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A novel and more efficient biosynthesis approach for human insulin production in *Escherichia coli* (*E. coli*).

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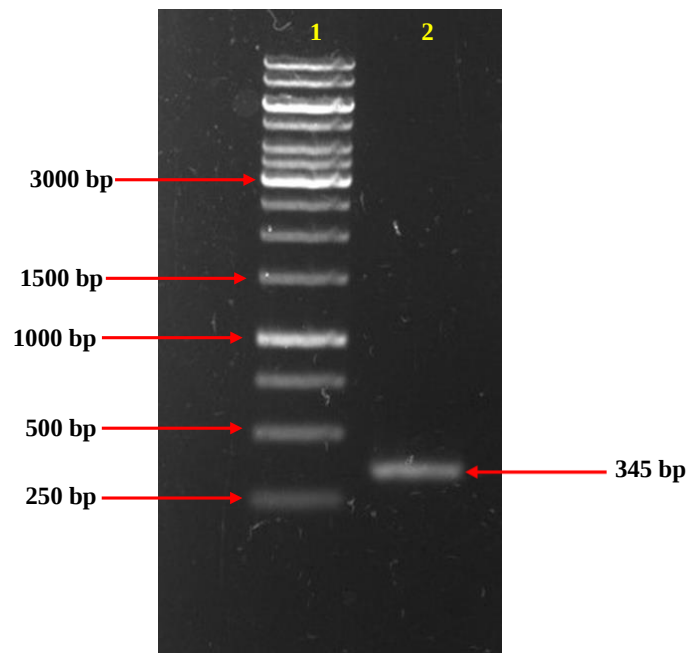


Figure S1a: An image depicting a purified PCR product of human proinsulin gene. Lane 1 contains the one kb molecular weight marker, and lane 2 contains the purified *Bam*HI, and an *Xho*I ended PCR product of human proinsulin gene (345 bp).

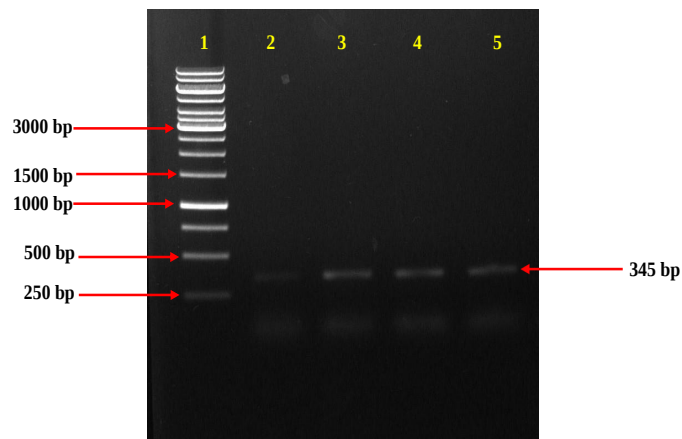


Figure S1b: An image displaying colony PCR products of positive transformants obtained from clones that contain the pET21b-hPin vector. Lane 1 contains the one kb molecular weight marker, and lanes 2-5 contain colony PCR amplicons (345 bp).

