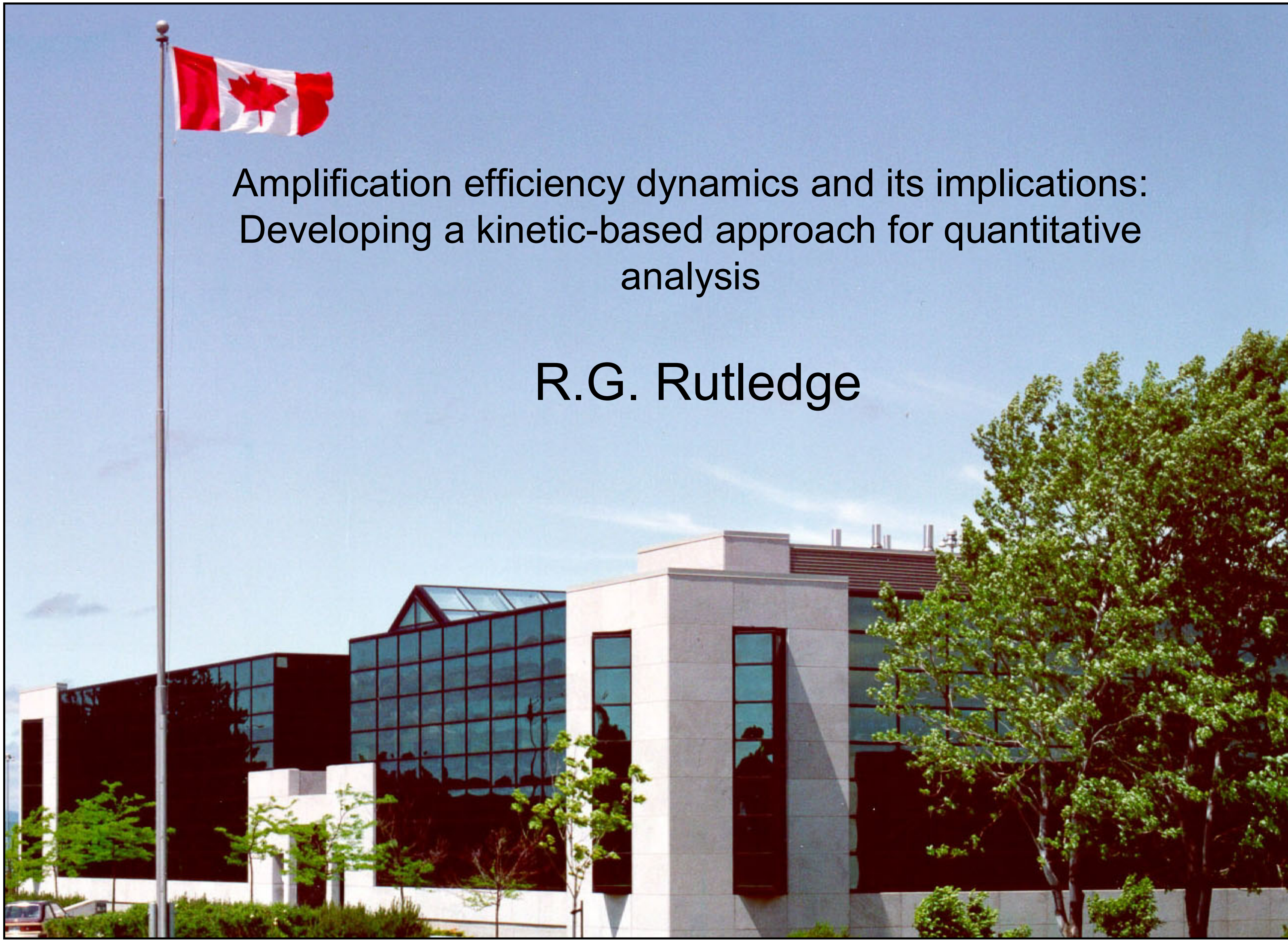


Amplification efficiency dynamics and its implications: Developing a kinetic-based approach for quantitative analysis

R.G. Rutledge

qPCR 2005,
Freising-Weihenstephan, Germany

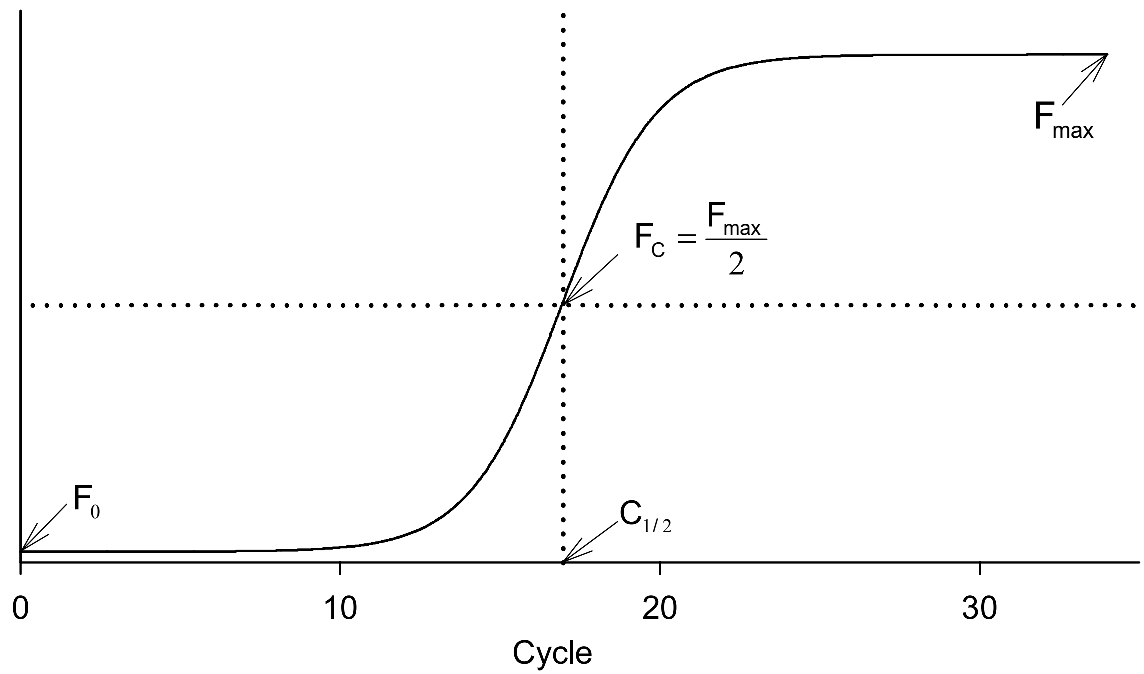
Natural Resources Canada
Ressources naturelles Canada

