

Saccharomyces cerevisiae as a host for chondroitin production

Márcia R. Couto, Joana L. Rodrigues, Oscar Dias and Lígia R. Rodrigues

Table S1. Primers used in this study.

Purpose	Name	Template	Sequence (5' → 3')	Restriction enzyme
Cloning of <i>Giuae</i> in pSP-GM1 (PGK1)	Gi_uae_Fw1	pUC57_Giuae	AAAGGATCCAAAAAATGCATCACCATCAC CATCACCAGAATCATTCTTCTTGG	<i>Bam</i> HI
	Gi_uae_Rv1		AAAAC TCGAGCTATAATCCAATACCTTTTAC ATC	<i>Xho</i> I
Cloning of <i>Btchsy</i> in pSP-GM1 (PGK1)	Bt_chsy1_Fw	pUC57_Btchsy1	AAAGGATCCAAAAAATGCATCACCATCAC CATCACGCTGTCCGAGGTCTGAAGAGC	<i>Bam</i> HI
	Bt_chsy1_Rv		AAAAC TCGAGTTAAGCCGTGCGGACTGAGC	<i>Xho</i> I
Cloning of <i>Giuae</i> in pSP-GM1_(PTEF)	Giuae_tefp_Fw	pSP-GM1_Giuae	ATTTGCGGCCGCAAAACAATGCATCACCAT CACCAT	<i>Not</i> I
	Giuae_tefp_Rv		AACGAGCTCCTATAATCCAATACCTTTTACA TC	<i>Sac</i> I
<i>Zmugd</i> cloning in pSP-GM1_tefp_Giuae	Zm_psp_Fw	pSP-GM1_Zmugd	AAAGGATCCAAAAAATGCATCACCATCAC CATCACC GCATTACCATGATCGGTTCC	<i>Bam</i> HI
	Zm_psp_Rv		AAAGCTAGCTTACCCTGCATTGGGTCGAC	<i>Nhe</i> I
<i>Btchsy</i> cloning in pBEVY	Btchsy_pBEVY_Fw	pSP-GM1_Btchsy	AACGCGGATCCAAAACAATGCATCACCATC AC	<i>Bam</i> HI
	Btchsy_pBEVY_Rv		AACGCCGTCGACTTAAGCCGTGCGGACTGA GC	<i>Sal</i> I
<i>kfoA</i> cloning in pSP-GM1_Zmugd	kfoA_psp_Fw	pETM6_kfoCA	ATTTGCGGCCGCAAAACAATGAATATATTA GTTACAGGTG	<i>Not</i> I
	kfoA_psp_Rv		AACGAGCTCTTAAATATAACCATTG GGGTTT	<i>Sac</i> I
<i>kfoC</i> cloning in pBEVY-L	kfoC_pBEVY_Fw	pETM6_kfoCA	AACGCGGATCCAAAACAATGAGTATTCTTA ATCAAGC	<i>Bam</i> HI
	kfoC_pBEVY_Rv		AACGCCGTCGACTTATAAATCATTCTCTATT TTTTCCC	<i>Sal</i> I
Colony PCR and sequencing				
	tADH1_Fw	pSP-GM1_tefp_Giuae	CCTAAGAGTCACTTTAAAATTTGT	
	TEF1p_Rv	/ pSP-GM1_Zmugd_kfoA	CGTACCACTTCAAAACACC	
	PGK1p_FW	pSP-GM1_Zmugd /	GTGATCTCCAGAGCAAAGT	
	CYCt_REV	pSP-GM1_Giuae_Zmugd	CTTCGAGCGTCCCAAAC	
	tADH1_Fw	pBEVY_Btchsy /	CCTAAGAGTCACTTTAAAATTTGT	
	pGAL_middle_Rv	pBEVY_kfoC	ATTATTCCTACTTGACTAATAAG	
	Bt_chsy1_mid dle	pBEVY_Btchsy	CGGTAAATGTTTAAGAGAGATG	
	kfoC_pBEVY_Rv	pBEVY_kfoC	AACGCCGTCGACTTATAAATCATTCTCTATT TTTTCCC	

Table S2. Codon-optimized gene sequences (5' → 3') for *Saccharomyces cerevisiae*.

