

# Comparison of the different real-time PCR machines

Bruno Verhasselt

Ghent University Hospital,  
Belgium

# Importance of PCR

- Hematology and microbiology (more after lunch!).
- Superior detection sensitivity and specificity
- Molecular diagnostic gains impact in diverse applications:
  - \* detection of viruses and fastidious organisms, e.g. useful in immunosuppressed patients:
    - Early diagnosis (life-threatening infections)
    - Monitoring (EBV-lymphoproliferative disease)
  - \* diagnosis and follow-up in leukemia and lymphoma
  - \* genetic counseling

# PCR methodology

- Traditional PCR: endpoint – post-PCR manipulation to visualise the product
- Quantitative PCR:
  - Competitive PCR: internal standard template
  - Real time-PCR:
    - measuring fluorescence during each cycle
    - threshold cycle proportional to initial amount of template
    - no post-PCR processing

# New cyclers

- Low budget end ( $< 60$  k€) real-time cyclers are being introduced on the market
- How do they perform compared to earlier generation expensive real-time cyclers?

1993 First publication: Higuchi et al. Bio-technology 11, 1026-1030



1996 Idaho Technologies Light Cycler, licensed to Roche in 1998



1997 Perkin-Elmer launches ABI Prism 7700, first high-throughput real-time cycler. Applied Biosystems will become biggest player in the market



1999 Bio-Rad commercialises iCycler iQ, first 96-well 4 colour low budget-end cycler



1999 Idaho Technologies Ruggedized Advanced Pathogen Identification Device (RAPID)

2000 and onwards: more "cheaper" real-time cyclers reach the market, high throughput solutions are introduced (384 wells, robotics, ...)

# ABI Prism 7000



Source	Tungsten-halogen
Detector: 4 colours	CCD camera
Applications	SYBR-green, FAM HEX, TET, TAMRA, VIC
Samples	96
Footprint	39 x 51 (cm x cm)



Range of cyclers



# Bio-Rad iCycler iQ



Source	Tungsten-halogen
Detector: 4 colours	CCD camera
Applications	SYBR-green, FAM HEX, TET, TAMRA, VIC
Samples	96
Footprint	33 x 62 (cm x cm)



Real-time upgrade of iCycler; single colour version: MyiQ

# Cepheid SmartCycler



Source	LED
Detector: 4 colours	Silicon photodetectors
Applications	SYBR-green, FAM, TET, ROX, Cy3, Cy5
Samples	16, independent
Footprint	30 x 25 (cm x cm)

96 samples by connecting 6 blocks



# Corbett Research Rotor-Gene 3000



Source	LED
Detector: 4 colours	PMT
Applications	SYBR-green, FAM HEX, TET, TAMRA, VIC
Samples	72
Footprint	38 x 48 (cm x cm)



# MJ Research DNA Engine Opticon 2

Source	LED
Detector: 2 colours	2 PMTs (523-543; 540-570 nm)
Applications	SYBR-green, FAM HEX, TET, TAMRA, VIC
Samples	96
Footprint	34 x 47 (cm x cm)



Chromo 4 module to convert DNA engine to real-time cycler

# Roche LightCycler 2



Source	LED
Detector: 6 colours	Fluorimeters
Applications	SYBR-green, FAM HEX, VIC, LightCycler Red stains
Samples	32
Footprint	30 x 45 (cm x cm)



New version of original 2 colour machine

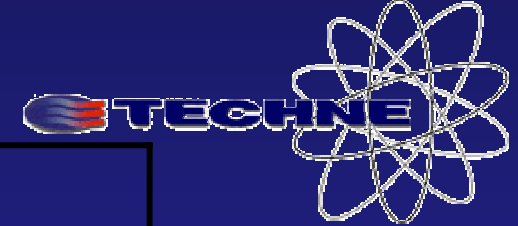
# Stratagene Mx3000P



Source	Tungsten-halogen
Detector: 4 colours	1 PMT scanner
Applications	SYBR-green, FAM HEX, TET, TAMRA, VIC
Samples	96
Footprint	33 x 46 (cm x cm)



# Techne Quantica





Source	Halogen
Detector: 4 colours	PMT (500 to 710 nm)
Applications	SYBR-green, FAM HEX, TET, TAMRA, VIC
Samples	96
Footprint	45 x 50 (cm x cm)