Canada

Sigmoidal Modeling of PCR Amplification

Classic Boltzmann Sigmoid Function (ca. 1875)

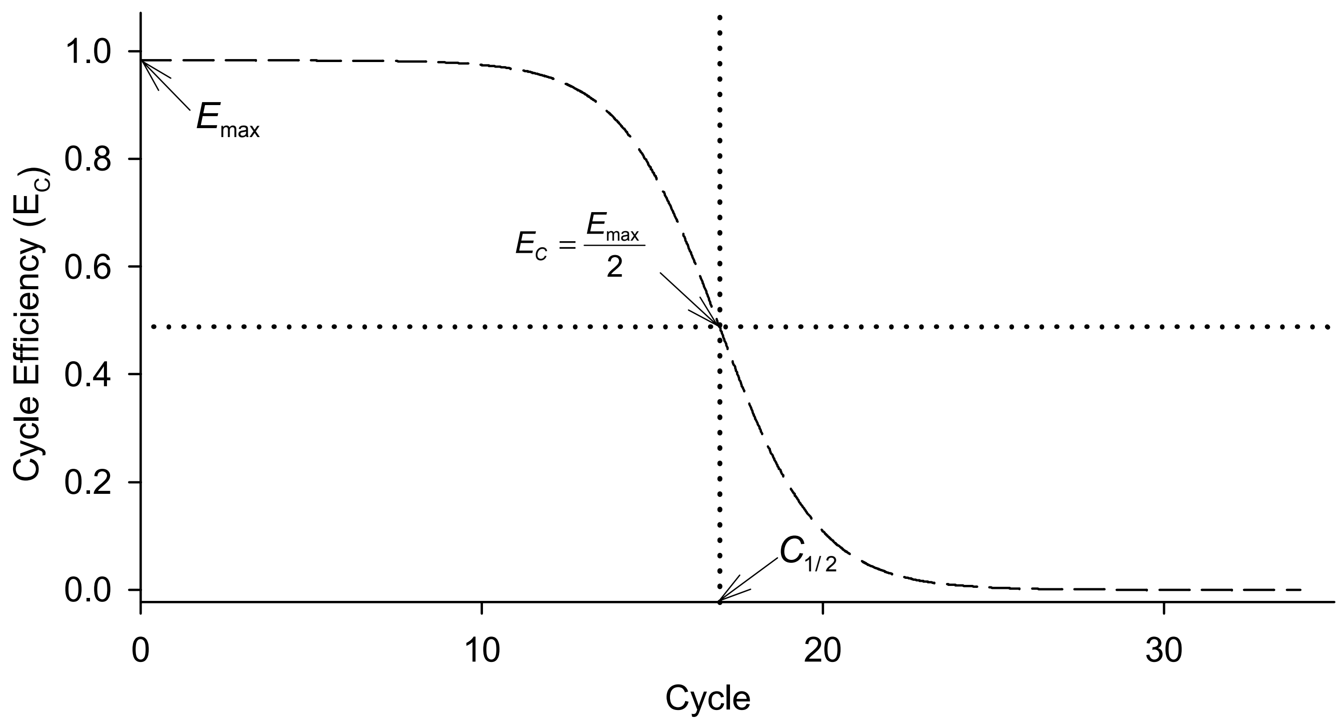
$$F_C = \frac{F_{max}}{1+e^{-\left(\frac{C-C_{1/2}}{k}\right)}} - F_b$$



