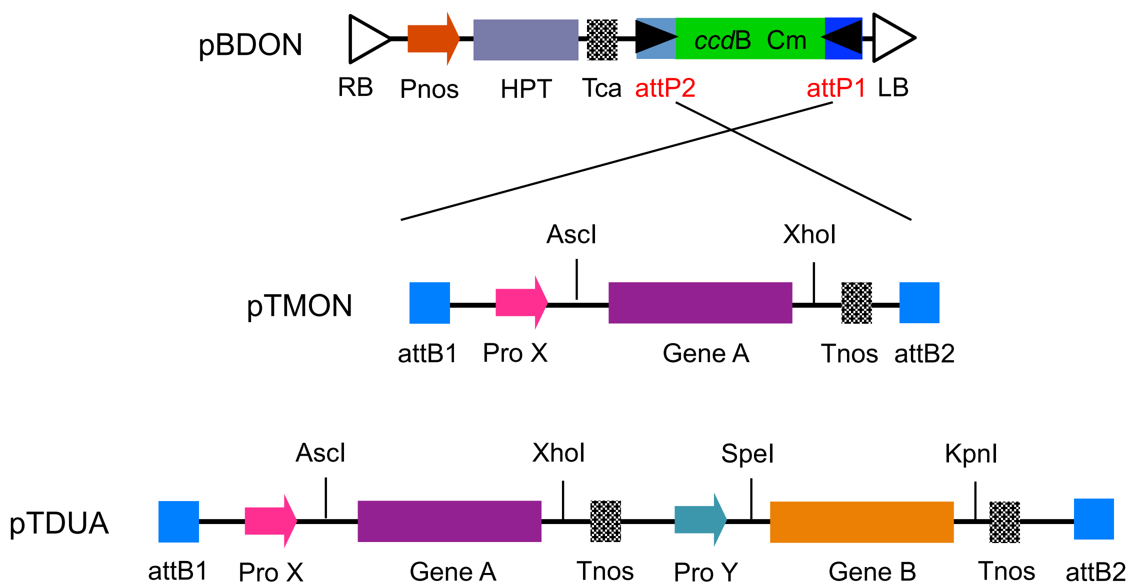
Online Resources

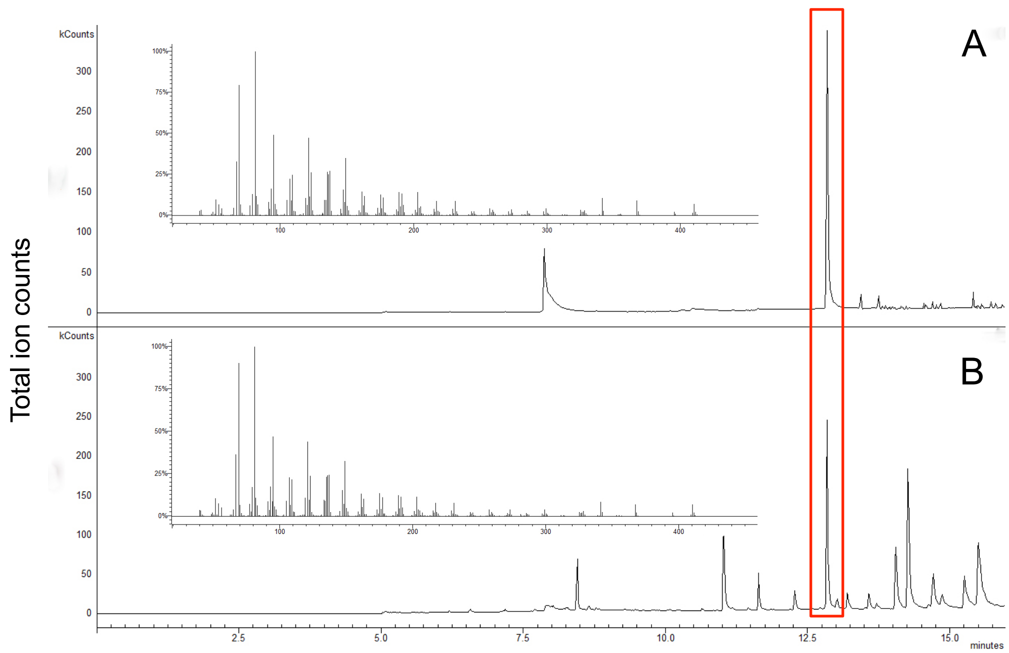
Ti vector design (**Supplementary Fig. 1**)

GC-MS of isolated squalene (**Supplementary Fig. 2**)

NMR files (**Supplementary Figs. 3 and 4**)

