This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 727459

TPS code	TrSafe - GY LAMP
Scope of TPS	Detection of Grapevine Yellows phytoplasma from South Africa

Report on the results of the test performance study for molecular detection South African aster yellows

Version of report: V1.0

Elaborated by:

Name:	Špela Alič
Organisation:	National Institute of Biology
Date:	17.09.2020

1. INTRODUCTION

Grapevine yellows is a widespread phytoplasma disease. Symptoms of the disease on grapevine caused by AY normally lead to the abortion of immature berries (Engelbrecht et al. 2010) resulteding in high levels of yield loss. In South Africa the symptoms of the disease were observed for the first time in 2006. The main agent pathogen causing the South African grapevine yellows (GY) are phytoplasmas classified in 16SrI-B soubgroup, corresponding to 'Candidatus Phytoplasma asteris', or Aster Yellows (AY) (Engelbrecht et al., 2010; Carsterns et al., 2011). No permanent cure for AY or other phytoplasma have been reported yet. Therefore, vector control, monitoring and eliminating infected plants represented remain the main management strategy to control the epidemic. Therefore, a novel LAMP test, namely TrSafe_Seq11_ID58 assay, for specific detection of Grapevine Yellows from South Africa was developed and validated in the framework of an EU funded research project, TROPICSAFE (http://www.tropicsafe.eu/). The project aims at developing innovative tools and solutions to manage and reduce the impact of these diseases due to insect-transmitted bacteria that are affecting and threatening tropical and subtropical agricultural relevant species. To obtain additional information regarding performance of the novel LAMP assay, interlaboratory comparison through test performance study (TPS) was organised.
National Institute of Biology, Department of Biotechnology and Systems Biology organised the TPS for the detection of Grapevine Aster Yellows from South African (SAAY). Evaluation of the methods was conducted on synthetic DNA and DNA from naturally infected plant matrix.

2. METHODOLOGY AND RESULTS

2.1. Samples

The TPS sample panel was composed of 12 test items: positive samples containing synthetic dsDNA of SAAY, positive samples containing naturally contaminated sample SAAY, and negative samples containing other Aster yellows or host plant *Vitis vinifera* DNA (Table 1). Also, a set of controls were provided.

Stability

Stability of test items and controls was tested with LAMP TrSafe_Seq11_ID58 assay. Stability testing was conducted after results from both participants were obtained. One sample panel and controls stored at stored < -15 °C were tested in two technical repeats accordingly to TPS protocol. Results were in concordance with the samples target concentration.

Assigned reference values

Reference values were assigned to the test items based on the results of LAMP TrSafe_Seq11_ID58 test (qualitative assigned reference values). Quantitative reference values (target concentrations) were determined for test using digital PCR analysis of test items and controls (Table 1).

Quantitative reference values were determined using LAMP test TrSafe_Seq11_ID58 according to TPS instructions (see 2.3 Methods) prior TPS.

For digital PCR, the real-time PCR TrSafe_Seq11 was transferred to digital PCR format and used to determine the concentration of the target copy numbers in test items and controls. Digital PCR was performed on the QX100™ Droplet Digital™ PCR system (Bio-Rad, Pleasanton, CA, USA). dPCR Supermix for probes (12 µL, Bio-Rad) and 8 µL sample DNA were used, with the primer and probe concentrations of 900 nmol, 900 nmol and 200 nmol per reaction, respectively. After droplet generation, 40 µL of the generated droplet emulsion were transferred to a new 96-well PCR plate (Eppendorf) and amplified in a Thermal Cycler (Bio-Rad). The amplification conditions were 10 min DNA polymerase activation at 95 °C, followed by 45 cycles of a two-step thermal profile of 15 s at 95 °C for denaturation, and 60 s at 60 °C for annealing and extension, followed by a final hold of 10 min at 98 °C for droplet stabilisation and cooling to 4 °C. The temperature ramp rate was set to 3 °C/s, and the lid was heated to 105 °C, according to the Bio-Rad recommendations. After the thermal cycling, the plates were transferred to a droplet reader QX100 ™ (Bio-Rad). The software package provided with the dPCR system (QuantaSoft™ Software, version 17.4.0917, Bio-Rad) was used for data acquisition. A minimum of 10.000 accepted droplets per reaction was required for the reaction to be considered valid and no non-valid reactions were observed. A fixed manual global threshold discriminating between negative and positive droplets was selected. A reaction was interpreted as positive if the number of positive droplets was ≥3. Positive and no template controls were used in each run. Each sample was analysed in 3 technical repeats. Standard measurement uncertainty was calculated for each sample.

Table 1: Determination of concentrations of target DNA copies in test items and controls with digital PCR. Legend: cps = DNA copies, NA = not applicable.

Sample ID	Sample	Sample description	Digital PCR analysis; 95 % confidence log(cps/mL)*	Contains target?	Expected qualitative result
GY-1	gBlock target	synthetic dsDNA of SAAY	2,6 ± 2,4	yes	positive
GY-2	gBlock target	synthetic dsDNA of SAAY	3,4 ± 2,7	yes	positive
GY-3	gBlock target	synthetic dsDNA of SAAY	4,5 ± 3,8	yes	positive
GY-4	gBlock target	synthetic dsDNA of SAAY	5,6 ± 4,6	yes	positive
GY-5	AY 43(+)	naturally contaminated sample SAAY	5,6 ± 4,6	yes	positive
GY-6	AY 43(+)	naturally contaminated sample SAAY	4,5 ± 3,9	yes	positive
GY-7	Small Bag B(+)	naturally contaminated sample SAAY	5,5 ± 4,6	yes	positive
GY-8	Small Bag B(+)	naturally contaminated sample SAAY	4,4 ± 3,8	yes	positive
GY-9	Small Bag B(+)	naturally contaminated sample SAAY	3,4 ± 2,6	yes	positive
GY-10	D310/17 A	DNA of carrot containing AY (other)	NA	no	negative
GY-11	D322/17 B	DNA of carrot containing AY (other)	NA	no	negative
GY-12	Vitis neg	DNA of Vitis vinifera	NA	no	negative
PAC	PAC	synthetic dsDNA of SAAY	6,5 ± 5,7	yes	positive
NAC	NTC	molecular grade water	NA	no	negative

*Target concentration was measured by digital droplet PCR using TrSafe_Seq11 assay. Measurements were conducted in 3 technical repeats and measurement uncertanty was calculated.

2.2. Consumables

The LAMP primers were provided by the organizer as a mixture and dispatched with the panel The TPS organizer also provided all the controls needed. The samples were coded in such a ways to ensure a l blind testing of samples.

2.3. Methods

The organisers suppled the primers, samples and controls (PAC and NAC). The participant had to provide other required reagents, disposables and equipment.
The preliminary studies conducted by the TPS organizer used Isothermal Master Mix ISO 001 (Optigene Ltd., Horsham, UK) as a mastermix.

Table 2: Primers and probe information for test LAMP TrSafe_Seq11_ID58

Oligonucleotides name	Size (nt)	Sequence (5' – 3')
TrSafe_AY_SA_Seq11_ID58_F3	18	TGAAGCAGGACACGCTAT
TrSafe_AY_SA_Seq11_ID58_B3	23	CAAAAATTAATTCTTCAGCCACA
TrSafe_AY_SA_Seq11_ID58_FIP*	40	CCGAATTCCCACACGGAATAATTAAGTTGGAACATGCCCA
TrSafe_AY_SA_Seq11_ID58_BIP*	45	AATGACACCAGAAACAGAAACTTTCCGTCCCCCTAAATAAGATGT

*HPLC purified

Experimental protocol

Important: the reaction mixtures should be prepared and kept on ice until analysis (through the addition of sample and control DNA).

Table 3: qPCR mix composition

Reagent (name)	Final concentration/ volume
Isothermal Master Mix ISO 001 (Optigene Ltd., Horsham, UK)	1x
TrSafe_AY_SA_Seq11_ID58_F3	0,20 µM
TrSafe_AY_SA_Seq11_ID58_B3	0,20 µM
TrSafe_AY_SA_Seq11_ID58_FIP	1,60 µM
TrSafe_AY_SA_Seq11_ID58_BIP	1,60 µM
Molecular grade water	/
Volume of the DNA extract	5 µL
Final volume	25 µL

Table 4: LAMP reaction program

LAMP reaction program:	
Amplification	63 °C 30 min
Melting curve	60 - 98 °C, 0.05 °C/s

Note: Each sample and control was analysed in one reaction per strip.

Controls

Controls included:

- **PAC (Positive amplification control):** to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This includes nucleic acid isolated from the target organism. A PAC control was provided.
- **NAC (Negative amplification control or no template control):** to rule out false positives due to contamination during the preparation of the reaction mix: amplification of molecular grade water will be provided to prepare the reaction mix.

Interpretation of results

Verification of the controls:

- NAC should produce no fluorescence or fluorescence with non-characteristic Tm.
- PAC: a positive reaction is defined by a curve of sigmoidal shape above the background, time of positivity below 30 min and Tm in the interval between 82 - 83 °C.

When these conditions are met and controls have valid results, the test is considered valid and the samples interpreted as follows:

- A test is considered positive as defined for PAC reactions (see above).
- A test is considered negative if it produces no fluorescence or fluorescence with non-characteristic Tm.
- Tests should be repeated if any contradictory or unclear results are obtained.
- In case of analysis of duplicate reactions, a test is considered positive if one or more reactions is positive, and negative if both reactions are negative.

2.4. Results

Table 5 Results of the LAMP test TrSafe_Seq11_ID58 evaluated in the TPS. The table presents TPS results of both participants and results of the stability testing. Stability testing was conducted accordingly to TPS protocol after TPS submission of the TPS results. All TPS results were valid and 100 % in concordance with expected results.

Sample ID	Expected qualitative result	Participant						Stability testing		
		1			2					
		Result	Average Tp (min)	Average Tm of positive samples (°C)	Result	Average Tp (min)	Average Tm of positive samples (°C)	Result stability	Average Tp (min)	Average Tm of positive samples (°C)
GY-1[a]	positive/ negative	positive	23.0	82.4	negative	NA	NA	positive	29.0	82.1
GY-2	positive	positive	19.7	82.3	positive	19.9	81.9	positive	23.5	82.2
GY-3	positive	positive	16.5	82.3	positive	15.8	81.8	positive	15.6	82.3
GY-4	positive	positive	13.7	82.3	positive	13.3	82.0	positive	12.9	82.3
GY-5	positive	positive	13.2	82.2	positive	13.6	82.1	positive	13.3	82.2
GY-6	positive	positive	15.8	82.3	positive	15.7	82.1	positive	15.3	82.3
GY-7	positive	positive	13.7	82.3	positive	13.2	81.9	positive	13.7	82.3
GY-8	positive	positive	16.2	82.3	positive	15.7	82.0	positive	16.1	82.4
GY-9	positive	positive	19.8	82.1	positive	17.3	82.2	positive	23.0	82.1
GY-10	negative	negative	NA	NA	negative	NA	NA	negative	NA	NA
GY-11	negative	negative	NA	NA	negative	NA	NA	negative	NA	NA
GY-12	negative	negative	NA	NA	negative	NA	NA	negative	NA	NA
PAC	positive	positive	11.9	82.2	positive	11.7	82.1	positive	11.4	82.2
NAC	negative	negative	NA	NA	negative	NA	NA	negative	NA	NA

[a]Target concentration in the sample GY-1 was at the limit of detection (LOD) of the LAMP test, therefore either, positive or negative results were expected.

The controls provided with the test panel were used as a quality check of the data set. If both controls results were concordant with the expected results, test panel data was considered as valid. All TPS results were valid and 100 % in concordance with expected results. Target concentration in the sample GY-1 was at the limit of detection (LOD) of the LAMP test, therefore either, positive or negative results were expected.

Analytical specificity

LAMP test TrSafe_Seq11_ID58 was designed to specifically detect only Aster yellows phytoplasma from South Africa. Two test item were comprised of naturally infected carrot samples with aster yellows phytoplasma and one test item contained DNA of healthy host plant *Vitis vinifera*. The TPS confirmed that the LAMP test TrSafe_Seq11_ID58 does not cross react with other Aster yellow phytoplasma or host plant DNA.

Analytical sensitivity of the test determined synthetic dsDNA of SAAY

The analytical sensitivity of the tests was determined on serial dilution of synthetic target DNA. Each test panel contained PAC, GY-4, GY-3, GY-2 and GY-1 test items that formed 10 fold serial dilutions ranging from 6.5 to 2.6 log(copies/mL). Results of those sample items were used to determine limit of detection by non-linear modelling. Synthetic dsDNA samples did not contain any host background DNA.

Based on the results, host DNA does not affect sensitivity of the LAMP test.

For non-linear modelling of the TPS results, the best fit models for the test were two-parameter Weibull function (W2.2) and asymptotic regression (AR.2). The concentration (log(copies/mL) detected with 95 % probability is shown in Figure 1 (written in red).

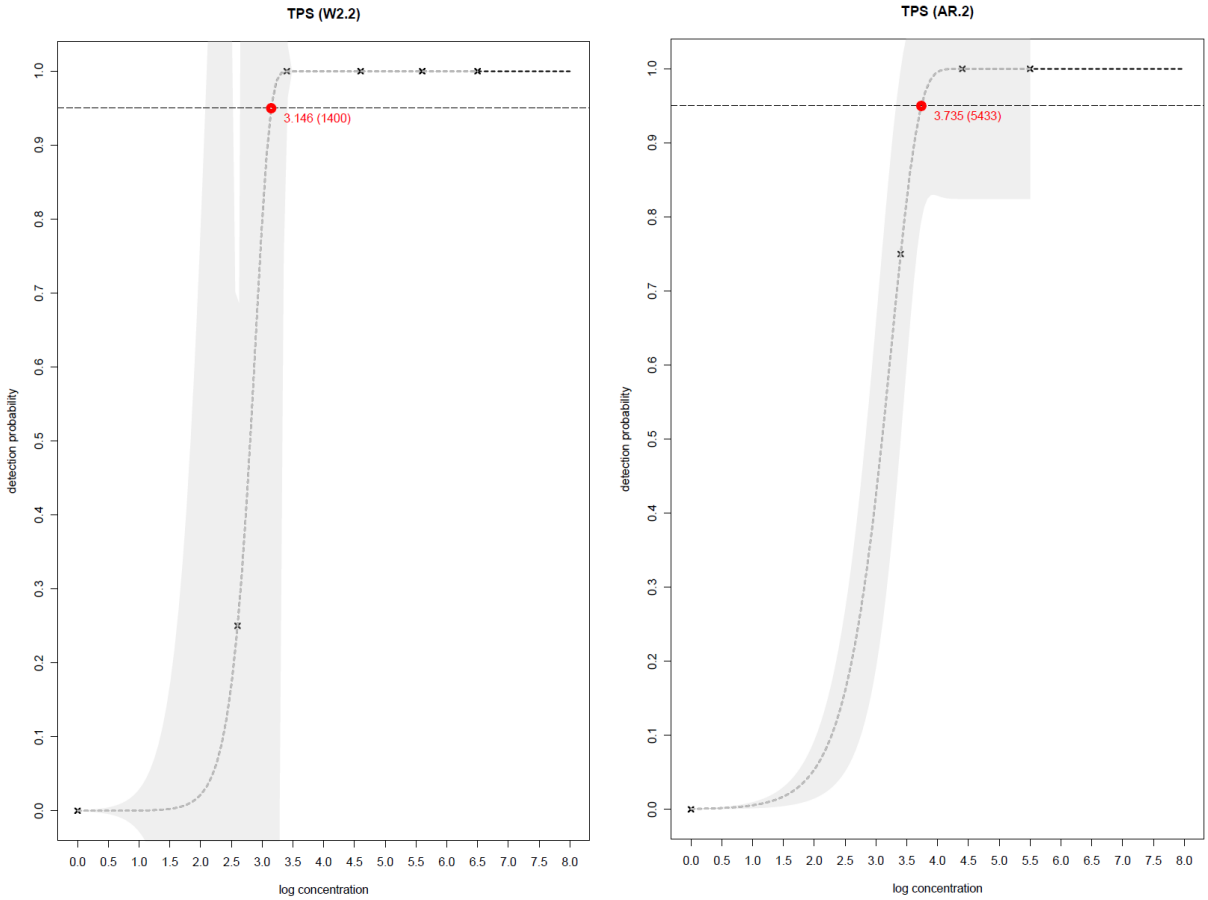


Figure 1 Non-linear modelling of probability of detection for LAMP test TrSafe_Seq11_ID58. The Left model shows probabilty of detection determined on serial dilution of synthetic target DNA without host background and the right model shows probability of detection for dilution of naturally contaminated sample SAAY. Concentrations shown are expressed as log(copies/mL) and were measured by digital PCR assay.

Legend: W2.2 = two-parameter Weibull function; AR.2 = asymptotic regression. The dotted line denotes 95 % probability of detection

3. CONCLUSION

The scope of this TPS was detection of South African Aster yellows using LAMP test TrSafe_Seq11_ID58. The following can be concluded based on the results of the TPS:

- Newly developed LAMP test TrSafe_Seq11_ID58 showed high sensitivity on synthetic dsDNA of SAAY and naturally contaminated samples SAAY.
- LAMP test TrSafe_Seq11_ID58 does not cross react with other tested Aster yellow phytoplasma or *Vitis vinifera* host plant DNA.

4. REFERENCES

Engelbrecht, M., Joubert, J., & Burger, J. T. (2010). First report of aster yellows phytoplasma in grapevines in South Africa. *Plant disease*, *94*(3), 373-373.

Carstens, R. (2014). *The incidence and distribution of grapevine yellows disease in South African vineyards* (Doctoral dissertation, Stellenbosch: Stellenbosch University).

