Real-time Fluorescence PCR assays for the Detection and Characterization of Heat-labile and Heat-stable Enterotoxin Genes from Enterotoxigenic *Escherichia coli*

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Enterotoxigenic *Escherichia coli* (ETEC)

- Recognized as a pathogen in 1968, Calcutta, India
- Cause watery (cholera-like) diarrhea
  - Developing world
  - Travelers to endemic areas
- 26 outbreaks in US from 1975-present
- Produce plasmid-encoded enterotoxins
  - Heat-labile (LT)
  - Heat-stable (ST)

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Detection of ETEC

- **Phenotypically heterogeneous**
  - **No bacteriologic culture methods**
- **Identified by detecting enterotoxins**
  - **Animal and cell culture**
  - **Immunological**
  - **DNA-hybridization**
  - **PCR**

Conventional PCR challenges

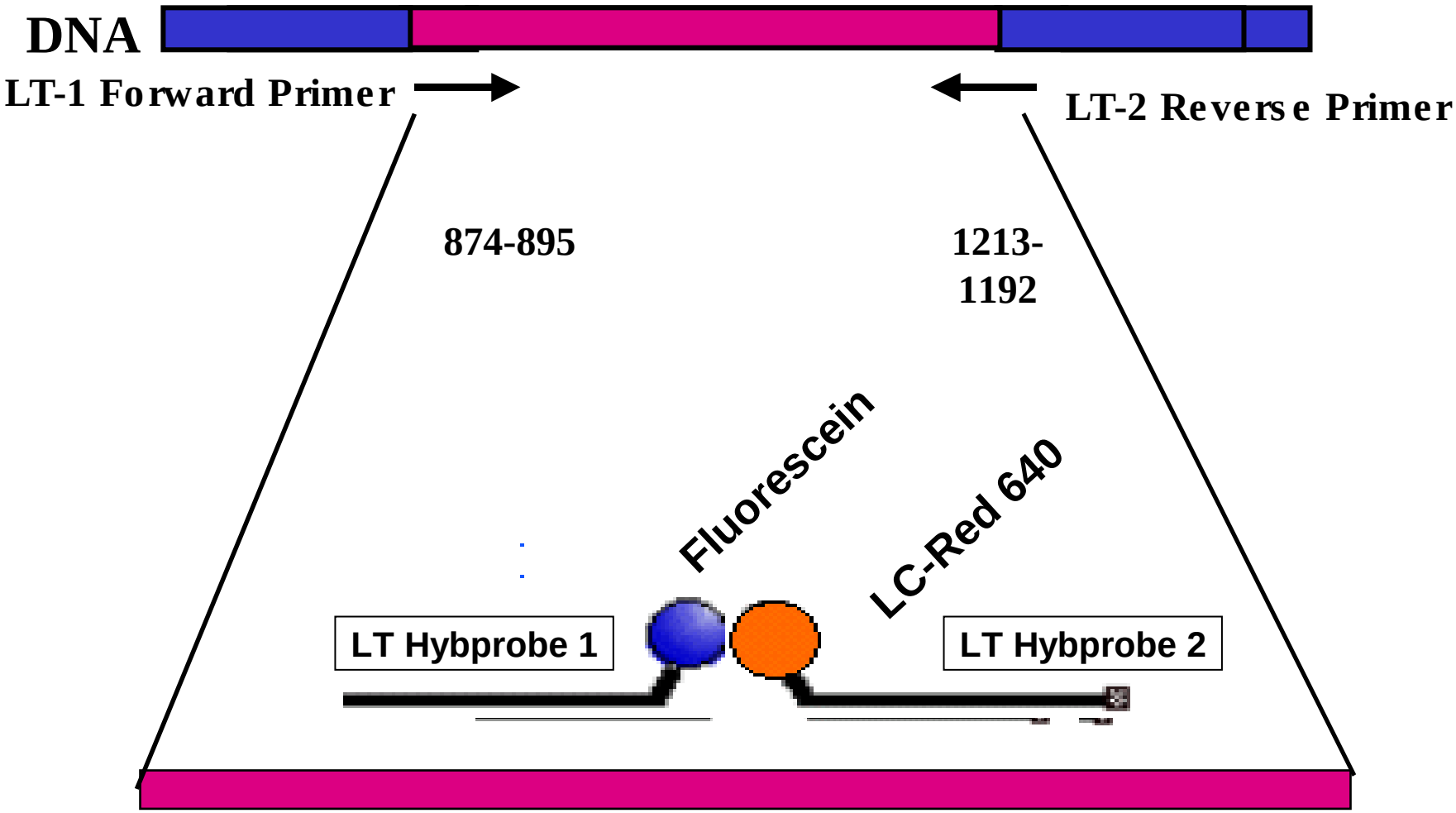
Gel-based detection format

- Labor-intensive
- Slow – gels required several hours
- Non-specific bands complicate interpretation
- Sequence confirmation of products not practical for most laboratories
- Opportunities to contaminate lab with PCR products