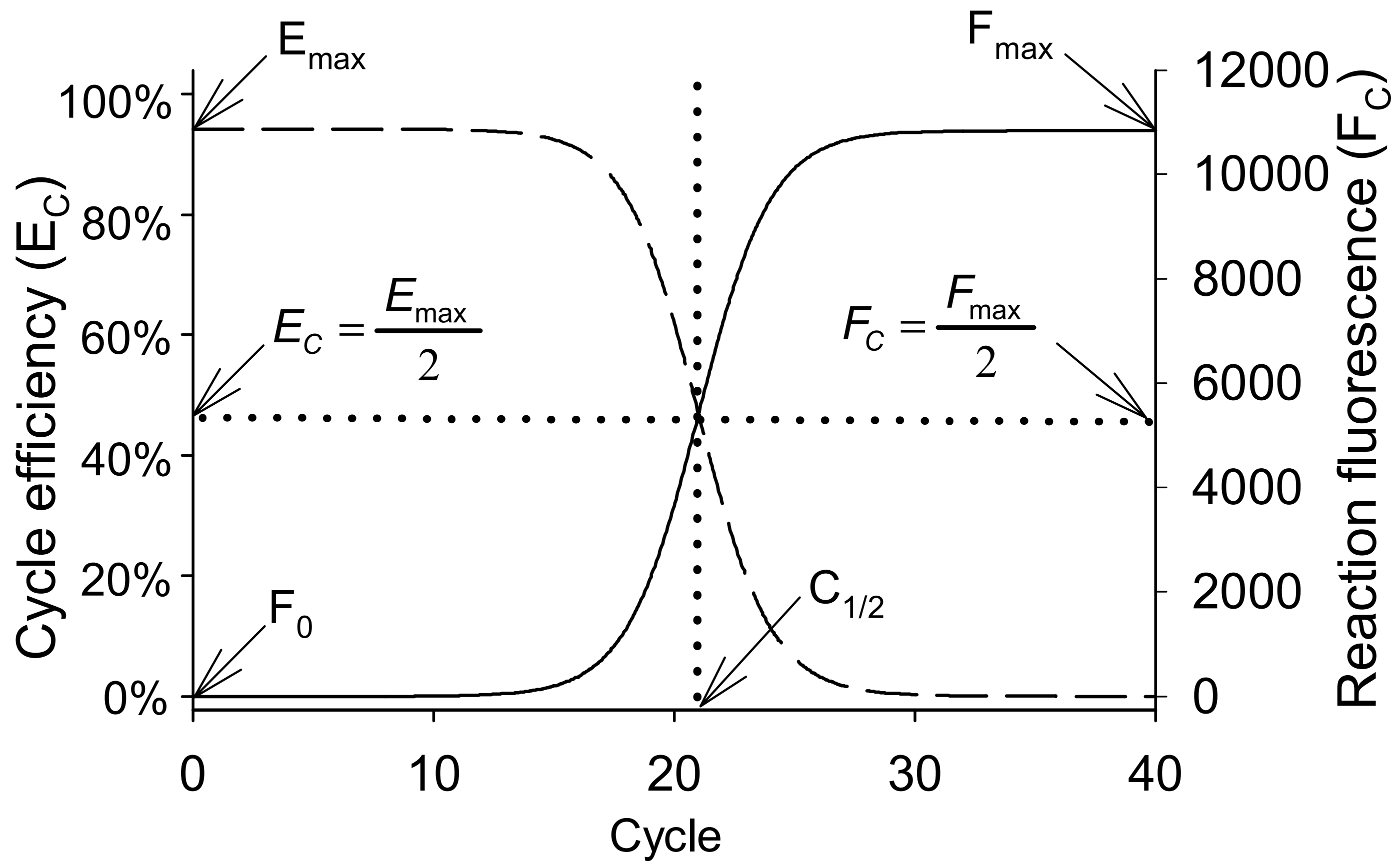
Each Cycle has a Unique Amplification Efficiency (E_C)

Liu and Saint *Biochem. Biophys. Res. Commun.* **294**, 347-53 (2002)

$$E_C = \frac{1 + e^{-\left(\frac{C-1-C_{1/2}}{k}\right)}}{1 + e^{-\left(\frac{C-C_{1/2}}{k}\right)}} - 1$$

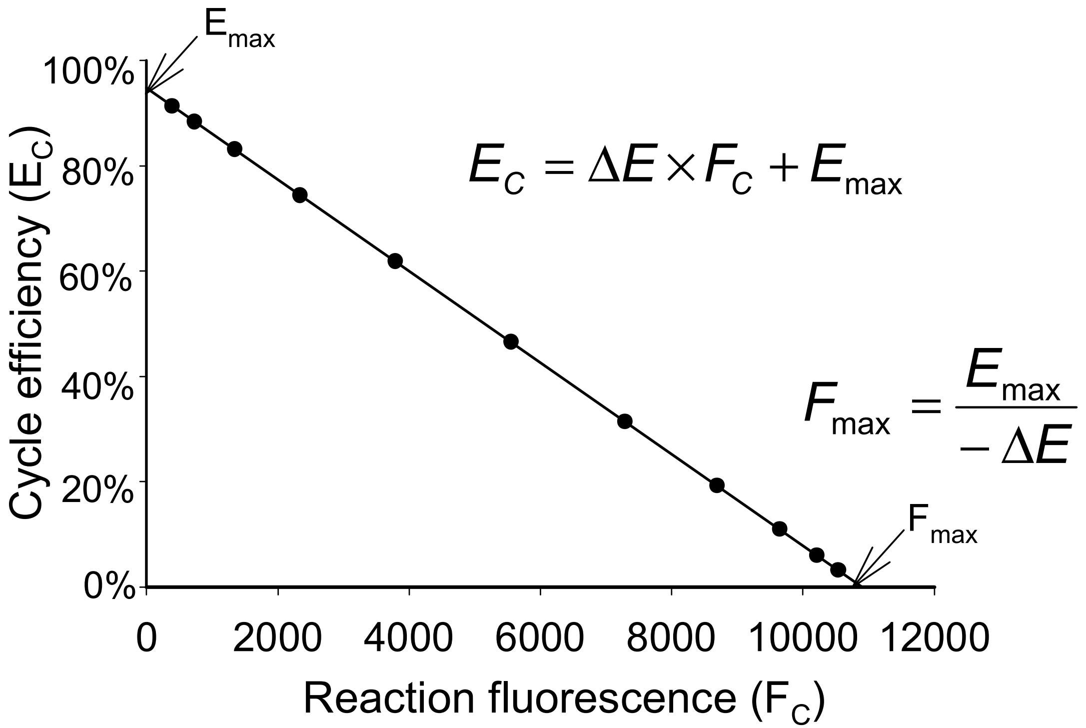


Mirror Symmetry Between Amplicon Accumulation and Amplification Rate



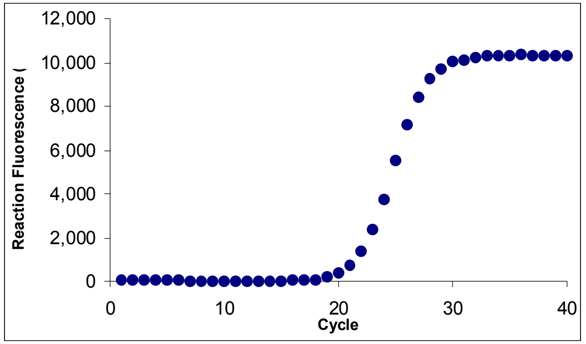
Linearity between Amplification Rate and Amplicon Accumulation

