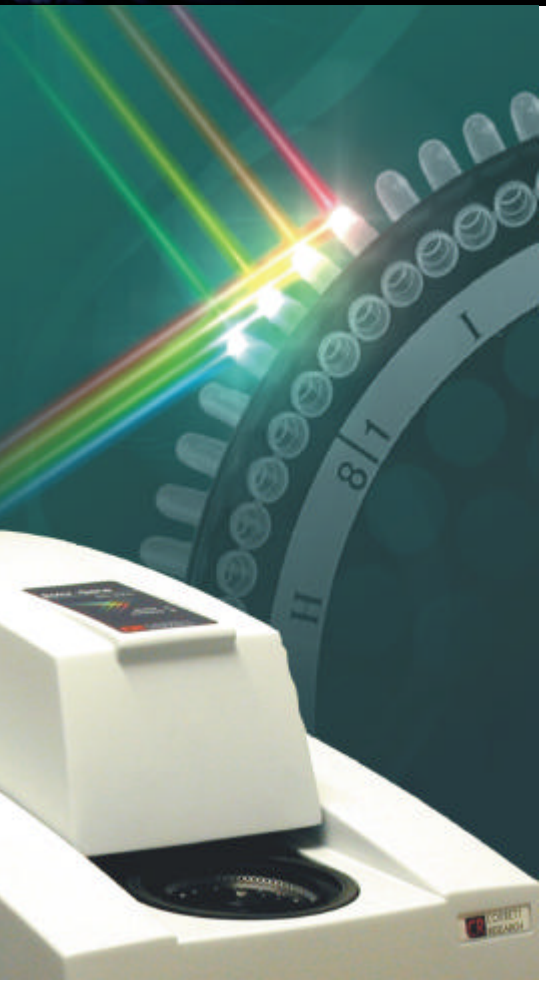
CORBETT RESEARCH
Innovative instrumentation for life sciences



Rotor-Gene 3000

Real-time Amplification

CAS-1200

Liquid Handling System

Miniaturisation and Reproducibility

Reduction of running times using "Optical Denaturation"?

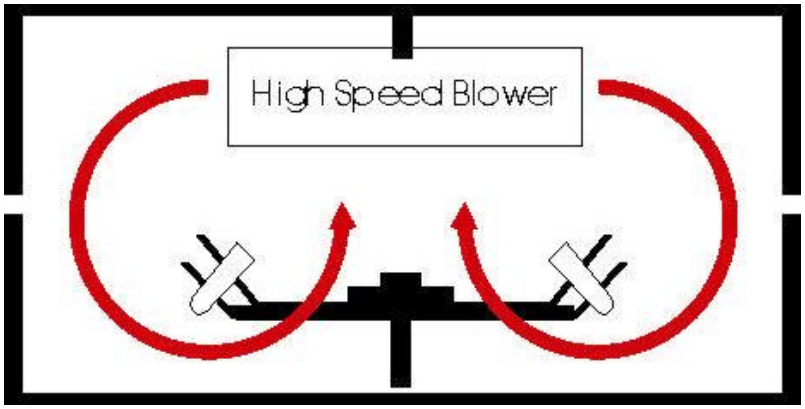
Reduction of the final probe concentrations to 5nM?

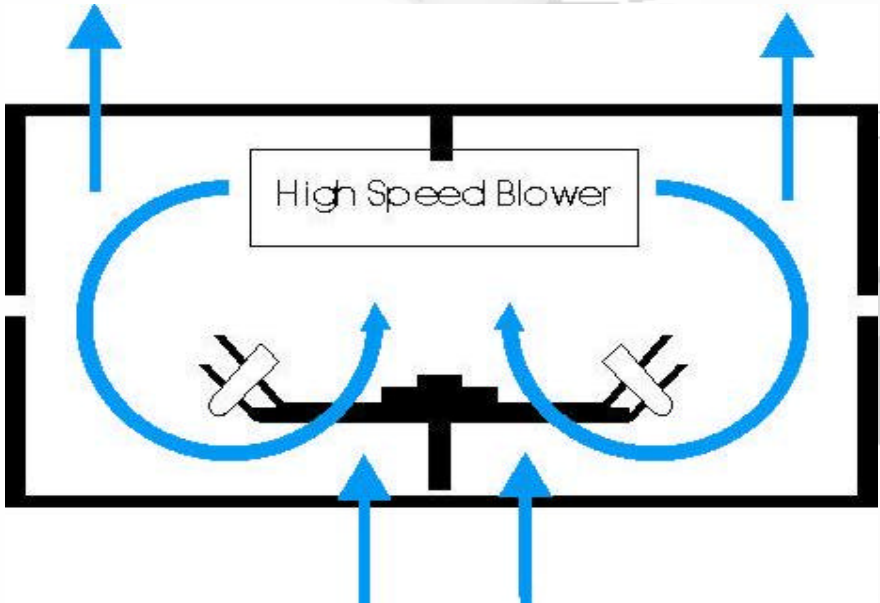
Minimal running volumes using the CAS-1200?

Reproducibility due to the design of the Rotor-Gene.

Heating and Cooling the Chamber

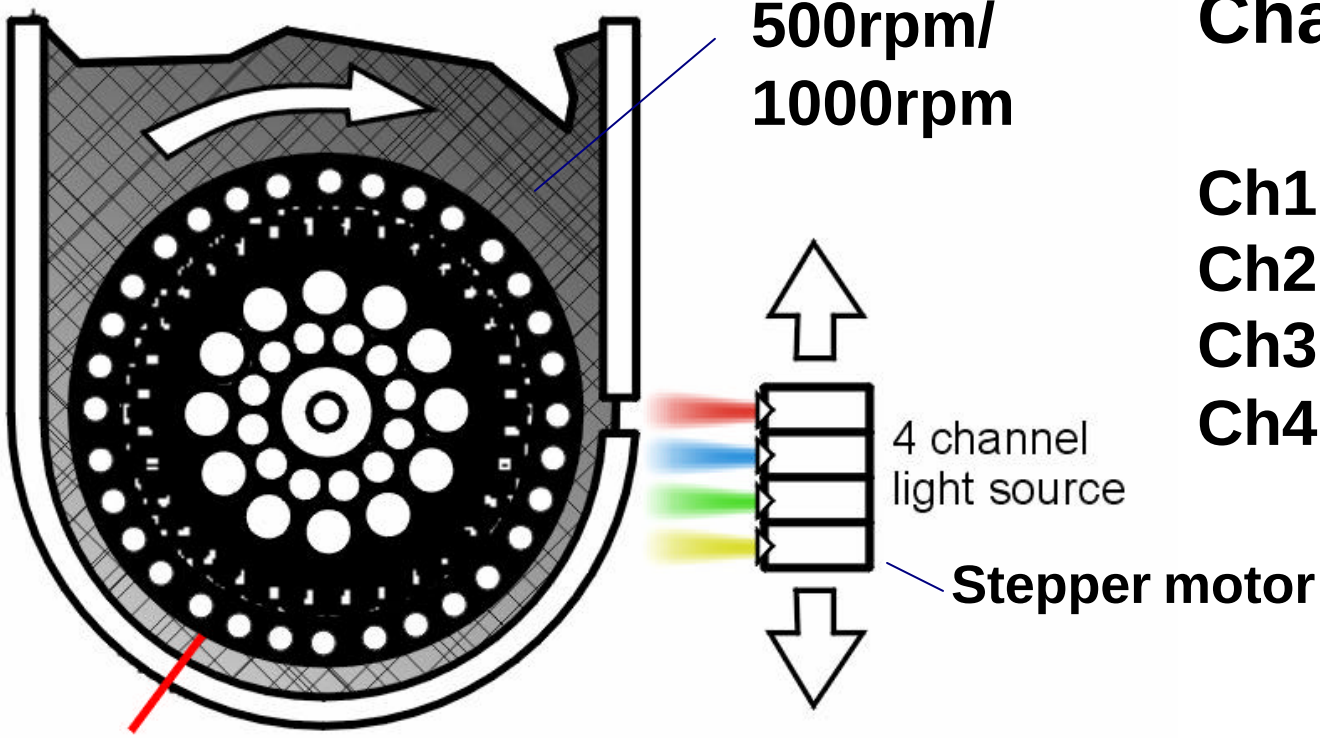
- Heated by a circular Ni-chrome element in the lid.
- Samples spin continually at 500rpm or 1000rpm.
- Cooling: a vent is opened in the top of the chamber.





