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PikoREAL® qPCR Thermal Cycler

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Introduction to the PikoREAL qPCR System

- **Thermo Fisher Scientifics' first instrument in the qPCR market**

- Key features:
  - Built on Piko technology, providing the same thermal performance, speed and low power and reagent consumption
  - Smaller footprint than any competing system
  - Five optical detection channels

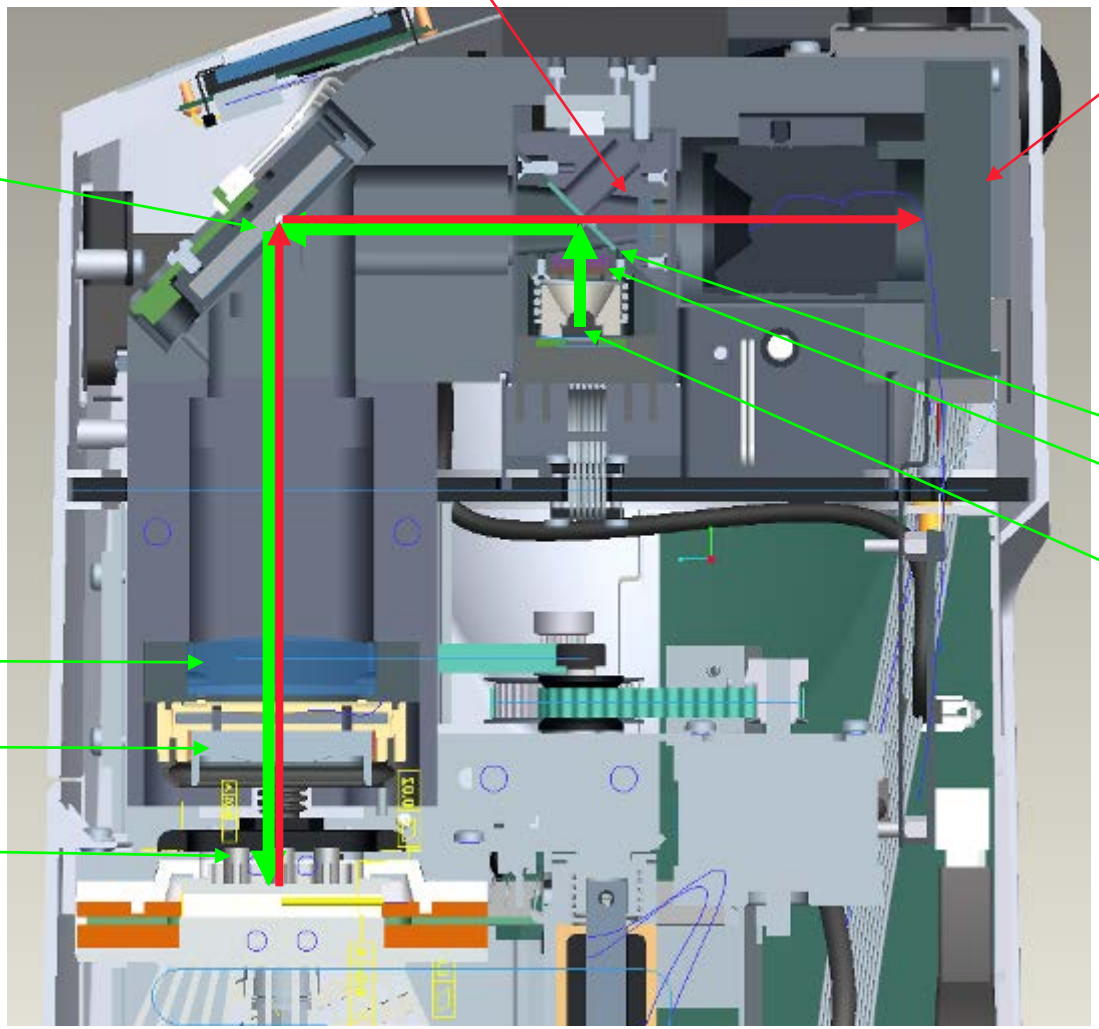
PikoREAL Optical System

- Three major components for the optical system:
  - Light source: 5x LED
    - Long lasting, constant and stable signal throughout the qPCR experiment and life of the instrument
  - Detector: CCD camera
    - Collect data from all wells and dyes simultaneously and fast enough to allow multiple reads of the plate to be taken at each cycle of the qPCR
  - Separation of light into specific wavelengths: excitation and emission filters
    - Ensures only the correct wavelength of light reaches the sample and only the emitted light from the dye molecule reaches the detector.

PikoREAL Dye Compatibility

Channel	Compatible Dyes
1	FAM, fluorescein, SYBR Green
2	HEX, Yakima Yellow
3	ROX, Texas Red
4	Cy5
5	HRM: coming soon