Kinetic analysis via linear regression analysis: "Linear Regression of Efficiency" or LRE Analysis



Application to Experimental Data

Cycle	Fc
1	79
2	70
3	59
4	48
5	42
6	38
7	22
8	26
9	-1
10	0
11	0
12	2
13	-1
14	2
15	4
16	44
17	43
18	73
19	192
20	385
21	741
22	1,351
23	2,336
24	3,770
25	5,501
26	7,151
27	8,424
28	9,234
29	9,726
30	10,013
31	10,095
32	10,223
33	10,314
34	10,275
35	10,304
36	10,338
37	10,276
38	10,288
39	10,322
40	10,279



Five optical reads per cycle

F_C readings from four replicated amplifications were averaged

NO curve smoothing (running average)

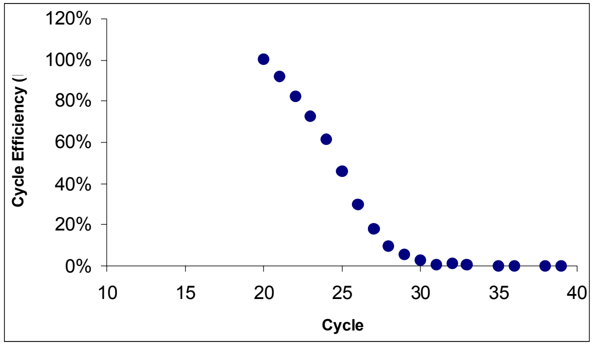
Classic definition of amplification efficiency

$$E_C = \frac{F_C}{F_{C-1}} - 1$$

Ratio-derived Estimation of Cycle Efficiency

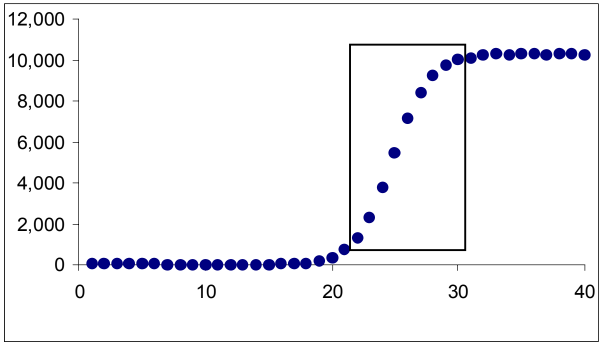
Cycle	Fc	Ec
1	79	
2	70	
3	59	
4	48	
5	42	
6	38	
7	22	
8	26	
9	-1	
10	0	
11	0	
12	2	
13	-1	
14	2	
15	4	58.62%
16	44	1091.30%
17	43	-1.46%
18	73	68.89%
19	192	163.05%
20	385	100.75%
21	741	92.29%
22	1,351	82.33%
23	2,336	72.90%
24	3,770	61.43%
25	5,501	45.91%
26	7,151	29.99%
27	8,424	17.80%
28	9,234	9.62%
29	9,726	5.32%
30	10,013	2.96%
31	10,095	0.82%
32	10,223	1.27%
33	10,314	0.88%
34	10,275	-0.38%
35	10,304	0.29%
36	10,338	0.33%
37	10,276	-0.60%
38	10,288	0.12%
39	10,322	0.33%
40	10,279	-0.41%

$$E_C = \frac{F_C}{F_{C-1}} - 1$$



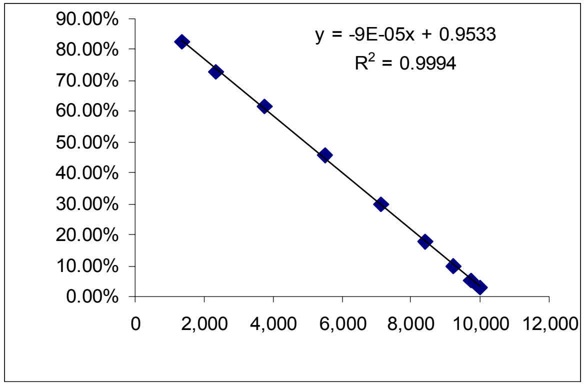
Kinetic Analysis via Linear Regression (LRE Analysis)

Cycle	Fc	Ec
1	79	
2	70	
3	59	
4	48	
5	42	
6	38	
7	22	
8	26	
9	-1	
10	0	
11	0	
12	2	
13	-1	
14	2	
15	4	58.62%
16	44	1091.30%
17	43	-1.46%
18	73	68.89%
19	192	163.05%
20	385	100.75%
21	741	92.29%
22	1,351	82.33%
23	2,336	72.90%
24	3,770	61.43%
25	5,501	45.91%
26	7,151	29.99%
27	8,424	17.80%
28	9,234	9.62%
29	9,726	5.32%
30	10,013	2.96%
31	10,095	0.82%
32	10,223	1.27%
33	10,314	0.88%
34	10,275	-0.38%
35	10,304	0.29%
36	10,338	0.33%
37	10,276	-0.60%
38	10,288	0.12%
39	10,322	0.33%
40	10,279	-0.41%

