

First steps of the National Reference Laboratory for *Escherichia coli* including Verotoxin-producing *E. coli* in Austria

Austrian Agency of Health and Food Safety

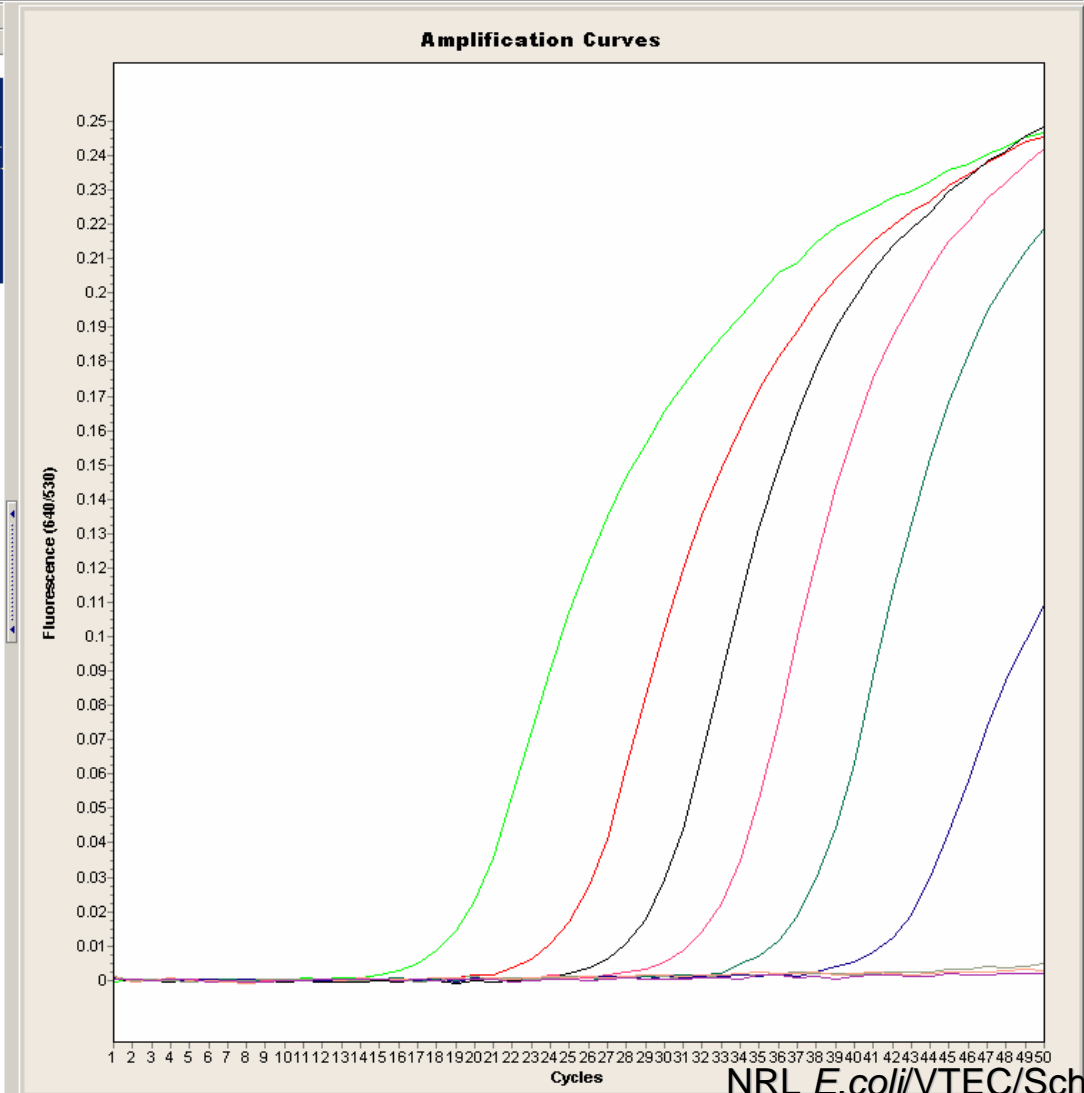
Institute of Medical Microbiology and Hygiene, Graz

Duplex-PCR: *stx1*-Sensitivity

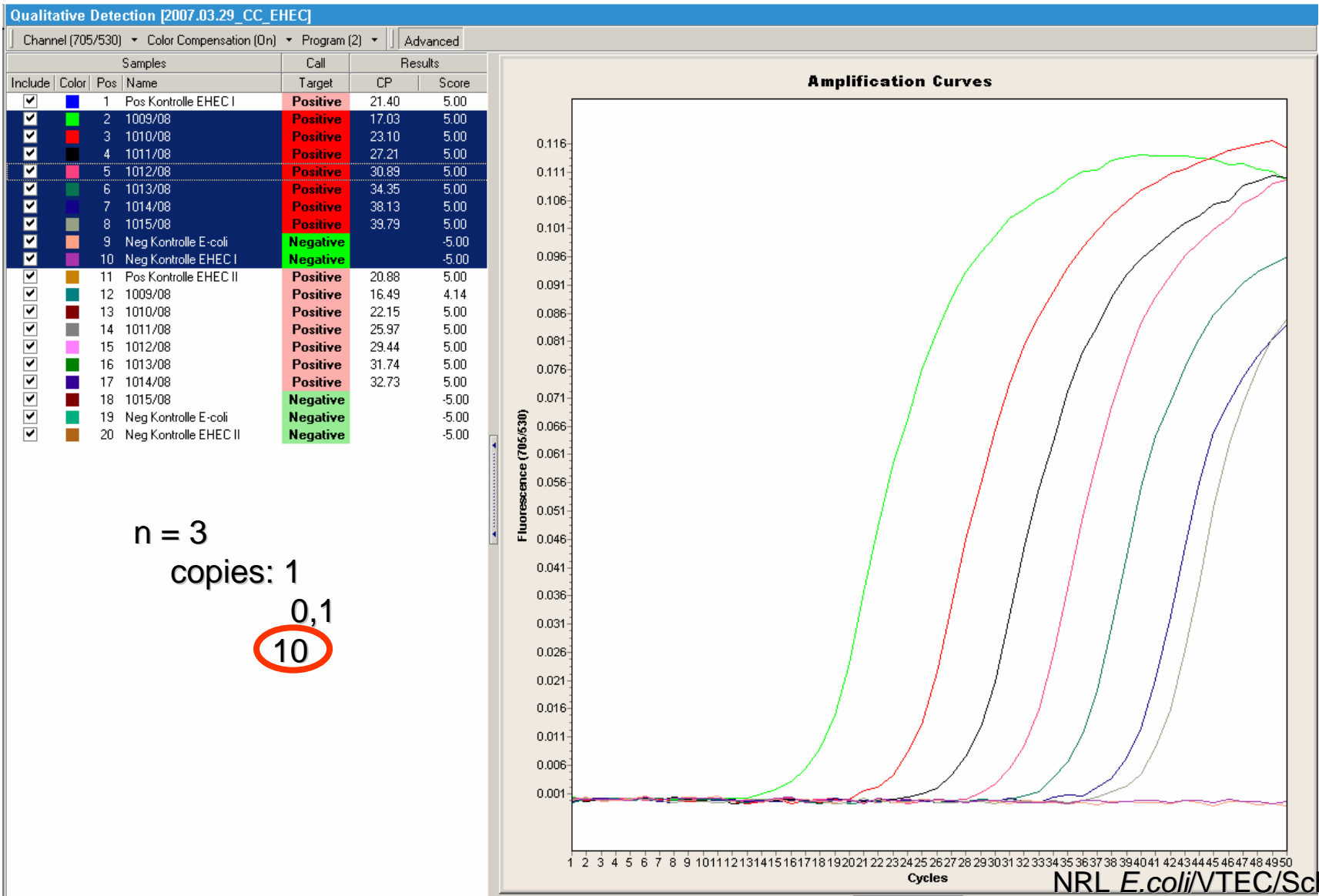
Qualitative Detection [2007.03.29_CC_EHEC]

Samples				Call	Results	
Include	Color	Pos	Name	Target	CP	Score
<input checked="" type="checkbox"/>		1	Pos Kontrolle EHEC I	Positive	22.92	5.00
<input checked="" type="checkbox"/>		2	1009/08	Positive	18.37	4.93
<input checked="" type="checkbox"/>		3	1010/08	Positive	24.24	5.00
<input checked="" type="checkbox"/>		4	1011/08	Positive	28.45	5.00
<input checked="" type="checkbox"/>		5	1012/08	Positive	32.13	5.00
<input checked="" type="checkbox"/>		6	1013/08	Positive	36.63	5.00
<input checked="" type="checkbox"/>		7	1014/08	Positive	41.31	5.00
<input checked="" type="checkbox"/>		8	1015/08	Negative		-1.73
<input checked="" type="checkbox"/>		9	Neg Kontrolle E-coli	Negative		-5.00
<input checked="" type="checkbox"/>		10	Neg Kontrolle EHEC I	Negative		-3.34
<input checked="" type="checkbox"/>		11	Pos Kontrolle EHEC II	Positive	20.82	5.00
<input checked="" type="checkbox"/>		12	1009/08	Positive	16.57	3.80
<input checked="" type="checkbox"/>		13	1010/08	Positive	22.16	5.00
<input checked="" type="checkbox"/>		14	1011/08	Positive	26.00	5.00
<input checked="" type="checkbox"/>		15	1012/08	Positive	29.53	5.00
<input checked="" type="checkbox"/>		16	1013/08	Positive	31.92	5.00
<input checked="" type="checkbox"/>		17	1014/08	Positive	33.16	5.00
<input checked="" type="checkbox"/>		18	1015/08	Negative		-5.00
<input checked="" type="checkbox"/>		19	Neg Kontrolle E-coli	Negative		-5.00
<input checked="" type="checkbox"/>		20	Neg Kontrolle EHEC II	Negative		-5.00