Chamber Layout – Top

Four Channel System

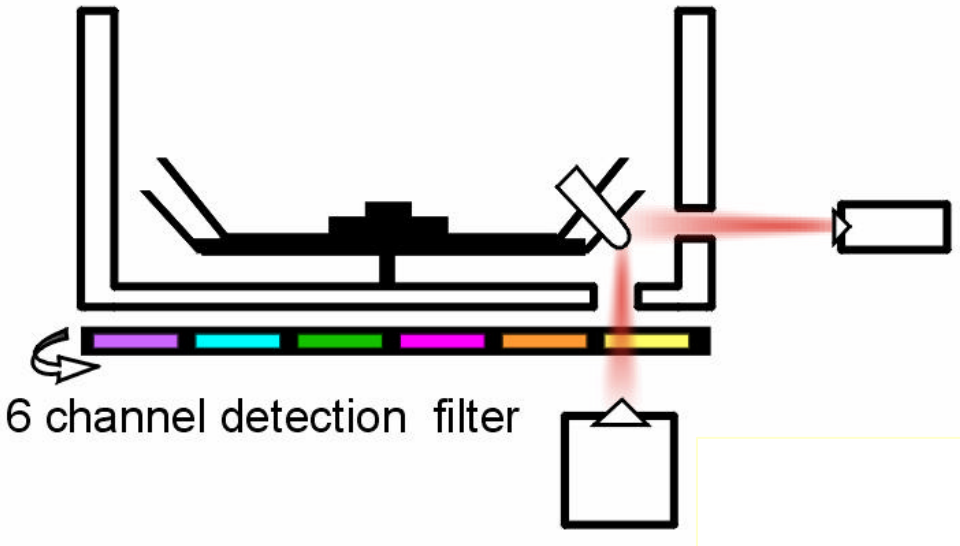


Excitation Channels

Ch1: 470nm
Ch2: 530nm
Ch3: 585nm
Ch4: 625nm

36/72 well Rotor

Fixed optical path length



Hamamatsu Photomultiplier

- LED excites from the side of the chamber.
- PMT detects at the base of the chamber.
- Energy transmitted through the thin walls at the base of the tube.

Fixed optical path length ensures no variation sample to sample.

Optical Denaturation

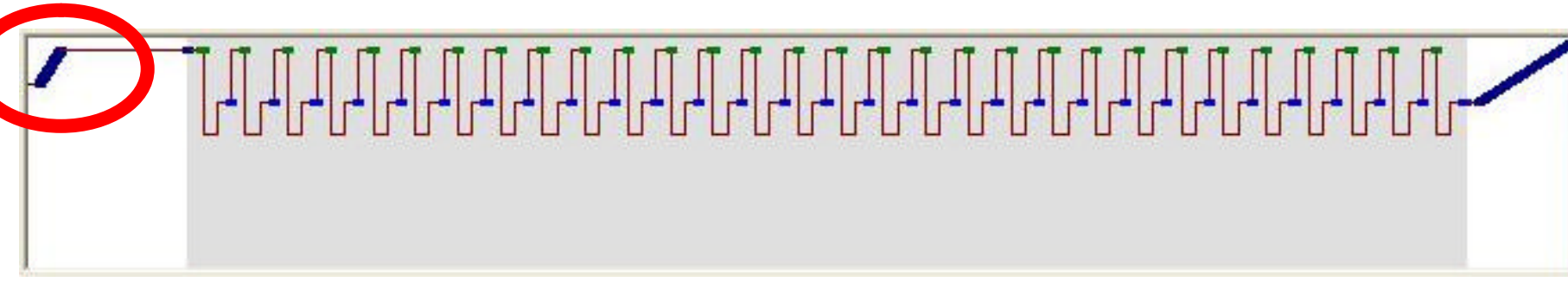
Conventional amplification relies on setting temperatures and hold times at each step of the reaction profile.

Optical Denaturation monitors, in real time, the dissociation of product (i.e. denaturation or melt point) during an amplification reaction.

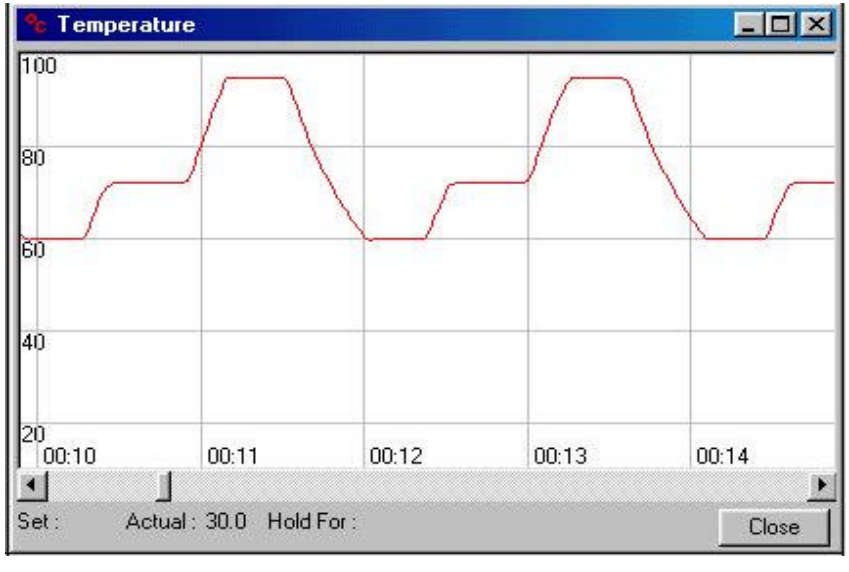
Requirements:

- Reference sample
- Optical denature profile
- Temperature uniformity of the instrument