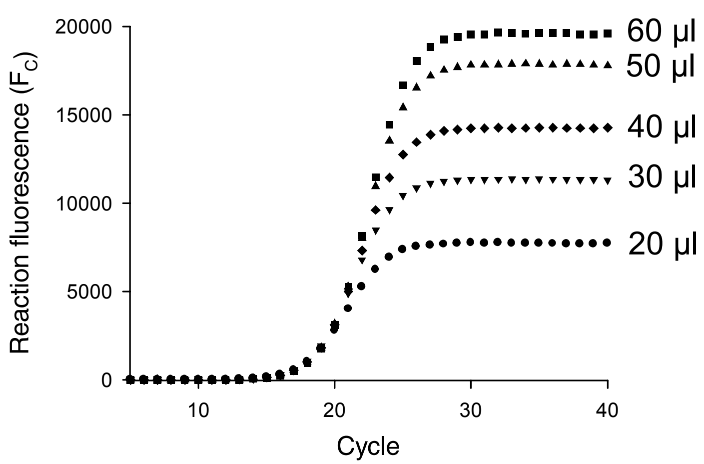


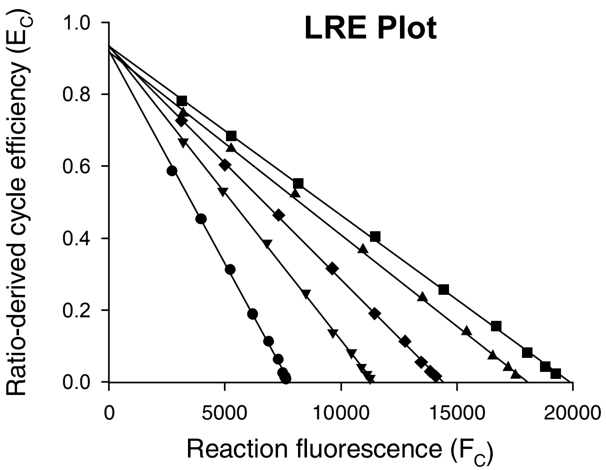
r2:	0.9994
ΔE:	**-9.21E-05**
Emax:	**95.33%**
Fmax:	10,349

$$F_{max} = \frac{E_{max}}{-\Delta E}$$



Reaction Volume is a Determinant of F_{max}

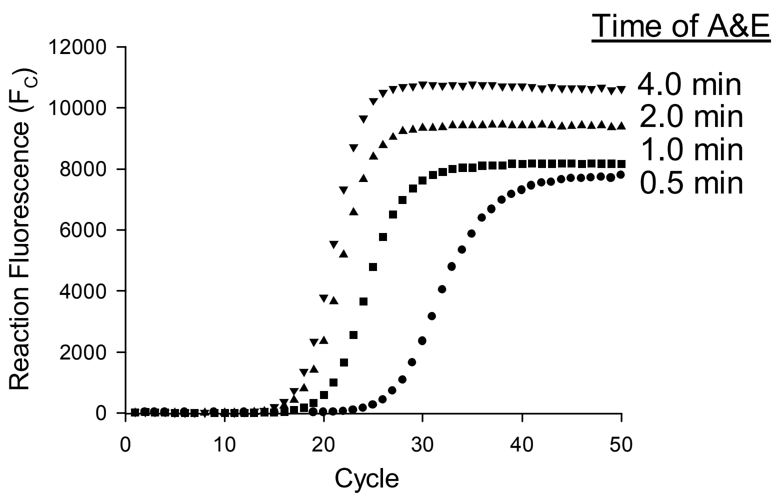


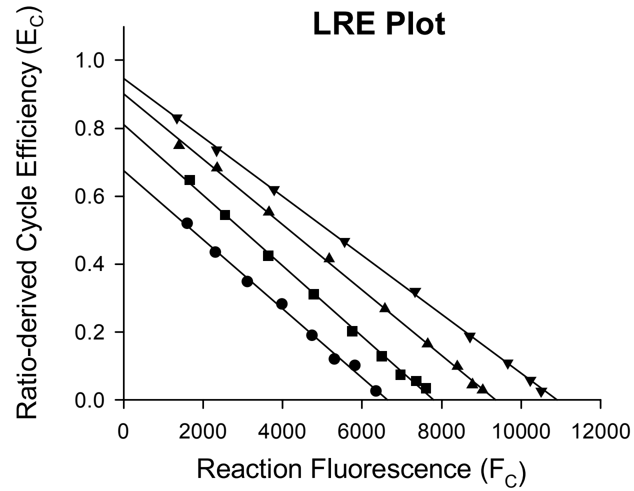


Vol. (µl)	r^2	E_{max}	ΔE	F_{max}
20	0.9984	92.2%	$-1.18x10^{-4}$	7,810
30	0.9995	93.5%	$-8.19x10^{-5}$	11,400
40	0.9996	93.1%	$-6.47x10^{-5}$	14,400
50	0.9994	91.8%	$-5.09x10^{-5}$	18,000
60	0.9996	93.4%	$-4.70x10^{-5}$	19,900
	Average:	92.8%		
	SD:	+/-0.8%		

$$F_{max} = \frac{E_{max}}{-\Delta E}$$

Time of Annealing and Elongation is a Determination of E_{max}





A&E (min)	r^2	E_{max}	ΔE	F_{max}
4.0	0.9996	94.7%	$-8.69\text{x}10^{-5}$	10,800
2.0	0.9988	90.1%	$-9.63\text{x}10^{-5}$	9,360
1.0	0.9981	81.0%	$-1.04\text{x}10^{-4}$	7,790
0.5	0.9892	65.7%	$-9.28\text{x}10^{-5}$	6,840
		Average:	$-9.50\text{x}10^{-}$	
		CV:	+/-7.8%	

$$F_{max} = \frac{E_{max}}{-\Delta E}$$

Kinetic-based Sigmoidal Modeling of PCR Amplification

Derivations based upon the Classic Boltzmann Sigmoid Function

$$F_C = \frac{F_{max}}{1 + e^{-\left(\frac{C - C_{1/2}}{k}\right)}} - F_b$$

Target quantity in FU ➔ $$F_0 = \frac{F_{\max}}{1 + \left(\frac{F_{max}}{F_C} - 1\right)(E_{\max} + 1)^C}$$

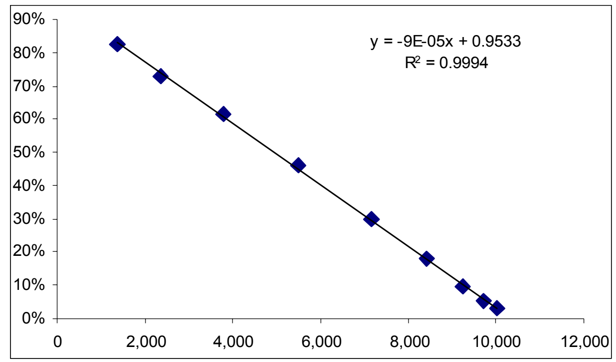
Predicted reaction fluorescence ➔ $$F_C = \frac{F_{\max}}{1 + \left(\frac{F_{max}}{F_0} - 1\right)(E_{\max} + 1)^{-C}}$$