n = 3
copies: 0,1
1
0,1



Duplex-PCR: *stx2*-Sensitivity



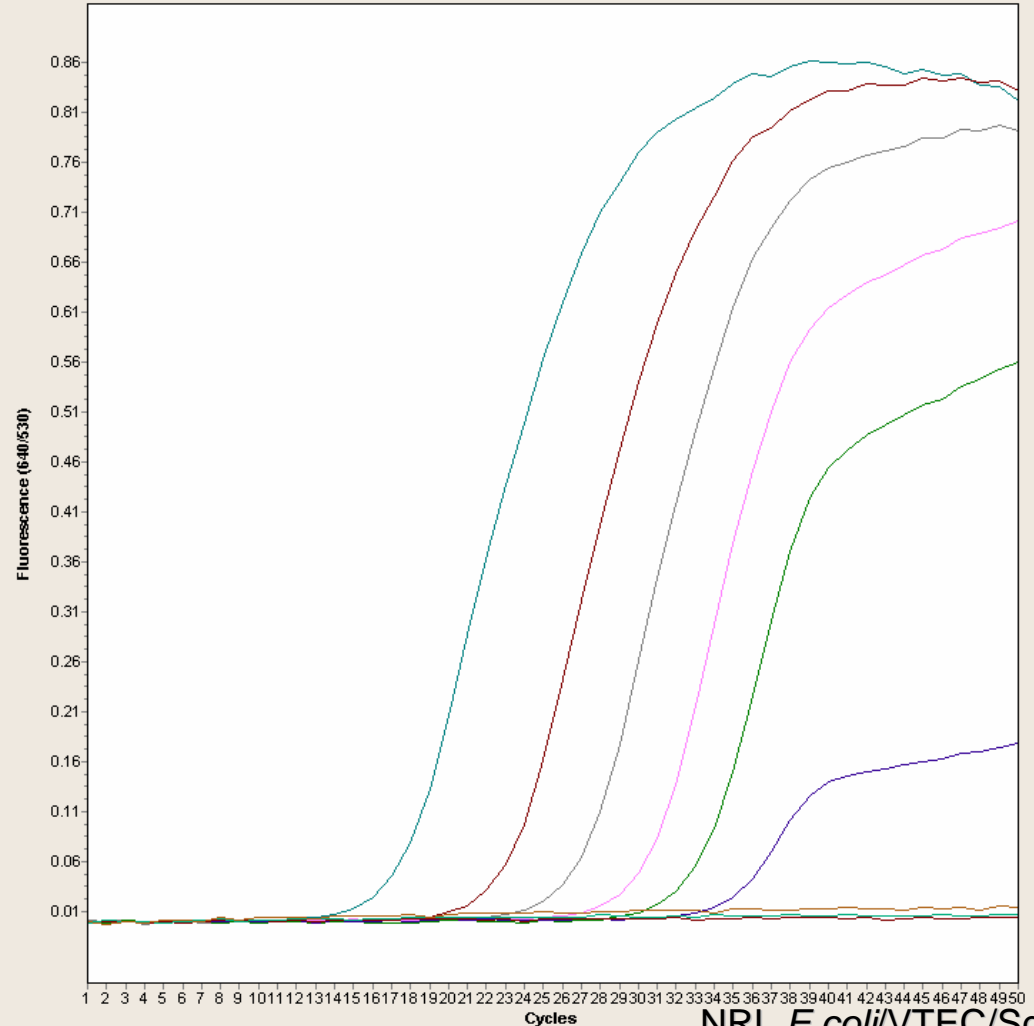
Duplex-PCR: eae-Sensitivity

Qualitative Detection [2007.03.29_CC_EHEC]

Channel (640/530) Color Compensation (On) Program (2) Advanced

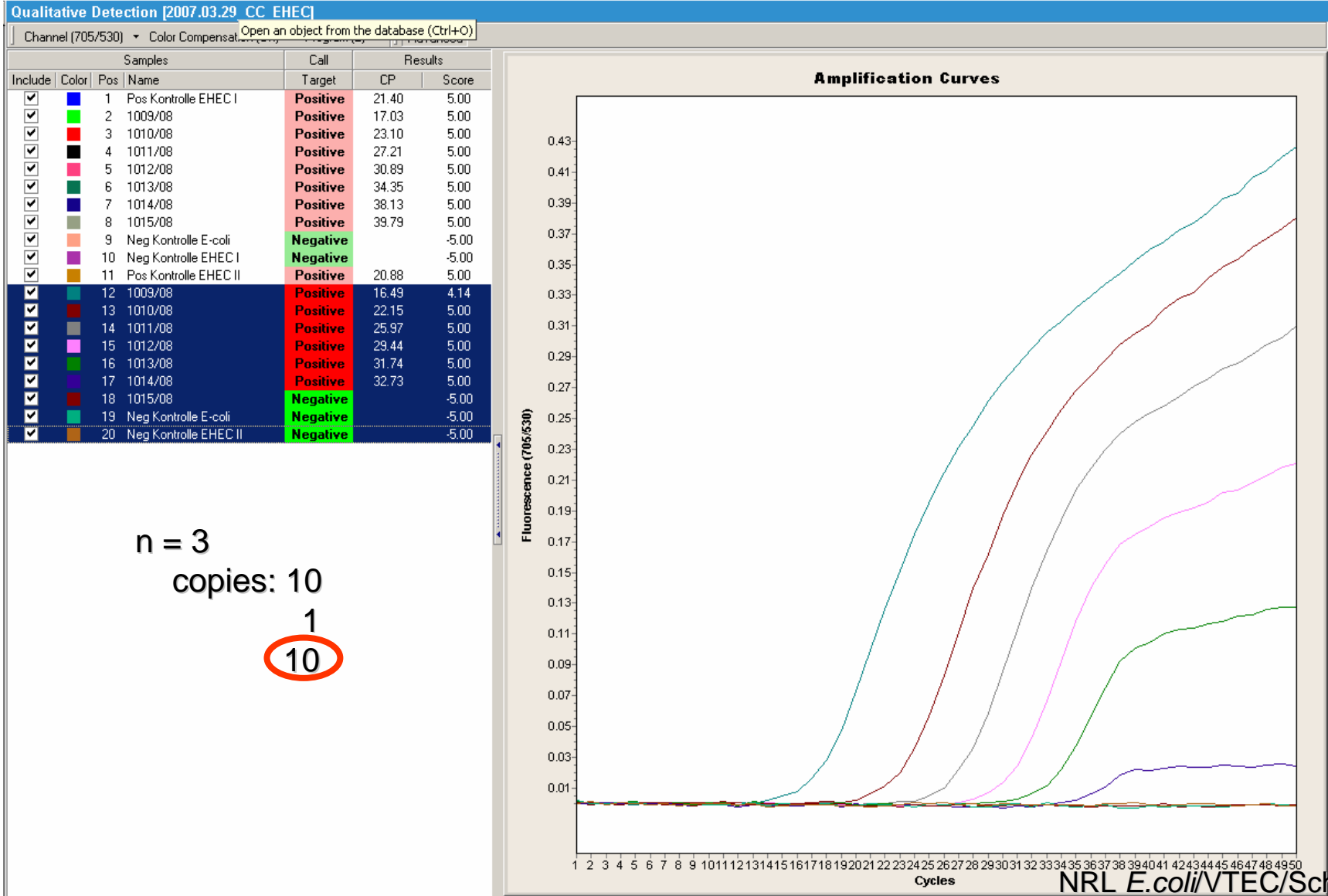
Samples				Call	Results	
Include	Color	Pos	Name	Target	CP	Score
<input checked="" type="checkbox"/>	Blue	1	Pos Kontrolle EHEC I	Positive	22.92	5.00
<input checked="" type="checkbox"/>	Green	2	1009/08	Positive	18.37	4.93
<input checked="" type="checkbox"/>	Red	3	1010/08	Positive	24.24	5.00
<input checked="" type="checkbox"/>	Black	4	1011/08	Positive	28.45	5.00
<input checked="" type="checkbox"/>	Pink	5	1012/08	Positive	32.13	5.00
<input checked="" type="checkbox"/>	Teal	6	1013/08	Positive	36.63	5.00
<input checked="" type="checkbox"/>	Dark Blue	7	1014/08	Positive	41.31	5.00
<input checked="" type="checkbox"/>	Grey	8	1015/08	Negative		-1.73
<input checked="" type="checkbox"/>	Orange	9	Neg Kontrolle E-coli	Negative		-5.00
<input checked="" type="checkbox"/>	Purple	10	Neg Kontrolle EHEC I	Negative		-3.34
<input checked="" type="checkbox"/>	Brown	11	Pos Kontrolle EHEC II	Positive	20.82	5.00
<input checked="" type="checkbox"/>	Light Blue	12	1009/08	Positive	16.57	3.80
<input checked="" type="checkbox"/>	Dark Red	13	1010/08	Positive	22.16	5.00
<input checked="" type="checkbox"/>	Light Grey	14	1011/08	Positive	26.00	5.00
<input checked="" type="checkbox"/>	Pink	15	1012/08	Positive	29.53	5.00
<input checked="" type="checkbox"/>	Green	16	1013/08	Positive	31.92	5.00
<input checked="" type="checkbox"/>	Purple	17	1014/08	Positive	33.16	5.00
<input checked="" type="checkbox"/>	Dark Red	18	1015/08	Negative		-5.00
<input checked="" type="checkbox"/>	Teal	19	Neg Kontrolle E-coli	Negative		-5.00
<input checked="" type="checkbox"/>	Brown	20	Neg Kontrolle EHEC II	Negative		-5.00

