Supplementary Information for

A GPCR-based yeast biosensor for biomedical, biotechnological, and point-of-use cannabinoid determination

Karel Miettinen[1], Nattawat Leelahakorn[1], Aldo Almeida[1,2], Yong Zhao[1], Lukas Ronild Hansen[1], Iben Egebæk Nikolajsen[1], Jens B. Andersen[3], Michael Givskov[3], Dan Staerk[4], Søren Bak[1], and Sotirios C. Kampranis[1, *]

[1]Biochemical Engineering Group, Plant Biochemistry Section, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark.
[2]Bioremediation Laboratory, Faculty of Biological Sciences, Autonomous University of Coahuila, Carretera Torreón-Matamoros km. 7.5, Torreón, Coahuila, 27000, Mexico
[3]Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen, Denmark
[4]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

*Corresponding author: Email: soka@plen.ku.dk

This PDF file includes: Supplementary Methods Figures S1 to S13 and Tables S1 to S9

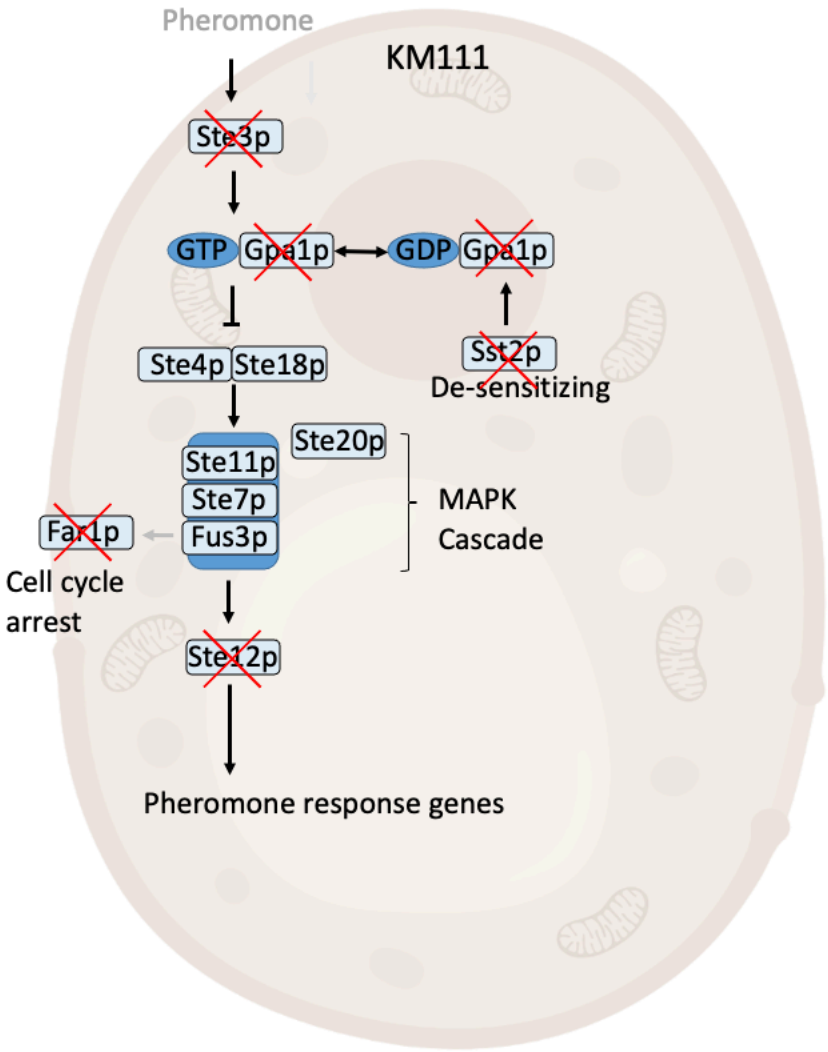


Figure S1. Engineering the chassis strain KM111. The biosensor chassis strain KM111 was constructed by sequentially knocking out three genes (*STE3*, *STE12* and *GPA1*) that encode pheromone pathway components so that they can be replaced by custom parts. In addition, two genes (*SST2* and *FAR1*) that are detrimental to biosensor function were also deleted. First, *STE3* was knocked out by replacing it with the selection marker *URA3* by homologous recombination. Then the marker was removed using Cre/Lox recombination (LoxP sites flanking the selection marker). This was repeated with SST2, then FAR1, then STE12, and finally, GPA1.

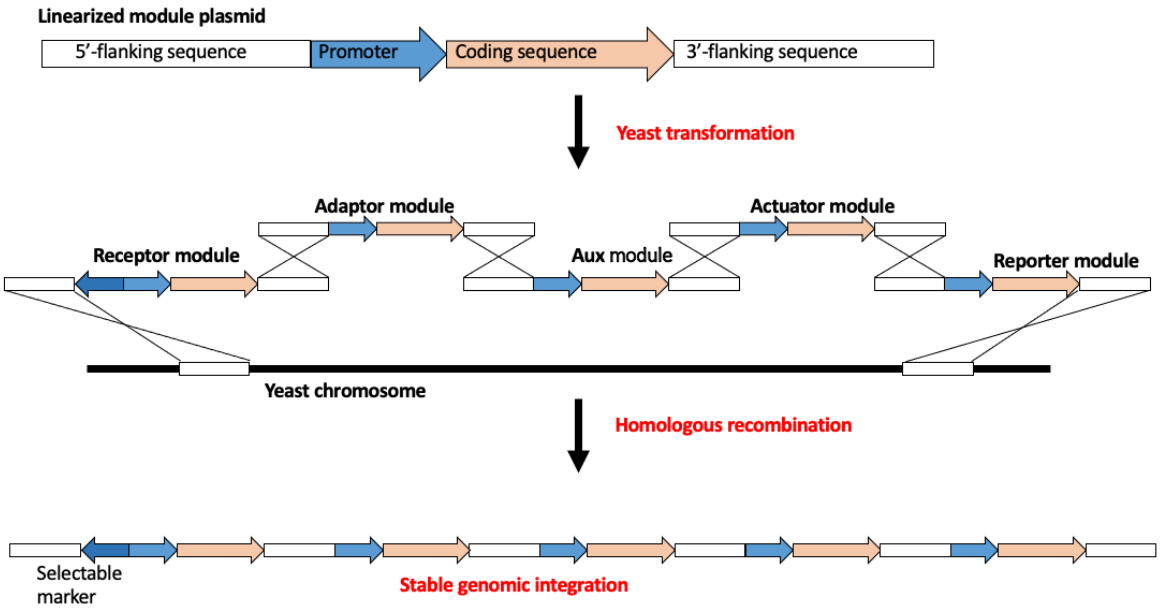


Figure S2. Genetic integration of biosensor parts. Each biosensor was constructed by integrating parts into four modules using multipart modular genomic integration. A set of five plasmids containing a receptor part, adaptor part, actuator part, reporter part, and aux part (an empty vector that can be used for the integration of additional parts) were chosen according to the desired biosensor design. These plasmids were linearized and transformed into the chassis strain, where they were orderly integrated into the genome based on matching HR regions in each part. Integration positive colonies were selected using the *URA3* selection marker.

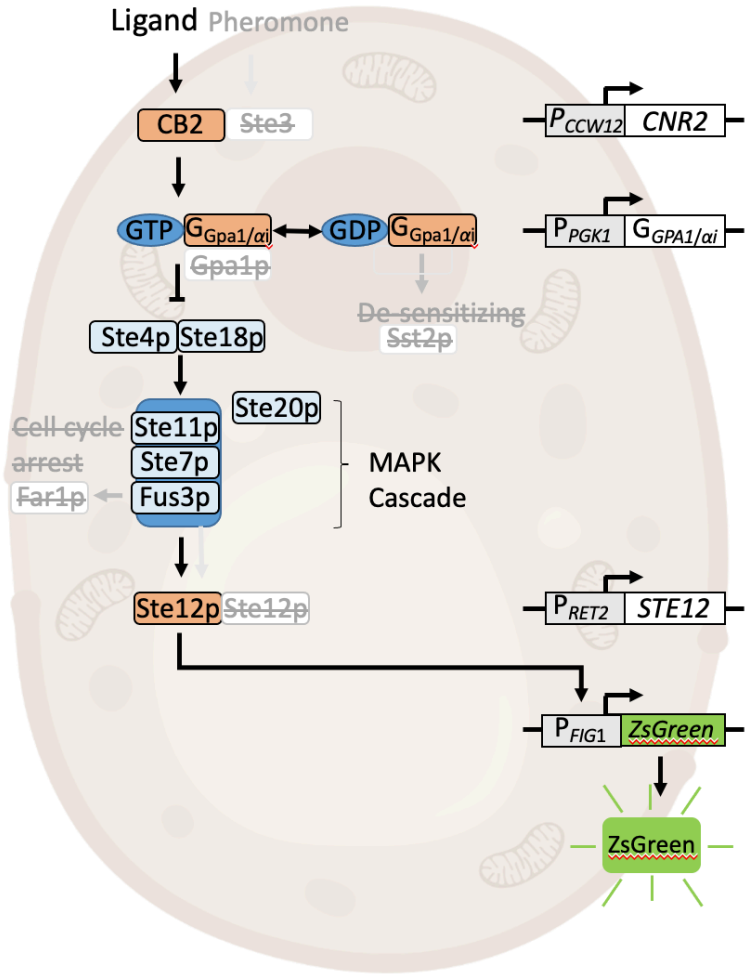


Figure S3. The initial biosensor. The biosensor strain KM202 was constructed by integrating the *CNR2* coding sequence (gene coding for the CB2 receptor) controlled by the P_{CCW12} promoter into the receptor module, the Gpa1/Gαi1 chimeric protein coding sequence with the P_{PGK1} promoter into the adaptor module, *STE12* driven by the P_{RET2} promoter into the actuator module, and ZsGREEN with the P_{FIG1} promoter into the reporter module. The rest of the biosensors used in this work were constructed in a similar manner except for using different reporter or receptor module parts.

