

## Supplementary file 1

# Designing a new generation of expression toolkits for engineering of green microalgae; robust production of human interleukin-2

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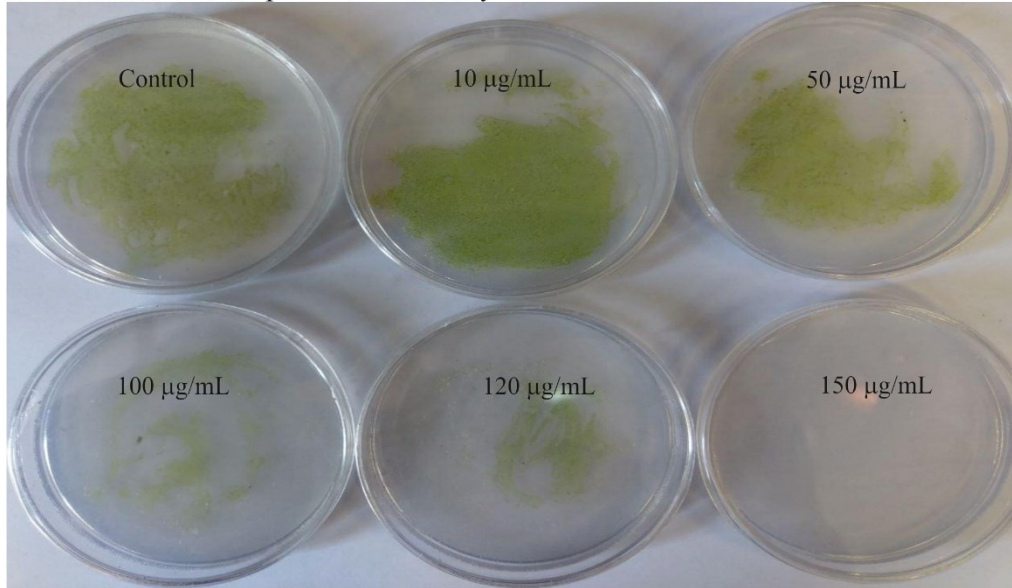
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**Table S1.** The results of the ELISA assay from the total extracted soluble proteins from the transformed and untransformed *C. reinhardtii* cells.

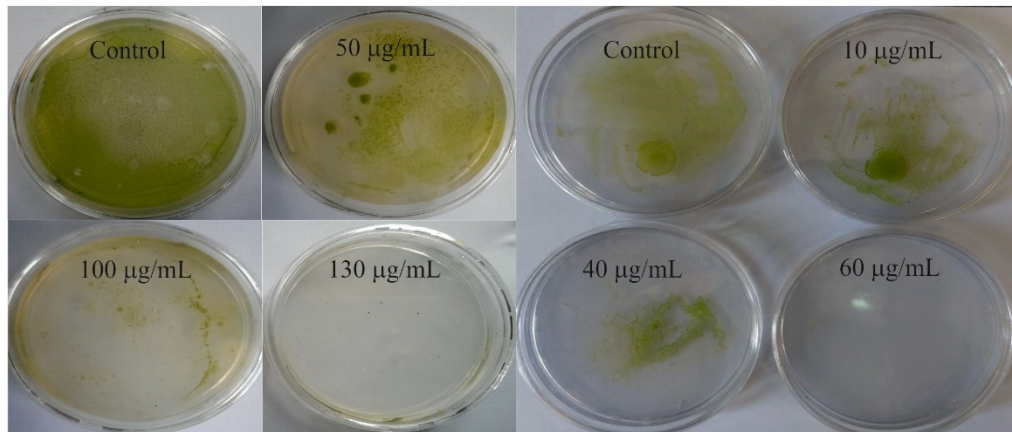
	Blank			Sample 1			Sample 2			Sample 3		
	B-1	B-2	B-3	S1-1	S-1-2	S1-3	S2-1	S2-2	S2-3	S3-1	S3-2	S3-3
<b>OD (nm)</b>	0.079	0.093	0.086	0.113	0.11	0.114	0.155	0.154	0.156	0.198	0.203	0.202
<b>IL-2 Concentration (pg/mL)</b>	0	0	0	5.0526	4.2631	5.3157	16.1052	15.8421	16.3684	27.421	28.7368	28.4736

*C. reinhardtii* chloramphenicol sensitivity



*C. vulgaris* chloramphenicol sensitivity

*D. salina* chloramphenicol sensitivity



**Fig. S1.** Evaluating the sensitivity of the *C. reinhardtii*, *C. vulgaris*, and *D. salina* to the chloramphenicol for the selection of the transformants.