Amplification Curves

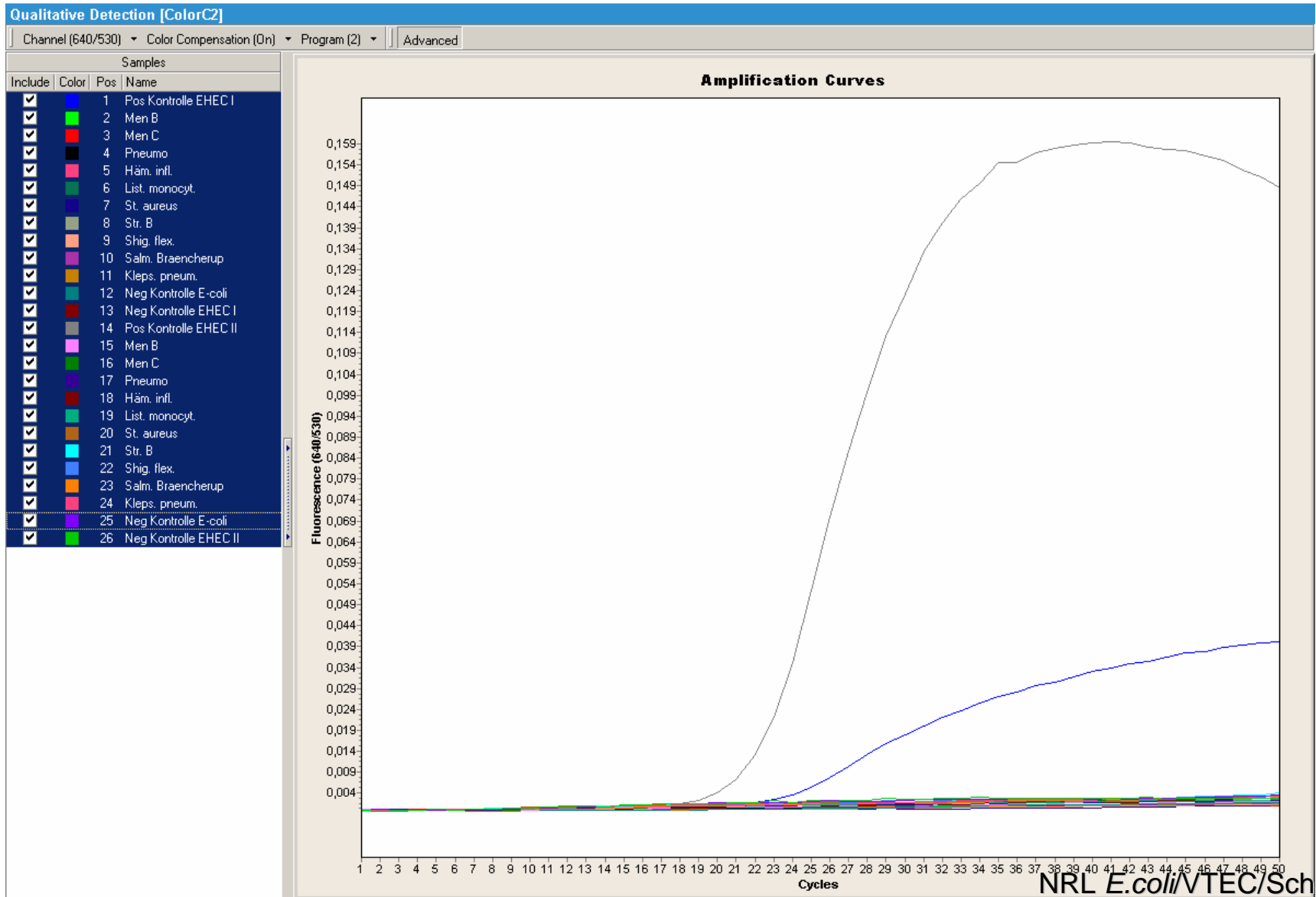


n = 3
copies: 0,1
1
10

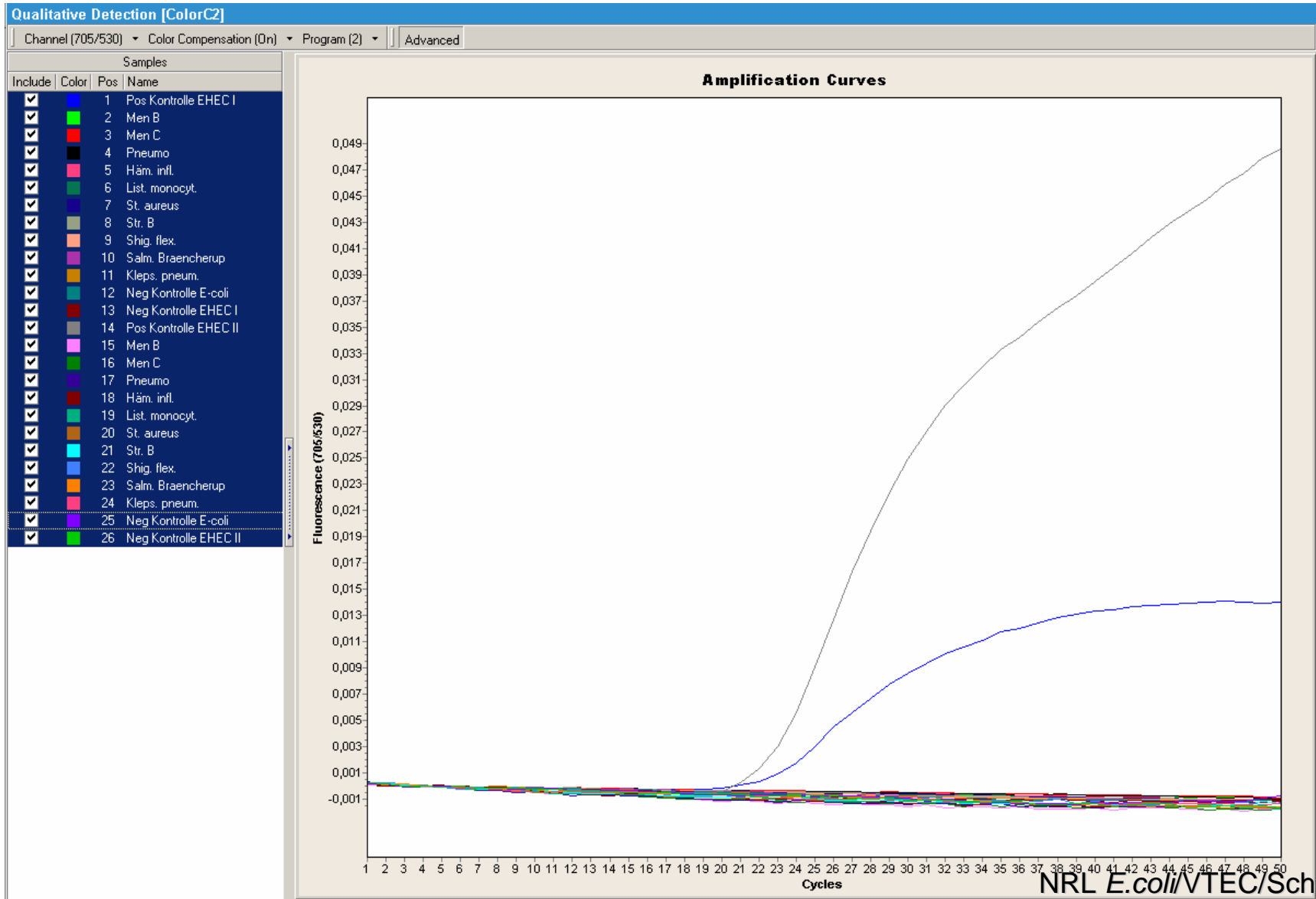
Duplex-PCR: E-*hly*-Sensitivity



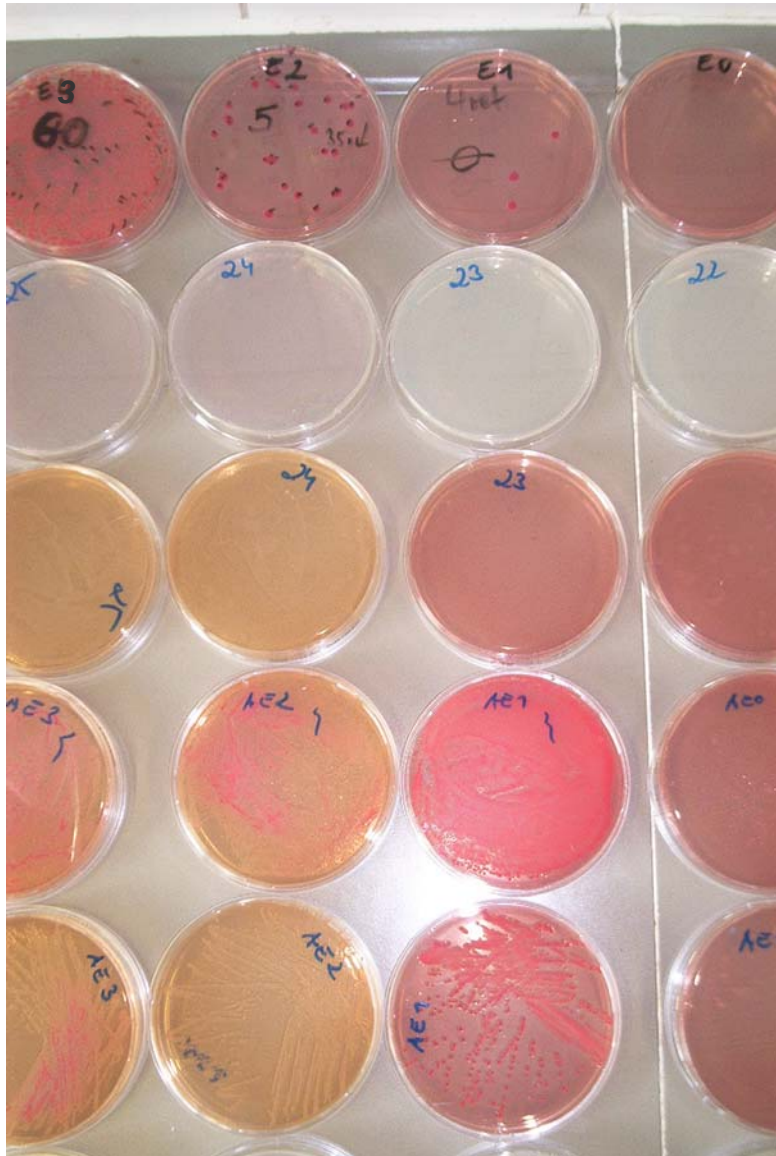
Duplex-PCR: Specificity *stx1* + *eae*



Duplex-PCR: Specificity *stx2* + *E-hly*



Validation: 10% *E. coli* O157 in 90% nonpathog. *E. coli* (ÖNORM EN ISO 16654)