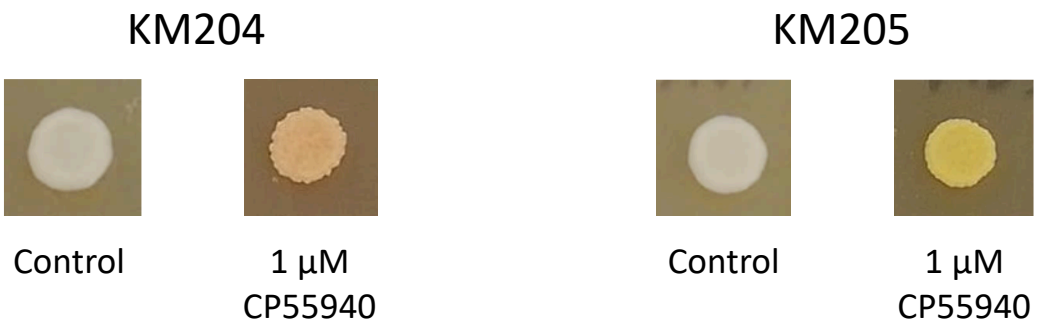


Figure S4. Color production of the betalain reporter strains. Betalain reporter strains KM204 (betainin, red) and KM205 (betaxanthis, yellow) were grown on plates containing 1 µM CP55940. KM204 shows a clear red color and KM205 shows a yellow color when induced with the cannabinoid. In the absence of cannabinoid induction (control), neither strain shows color build up.

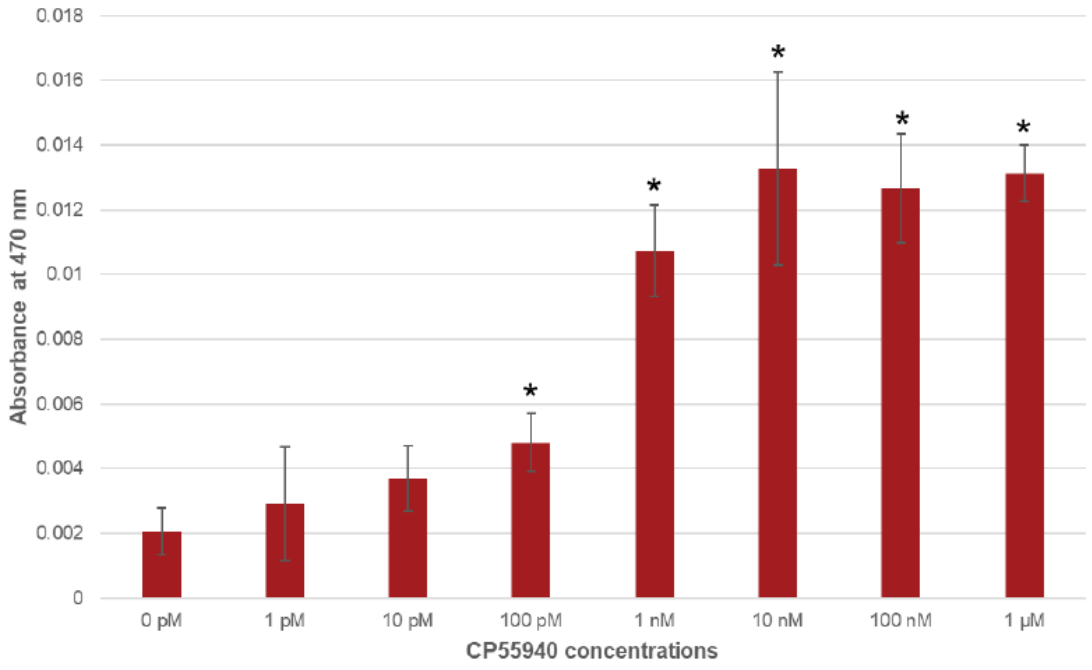


Figure S5. Dose response of CP55940 activation of the cannabinoid biosensor strain KM205 with betaxanthin output. Incubation of the stain KM205 with a CP55940 dilution series ranging from 0 to 1 µM results in a dose dependent increase in absorbance at 470 nm (betaxanthin absorbance maximum).

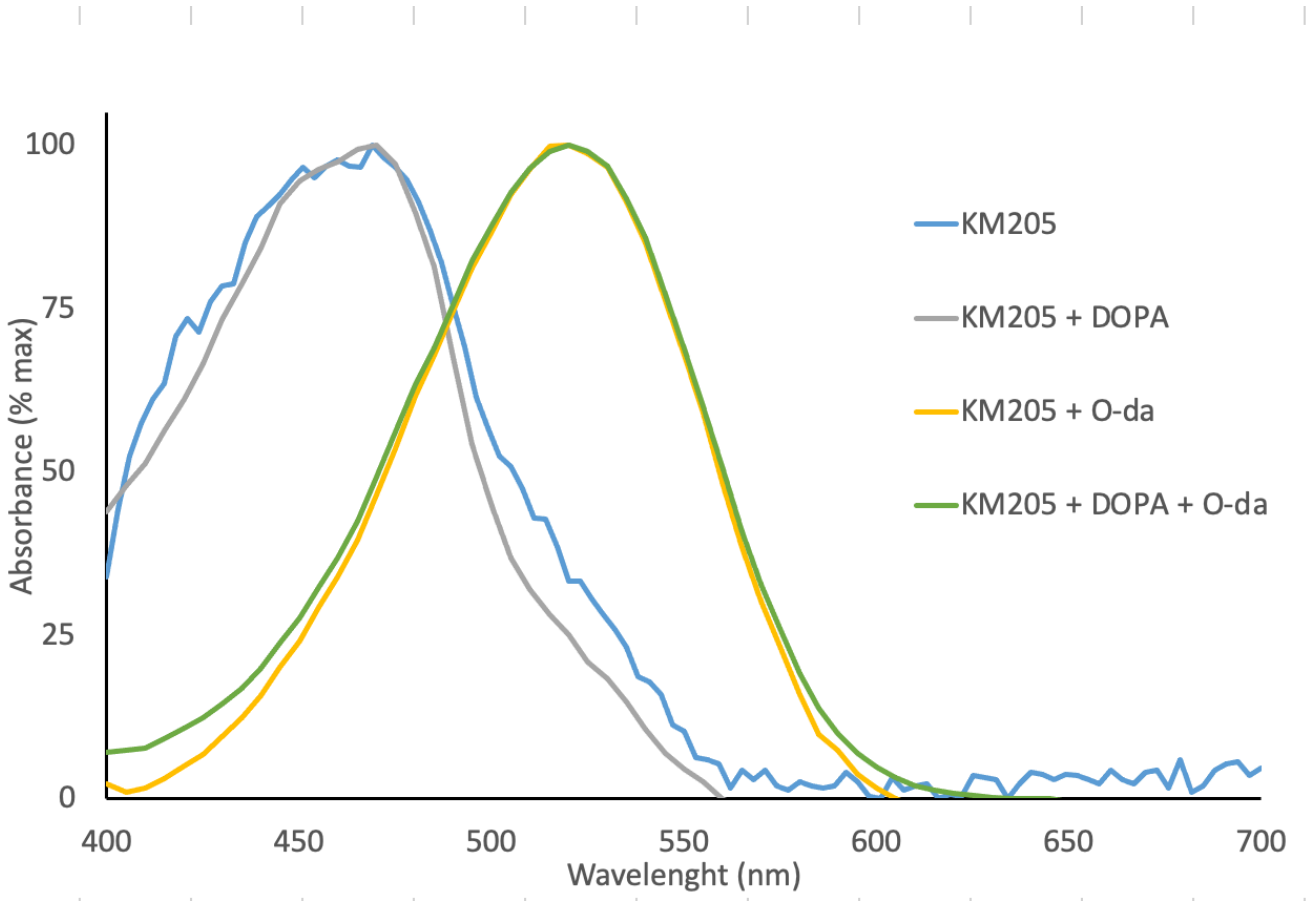


Figure S6. Absorbance spectra of the betalain reporter strain KM205 supplemented with L-DOPA and or O-da. Absorbance spectra of supernatant of strain KM205 culture induced with 1 µM CP55940 shows the presence of betaxanthins (peak absorbance wavelength 470 nm) but no betacyanins (peak absorbance wavelength 520 nm). On the other hand, when supplemented with O-da the spectra shows betacyanins (O-da-betacyanin) but no betaxanthins. This clear distinction facilitates the use of the betalain reporter for quantitative assays. Addition of L-DOPA did not have a marked effect on the spectra.

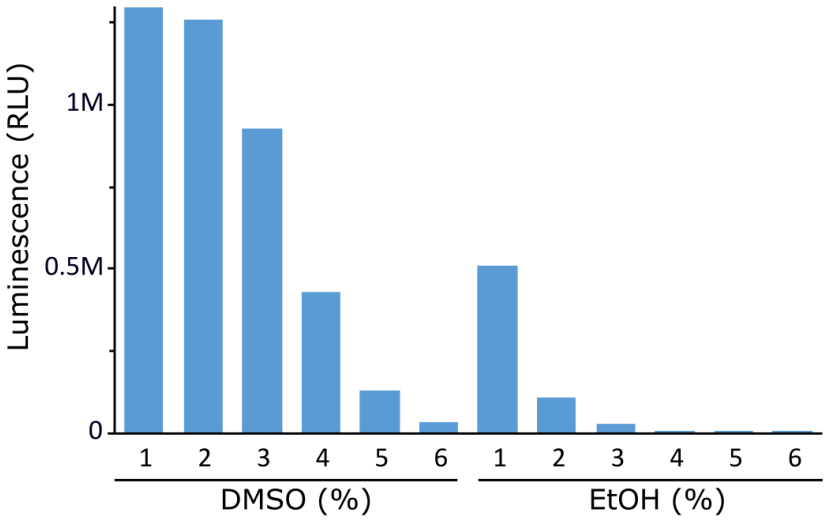


Figure S7. Effect of solvent concentration of biosensor output. The biosensor strain KM206 with luminescence reporter was induced with 1 µM CP55940 in the presence of 1 to 6 % ethanol or DMSO. This revealed that the presence of ethanol has a negative effect on biosensor output. However, the presence of 1 or 2 % DMSO resulted in similar output levels indicating that these concentrations are not significantly detrimental to biosensor output.