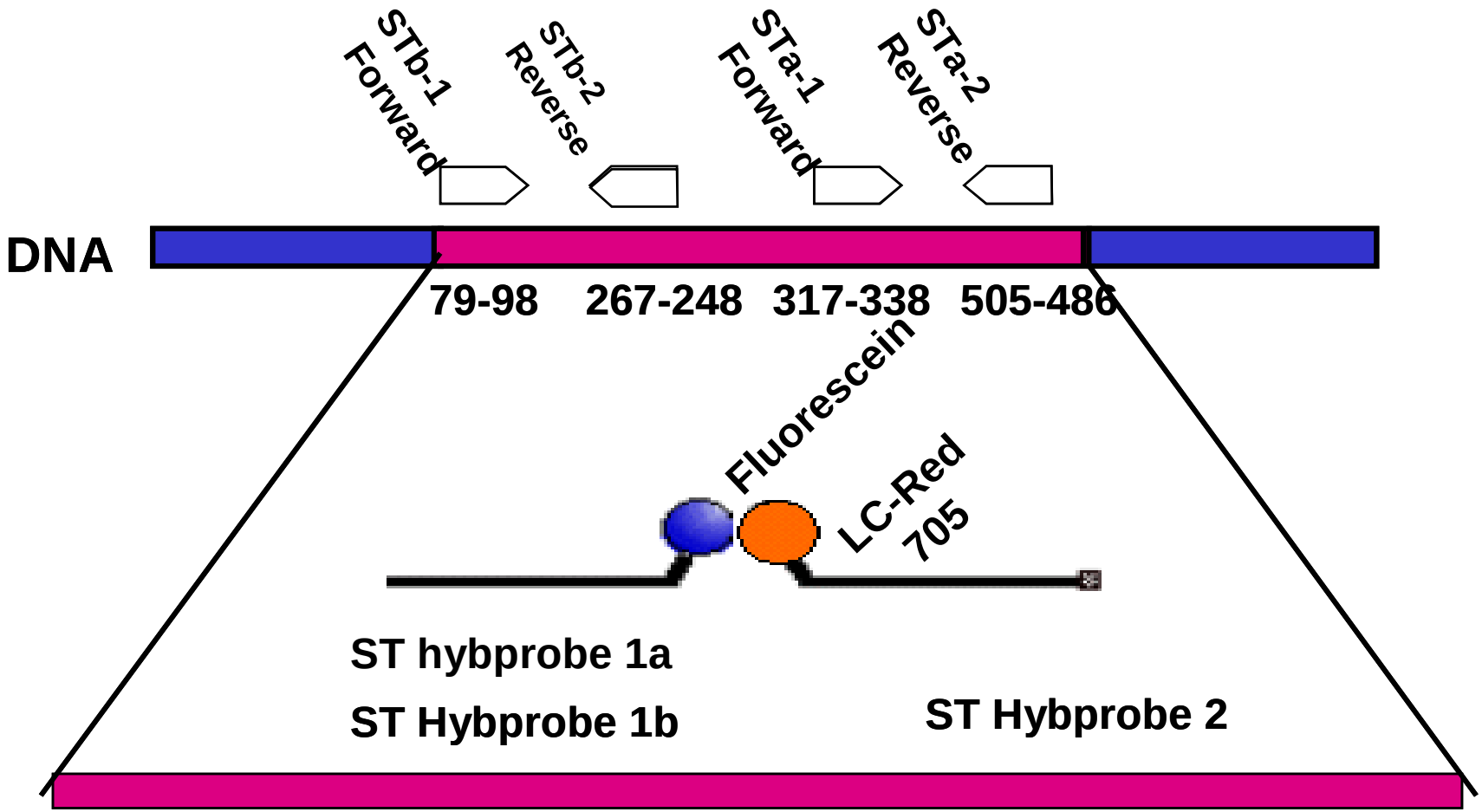
Objective

To develop a real-time fluorescence assay for the LightCycler to detect and characterize ETEC.