Spike: 10% *E. coli* O157 in 90% nonpathog. *E. coli*

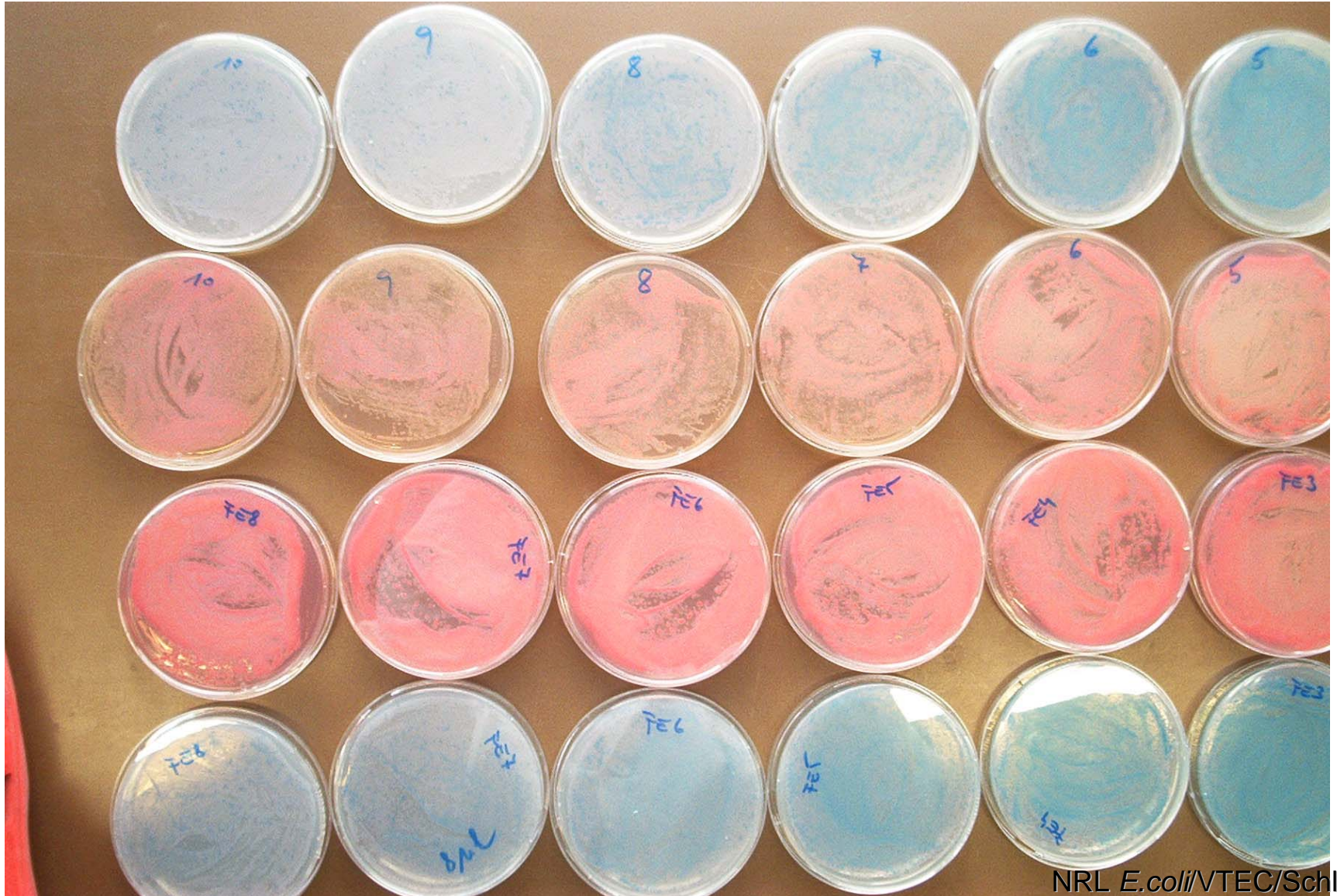
8 µl after IMS with O157 Dynabeads

8 µl after IMS with O157 Dynabeads

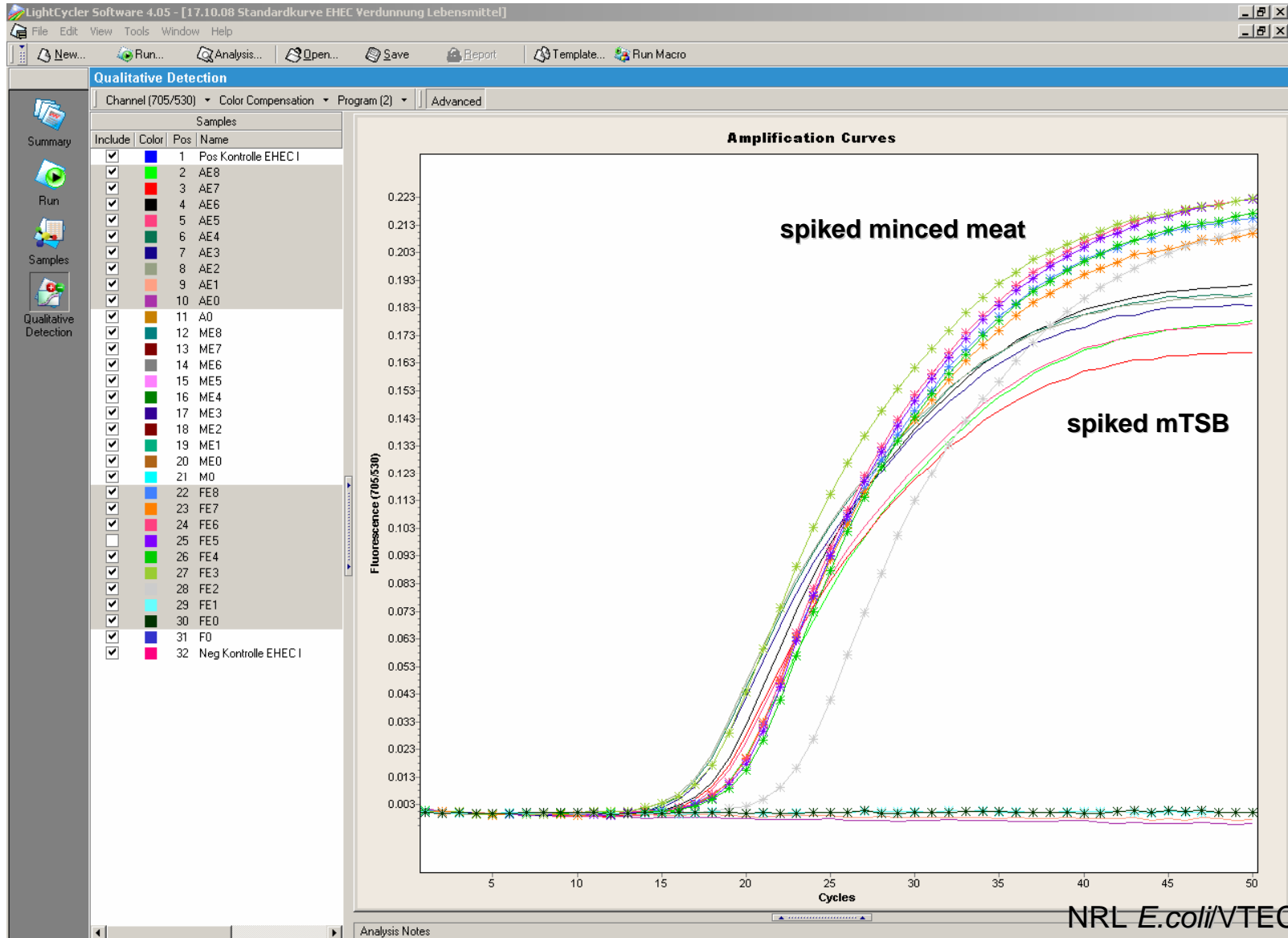
8 µl from 250 ml mTSB o.n. 42°C, spatula

8 µl from 250 ml mTSB o.n. 42°C, loop

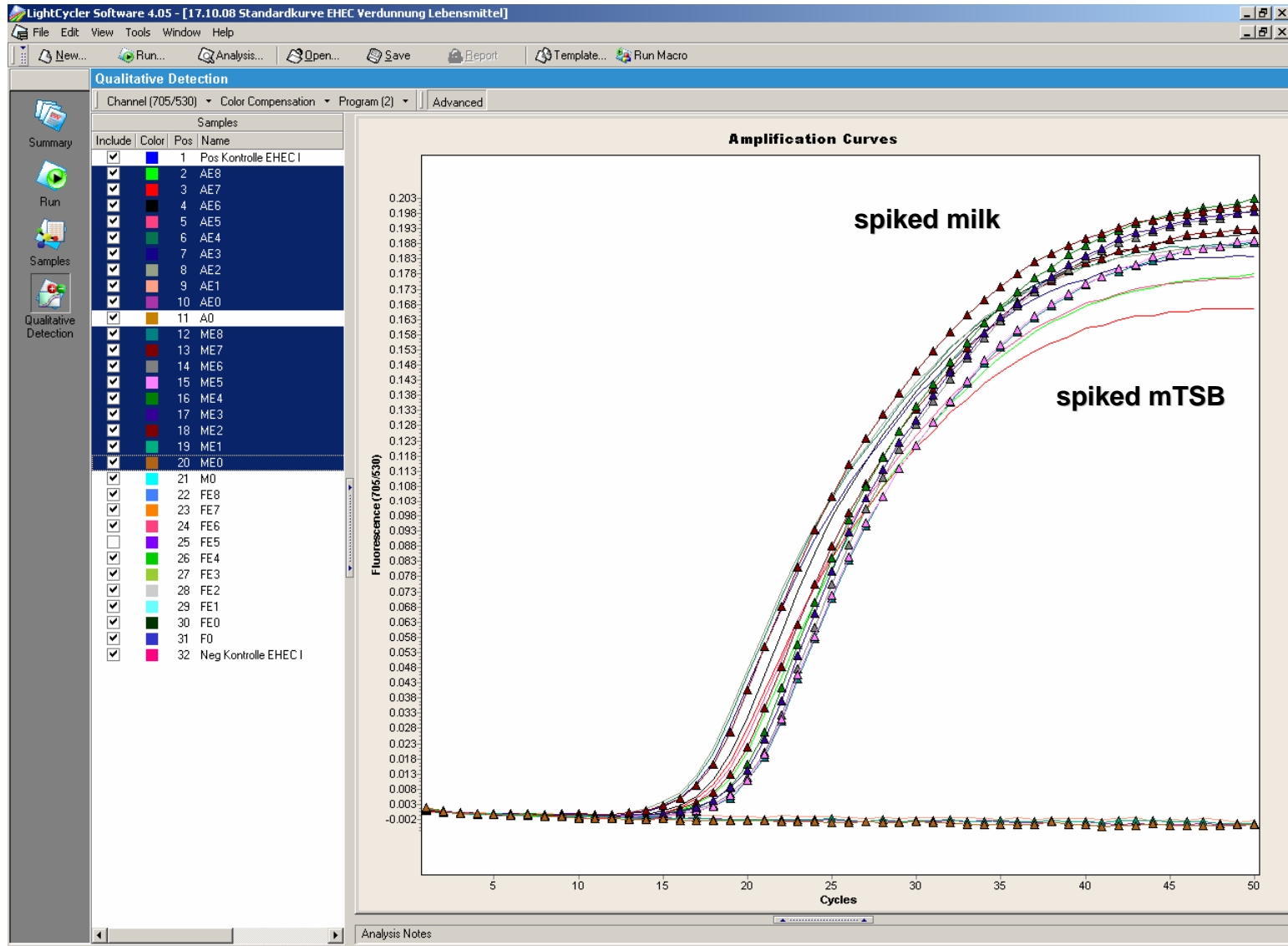
Validation: 10% O157 and 90% nonpathog. *E. coli* in minced meat (ÖNORM EN ISO 16654)