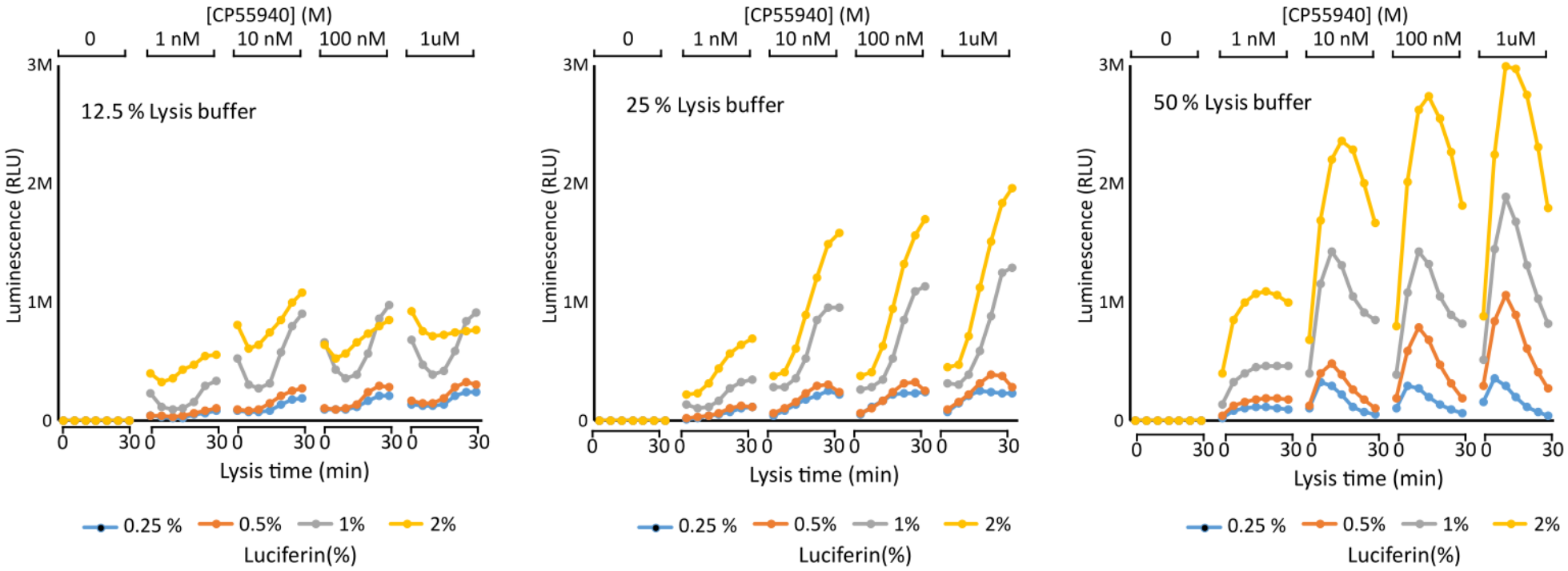


Figure S8. Effect assay conditions on the performance of the luminescence biosensor strain. Luminescence is a dynamic process, and the output of the biosensor depends on several factors including concentration of luciferase, luciferin, and lysis buffer. In order to find the optimal parameters for our setup, we performed time series varying each parameter. The result indicated the optimal conditions for maximum output to be 2% luciferin and 50% lysis buffer for a 10-15 min incubation time. However, the biosensor showed workable output in a range of conditions, suggesting that, if required, a different set up can also be used.

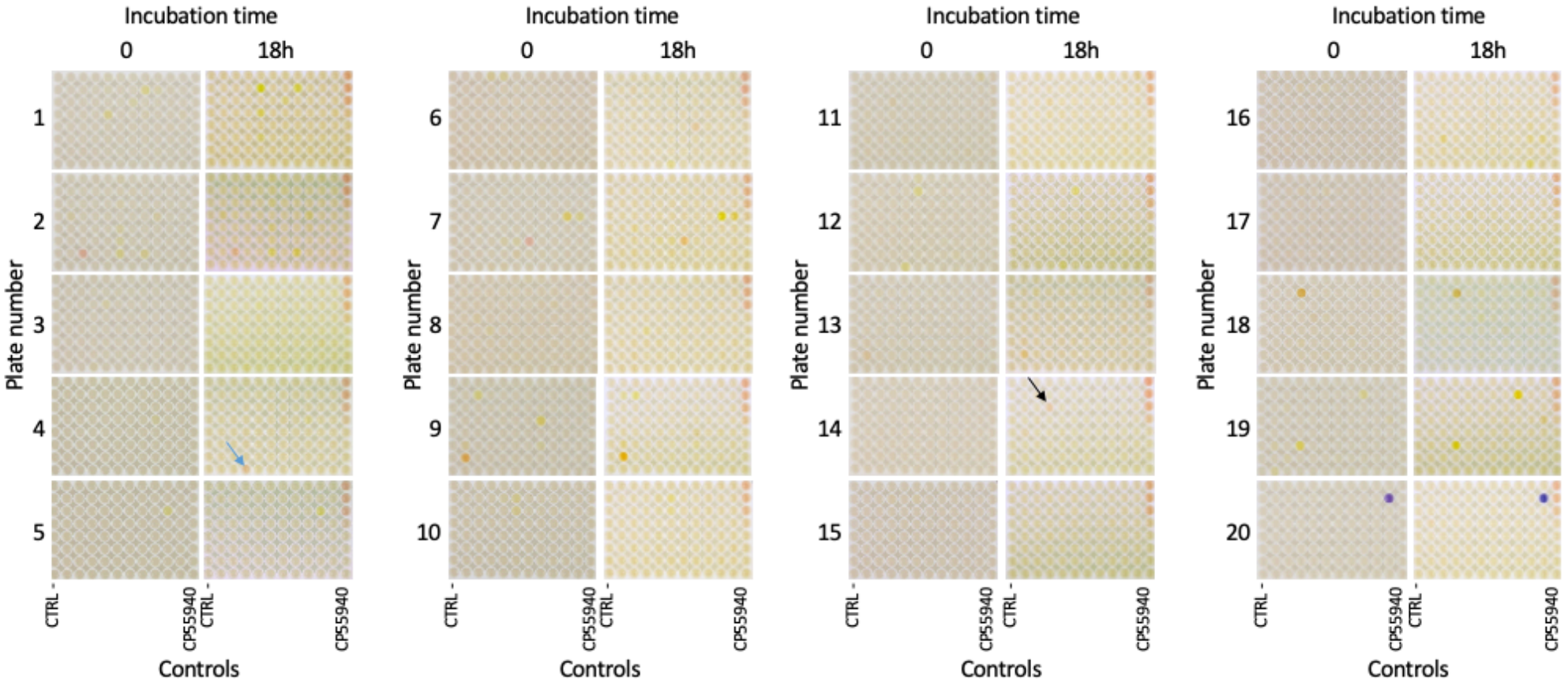


Figure S9. High-throughput screening with the biosensor strain KM205 that includes the betalain reporter (O-da-betacyanin). A 100 µl-aliquot of yeast culture (OD_{600} = 5) containing 0.1 mg/mL DOPA and 0.5 mM O-da was dispensed in each well. Then, 1 µL of the chemical library was dispensed in rows 1 to 11 of each plate. Additionally, DMSO was dispensed in each well of row 1 of each plate (negative control, CTRL-) and a dilution series of CP55940 (1 pM – 1 µM) to row 12 (positive control, CTRL+). The plates were incubated for 18 h at 30°C and then photographed. Two visible hits, in plate 4 blue arrow and plate 14 black arrow, appeared after the incubation.