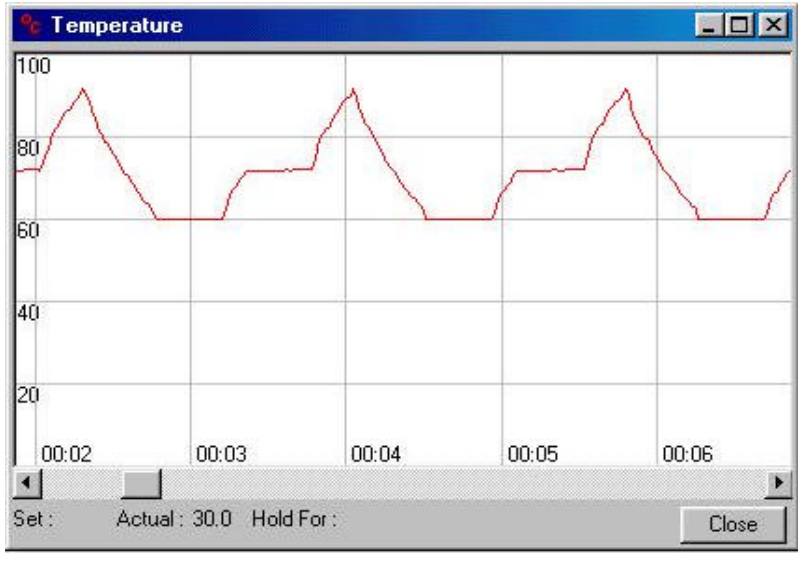
Optical Denaturation



Conventional



Optical Denaturation



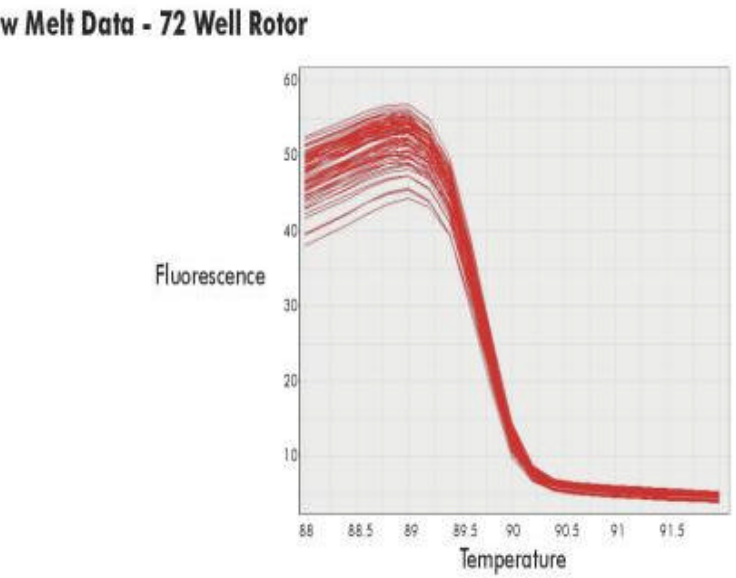
Optical Denaturation

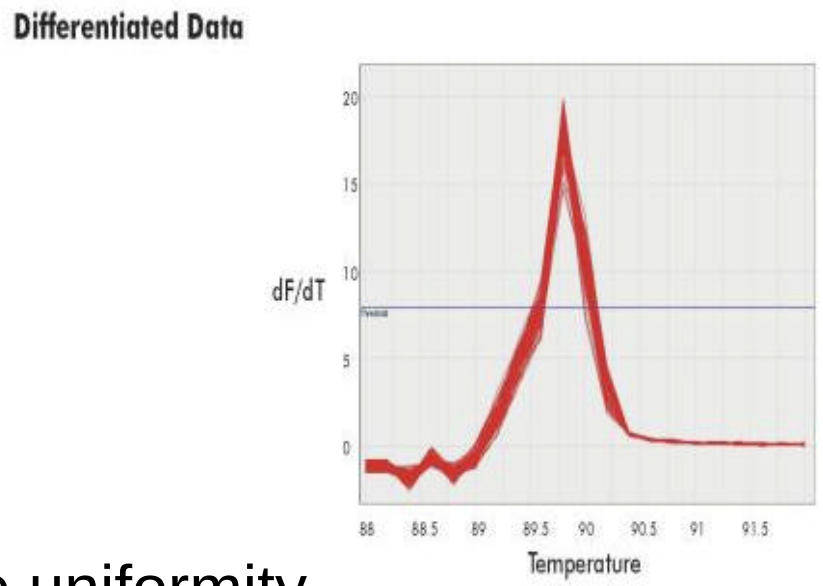
Significant reduction of running times

Automatic detection of the reference melt point

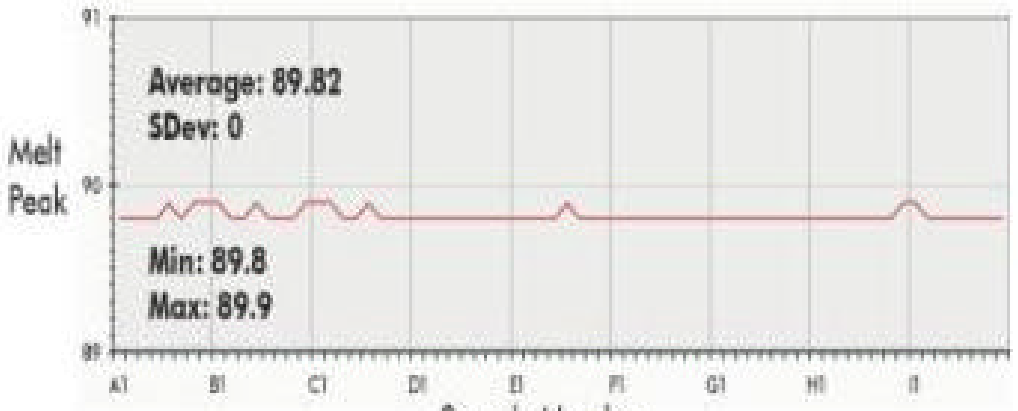
Increased lifetime of the enzym

Temperature uniformity of the Rotor-Gene





Sample uniformity



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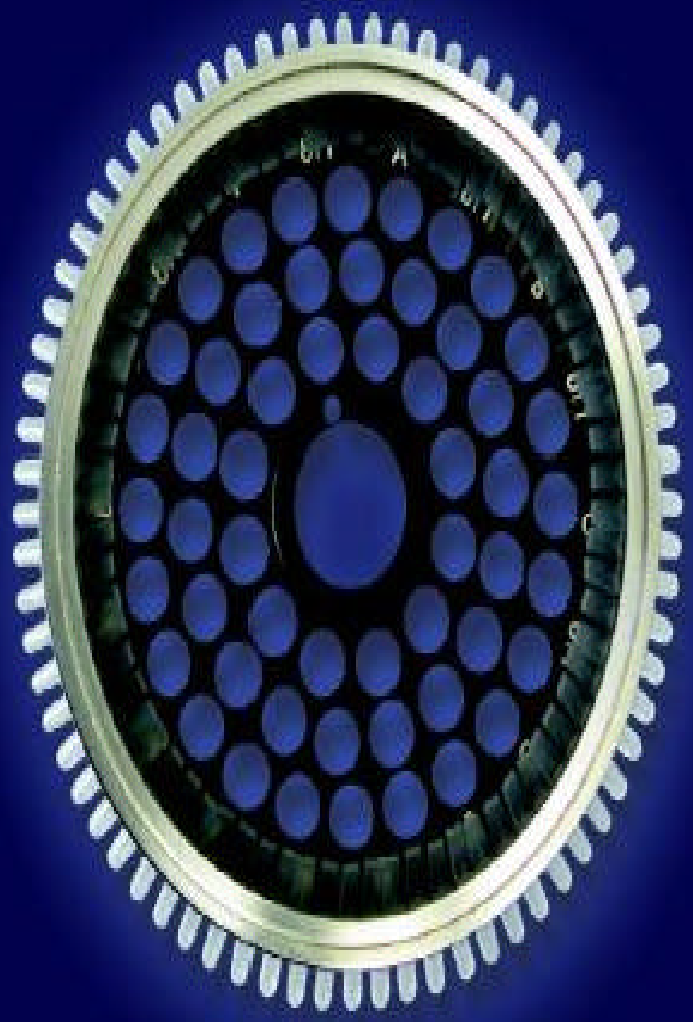
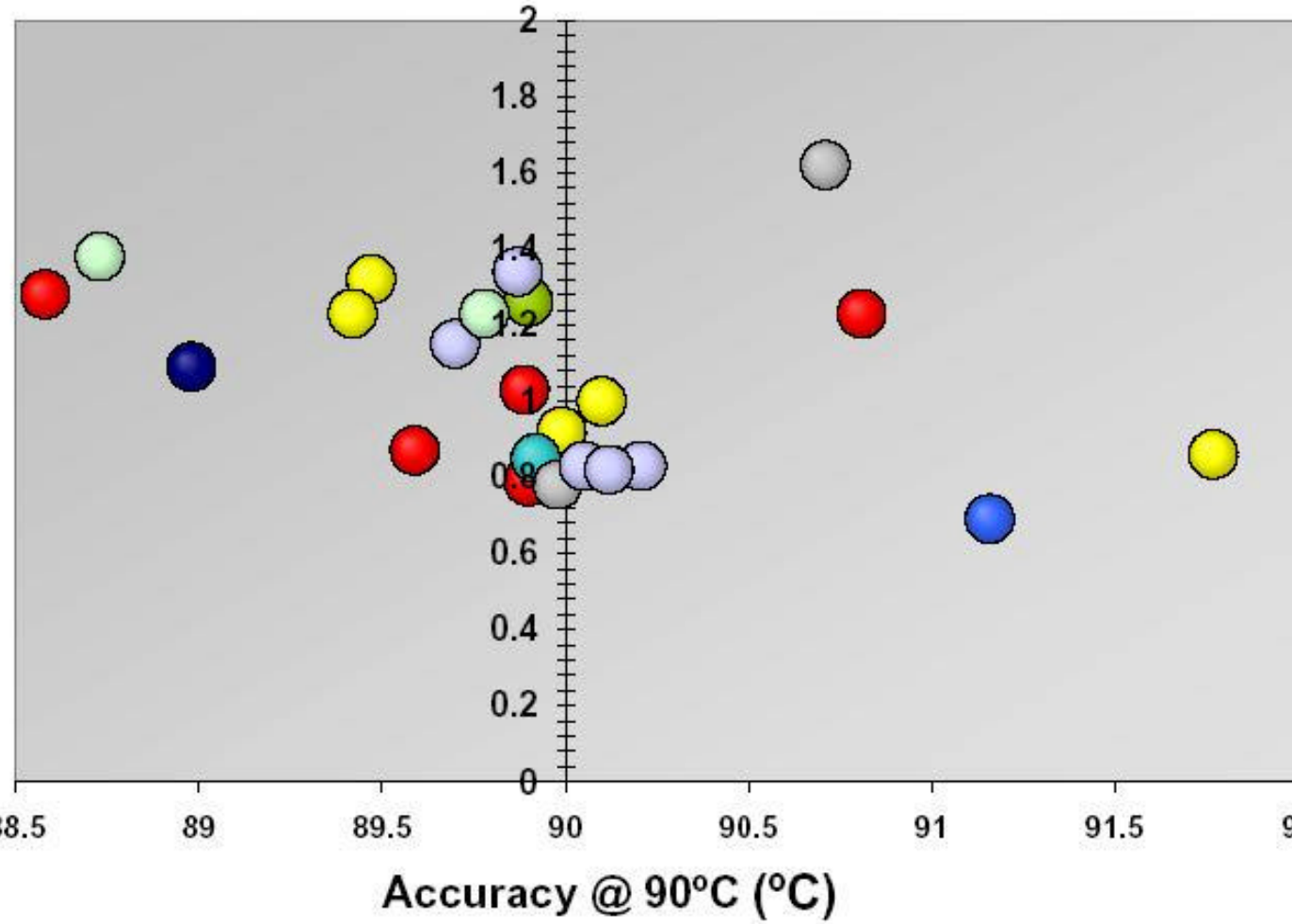