PikoREAL Optical Light Path



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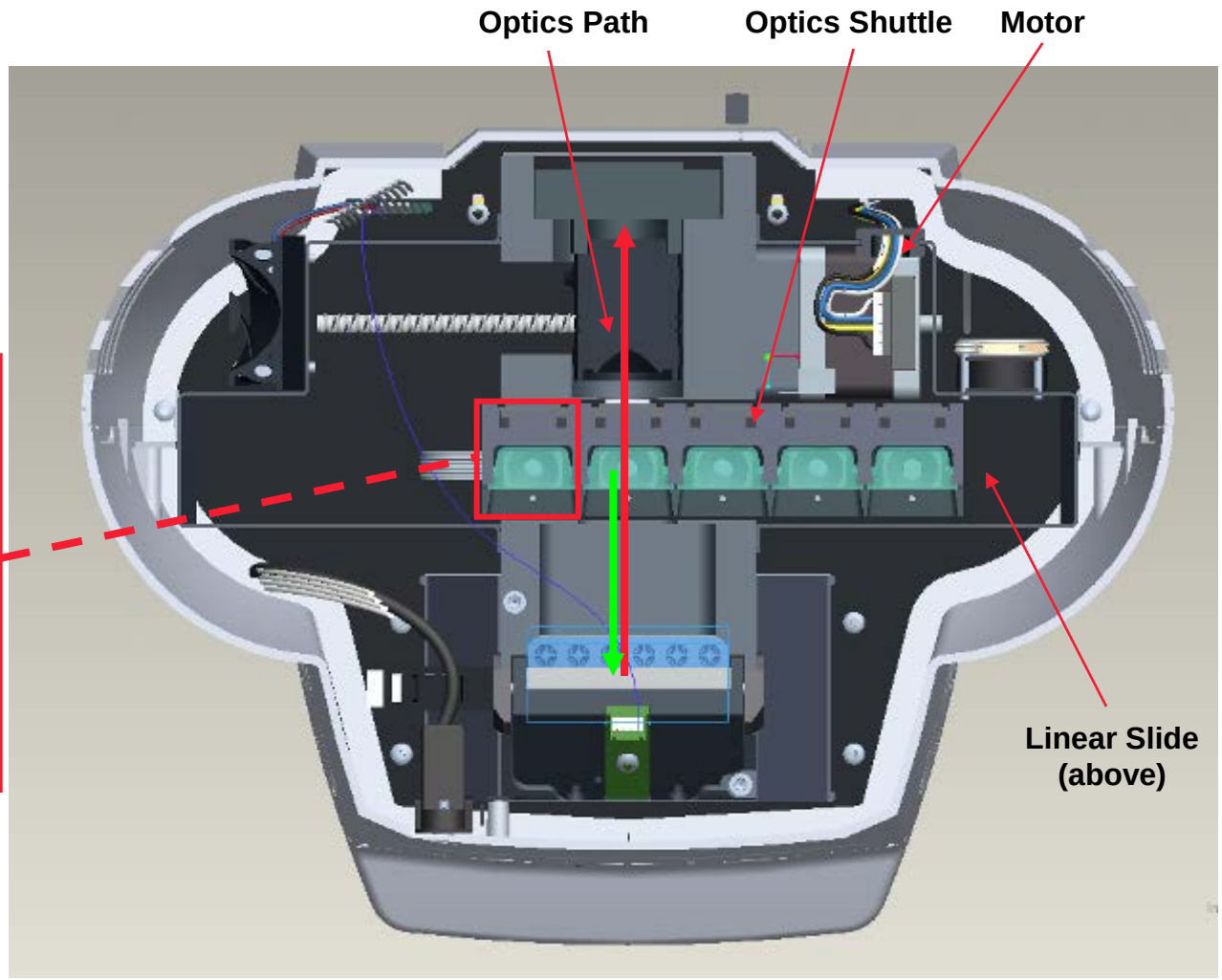
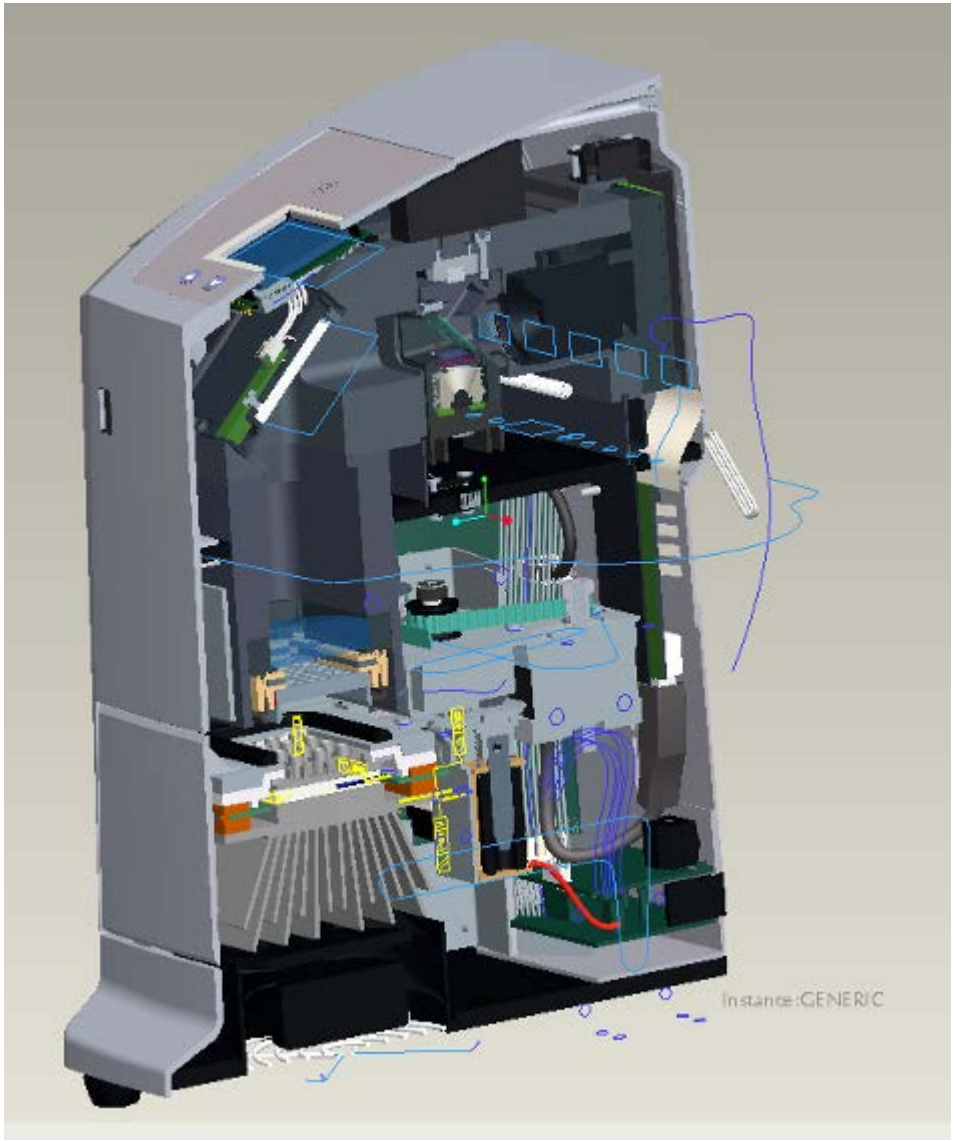
Optics System – Channel Selection

- Linear optics shuttle moves to any of the 5 positions
- Shuttle contains separate LED, reflector, excitation filter, dichroic mirror and emission filter for each of the 5 channels