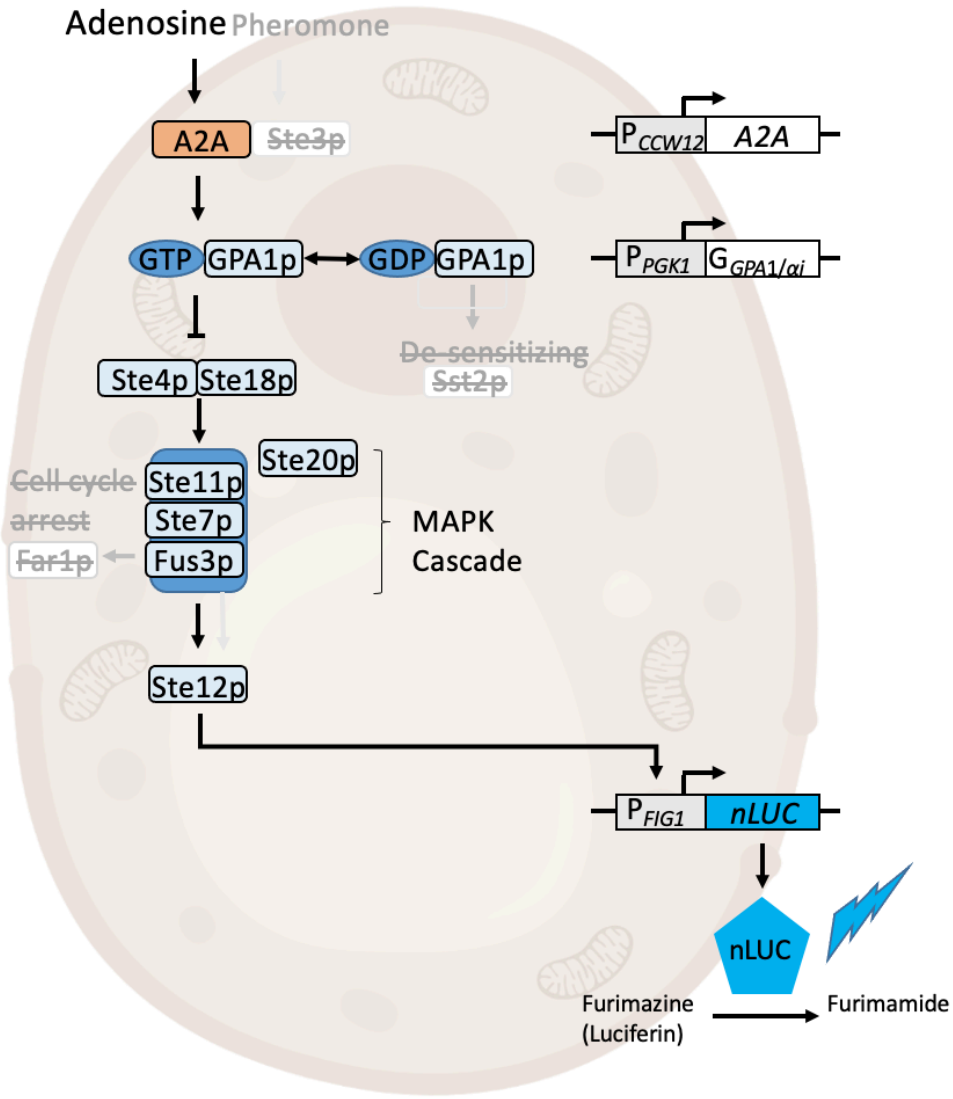


Figure S10. The A2A adenosine receptor-based biosensor for monitoring non-specific effect of compounds. This biosensor strain, KM207 was constructed to test potential non-specific inhibitory or activating effects of compounds (i.e. compounds that do not target specifically the CB2 receptor but have a non-specific effect in the yeast cells or the rest of the engineered sensing mechanism). It was constructed by integrating the coding sequence (gene coding for the A2A adenosine receptor) controlled by the P_{CCW12} promoter and the NanoLuc reporter into the receptor module of the chassis strain KM108. This strain has the native *STE12* and *GPA1*.

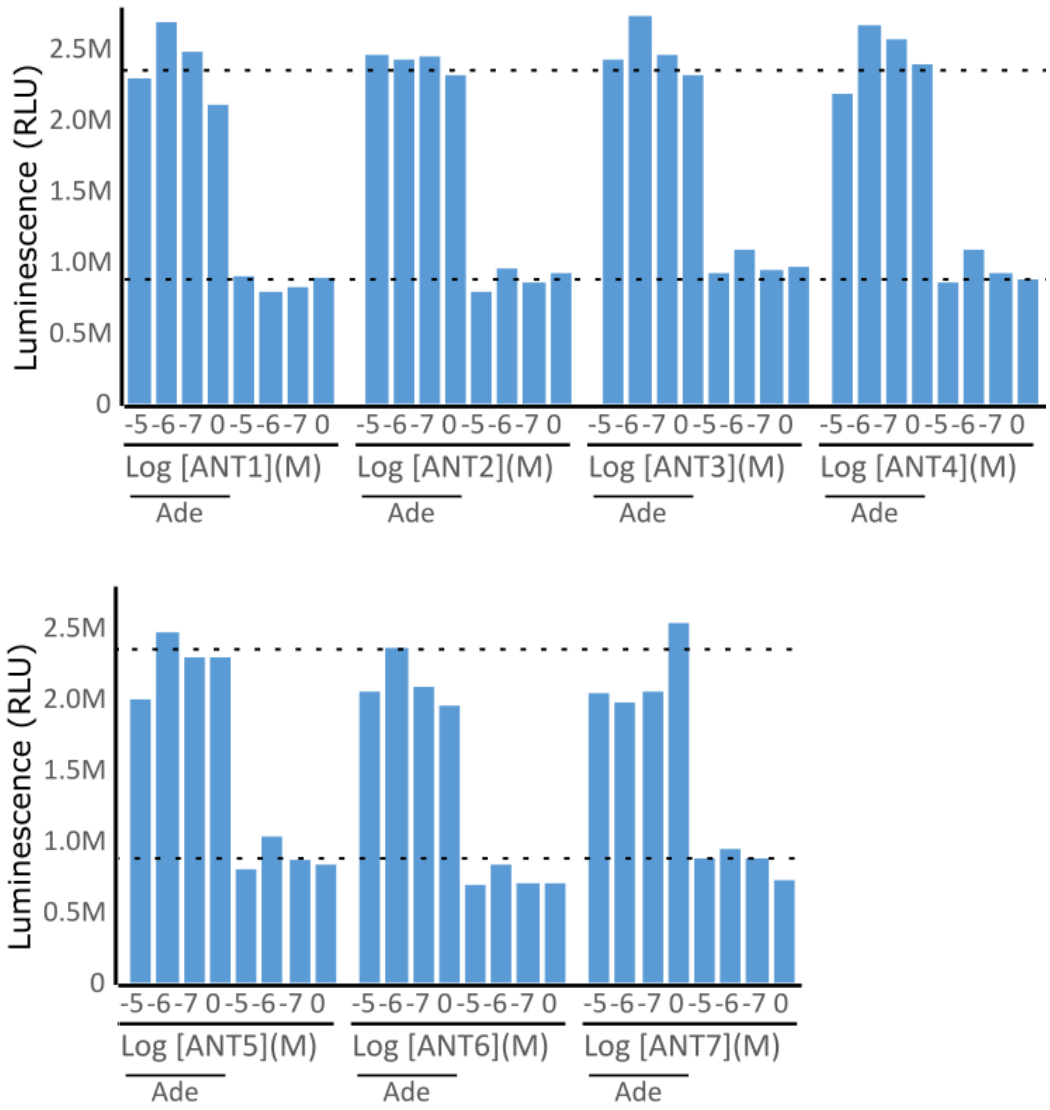


Figure S11. Effect of the compounds identified as hits during the CB2 antagonist screen on the function of the A2A biosensor strain KM207. Dilutions (100 pM to 10 µM) of each compound (ANT1-7) were incubated with KM207 with or without the A2A ligand adenosine. Each assay resulted in activation of the biosensor by adenosine, suggesting no effect on biosensor function.

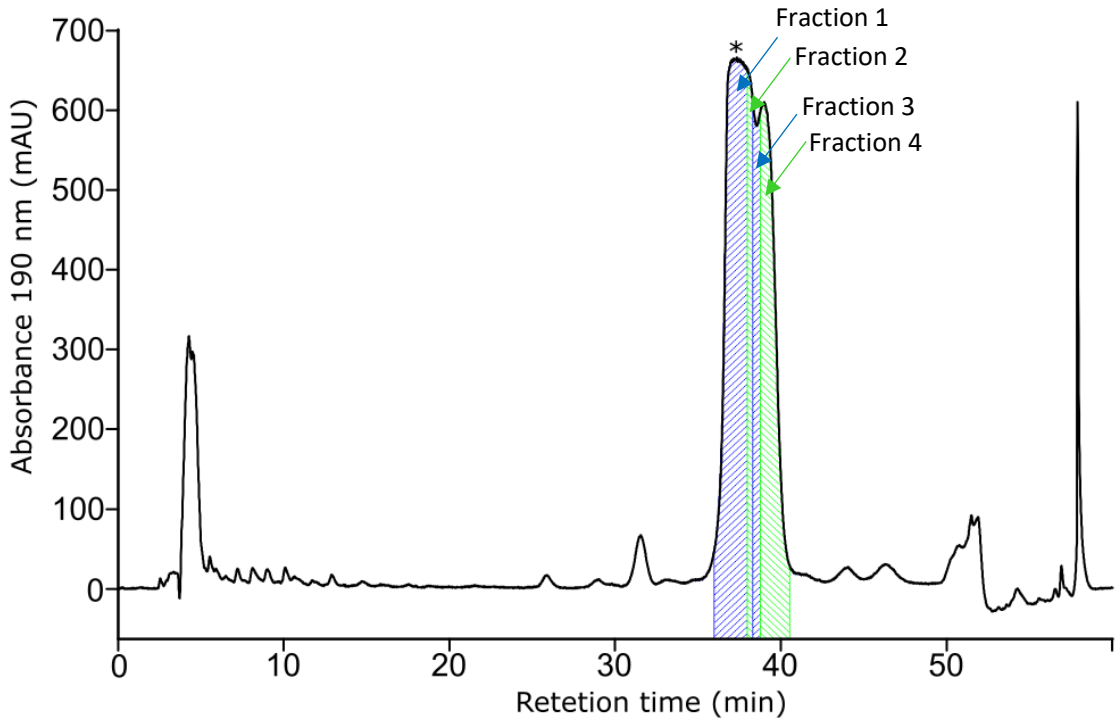


Figure S12. Purification of the main cannabinoid from *R. columnifera*. In order to obtain the high degree of purity necessary for NMR structure determination, the cannabinoid containing fraction from the initial preparative HPLC run (Fig. 7C) was further purified by additional preparative HPLC fractionation and biosensor-based cannabinoid determination. Four fractions were collected (green and blue) and fraction 1 was used for NMR.

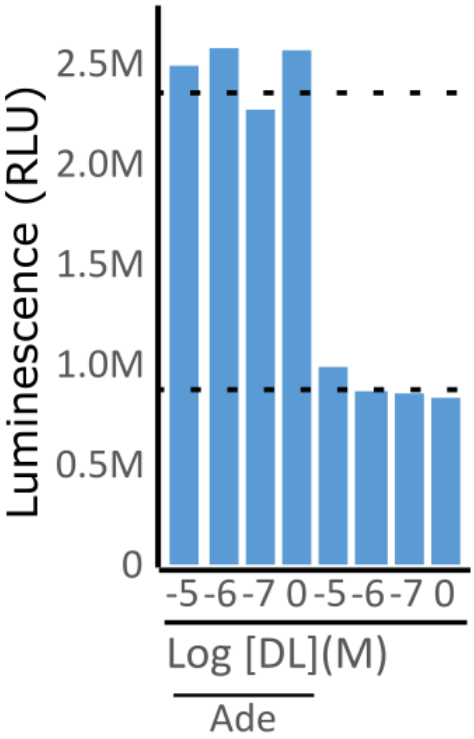


Figure S13. Effect of dugesialactone on the function of the A2A biosensor strain KM207. Dilutions (100 pM to 10 μM) dugesialactone were incubated with KM207 with or without the A2A ligand adenosine. Each assay resulted in activation of the biosensor by adenosine suggesting no effect on biosensor function.