Calibration Rotor

The calibration rotor is supplied pre-loaded with 0.1mL tubes that are fixed and cannot be removed.
The rotor is placed into the Rotor-Gene and a melt template is run to produce three distinct melt curves.
These curves are analyzed to generate a temperature calibration report.



Uniformity of conventional block cyclers

Mean values of multiple measurements of tested cyclers



More information can be found at www.cyclertest.com

Reduced probe concentration

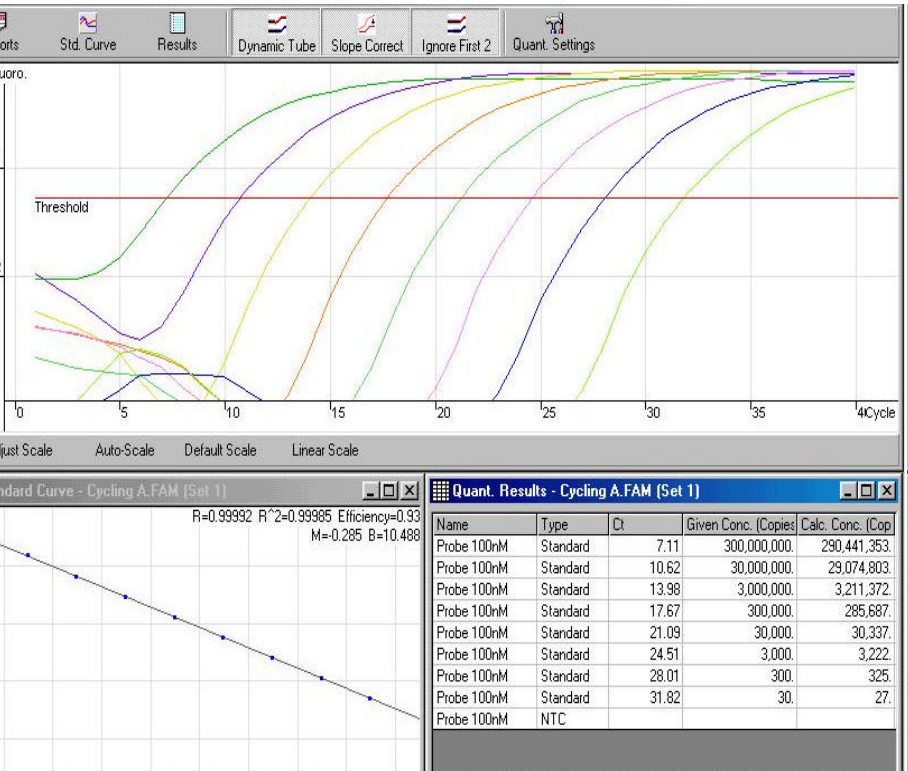
In many cases, researchers use ~200nM final probe concentrations.