Internal Design of PikoREAL



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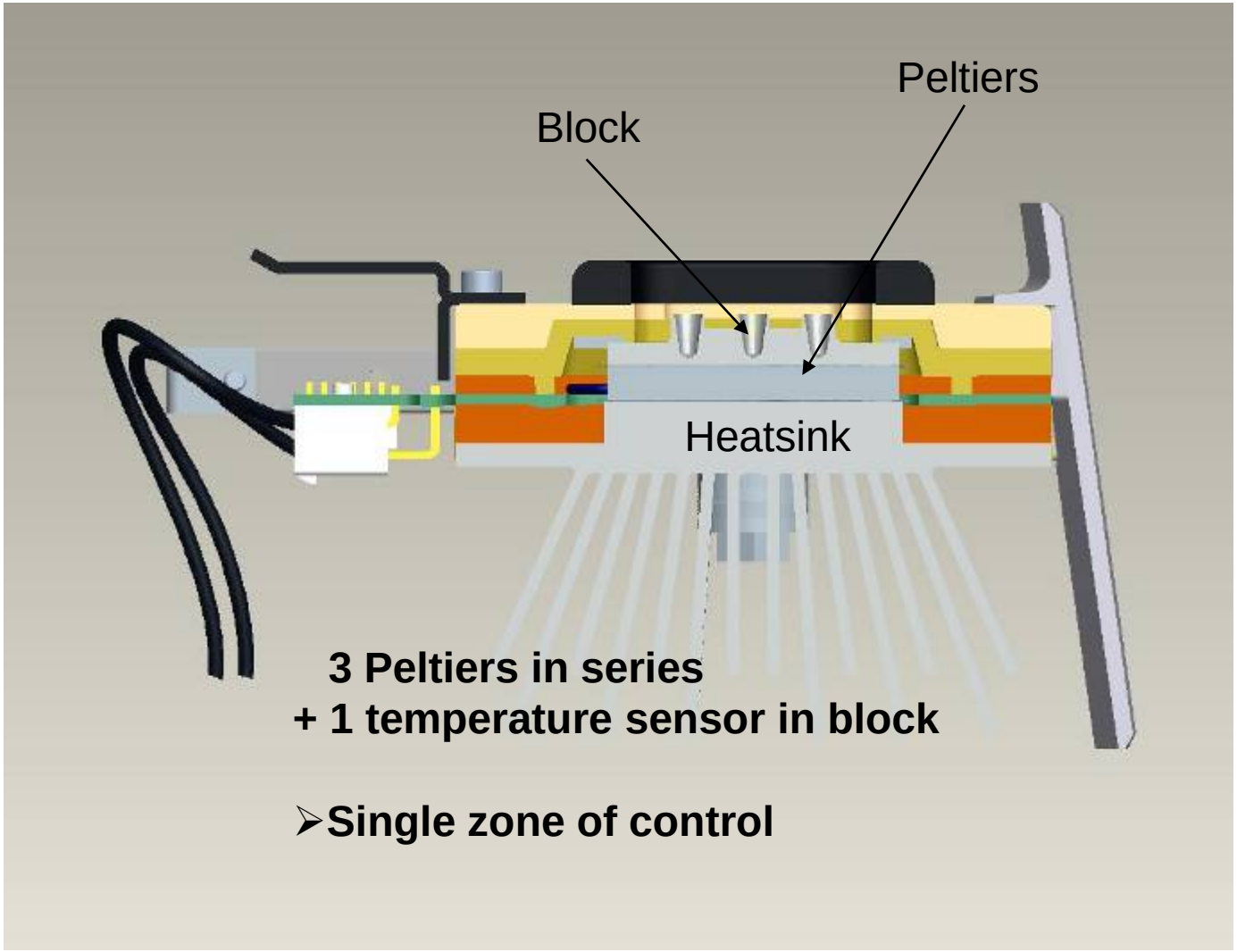
PikoREAL Block Specifications

- Tremendous thermal performance:
  - Thermal accuracy ± 0.2 °C
  - Thermal uniformity ± 0.3 °C at 95 °C
  - Average ramp rate 5 °C/s heating and 4.5 °C/s cooling, Max ramp rate 7 °C/s
  - <=1 sec for all samples to reach same Temp.
  - Thermal range 4°C - 99.9°C
- Block configurations
  - 24-well and 96-well
- Based around the Piko PCR Plate which is ¼ the size of conventional microplates
  - Maintains industry-standard sample capacity and well-to-well spacing routinely used in research and diagnostic labs

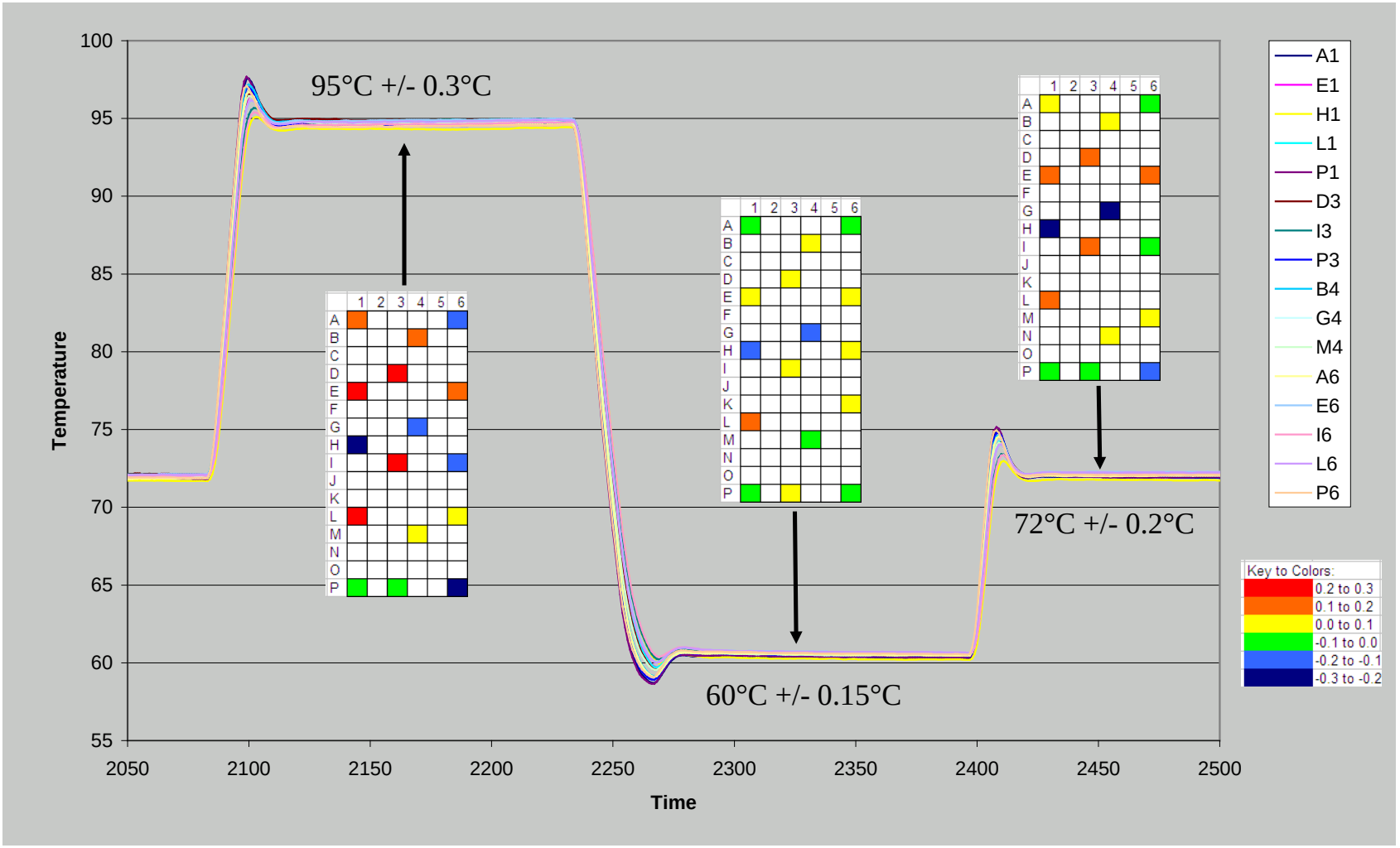
Block Formats

- 24-well block
  - ¼ of a standard SBS 96-well plate
  - Well volume 0.225 ml
  - Sample volumes of 5-50µl
  - Fits Thermo UTW 24 well Piko plates and strip tubes
    - Standard PCR products can also be used
- 96-well block
  - ¼ of a standard SBS 384-well plate
  - Well volume 50µl
  - Sample volumes of 5-20µl
  - Fits Thermo UTW 96 well Piko plates