TGGCAGGATATATTGTGGTGTAAACA<sup>>LB</sup>GCAAATTACATATGTCTGCGTGACGGCGGGGA  
 GCTCGCTGAGGCTTGACATGATTGGTGCATGTTTGTATGAAGCTACAGGACTGATTT  
 GGCGGGCTATGAGGGCGGGGAAGCTCTGGAAGGGCCGCGATGGGGCGCGCGCGT  
 CCAGAAGGCGCCATACGGCCCGCTGGCGGCACCCATCCGGTATAAAAGCCCGCGACCCC  
 GAACGGTGACCTCCACTTTCAGCGACAAACGAGCACTTATACATACGCGACTATTCTGCC  
 GCTATACATAACCACTCAGCTAGCTTAAGAGTCAGTAAAC<sup>>hsp70</sup>GTGAGTCGACGAGCAAGCC  
 CGGCGGATCAGGCAGCGTGCTTGCAGATTTGACTTGCAACGCCCGCATTGTGTCGACGA  
 AGGCTTTGGCTCCTCTGTCGCTGTCTCAAGCAGCATCTAACCTGCGTCGCCGTTTCCAT  
<sup>>Int-1</sup>  
 TTGCAGTCCCATCAAGCTTGCATGCCGGGCGCGCCAGAAGGAGCGCAGCCAAACCAGG  
 ATGATGTTTGATGGGGTATTTGAGCACTTGCAACCCTTATCCGGAAGCCCCCTGGCCAC  
 AAAGGCTAGGCGCCAATGCAAGCAGTTCGCATGCAGCCCCTGGAGCGGTGCCCTCCTGA  
<sup>>rbcS2</sup>  
 TAAACCGGCCAGGGGCCTATGTTCTTTACTTTTTACAAGAGAAGTCACTCAACATCTT  
<sup>>5-UTR</sup>  
 AAA

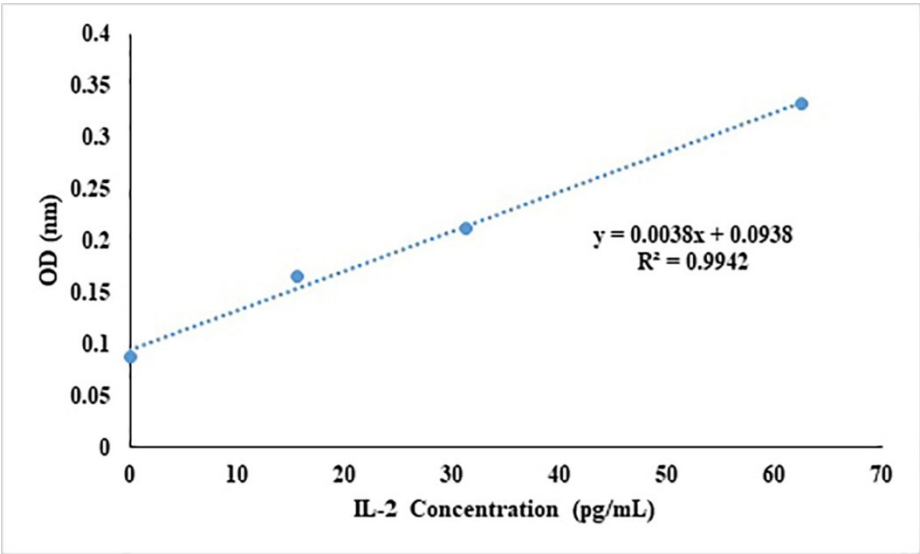
**Fig. S2.** The sequence of the promoter cassette composed of hsp70 and rbcS2 regulatory elements, Int-1, and 5-UTR of the rbcS2 gene. The elements were determined in different colors.