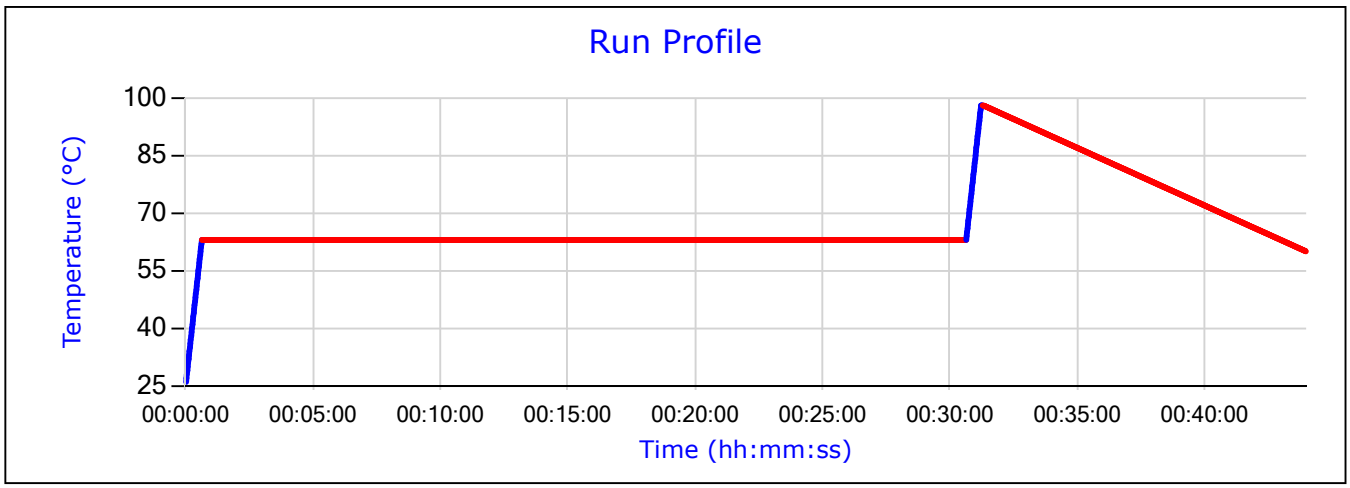
Nicolaisen, M., Dermastia, M., Dreo, T., Alič, Š., Contaldo, N., Satta, E., Bertaccini, A., Dickinson, M., Angelini, E., Burger, J., Campa, M., Pietersen, G., Formica, L., Pietersen, G., Oropeza, C., Narvaez, M., Yankey, N. (2019). New diagnostic protocols (LAMP and ELISA, lateral flow) for detection of GY, LY and HLB : deliverable number D4.3 : work package WP4.

5. APPENDENCES

5.1. Appendix 1: Gene Experiment report – participant 1, run 1

Genie Experiment Report

Profile Name	
Start Time	13:01:58 - petek, 14. avgust 2020
End Time	13:45:25 - petek, 14. avgust 2020
Serial Number	GEN2-1047



Preheat	No
Temperature	45 °C
Time	3 min(s)

Isothermal	Yes
Temperature	63 °C
Time	30 min
Gradient	0 °C

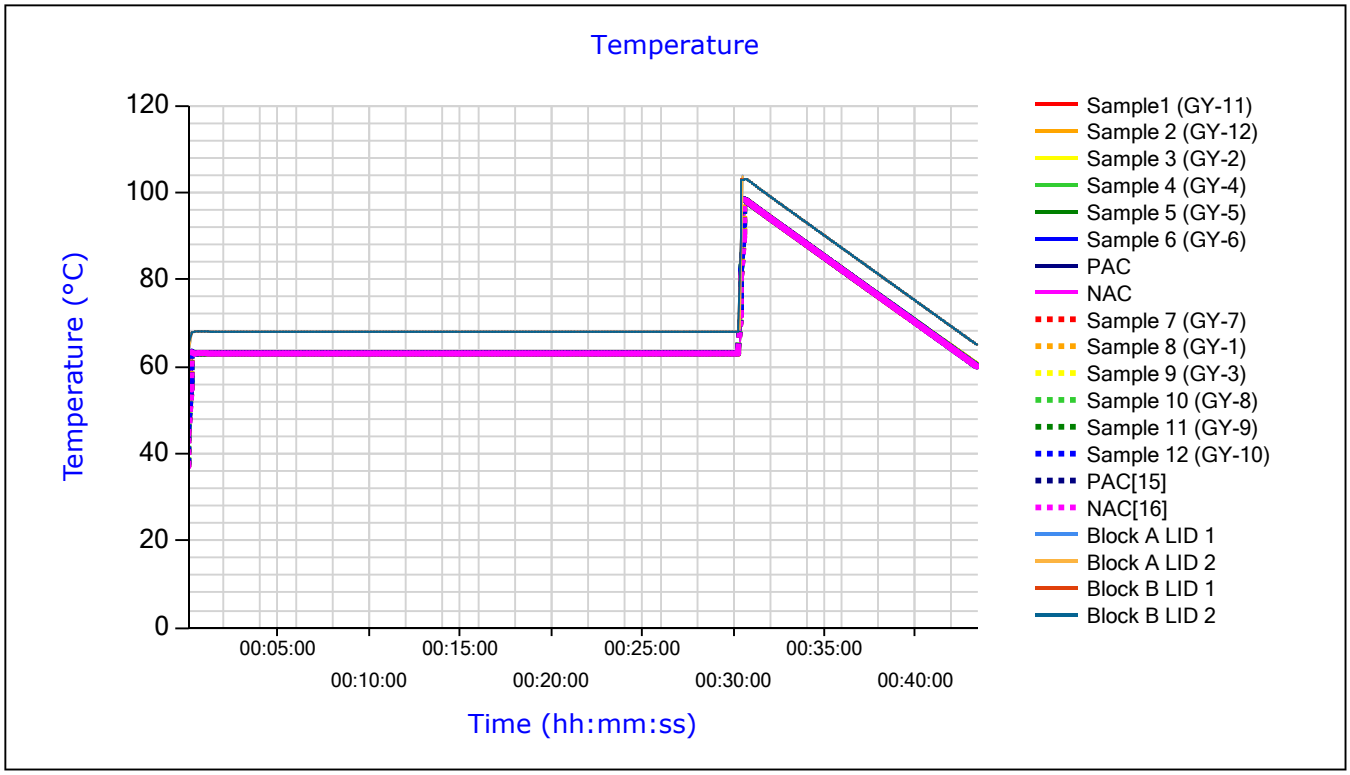
Anneal	Yes
Start Temp	98 °C
End Temp	60 °C
Ramp Rate	0,05 °C/sec

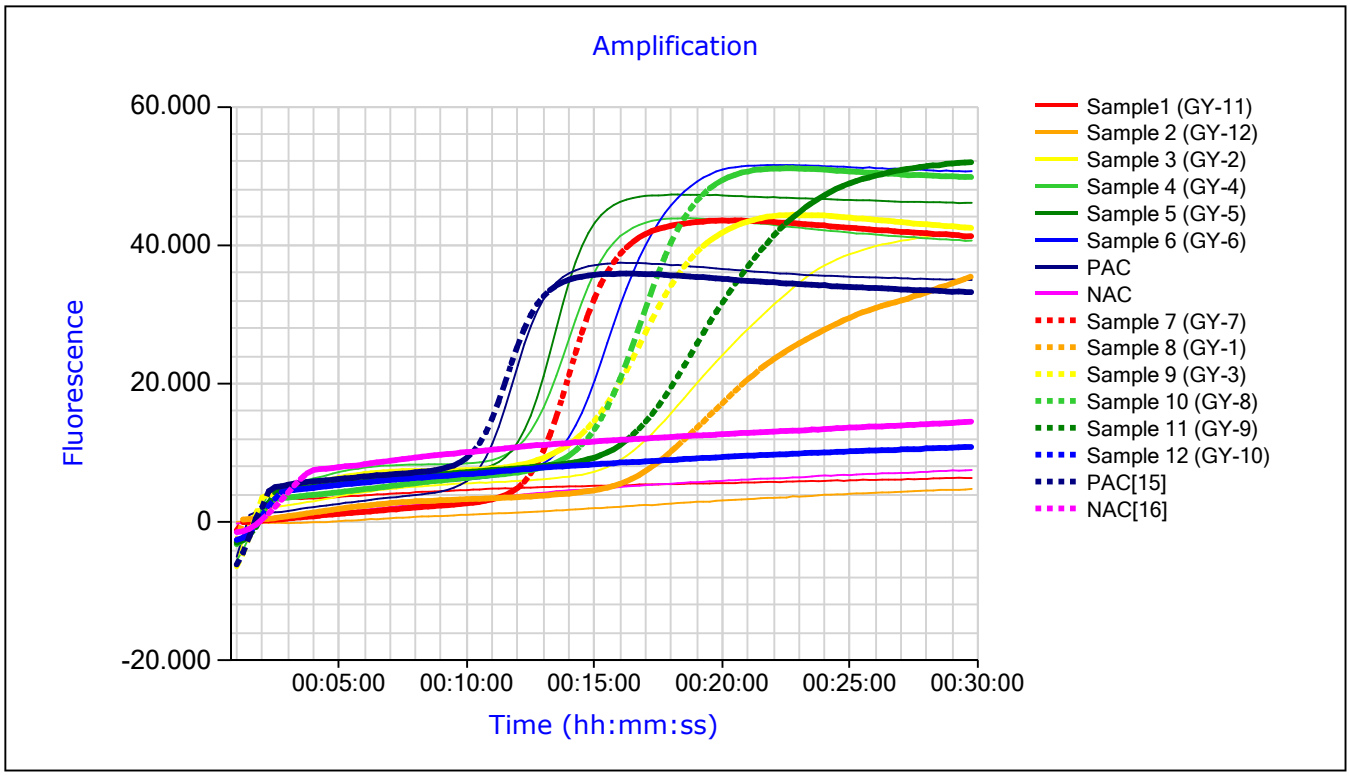
Estimated Run time	43:58 (mm:ss)

Advanced Options:

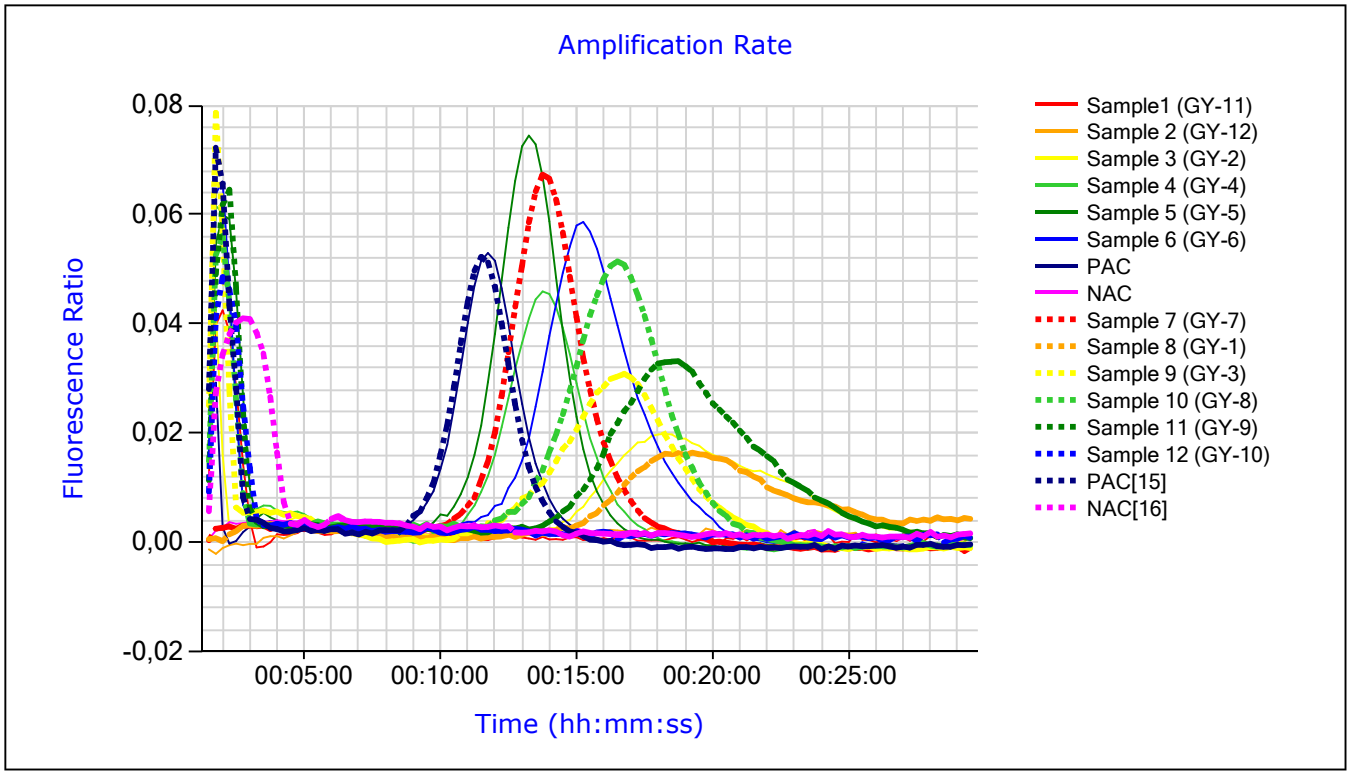
Preheat Ramp Rate	1 °C/Sec
Isothermal Ramp Rate	1 °C/Sec
Isothermal period	15 Sec
Anneal initial rate	1 °C/Sec
Anneal period	0 Sec
Anneal settling time	5 Sec

Notes:





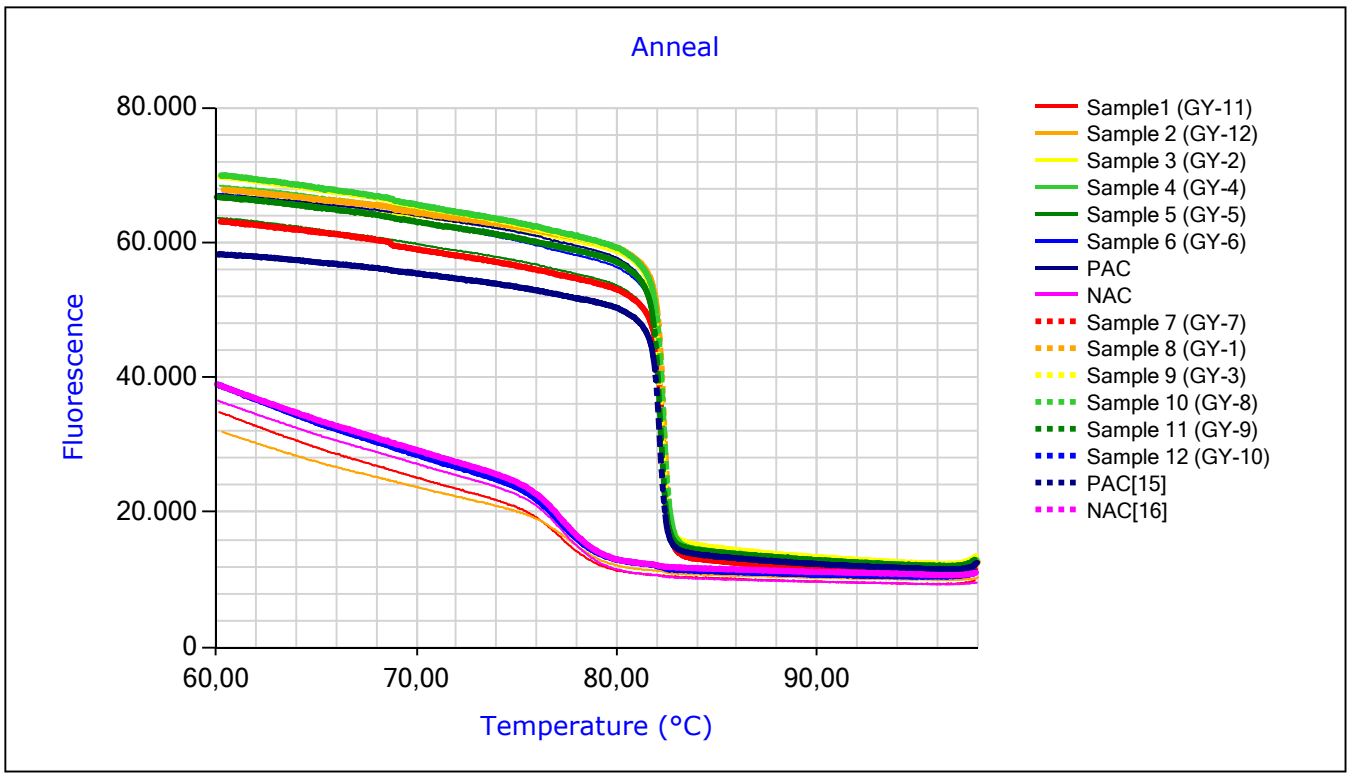
Smoothing Points	3	**Normalization**	From =0 sec To =180 sec



