

Service Report: Order 35000

C203: RNA Isolation

C204: cDNA Synthesis

C130: Primer Design Service

C132: Primer Synthesis (10 nmol)

C533: qPCR Service

January 10, 2019



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Order Details Summary

C203 -- RNA Isolation
 C204 – cDNA Synthesis
 C130 – Primer Design Service
 C132 – Primer Synthesis (10 nmol)
 C533 – qPCR Service

Service Summary

24 samples were received for RNA isolation. The isolated RNA was quantified by nanodrop before proceeding to cDNA synthesis with 5X All-In-One RT MasterMix (**abm** G485). 1 pair of forward and reverse primers was designed and synthesized for the detection of target gene TGM1. The target gene (TGM1) and a housekeeping gene (H GAPDH, provided in-house) were amplified with BrightGreen 2X qPCR MasterMix-No Dye (**abm** MasterMix-S).

RNA Isolation

Sample Name	Nanodrop Concentration
#1	500 ng/μl
#2	450 ng/μl
#3	480 ng/μl
#4	350 ng/μl
#5	550 ng/μl
#6	280 ng/μl
#7	350 ng/μl
#8	370 ng/μl
#9	450 ng/μl
#10	430 ng/μl
#11	520 ng/μl
#12	520 ng/μl
#13	450 ng/μl
#14	480 ng/μl
#15	480 ng/μl
#16	350 ng/μl
#17	450 ng/μl
#18	440 ng/μl
#19	520 ng/μl
#20	520 ng/μl
#21	450 ng/μl
#22	480 ng/μl
#23	500 ng/μl
#24	450 ng/μl

Primer Design

1 pair of forward and reverse primers was designed based on the specification below:

- 1) Primers targeting Human TGM1 (NM_000359.2):

Target sequence:

ATGATGGATGGGCCACGTTCCGATGTGGGCCGTTGGGGTGGCAACCCCTTGCAGCCCCCTACCACGCCATCTC
 CAGAGCCAGAGCCAGAGCCAGACGGACGCTCTCGCAGAGGAGGAGGCCGTTCTTCTGGGCTCGCTGCTGTG
 GCTGCTGTTTCATGCCGAAATGCGGCAGATGACGACTGGG**GACCTGAACCCTCTGACT**CCAGGGGTCGAGGGTC
 CAGCTCTGGCACTCGAAGACCTGGCTCCCGGGGCTCAGACTCCCGCCGGCCTGTATCCCGGGGCAGCGGTGT
 CAATGCAGCTGGAGATGGCACCATCCGAGAGGGCATGCTAGTAGTGAACGGTGTGGACTTGTGAGCTCGCGC
 TCGGACCAGAACCGCCGAGAGCACCACACAGACGAGTATGAGTACGACGAGCTGATAGTGCGCCGCGGGCAG
 CCTTTCCATATGCTCCTCCTCTGTCCCGGACCTATGAATCCTCTGATCGCATCACCTTGAGTTACTCATCGGA
 AACAAACCCCGAGGTGGGCAAGGGCACGCACGTGATCATCCAGTGGGCAAGGGGGGCAGTGGAGGCTGGAAA
 GCCCAGGTGGTCAAGGCCAGTGGGCAGAATCTGAACCTGCGGGTCCACACTTCCCCAACGCCATCATCGGCA
 AG**TTTCAGTTCACAGTCCGC**ACACAATCAGACGCTGGGGAGTTCCAGTTGCCCTTTGACCCCCGCAATGAGATC
 TACATCCTCTTCAACCCCTGGTGGCCAGAGGACATTGTGTACGTGGACCATGAGGATTGGCGGCAGGAGTATGT
 TCTTAATGAGTCTGGGAGAATTTACTACGGGACCGAAGCACAGATTGGTGAGCGGACCTGGAACACTCGGCCAGT
 TTGACCACGGGGTGTGGATGCCTGCTTATACATCCTGGACCGGCGGGGATGCCATATGGAGGCCGTGGAGA
 CCCAGTCAATGTCTCCCGGGTCTCTGCCATGGTGAACCTCCCTGGATGACAATGGAGTCTGATTGGGAACT
 GGTCTGGTGATTACTCCCGAGGCACCAACCCATGCTGGGTGGGTCAGCGTGGGAGCGTGGAGATGCTTAGCTACCT
 ACGCAGCGGATATTCCGTCGCCATGGCCAGTGTGGGCTTTGCTGGCGTGACCACCACAGTGTGCGCTGC
 CTGGTCTGGCCACCCGTACTGTCACCAACTTCAACTCCGCCACGACACAGACACATCCCTTACCATGGACAT
 CTACTTCGACGAGAACATGAAGCCCCTGGAGCACCTGAACCATGATTCTGTCTGGAACCTCCATGTGTGGAACG
 ACTGCTGGATGAAGAGGCCGGATCTGCCCTCGGGCTTTGATGGGTGGCAGGTGGTGGATGCCACACCCCAAGA
 GACTAGCAGTGGCATCTTCTGCTGCGGCCCTGCTCTGTGGAGTCCATCAAGAATGGCCTGGTCTACATGAAGT
 ACGACACGCCTTTTCATTTTTGCTGAGGTGAATAGTGACAAGGTGTACTGGCAGCGGCAGGATGATGGCAGCTTC
 AAGATTGTTTATGTGGAGGAGAAGGCCATCGGCACACTCATTGTCAAAAGGCCATCAGCTCCAACATGCGGGA
 GGACATCACCTACCTCTATAAGCACCCAGAAGGCTCAGACGCAGAGCGGAAGGCAGTAGAGACAGCAGCAGCC
 CACGGCAGCAAACCCAAATGTGTATGCCAACCGGGGCTCAGCGGAGGATGTGGCCATGCAGGTGGAGGCACAG
 GACGCGGTGATGGGGCAGGATCTGATGGTCTCTGTGATGCTGATCAATCACAGCAGCAGCCGCCGCACAGTGA
 AACTGCACCTCTACCTCTCAGTCACTTTCTATACTGGTGTGAGTGGTACCATCTTCAAGGAGACCAAGAAGGAAG
 TGGAGCTGGCACCAGGGCCTCGGACCGTGTACCATGCCAGTGGCCTACAAGGAATACCGGCCCATCTTGT
 GGACCAGGGGGCCATGCTGCTCAATGTCTCAGGCCAGTCAAGGAGAGCGGGCAGGTGCTGGCCAAGCAGCA
 CACCTTCCGTCTGCGCACCCAGACCTCTCCCTCACGTTACTGGGAGCAGCAGTGGTTGGCCAGGAGTGTGAA
 GTACAGATTGCTTCAAGAACCCCTTCCCGTACCCCTACCAATGTGCTTCCGGCTCGAAGGCTCTGGGTTA
 CAGAGGCCAAAGATCCTCAACGTTGGGACATTGGAGGCAATGAAACAGTGACACTGCGCCAGTCGTTTGTGC
 CTGTGCGACCAGGCCCCCGCCAGCTCATTGCCAGCTTGGACAGCCACAGCTCTCCAGGTGCACGGTGTGAT
 CCAGGTGGATGTGGCCCCAGCCCTGGGGATGGGGCTTCTTCTCAGACGCTGGAGGTGACAGTCACTTAGGA
 GAGACCATCCCTATGGCATCTCGAGGTGGAGCTTAG

	Primer Sequence	GC %
Forward Primer	5'- GACCTGAACCCTCTGACT-3'	55.6%
Reverse Primer	5'- GCGGACTGTGAACTGAAA-3'	50%

Primer Synthesis

Lot Number: 0019844635001
Date of Manufacture: January 10, 2019
Storage condition: -25°C to -15°C
Deliverable Form: Lyophilized
 Recommend re-suspending the lyophilized primers using
Reconstitution: DNase and RNase-free ddH₂O.