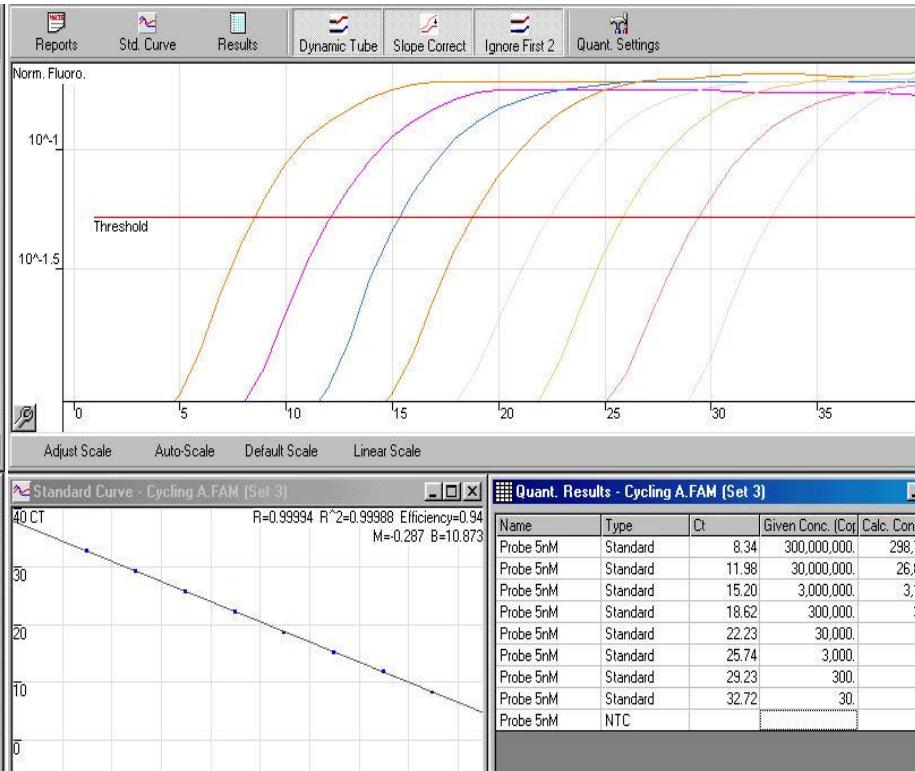
Can lower probe concentrations be used (how low?) and does it influence the performance of the assay?

Reduced probe concentration

100 nM

5 nM



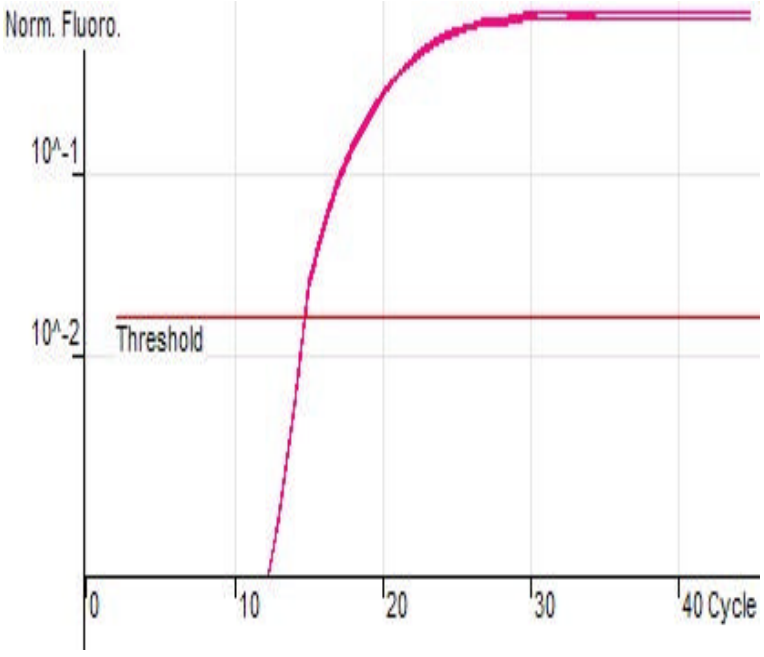


Given Conc. Copies	100nM Ct-value	75nM Ct-value	50nM Ct-value	25nM Ct-value
1:1	**7.11**	**7.02**	**7.55**	**7.82**
1:10	10.62	10.44	10.52	10.88
1:100	13.98	13.85	14.14	14.06
1:1000	17.67	17.54	17.89	17.92
1:10000	21.09	20.99	21.23	21.2
1:100000	24.51	24.37	24.64	24.81
1:1000000	28.01	27.87	28.15	28.26
1:10000000	31.82	31.69	31.59	31.71
R-value (R)	0.99994	0.99992	0.99983	0.99975
R^2-value	0.99987	0.99983	0.99967	0.99950
Reaction eff	93%	93%	94%	95%
Slope (m)	0.285	0.285	0.288	0.290
Intercept (b)	10.499	10.452	10.588	10.653

Given Conc. Copies	15nM Ct-value	10nM Ct-value	5nM Ct-value	
1:1	**7.2**	**7.53**	**8.34**	
1:10	10.2	10.76	11.86	
1:100	13.6	14.12	15.20	
1:1000	16.94	17.66	18.86	
1:10000	20.76	20.98	22.23	
1:100000	24.09	24.54	25.60	
1:1000000	27.68	28.05	29.23	
1:10000000	30.96	31.58	32.72	
R-value (R)	0.99975	0.99991	0.99996	
R^2-value	0.99950	0.99983	0.99993	
Reaction eff.	95%	95%	94%	
Slope (m)	0.291	0.290	0.288	
Intercept (b)	10.476	10.608	10.874	

