

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

Mohammad T. Youssef

Nancy Strockbine

N. Lehn

Udo Reischl

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)**

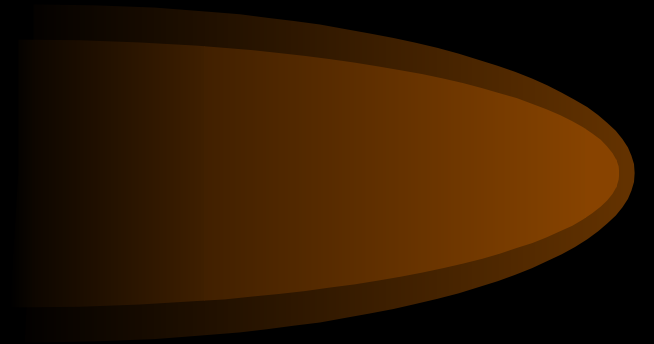
Detection of ETEC

- **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**

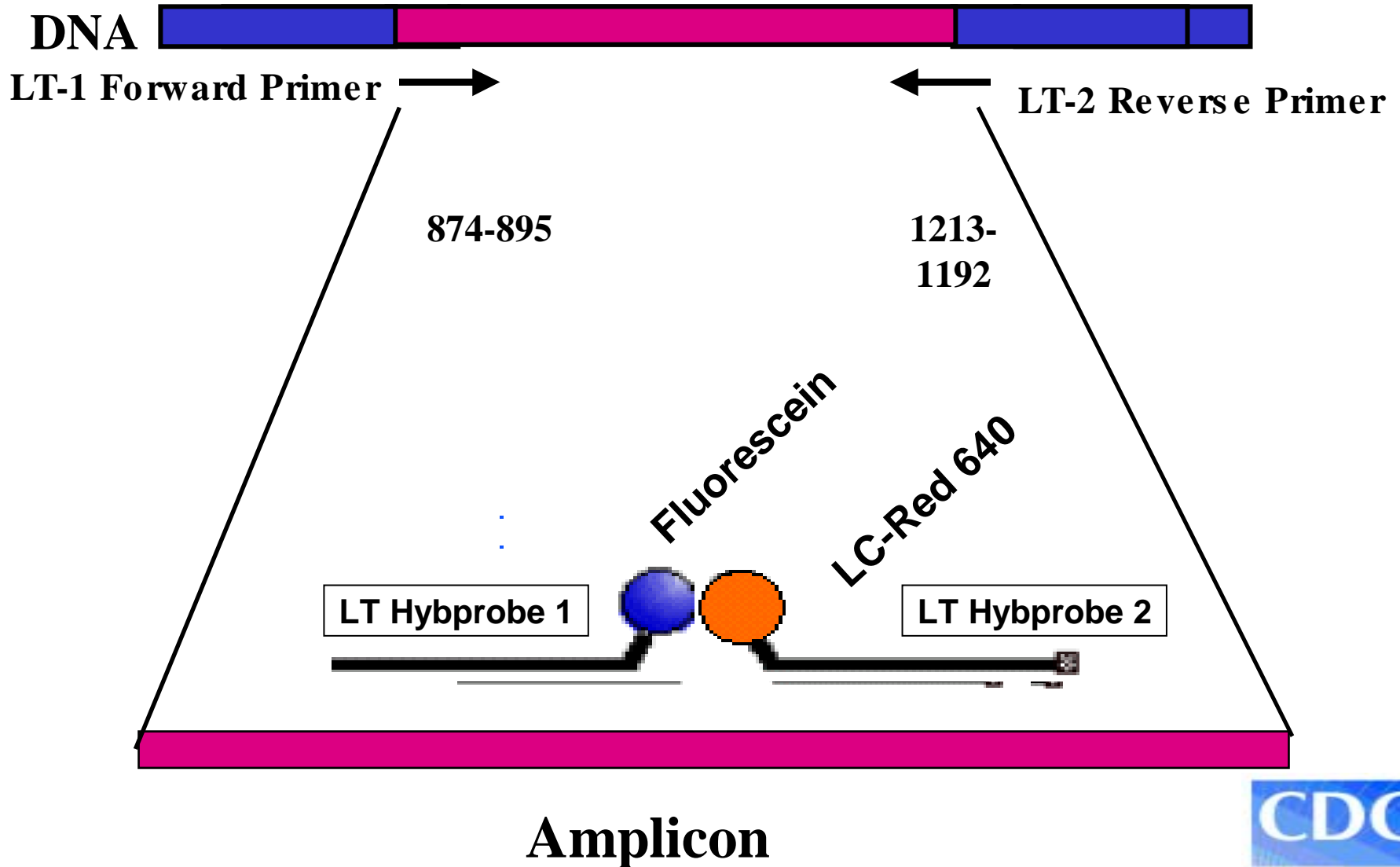


Objective

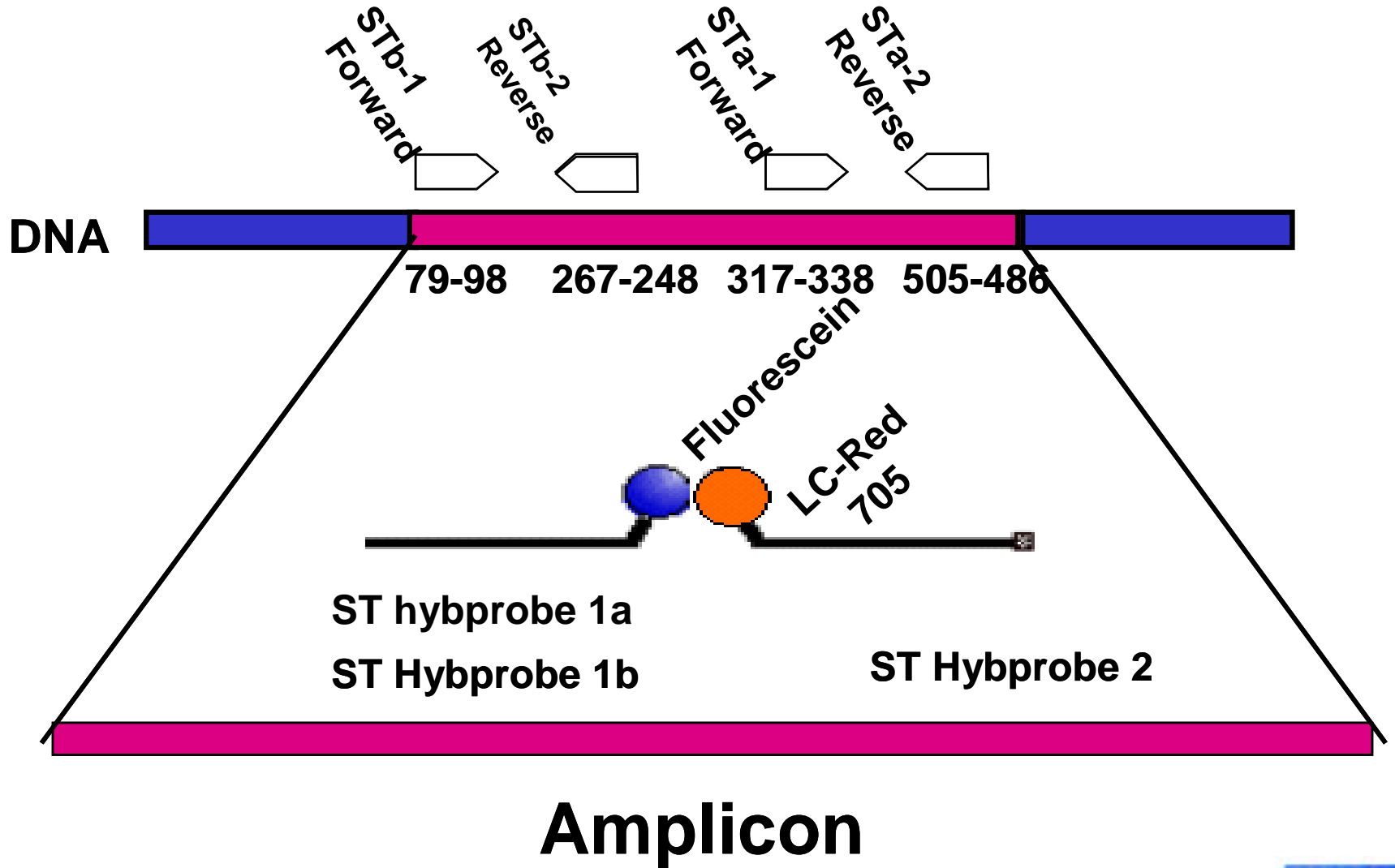


