molecules
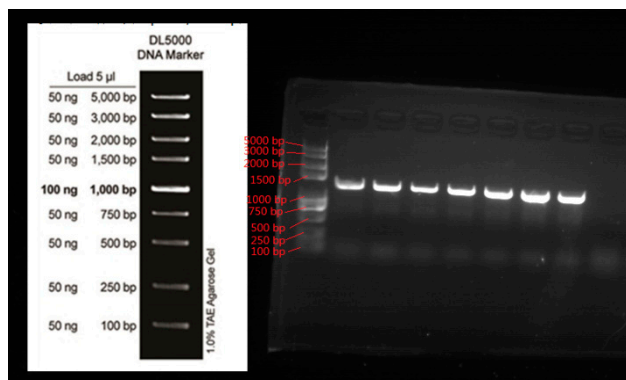
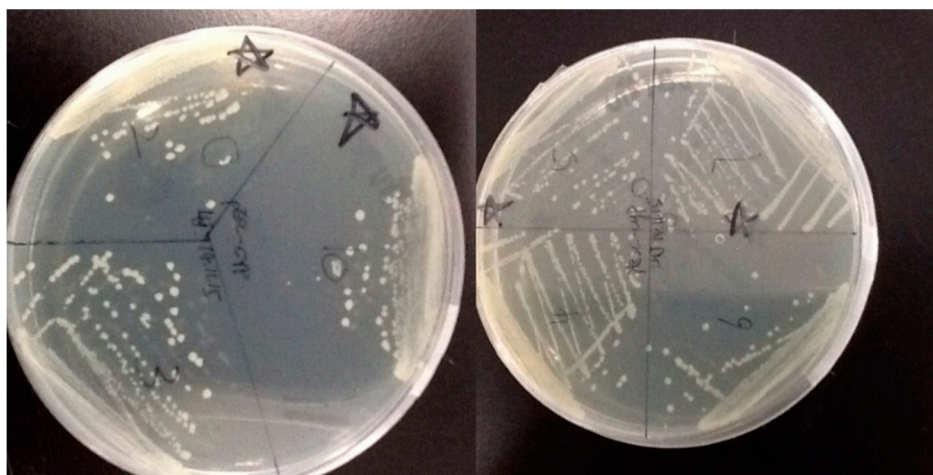# Supplementary Materials (Figures and Tables)

Figure S1. Agarose gel electrophoresis results of CYP716A12.

Figure S2. Agarose gel electrophoresis results of ATR1.

Figure S3. Purification and separation of a pESC-ura-CYP716A12 DH5α single colony.
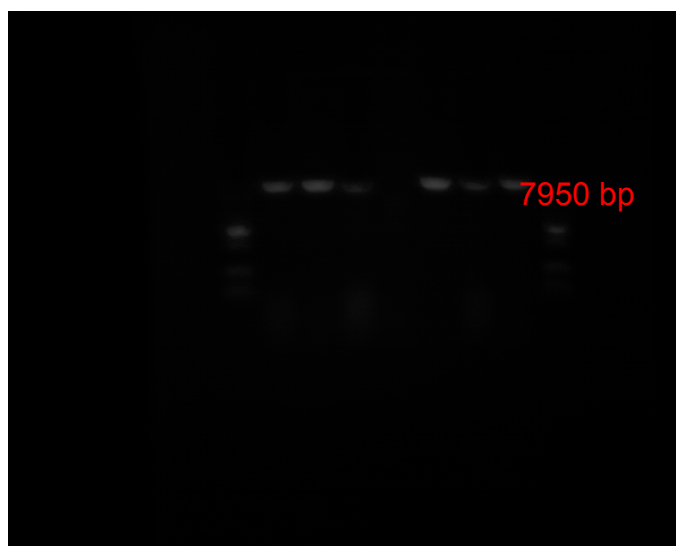
8

9   **Figure S4.** Purification and separation of a pESC-ura-CYP716A12-ATR1 DH5α single colony.
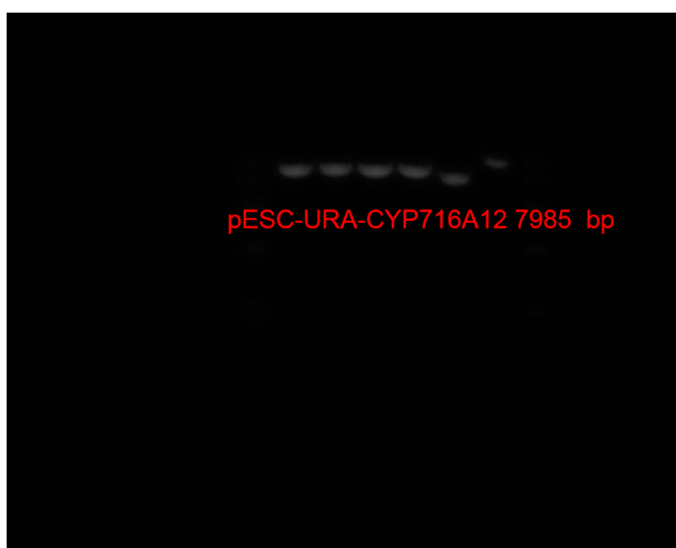

