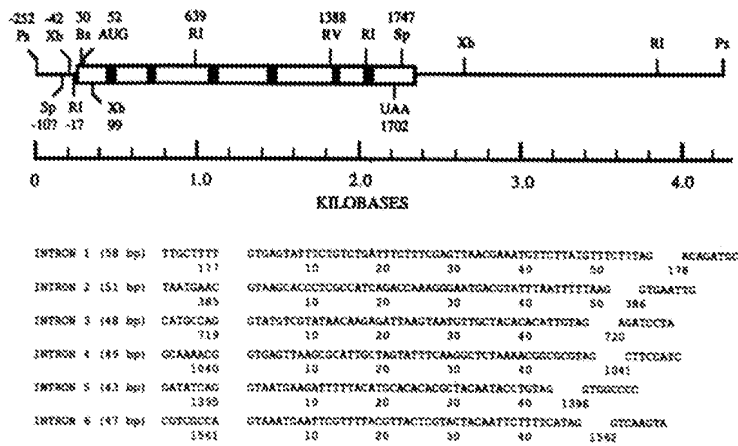
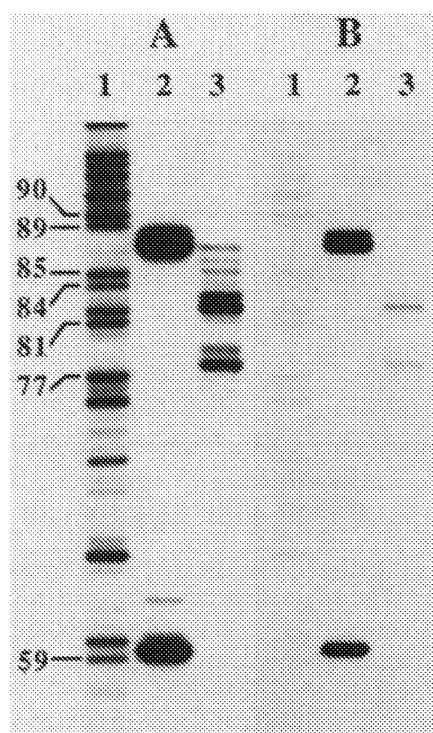


Figure 11

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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

A

(4361/1782)
Eco RI
amp^r
pBR
pSV0
Pvu II (2065)
Hind III linker
Bam HI (2533)
An
Bcl I (2770)
Mbo I (4190)
small-t intron
Bgl II (4710)

(4361/1782)
Eco RI
amp^r
pBR
pSVOA
Bam HI linker (2065)
Bcl I (2770)
Bam HI (2533)
Bcl I (2770)
Bam HI (2533)
Hind III linker
An
Bgl II (4710)

(4361/1782)
Eco RI
amp^r
pBR
pSV2
Pvu II (2065/270)
SV40 ori, promoter
Hind III (5171)
Bgl II (4710)
An

(4361/1782)
Eco RI
amp^r
pBR
pSV2A
An
An
Bam HI (2533/270)
SV40 ori, promoter
Hind III (5171)
Bgl II (4710)
An

(4361/1782)
Eco RI
amp^r
pBR
pSV232A
An
An
Bam HI (2533/270)
SV40 ori, enhancerless promoter
Hind III (5171)
Bgl II (4710)
An

(4361/1782)
Eco RI
amp^r
pBR
pRSV
Acc I (2246)
Pvu II
RSV LTR
BasNI
Hind III (4710) linker
An
Bgl II (4710)

B

RI AUG RI H2 UAA (A)_n RI AUG RI H2 UAA
-17 52 639 1354 1702 1810 -17 52 639 1354 1702
H3 30 99 Bs Xb L-pJG201 1747 1841 Sa Kp Sm Ba H3 30 99 Bs Xb L-A-pJG202 1747 Sp-H2

AUG RI H2 UAA (A)_n AUG RI H2 UAA
52 639 1354 1702 1810 52 639 1354 1702
30 99 LΔ5'-pJG204 1747 1841 Sa Kp Sm Ba 30 99 L-ΔΔ5'-pJG206 1747
H3 30 99 H3 Xb So RI H3 Xb So-H2

Figure 13

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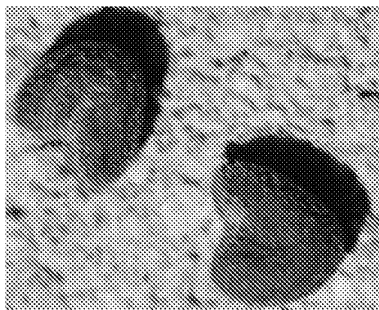


Figure 14(a)



Figure 14(b)

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T1: THC synthase gene wildtype seed
T2: THC synthase gene wildtype plant
K1: THC synthase gene knockout seed
K2: THC synthase gene knockout plant

Figure 15(a)

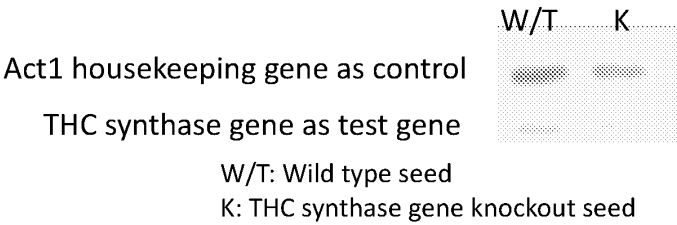
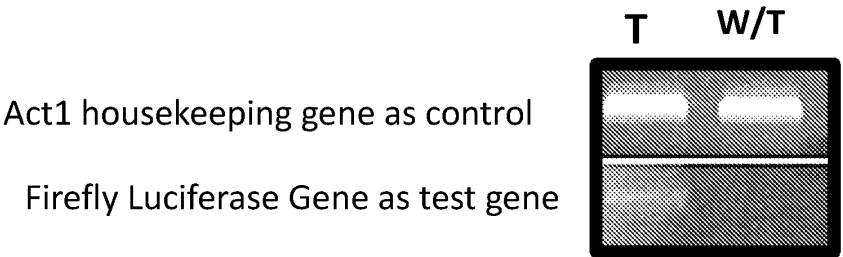


Figure 15(b)



W/T: Wild type seed
T: Transgenic seed with Firefly Luciferase Gene

Figure 15(c)