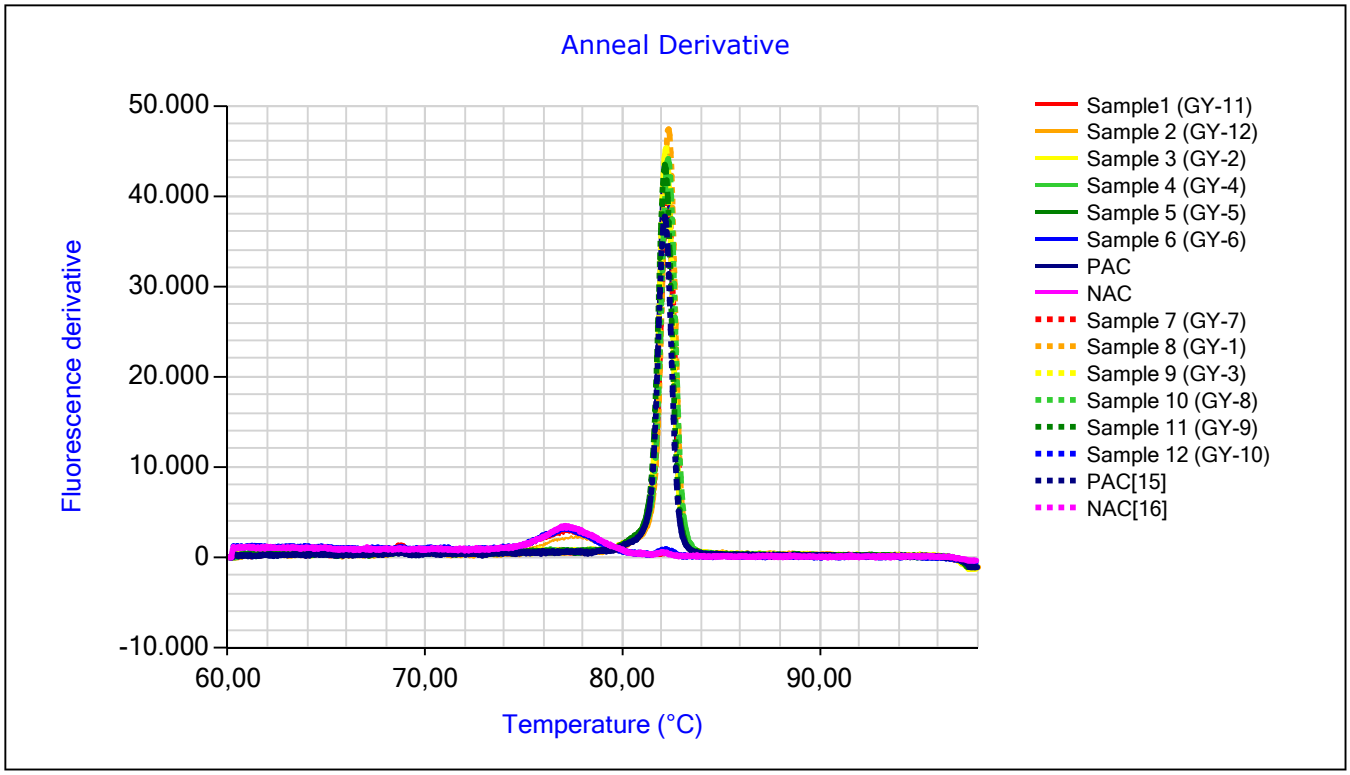
Smoothing Points	2

Peak	Width	Threshold
Simple	0(Sec)	0,02(ratio)

Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	Sample1 (GY-11)	02:00
2	Sample 2 (GY-12)	
3	Sample 3 (GY-2)	01:45
4	Sample 4 (GY-4)	01:45
5	Sample 5 (GY-5)	13:15
6	Sample 6 (GY-6)	02:00
7	PAC	11:45
8	NAC	
9	Sample 7 (GY-7)	13:45
10	Sample 8 (GY-1)	
11	Sample 9 (GY-3)	01:45
12	Sample 10 (GY-8)	02:00
13	Sample 11 (GY-9)	02:15
14	Sample 12 (GY-10)	02:00
15	PAC[15]	01:45
16	NAC[16]	02:45



Smoothing Points	3



Smoothing Points	2

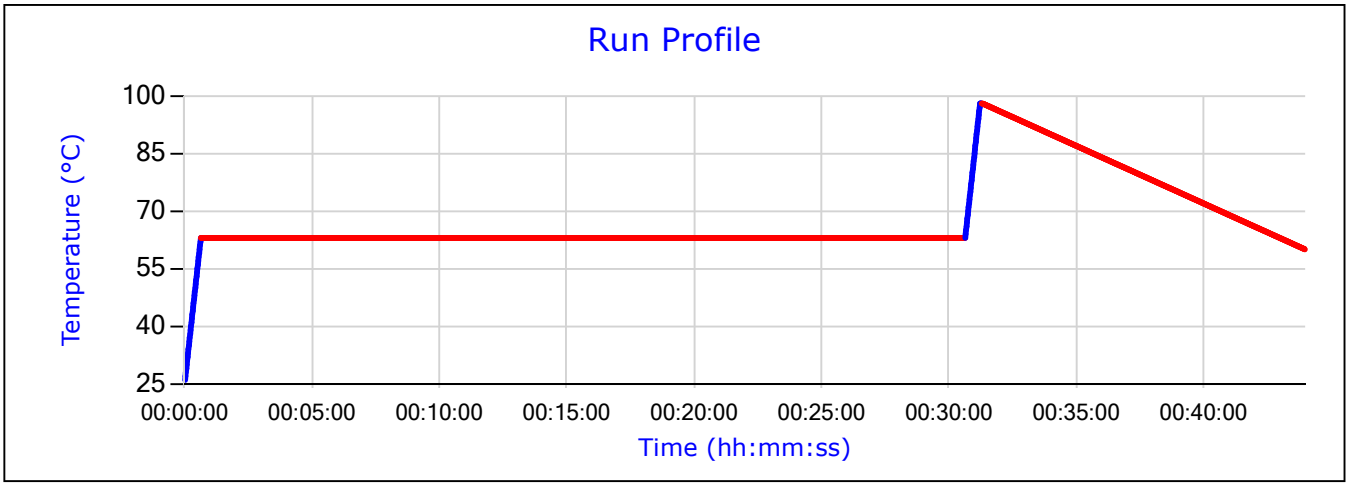
Peak	Width	Threshold
Simple	4(mm:ss)	5000

Well Number	Well Name(°C)	Peak Value(°C)
1	Sample1 (GY-11)	
2	Sample 2 (GY-12)	
3	Sample 3 (GY-2)	82,3
4	Sample 4 (GY-4)	82,3
5	Sample 5 (GY-5)	82,2
6	Sample 6 (GY-6)	82,2
7	PAC	82,1
8	NAC	
9	Sample 7 (GY-7)	82,3
10	Sample 8 (GY-1)	82,3
11	Sample 9 (GY-3)	82,2
12	Sample 10 (GY-8)	82,3
13	Sample 11 (GY-9)	82,1
14	Sample 12 (GY-10)	
15	PAC[15]	82,1
16	NAC[16]	

5.2 Appendix 2: Gene Experiment report – participant 1, run 2

Genie Experiment Report

Profile Name	
Start Time	14:05:19 - petek, 14. avgust 2020
End Time	14:48:43 - petek, 14. avgust 2020
Serial Number	GEN2-1047



Preheat	No
Temperature	45 °C
Time	3 min(s)

Isothermal	Yes
Temperature	63 °C
Time	30 min
Gradient	0 °C

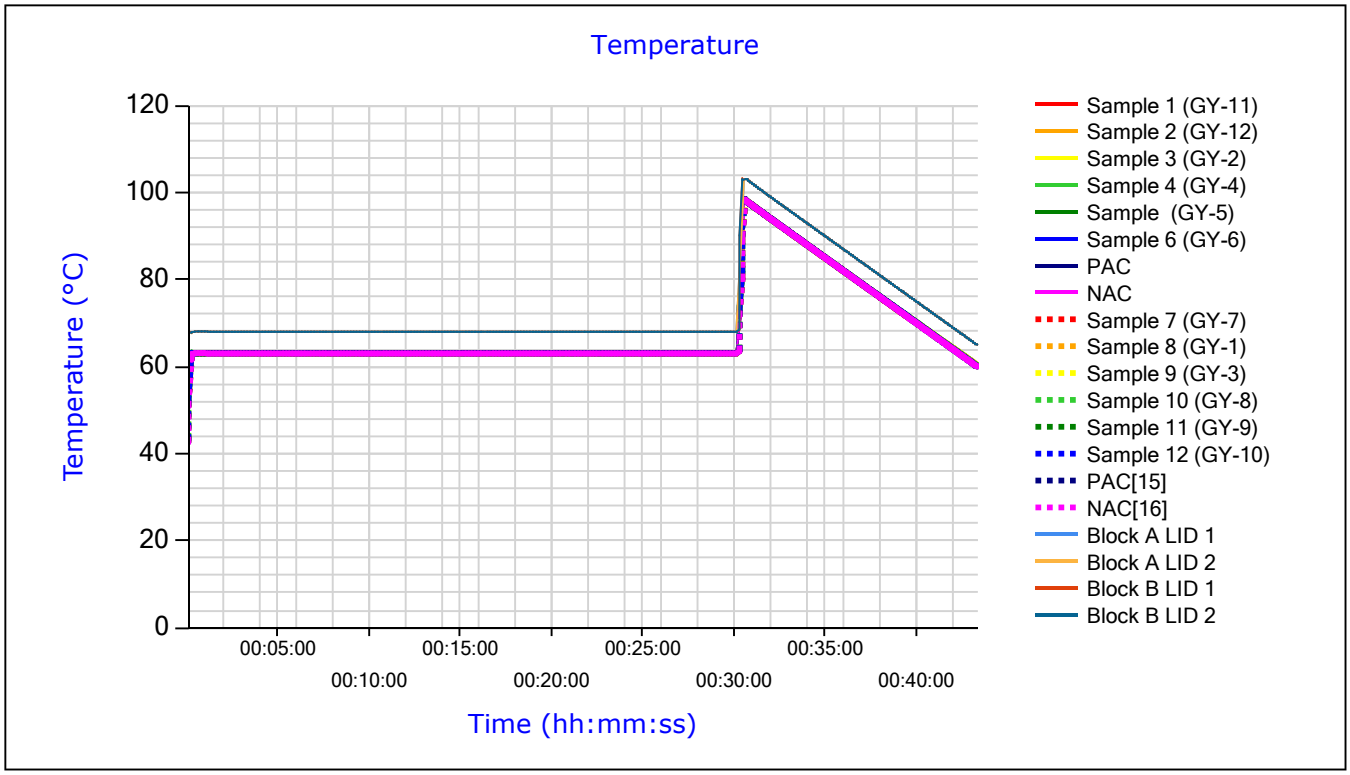
Anneal	Yes
Start Temp	98 °C
End Temp	60 °C
Ramp Rate	0,05 °C/sec

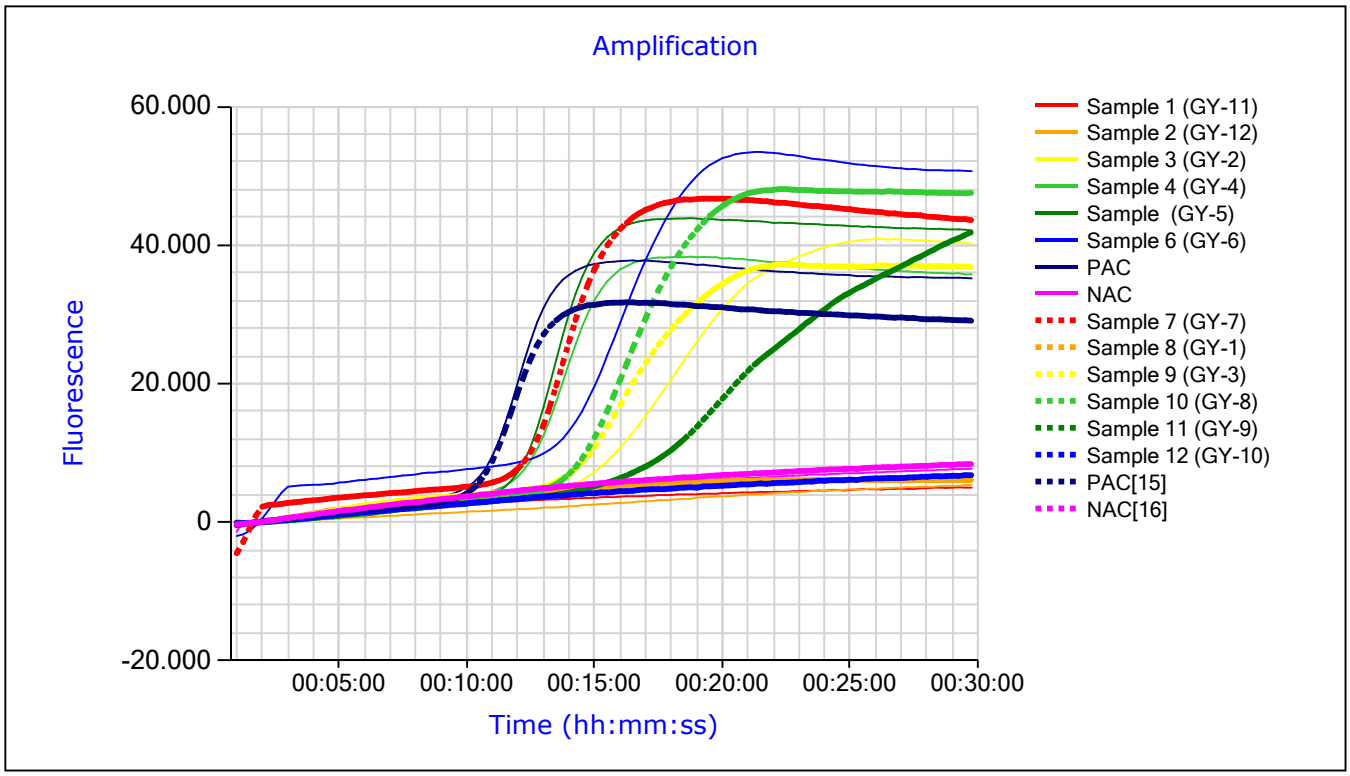
Estimated Run time	43:58 (mm:ss)

Advanced Options:

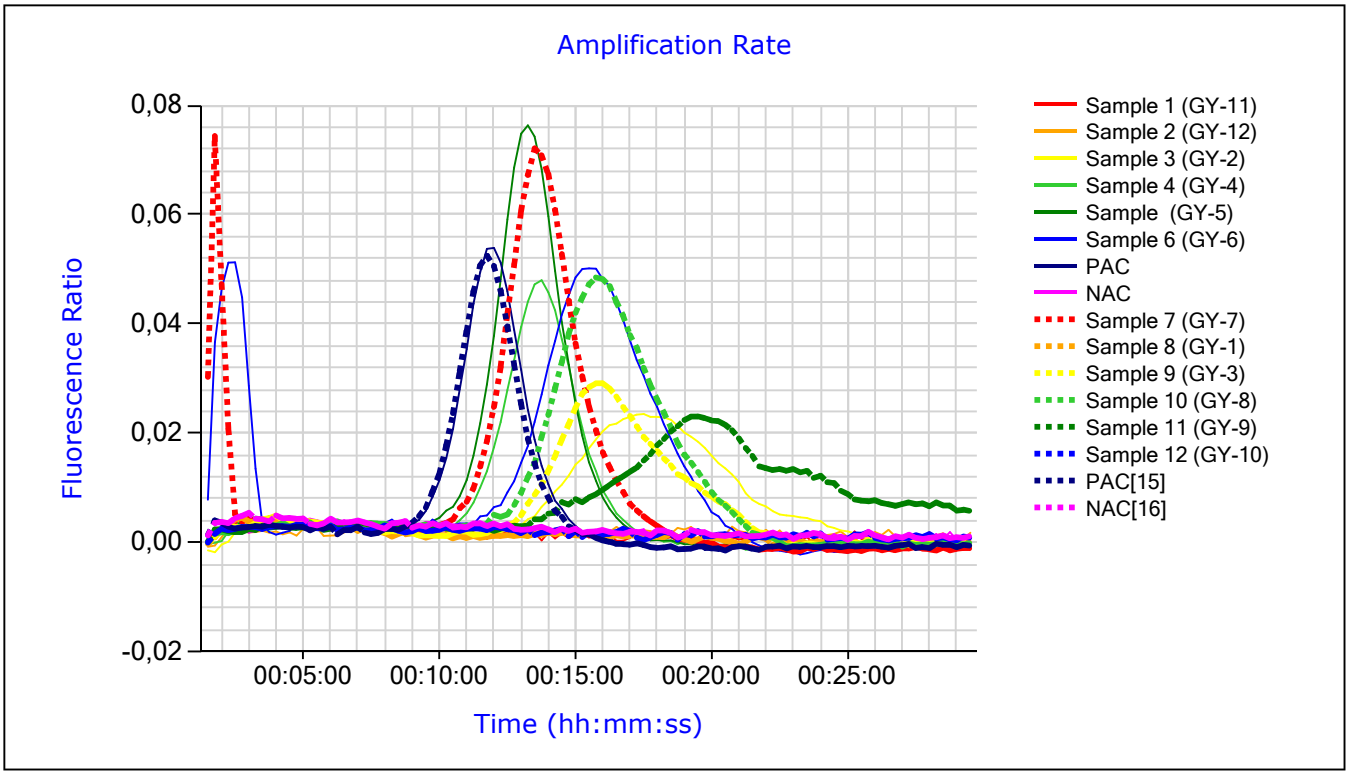
Preheat Ramp Rate	1 °C/Sec
Isothermal Ramp Rate	1 °C/Sec
Isothermal period	15 Sec
Anneal initial rate	1 °C/Sec
Anneal period	0 Sec
Anneal settling time	5 Sec

Notes:





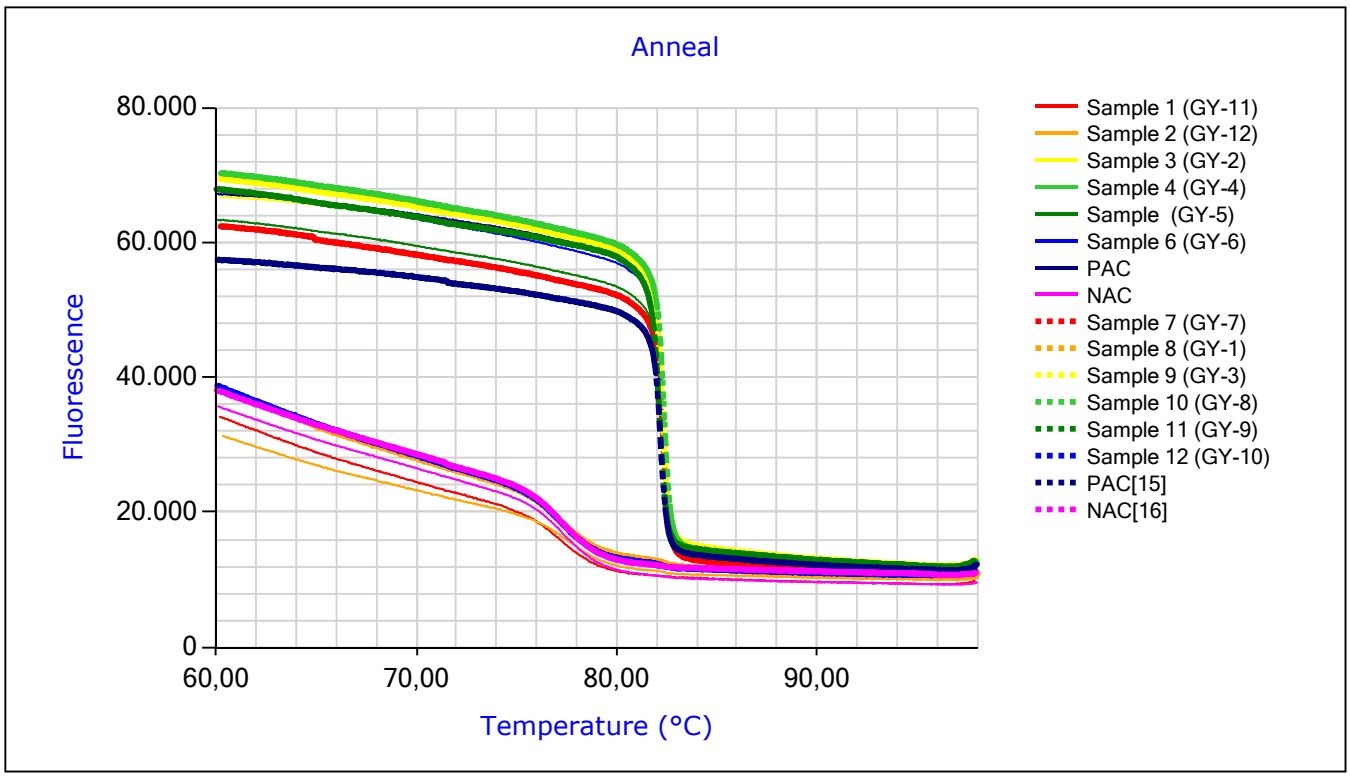
Smoothing Points	3	**Normalization**	From =0 sec To =180 sec



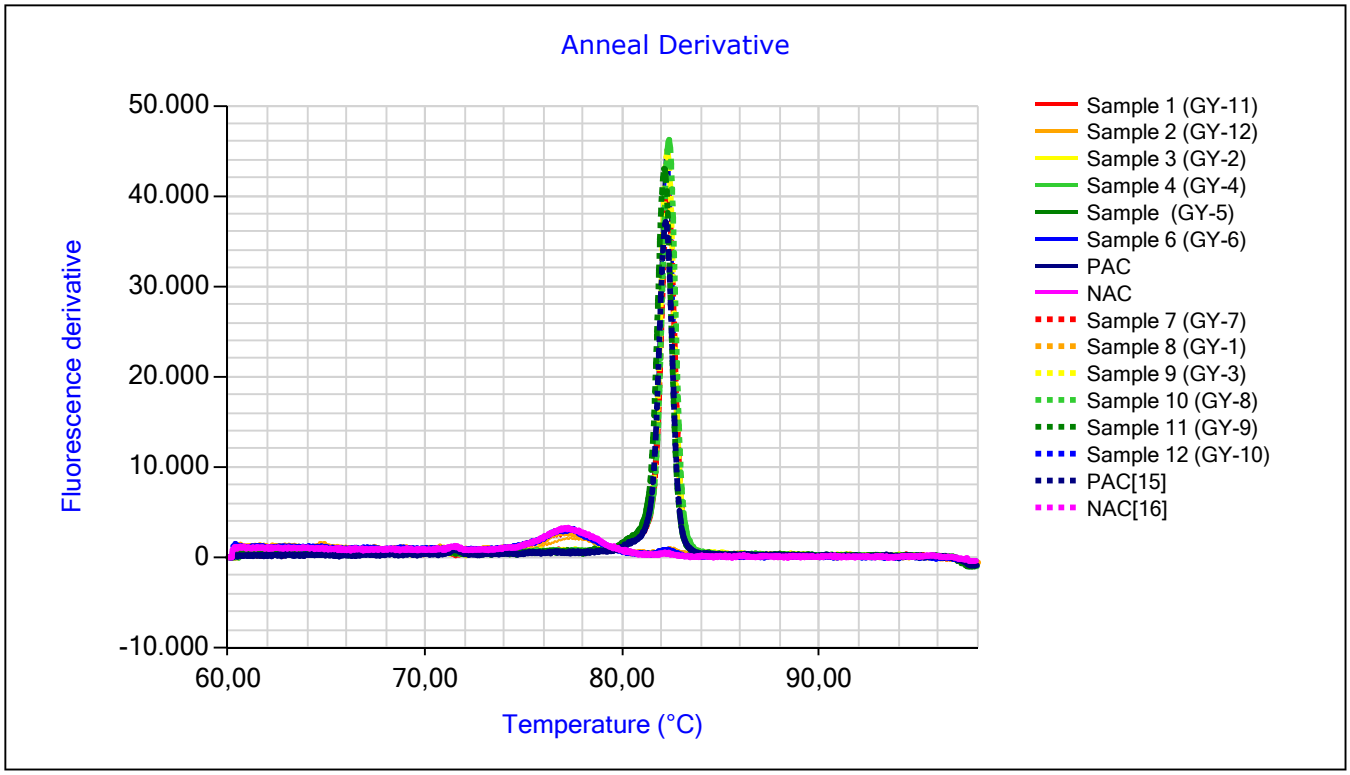
Smoothing Points	2

Peak	Width	Threshold
Simple	0(Sec)	0,02(ratio)

Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	Sample 1 (GY-11)	
2	Sample 2 (GY-12)	
3	Sample 3 (GY-2)	17:30
4	Sample 4 (GY-4)	13:45
5	Sample (GY-5)	13:15
6	Sample 6 (GY-6)	02:30
7	PAC	12:00
8	NAC	
9	Sample 7 (GY-7)	01:45
10	Sample 8 (GY-1)	
11	Sample 9 (GY-3)	16:00
12	Sample 10 (GY-8)	15:45
13	Sample 11 (GY-9)	19:30
14	Sample 12 (GY-10)	
15	PAC[15]	11:45
16	NAC[16]	



Smoothing Points	3



Smoothing Points	2

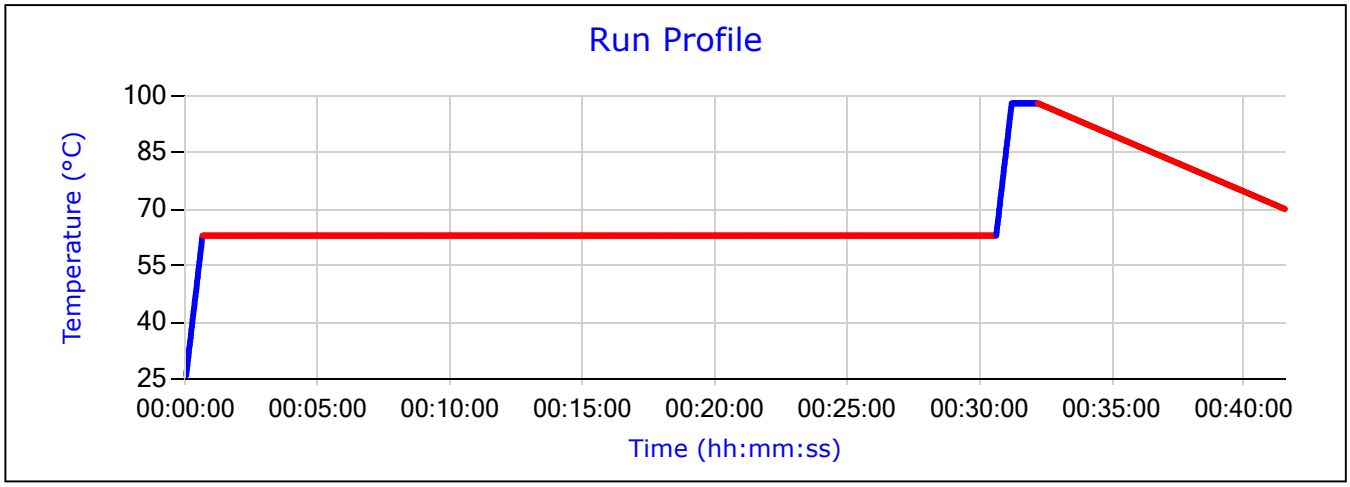
Peak	Width	Threshold
Simple	4(mm:ss)	5000

Well Number	Well Name(°C)	Peak Value(°C)
1	Sample 1 (GY-11)	
2	Sample 2 (GY-12)	
3	Sample 3 (GY-2)	82,2
4	Sample 4 (GY-4)	82,3
5	Sample (GY-5)	82,2
6	Sample 6 (GY-6)	82,3
7	PAC	82,2
8	NAC	
9	Sample 7 (GY-7)	82,3
10	Sample 8 (GY-1)	
11	Sample 9 (GY-3)	82,3
12	Sample 10 (GY-8)	82,4
13	Sample 11 (GY-9)	82,1
14	Sample 12 (GY-10)	
15	PAC[15]	82,2
16	NAC[16]	

5.3 Appendix 3: Gene Experiment report – participant 2, run 1

Genie Experiment Report

Profile Name	tropicsafe gy samples test 1 matt
Start Time	11:48:40 - 10 August 2020
End Time	12:28:00 - 10 August 2020
Serial Number	GEN2-1040



Preheat	No
Temperature	45 °C
Time	3 min(s)

Isothermal	Yes
Temperature	63 °C
Time	30 min
Gradient	0 °C

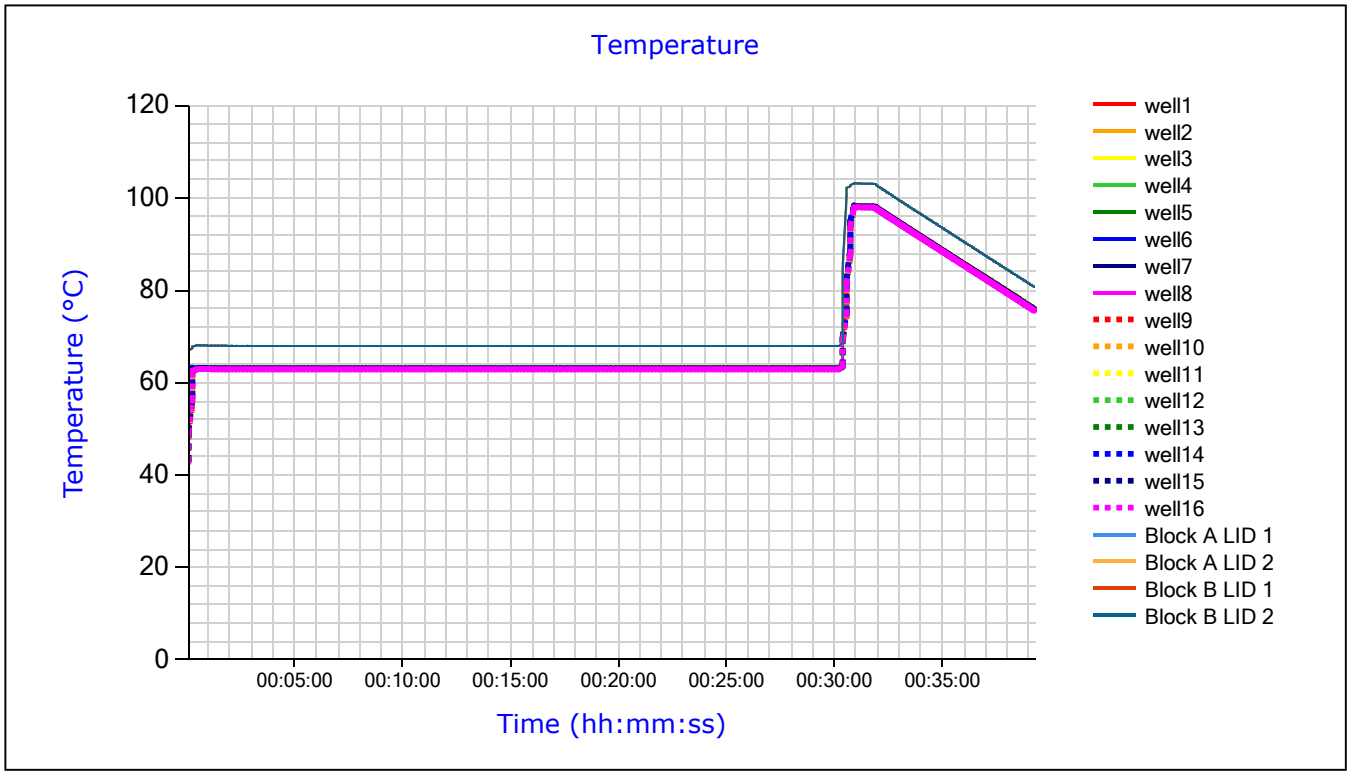
Anneal	Yes
Start Temp	98 °C
End Temp	70 °C
Ramp Rate	0.05 °C/sec

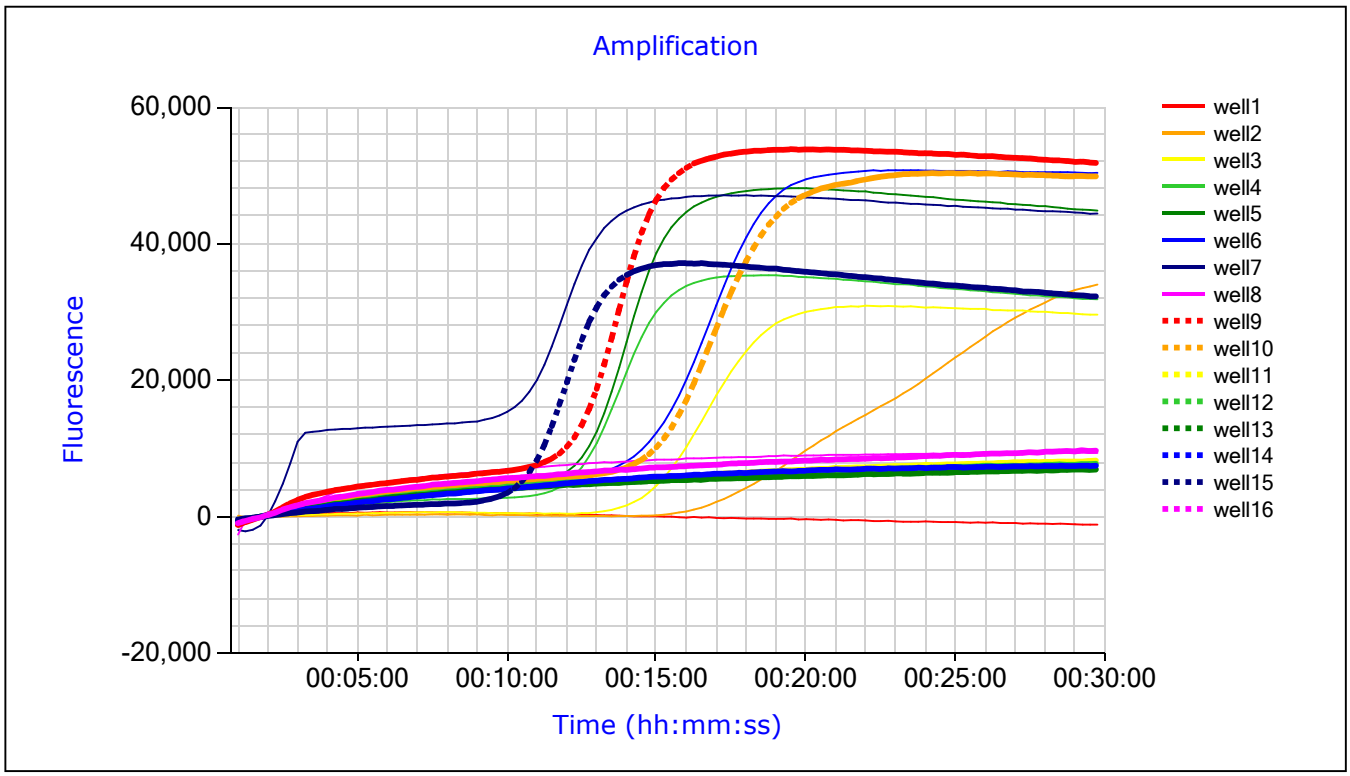
Estimated Run time	41:33 (mm:ss)

Advanced Options:

Preheat Ramp Rate	1 °C/Sec
Isothermal Ramp Rate	1 °C/Sec
Isothermal period	15 Sec
Anneal initial rate	1 °C/Sec
Anneal period	0 Sec
Anneal settling time	60 Sec