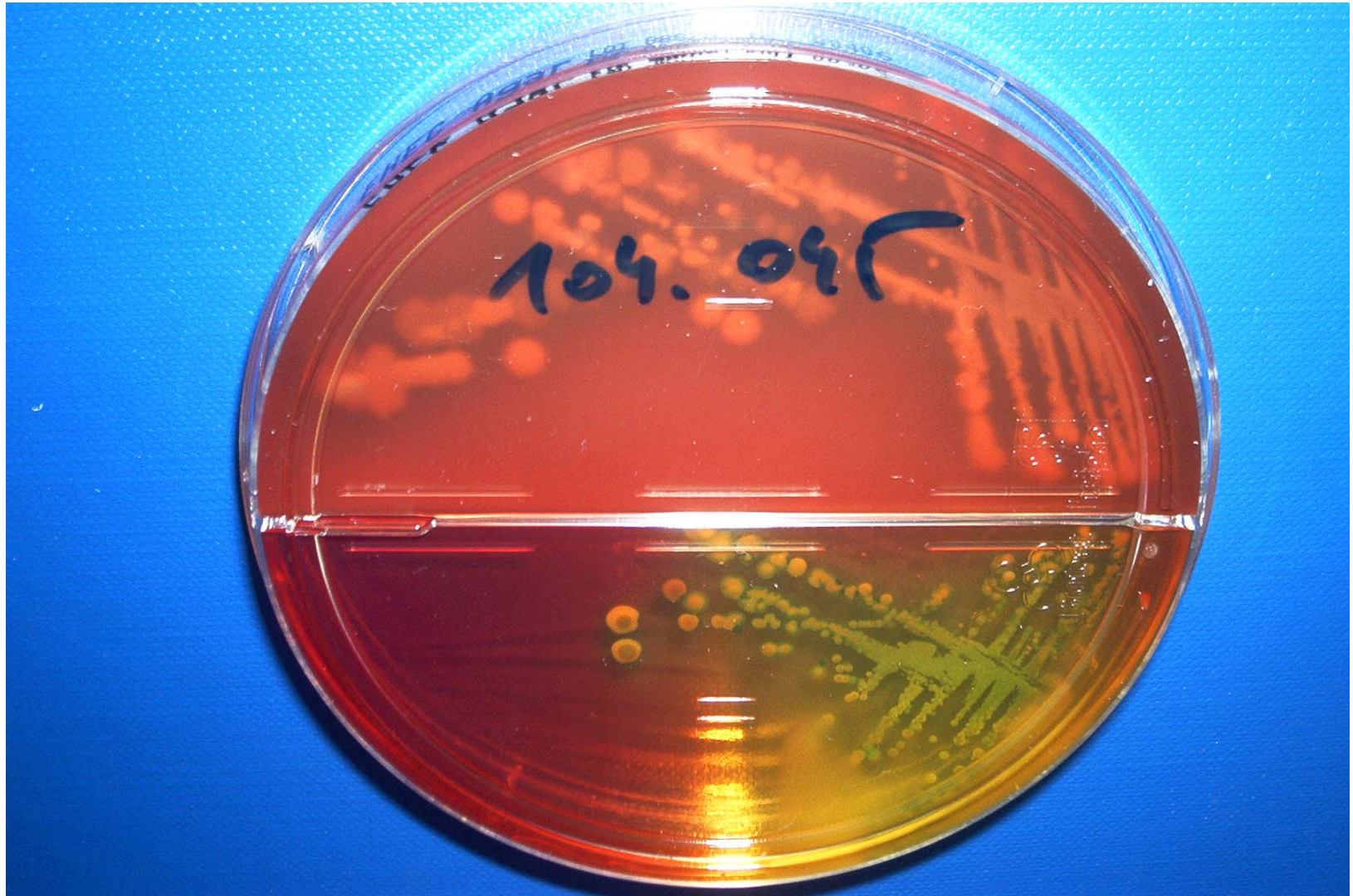
Validation: spiked mTSB vs. spiked minced meat

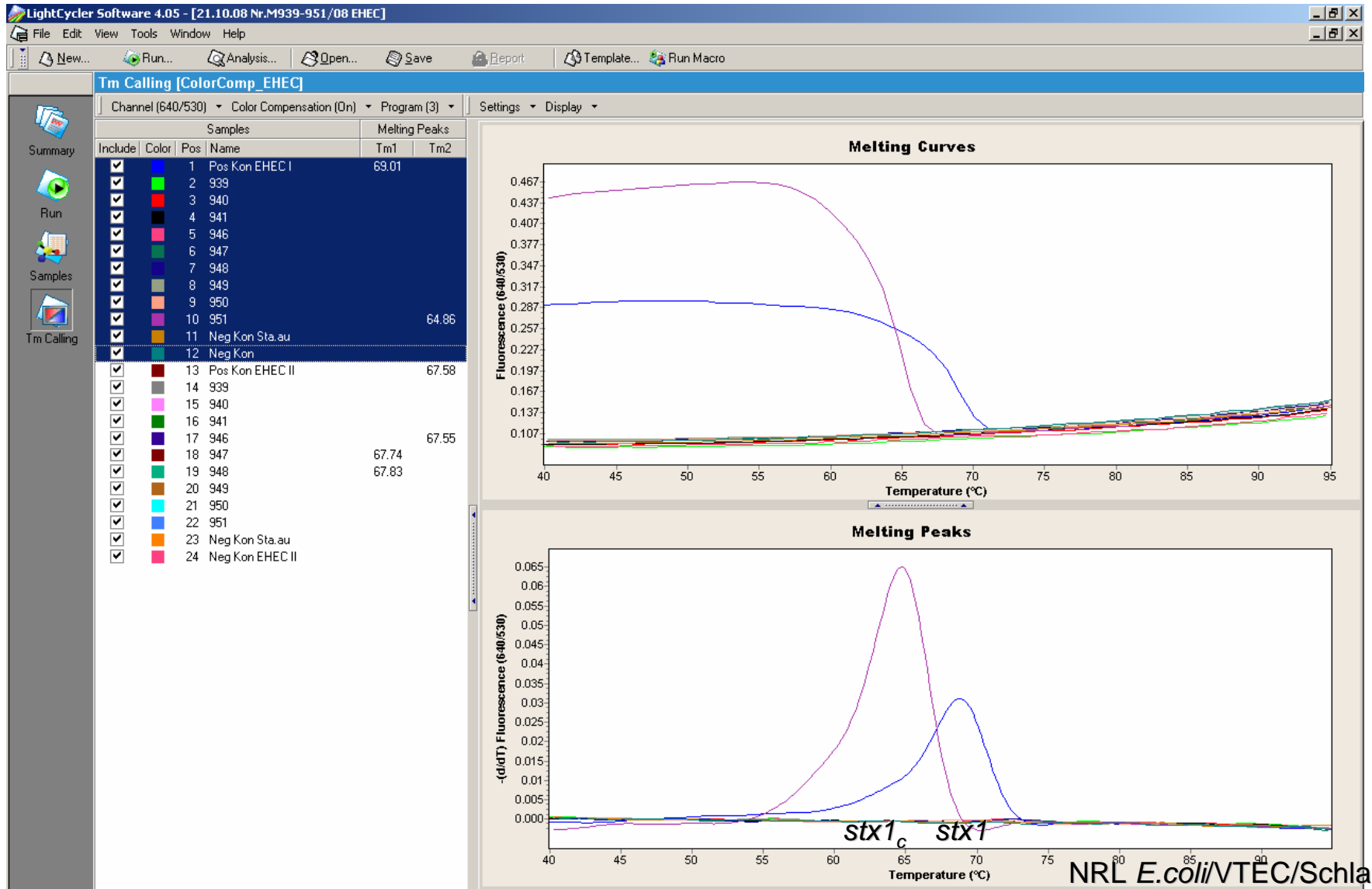


Validation: spiked mTSB vs. spiked raw milk



Enterohemolysin-Agar







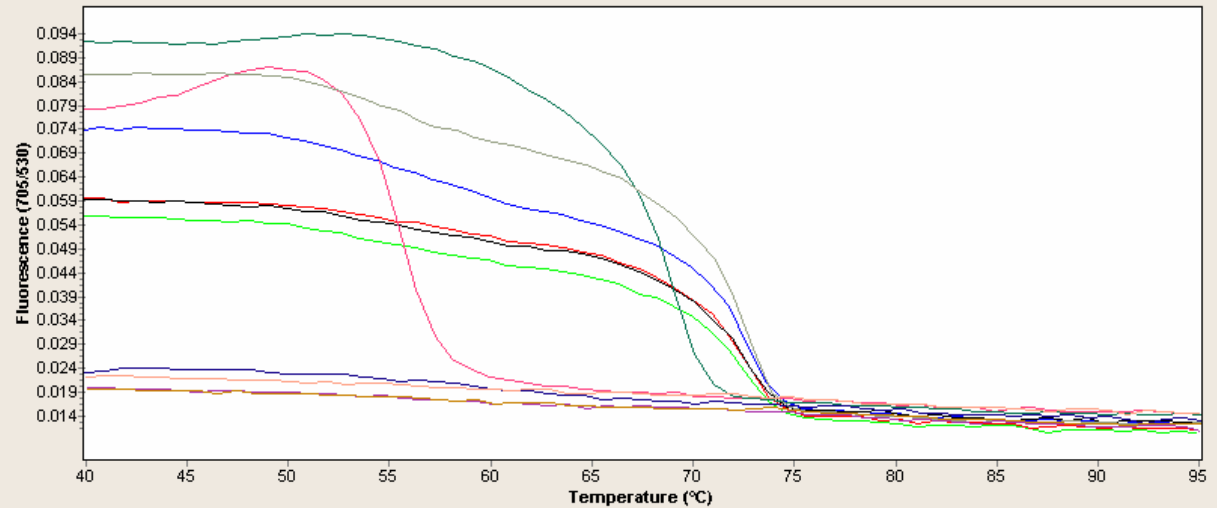
Duplex-PCR: *stx2* Tm-Analysis

Tm Calling [CC23.06.08]