Available soon

# Overview of the cyclers presented

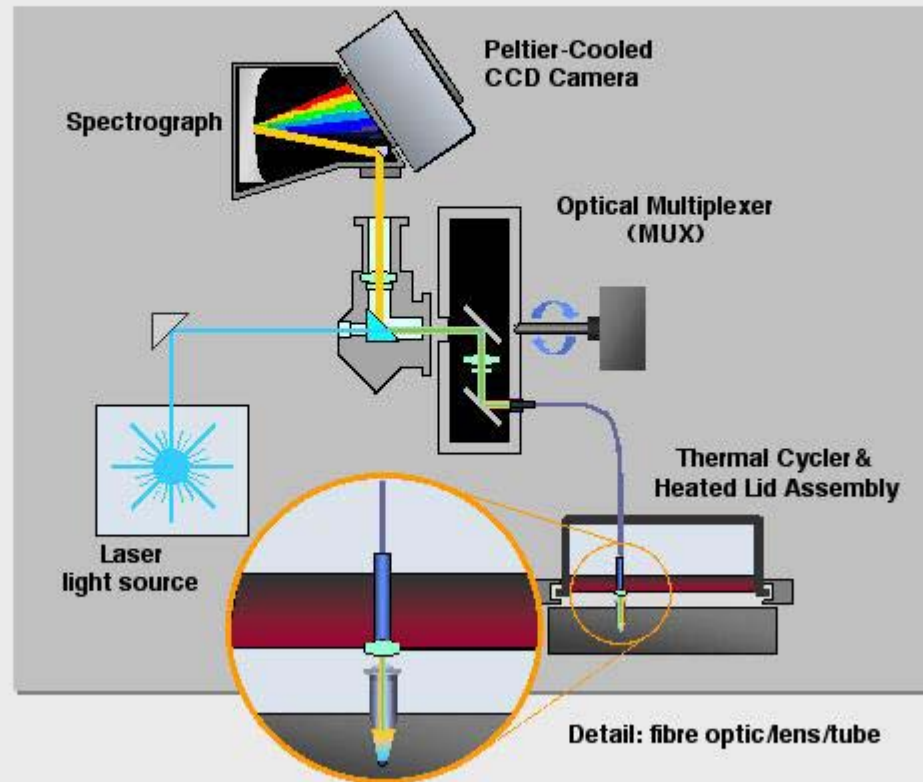
Cycler	Source	Detector	Applications	Smples	Footprint
<b>ABI Prism 7000</b> 	Tungsten-halogen	CCD camera	SYBR, F, H, TE, TA, V, ...	96	39 x 51
<b>Bio-Rad iCycler iQ</b> 	Tungsten-halogen	CCD camera	SYBR, F, H, TE, TA, V, ...	96	33 x 62
<b>Cepheid SmartCycler</b> 	LED	Silicon detectors	SYBR, F, T, R, C3, C5, ...	16	30 x 25
<b>Corbett Res. Rotor-Gene</b> 	LED	PMT	SYBR, F, H, TE, TA, V, ...	72	38 x 48
<b>MJ Res. DNA EngineOpticon 2</b> 	LED	2 PMTs	SYBR, F, H, TE, TA, V, ...	96	34 x 47
<b>Roche LightCycler 2</b> 	LED	Fluorimeters	SYBR, F, H, V, LightC R	32	30 x 45
<b>Stratagene Mx3000P</b> 	Tungsten-halogen	1 PMT scanner	SYBR, F, H, TE, TA, V, ...	96	33 x 46
<b>Techne Quantica</b> 	Halogen	PMT	SYBR, F, H, TE, TA, V, ...	96	45 x 50