PikoREAL Thermal System



PikoREAL – Superior Thermal Performance



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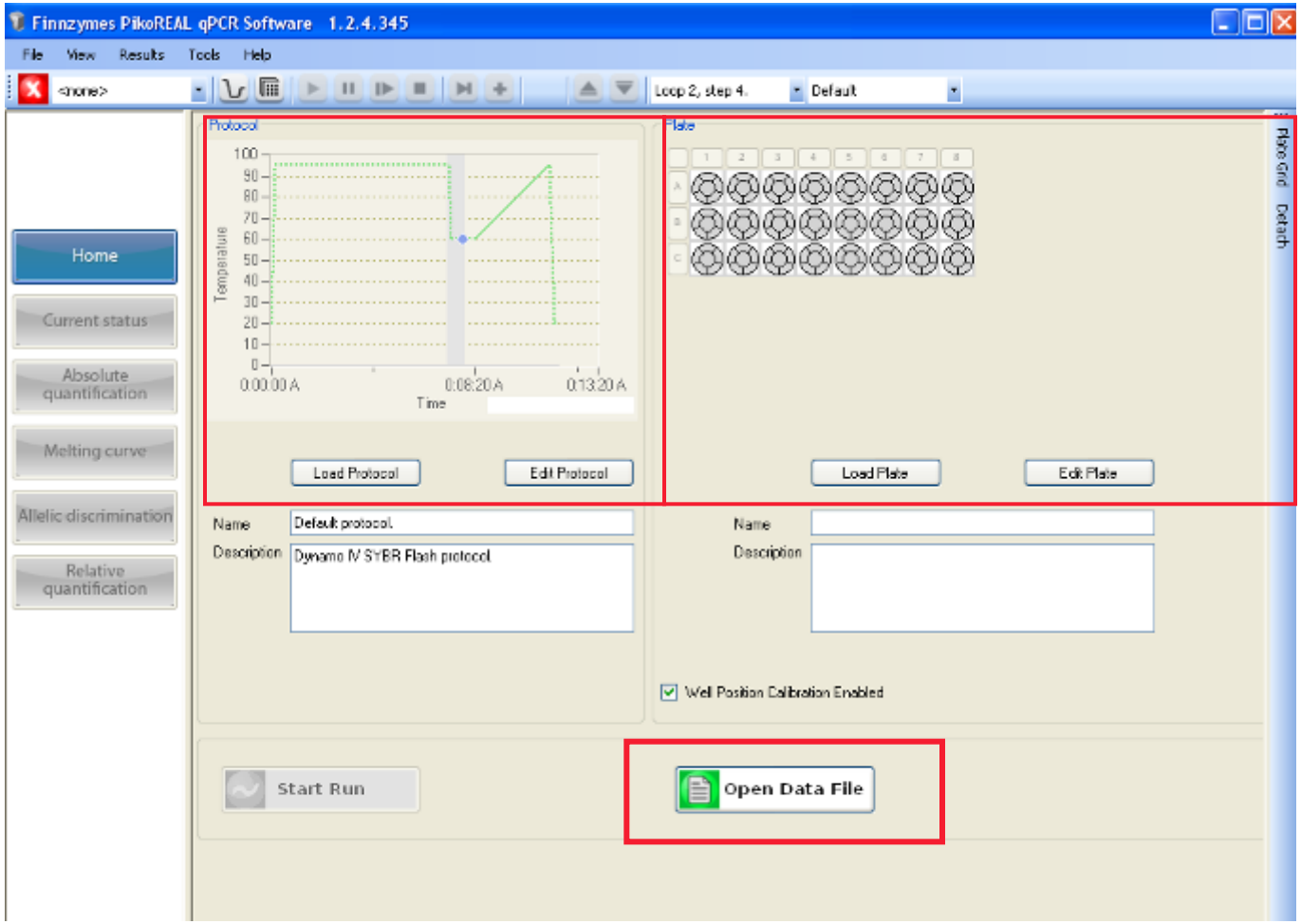
Automated Drawer and Heated Lid Mechanisms

- Fully-automated and motorized drawer function
  - CD drivelike loading mechanism contributes to the tiny footprint, and simplifies the loading process to a single push of a button
- Automated lid has both temperature and pressure control
  - Computer-controlled lid pressure, so the process of keeping samples sealed and free from evaporation is simple and repeatable.
  - The temperature and pressure are automatically applied when the consumables and sample volume are selected.