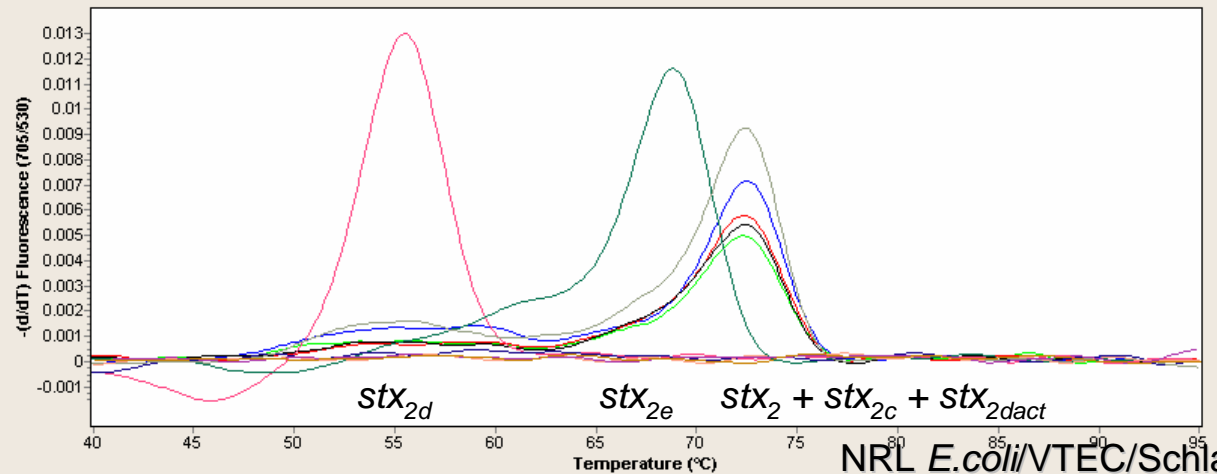
Channel (705/530) Color Compensation (On) Program (3) Settings Display

Samples			Melting Peaks	
Include	Color	Pos Name	Tm1	Tm2
<input checked="" type="checkbox"/>	Blue	1 Pos Kon EHEC I	72.60	
<input checked="" type="checkbox"/>	Green	2 M 946	72.28	
<input checked="" type="checkbox"/>	Red	3 M 947		72.63
<input checked="" type="checkbox"/>	Black	4 M 948	72.20	
<input checked="" type="checkbox"/>	Pink	5 M 949	55.31	
<input checked="" type="checkbox"/>	Dark Green	6 M 950	68.80	
<input checked="" type="checkbox"/>	Dark Blue	7 M 951		
<input checked="" type="checkbox"/>	Grey	8 M 989	72.68	
<input checked="" type="checkbox"/>	Orange	9 M 990		
<input checked="" type="checkbox"/>	Purple	10 Neg Kon Sta.au		
<input checked="" type="checkbox"/>	Brown	11 Neg Kon		
<input checked="" type="checkbox"/>	Teal	12 Pos Kon EHEC II	67.67	
<input checked="" type="checkbox"/>	Dark Red	13 M 946	67.62	
<input checked="" type="checkbox"/>	Grey	14 M 947	67.40	
<input checked="" type="checkbox"/>	Pink	15 M 948	67.61	
<input checked="" type="checkbox"/>	Green	16 M 949		
<input checked="" type="checkbox"/>	Dark Blue	17 M 950		
<input checked="" type="checkbox"/>	Dark Red	18 M 951		
<input checked="" type="checkbox"/>	Teal	19 M 989		
<input checked="" type="checkbox"/>	Orange	20 M 990		
<input checked="" type="checkbox"/>	Cyan	21 Neg Kon Sta.au		
<input checked="" type="checkbox"/>	Blue	22 Neg Kon		