LightCycler PCR for the detection of LT

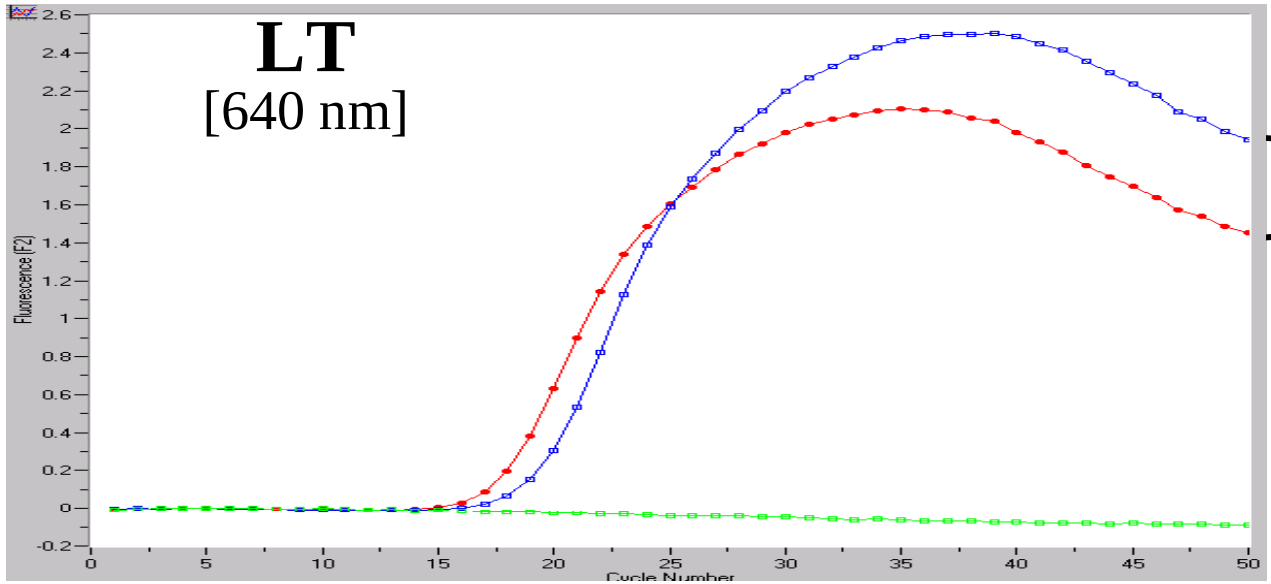


LightCycler PCR for the detection of ST

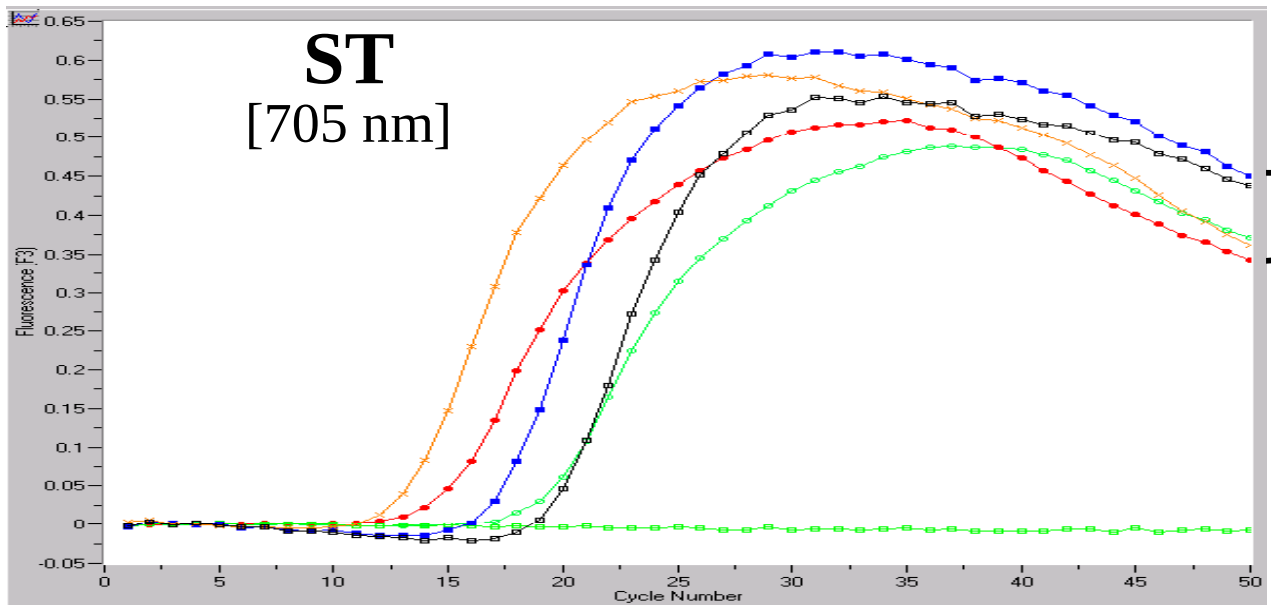


