

Amplification Efficiency: linking baseline and bias in the analysis of qPCR data

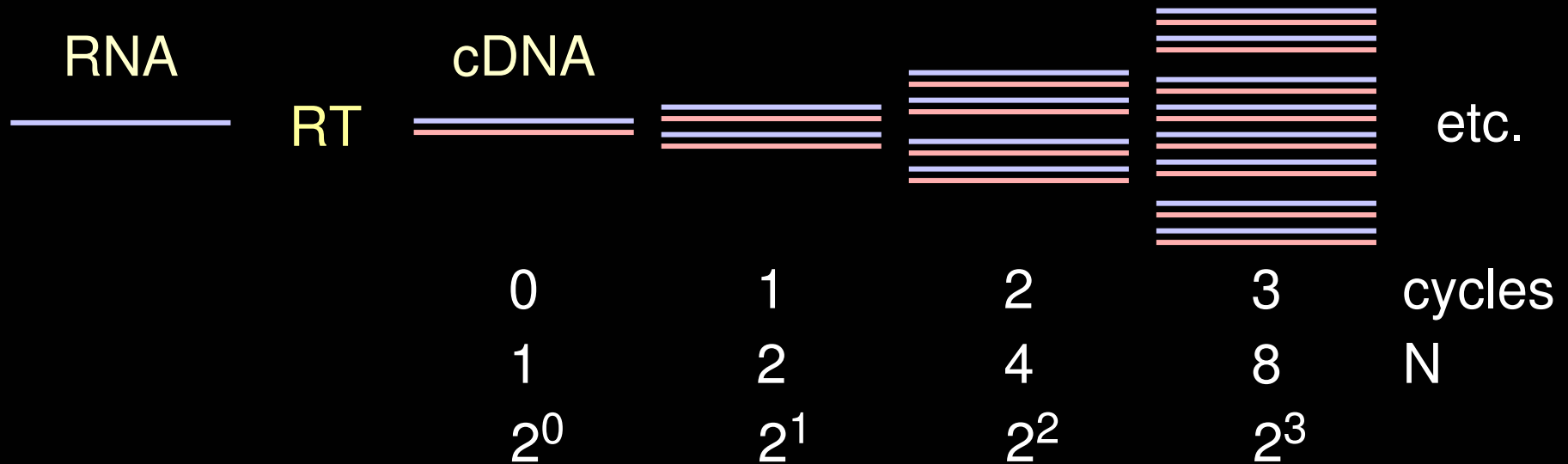
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with:

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Willem Hoogaars
Yann Karlen
Onno Bakker
Maurice van den Hoff
Antoon Moorman**