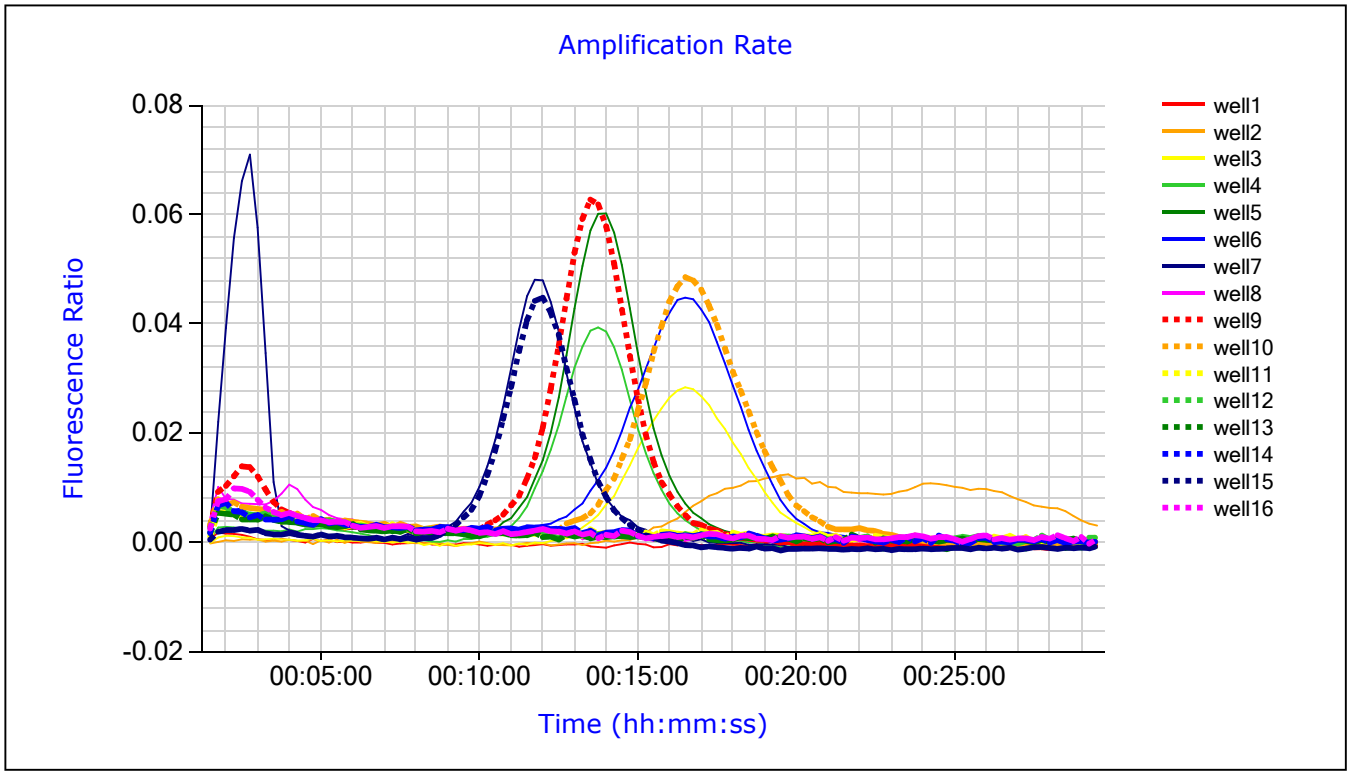
Notes:





Smoothing Points	3

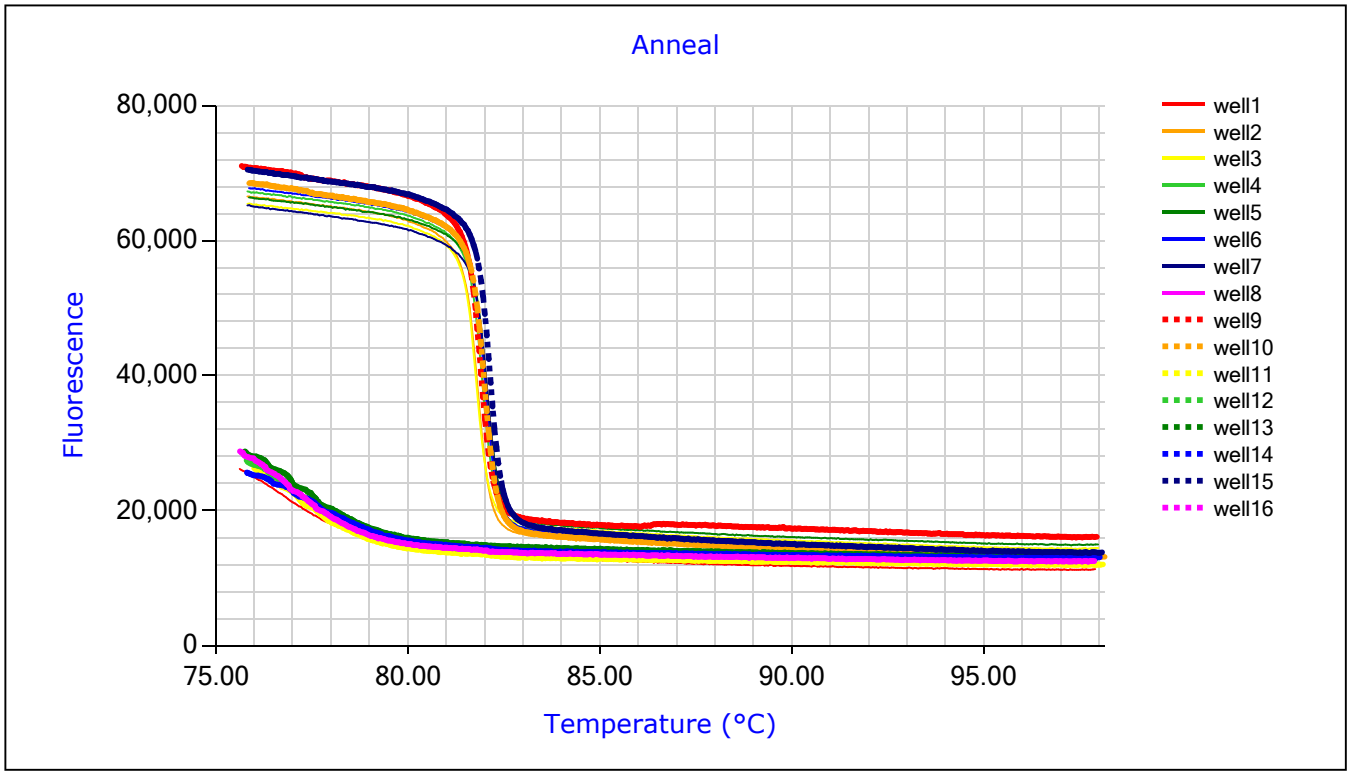
Normalization	From =0 sec To =180 sec



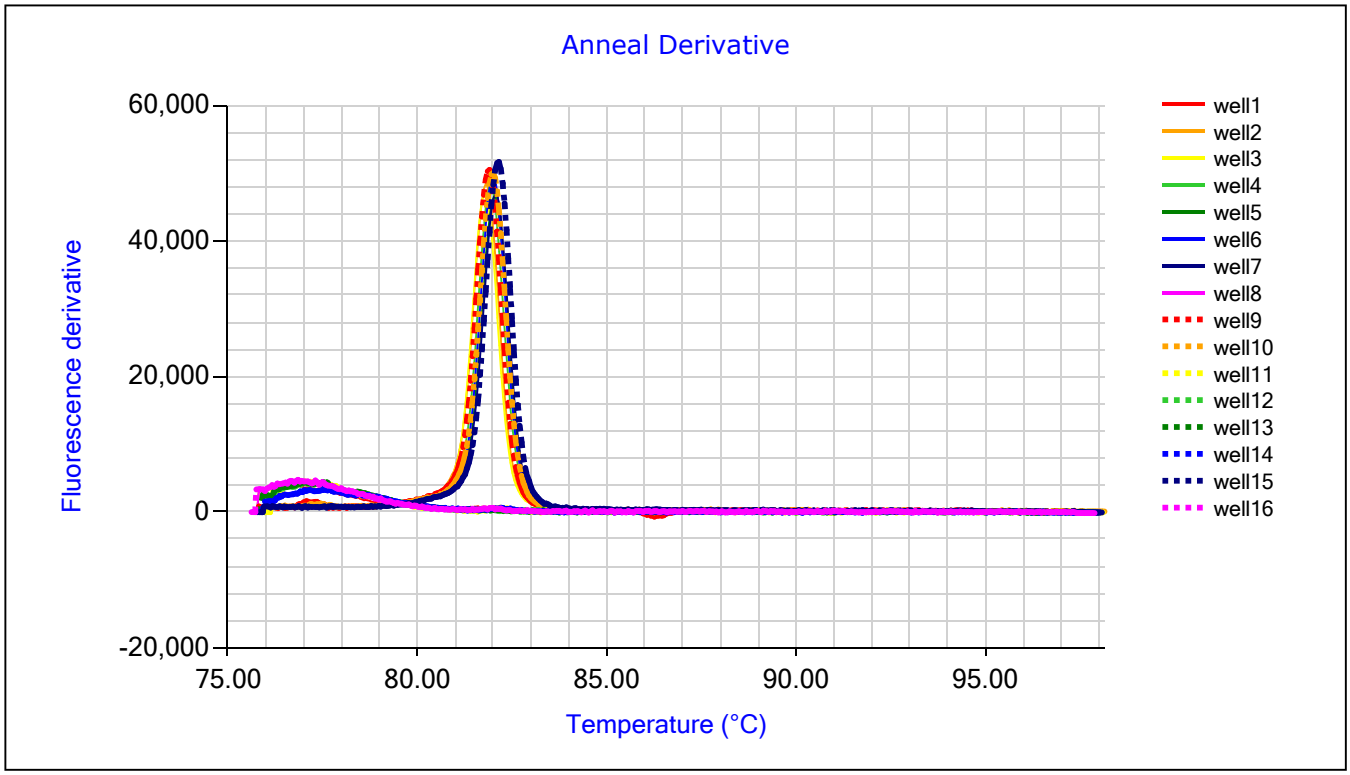
Smoothing Points	2

Peak	Width	Threshold
Simple	0(Sec)	0.02(ratio)

Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	well1	
2	well2	
3	well3	16:30
4	well4	13:45
5	well5	14:00
6	well6	16:30
7	well7	02:45
8	well8	
9	well9	13:30
10	well10	16:30
11	well11	
12	well12	
13	well13	
14	well14	
15	well15	12:00
16	well16	



Smoothing Points	3



Smoothing Points	2

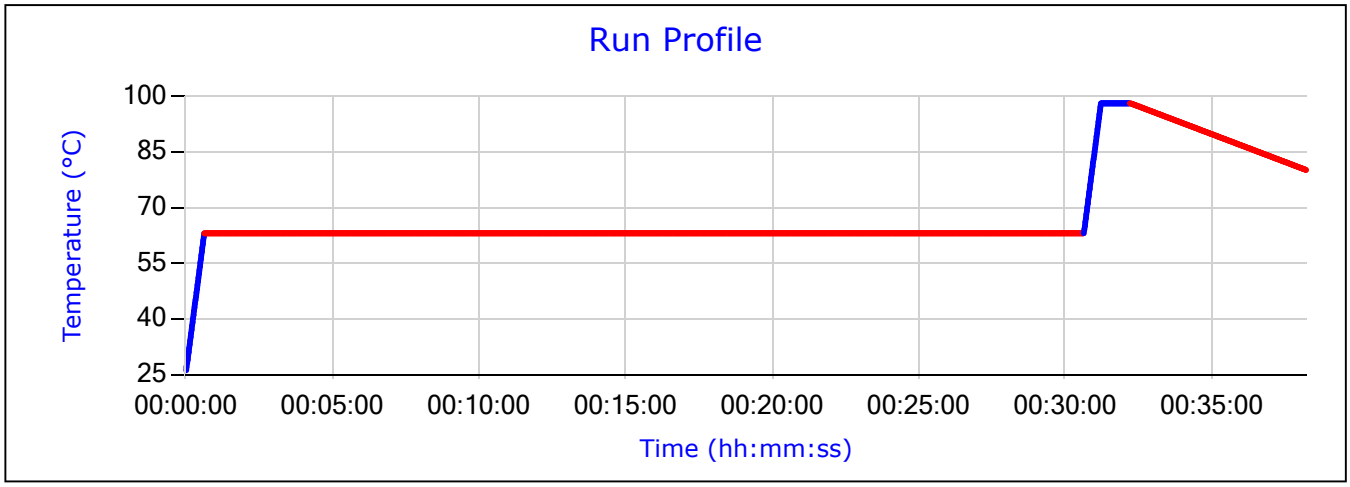
Peak	Width	Threshold
Simple	4(mm:ss)	5000

Well Number	Well Name(°C)	Peak Value(°C)
1	well1	
2	well2	81.8
3	well3	81.8
4	well4	81.9
5	well5	82.0
6	well6	82.0
7	well7	82.0
8	well8	
9	well9	81.9
10	well10	82.0
11	well11	
12	well12	
13	well13	
14	well14	
15	well15	82.1
16	well16	

5.4 Appendix 4: Gene Experiment report – participant 2, run 2

Genie Experiment Report

Profile Name	SLOVENIA TEST
Start Time	13:38:21 - 11 August 2020
End Time	14:16:24 - 11 August 2020
Serial Number	GEN2-1040



Preheat	No
Temperature	45 °C
Time	3 min(s)

Isothermal	Yes
Temperature	63 °C
Time	30 min
Gradient	0 °C

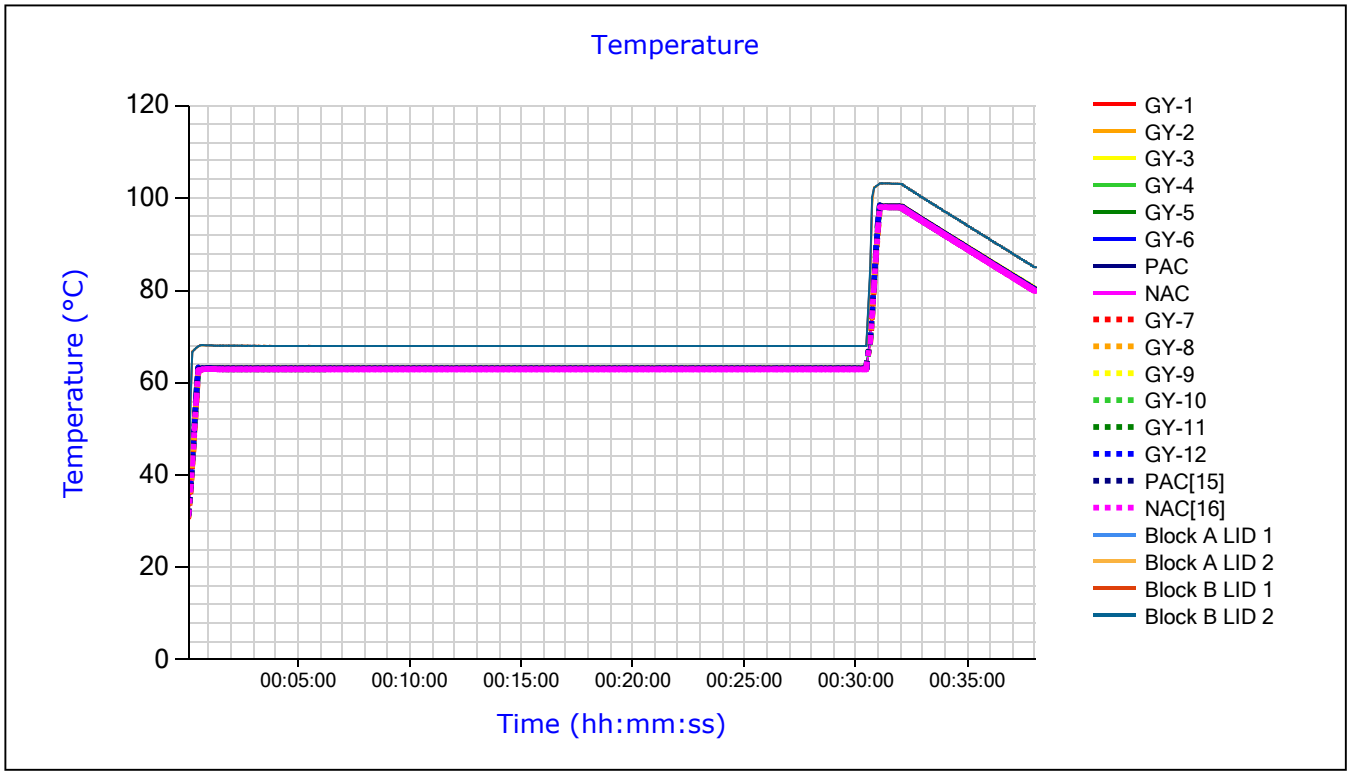
Anneal	Yes
Start Temp	98 °C
End Temp	80 °C
Ramp Rate	0.05 °C/sec

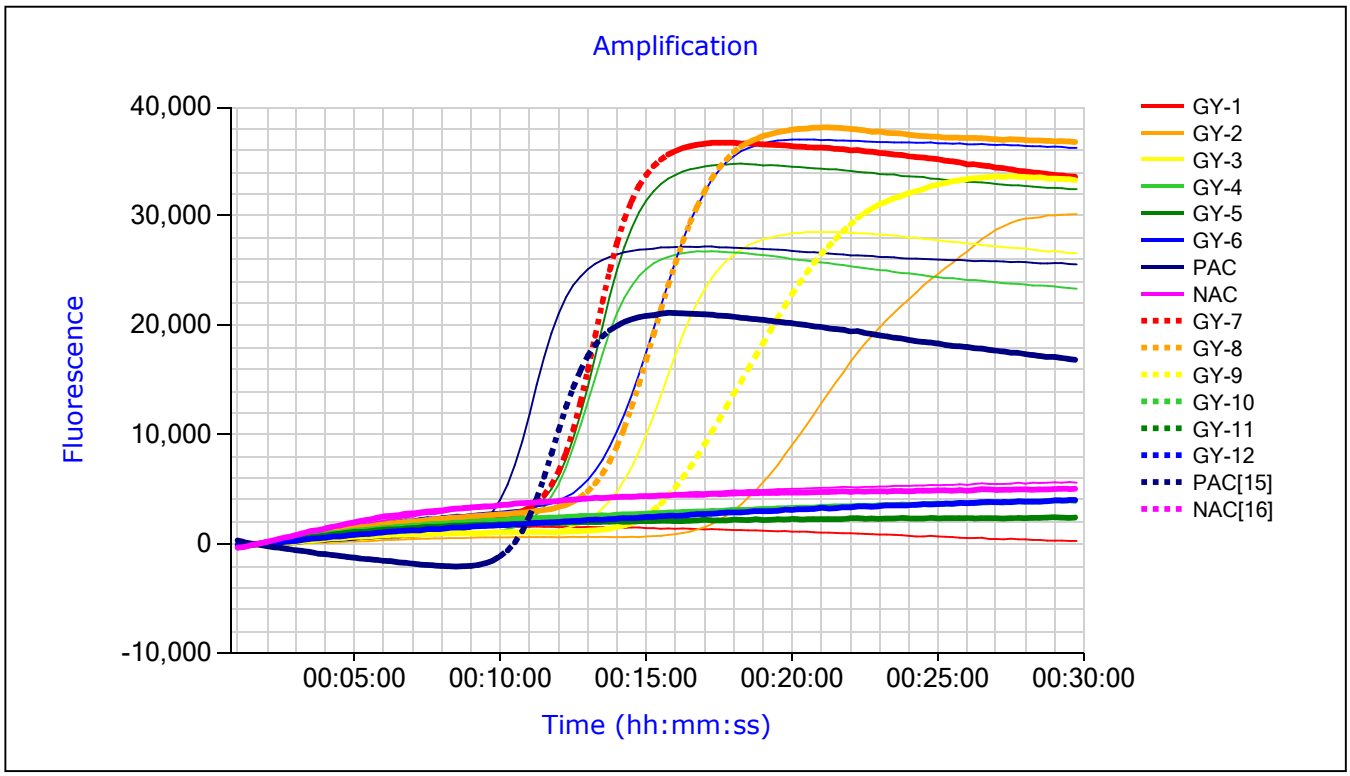
Estimated Run time	38:13 (mm:ss)