Purification	Sequence Name	Sequence	Tm °C	nmoles
Standard Desalting	TGM1 Forward Primer	GACCTGAACCCTCTGACT	53.4	30.5
Standard Desalting	TGM1 Reverse Primer	GCGGACTGTGAACTGAAA	52.7	30.1

Deliverables

1. qPCR Service Raw Data

Target Gene	H GAPDH		TGM1	
	Ct values	Avg. Ct	Ct values	Avg. Ct
Sample #1	20.5	20.4	20.6	20.4
	20.3		20.2	
	20.4		20.4	
Sample #2	16.2	16.4	28.6	28.4
	16.4		28.4	
	16.6		28.2	
Sample #3	16.7	16.43	25.6	25.4
	16.4		25.4	
	16.2		25.2	
Sample #4	17.2	17.4	29.3	29.3
	17.4		29.4	
	17.6		29.2	
Sample #5	19.3	19.4	20.5	20.3
	19.4		20.3	
	19.5		20.1	
Sample #6	21.3	21.5	28.6	28.4
	21.5		28.4	
	21.7		28.2	
Sample #7	17.1	17.4	25.3	25.2
	17.4		25.2	
	17.7		25.1	

Sample #8	23.2	23	19.5	19.7
	23		19.7	
	22.8		19.9	
Sample #9	24.5	24.37	23.2	23
	24.4		23	
	24.2		22.8	
Sample #10	16.3	16.5	19.2	19
	16.5		19	
	16.7		18.8	
Sample #11	19.2	19	18.1	17.9
	19		18	
	18.8		17.6	
Sample #12	20.3	20.2	27.6	27.47
	20.2		27.5	
	20.1		27.3	
Sample #13	17.1	16.97	19.2	18.9
	17		18.9	
	16.8		18.6	
Sample #14	16.5	16.3	25.6	25.9
	16.3		25.9	
	16.1		26.2	
Sample #15	18.2	18.47	15.8	15.67
	18.5		15.7	
	18.7		15.5	
Sample #16	19.2	18.9	32.5	32.2
	18.9		32.2	
	18.6		31.9	
Sample #17	16.2	16.3	33.8	33.63
	16.3		33.6	
	16.4		33.5	
Sample #18	19.1	19.37	21.6	21.9
	19.4		21.9	
	19.6		22.2	
Sample #19	18.1	17.87	16.3	16.5
	17.9		16.5	
	17.6		16.7	
Sample #20	16.1	16.3	29.8	30
	16.3		30	
	16.5		30.2	
Sample #21	25.2	25.4	17.8	17
	25.4		17	
	25.6		18.2	

Sample #22	24.1	24.4	23.5	23.3
	24.4		23.3	
	24.7		23.1	
Sample #23	16.3	16.53	28.7	28.43
	16.5		28.4	
	16.8		28.2	
Sample #24	26.7	26.9	20.5	20.17
	26.9		20.2	
	27.1		19.8	

2. qPCR Service Data Analysis

Target Gene	TGM1
Expression Level of Sample #1	1
Expression Level of Sample #2	0.00024
Expression Level of Sample #3	0.00202
Expression Level of Sample #4	0.00027
Expression Level of Sample #5	0.53589
Expression Level of Sample #6	0.00837
Expression Level of Sample #7	0.00448
Expression Level of Sample #8	9.84916
Expression Level of Sample #9	2.54912
Expression Level of Sample #10	0.17678
Expression Level of Sample #11	2.21914
Expression Level of Sample #12	0.00657
Expression Level of Sample #13	0.25882
Expression Level of Sample #14	0.00129
Expression Level of Sample #15	6.96440
Expression Level of Sample #16	0.00010
Expression Level of Sample #17	0.000006
Expression Level of Sample #18	0.17076
Expression Level of Sample #19	2.54912
Expression Level of Sample #20	0.00008
Expression Level of Sample #21	337.79403
Expression Level of Sample #22	2.14355
Expression Level of Sample #23	0.00026
Expression Level of Sample #24	107.63474

$$\text{Expression Level} = 2^{-\Delta\text{Ct}}$$

$$\Delta\text{Ct} = \text{Ct (GOI)} - \text{Ct (HKG)}$$

GOI = gene of interest

HKG = housekeeping gene

Acknowledgement

Thank you for choosing abm as your qPCR service provider. It is our goal to provide you with the best customer experience in the world. Please do not hesitate to contact us should you need further assistance analyzing your data. We are grateful to be a part of your scientific exploration and we look forward to serving you again.