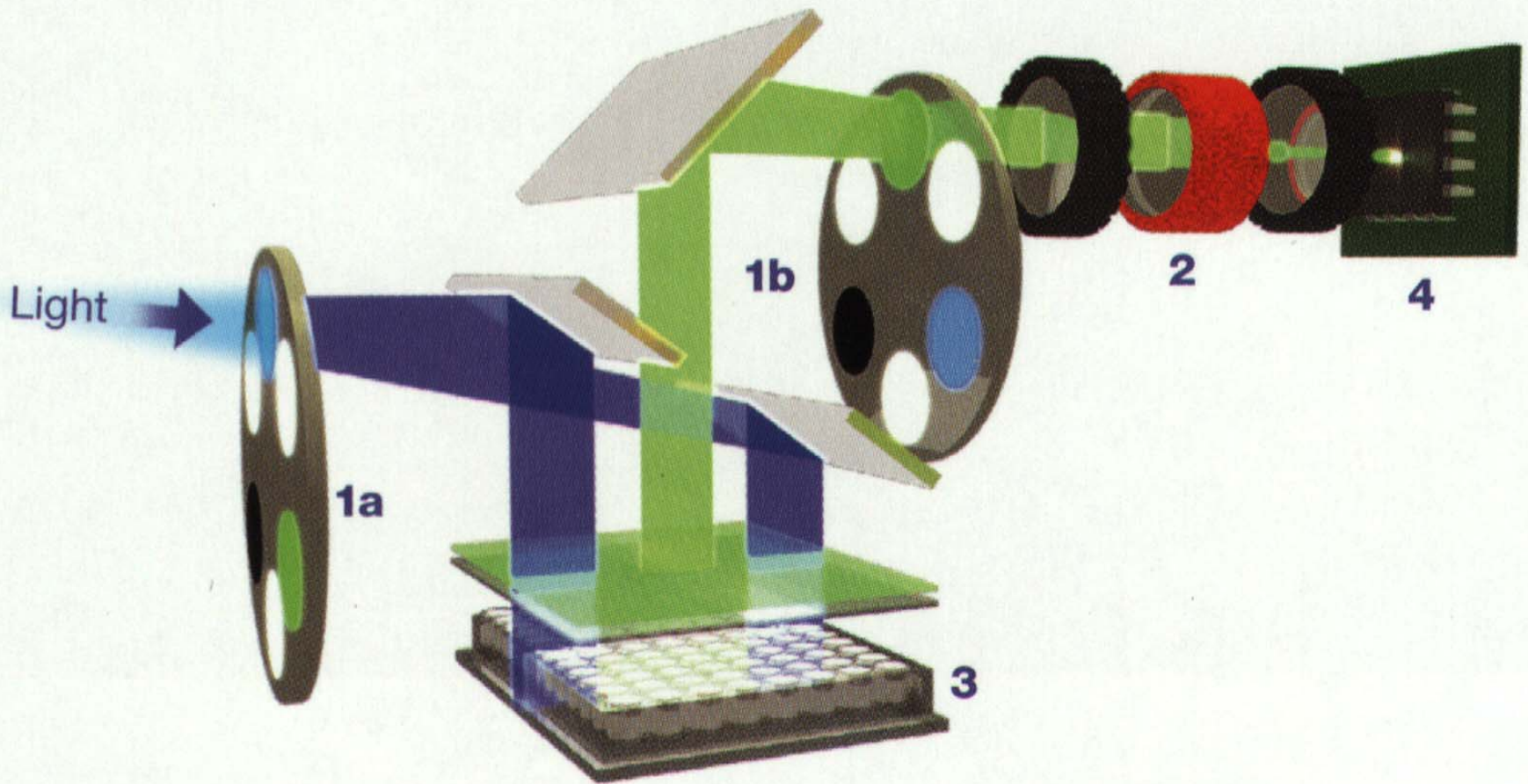
# Machines and methods compared (1)

- High end: Applied Biosystems (ABI) 7700
  - Light source: laser
- Low end: ABI 7000, Biorad iCycler iQ, Stratagene Mx3000P
  - Light source: tungsten halogen lamp