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11   **Figure S5.** Agarose gel electrophoresis results of the added concentration of vector and targeting gene.

12

13   **Figure S6.** Colony PCR of a pESC-ura-CYP716A12 DH5α single colony.

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15   **Figure S7.** Agarose gel electrophoresis results of plasmid from pESC-ura-CYP716A12 DH5α.
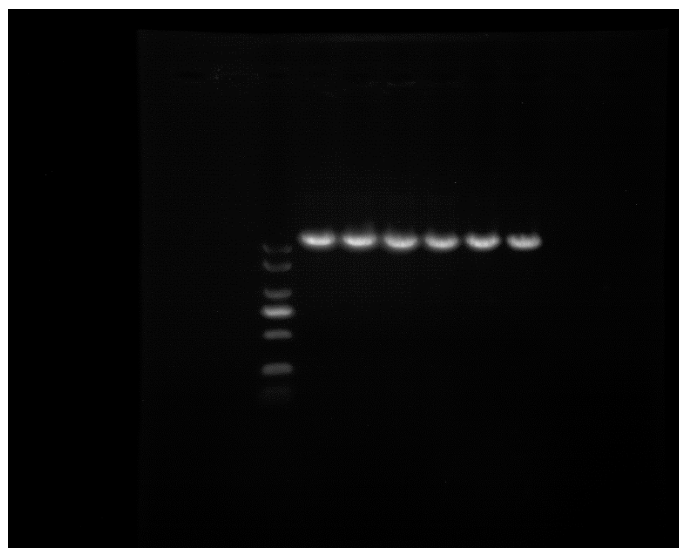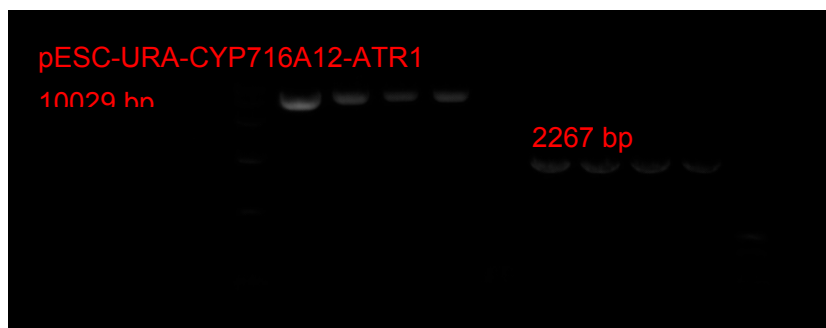
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18　　　　　　　**Figure S8.** Agarose gel electrophoresis results of linearized pESC-ura-CYP716A12.
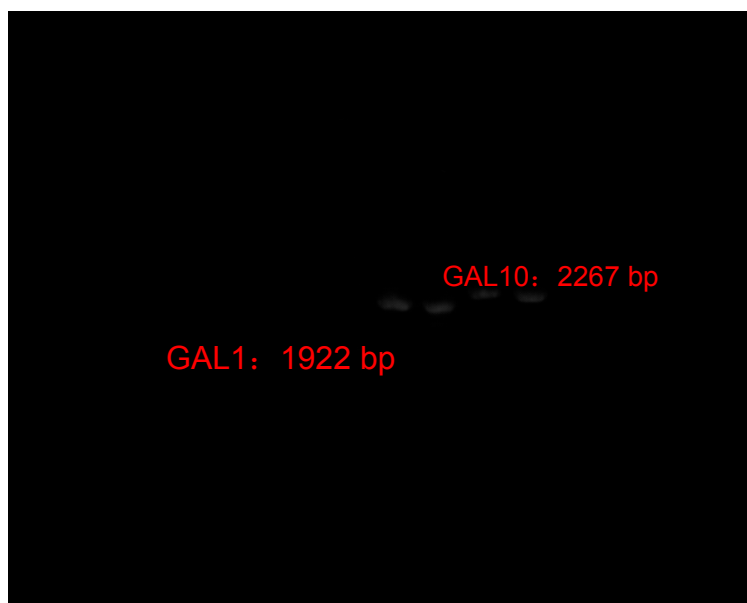

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20　　　　　　　**Figure S9.** Colony PCR of pESC-ura-CYP716A12-ATR1 DH5α.