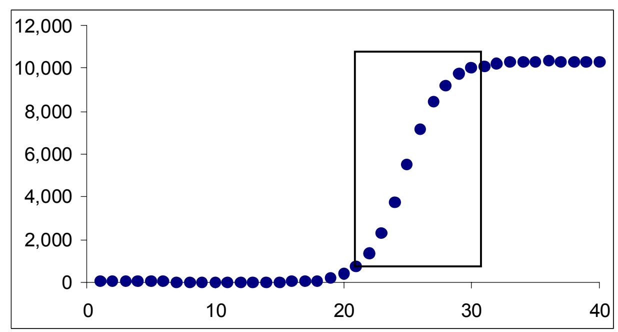
Multiple Determinations of Target Quantity (F_0)

$$F_0 = \frac{F_{max}}{1 + \left(\frac{F_{max}}{F_C} - 1 \right) (E_{max} + 1)^C}$$

r2:	0.9994
ΔE:	9.21E-05
Emax:	95.33%
Fmax:	10349
Av. Fo:	6.19E-04
CV:	2.95%

Cycle	Fc	Ec	Fo
1	79		
2	70		
3	59		
4	48		
5	42		
6	38		
7	22		
8	26		
9	-1		
10	0		
11	0		
12	2		
13	-1		
14	2		
15	4	58.62%	1.60E-04
16	44	1091.30%	9.80E-04
17	43	-1.46%	4.95E-04
18	73	68.89%	4.29E-04
19	192	163.05%	5.84E-04
20	385	100.75%	6.12E-04
21	741	92.29%	6.25E-04
22	1,351	82.33%	6.23E-04
23	2,336	72.90%	6.19E-04
24	3,770	61.43%	6.23E-04
25	5,501	45.91%	6.32E-04
26	7,151	29.99%	6.37E-04
27	8,424	17.80%	6.38E-04
28	9,234	9.62%	6.19E-04
29	9,726	5.32%	5.97E-04
30	10,013	2.96%	5.84E-04
31	10,095	0.82%	3.99E-04
32	10,223	1.27%	4.17E-04
33	10,314	0.88%	7.60E-04
34	10,275	-0.38%	1.86E-04
35	10,304	0.29%	1.57E-04
36	10,338	0.33%	3.26E-04
37	10,276	-0.60%	2.52E-05
38	10,288	0.12%	1.55E-05
39	10,322	0.33%	1.76E-05
40	10,279	-0.41%	3.57E-06

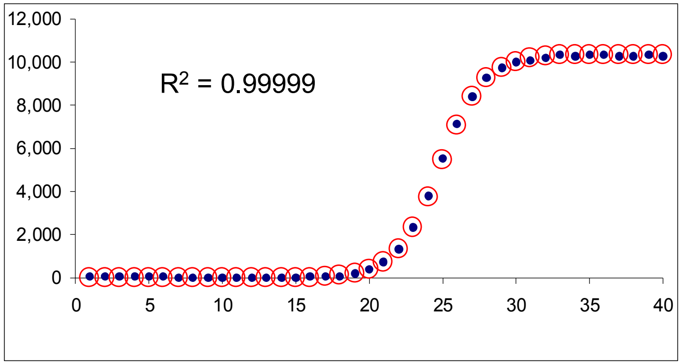




Correlation of Predicted to Actual Reaction Fluorescence

Cycle	Fc			pFc
1	79			0
2	70			0
3	59			0
4	48			0
5	42			0
6	38	**r2:**	0.9994	0
7	22	**ΔE:**	9.21E-05	0
8	26	**Emax:**	95.3%	0
9	-1	**Fmax:**	10349	0
10	0			1
11	0	**Av. Fo:**	6.19E-04	1
12	2	**CV:**	2.95%	2
13	-1			4
14	2	**Ec**	**Fo**	7
15	4	58.62%	1.60E-04	14
16	44	1091.30%	9.80E-04	28
17	43	-1.46%	4.95E-04	54
18	73	68.89%	4.29E-04	105
19	192	163.05%	5.84E-04	203
20	385	100.75%	6.12E-04	389
21	741	92.29%	6.25E-04	734
22	1,351	82.33%	6.23E-04	1,344
23	2,336	72.90%	6.19E-04	2,336
24	3,770	61.43%	6.23E-04	3,754
25	5,501	45.91%	6.32E-04	5,449
26	7,151	29.99%	6.37E-04	7,087
27	8,424	17.80%	6.38E-04	8,375
28	9,234	9.62%	6.19E-04	9,235
29	9,726	5.32%	5.97E-04	9,747
30	10,013	2.96%	5.84E-04	10,032
31	10,095	0.82%	3.99E-04	10,184
32	10,223	1.27%	4.17E-04	10,264
33	10,314	0.88%	7.60E-04	10,305
34	10,275	-0.38%	1.86E-04	10,327
35	10,304	0.29%	1.57E-04	10,338
36	10,338	0.33%	3.26E-04	10,343
37	10,276	-0.60%	2.52E-05	10,346
38	10,288	0.12%	1.55E-05	10,348
39	10,322	0.33%	1.76E-05	10,348
40	10,279	-0.41%	3.57E-06	10,349