qPCR data

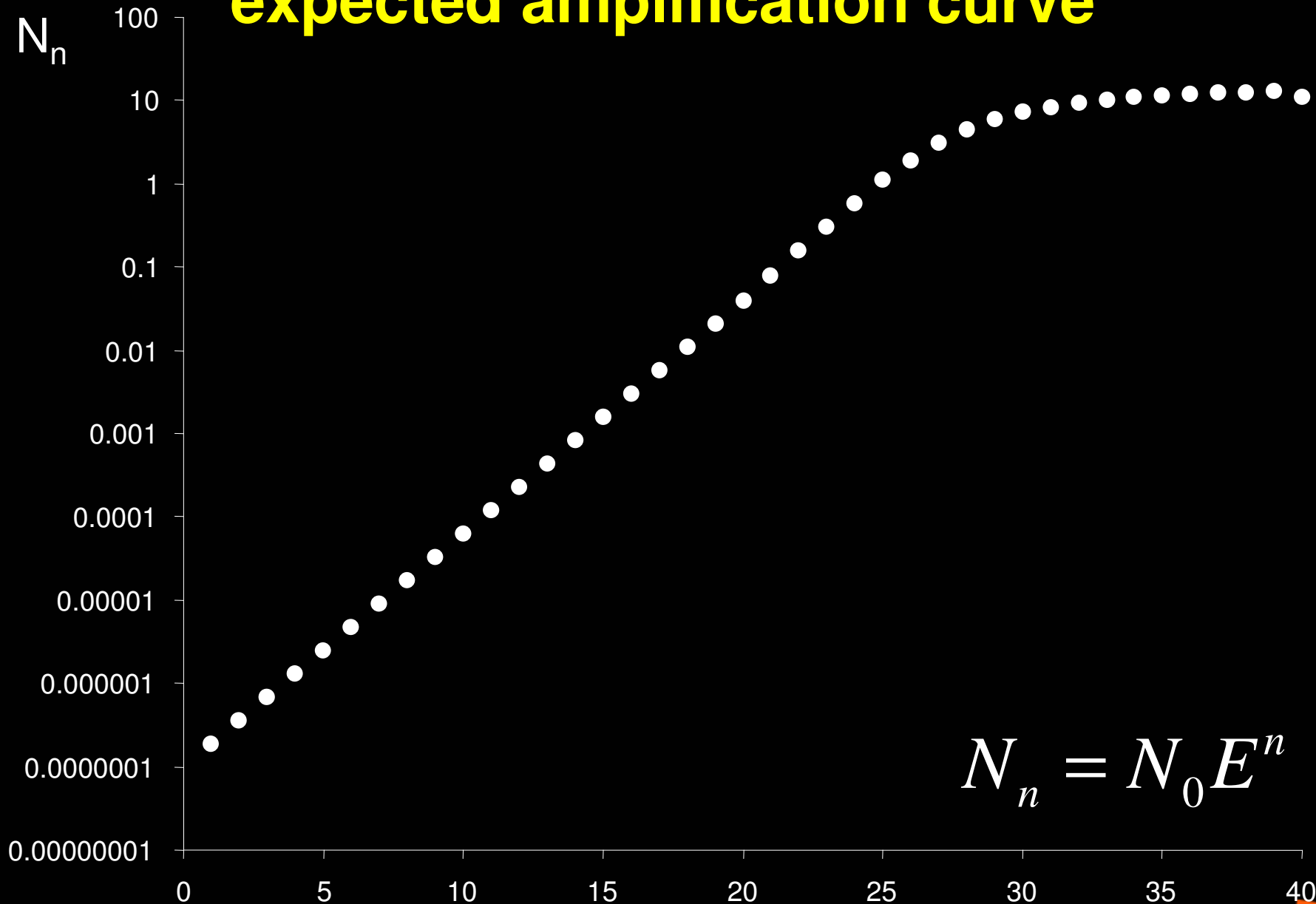


PCR product after n cycles

$$N_n = N_0 E^n$$

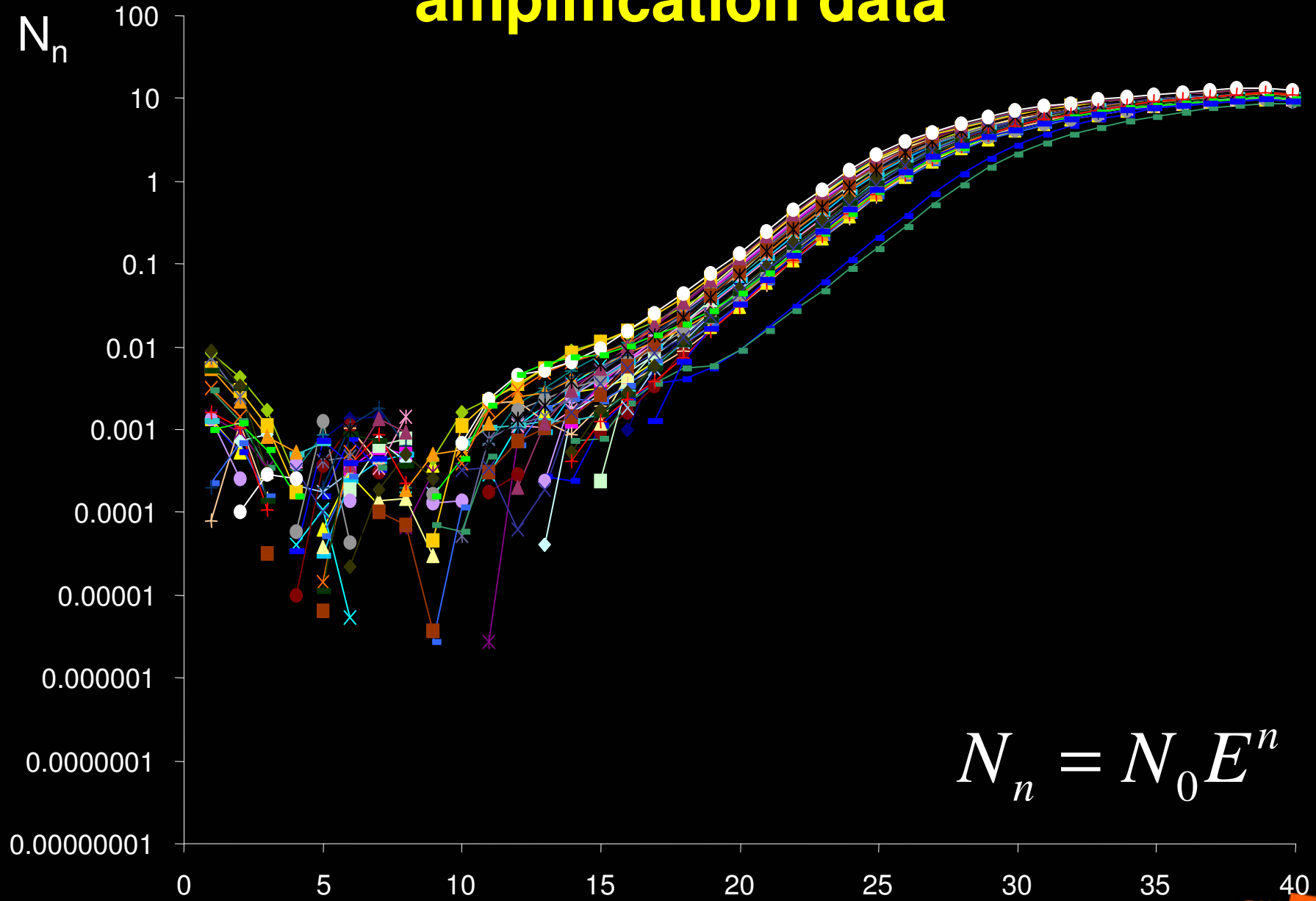
N_n → PCR product after n cycles
 N_0 → start concentration
 E^n → Efficiency (1-2; 2=100%)
 n → number of PCR cycles