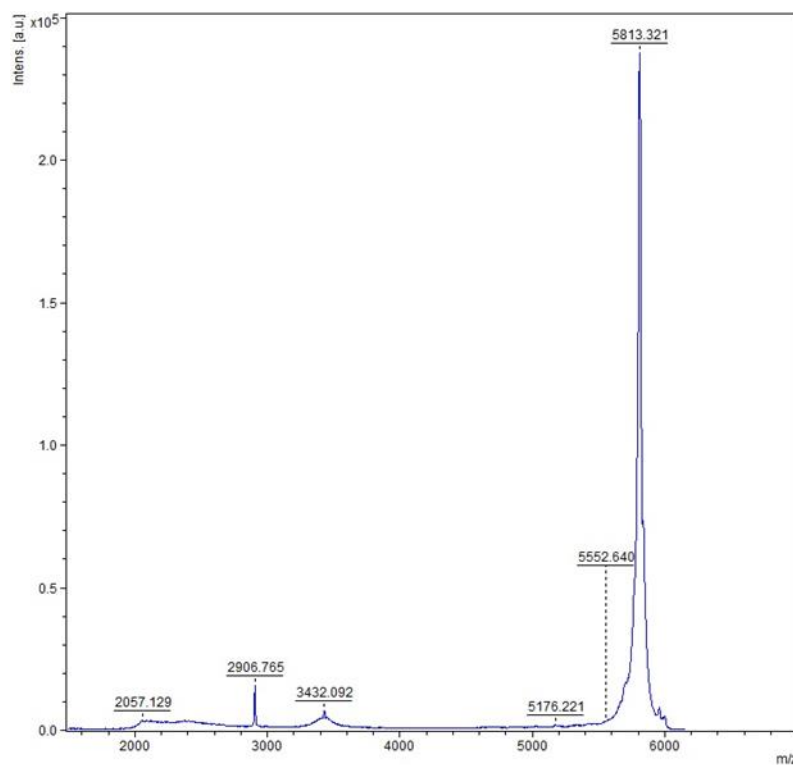


Figure S2: An image depicting a MALDI-TOF spectrum of standard human insulin as a positive control.

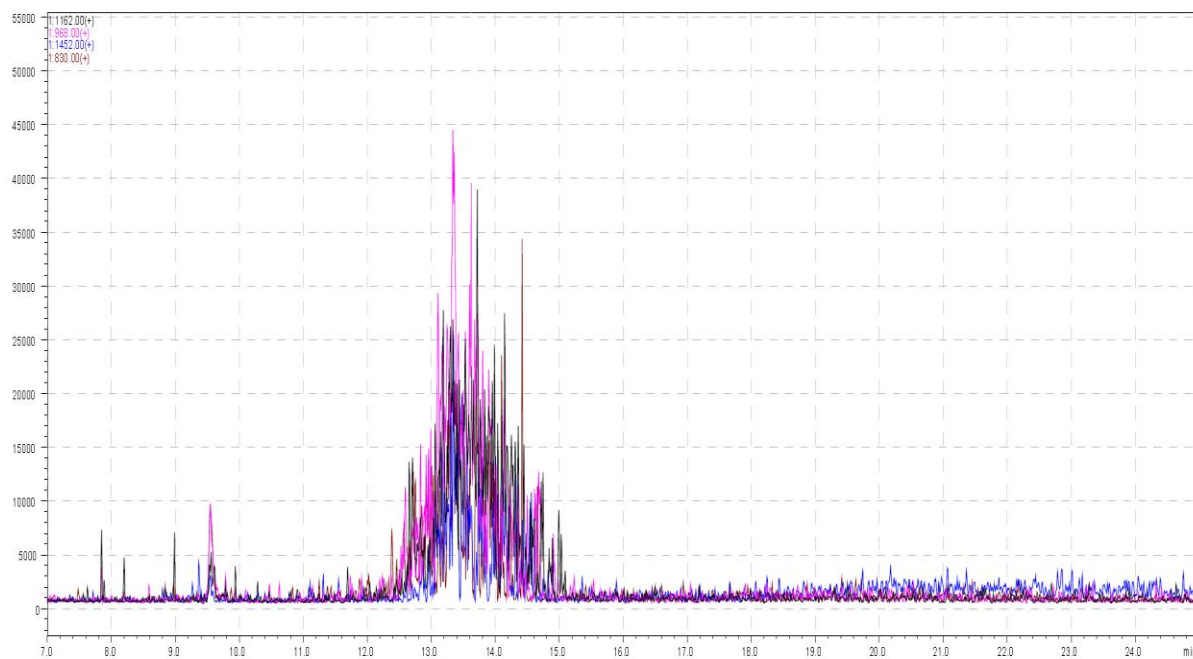


Figure S3: A LC-MS chromatogram illustrating 1 mM IPTG induced biosynthesised human insulin sample.