>Chondroitin synthase (chsy1) (2412 bp GC%: 41,54; CAI: 0,74)

```

ATGGCTGTCCGAGGTCTGAAGAGCATGGTTAAGCGTTTTGCTGGGTTTAGTTCTTGTTT
TGTCTTAGCCTCTAGACTCGTTTTGCCGAGGGCTTCTGAGTTAAAAAGAGCAGGCCCA
CGGAGAAGAGCTAGTCCAGAGGCTTGTAGGCCGGGTACAGGCAGCAGCCGTACCATT
AGCTGGAGGTGCTAGAGGAGATGCGAGAGGCCAAAGATTATGGCCCCACGGGGAAG
CTCAAGACGGTGTTCCTCCGCGACCGCAATTTCTTATTCGTAGGAGTCATGACCGCTCA
AAAGTATCTACAAACGAGAGCTGTGGCTGCTTTCAGAACTTGGTCAAAAACCTATCCC
AGGCAAGGTCTGAATTTTCTCATCTGAAGGTTCTGATACCAGTATTAGTATTCCTGTG
GTCCCATTTGCGAGGTGTTGATGATTCATATCCGCCTCAGAAAAAATCATTATGATGT
TAAAATATATGCATGATCATTACTTGGATAAGTACGAATGGTTCATGAGAGCTGACG
ATGATGTTTATATTAAGGTGACAGATTGGAATCATTCTTAAGATCGCTCAACAGCAG
CGAGCCTTTGTTCTTGGGACAGACCGGCTTAGGAACCACTGAAGAAATGGGAAAAC
TGCTCTAGAACCCGGTGAAAACTTTGCATGGGAGGCCCGGGCGTTATTATGAGCAG
AGAAGTATTGAGAAGAATGGTACCTCACATCGGTAAATGTTTAAGAGAGATGTACAC
TACTCACGAGGATGTAGAGGTGGGTCGCTGCGTTAGAAGATTTGCTGGTGTACAATG
CGTCTGGTCGTACGAAATGCAACAACCTCTTTATGAAAATTATGAACAAAATAAAAA
AGGTTACATACGTGATTTACACAATTCCAAGATACATAGAGCCATCACCTTACATCCT
AACAAAAATCCTCCATATCAATATCGTTTGCACAGCTATATGCTTTCTAGAAAAATCG
CTGAACCTGAGACATCGTACTATCCAACCTGCATCGCGAGATTGTCCTTATGTCCAAGTA
CAGTAATACGGAAGTTCATAAAGAGGATTTGCAATTAGGTATTCCACCGTCTTTCATG
AGATTTCAACCAAGACAAAGAGAAGAAATTTTGGAATGGGAATTTCTAACCGGTAA
GTATTTATATAGTGCAGCCGATAGCCAGCCCCACGTAGAGGTATGGACAGCGCCCA
AAGAGAAGCATTGGATGATATTGTTATGCAAGTTATGGAAATGATAAATGCCAATGC
CAAACTAGAGGGAGAATTATAGATTTCAAAGAAATTCAATACGGTTATCGCCGTGT
TAATCCGATGTACGGGGCTGAATATATACTGGATTTGTTGTTGCTTTACAAGAAACAT
AAAGGTAAGAAAATGACAGTTCAGTTAGAAGACATGCCTATTTGCAACAACTTTT
TCAAAAATTCAGTTTGTGTAACATGAAGAACTTGATGCAAAGGAATTGGCGAATAAA
ATTAATCAAGAATCCGGCTCCCTGTCTTCTGTCCAATTCATTGAAAAAGTTGGTGC
CATTTCAACTTCCCGGGAGTAAAAACGAACACAAAGAACCTAAGGAGAAAAAGATT
AATATTTTAATCCCCCTGAGCGGAAGATTTGACATGTTTGTCCGTTTCATGGGAACT
TTGAAAAGACATGTTTAATTCCGAATCAAAACGTGAAACTGGTTGTCTTATTGTTTAA
TTCTGATTCGAACCCTGACAAAGCCAAGCAAGTGGAACCTATGCGTGATTACAGAAT
TAAATATCCCAAGGCCGATATGCAAATTTTGCCGGTGTGAGGTGAGTTTTCCAGAGCC
TTGGCATTGGAGGTGGGCAGTTCTCAATTTAACAATGAGAGTCTTTTGTTTTCTGTGA
TGTTGACTTAGTCTTCACGGCCGAGTTTTTGCAACGTTGTAGAGCTAACACTGTCTTAG
GCCAACAAATTTACTTCCCTATTATCTTCTCACAATATGATCCTAAAATTGTCTACTCC
GGCAAAGTTCCAAGTGATAATCACTTTGCTTTTACTCAGAAAACTGGTTTTTGGAGGA
ATTATGGATTTGGAATTACGTGTATATACAAAGGGGATTTAGTGAGGGTGGGTGGCTT
TGATGTGTCCATCCAGGGTTGGGGTCTGGAAGATGTGCGATTTATTCAACAAAGTCGTT
CAAGCAGGGCTTAAACTTTTCAGGAGCCAGGAAGTTGGCGTGGTCCACGTACACCAT
CCTGTTTTTTGTGATCCGAACCTAGATCCTAAGCAATACAAAATGTGTTTGGGCAGTA
AAGCCTCCACTTATGGTAGCACTCATCAATTGGCAGAAATGTGGTTAGAAAAAACG
ATCCCAATTACTCTAAGTCGAGTAACAACAACGGCTCAGTCCGCACGGCTTAA

