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Supporting Information

Nanoscale Advances

Magnetic Nanoparticles Mediated-Gene Delivery for Simpler and More

Effective Transformation of Pichia pastoris

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Table S 1. Primer sequences used in this study

Specific Primers	Name	Sequencing
Gene	GFP Forward GFP Reverse	5'-CGCGAATTCATGTCTAAAGGTGAAGA-3' 5'-GGGGTACCTTTGTACAATTCATCCAT-3'
Vector	pGAP Forward 3'AOX1	5'-GTCCCTATTTCAATCAATTGAA-3' 5'-GCAAATGGCATTCTGACATCC-3'



Fig S 1. The photos of the time-dependent magnetic accumulation of non-acidified and acidified Fe₃O₄@PEI MNPs after 0, 5, 15 and 55 seconds.



Fig S 2. A. TEM image of non-acidified Fe $_3O_4@PEI$ MNPs and B. SEM image of Fe $_3O_4@PEI$ MNPs.



Fig S 3. Agarose gel electrophoresis results belong to **A**) the GFP amplification by PCR (1: reaction with 50 mM MgCl₂ 2: reaction without MgCl₂), the colony PCR from selected *E. coli* colonies with **B**) gene-specific primers (1-8: numbers of colonies) and **C**) vector-specific primers (1-6: numbers of colonies).

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Sequence ID: Query_59613 Length: 1186 Number of Matches: 1

Range 1: 170 to 883 Graphics

Vext Match

Score	its(71	4)	Expect	Identities 714/714(100%)	Gaps 0/714(0%)	Strand Plus/Minu	JS
Query	1	ATGTCTA	AGGTGAAG	AATIGIICACIGGIGII	STICCAALTIGGTIGAA	TIGGATOOT	60
Christ	1						00
SDJCT	883	AIGICIA	AAGGTGAAG	AATIGITCACIGGIGITC	GITCCAATTIIGGITGAA	TIGGATGGT	824
Query	61	GATGTTA/	ATGGTCAAA	AATTCTCTGTTTCTGGT	5AGGGTGAAGGTGATGCA	ACATACGGT	120
Sbjct	823	GATGTTA	ATGGTCAAA	AATTCTCTGTTTCTGGT	SAGGGTGAAGGTGATGCA	ACATACGGT	764
Query	121	AAATTGAO	CTTGAAAT	TTATTTGCACTACTGGT/	AAGTTGCCTGTTCCATGG	CCAACATTG	180
Sbjct	763	AAATTGA	CTTGAAAT	TTATTTGCACTACTGGT/	AAGTTGCCTGTTCCATGG	CCAACATTG	704
Query	181	GTTACTAC	CTTTCTCTT	ATGGTGTTCAATGCTTT	TCTAGATACCCAGATCAT	ATGAAACAG	240
Sbjct	703	GTTACTAC	CTTTCTCTT	ATGGTGTTCAATGCTTT	TCTAGATACCCAGATCAT	ATGAAACAG	644
Query	241	CATGACT	TTTTCAAGT	CTGCCATGCCCGAAGGT	TATGTTCAGGAAAGAACT	ATATTTTAC	300
Sbjct	643	CATGACT	TTTTCAAGT	CTGCCATGCCCGAAGGT	TATGTTCAGGAAAGAACT	ATATTTTAC	584
Query	301	AAAGATG	ACGGTAACT	ACAAGACACGTGCTGAAG	STTAAGTTTGAAGGTGAT	ACCTTGGTT	360
Sbjct	583	AAAGATG	ACGGTAACT	ACAAGACACGTGCTGAAG	STTAAGTTTGAAGGTGAT	ACCTTGGTT	524
Query	361	AATAGAAT	TCGAGTTGA	AAGGTATTGATTTTAAAG	SAAGATGGTAACATTTTG	GGTCACAAA	420
Sbjct	523	AATAGAAT	TCGAGTTGA	AAGGTATTGATTTTAAAG	SAAGATGGTAACATTTTG	GGTCACAAA	464
Query	421	ATGGAATA		ACTCTCATAATGTTTAC	ATCATGGCAGACAAACCA	AAGAATGGT	480
Sbjct	463	ATGGAAT	ACAACTATA	ACTCTCATAATGTTTAC	ATCATGGCAGACAAACCA	AAGAATGGT	404
Query	481	ATCAAAG	TTAACTTCA	AAATTAGACACAACATT/	AAAGATGGTTCTGTTCAA	TTGGCAGAC	540
Sbjct	403	ATCAAAG	TTAACTTCA	AAATTAGACACAACATT	AAAGATGGTTCTGTTCAA	TTGGCAGAC	344
Query	541	CATTATCA	ААСААААТА	CTCCAATTGGTGATGGT	CTGTTTTGTTGCCAGAC	AACCATTAC	600
Sbjct	343	CATTATC/	AACAAAATA	CTCCAATTGGTGATGGTG	CTGTTTTGTTGCCAGAC	AACCATTAC	284
Query	601	TTGTCTA	CACAATCTO	CCTTGTCTAAAGATCCC/	AACGAAAAGAGAGATCAC	ATGATCTTG	660
Sbjct	283	TTGTCTAG	CACAATCTO	CCTTGTCTAAAGATCCC	AACGAAAAGAGAGATCAC	ATGATCTTG	224
Query	661	TTGGAGT	TTGTTACAG	CTGCTGGTATTACACATO	GTATGGATGAATTGTAC	AAA 714	
Sbjct	223	TTGGAGT	TTGTTACAG	CTGCTGGTATTACACATO	GTATGGATGAATTGTAC	AAA 170	

Fig S 4. Nucleotide BLAST analysis result of pGKB-GFP expression cassette (sequencing with pGAP forward and 3'AOX1 primer)



Fig S 5. Construction map of pGKB-GFP which is used in this study (vector map was visualized by SnapGene (SnapGene[®] software, www.snapgene.com).



Fig S 6. A) Growing of the cells exposed to $Fe_3O_4@PEI$ MNPs and magnetofectins prepared with both pGKB and pGKB-GFP ($Fe_3O_4@PEI+pGKB+PEI$ and $Fe_3O_4@PEI+pGKB-GFP+PEI$) in YPD agar and, **B)** Second selection of the transformed cells in YPD plates containing geneticin (G418, 200 µg/mL).



Fig S 7. DNA ladder used in the study (GeneRuler 100 bp Plus DNA Ladder, Thermo Scientific™).