Amplicon

A



B



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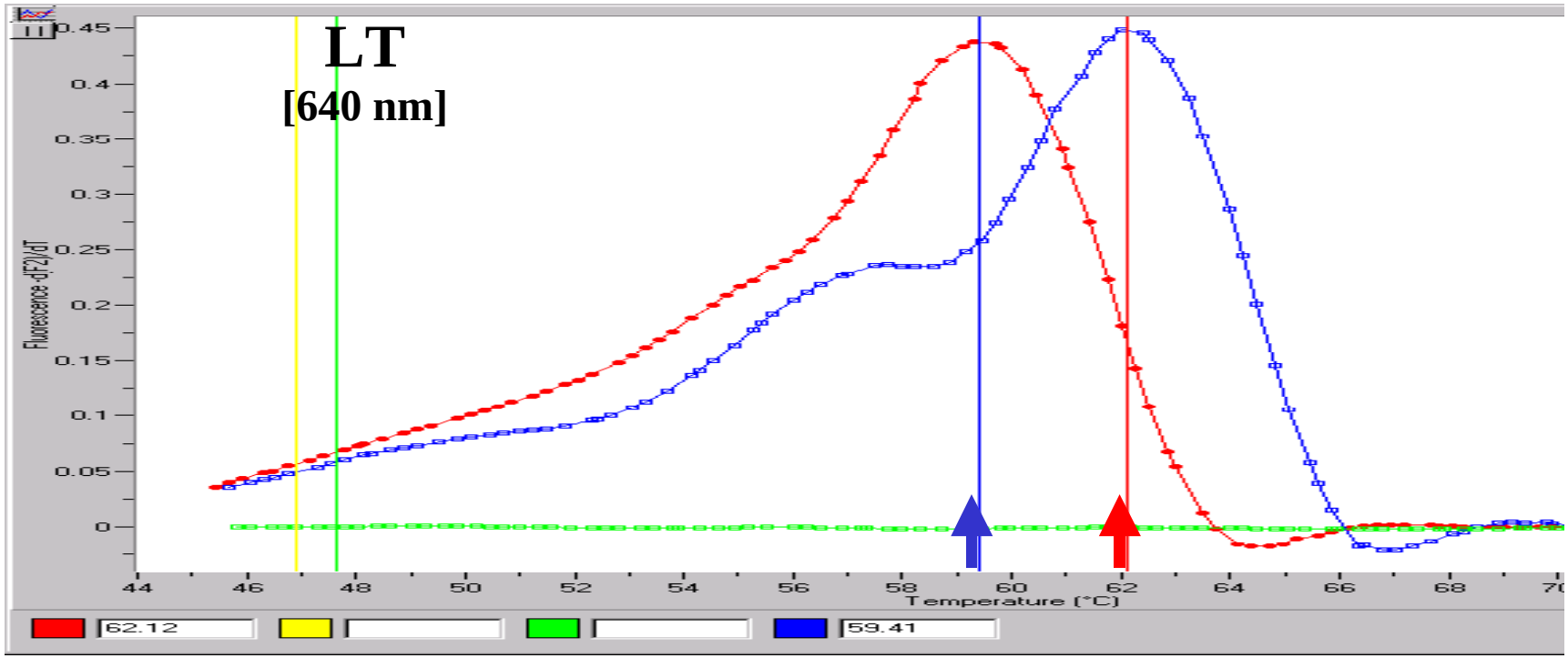
Melting curve analysis of LT PCR products