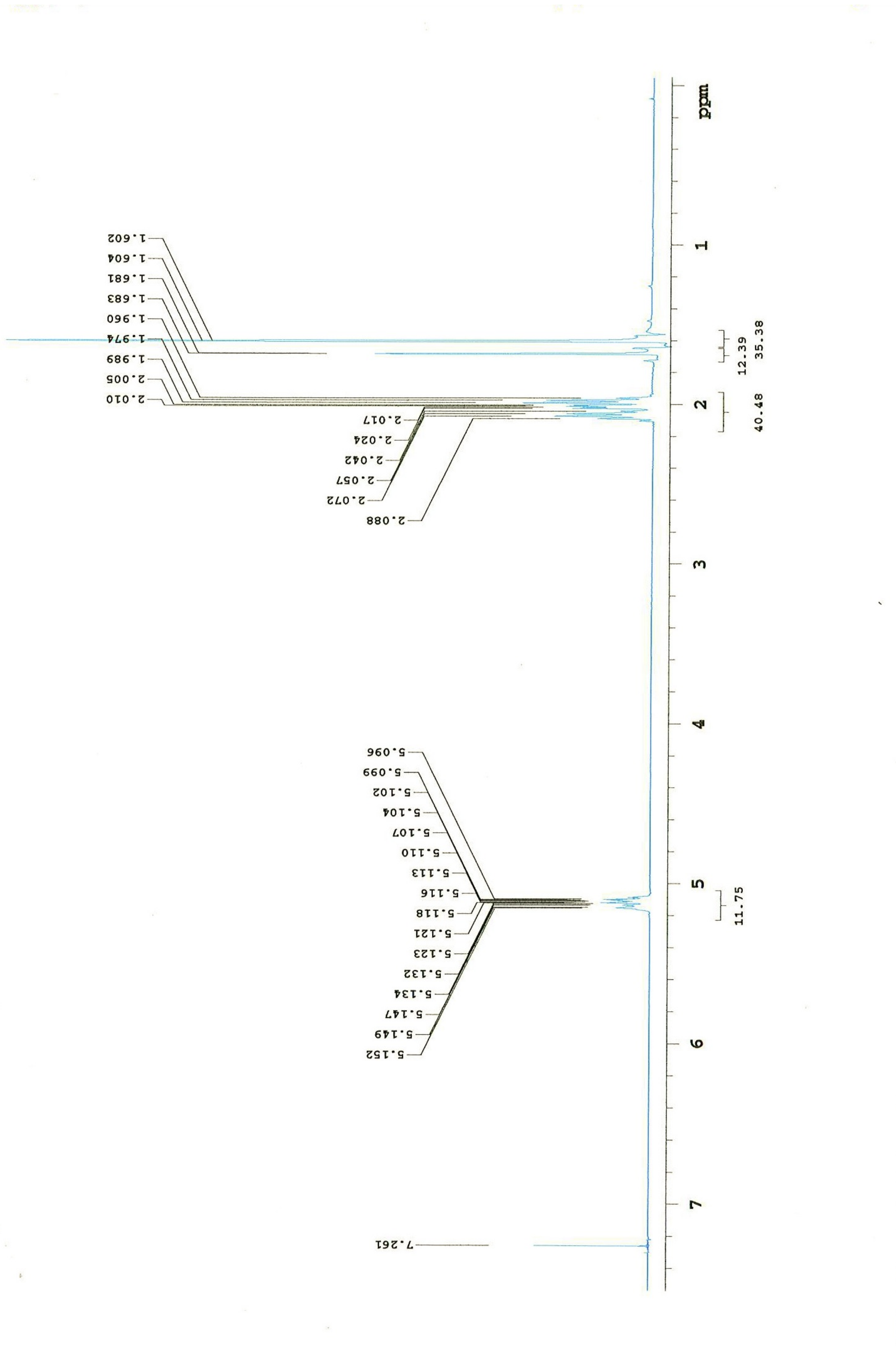


Supplementary Figure 1. A Ti vector system compatible for recombination cloning was developed to facilitate vector construction (pBDON). Helper vectors were designed to accommodate single (pTMON) or multiple gene (pTDUA) insertions.