expected amplification curve



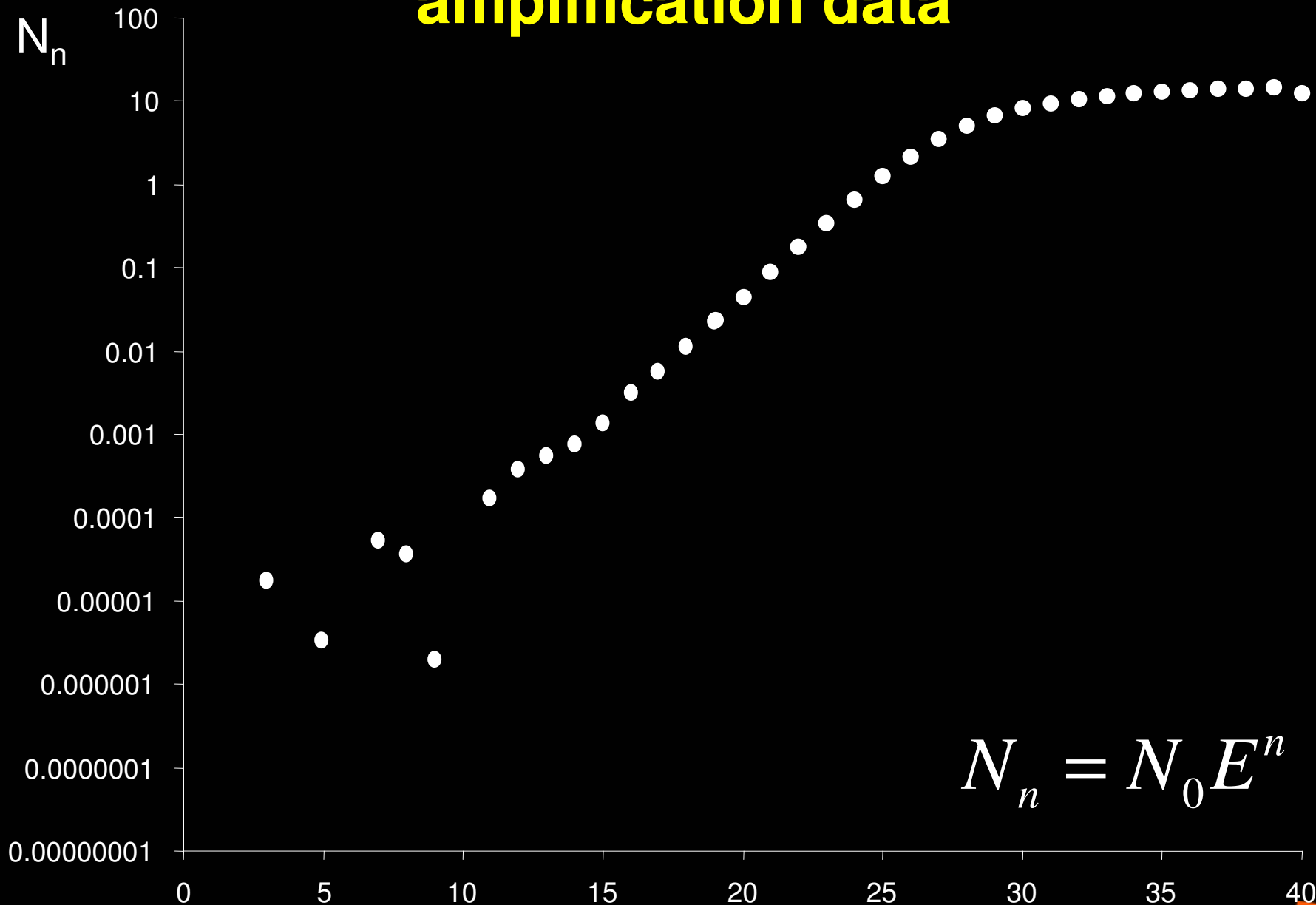
$$N_n = N_0 E^n$$

amplification data



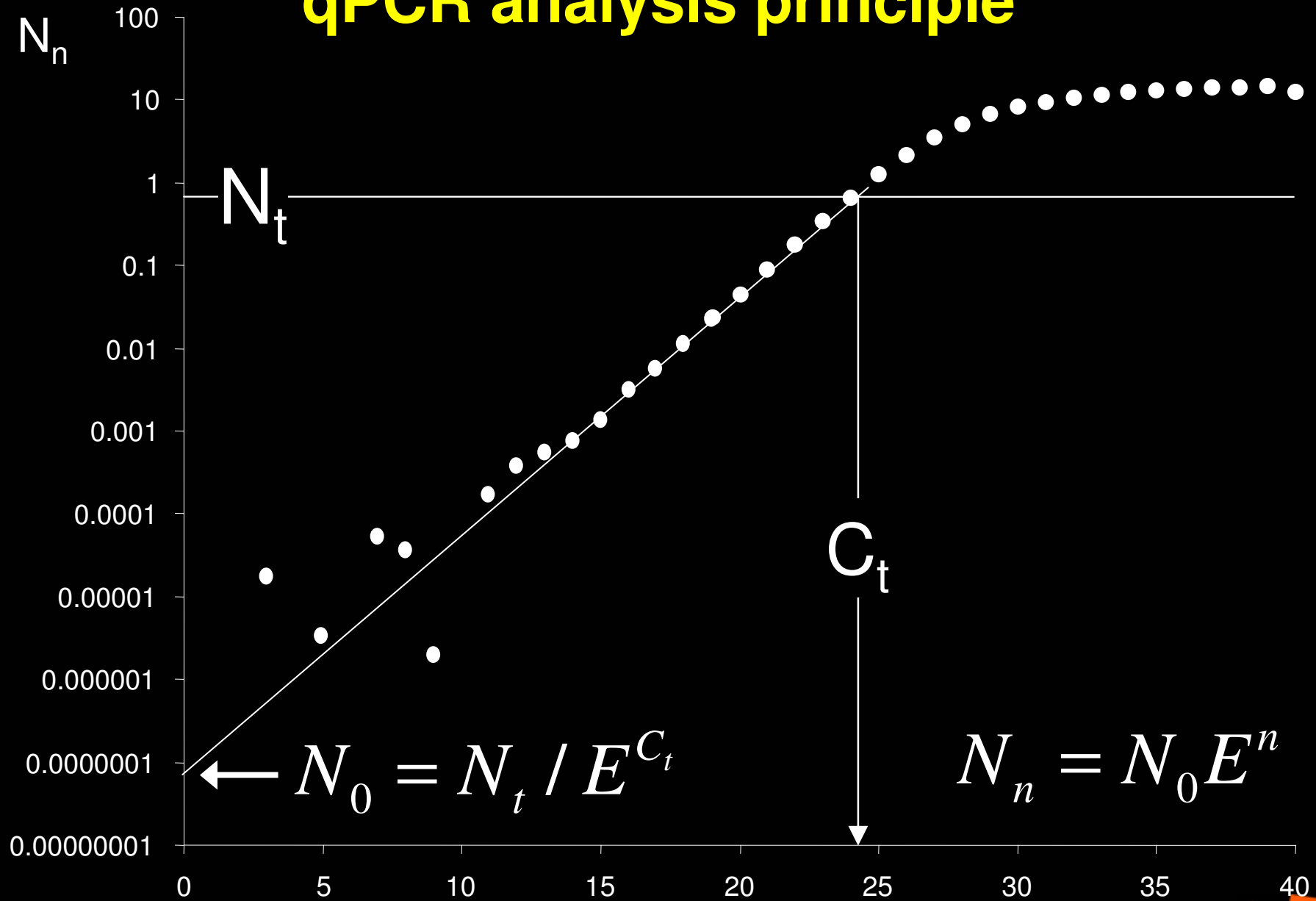
$$N_n = N_0 E^n$$

amplification data

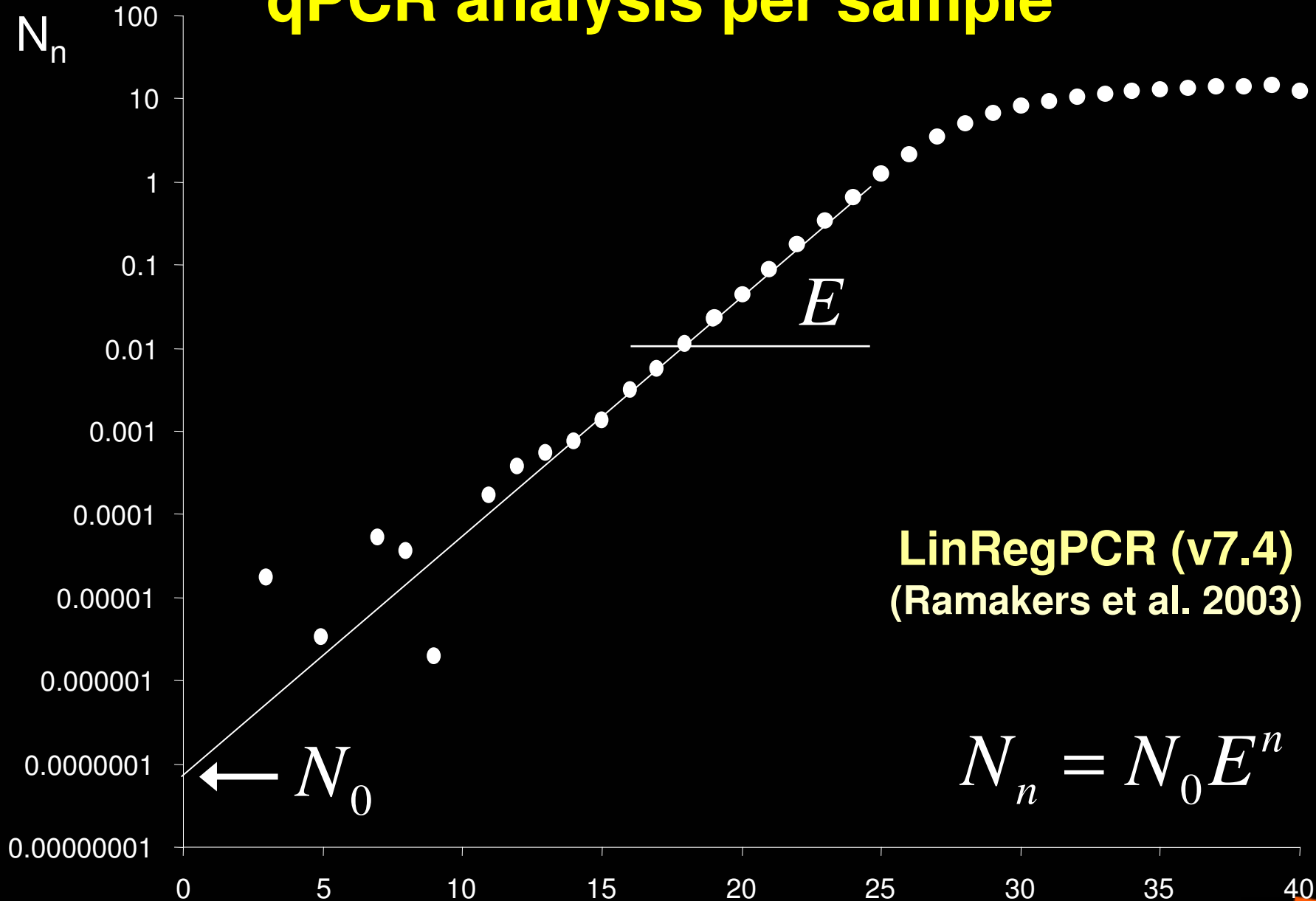


