2025.5.12 80°C samples re-seq with Azenta Amplicon-EZ

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Entry Created On: 12 May 2025 20:32:02 UTC
Entry Last Modified: 19 May 2025 22:42:58 UTC
Export Generated On: 12 Jun 2025 01:45:06 UTC

MONDAY, 5/12/2025

PCR amplification of the amplicon for Amplicon-EZ:

System-SL5

	Component	Vol (ul)	7x (ul)	Note
1	5x Phusion HF buff	40	280	
2	10mM dNTP	4	28	
3	100uM F	1	7	COVID_6-28_F_56C
4	100uM R	1	7	CMV-5UTR_SHAPE_Rv
5	Phusion Pol	2	14	
6	cDNA+water	152/EA	152/EA	5/9/25: prepared 200ng DNA in 152ul/EA
7	Total	200	200/EA	

Program-SL5

	A	B	C
1	98°C	30s	
2	98°C	10s	
3	62°C	10s	
4	72°C	15s	35x
5	72°C	5min	
6	4°C	hold	

System-SMN2

	Component	Vol (ul)	3.5x (ul)	Note
1	5x Phusion HF buff	40	140	
2	10mM dNTP	4	14	
3	2uM F+R	50	175	"148 PCR primer" (labeled on tube, from Zhichao); SMN2-GT-PCR-FW (GT2) + SMN1/2-GT-PCR-Rv (on map)
4	Water	103	360.5	
5	Phusion Pol	2	7	
6	cDNA	1	1/EA	
7	Total	200	50/EA	

Program-SMN2

	A	B	C
1	98°C	30s	
2	98°C	10s	35x
3	58°C	10s	
4	72°C	15s	
5	72°C	5min	
6	4°C	hold	

PCR purification:

- 1000ul DNA Binding Buffer to 200ul PCR product (5:1)
- Transfer 600ul to Zymo-Spin Column, 10000xg 30s, repeat
- Add 500 ul DNA Wash Buffer, 10000xg 30s
- Repeat washing step
- Spin at full speed 2min
- Elute = **30ul** water
- Nanodrop & Qubit:

Quantification

	#	Nanodrop (ng/ul)	260/280	Qubit (ng/ul)	500ng (ul)	to 25ul
1	7	156	1.89	97.6	5.1	19.9
2	8	172.5	1.9	98.2	5.1	19.9
3	9	167.4	1.88	104.0	4.8	20.2
4	10	174.7	1.86	105.0	4.8	20.2
5	11	165.3	1.9	104.0	4.8	20.2
6	12	160.8	1.91	114.0	4.4	20.6
7	13	162.9	1.87	96.4	5.2	19.8
8	14	165.8	1.88	99.2	5.0	20.0
9	15	150	1.86	108.0	4.6	20.4

Size verification:

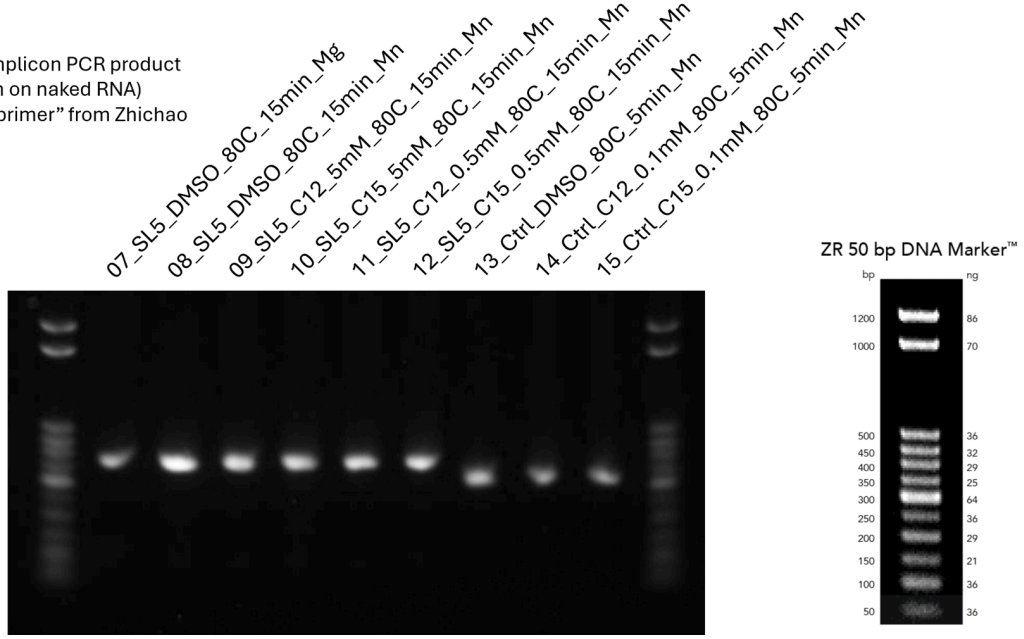
- Gel: 1% agarose in 1x TBE, 1:20000 APExBIO Safe DNA Gel Stain
- Ladder: Zymo ZR 50bp DNA Marker
- Loading dye: Invitrogen 10x Blue Juice (200ng sample or 1ug 50bp Marker)
- Amplicon size=378bp/324bp
- Gel running: 100v 30min

Gel result

2025.5.12 Amplicon-EZ gel check

- Template: previous amplicon PCR product
- F primer: COVID_6-28_F_56C
- R primer: CMV-5UTR_SHAPE_Rv
- Expected size: 378bp

- Template: previous amplicon PCR product (covalent modification on naked RNA)
- F/R primer: “148 PCR primer” from Zhichao
- Expected size: 324bp



Submit Amplicon-EZ order:

20250512 Amplicon EZ HC.pdf