## ABI Prism™ 7700 Sequence Detection System

optics of the virtual filter system



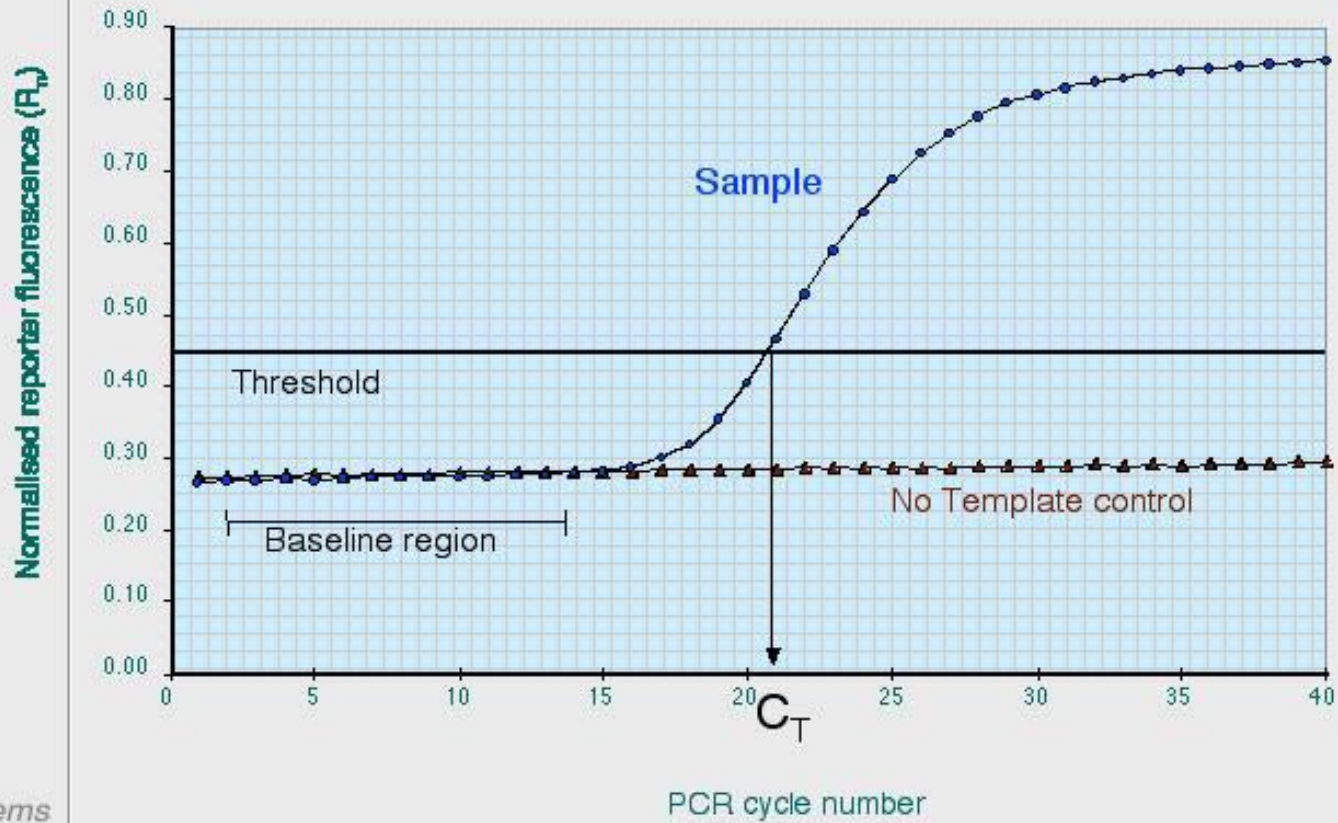


iCycler iQ (Biorad)