$$N_n = N_0 E^n$$

qPCR analysis principle



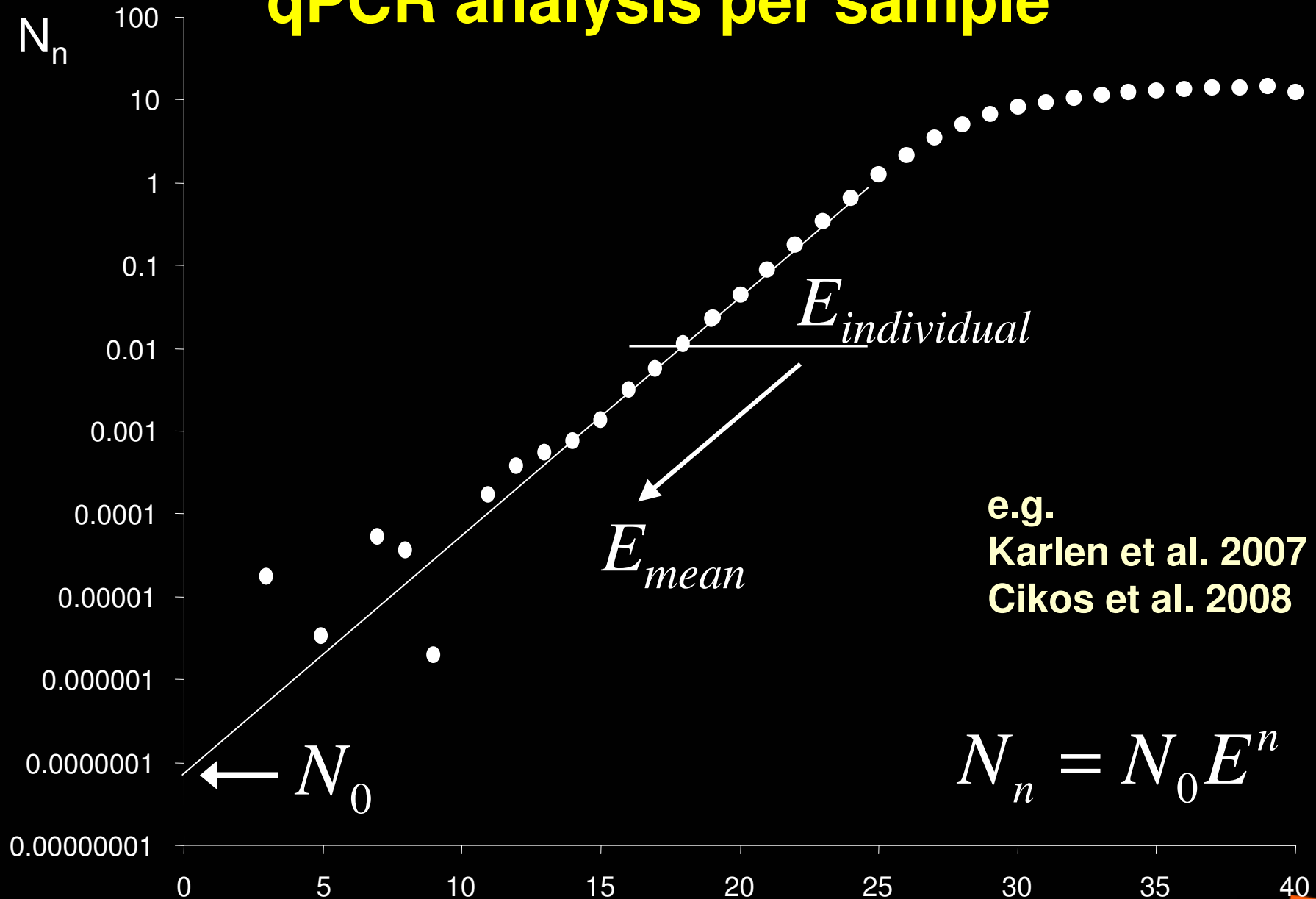
qPCR analysis per sample



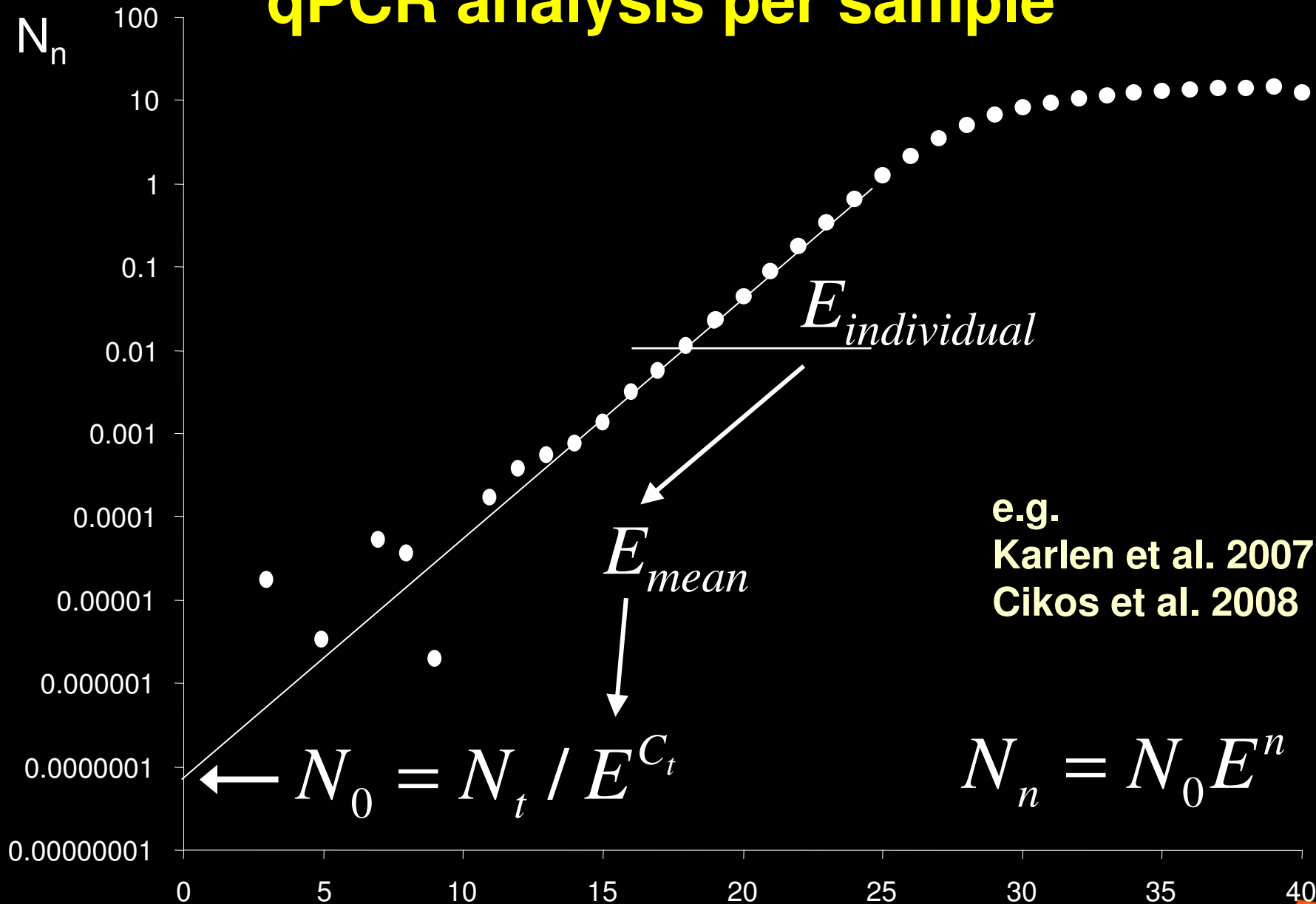
LinRegPCR (v7.4)
(Ramakers et al. 2003)