```

>UDP-N-acetylglucosamine 4-epimerase (UAE) (1158 bp GC%: 42,31; CAI: 0,77)

```
ATGCAGAATCATTCTTCTTGGGTAAAACGGTCTTGATCACAGGCGGTTGTGGTTTCA
TTGGATCTCATTTTGTAGAGGCTTGTCATGTTCTCGGTATGACCGTCTATGTTTTAGAT
AACCTCAGTTCTGGTAAGAATGTATTTAAGACAACATCTGATTGTTCTTCATCATTGGT
TTAACTATCGGTGACATTAGAGACAAGGCTATCTTCTCTAGACTTCCGCAGAAAATT
GACTTCGTCATTCATTTGGCCGCAGCTGTTTCTGTTGCCGAATCAGTAACAAATCCGC
AGAAATATATGTTAACCAATGTTGAGGGTAGTAGAAACGTTTTCCAGTATGCTGTTGA
CGCAAAGGCTTCAGCCGTCTTAAGTGCTTCTACTGCTGCATATTACGGCGATTGTGGT
AAGTCAGCTATTACCGAGGCTTTCCCCTATGGTGGCATTCTCCATACGCTGAAAGTA
AGATGGAGATGGAGAGATTAGGAGCCGAATTTCAAAAAGACATCTCGTTGCAGATTCA
TTTTCTGCAGATTTTTCAATGTCTACGGCCCTAGACAGGACCCCTCCTCGCCCTACACT
GGAGTTATGAGTATTTTTATGGACAGGTGCGCCGCTAGAAAACCGATTACAATCTTTG
GTACCGGCGAGCAAACAAGAGATTTTGTTCATCAAGGACTTAATTGTCGCTGCCAT
TAACTTATTAGGTCAATTGGATAAGTTTCCTATTGGAGCAGATGCCGTGCAACAGAAC
GATCCAGAAGAAGTCCAAAGGTCCGCATATACCGGTGAAGGTGTGTACCCAACTGTT
TTCAACATCGGATCGGGAATTTGATCAGTGTCAATGAGCTTGCTGAATTAGCCAAA
ATTGTTTCTGGTAGACATGAAGTGGAATTTGTTTCATGGAGAACCACGTAGTGGAGAC
ATATTGCATTCATTATCCGATTGCACTAGGATTAGAAACGCTACTGGATGGAGCGCAT
CTACTACTCTTAGAGTTGGTATGTCTGAAACTTGGGGATGGGCTGCTGGCGAAATTAG
TTACTTGTCTGGTGACTTGGTAAAGAGTGTGGAAAACGAATTAAAGATTGATGGGGTT
TCAGTAGCAAAATCTCTATGTGGTAAAGATGCAGATGTAAAAGGTATTGGATTATAG
```