PC Applications Software Homepage

Data Analysis options



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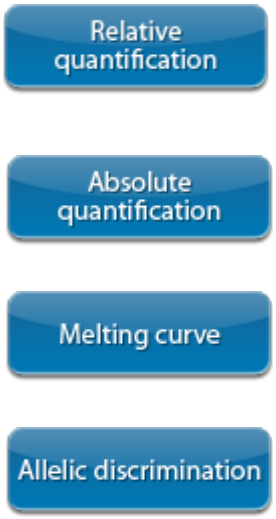
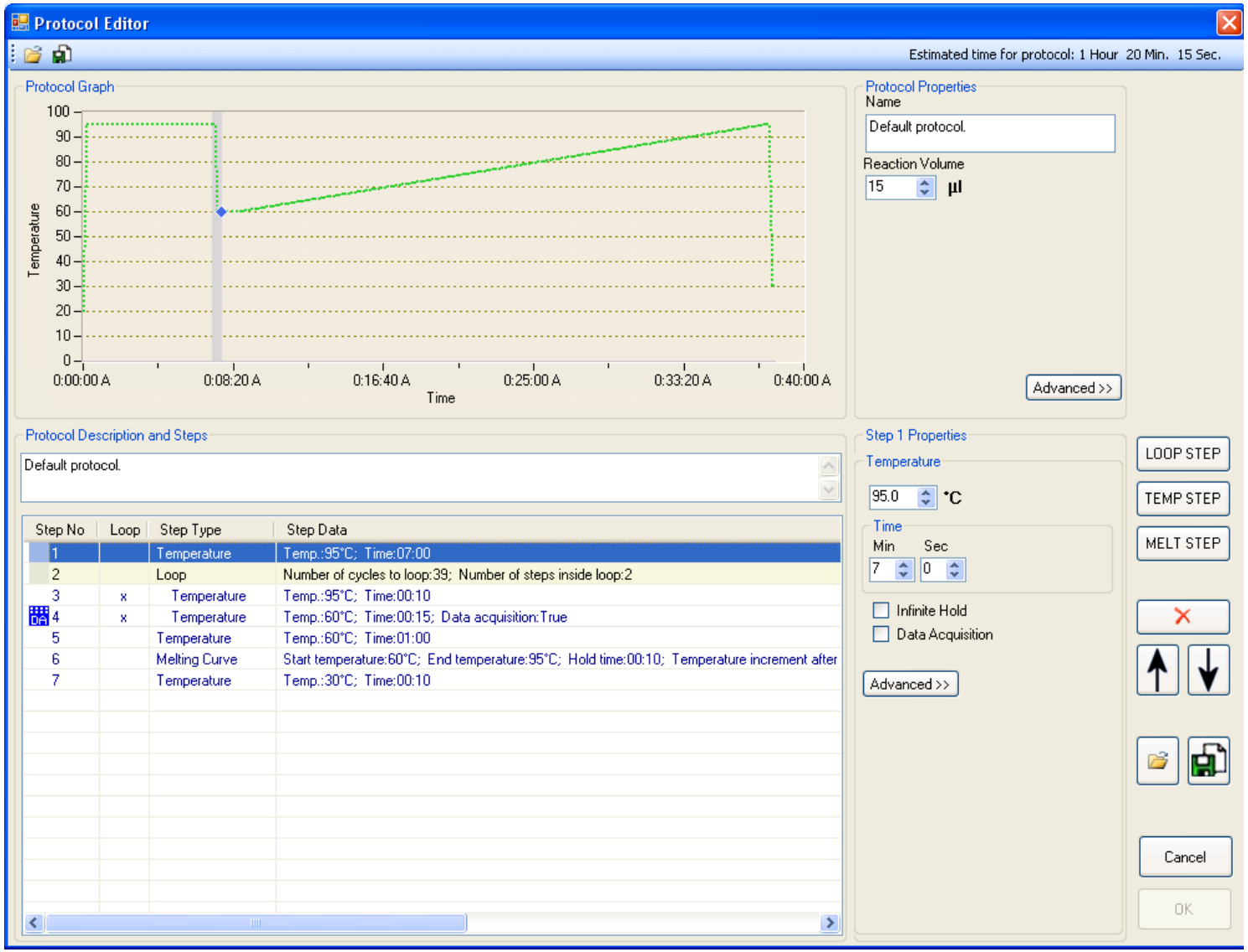
Instrument User Interface

- Applications software specific for the PikoReal
  - Can be run remotely or via a PC
- Data analysis options for:
  - Relative quantification
  - Absolute quantification
  - Melt curve
  - Allelic discrimination



Programming the Thermal Cycler



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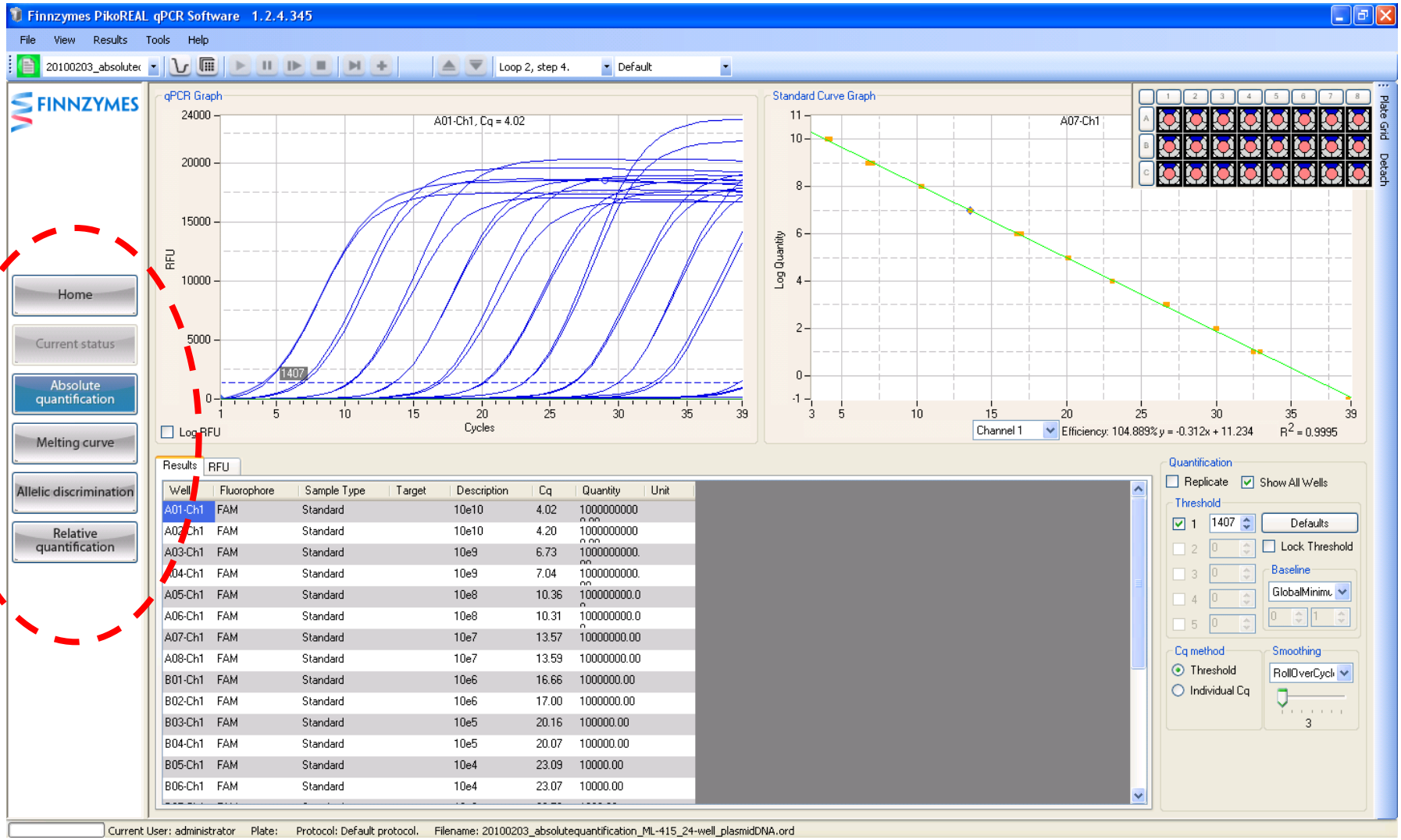
qPCR in Many Forms