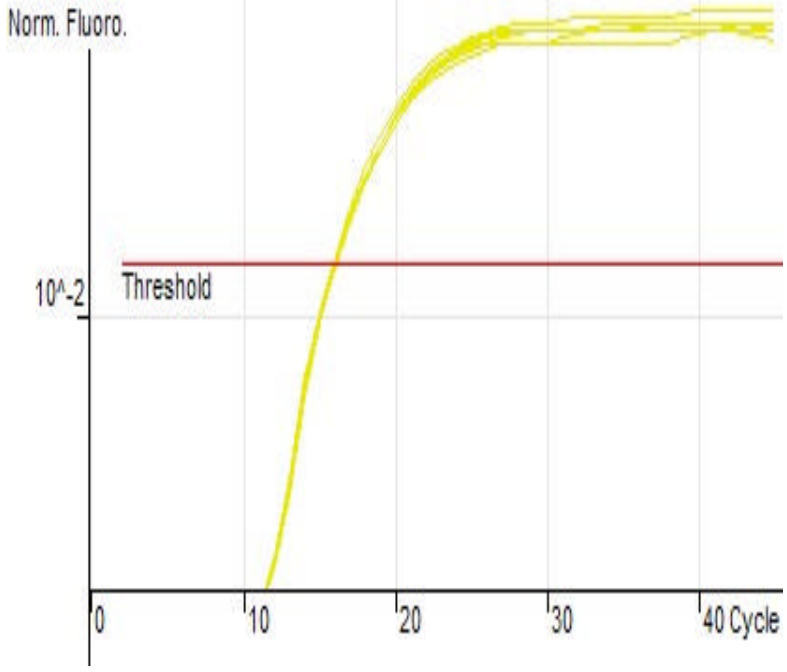
Reduced probe concentration

100 nM



Std dev: 0.05

5 nM



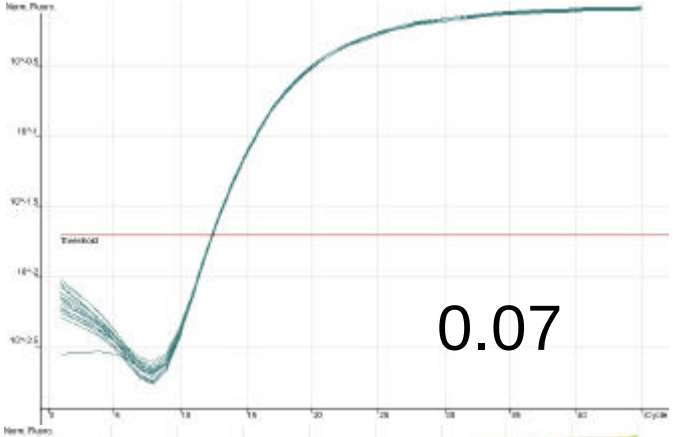
Std dev: 0.09

CAS-1200

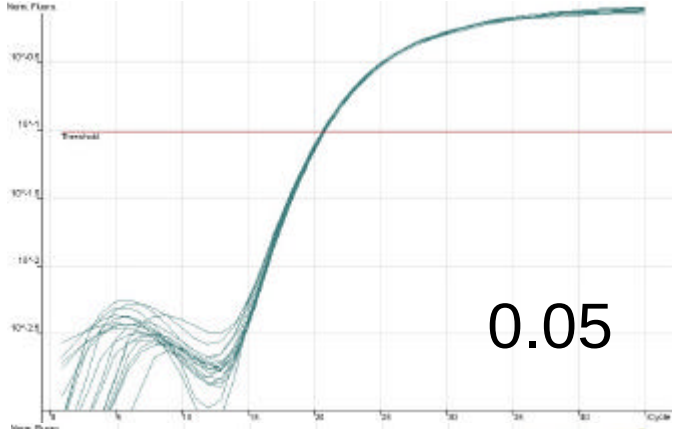
Minimal volumes run on the Rotor-Gene

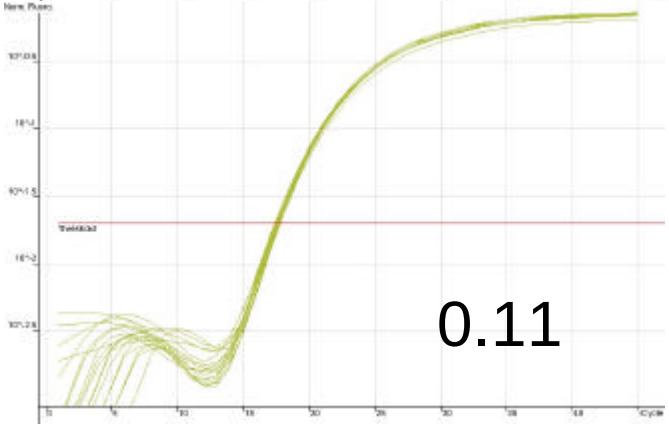
hand pipetting

CAS-1200



20ul





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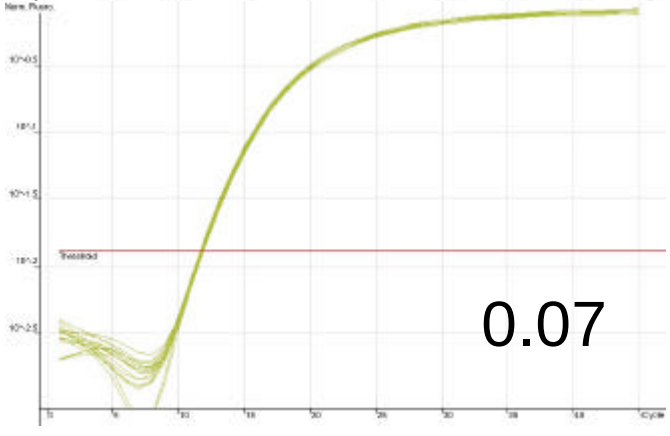
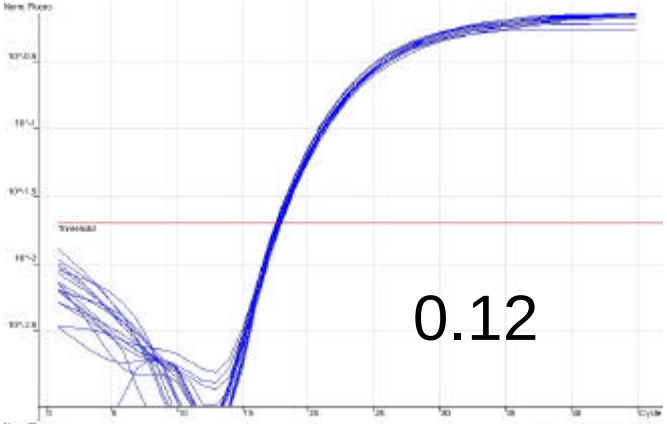


Minimal volumes run on the Rotor-Gene

hand pipetting

CAS-1200



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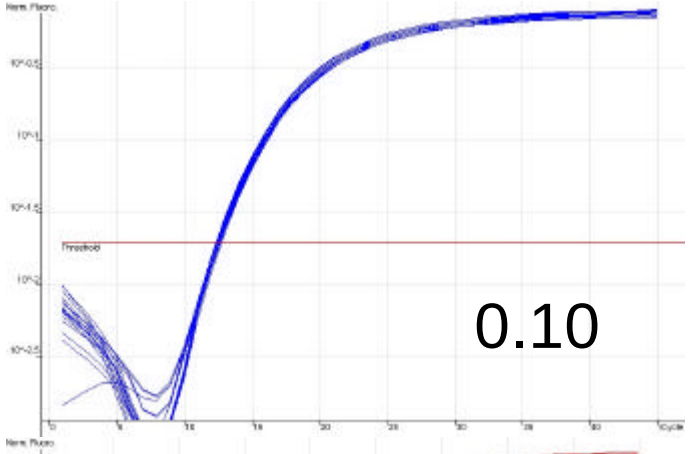