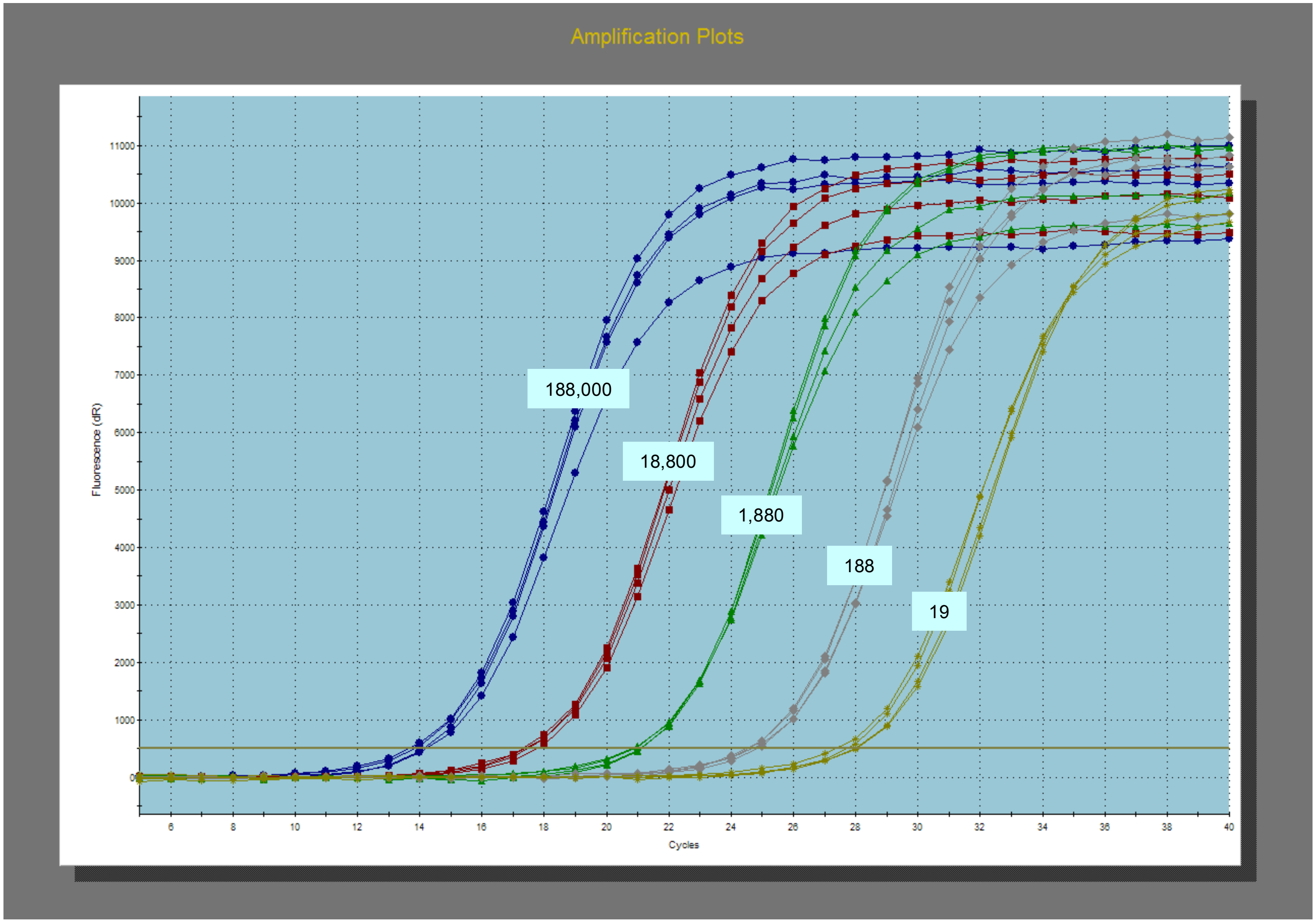
$$F_C = \frac{F_{max}}{1 + \left(\frac{F_{max}}{F_0} - 1\right)(E_{max} + 1)^{-C}}$$



$$R^2 = 1 - \frac{\sum (F_C - F_P)^2}{\sum (F_C - F_{av})^2}$$

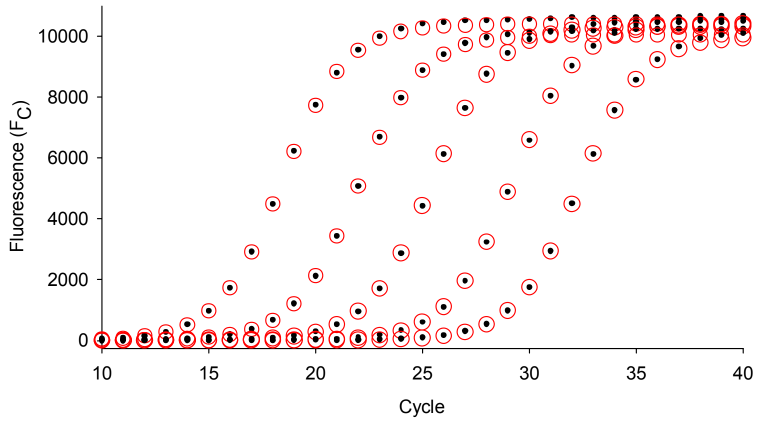
Lambda Genomic DNA 10 Fold Dilution Series

LamK7K12 10pg to 1fg Lambda gDNA



qPCR 2005,
Freising-Weihenstephan, Germany

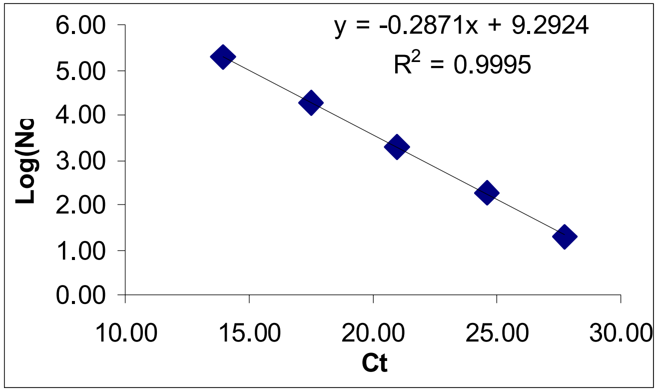
Quantitative Precision of Kinetic-based Sigmoidal Modeling