21

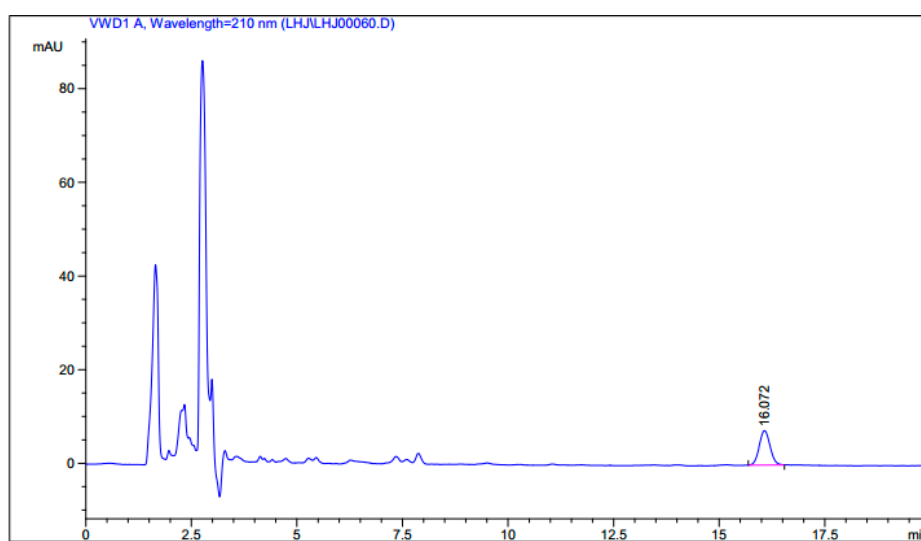22　　　　　**Figure S10.** Agarose gel electrophoresis results of plasmid from pESC-ura-CYP716A12-ATR1 DH5α.