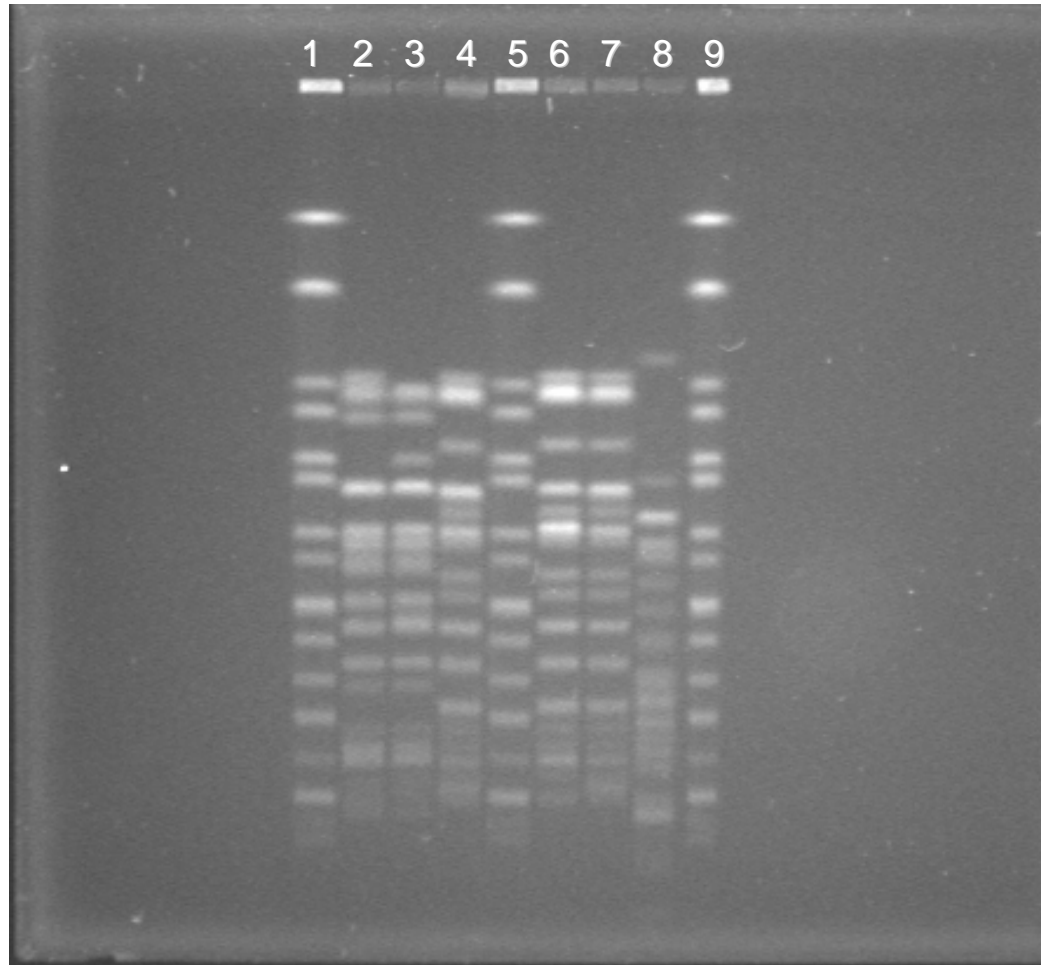
Melting Curves

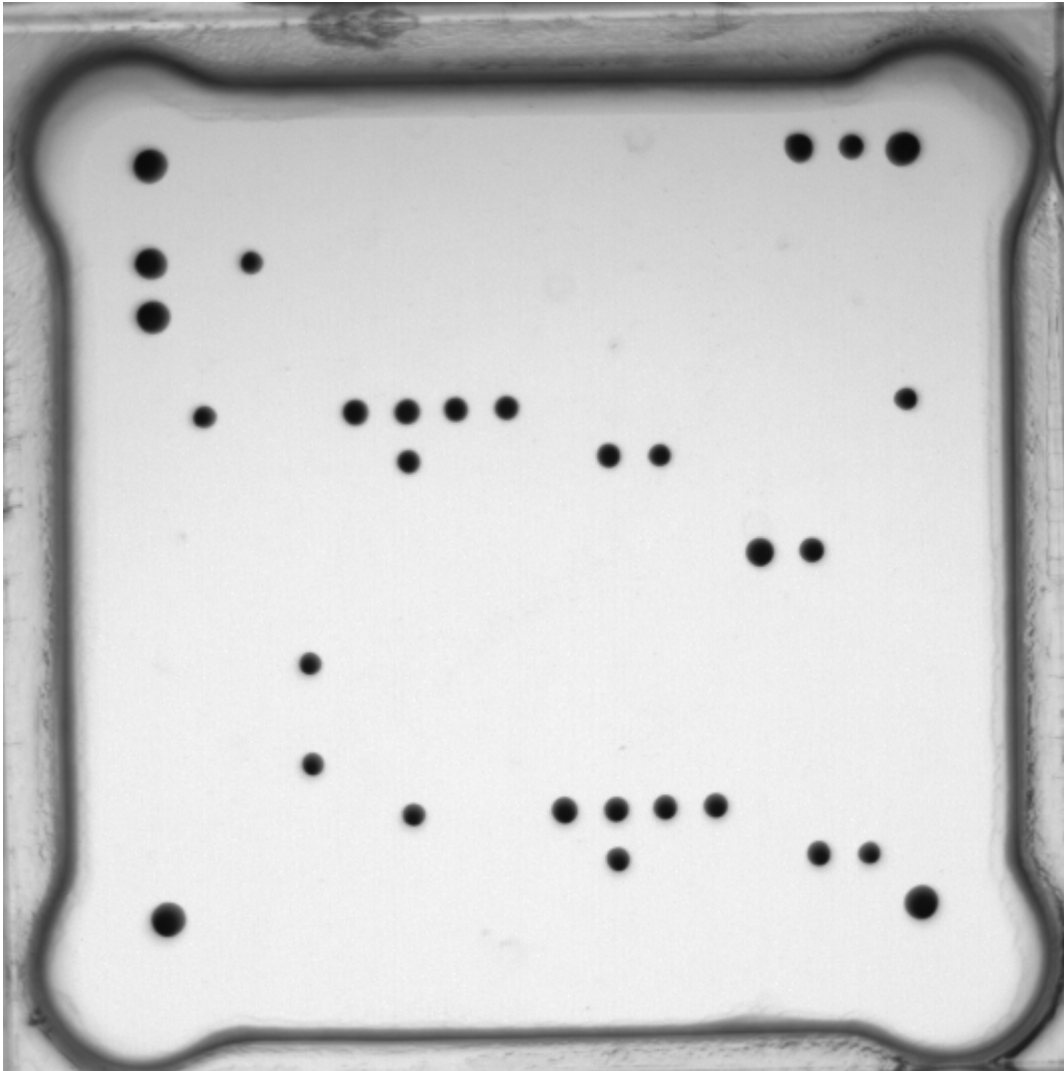


Melting Peaks



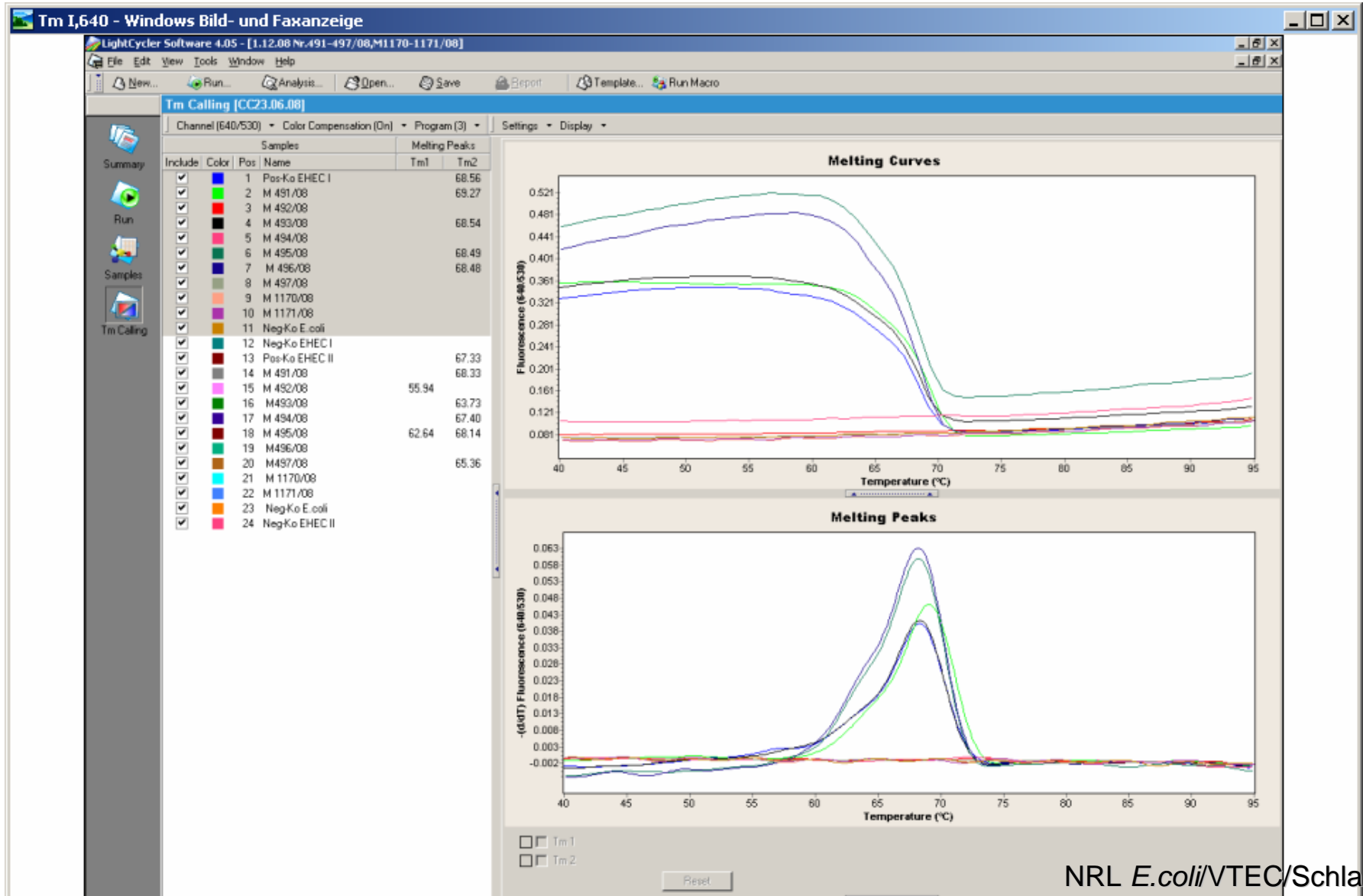
Escherichia coli - PFGE



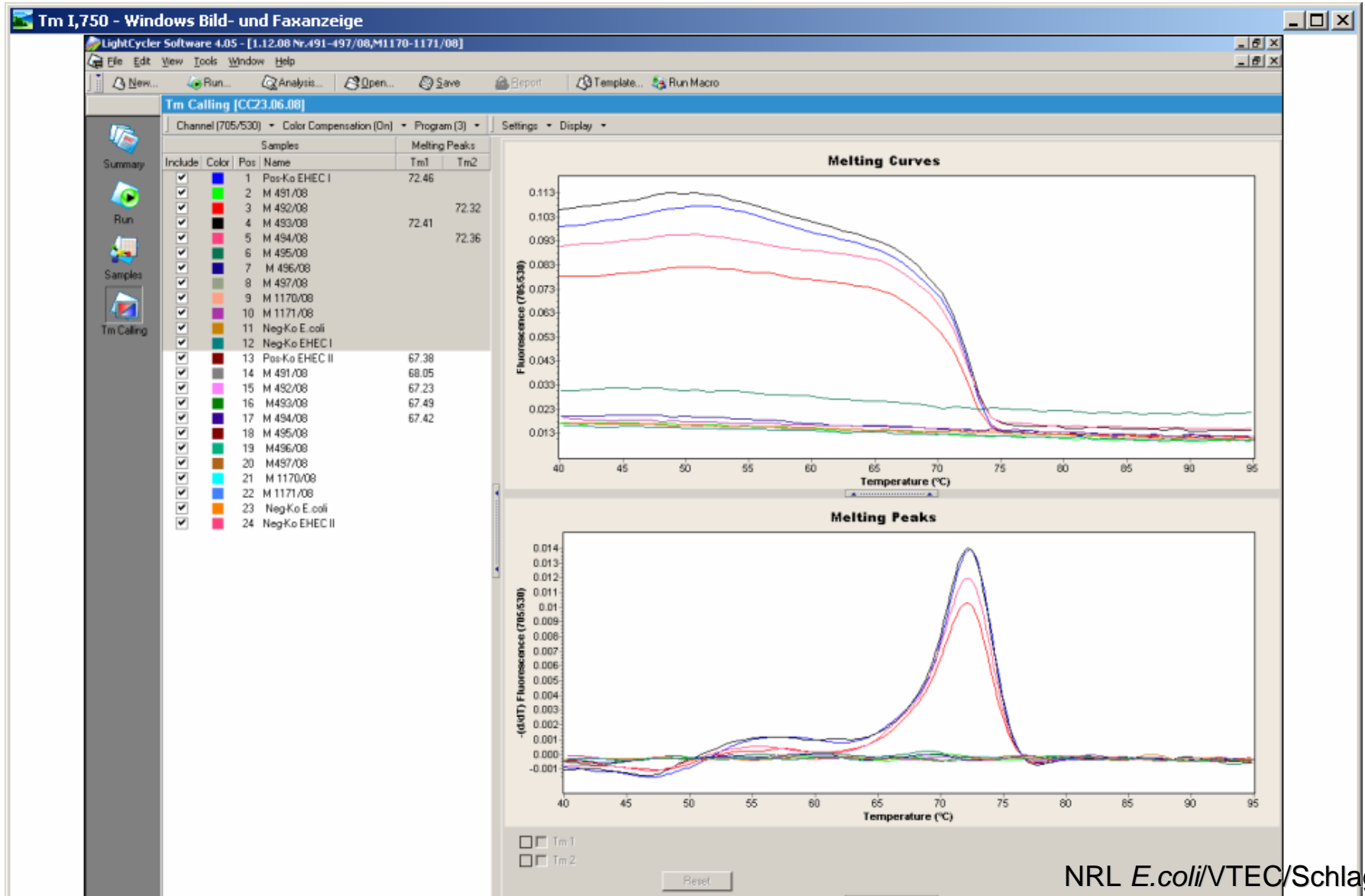


135	celb_10	0,994975	0,990469
10	celb_10	0,992824	0,990194
5	cba_10	0,992057	0,98908
137	cma_20	0,991677	0,992255
125	prob_ihfA_611	0,991503	0,994274
138	cnf1_20	0,989339	0,982057
126	prob_gapA_611	0,986135	0,978224
82	hp_iha_611	0,985649	0,979832
211	hp_lpfA_611	0,985623	0,973836
162	iss_10	0,984894	0,977603
12	cma_20	0,983843	0,977822
132	cdtB_40	0,981928	0,983477
13	cnf1_20	0,979953	0,968043
155	gad_10	0,978965	0,972083
84	hp_lpfA_611	0,973715	0,96577
38	iss_10	0,973466	0,975374
7	cdtB_40	0,97192	0,966627
31	gad_10	0,958199	0,935029
222	hp_saa_611	0,951166	0,93983
95	hp_saa_611	0,944778	0,934323
133	cdtB_50	0,928444	0,930863
8	cdtB_50	0,903975	0,898321
188	hp_espA_Crod_611	0,154942	0,187948
63	hp_espA_Crod_611	0,149681	0,124301