61°C	63°C
LT variant #F5176	LT H10407

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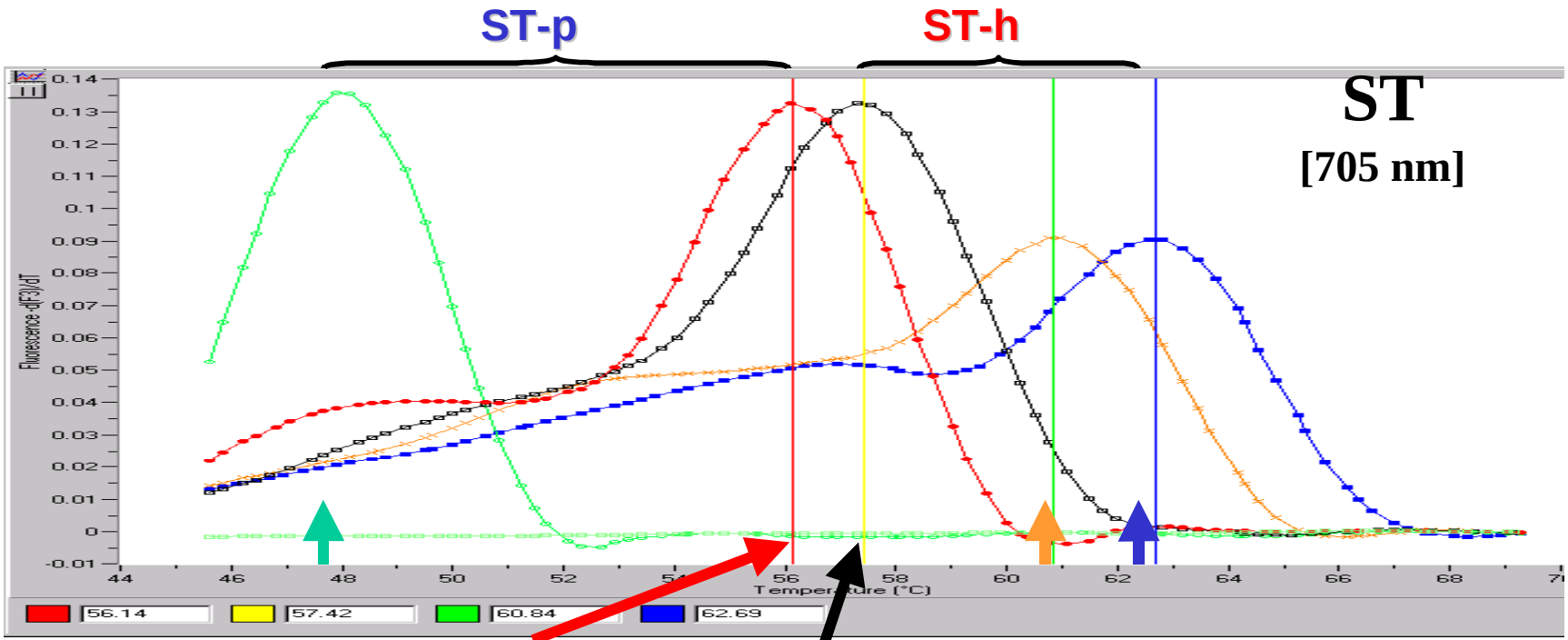
Melting curve analysis of ST PCR products



48°C	57°C	59°C	62°C	64°C
ST Ia variant	ST Ia	ST Ib variant	ST Ib variant	ST Ib
#F7682		#R 554	#C4046	

H10407 (Tx-1)

E. coli strains tested by conventional and LightCycler PCR assays (n = 160)

	Number of Strains
ETEC (45 serotypes)	137
LT	74
ST1a (STp)	48
ST1b (STh)	66
Non-ETEC (13 serotypes)	23

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Conventional PCR assays

Olive, M. 1989. J. Clin. Microbiol. *27*:261-265.

Olsvik et al. 1991. J. Clin. Microbiol. *29*:2375-2379.

Schultsz *et al*. 1994. J. Clin. Microbiol. *32*:2393-2397.

Correlation of conventional and LightCycler PCR results
n = 160 bacterial isolates

Target gene	Conventional PCR / LightCycler				Sensitivity	Specificity
	+/ +	-/ +	+/-	-/-		
ST Ia	48	0	0	122	100 %	100 %
ST Ib	66	0	0	105	100 %	100 %
LT	74	0	0	96	100 %	100 %

Summary

- **LightCycler ETEC PCR**
  - **Equals conventional PCR in sensitivity and specificity**
  - **Faster (60 min *vs* 4.5-5 hrs)**
  - **Sequence-specific product identification**
  - **Detection of sequence variants**
  - **Limited multiplexing**

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Conclusion

LightCycler PCR is a good alternative to conventional PCR for ETEC.

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