Advanced Options:

Preheat Ramp Rate	1 °C/Sec
Isothermal Ramp Rate	1 °C/Sec
Isothermal period	15 Sec
Anneal initial rate	1 °C/Sec
Anneal period	0 Sec
Anneal settling time	60 Sec

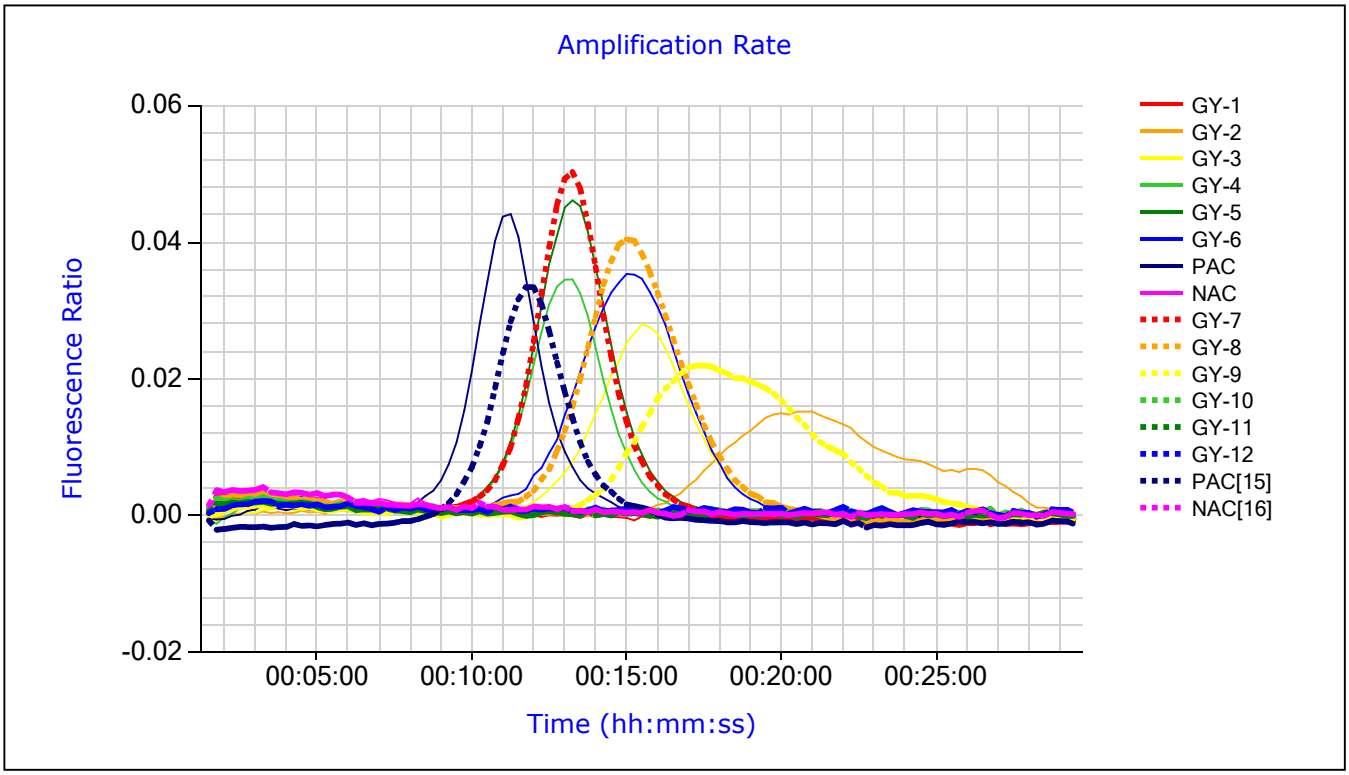
Notes:





Smoothing Points	3

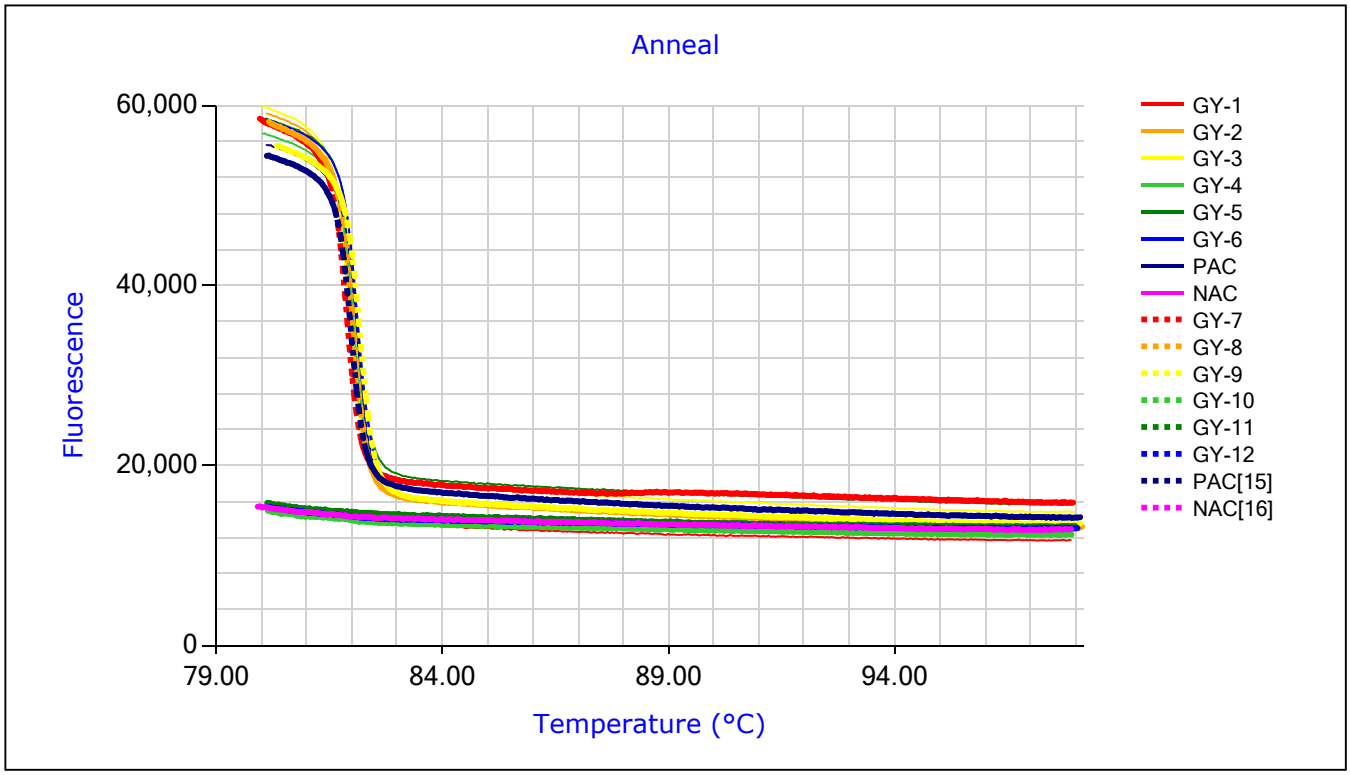
Normalization	From =0 sec To =180 sec



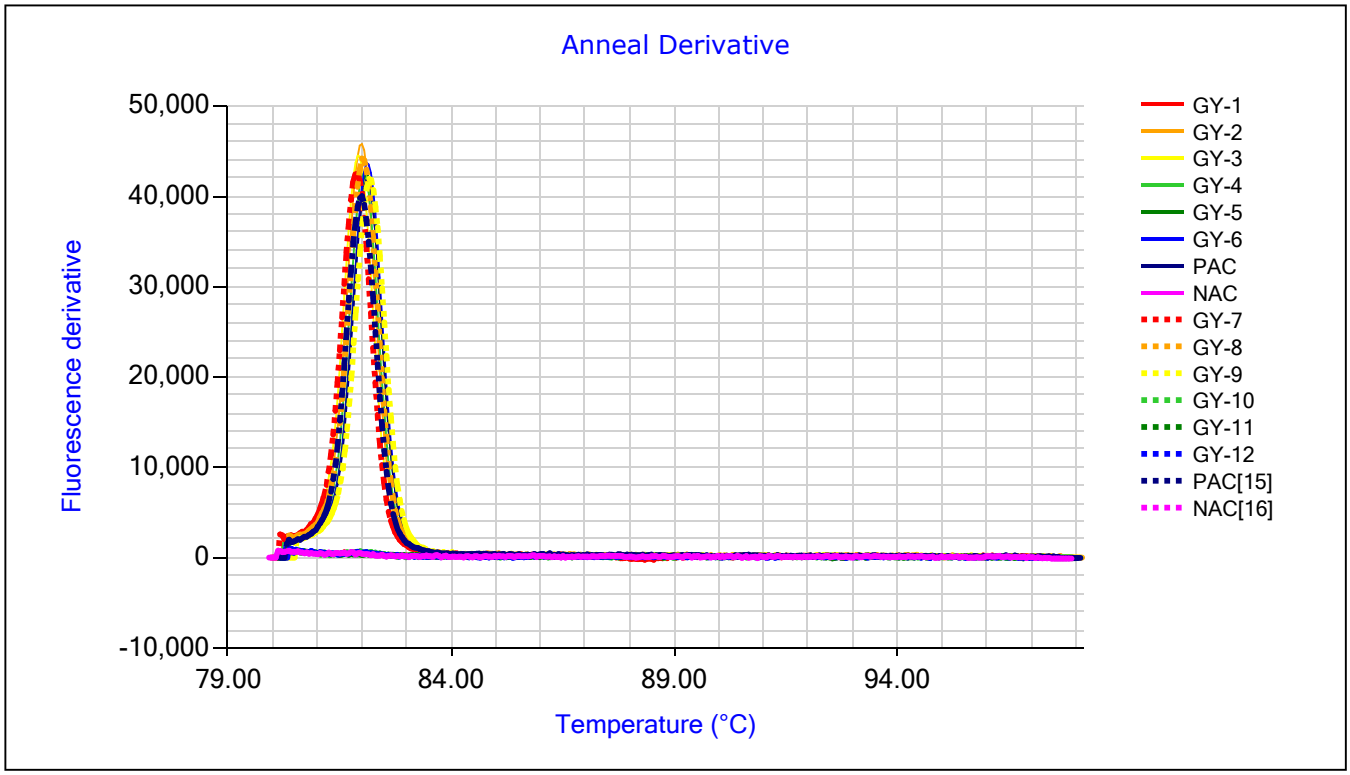
Smoothing Points	2

Peak	Width	Threshold
Simple	0(Sec)	0.02(ratio)

Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	GY-1	
2	GY-2	
3	GY-3	15:30
4	GY-4	13:15
5	GY-5	13:15
6	GY-6	15:00
7	PAC	11:15
8	NAC	
9	GY-7	13:15
10	GY-8	15:00
11	GY-9	17:30
12	GY-10	
13	GY-11	
14	GY-12	
15	PAC[15]	11:45
16	NAC[16]	



Smoothing Points	3



Smoothing Points	2

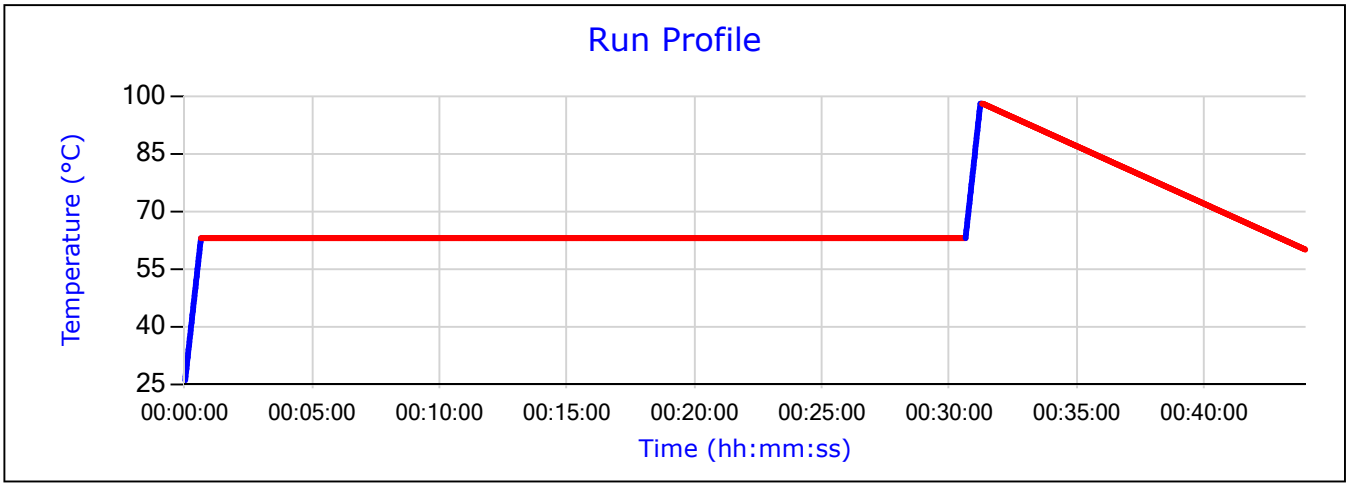
Peak	Width	Threshold
Simple	4(mm:ss)	5000

Well Number	Well Name(°C)	Peak Value(°C)
1	GY-1	
2	GY-2	82.0
3	GY-3	81.9
4	GY-4	82.0
5	GY-5	82.1
6	GY-6	82.1
7	PAC	82.1
8	NAC	
9	GY-7	81.9
10	GY-8	82.0
11	GY-9	82.2
12	GY-10	
13	GY-11	
14	GY-12	
15	PAC[15]	82.0
16	NAC[16]	

5.5 Appendix 5: Gene Experiment report – stability testing, run 1

Genie Experiment Report

Profile Name	
Start Time	16:16:29 - četrtek, 27. avgust 2020
End Time	16:59:58 - četrtek, 27. avgust 2020
Serial Number	GEN2-1047



Preheat	No
Temperature	45 °C
Time	3 min(s)

Isothermal	Yes
Temperature	63 °C
Time	30 min
Gradient	0 °C

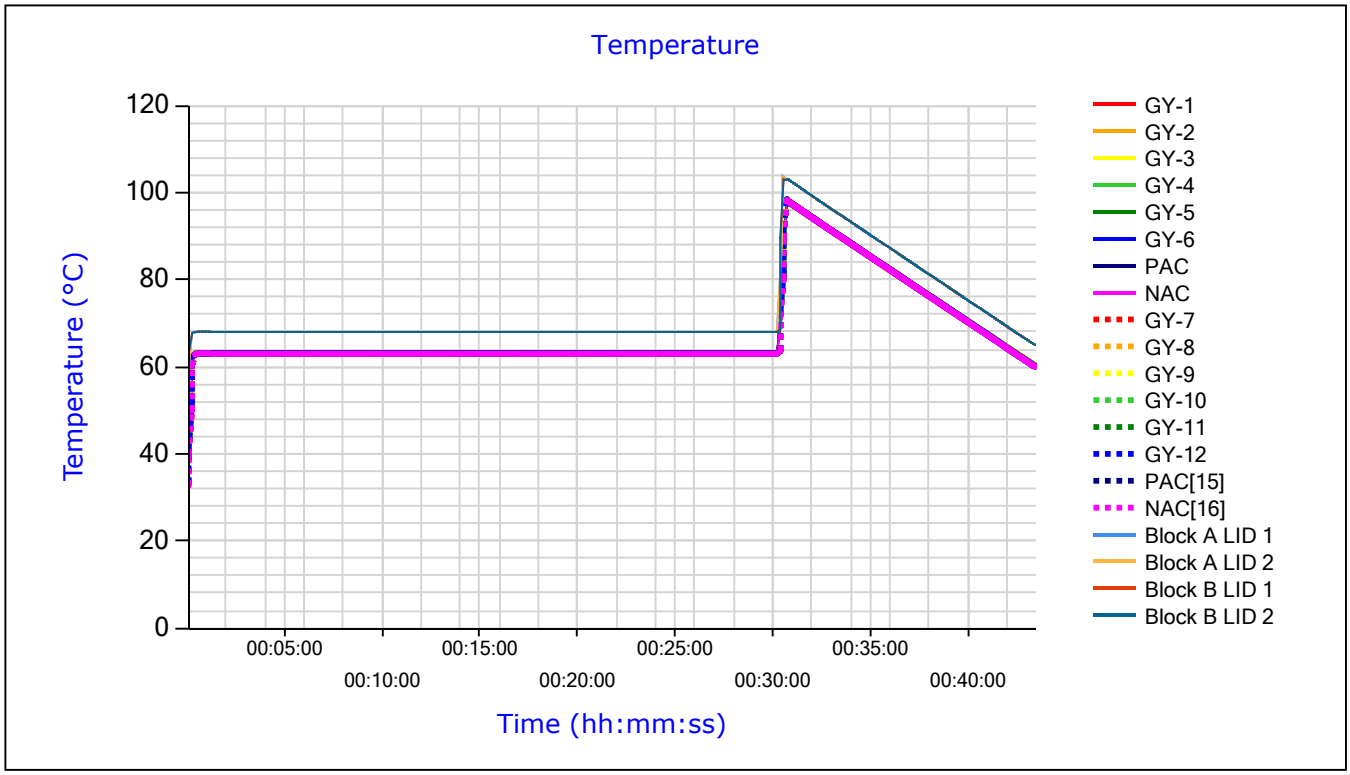
Anneal	Yes
Start Temp	98 °C
End Temp	60 °C
Ramp Rate	0,05 °C/sec