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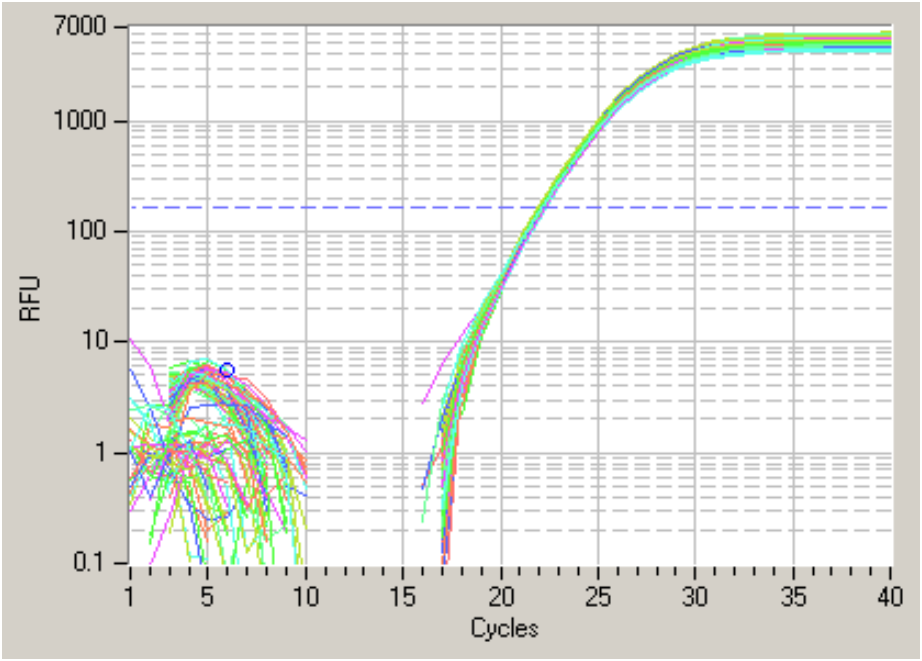
Additional Features of PikoREAL

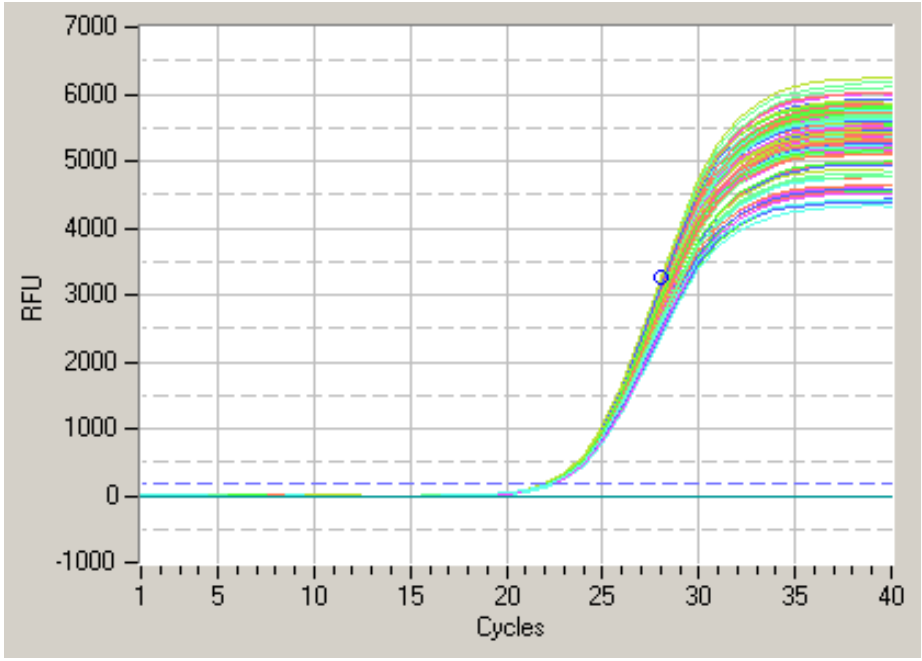
- Size.....
  - Only 20cm x 30cm x 32cm (W x D x H)
  - Footprint of 600cm^2

- Weight 10kg
- Auto restart on power failure
- Communication
  - USB port for data and protocol transfer
  - Ethernet for connection to PC
- Universal power supply
- Very quiet operation, no noisy fans
- Low energy requirements compared to competitors
- 1 year warranty