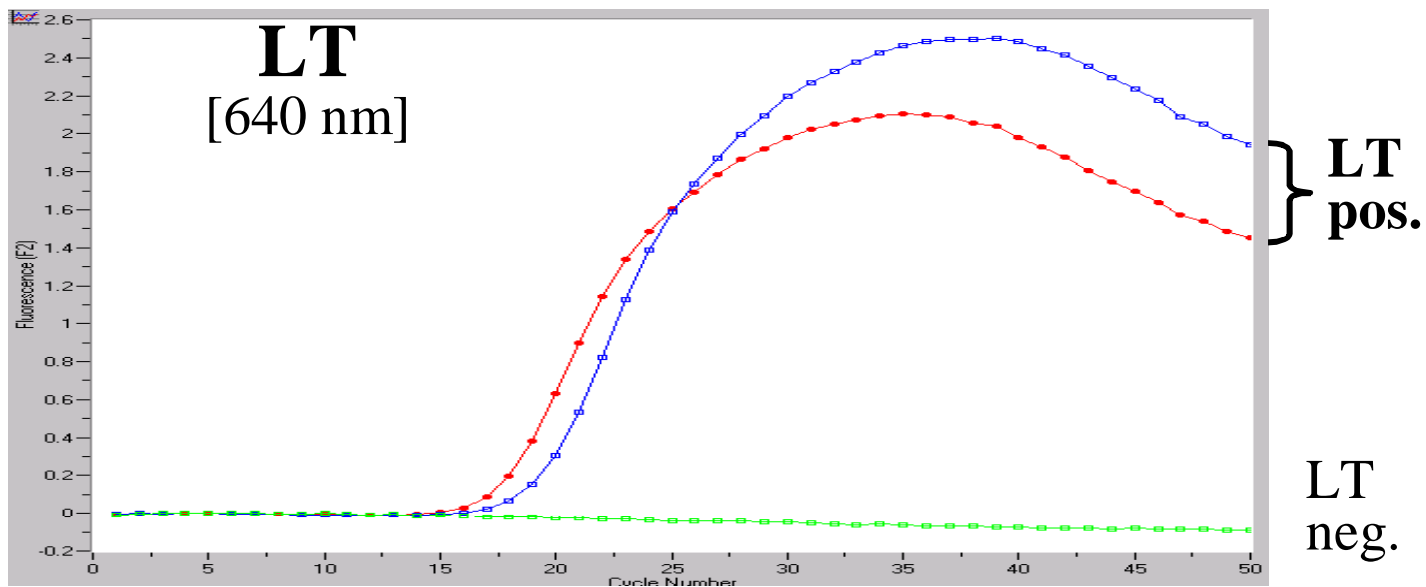
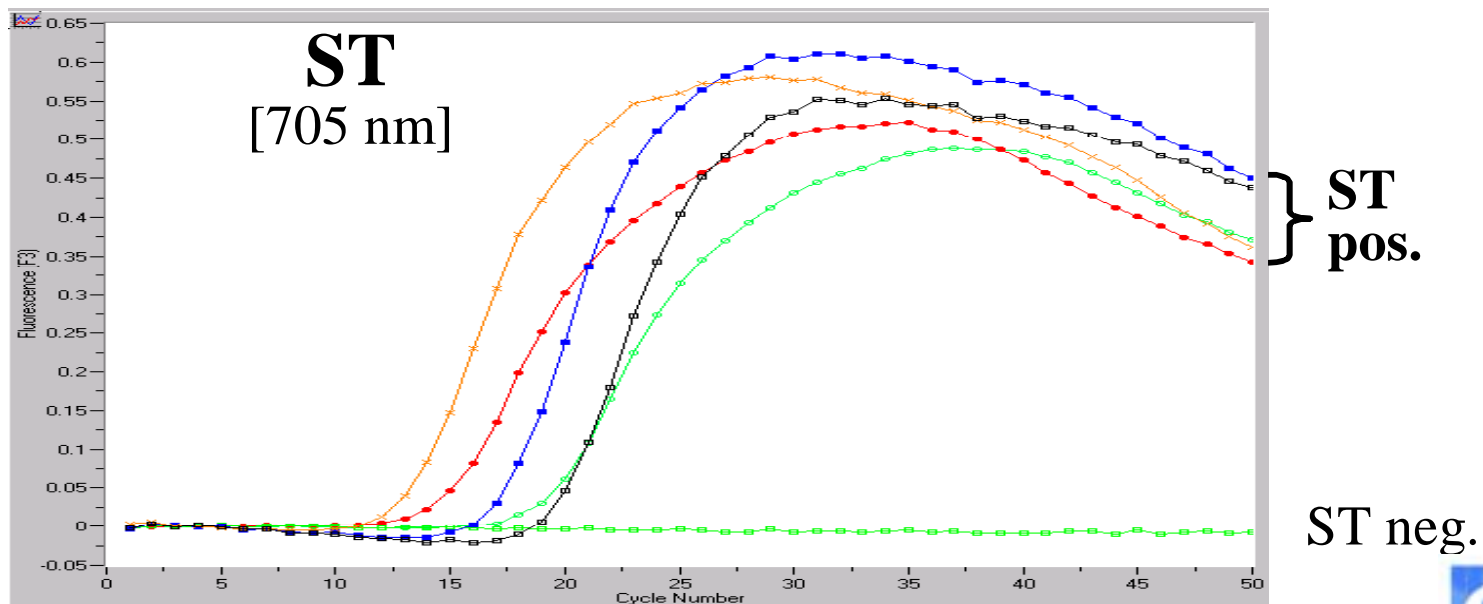
To develop a real-time fluorescence assay for the LightCycler to detect and characterize ETEC.