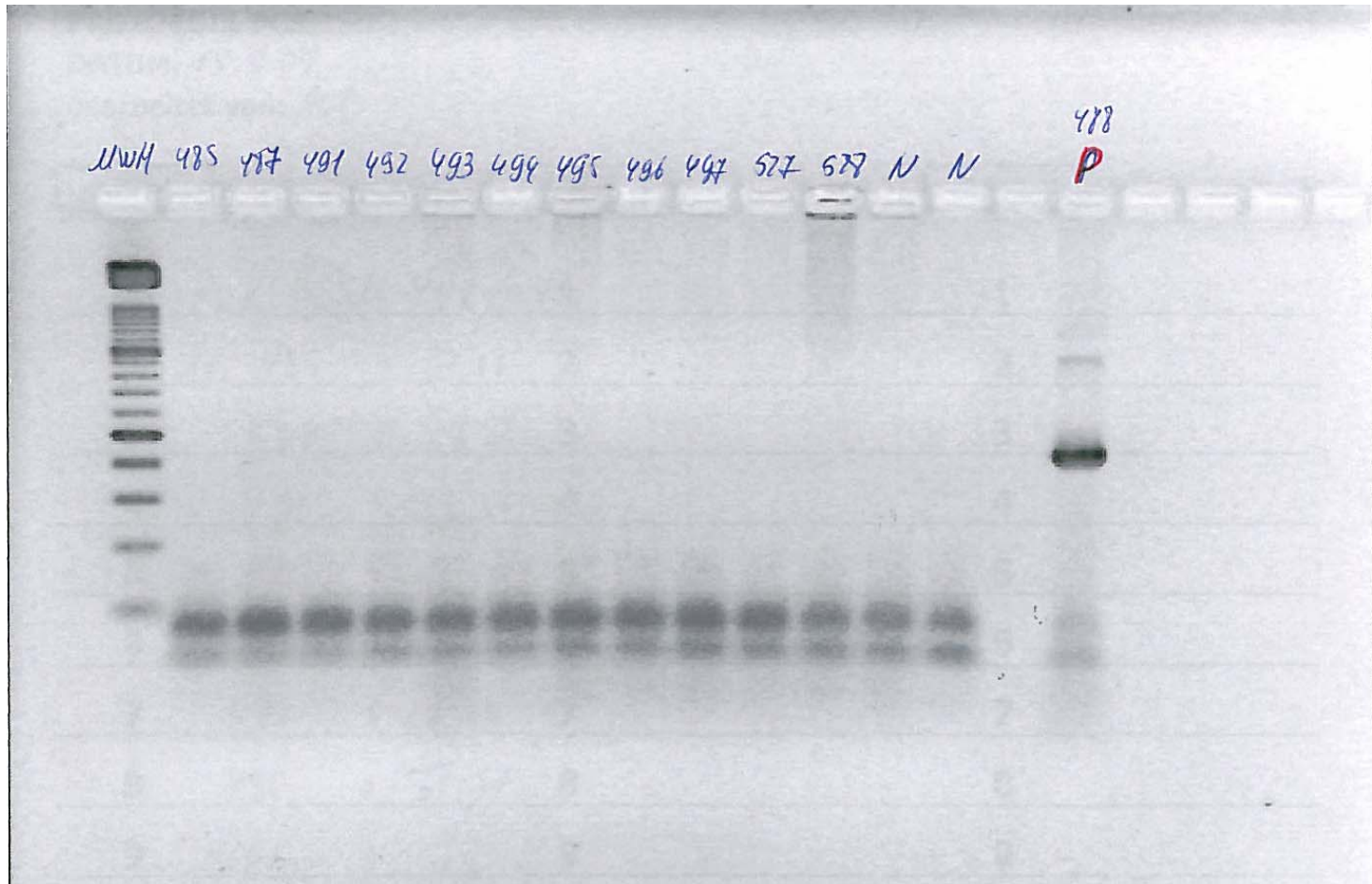
crl – Inter-laboratory study on VTEC identification and typing 2008 – stx1



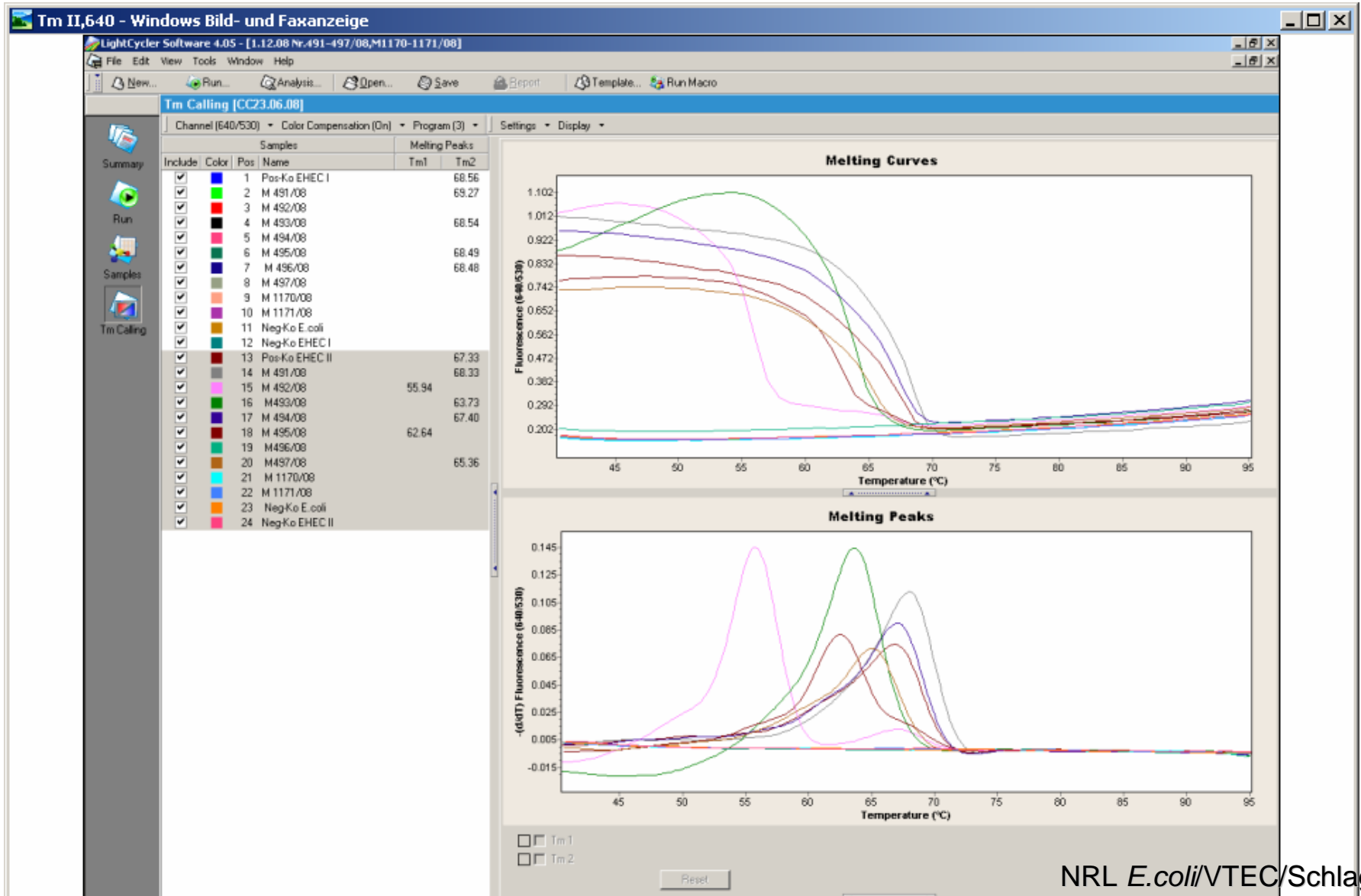
crl – Inter-laboratory study on VTEC identification and typing 2008 – stx2



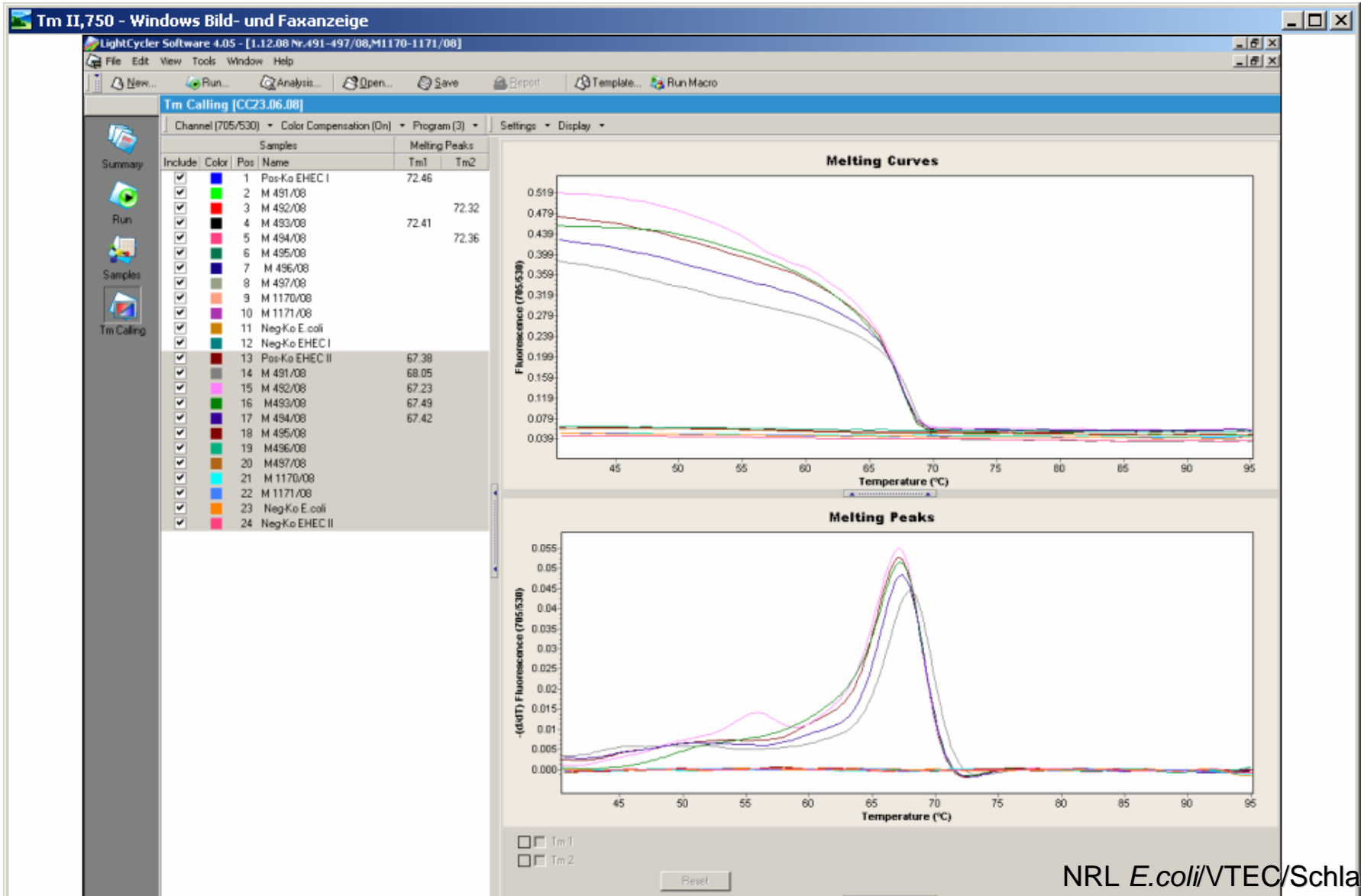
crl – Inter-laboratory study on VTEC identification and typing 2008 – stx2f



crl – Inter-laboratory study on VTEC identification and typing 2008 – Intimin



crl – Inter-laboratory study on VTEC identification and typing 2008 – E-hly



**Austrian National Reference Laboratory for
Escherichia coli including Verotoxin-
producing *E. coli***

**Sabine Schlager
Sabine Neubauer
Daniela Arnhold**

**Brigitte Reichl
Oksana Ableitner
Parwin Taghawi-
Donjai**

Stool directly vs. Enrichment broth