$$N_n = N_0 E^n$$

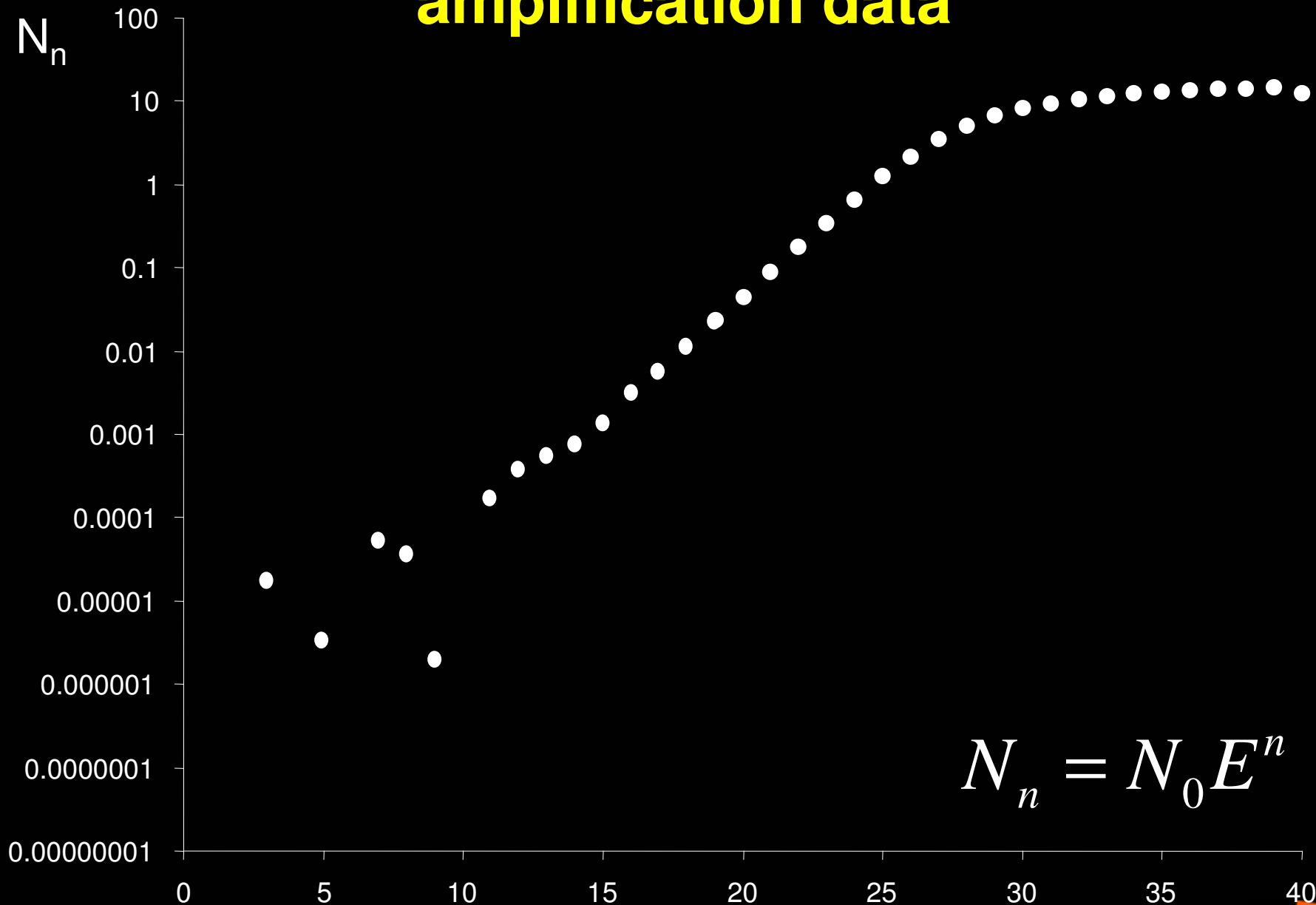
qPCR analysis per sample



qPCR analysis per sample

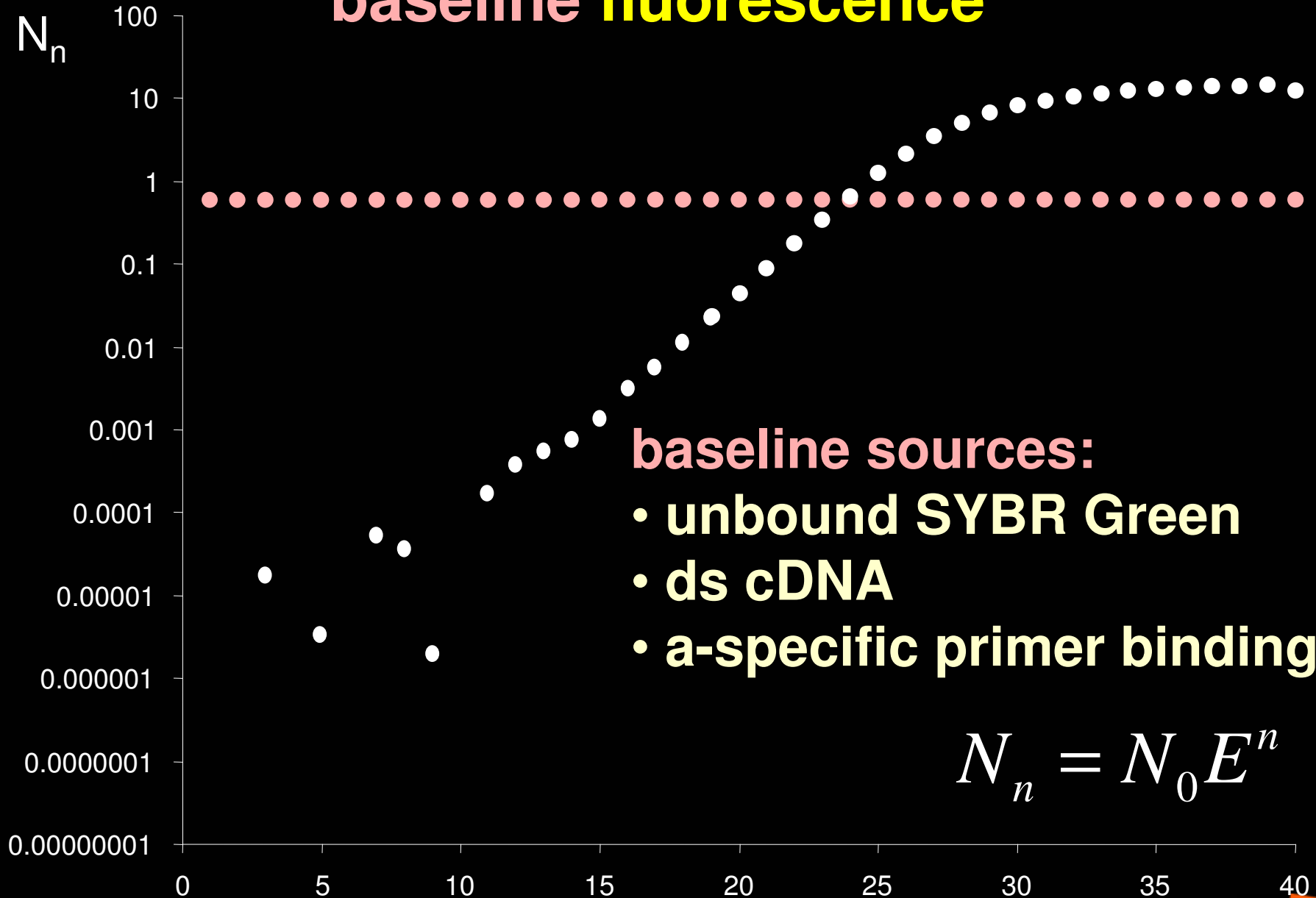


amplification data