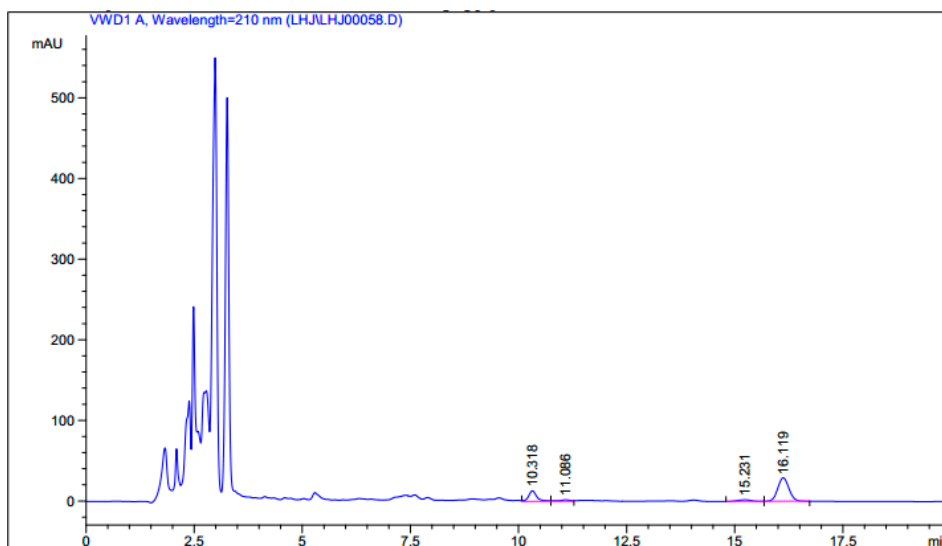23

24   **Figure S11.** Agarose gel electrophoresis results of specific primer PCR of plasmid from *Saccharomyces*
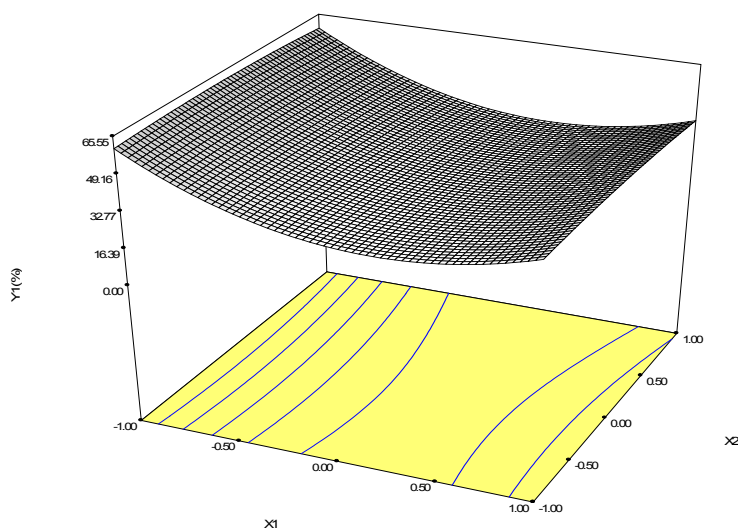25   *cerevisiae* ZJUQH311.

26

27   **Figure S12.** HPLC chromatograms of microsome protein from the total protein extraction.
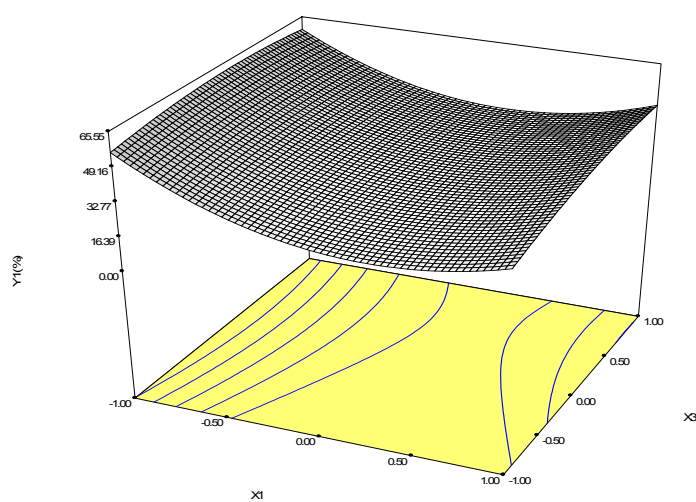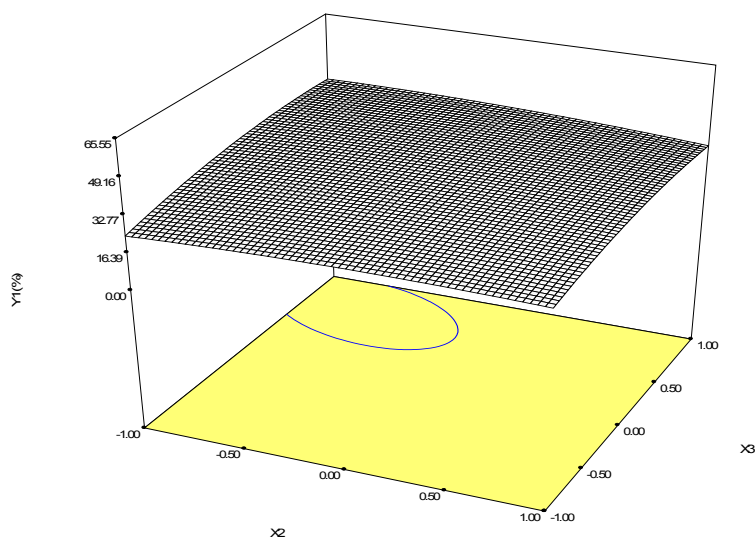
28