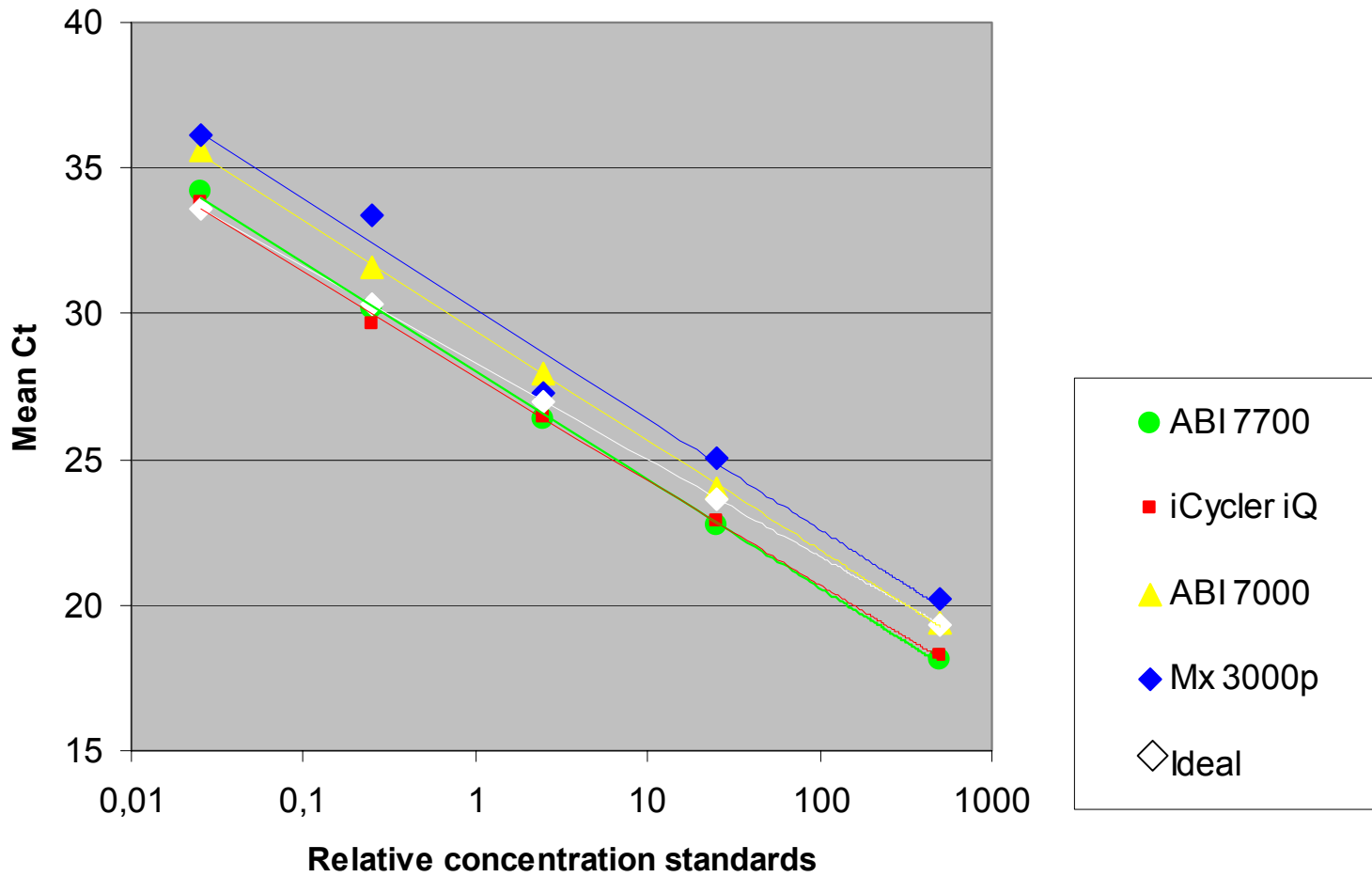
# Machines and methods compared (2)

- Uniplex: 6-FAM labeled Taqman probe, one-step reverse transcription-PCR, target: PBGD mRNA from K562 cells
- Multiplex: 6-FAM/VIC Taqman probes, co-amplification of EBV- and CRP-DNA from Namalwa-cells (2 copies/cell)