Table S1. List of strains used in this work.

#	Name	Genotype				
1	AM254	MAT α, his3, ura3, trp1, lexO:LEU2, rox1, dos2, yer134c, vba5, ygr259c, ynr063w, ubc7				
	Chassis strains					
	Name	**Parent; Mutations**				
2	KM102	AM254; ΔSTE3-0				
3	KM106	AM254; ΔSTE3-0, ΔSST2-0				
4	KM108	AM254; ΔSTE3-0, ΔSST2-0, ΔFAR1-0				
5	KM109	AM254; ΔSTE3-0, ΔSST2-0, ΔFAR1-0, ΔSTE12-0				
6	KM111	AM254; ΔSTE3-0, ΔSST2-0, ΔFAR1-0, ΔSTE12-0, ΔGPA1-0				
	Biosensor strains					
	Name	**Parent**	**Assembler** 1	**Assembler 2**	**Assembler 3**	**Assembler 4**	**Assembler 5**
7	KM201	KM111;	pX3A EV	pASS2A - PPGK1- Gpa1-Gαi1	pASS2B-PRET2-STE12	pASS2C -EV	pX3C - PFig1-ZsGREEN
8	KM202	KM111;	pX3A - PCcw12-CB2	pASS2A - PPGK1- Gpa1-Gαi1	pASS2B-PRET2-STE12	pASS2C -EV	pX3C - PFig1-ZsGREEN
9	KM203	KM111;	pX3A - PCcw12-MFαSS-CB2	pASS2A - PPGK1- Gpa1-Gαi1	pASS2B-PRET2-STE12	pASS2C -EV	pX3C - PFig1-ZsGREEN
10	KM204	KM111;	pX3A - PCcw12-MFαSS-CB2	pASS2A - PPGK1- Gpa1-Gαi1	pASS2B-PRET2-STE12	pASS2C - BvCYP76AD1-pSED1/PTDH3-BvDOPA5GT	pX3C - PFig1-DOD
11	KM205	KM111;	pX3A - PCcw12-MFαSS-CB2	pASS2A - PPGK1- Gpa1-Gαi1	pASS2B-PRET2-STE12	pASS2C - pHHF2-BvCYP76AD5	pX3C - PFig1-DOD
12	KM206	KM111;	pX3A - PCcw12-MFαSS-CB2	pASS2A - PPGK1- Gpa1-Gαi1	pASS2B-PRET2-STE12	pASS2C -EV	pX3C - PFig1-NanoLuc
13	KM207	KM108;	PX3A – PCCW12-A2A	pB - EV			pX3C - PFig1-NanoLuc

Table S2. List of screening hits[a].

Antagonists	**Label**	**SMILES**	**Manufacturer**
AGO1	P1-H4	Cc1c2cc(ccc2oc1C(Nc1ccc(c(c1)OC)NC(c1ccco1)=O)=O)[Cl]	ChemDiv
AGO2	P4-C4	CC(C)C(NC(C([Cl])([Cl])[Cl])NC(Nc1ccccc1C(O)=O)=S)=O	Chembridge
Agonists			
ANT1	P1-C10	C1CC2=C(C=C(C#N)C(N2)=O)C(C1)=NNc1ccccc1	Chembridge
ANT2	P3-B11	CCOCc1c(C)cc(C)c(C2C(C#N)=C(N)Oc3c2c(C)n[nH]3)c1C	Chembridge
ANT3	P5-C16	C(CC(O)=O)CNC(c1ccncc1)=O	Chembridge
ANT4	P1-D23	CCc1cc(C(=O)OCC)c(NC(CC2C(Nc3ccccc3N2)=O)=O)s1	ChemDiv
ANT5	P1-F23	CCc1ccc(cc1)NC(CN1C=Nc2c(C1=O)c(cs2)c1ccccc1)=O	ChemDiv
ANT6	P1-N23	Cc1ccc(c(c1)N(=O)=O)N1C(C2C3CCC(C=C3)C2C1=O)=O	ChemDiv
ANT7	P2-I17	CC(c1ccc(cc1)OCCOc1ccc(C#N)cc1)=NO	Chembridge

[a]The label describes the position of each hit in the screening plate. SMILES is a textual representation of the chemical structure that can be used to find the compounds from the manufacturer's web page.

Table S3. List of plant extracts used for bioprospecting. Label indicates the position of the extract in the screening plate.

#	Label	Species name	Tissue	Source
1	A01	Agastache mexicana (Kunth) Lint & Epling (Toronjil morado)	Aerial	1
2	A02	Annona muricata L. (Guanabana)	Leaves	1
3	A03	Artemisia ludoviciana Nutt. (Estafiate)	Aerial	1
4	A04	Bougainvillea spectabilis Wild. (Bugambilia)	Flowers & sepals	2
5	A05	Calendula officinalis L. (Pot marigold)	Aerial	3
6	A06	Calendula officinalis L. (Pot marigold)	Flowers	3
7	A07	Calendula officinalis L. (Pot marigold)	Roots	3
8	A08	Carica papaya L.	Leaves	4
9	A09	Cascabela thevetia (L.) Lippold	Leaves	4
10	A10	Casimiroa edulis La Llave & Lex.	Leaves	1
11	A11	Ceiba pentandra (L.) Gaertn.	Leaves	5
12	A12	Celosia argentea var. cristata (L.) Kuntze	Aerial	6
13	B01	Cobaea scandens Cav.	Leaves	3
14	B02	Cobaea scandens Cav.	Flower buds	3
15	B03	Coreopsis tinctoria Nutt.	Aerial	3
16	B04	Coreopsis tinctoria Nutt.	Roots	3
17	B05	Cosmos bipinnatus Cav. (Cosmos)	Aerial	3
18	B06	Cosmos bipinnatus Cav. (Cosmos)	Roots	3
19	B07	Croton incanus Kunth (Torrey)	Aerial	1
20	B08	Cuphea ignea A.DC.	Aerial	3
21	B09	Chenopodium graveolens Wild. (Epazote)	Aerial	1
22	B10	Echinacea purpurea (L.) Moench	Stem	6
23	B11	Echinacea purpurea (L.) Moench	Roots	6
24	B12	Echinacea purpurea (L.) Moench	Leaves	6
25	C01	Equisetum hyemale L.	Aerial	1
26	C02	Eschscholzia californica Cham.	Fruits	3
27	C03	Eschscholzia californica Cham.	Leaves	3
28	C04	species of Euphorbia	Aerial	1
29	C05	Pseudognaphalium canescens (DC.) W.A.Weber	Flowers	1
30	C06	Pseudognaphalium canescens (DC.) W.A.Weber	Stem	1
31	C07	Clarkia amoena (Lehm.) A.Nelson & J.F.Macbr.	Leaves	3
32	C08	Clarkia amoena (Lehm.) A.Nelson & J.F.Macbr.	Flowers	3
33	C09	Ipomoea purpurea L.	Aerial	6
34	C10	Ipomoea purpurea L.	Roots	6
35	C11	Ipomoea tricolor Cav.	Leaves	6
36	C12	Ipomoea tricolor Cav.	Stem	6
37	D01	Ipomoea tricolor Cav.	Roots	6
38	D02	Amphipterygium adstringens (Schltdl.) Standl.	Bark	1
39	D03	Justicia spicigera Schlechtendal	Aerial	1

40	D04	Lippia graveolens H. B. K.	Aerial	[1]
41	D05	Melothria scabra Naudin	Leaves	[6]
42	D06	Mirabilis jalapa L.	Leaves	[3]
43	D07	Mirabilis jalapa L.	Flowers	[3]
44	D08	Nemophila menziesii Hook. & Arn.	Aerial	[6]
45	D09	Persea americana Mill. (Aguacate)	Leaves	[1]
46	D10	Piper auritium Kunth (Hierba santa)	Leaves	[7]
47	D11	Plumeria rubra L. (Flor de mayo)	Leaves	[8]
48	D12	Prosopis laevigata (Humb. & Bonpl. ex Willd.) M.C.Johnst.	Leaves	[9]
49	E01	Pseudobombax ellipticum (Kunth) Dugand (Coquito/Xiloxochitl)	Leaves	[10]
50	E02	Psidium guajava L. (Guayaba)	Leaves	[1]
51	E03	Rhizophora mangle L. (Mangle)	Stem	[1]
52	E04	Rosa ×centifolia L. (Rosa de castilla)	Flowers	[1]
53	E05	Ratibida columnifera (Nutt.) Wooton & Standl.	Aerial	[6]
54	E06	Ratibida columnifera (Nutt.) Wooton & Standl.	Roots	[6]
55	E07	Selaginella lepidophylia (Hook & Grev.) Spring. (Doradilla)	plant	[1]
56	E08	Smilax aristolochiifolia Mill. (Zarzaparrilla)	Roots	[1]
57	E09	Swietenia humilis Zucc. (Zopilote)	Seeds	[1]
58	E10	Tagetes patula L. (Cempaxochitl)	Leaves	[3]
59	E11	Tagetes patula L. (Cempaxochitl)	Flowers	[3]
60	E12	Tecoma stans (L.) Juss. ex Kunth (Nixtamaxochitl)	Leaves	[11]
61	F01	Theobroma cacao L. (Cacao)	Seeds	[12]
62	F02	Tithonia rotundifolia (Mill.) S.F.Blake (Girasol mexicano)	Stem	[6]
63	F03	Tithonia rotundifolia (Mill.) S.F.Blake (Girasol mexicano)	Roots	[6]
64	F04	Tithonia rotundifolia (Mill.) S.F.Blake (Girasol mexicano)	Leaves	[6]
65	F05	Urtica dioica L. (Ortiga)	Aerial	[1]
66	F06	Vachellia farnesiana (L.) Wight & Arn. (Huizache)	Flowers	[9]
67	F07	Zinnia elegans Jacq. (Cinia)	Flowers	[6]
68	F08	Zinnia elegans Jacq. (Cinia)	Aerial	[6]
69	F09	Zinnia elegans Jacq. (Cinia)	Roots	[6]
70	F10	species of Allionia (Hierba de la hormiga)	Aerial	[1]
71	F11	species of Kohleria (Tlachichinoli)	Leaves	[1]

[1] Herbolaria El Sauz de Silva

[2] Collected in surroundings of Cd. Lerdo, Durango, Mexico.