Excellent Uniformity Across 96 Wells



Log scale

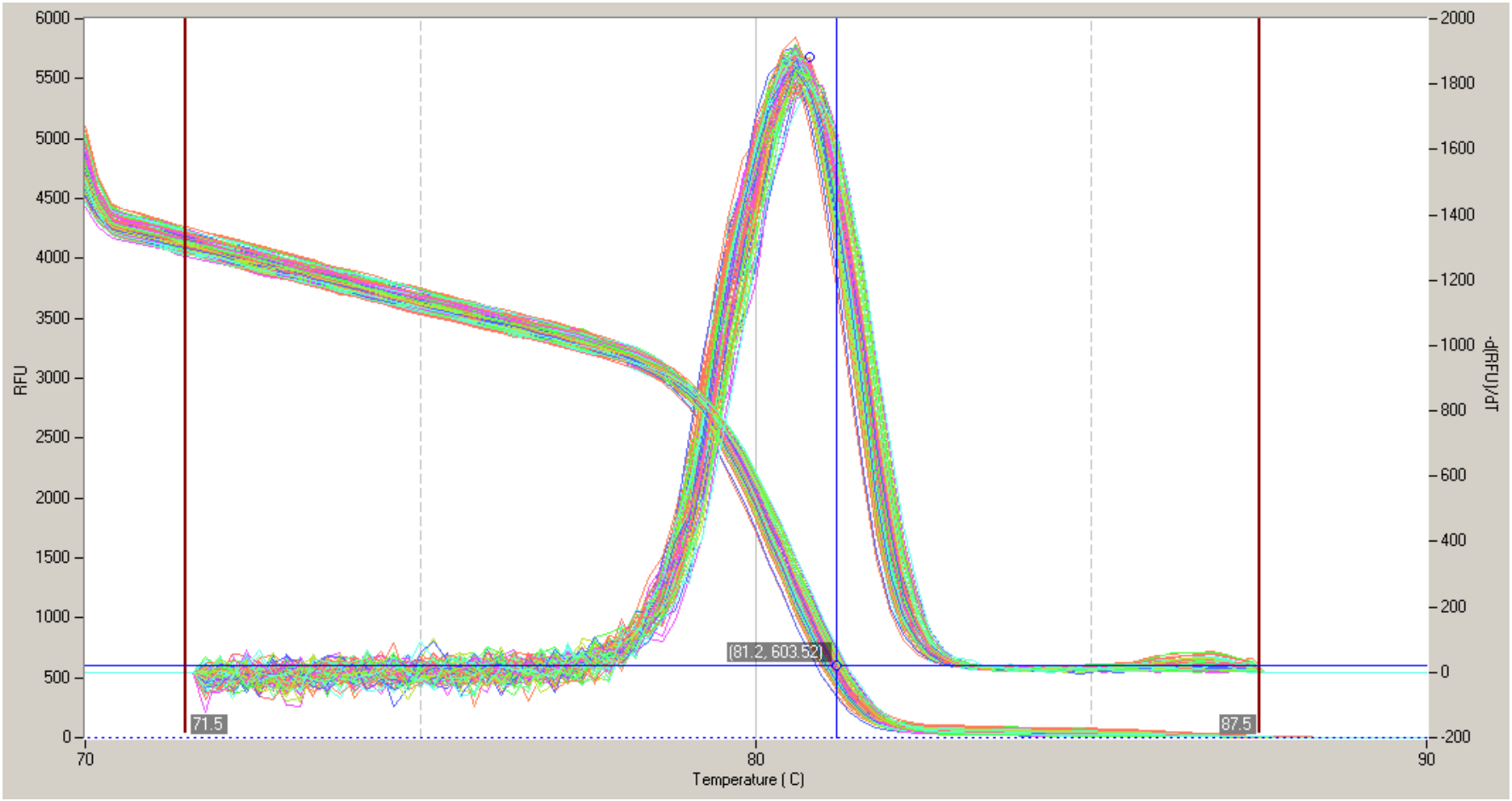


Linear scale

Dynamo SYBR Flash in all 96 wells

Ave Cq	21.082
Max Cq	21.28
Min Cq	20.88
d Cq	0.4
SD Cq	0.090

Melt Curve Analysis: Dynamo SYBR Flash



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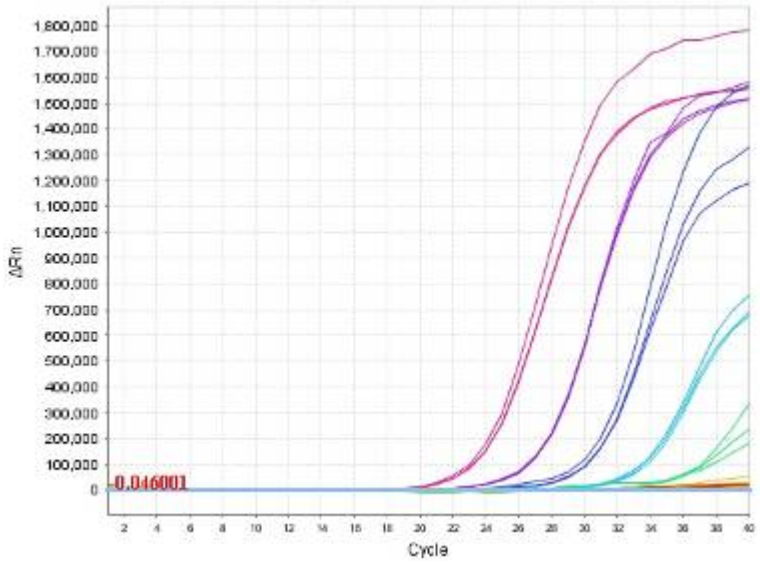
PikoREAL vs LifeTech 7500 Fast

ABi 7500



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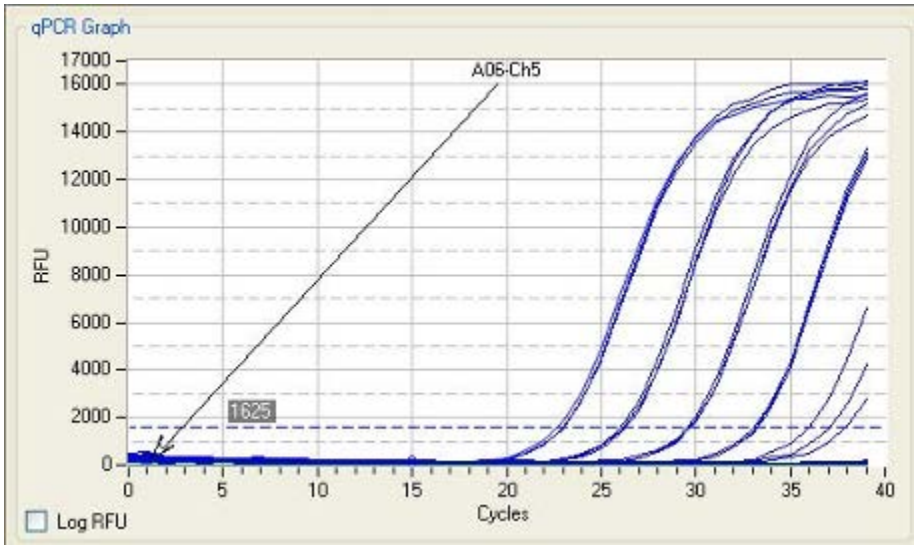

TaqMan Hydrolysis probe with FAM label, identical cycling conditions were used.

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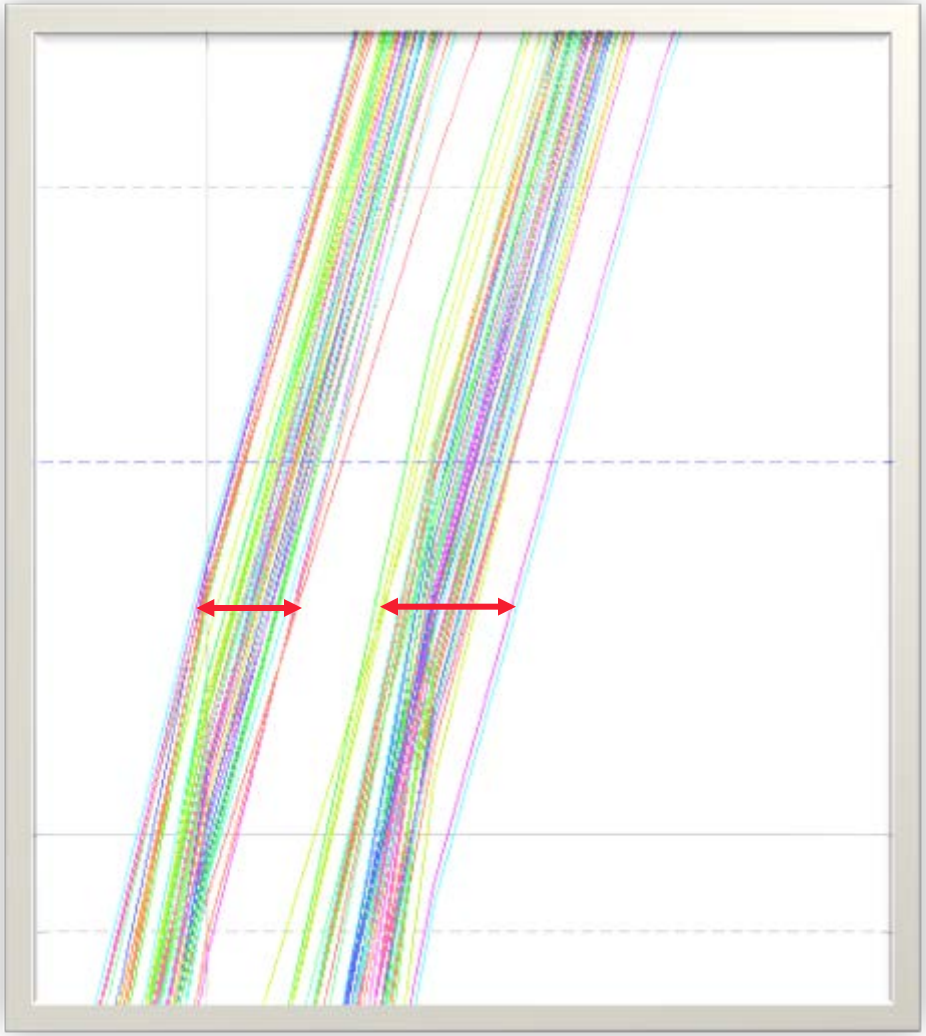
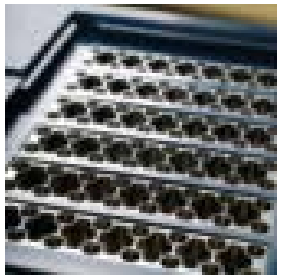
2-Fold Discrimination