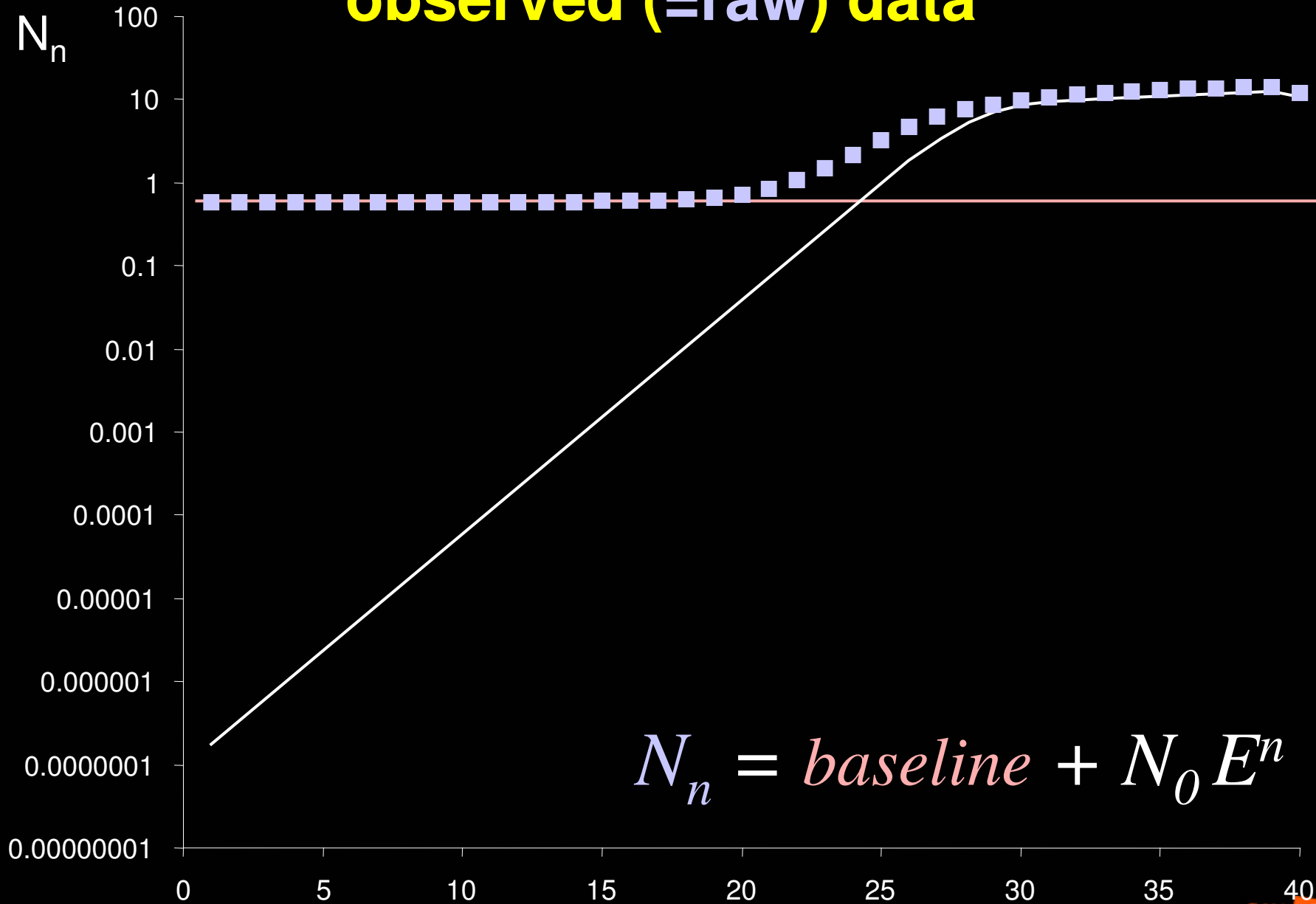


$$N_n = N_0 E^n$$

baseline fluorescence

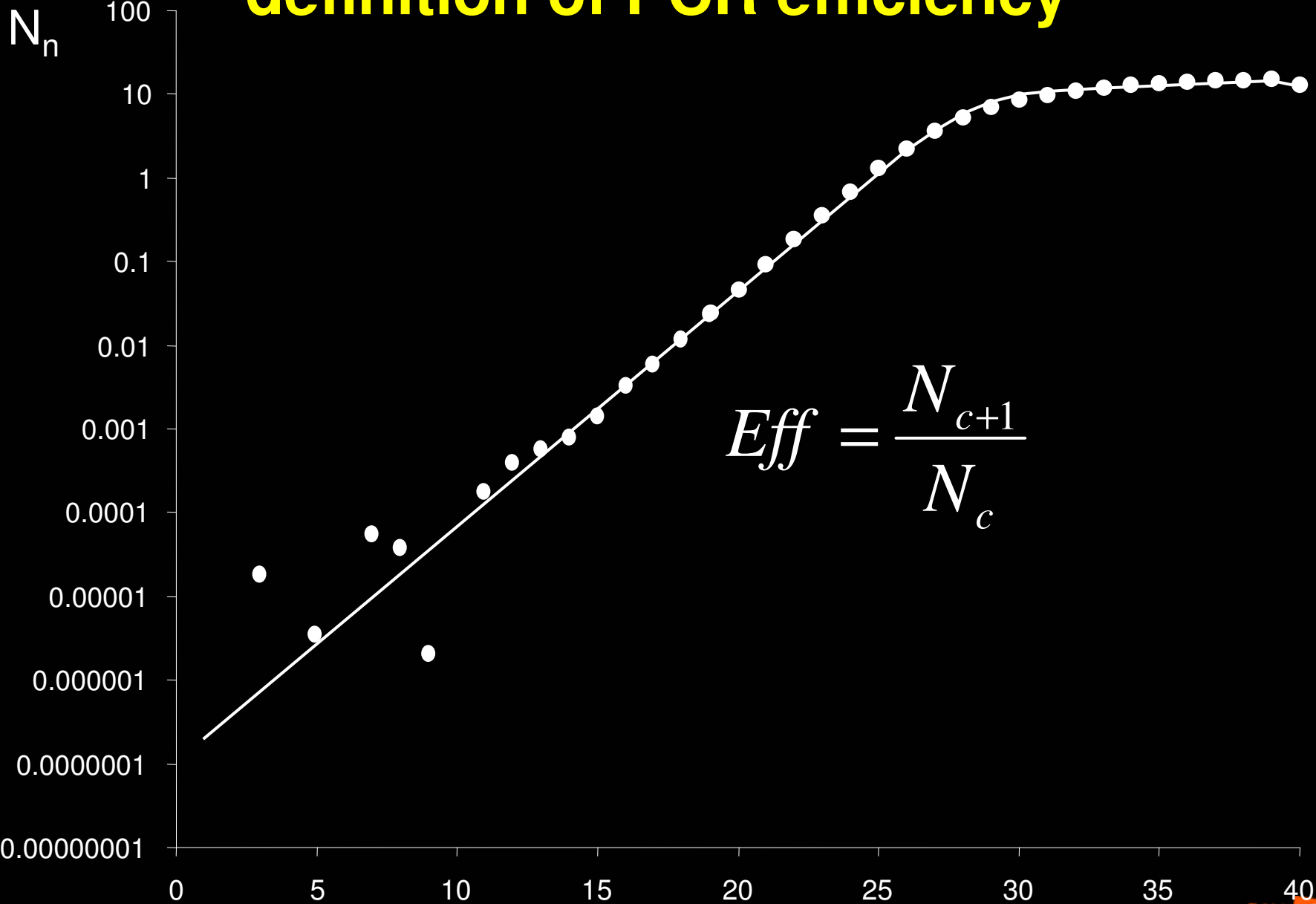


observed (=raw) data

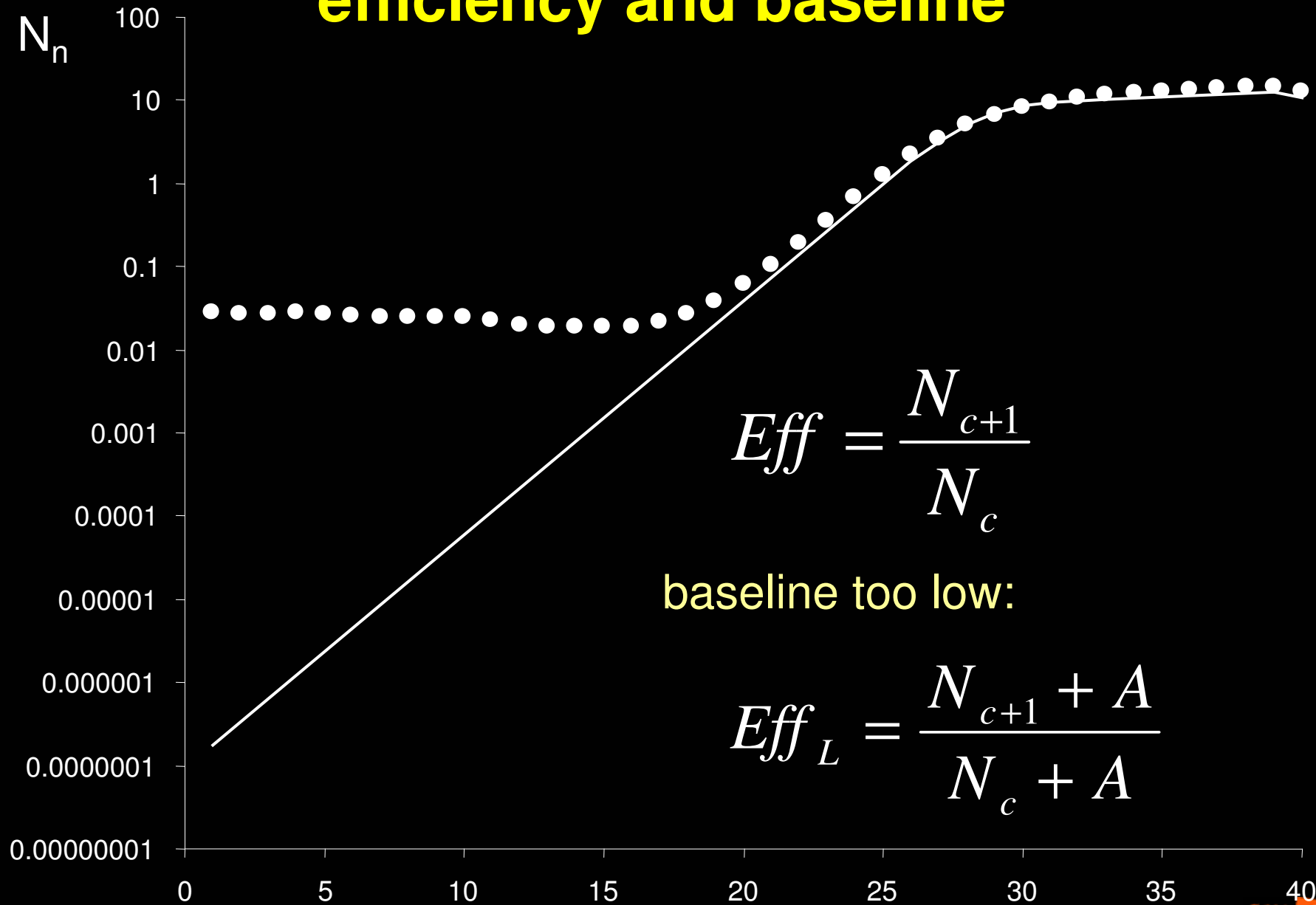


$$N_n = \text{baseline} + N_0 E^n$$