29 **Figure S13.** HPLC chromatograms of microsome protein from the microsome protein extraction.
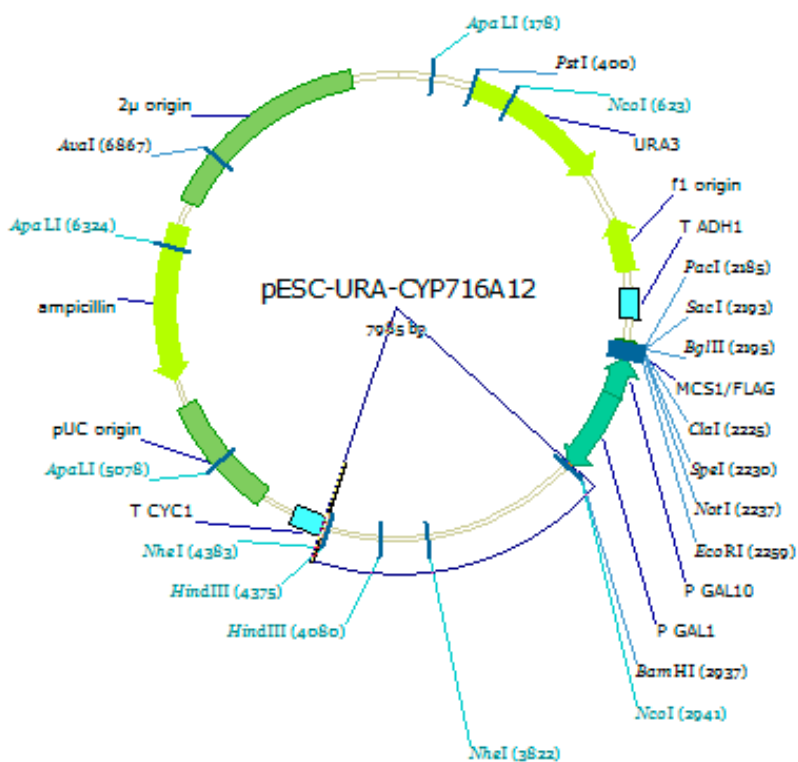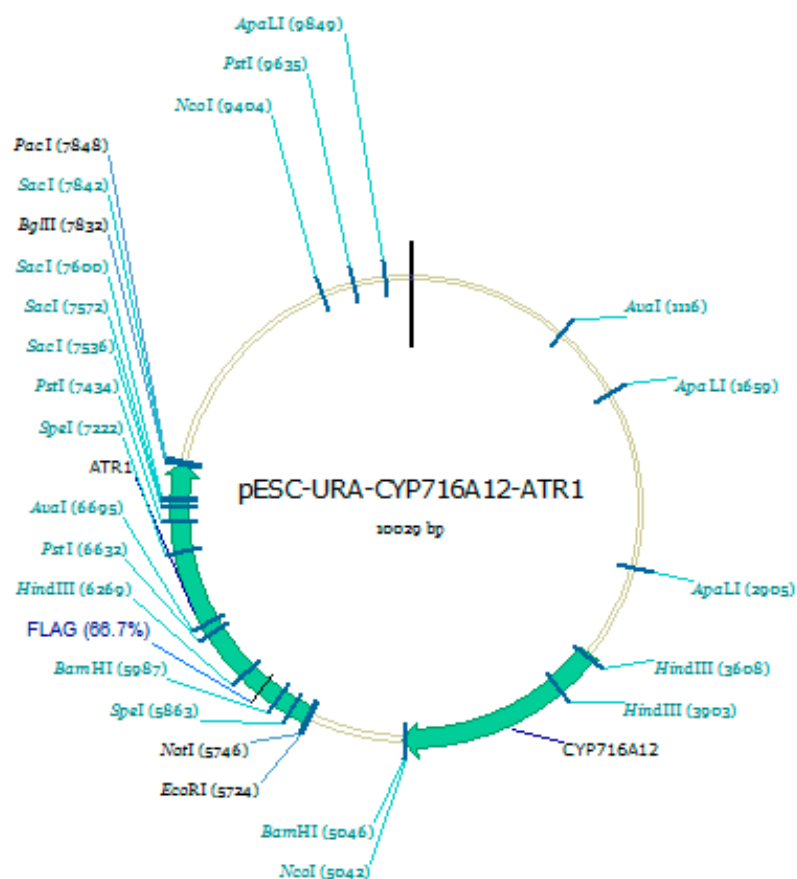
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33 **Figure S14.** The response surface plot of conversion time (X₁), betulin (X₂), and NADPH (X₃) against
34 betulin conversion.

35

36 **Figure S15.** Schematic diagram of pESC-ura-CYP716A12.

Figure S16. Schematic diagram of pESC-ura-CYP716A12-ATR1.

Table S1. Results of the response surface methodology regression analysis for betulin conversion (Y_1).

Source	Sum of squares	DF	Mean Square	F value	Prob > F
Model	2269.72	9	252.19	9.74	0.0033
X_1	645.31	1	645.31	24.92	0.0016
X_2	11.08	1	11.08	0.43	0.5340
X_3	113.74	1	113.74	4.39	0.0743
X_1^2	1430.38	1	1430.38	55.23	0.0001
X_2^2	13.18	1	13.18	0.51	0.4987
X_3^2	61.40	1	61.40	2.37	0.1675
X_1X_2	1.15	1	1.15	0.04	0.8394
X_1X_3	18.11	1	18.11	0.70	0.4307
X_2X_3	11.82	1	11.82	0.46	0.5210
Residual	181.29	7	25.90		
Lack of Fit	148.86	3	49.62	6.12	0.0563
Pure error	32.43	4	8.11		
Cor Total	2451.01	16			