2-fold discrimination of 5,000 and 10,000 copies is easily achievable

StepOne and StepOne Plus

- PikoREAL has both 24 and 96-well options, StepOne has 48-well capacity and Plus has 96-well
- PikoREAL has superior thermal performance (±0.3ºC vs. ±0.5ºC)
  - Increased reproducibility across plate
  - Faster run time, 10-30min for PikoREAL
- PikoREAL can truly multiplex 4 assays (4th one for Plus is TAMRA (quencher and StepOne only 3 dye)
- PikoREAL has wider dynamic range and greater senstivity
- HRM available soon on PikoREAL
- Size and price: 2 for 1!
- StepOne/Plus can be programmed directly via the colour touchscreen but market feedback indicates the software is not user-friendly



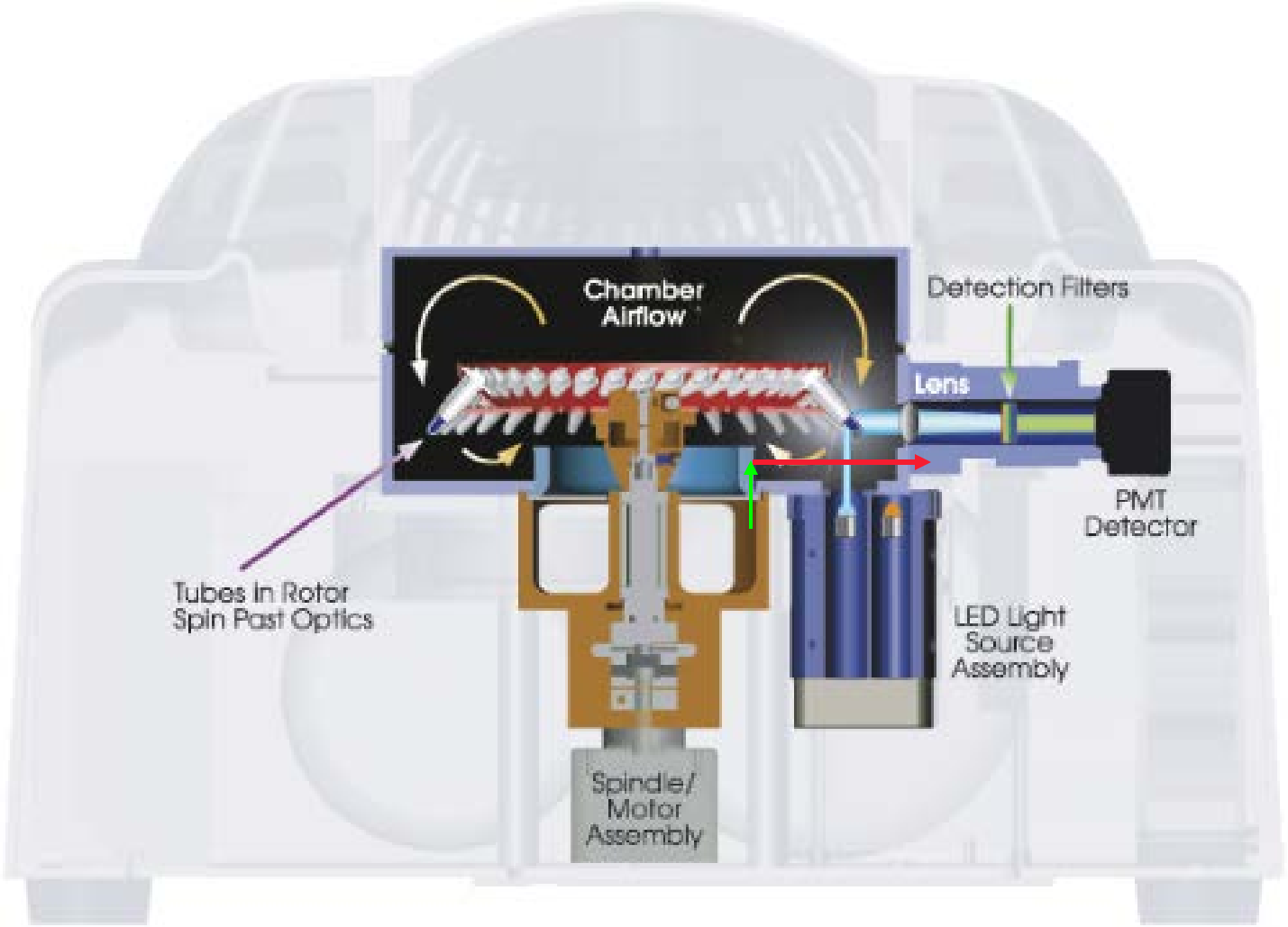
Bio-Rad CFX 96

- The CFX units are positioned as a standard to mid throughput unit
  - 96 and 384-well blocks with optional robotic plate loading
- Thermal performance of the two instruments is similar for speed and uniformity
  - If we add in UTW and Thermo fast reagents then PikoREAL is faster
- The optics of the CFX are LED/APD shuttle so the each well is scanned in turn
  - Takes 20 seconds to scan the plate; in 40 cycle qPCR this is 13mins and the PikoREAL could be finished the entire PCR program and sample reading!
- The CFX has an upgradeable thermal cycler base and gradient function
- Customer like the software although it is not easy to export the raw data
- PikoREAL is much smaller and is more energy efficient
- In the market often quotations for incomplete systems

Bio-Rad MiniOpticon

- PikoREAL and MiniOpticon are placed similarly in the market
- The thermal performance of the PikoREAL is superior: speed, uniformity and temperature range (MiniOpticon only 35 -99^{o}C, so no RT assays)
- The PikoREAL has 24 or 96-well formats compared to 48-well of Bio-Rad (but it does have a gradient)
- It's possible to multiplex 4 assays on PikoREAL, only 2 on MiniOpticon
  - 48 blue LEDs as light source and 2 photodiodes for detectors
- PikoREAL will have HRM soon
- MinOpticon is the only other very small instrument on the market.

Qiagen Rotorgene Q



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