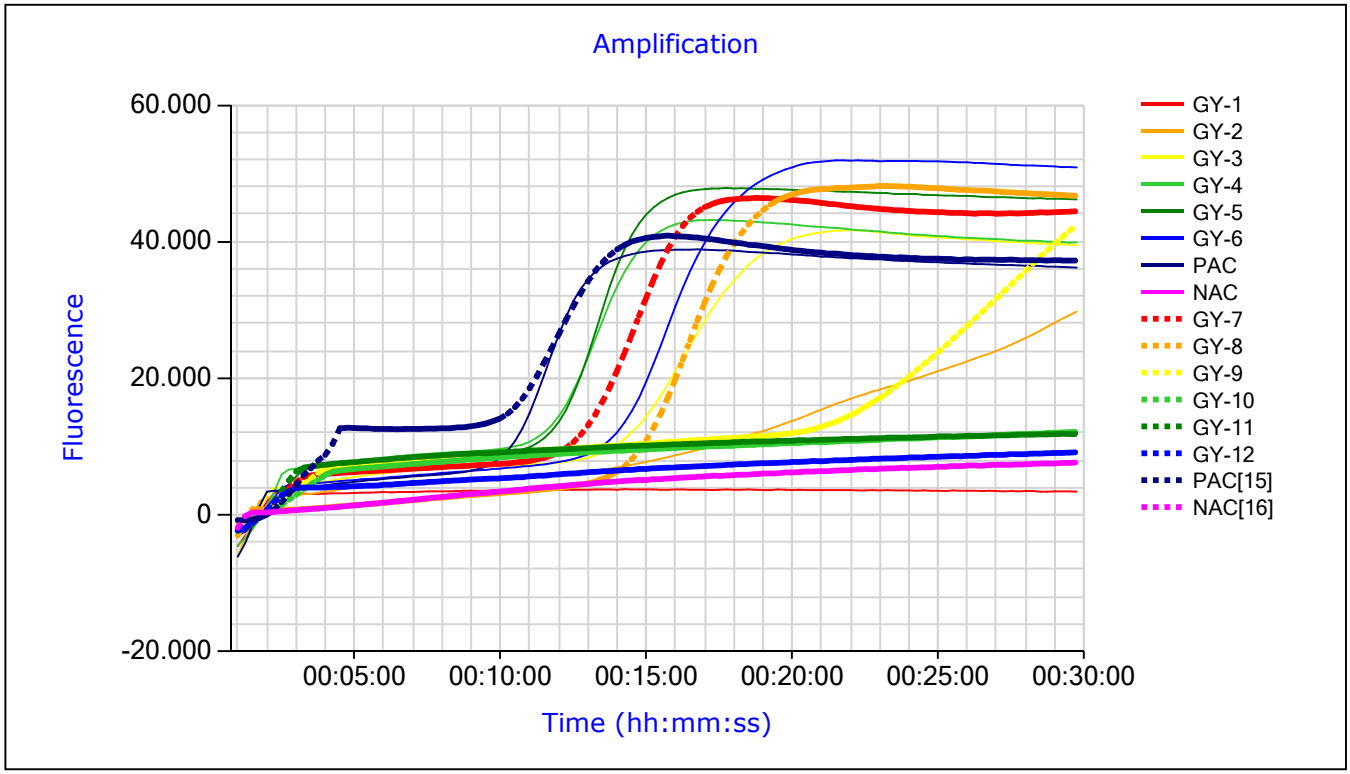
Estimated Run time	43:58 (mm:ss)

Advanced Options:

Preheat Ramp Rate	1 °C/Sec
Isothermal Ramp Rate	1 °C/Sec
Isothermal period	15 Sec
Anneal initial rate	1 °C/Sec
Anneal period	0 Sec
Anneal settling time	5 Sec

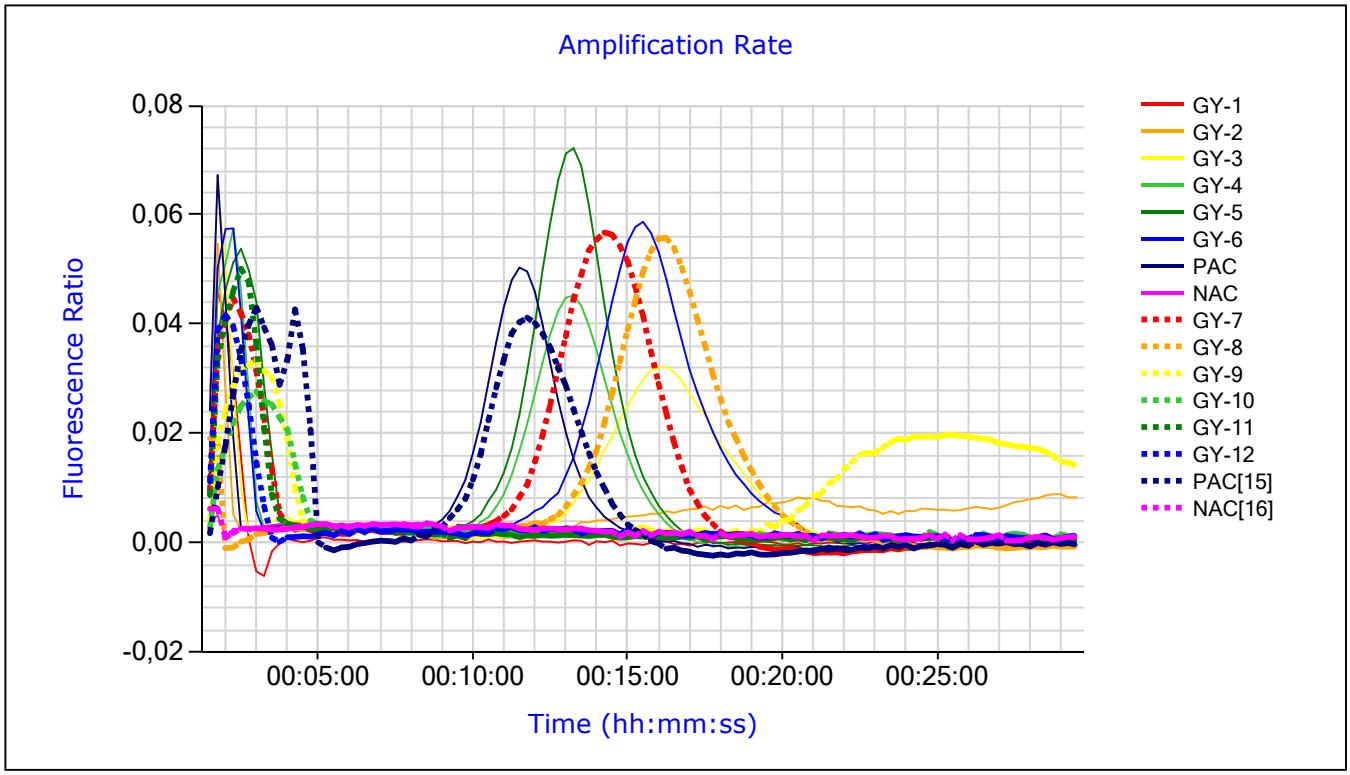
Notes:





Smoothing Points	3

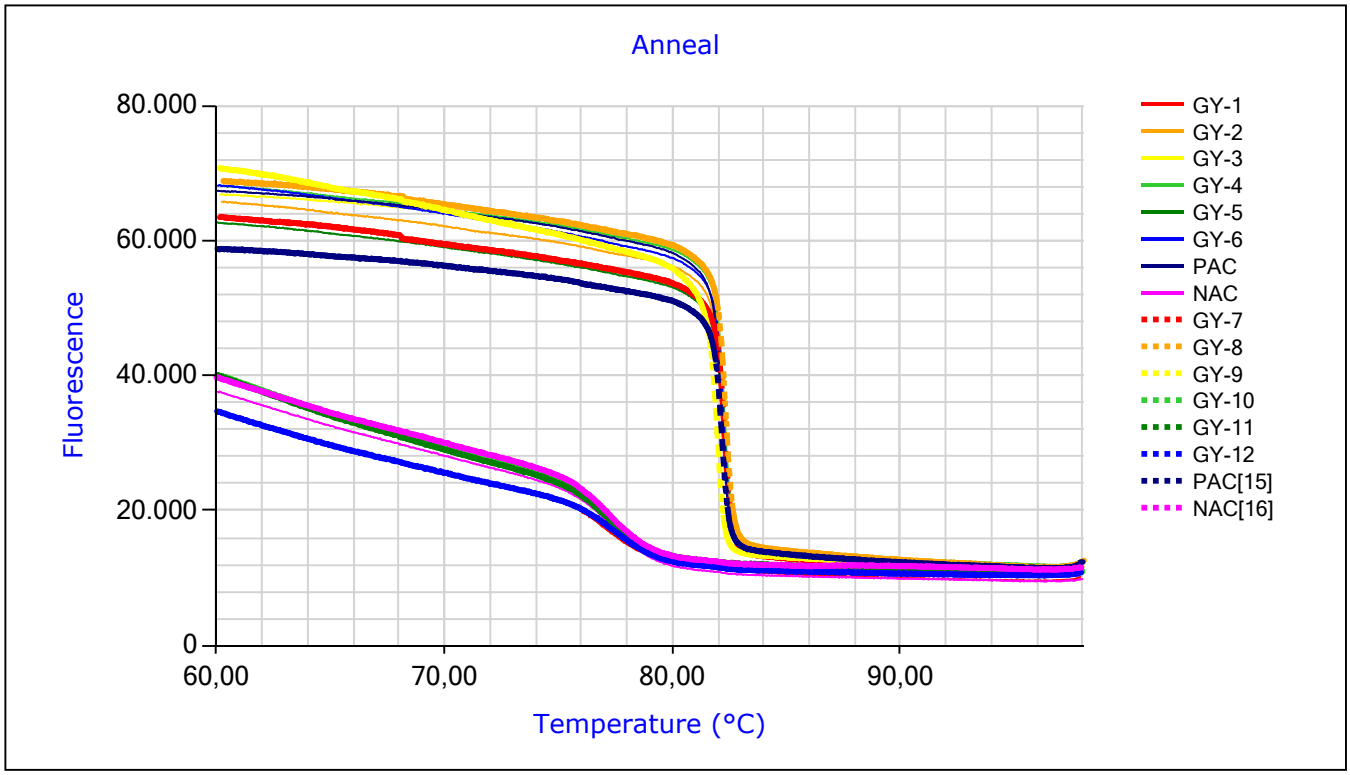
Normalization	From =0 sec To =180 sec



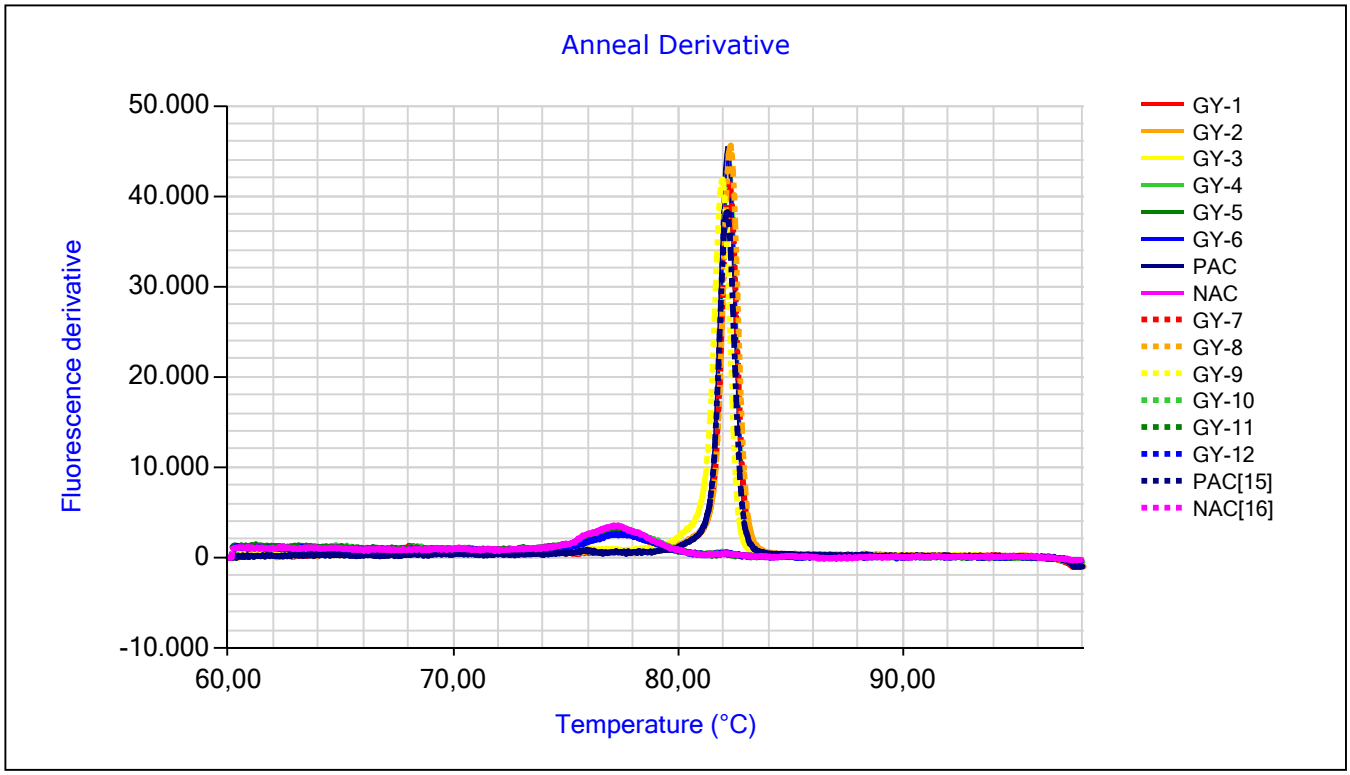
Smoothing Points	2

Peak	Width	Threshold
Simple	0(Sec)	0,02(ratio)

Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	GY-1	01:45
2	GY-2	01:45
3	GY-3	01:45
4	GY-4	02:15
5	GY-5	13:15
6	GY-6	15:30
7	PAC	01:45
8	NAC	
9	GY-7	14:15
10	GY-8	16:15
11	GY-9	03:00
12	GY-10	03:00
13	GY-11	02:30
14	GY-12	02:00
15	PAC[15]	03:00
16	NAC[16]	



Smoothing Points	3



Smoothing Points	2

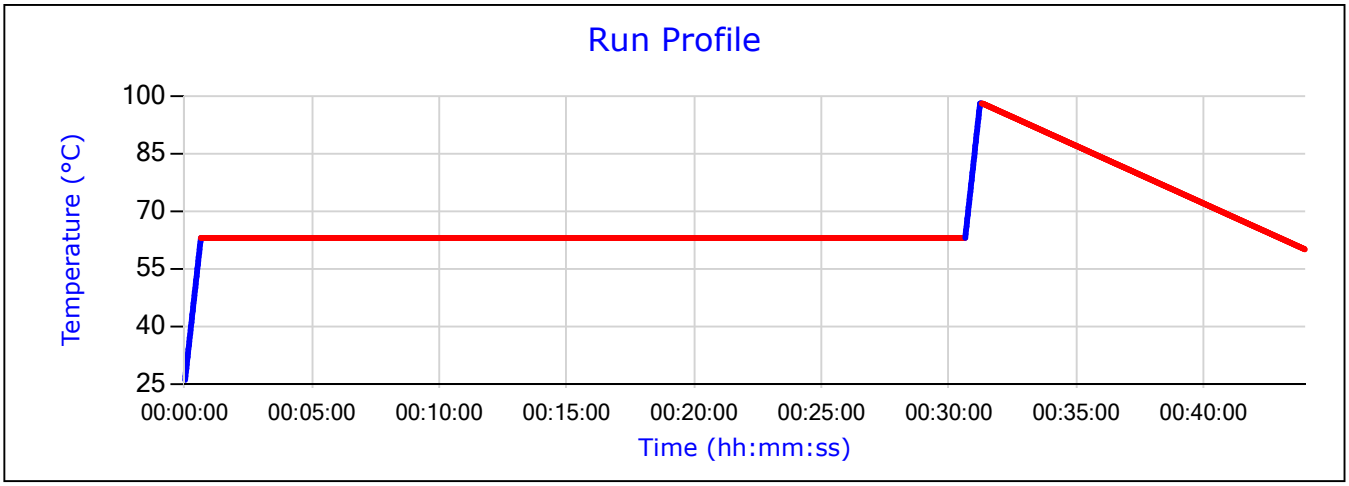
Peak	Width	Threshold
Simple	4(mm:ss)	5000

Well Number	Well Name(°C)	Peak Value(°C)
1	GY-1	
2	GY-2	82,2
3	GY-3	82,3
4	GY-4	82,3
5	GY-5	82,2
6	GY-6	82,2
7	PAC	82,1
8	NAC	
9	GY-7	82,3
10	GY-8	82,3
11	GY-9	82,0
12	GY-10	
13	GY-11	
14	GY-12	
15	PAC[15]	82,2
16	NAC[16]	

5.6 Appendix 5: Gene Experiment report – stability testing, run 2

Genie Experiment Report

Profile Name	
Start Time	17:38:30 - četrtek, 27. avgust 2020
End Time	18:21:55 - četrtek, 27. avgust 2020
Serial Number	GEN2-1047



Preheat	No
Temperature	45 °C
Time	3 min(s)