definition of PCR efficiency



efficiency and baseline

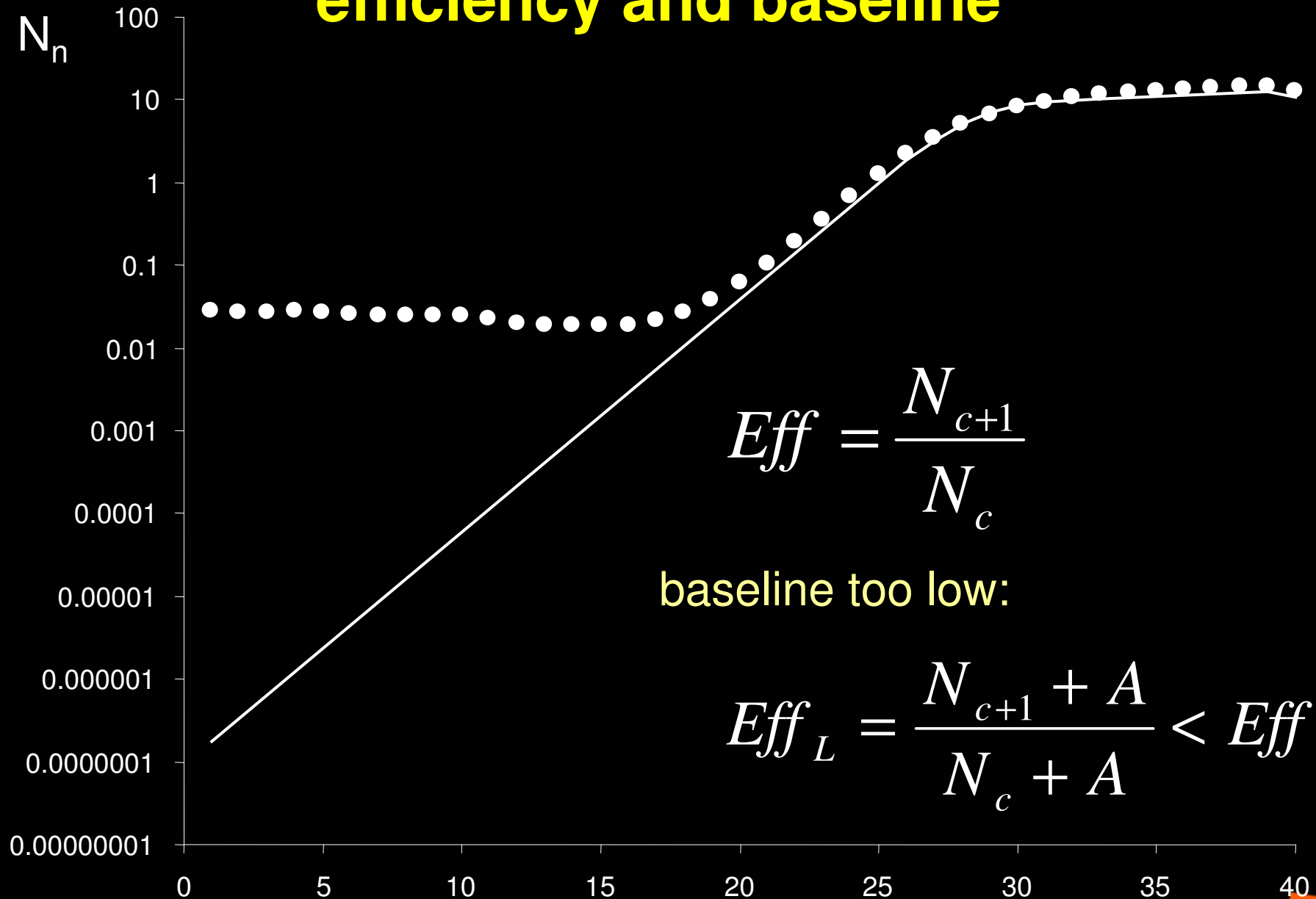


$$Eff = \frac{N_{c+1}}{N_c}$$

baseline too low:

$$Eff_L = \frac{N_{c+1} + A}{N_c + A}$$