LightCycler PCR for the detection of LT

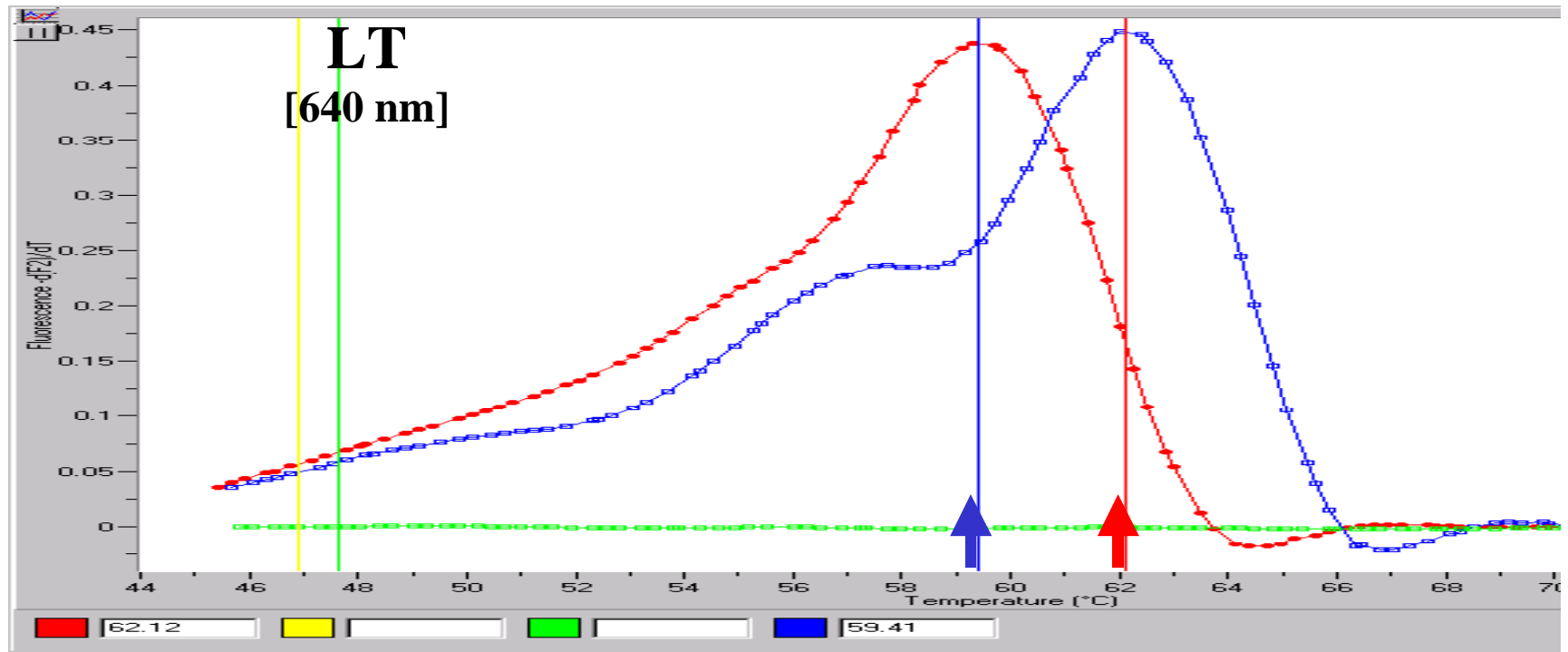


LightCycler PCR for the detection of ST



A**B**

Melting curve analysis of LT PCR products



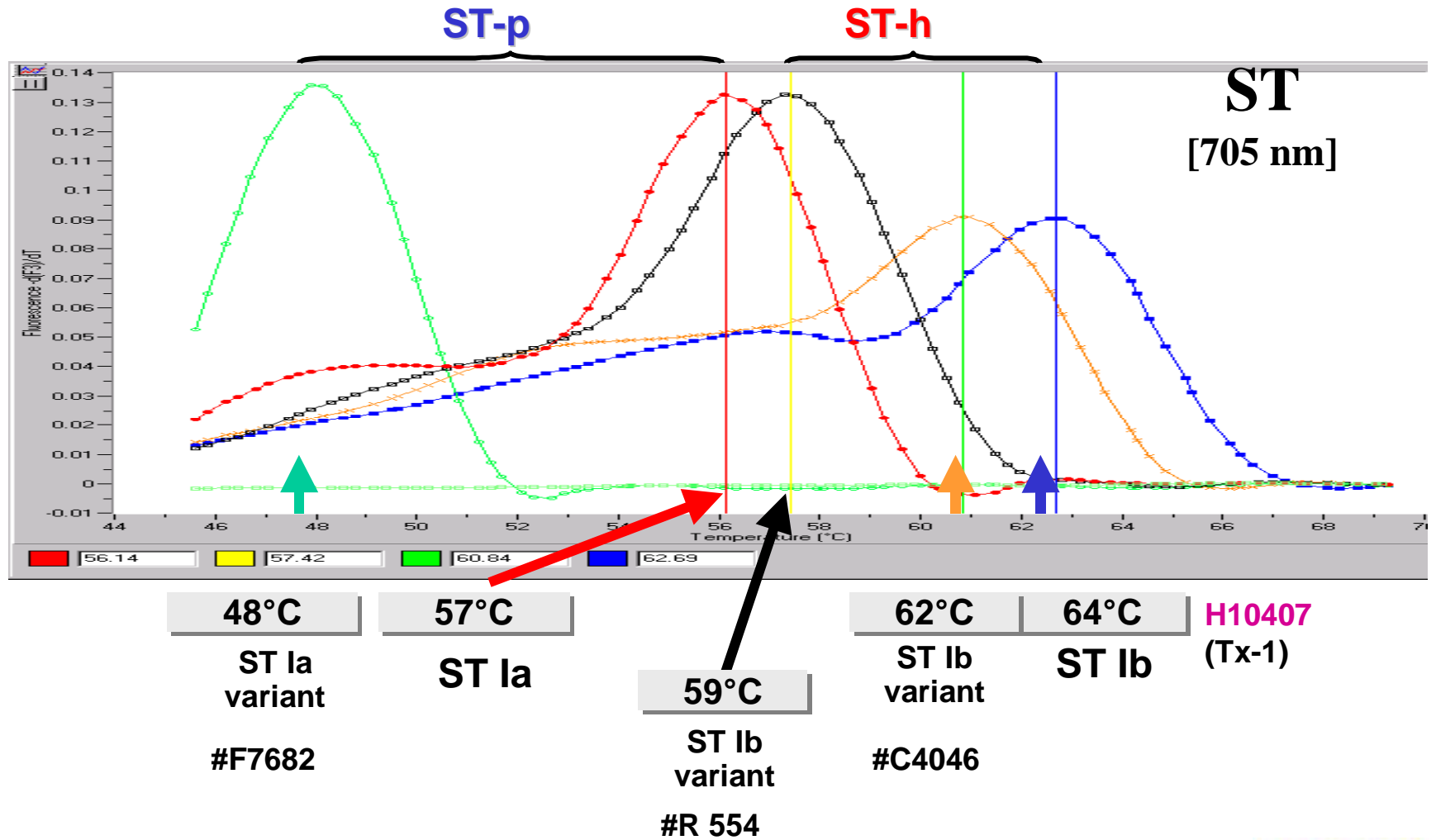
61°C

LT
variant
#F5176

63°C

LT
H10407

Melting curve analysis of ST PCR products



***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

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**

Conclusion

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