# Lambda Genomes	R^2	E_{max}	ΔE	F_0	CV	N_0	% of Predicted
188,000	0.99996	95.6%	-9.51×10^{-5}	4.30×10^{-2}	-/+1.50%	206,000	109.9%
18,800	0.99999	95.3%	-9.49×10^{-5}	4.10×10^{-3}	-/+0.45%	19,900	106.2%
1,880	0.99999	94.9%	-9.25×10^{-5}	4.41×10^{-4}	-/+0.61%	2,110	112.7%
188	0.99999	95.7%	-9.23×10^{-5}	3.22×10^{-5}	-/+0.80%	155	82.4%
19	0.99997	96.1%	-9.86×10^{-5}	3.73×10^{-6}	-/+1.39%	18	95.4%
Average:	0.99998	95.5%	-9.47×10^{-5}		-/+0.95%		101.3%
SD/CV:	+/-0.00001	+/-0.5%	+/-2.7%				+/-12.4%

C_t-derived Amplification Efficiency vs. LRE-derived E_{max}

# of Lambda Genomes (No)	Log(No)	Av. Ct
188,000	5.274	13.95
18,800	4.274	17.48
1,880	3.274	20.99
188	2.274	24.63
19	1.274	27.78
	r2:	0.9995
	Nt:	1.96E+09
	Es:	93.7%
	Emax:	95.5%
	Difference:	1.83%



$$N_t = 10^{\text{Intercept}}$$

$$E_S = 10^{\text{-Slope}} - 1$$

E_{max} closely correlates to a slope-derived amplification efficiency via C_t

Correlation to C_t-based Quantification via F_0

$$F_0 = \frac{F_t}{(E+1)^{C_t}}$$

Ft.: 500
Emax: 95.5%

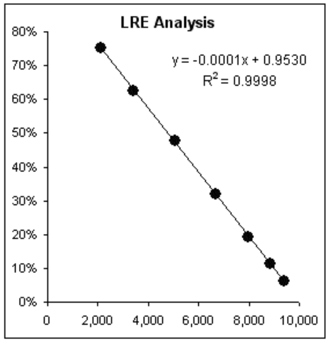
# Lambda Genomes	Av. Ct	Ct-Fo (FU)	LRE-Fo (FU)	Ct vs. LRE
188,000	13.95	4.35E-02	4.30E-02	1.27%
18,000	17.48	4.07E-03	4.10E-03	-0.70%
1,800	20.99	3.88E-04	4.41E-04	-12.08%
188	24.63	3.37E-05	3.22E-05	4.58%
19	27.78	4.09E-06	3.73E-06	9.63%
			Average:	0.54%
			SD:	8.07%

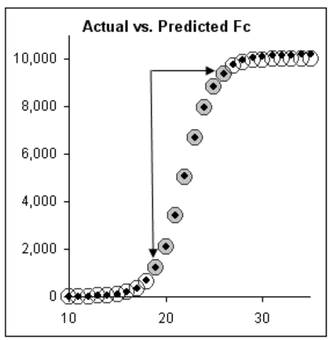
LRE-based quantification correlates closely to C_t quantification

Computational Simplicity and Amiability to Automation

Modeling based upon the fluorescence data generated by an individual amplification reaction

Lam K7-K12
1pg lambda (18,800 genomes)





Cycle	Fc			Fp
1	73	**Emax:**	95.30%	0
2	20	**ΔE:**	-9.49E-05	0
3	14	**Fmax:**	10,044	0
4	-12	**r2:**	0.99984	0
5	-11			0
6	5			0
7	-5	**Av. Fo:**	4.10E-03	0
8	-4	**CV:**	0.45%	1
9	-5			2
10	9	**Ec**	**Fo**	3
11	16	83.43%	1.03E-02	6
12	10	-36.88%	3.34E-03	13
13	32	211.30%	5.33E-03	25
14	52	63.18%	4.46E-03	48
15	103	97.66%	4.54E-03	93
16	192	86.55%	4.38E-03	180
17	364	89.28%	4.32E-03	346
18	671	84.26%	4.21E-03	655
19	1204	79.50%	4.10E-03	1204
20	2111	75.25%	4.10E-03	2110
21	3430	62.50%	4.09E-03	3434
22	5067	47.75%	4.11E-03	5058
23	6685	31.91%	4.12E-03	6675
24	7967	19.18%	4.06E-03	7981
25	8868	11.31%	4.09E-03	8870
26	9410	6.11%	4.12E-03	9407
27	9772	3.85%	5.11E-03	9707
28	9961	1.93%	8.72E-03	9869
29	10059	0.98%	-2.52E-02	9953
30	10109	0.50%	-2.98E-03	9997
31	10149	0.40%	-9.46E-04	10020
32	10162	0.13%	-4.30E-04	10032
33	10174	0.12%	-2.00E-04	10038
34	10203	0.28%	-8.43E-05	10041
35	10232	0.29%	-3.65E-05	10042

$$E_C = \frac{F_C}{F_{C-1}} - 1$$

$$E_C = \Delta E \times F_C + E_{max}$$

$$F_{max} = \frac{E_{max}}{-\Delta E}$$

$$F_0 = \frac{F_{max}}{1 + \left(\frac{F_{max}}{F_C} - 1\right)(E_{max} + 1)^C}$$

$$F_C = \frac{F_{max}}{1 + \left(\frac{F_{max}}{F_0} - 1\right)(E_{max} + 1)^{-C}}$$

- Kinetic-based sigmoidal modeling is a five step process
  1. Estimation of cycle efficiency (E_C)
  2. Linear regression analysis (LRE) generating values for E_{max} and ΔE
  3. F_{max} calculation
  4. Calculation of an average F_0 based upon multiple cycles and F_C
  5. Calculating predicted F_C