[3] Det Biovidenskabelige Fakultets Have, Botanical Garden, University of Copenhagen, Frederiksberg, Denmark

[4] Collected in surroundings of Zona Arquelogica Bocana del Rio Copalita, Oaxaca, Mexico.

[5] Collected in surroundings of Aeropuerto internacional bahias de Huatulco, Oaxaca, Mexico.

[6] Grown from seeds in greenhouse at the Department of Plant and Environmental Sciences, University of Copenhagen Denmark

[7] Collected in surroundings of Crucecita, Oaxaca, Mexico.

[8] Collected in surroundings of San Isidro Roaguia, Hierve el Agua, Oaxaca, Mexico.

[9] Collected in surroundings of Torreon, Coahuila, Mexico.

[10] Collected in surroundings of Oaxaca city, Oaxaca, Mexico.

[11] Collected in surroundings of Playa la entrega, Crucecita, Oaxaca, Mexico.

[12] Raw seeds of Cacao were purchased from distributor TrancePlants in Canada

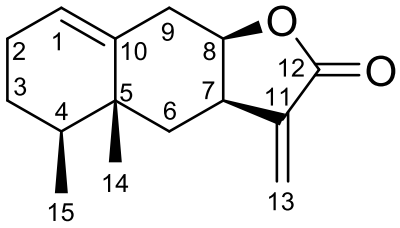
Table S4. Biosensor signal intensity of *R. columnifera* fraction dilutions[a].

Fraction	log dilution 0	-1	-2	-3	-4	-5
1	4832.349	3589.745	3059.277	2608.742	2458.564	1678.606
2	6280.843	3606.701	2829.165	2264.785	2318.074	1773.073
3	8894.43	3737.501	2855.81	2281.741	2536.075	2104.918
4	8506.873	3962.769	2637.809	2245.407	2204.229	2056.473
5	9422.476	3485.589	2666.875	2204.229	2167.896	2008.029
6	24856.93	5985.331	2739.542	2356.83	2274.474	2141.251
7	1576.872	4660.371	2800.098	2390.741	2475.519	2078.273
8	903.492	11251.26	3846.502	2727.431	2041.94	2201.807
9	462.646	8538.361	17379.5	2943.01	1664.072	1470.294
10	7164.957	3880.413	1751.272	1455.76	1567.183	1564.761
11	4289.77	2153.363	1823.939	1412.16	1320.115	1499.36
12	3262.744	1918.406	1727.05	1516.316	1455.76	1404.894
13	2395.586	2027.407	1804.562	1576.872	1588.983	1356.449
14	3369.322	1995.918	1656.806	1492.094	1605.939	1019.759
15	5689.819	1988.651	1891.762	1555.072	1448.494	1235.337
16	3279.7	1610.783	1957.162	1685.872	1753.695	1361.293

[a]The R. columnifera extract was fractionated into 16 fractions and 1-to-10^5-fold dilutions were made of each fraction. The presence of cannabinoids was assayed by incubating each dilution with the strain KM206 (luciferase reporter). Values are in RLU.

Table S5. NMR spectroscopic data (600 MHz, MeOD) of dugesialactone (δ in ppm).

Position	δ_H, mult (J in Hz)	δ_C, type [a]
1	5.51, br. s	125.2, CH
2	2.06, m	25.9, CH_2
3	1.56, m	27.7, CH_2
4	1.67, m	35.8, CH
5	-	37.9, C
6	1.79, dd, J = 5.2, 14.1	39.5, CH_2
	1.63, m	
7	3.02, m	37.5, CH
8	4.56, m	80.5, CH
9	2.56, dd, J = 7.8, 12.1	35.3, CH_2
	2.30	
10	-	139.5, C
11	-	141.0, C
12	-	172.4, C
13	6.16, d, J = 2.3	122.3, CH_2
	5.70, d, J = 2.0	
14	0.94, s	20.7, CH_3
15	0.96, d, J = 6.8	16.0, CH_3



[a] ^{13}C NMR shifts were obtained from HSQC and HMBC spectra.

Table S6. Plasmid construction. All plasmids were construction by USER cloning combining one single-promoter and one insert ORF or a two-sided promoter and two inserts. PCR fragments used here are described in Table S7.

plasmid name	Backbone	Insert 1	promoter	Insert 2
pX3A -EV[a]	pX3A			
pX3A - P_{CCW12}-CB2	pX3A	CB2	P_{CCW12}	
pX3A - P_{CCW12}-MFαSS-CB2	pX3A	MFαSS-CB2	P_{CCW12}	
pASS2A- EV[a]				
pASS2A - P_{PGK1}- Gpa1-Gαi1	pASS2A	Gpa1-Gai1	P_{PGK1}	
pASS2B-EV[a]				
pASS2B- P_{RET2}-STE12	pASS2B	Ste12	P_{RET2}	
pASS2C -EV[a]	pASS2C			
pASS2C - P_{HHF2}-BvCYP76AD5	pASS2C	*Bv*CYP76AD5	P_{HHF2}	
pASS2C - BvCYP76AD1- P_{SED1}/P_{TDH3}-BvDOPA5GT	pASS2C	*Bv*CYP76AD1	P_{SED1}/P_{TDH3}	*Bv*DOPAGT
pX3C- EV[a]				
pX3C - P_{FIG1}-ZsGREEN	pX3C	*Zs*GREEN	P_{FIG1}	
pX3C - P_{FIG1}-DOD	pX3C	*Mj*DOD	P_{FIG1}	
pX3C - P_{FIG1}-NanoLuc	pX3C	NanoLuc	P_{FIG1}	
pB-EV[a]	pB			

[a] Forman et al., unpublished, expected publication 2021.

Table S7. USER fragments used for plasmid construction. These fragments were amplified using primers listed in (Table S8)

Fragment	Primer-F	Primer-R	Template
CB2	CB2-F	CB2-R	pYX222-P_{TPI}-CNR2
MFαSS-CB2	MFα-F	CB2-R	pYX222-P_{TPI}-CNR2
GPA1-Gαi1	GPA1-F	GPA1/Gα1-R	yeast genomic DNA
STE2	STE12-F	STE12-R	yeast genomic DNA
BvCYP76AD5	CYP76AD5-F	CYP76AD5-R	Synthetic DNA
BvCYP76AD1	CYP76AD1-F	CYP76AD1-R	Synthetic DNA
ZsGREEN	ZsGREEN-F	ZsGREEN-R	Synthetic DNA
MjDOD	USER-DOD-F	USER-DOD-R	Synthetic DNA
BvDOPA5GT	DOPA5GT-F	DOPA5GT-R	Synthetic DNA
NanoLuc	NanoLuc-F	NanoLuc-R	Synthetic DNA
P_{CCW12}	PCCW12-F	PCCW12-R	yeast genomic DNA
P_{PGK1}	PPGK1-F	PPGK1-R	yeast genomic DNA
P_{RET2}	PRET2-F	PRET2-R	yeast genomic DNA
P_{HHF2}	PHHF2-F	PHHF2-R	yeast genomic DNA
P_{SED1}/P_{TDH3}	PTDH3-F	PSED1-R	yeast genomic DNA
P_{FIG1}	PFIG1-F	PFIG1-R	yeast genomic DNA
STE3 KO	STE3-OL5	STE3-OL3	pUG72
SST2 KO	SST3-OL5	SST3-OL3	pUG72
FAR1 KO	FAR1-OL5	FAR1-OL3	pUG72
STE12 KO	STE12-OL5	STE12-OL3	pUG72
GPA1 KO	GPA1-OL5	GPA1-OL3	pUG72

Table S8. PCR primers used in this work

1	CB2-F	ATCAACGGGUAAAATGGAGGAATGCTGGGTGA
2	CB2-R	CGTGCGAUTTAGCAATCAGAGAGGTCTAGAT
3	MFα-F	ATCAACGGGUAAAATGAGATTTCCTTCAATTTTTACTGCAGTT
4	GPA1 F	ATCAACGGGUAAAATGGGGTGTACAGTGAGTACGCAAA
5	GPA1/Gα1 R	CGTGCGAUTCAAAACAAACCACAATCTTTAAGGTTTTGCTGGATGATTAGA
6	STE12 F	ATCAACGGGUAAAATGAAAGTCCAAATAACCAATAGTAGAAC
7	STE12 R	CGTGCGAUTCAGGTTGCATCTGGAAGGTTTTTAT
8	CYP76AD1F	ATCAACGGGUAAAATGGATCATGCTACTTTGGCTATGA
9	CYP76AD1R	CGTGCGAUCTATTAGTATCTTGGAATGGGGATCAACT
10	CYP76AD5F	ATCAACGGGUAAAATGGATAACACTACCTTGGCTTTGA
11	CYP76AD5R	CGTGCGAUCTATTATTTTCTTGGAACGGGAATAACTTG
12	DOPA5GT-F	AGCGATACGUAAAATGACTGCTATTAAGATGAACACT
13	DOPA5GT-R	CACGCGAUTTATTGTAAAGATGGTTCCAACTTGGCT
14	USER-DOD-F	ATCAACGGGUAAAATGAAGGGTACTTACTACATTAACCA
15	USER-DOD-R	CGTGCGAUTTAATCAGTCTTTTGAGTAGTGGGA
16	NanoLuc-F	ATCAACGGGUAAAATGGTTTTTACTTTGGAAGATTTCG
17	NanoLuc-R	CGTGCGAUTACAGGATCTCTTCGAATAATCTATAACCAGTAACGGAGTTAATGGTGACTC
18	ZsGREEN-F	ATCAACGGGUAAAATGGCTCAATCTAAGCATGGTTTGA
19	ZsGREEN-R	CGTGCGAUTTATGGCAAAGCAGAACCAGAAGC
20	PCCW12-F	CACGCGAUTGAACCACACGGTTAGTCCAAAAGG
21	PCCW12-R	ACCCGTTGAUTATTGATATAGTGTTTAAGCGAATGACAGAAG
22	PPGK1-F	CACGCGAUGTGAGTAAGGAAAGAGTGAGGAACT
23	PPGK1-R	ACCCGTTGAUTGTTTTATATTTGTTGTAAAAAGT
24	PRET2-F	CACGCGAUACGATGGCTTCTTATCTCACTTCAA
25	PRET2-R	ACCCGTTGAUTGTGTATTTCTTTTGATGGAGCTATAG
26	PHHF2-F	CACGCGAUTGTGGAGTGTTTGCTTGGATTCTTTAG
27	PHHF2-R	ACCCGTTGAUTATTTTATTGTATTGATTGTTGTTTTTGCTACTCT
28	PFIG1-F	CACGCGAUGAACTGGTTGATATTATTACTGGTG
29	PFIG1-R	ACCCGTTGAUTTTTTTTTTTTTTTTTTGTTTGTTTGTTTGTTTGTTTAC
30	PTDH3-F	CACGCGAUCAGTTCGAGTTTATCATTATCAATACTGCC
31	PSED1-R	ACCCGTTGAUCTTAATAGAGCGAACGTATTTTATTTTGCTTG
32	STE3-OL5	TAGAGGCAATTAAATTTGTGTAGGAAAGGCAAAATACTATCAAAATTTTCCAGCTGAAGCTTCGTACGC
33	STE3-OL3	GTAAAAATAAAATACTCCTAGTCCAGTAAATATAATGCGACACTCTTGTGGCATAGGCCACTAGTGGATCTG