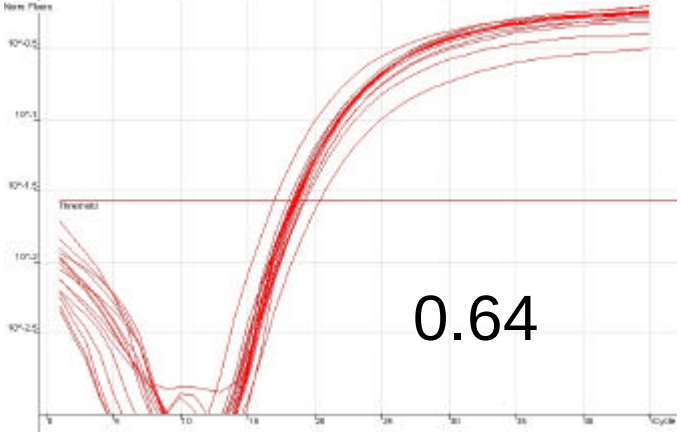

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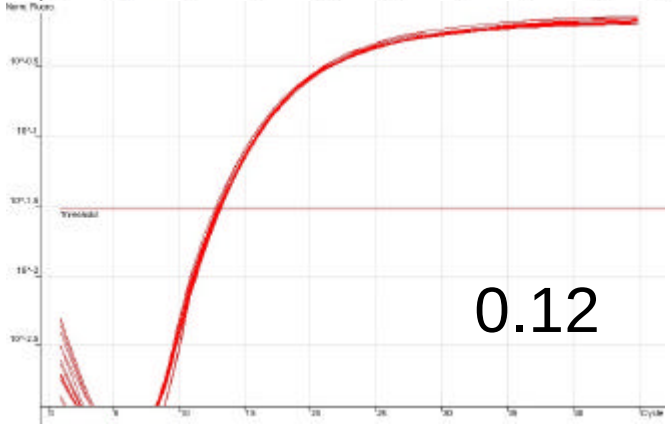
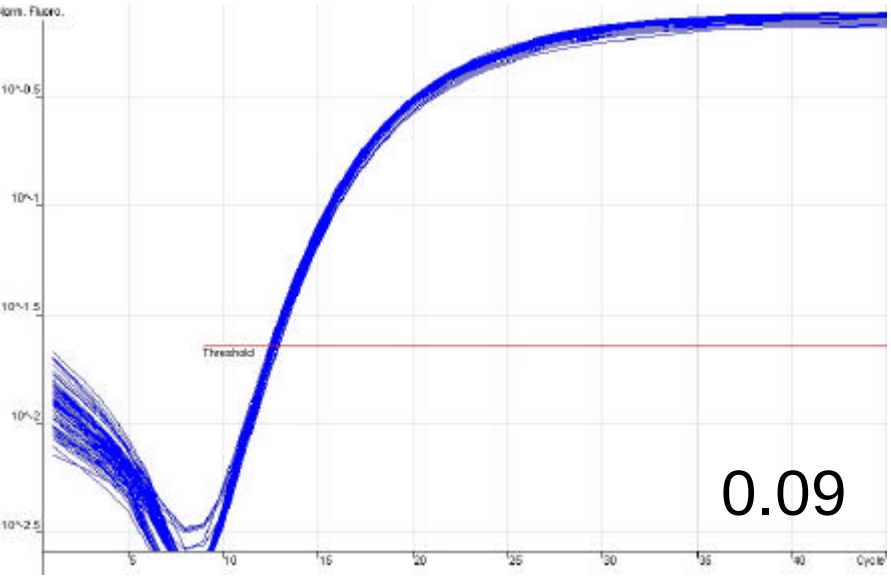


Minimal volumes run on the Rotor-Gene

72 replicates

10ul



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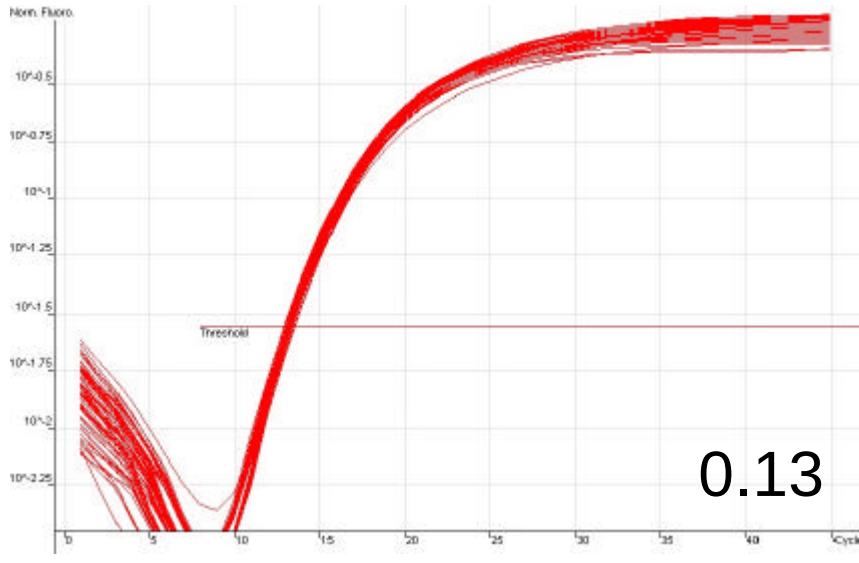
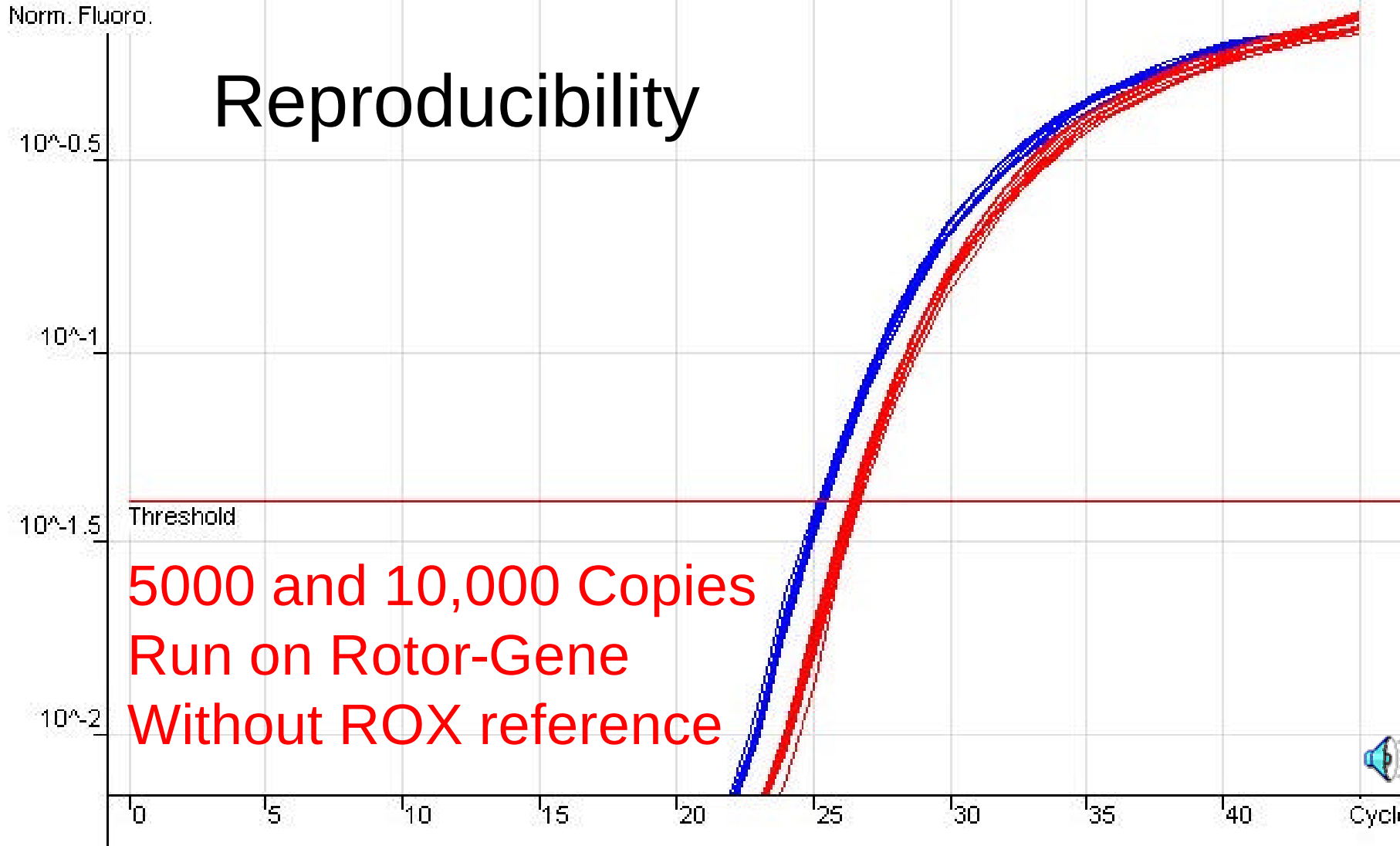