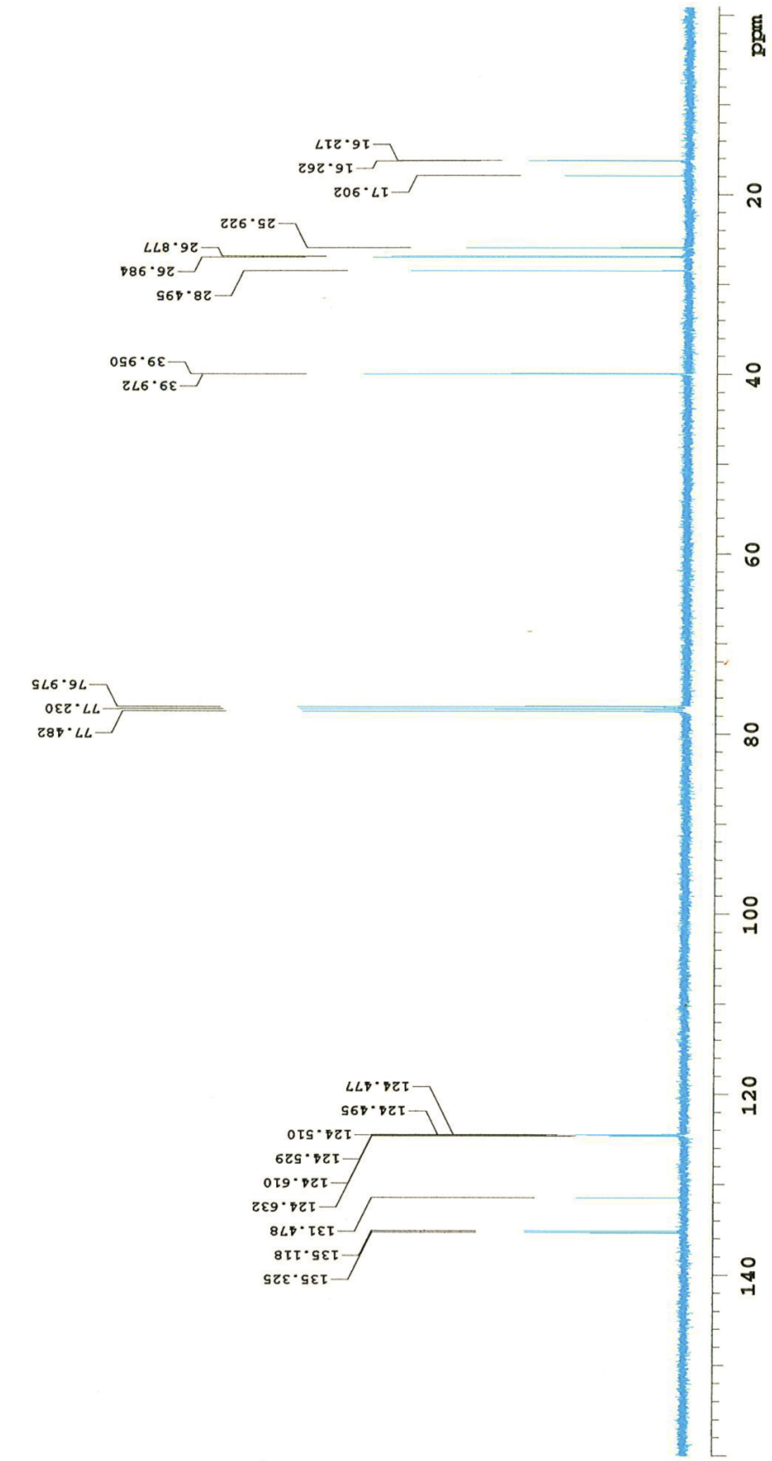


Supplementary Figure 2. GC-MS comparison of transgenic leaf hexane extract (B) to authentic squalene (A). Leaf material of homozygous line #5 expressing plastid target SQS and FPS under the direction of the constitutive promoters was ground in liquid nitrogen, extracted with hexane:ethyl acetate (85:15), the extract concentrated under nitrogen, then fractionated on a silica column. An aliquot of the flow through fraction was then analyzed by GC-MS (B) in comparison to a squalene standard (B). The MS for the 12.85 min peak in each sample is shown in the inset.

Structure identification of squalene. The structure of purified squalene from tobacco was determined ^{1}H-NMR and ^{13}C-NMR spectral analyses. ^{1}H-NMR and 13C-NMR spectra were recorded on a 500 MHz Varian J-NMR spectrometer at 300 K. Chemical shifts were referenced to solvent peaks, namely δ_H 7.24 and δ_C 77.0 for $CDCl_3$. (6*E*,10*E*,14*E*,18*E*)-squalene. Colorless oil. GC-MS mass: 410.5 *amu* (M^+). ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 1.60 (s, R-C$\underline{H}_3$, 18H), δ_H 1.68 (s, R-C$\underline{H}_3$, 6H), δ_H 1.99-2.09 (m, R-C$\underline{H}_2$-R', 20H), δ_H 5.10-5.15 (m, R=C$\underline{H}$, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 15.9 (=CH-$\underline{C}H_3$, 2C), δ_C 16.0 (=CH-$\underline{C}H_3$, 2C), δ_C 17.7 (=CH-$\underline{C}H_3$, 2C), δ_C 25.9 (=CH-$\underline{C}H_3$, 2C), δ_C 26.88 (=CH-$\underline{C}H_2$-R, 2C), δ_C 26.98 (=CH-$\underline{C}H_2$-CH_2, 2C), δ_C 28.5 (=CH-$\underline{C}H_2$-CH_2, 2C), δ_C 39.95 (=CH-$\underline{C}H_2$-CH_2, 2C), δ_C 39.97 (=CH-$\underline{C}H_2$-CH_2, 2C), δ_C 124.5 (=$\underline{C}$H, 2C), δ_C 124.6 (=$\underline{C}$H, 2C), δ_C 124.63 (=$\underline{C}$H, 2C), δ_C 131.5 (=$\underline{C}$H, 2C), δ_C 135.1 (=$\underline{C}$H, 2C), δ_C 135.3 (=$\underline{C}$H, 2C).



Supplementary Figure 3. ^{1}H-NMR spectrum of isolated squalene produced *in planta*. (500 MHz, $CDCl_3$).



Supplementary Figure 4. ^{13}C-NMR spectrum of isolated squalene produced *in planta*. (500 MHz, $CDCl_3$).