Isothermal	Yes
Temperature	63 °C
Time	30 min
Gradient	0 °C

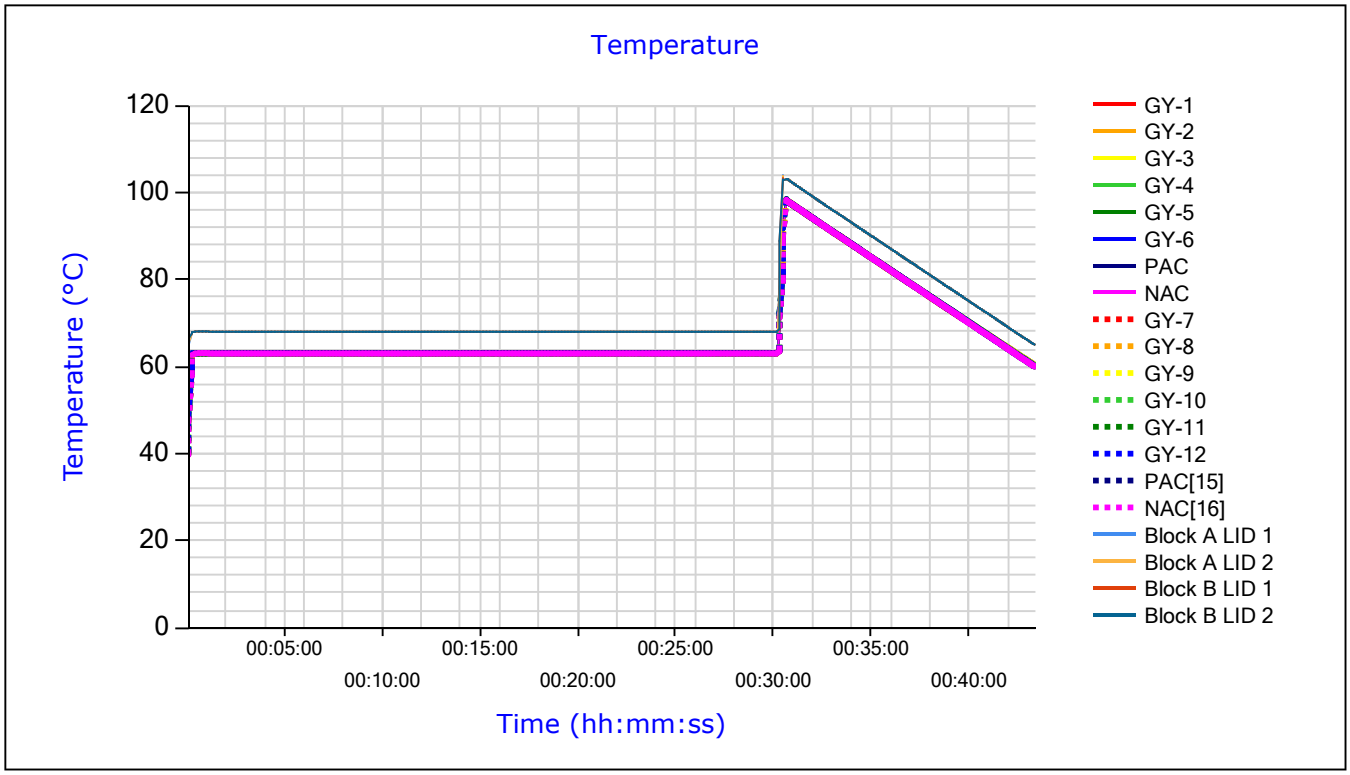
Anneal	Yes
Start Temp	98 °C
End Temp	60 °C
Ramp Rate	0,05 °C/sec

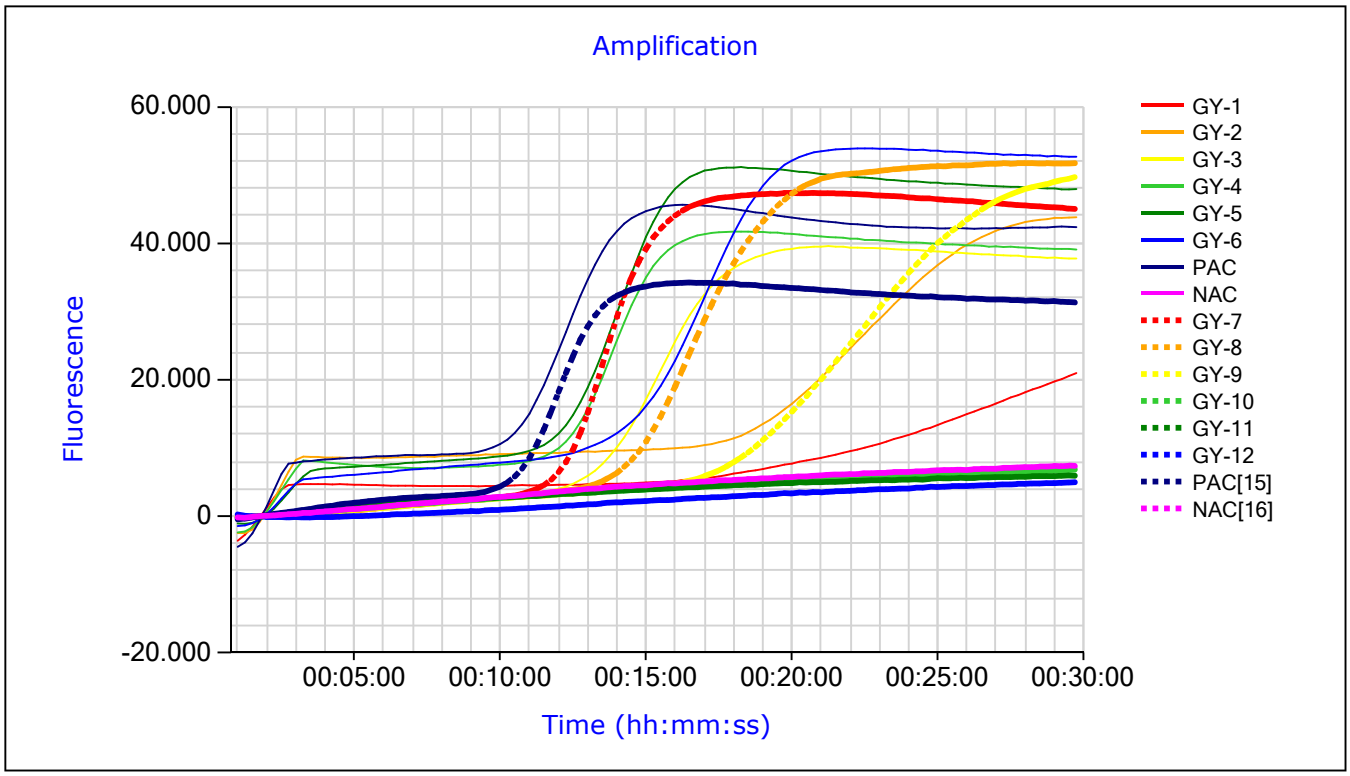
Estimated Run time	43:58 (mm:ss)

Advanced Options:

Preheat Ramp Rate	1 °C/Sec
Isothermal Ramp Rate	1 °C/Sec
Isothermal period	15 Sec
Anneal initial rate	1 °C/Sec
Anneal period	0 Sec
Anneal settling time	5 Sec

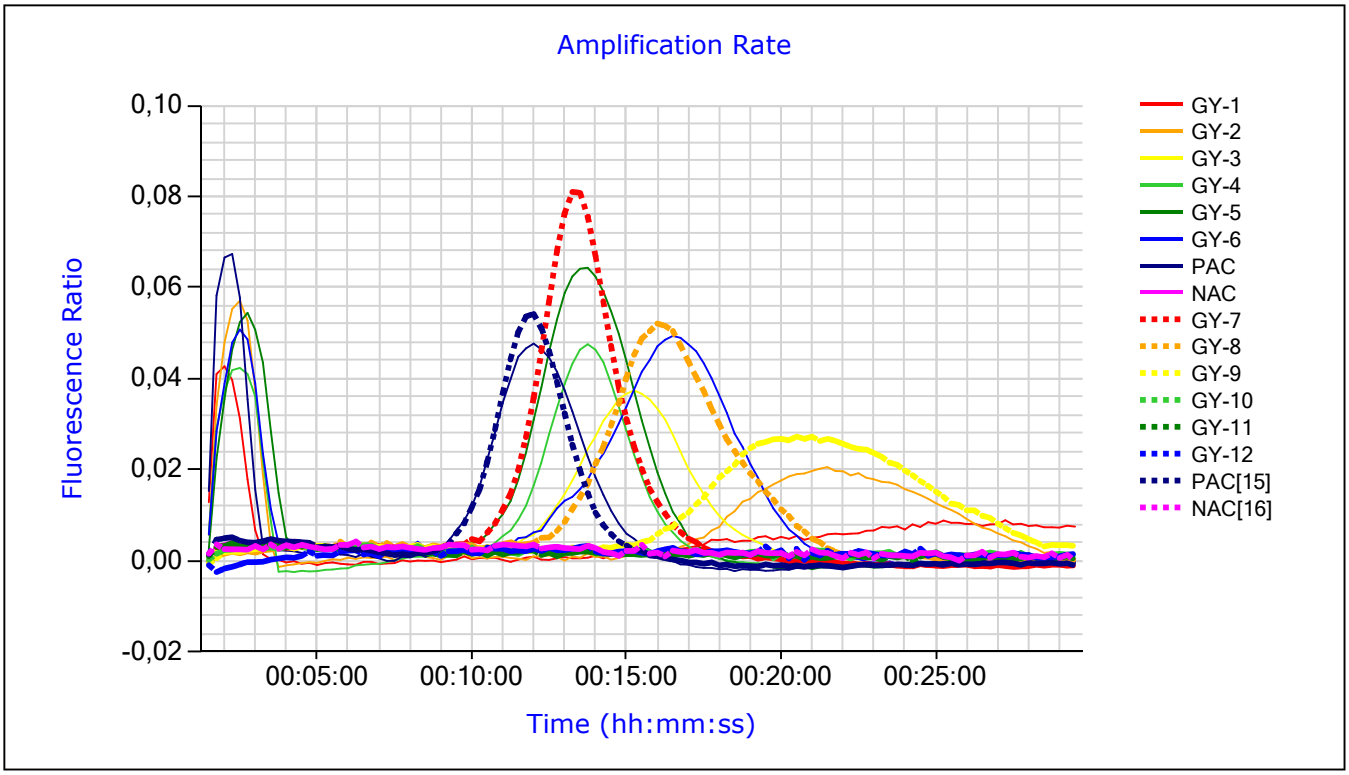
Notes:





Smoothing Points	3

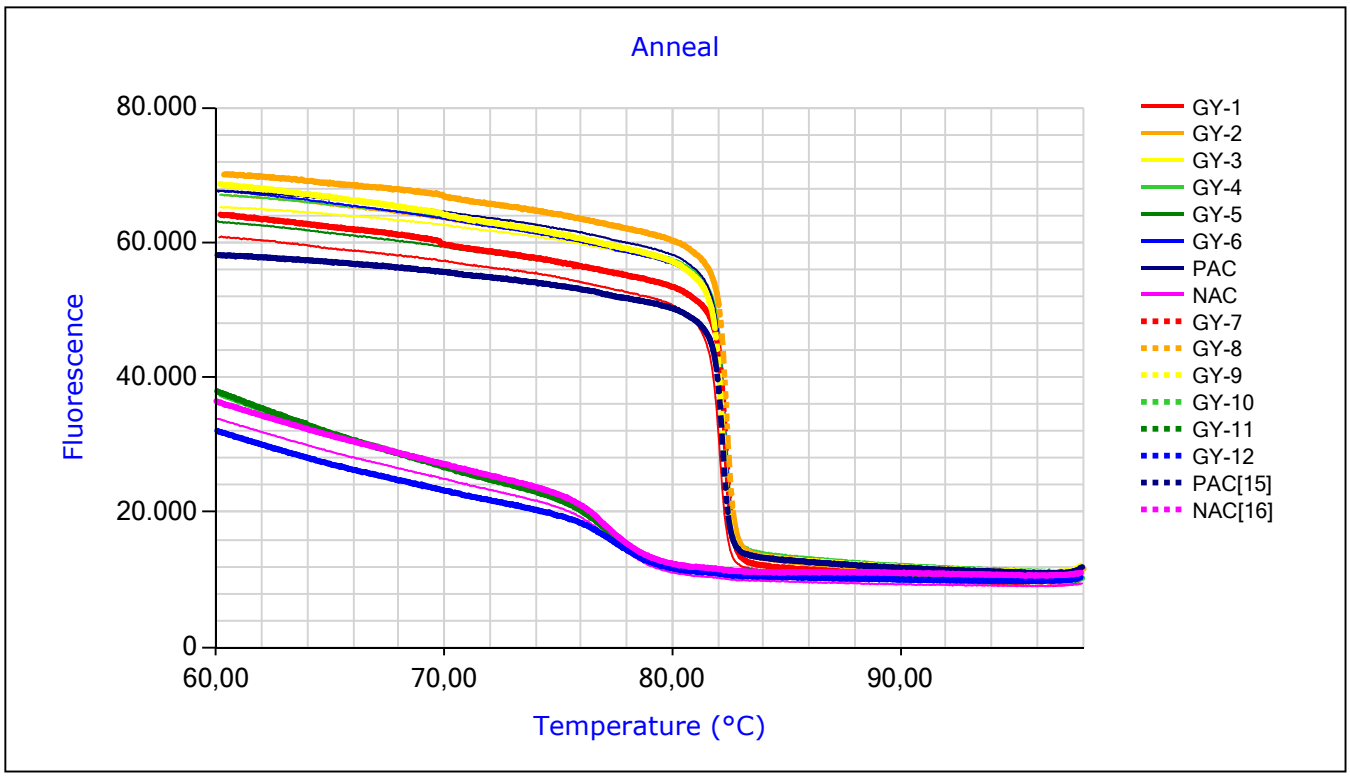
Normalization	From =0 sec To =180 sec



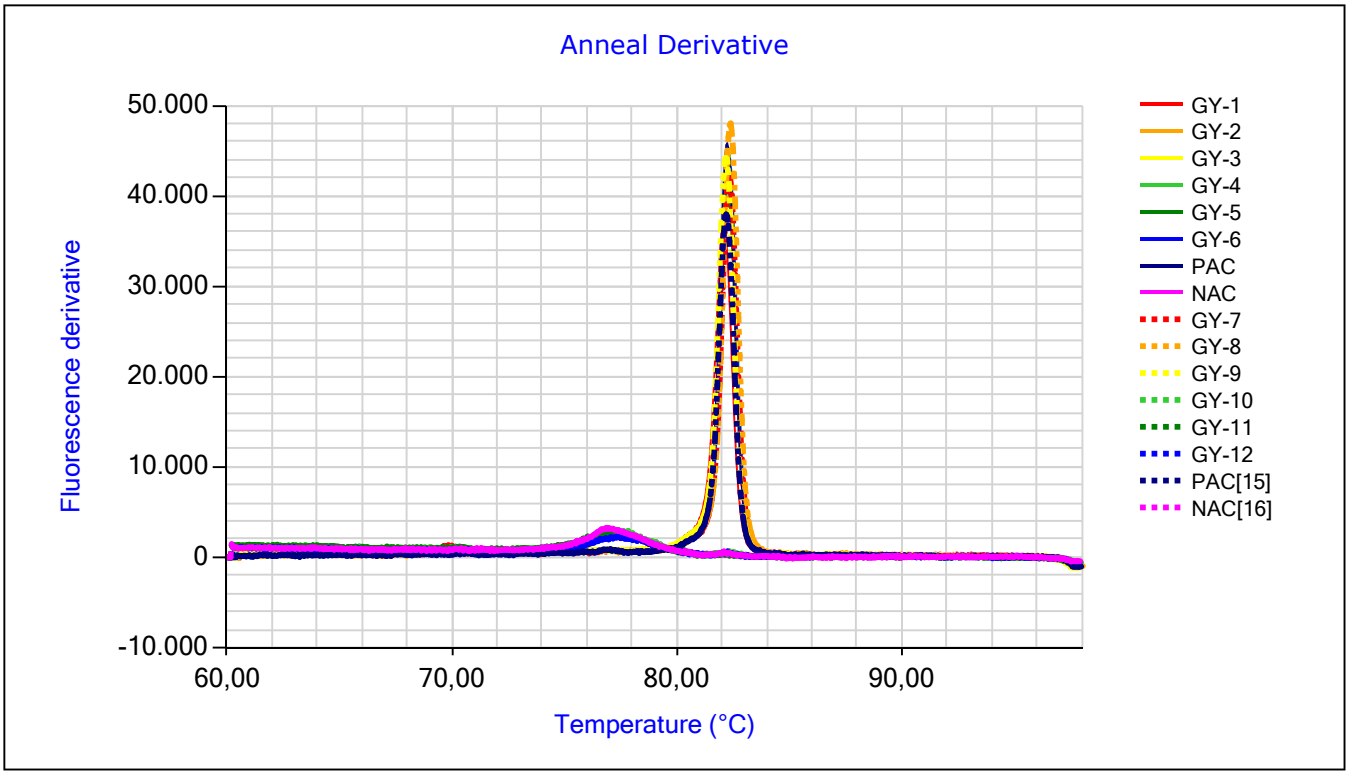
Smoothing Points	2

Peak	Width	Threshold
Simple	0(Sec)	0,02(ratio)

Well Number	Well Name(mm:ss)	Peak Value(mm:ss)
1	GY-1	02:00
2	GY-2	02:30
3	GY-3	15:15
4	GY-4	13:45
5	GY-5	13:45
6	GY-6	02:30
7	PAC	02:15
8	NAC	
9	GY-7	13:15
10	GY-8	16:00
11	GY-9	20:30
12	GY-10	
13	GY-11	
14	GY-12	
15	PAC[15]	12:00
16	NAC[16]	



Smoothing Points	3



Smoothing Points	2

Peak	Width	Threshold
Simple	4(mm:ss)	5000

Well Number	Well Name(°C)	Peak Value(°C)
1	GY-1	82,1
2	GY-2	82,3
3	GY-3	82,3
4	GY-4	82,3
5	GY-5	82,2
6	GY-6	82,3
7	PAC	82,2
8	NAC	
9	GY-7	82,3
10	GY-8	82,4
11	GY-9	82,2
12	GY-10	
13	GY-11	
14	GY-12	
15	PAC[15]	82,2
16	NAC[16]	