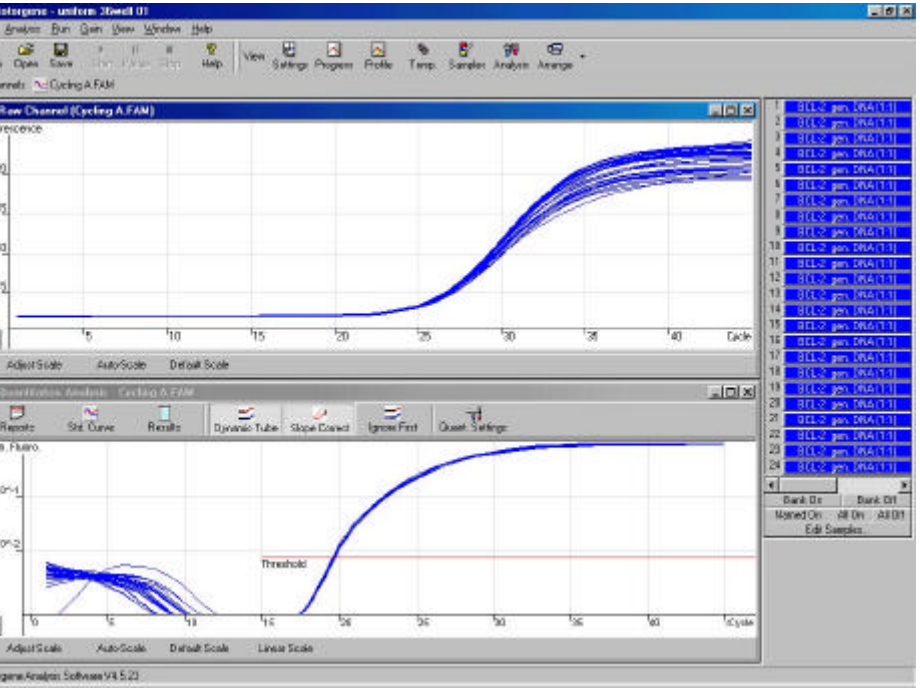

Norm. Fluoro.
Reproducibility
10^-0.5
10^-1
10^-1.5
10^-2
Threshold
5000 and 10,000 Copies
Run on Rotor-Gene
Without ROX reference
0
5
10
15
20
25
30
35
40
Cycl

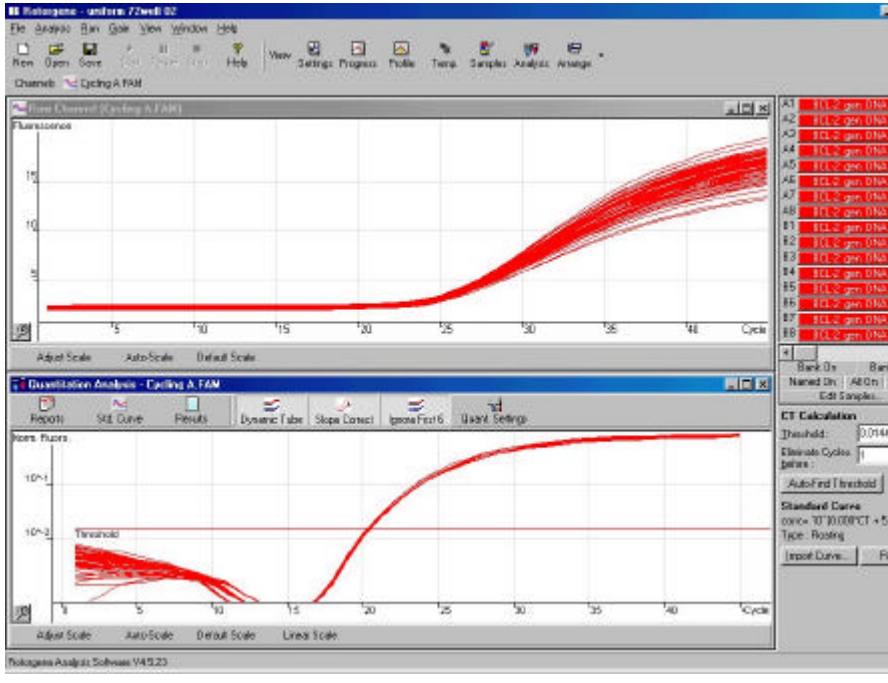
Replicates run on the Rotor-Gene

hand pipetting

full 36-well rotor

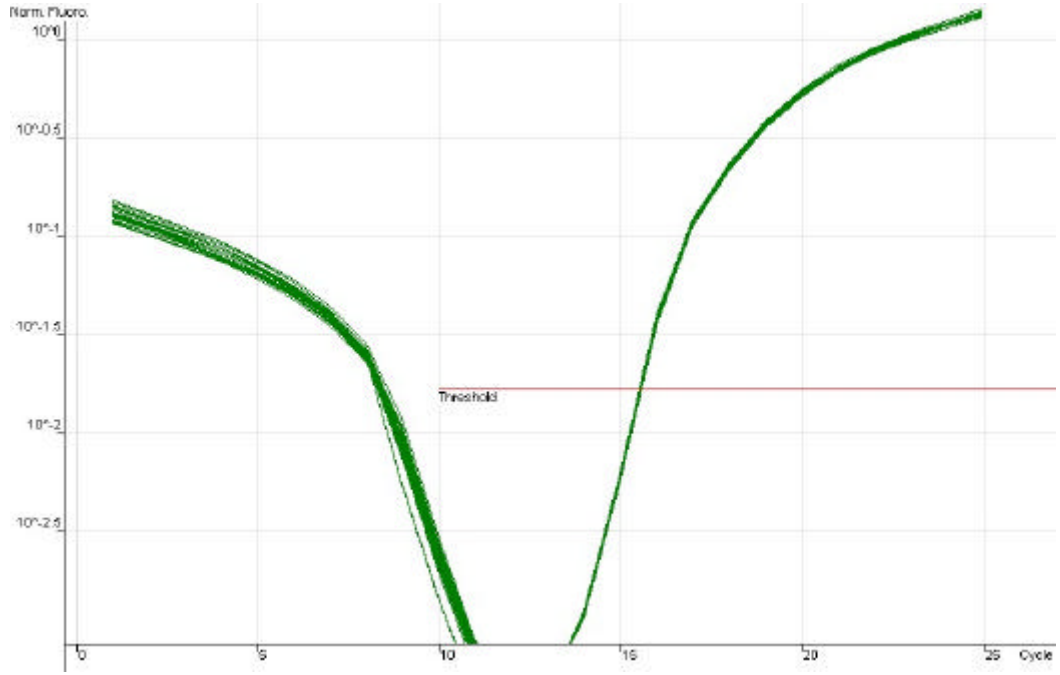
full 72-well rotor





Standard deviation 0.05

Replicates run on the Rotor-Gene
using CAS-1200



36-well rotor

Standard deviation 0.02 - Ct_{max}: 15.29 Ct_{min}: 15.24

Summary

Yes, times can be reduced using “opitical denaturation”

Yes, probe concentration could in some cases significantly be reduced

Yes, minimal volumes using the CAS-1200 can be run on the Rotor-Gene

Thanks !

Enjoy Your Evening…