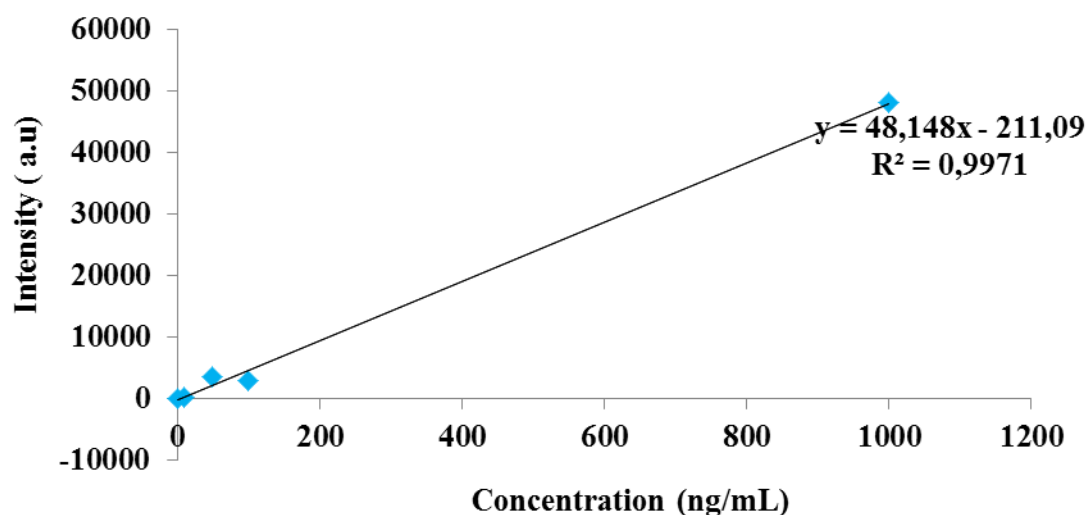


Figure S4: A MALDI-TOF standard curve of the human insulin standard at 0 ng, 10 ng, 50 ng, 100 ng, and 1000 ng.

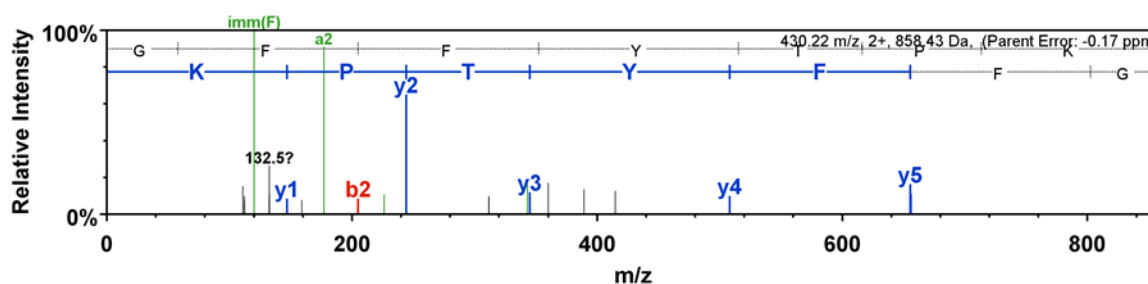


Figure S5: A peptide spectrum illustrating the protein sequence of standard human insulin, which was 90% similar to the human insulin sequence derived from Scaffold 1.4.4 software.

Table S1: Peptide fragmentation table of crude biosynthesised human insulin, which yielded a 100% match to human insulin in the protein database.

B	B Ions	B + 2H	B-NH3	B-H2O	AA	Y Ions	Y + 2H	Y-NH3	Y-H2O	Y
1	58.0	29.5			G	1702.6	851.9	1685.8	1684.8	15
2	145.1	73.0		127.1	S	1645.8	823.4	1628.8	1627.8	14
3	282.1	141.6		264.1	H	1558.8	779.9	1541.8	1540.8	13
4	395.2	198.1		377.2	L	1421.7	711.4	1404.7	1403.7	12
5	494.3	247.6		476.3	V	1308.7	654.8	1291.6	1290.7	11
6	623.3	312.2		605.3	E	1209.6	605.3	1192.6	1191.6	10
7	694.4	347.7		676.3	A	1080.6	540.8	1063.5	1062.5	9
8	807.4	404.2		789.4	L	1009.5	505.3	992.5	991.5	8
9	970.5	485.8		952.5	Y	896.4	448.7	879.4	878.4	7
10	1083.6	542.3		1065.6	L	733.4	367.2	716.3	715.4	6
11	1182.7	591.8		1164.6	V	620.3	310.6	603.3	602.3	5
12	1342.7	671.8		1324.7	C + 57	521.2	261.1	504.2	503.2	4
13	1399.7	700.4		1381.7	G	361.2	181.1	344.2	343.2	3
14	1528.7	764.9		1510.7	E	304.2	152.6	287.1	286.2	2
15	1702.9	851.9	1685.8	1684.8	R	175.1	88.1	158.1		1