34	FAR1-OL5	GTCTATAGATCCACTGGAAAGCTTCGTGGGCGTAAGAAGGCAATCTATTACAGCTGAAGCTTCGTACGC
35	FAR1-OL3	TTACGACGCATTATATATATTCAGTCATTGCGTAGTATAGACGTGGAGAAGCATAGGCCACTAGTGGATCTG
36	SST2-OL5	TATCTGAGGCGTTATAGGTTCAATTTGGTAATTAAAGATAGAGTTGTAAGCAGCTGAAGCTTCGTACGC
37	SST2-OL3	AGGACTGTTTGTGCAATTGTACCTGAAGATGAGTAAGACTCTCAATGAAAGCATAGGCCACTAGTGGATCTG
38	STE12-OL5	ATAATGAAAACGATGAAGTCAGTAAAGCTACTCCGGGCGAAGTTGAAGAACAGCTGAAGCTTCGTACGC
39	STE12-OL3	GAATGAGCTCCACCTTCTTCTGACTGGACCACCAAAGTATTGGAATCCGGGCATAGGCCACTAGTGGATCTG
40	GPA1-OL5	AGAACAAAAGAGCCAATGATGTCATCGAGCAATCGTTGCAGCTGGAGAAACAGCTGAAGCTTCGTACGC
41	GPA1-OL3	CTCAATACGAACTTCATAGTTTGGGTATCGGTAGCGCAGGTTCGTTTCACGCATAGGCCACTAGTGGATCTG

Table S9 Sequences of synthetic DNA fragments used in this work.

MfαSS-CNR2	ATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATTAGC TGCTCCAGTCAACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGC TGTCATCGGTTACTCAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGCCATTTT CCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCAGCATTGC TGCTAAAGAAGAAGGGGTATCTCTCGAAAAAAGAGAGGCTGGATCCATGGAGGA ATGCTGGGTGACAGAGATAGCCAATGGCTCCAAGGATGGCTTGGATTCCAACCCT ATGAAGGATTACATGATCCTGAGTGGTCCCCAGAAGACAGCTGTTGCTGTGTTGT GCACTCTTCTGGGCCTGCTAAGTGCCCTGGAGAACGTGGCTGTGCTCTATCTGAT CCTGTCCTCCCACCAACTCCGCCGGAAGCCCTCATACCTGTTCATTGGCAGCTTG GCTGGGGCTGACTTCCTGGCCAGTGTGGTCTTTGCATGCAGCTTTGTGAATTTCCA TGTTTTCCATGGTGTGGATTCCAAGGCTGTCTTCCTGCTGAAGATTGGCAGCGTG ACTATGACCTTCACAGCCTCTGTGGGTAGCCTCCTGCTGACCGCCATTGACCGAT ACCTCTGCCTGCGCTATCCACCTTCCTACAAAGCTCTGCTCACCCGTGGAAGGGC ACTGGTGACCCTGGGCATCATGTGGGTCCTCTCAGCACTAGTCTCCTACCTGCCC CTCATGGGATGGACTTGCTGTCCCAGGCCCTGCTCTGAGCTTTTCCCACTGATCCC CAATGACTACCTGCTGAGCTGGCTCCTGTTCATCGCCTTCCTCTTTTCCGGAATCA TCTACACCTATGGGCATGTTCTCTGGAAGGCCCATCAGCATGTGGCCAGCTTGTC TGGCCACCAGGACAGGCAGGTGCCAGGAATGGCCCGAATGAGGCTGGATGTGAG GTTGGCCAAGACCCTAGGGCTAGTGTTGGCTGTGCTCCTCATCTGTTGGTTCCCA GTGCTGGCCCTCATGGCCCACAGCCTGGCCACTACGCTCAGTGACCAGGTCAAGA AGGCCTTTGCTTTCTGCTCCATGCTGTGCCTCATCAACTCCATGGTCAACCCTGTC ATCTATGCTCTACGGAGTGGAGAGATCCGCTCCTCTGCCCATCACTGCCTGGCTC ACTGGAAGAAGTGTGTGAGGGGCCTTGGGTCAGAGGCAAAAGAAGAAGCCCCG AGATCCTCAGTCACCGAGACAGAGGCTGATGGGAAAATCACTCCGTGGCCAGAT TCCAGAGATCTAGACCTCTCTGATTGCTAA
NanoLuc	ATGGTTTTTACTTTGGAAGATTTCGTTGGTGATTGGGAACAAACTGCTGCTTACA ATTTGGATCAAGTCTTGGAACAAGGTGGTGTCTCTTCTTTATTGCAAAACTTGGCT GTTTCTGTCACCCCAATTCAAAGAATAGTTAGGTCTGGTGAAAACGCCTTGAAGA TCGATATTCATGTTATCATCCCATACGAAGGTTTGTCCGCTGATCAAATGGCTCA AATTGAAGAAGTTTTCAAGGTTGTTTACCCAGTTGATGATCACCACTTCAAGGTT ATTTTGCCATACGGTACTTTGGTTATTGATGGTGTTACTCCCAACATGCTGAATTA CTTTGGTAGACCTTATGAAGGTATCGCCGTTTTTGATGGTAAGAAGATTACTGTT ACTGGCACTTTGTGGAACGGTAACAAGATTATTGACGAAAGGTTGATCACTCCAG ACGGTTCTATGTTATTCAGAGTCACCATTAACTCCGTTACTGGTTATAGATTATTC GAAGAGATCCTGTAA
ZsGreen	ATGGCTCAATCTAAGCATGGTTTGACTAAGGAAATGACTATGAAGTACAGAATG GAAGGTTGTGTTGACGGTCATAAGTTCGTTATCACTGGTGAAGGAATTGGTTATC CATTCAAAGGTAAGCAGGCTATCAACTTGTGTGTTGTTGAAGGTGGTCCATTGCC ATTCGCTGAAGACATCTTGTCTGCTGCTTTCATGTACGGTAACAGAGTCTTCACTG AATACCCACAAGACATTGCTGATTACTTCAAGAACTCTTGTCCAGCTGGTTACAC TTGGGATAGATCCTTCTTGTTTGAAGATGGTGCTGTTTGTATCTGTAATGCTGATA TCACTGTTTCTGTTGAAGAAAACTGTATGTATCATGAATCTAAGTTCTATGGTGTC AACTTCCCAGCTGATGGTCCAGTTATGAAGAAGATGACTGATAACTGGGAACCAT CTTGTGAAAAGATCATTCCAGTTCCAAAGCAAGGTATCTTGAAAGGTGATGTTTC TATGTACTTGCTATTGAAAGATGGTGGTAGATTGAGATGTCAATTCGATACTGTC TACAAAGCTAAGTCTGTTCCAAGAAAGATGCCAGATTGGCATTTCATTCAACATA AGTTGACTAGAGAAGATAGATCTGATGCTAAGAATCAAAAGTGGCATCTAACTG AACATGCTATTGCTTCTGGTTCTGCTTTGCCATAA