## Amplification plot features



## Uniplex (pbgd)

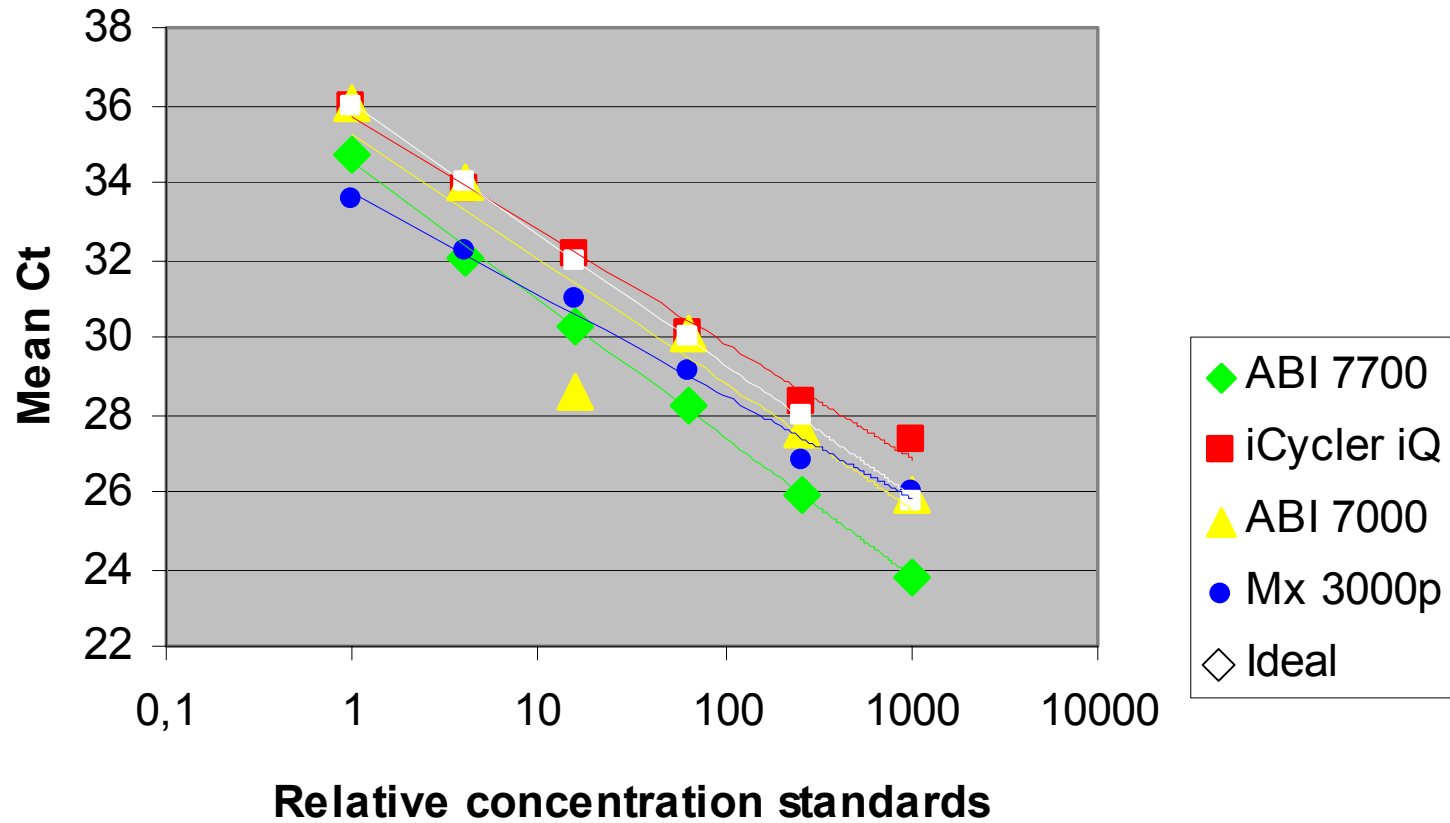


# Results 1

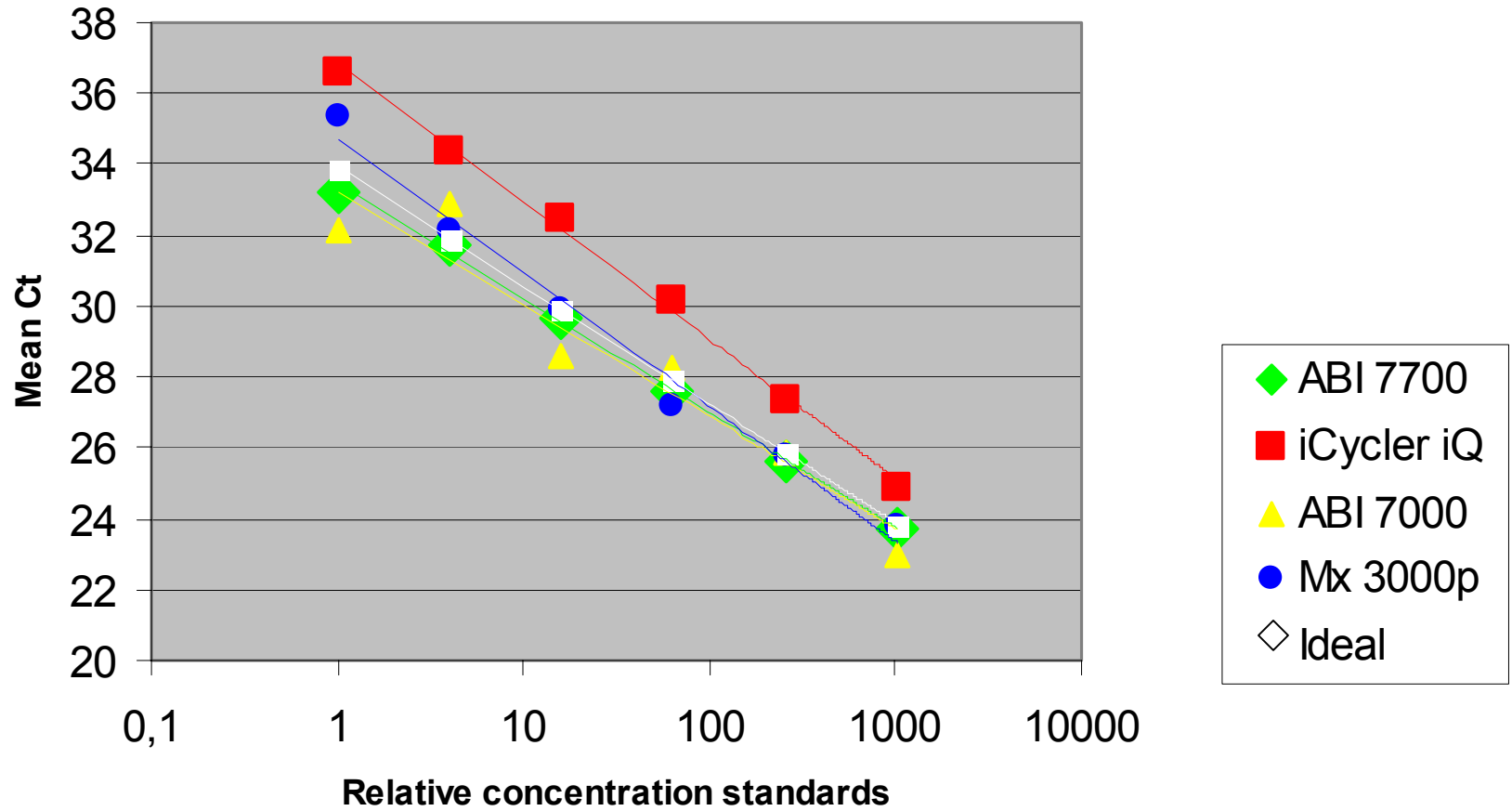


		<b>ABI7700</b>	<b>ABI7000</b>	<b>iCycler iQ</b>	<b>Mx 3000</b>
Uniplex	$C_T$ CV (%)	0.08 to 1.09	1.16 to 3.85	0.74 to 1.34	0.74 to 3.74
	Linearity (-3.29 ideal slope)	-3.73	-3.75	-3.57	-3.78
	Sensitivity (intercept $C_t$ , ideal 27,9)	28.0	29.4	27.8	30.1
	Lowest standard detected	0.025	0.025	0.025	0.025

## Multiplex: 6-FAM



## Multiplex: VIC



# Results 2



<b>Multiplex</b>		<b>ABI7700</b>	<b>ABI7000</b>	<b>iCycler iQ</b>	<b>Mx 3000</b>
C <sub>T</sub> CV (%)	FAM	0.49 to 2.14	0.49 to 3.29	0.42 to 1.70	0.61 to 6.77
	VIC	0.54 to 3.21	0.87 to 13.74	1.79 to 6.10	1.04 to 9.70
Slope (id -3.38)	FAM	-3.57	-3.25	-2.95	-2.65
	VIC	-3.22	-3.18	-3.89	-3.80
Intercept C <sub>T</sub>	FAM	34.5	34.3	35.7	33.8
	VIC	33.5	33.2	36.8	34.7
Lowest Standard	FAM	0.06	1	1	1
	VIC	0.06	0.06	1	1
FAM/VIC (id 1)		1,019	1,070	1,015	1,032
Price (€)		+/-120,000	+/-65,000	+/-45,000	+/-36,000

# Conclusion

- Laser based ABI 7700 is superior to low budget end cyclers using lamps
- Low budget end cyclers do however perform well enough compared to clinically acceptable error
- These cyclers are suited to bring along the molecular diagnostic boom