>Sc      >Kozak  
 ATG**GCCG**CGAGAAGAAGATCACCGGCTACACCACCGTGGACATCTCCCAGTGGCACCG  
 CAAGGAGCACTTCGAGGCGTTCCAGTCCGTGGCCCAGTGCACCTACAACCAGACCGTGC  
 AGCTGGACATCACCGCCTTCTTGAAGACCGTGAAGAAGAACAAGCACAAGTTCTACCCC  
 GCCTTCATCCACATCCTGGCCCGCCTGATGAACGCCACCCCGAGTTCCGCATGGCCATG  
 AAGGACGGCGAGCTGGTGATCTGGGACAGCGTGCACCCCTGCTACACCGTGTTCACGA  
 GCAGACCGAGACCTTCTCCTCGCTGTGGAGCGAGTACCACGACGACTTCGCCAGTTCTT  
 GCACATCTACTCGCAGGACGTGGCCTGCTACGGCGAGAACCTGGCCTACTTCCCAAGG  
 GCTTCATCGAGAACATGTTCTTCGTGTCGGCCAACCCCTGGGTGAGCTTCACCAGCTTCG  
 ACTTGAACGTGGCCAACATGGACAATTCTTCGCCCCCGTGTTACCATGGGCAAGTACT  
 ACACCCAGGGCGACAAGGTGCTGATGCCCTGGCCATCCAGGTGCACCATGCCGTGTGC  
 GACGGCTTCCACGTGGGCAGGATGCTGAACGAGTTGCAGCAGTACTGCGACGAGTGGC  
 AGGGCGGCGCC**GGCTCCGGCGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGT**  
**GGACGAGAACCCCGGCCCGAGGGCAGGGGCAGCCTGCTGACCTGCGGCGACGTGGA**  
**GGAGAACCCCGGCC** >Hybrid 2A

**Fig. S3.** The detailed sequences of the Kozak, *CmR*, and 2A peptide. The elements were determined in different colors. Sc: Start codon, *CmR*: Coding sequence of the chloramphenicol acetyltransferase gene.

>XhoI  
 CTCGAGTACAGGATGCAGCTGCTGTCCTGCATCGCCCTGAGCCTGGCCCTGGTGACCAA  
 CAGCGCCCCACCTCGAGCTCCACCAAGAAGACCCAGCTGCAGCTGGAGCACTTGCTGC  
 >Ex-1  
 TGGACTTGCAGATGATCTTGAACGGCATCAACGTGAGTCGACGAGCAAGCCCGGCGGA  
 TCAGGCAGCGTGCTTGCAGATTTGACTTGCAACGCCGCATTGTGTGACGAAGGCTTTT  
 >Int-1  
 GGCTCCTCTGTCGCTGTCTCAAGCAGCATCTAACCTGCGTCGCCGTTTCCATTTGCAGA  
 >Ex-2  
 ACTACAAGAACCCCAAGCTGACCAGGATGCTGACCTTCAAGTTCTACATGCCCAAGGTG  
 AGTCGACGAGCAAGCCCGGCGGATCAGGCAGCGTGCTTGCAGATTTGACTTGCAACGC  
 CCGCATTGTGTGACGAAGGCTTTTGGCTCCTCTGTCGCTGTCTCAAGCAGCATCTAAC  
 >Int-1  
 CTGCGTCGCCGTTTCCATTTGCAGGCCACCGAGCTGAAGCACCTGCAGTGCCTGGAGGA  
 GGAGCTGAAGCCCCTGGAGGAGGTGCTGAACTTGGCCCAGAGCAAGAAGCTTCCACTTG  
 >Ex-3  
 AGGCCAGGGACTTGATCAGCAACATCAACGTGATCGTGCTGGAGCTGAAGGGCTCCG  
 AGACCACCTTCATGTGCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTCCTGAAC  
 >Ex-4  
 AGGTGGATCACCTTCTGCCAGAGCATCATCTCCACCCTGACCACCACCACCACCAC  
 >His  
 >Stc >BamHI  
 TAAGGATCGTAAGTCTGGCGAGAGCCCGACGGGTCCACTGTGGCACTGGGTTAGCTTT  
 TGGCACACGGGTCCACTGTGGCACTGGTTAGCTTGGCACCGGGACAGCGCCTATCTCAC  
 CGCGGGGAACTGACGCATACCCCTGCTCGTGCTTCAGCACGGAAAAGCAAGGGGCCCA  
 ATTCATCTTTGGTGGTTCTGTGCGCTGGTGACTGAACTCTTCTCCCTCCATTCCCGT  
 >Int-3  
 GCGCCCGCAG

**Fig. S4.** The detailed sequences of the microalgae expression cassette. The elements were determined in different colors. Ex-1-4: Exons 1-4 of the hIL-2, His: Histidine tag, Stc: Stop codon.



**Fig. S5.** The standard curve of the hIL-2 based on the four elution reactions.