BvCYP76AD1	ATGGATCATGCTACTTTGGCTATGATTTTGGCCATCTTGTTCATCTCATTCCACTT CATCAAGCTGTTGTTCTCTCAACAAACCACTAAGTTGTTGCCACCAGGTCCTAAA CCATTGCCAATTATTGGTAACATCTTGGAGGTTGGTAAAAAGCCACATAGATCCT TTGCTAACTTGGCTAAAATTCACGGTCCATTGATCTCATTGAGATTGGGTTCTGTT ACTACCATCGTTGTTTCTTCAGCTGATGTTGCCAAAGAGATGTTCTTGAAAAAGG ATCACCCATTGTCCAACAGAACCATTCCAAATTCTGTTACAGCTGGTGATCATCA CAAGTTGACTATGTCTTGGTTGCCAGTTTCTCCAAAGTGGCGTAATTTCAGAAAG ATTACCGCTGTTCATTTGTTGTCCCCACAAAGATTGGATGCTTGTCAAACTTTTAG ACACGCTAAGGTTCAACAGTTGTACGAATACGTTCAAGAATGTGCTCAAAAAGG TCAAGCCGTTGATATTGGTAAAGCTGCTTTTACTACCTCCTTGAACTTGCTGTCCA AGTTGTTTTTCTCTGTTGAATTGGCCCATCATAAGTCCCATACTTCTCAAGAGTTC AAAGAGTTGATCTGGAACATCATGGAAGATATCGGTAAGCCAAATTACGCTGAT TACTTCCCAATTTTGGGTTGCGTTGATCCATCTGGTATTAGAAGAAGATTGGCTTG CTCTTTCGATAAGTTGATTGCTGTTTTCCAAGGTATCATCTGTGAAAGATTAGCCC CAGATTCTTCTACTACTACAACTACTACCACTGATGATGTTTTGGATGTGTTGTTG CAGTTGTTCAAGCAAAACGAATTGACCATGGGTGAAATCAACCACTTGTTGGTTG ATATTTTCGATGCTGGTACTGATACCACTTCTTCTACATTTGAATGGGTTATGACC GAGTTGATCAGAAACCCAGAAATGATGGAAAAAGCCCAAGAAGAAATCAAGCA GGTTTTGGGTAAAGACAAGCAGATCCAAGAATCCGATATTATCAACTTGCCATAC TTGCAGGCCATCATCAAAGAAACTTTGAGATTGCATCCACCAACCGTTTTTTTGTT GCCAAGAAAAGCTGATACCGATGTTGAGTTGTATGGTTACATCGTTCCAAAGGAT GCCCAAATCTTGGTTAATTTGTGGGCTATTGGTAGAGATCCAAATGCTTGGCAAA ACGCCGATATTTTCTCACCAGAAAGGTTCATTGGTTGCGAAATTGATGTTAAGGG TAGAGACTTTGGCTTGTTGCCATTTGGTGCTGGTAGAAGGATTTGTCCAGGTATG AATTTGGCTATCAGAATGTTGACTTTGATGCTGGCTACTCTGTTGCAATTTTTCAA CTGGAAATTGGAGGGTGACATCTCACCAAAAGATTTGGATATGGACGAAAAGTT CGGTATCGCCTTGCAAAAAACTAAGCCATTGAAGTTGATCCCCATTCCAAGATAC GGTTCTTAG
BvCYP76AD5	ATGGATAACACTACCTTGGCTTTGATTCTGTCCTCTTTGTTCGTTTGCTTCCAATTG ATCAGGTCCTTCATTAACCATGCCAAGAAGTCTAACAAATTGCCACCAGGTCCTA AGAGAATGCCAATTTTTGGTAACATCTTCGACTTGGGTGAAAAGCCACATAGATC CTTTGCTAACTTGGCTAAAATTCACGGTCCATTGGTTTCATTGCAATTGGGTTCTG TTACTACCGTTGTTGTTTCTTCTGCTGATGTTGCCAAAGAAATGTTCTTGAAGAAC GATCAAGCTTTGGCCAACAGAACTATTCCAGATTCTGTTAGAGCTGGTGATCACG ATAAGTTGTCTATGTCTTGGTTGCCAGTTTCTGCTAAATGGCGTAACTTGAGAAA GATTTCTGCTGTCCAACTGTTGTCTACCCAAAGATTGGATGCTTCTCAAGCTCATA GACAATCTAAGGTTCAACAGTTGTTGGAATACGTTCACGATTGCTCTAAAAAGGG TCAACCAGTTGATATTGGTAGAGCTGCTTTTACTACCTCCTTGAACTTGTTGTCTA ACACCTTCTTCTCTGTTGAATTGGCCTCTCATGAATCTTCAGCTTCCCAAGAGTTT AAGCAATTGATGTGGAACATCATGGAAGAAATCGGTAGACCAAATTACGCCGAT TTTTTCCCAATCTTGGGTTACTTAGATCCATTCGGTATCAGAAGAAGATTGGCTGG TTATTTCGACCAATTGATTGCCGTTTTCCAAGACATTATTGGTGAGAGACAAAAG ATCAGATCCGCTAATTTGTCTGGTGGTAAGCAAACTACCAACGATATTTTGGATA CCCTGCTGAACTTGTACGACGAGAAAGAATTATCTATGGGTGAGATCAACCACCT GTTGGTTGATATTTTTGATGCTGGTACTGATACCACTGCTTCTACTTTGGAATGGG CTATGGCTGAATTGGTTAAGAATCCAGATATGATGGTTAAGGTCCAGGACGAAAT TGAACAAGCTATTGGTAAAGGTTGCTCCATGGTTCAAGAATCTGATATCTCTAAG TTGCCATACTTGCAGGCCATTATCAAAGAAACCTTGAGATTGCATCCACCAACCG TTTTTTTGTTGCCTAGAAAAGCTGATGCAGATGTTGAGTTGTATGGTTACGTTGTT CCAAAGAATGCTCAAGTCTTGGTTAACTTGTGGGCTATAGGTAGAGATCCAAAGG TTTGGAAAAACCCAGAAGTTTTCTCACCAGAAAGGTTCTTGGAATCCAACATTGA TTACAAGGGTAGAGACTTTGAGTTGTTGCCATTTGGTGCTGGTAGAAGAATTTGT CCAGGTTTGACTTTGGCTTACAGGATGTTGAATTTGATGATGGCCAACTTCTTGCA CTCTTACGATTGGAAATTGGAAGATGGTATGCACCCTAAGGATTTGGATATGGAC

	GAAAAGTTTGGTATCACCTTGCAAAAGGTTAAGCCATTGCAAGTTATTCCCGTTC CAAGAAAATAG
MjDOD	ATGAAGGGTACTTACTACATTAACCATGGTGACCCATTGATGTACTTGAAGAAGC ACATTAAGTTGAGGCAGTTTTTGGAAGGTTGGCAAGAAAACGTTGTTATCGAAAA GCCAAAGTCCATCTTGATTATTTCTGCTCATTGGGATACCAACGTTCCAACTGTTA ATTTCGTTGAACATTGCGATACCATCCACGATTTTGATGATTACCCAGATCCACTG TACCAAATTCAGTATAGAGCCCCAGGTGCTCCTAATTTGGCTAAAAAAGTTGAAG AGTTGCTGAAAGAATCCGGTATGGAATGTGAAATCGATACCAAAAGAGGTTTGG ATCACGCTGCTTGGTTTCCACTAATGTTTATGTATCCAGAAGCCAACATTCCAATC TGCGAATTGTCTGTTCAACCATCCAAAGATGGTATCCATCATTACAATGTTGGTA AGGCTTTGTCCCCATTATTGCAACAAGGTGTTTTGATTATTGGCTCCGGTGGTACT GTTCATCCATCTGATGATACTCCACATTGTCCAAATGGTGTTGCTCCATGGGCTAT TGAATTTGATAATTGGTTGGAAGATGCCTTGTTGTCCGGTAGATATGAAGATGTC AACAACTTCAAAAAGTTGGCCCCAAACTGGGAAATTTCTCATCCAGGTCAAGAA CACTTGTACCCATTGCATGTTGCTTTGGGTGCTGCTGGTAAAAATCCAAAGACTC AATTGATCCATAGATCCTGGGCTGCTAATGGTGTTTTTGGTTACTCTACTTACAAC TTCACTCCCACTACTCAAAAGACTGATGGTTCATAA
BvDOPA5GT	ATGACTGCTATTAAGATGAACACTAATGGTGAAGGTGAAACCCAACACATTTTGA TGATTCCATTCATGGCTCAAGGTCATTTGAGGCCATTTTTGGAATTGGCTATGTTC CTGTACAAGAGATCCCATGTTATTATCACCTTGTTGACTACTCCATTGAACGCTGG TTTTTTGAGACATTTGTTGCATCACCACTCCTACTCTTCATCTGGTATTAGAATAG TCGAGCTGCCATTCAATTCTACCAATCATGGTTTGCCACCAGGTATTGAAAACAC TGATAAGTTGACTTTGCCCCTGGTTGTTTCTTTGTTCCATTCCACCATTTCTTTGGA CCCACATTTGAGAGATTACATCTCCAGACATTTTTCACCAGCTAGACCACCATTG TGCGTTATTCATGATGTTTTCTTAGGTTGGGTTGATCAAGTTGCTAAGGATGTTGG TTCTACTGGTGTTGTTTTTACTACTGGTGGTGCTTATGGTACTTCTGCCTATGTTTC TATTTGGAACGATTTGCCACACCAGAACTACTCTGATGATCAAGAATTTCCATTG CCAGGTTTTCCAGAAAACCATAAGTTCAGAAGATCCCAGTTGCACAGATTTTTGA GATATGCTGATGGTTCCGATGACTGGTCTAAATACTTTCAACCACAATTGAGGCA GTCCATGAAGTCTTTTGGTTGGTTGTGTAACTCCGTGGAAGAAATTGAAACTTTG GGCTTCTCCATCTTGAGAAACTACACTAAGTTGCCAATTTGGGGTATTGGTCCATT GATTGCTTCTCCAGTTCAACATTCTTCCTCCGATAACAATTCTACAGGTGCCGAAT TTGTTCAGTGGTTGTCTTTGAAAGAACCAGACTCTGTCTTGTACATCTCATTCGGT TCTCAAAACACTATCTCCCCAACTCAAATGATGGAATTAGCTGCTGGTTTGGAAT CCTCTGAAAAACCATTTTTGTGGGTTATTAGAGCCCCATTCGGTTTCGATATTAAC GAAGAAATGAGGCCAGAATGGTTGCCAGAAGGTTTTGAAGAAAGAATGAAGGTC AAGAAGCAGGGCAAATTGGTTTACAAATTAGGTCCACAGCTGGAAATCTTGAAC CACGAATCTATTGGTGGTTTCTTGACTCATTGCGGTTGGAACTCTATTTTGGAGTC TTTGAGAGAAGGTGTCCCAATGTTAGGTTGGCCATTGGCTGCTGAACAAGCTTAC AATTTGAAATACTTGGAGGACGAAATGGGTGTTGCTGTTGAATTAGCTAGAGGTT TGGAGGGTGAAATCTCTAAAGAAAAGGTTAAGAGGATCGTCGAGATGATCTTGG AAAGAAACGAAGGTTCTAAAGGTTGGGAAATGAAGAACAGAGCTGTTGAGATGG GTAAAAAGTTGAAGGATGCCGTTAACGAGGAAAAAGAATTGAAAGGTTCTTCCG TTAAGGCCATCGATGATTTTTTGGATGCTGTTATGCAAGCCAAGTTGGAACCATC TTTACAATAA