efficiency and baseline

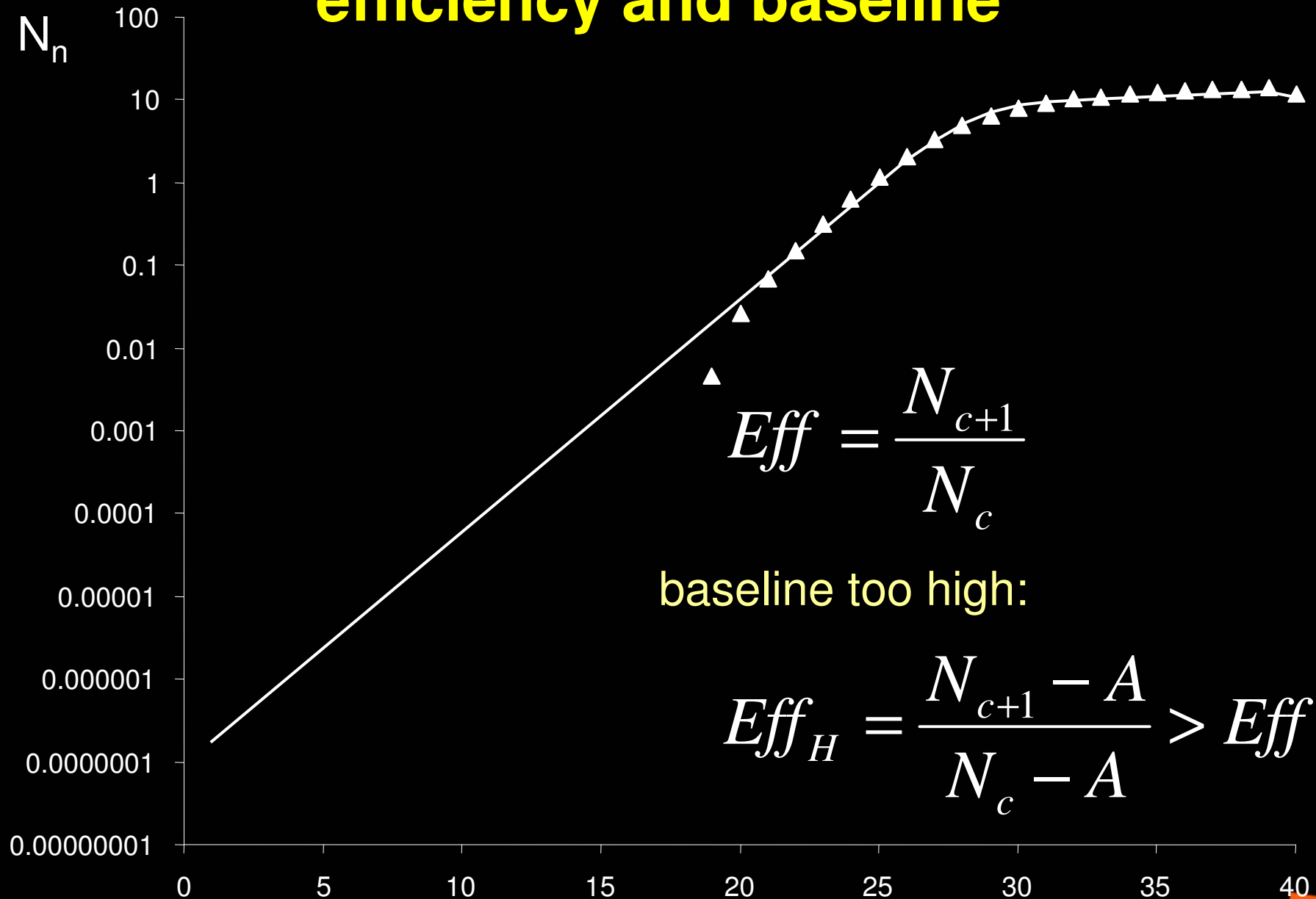


$$Eff = \frac{N_{c+1}}{N_c}$$

baseline too low:

$$Eff_L = \frac{N_{c+1} + A}{N_c + A} < Eff$$

efficiency and baseline

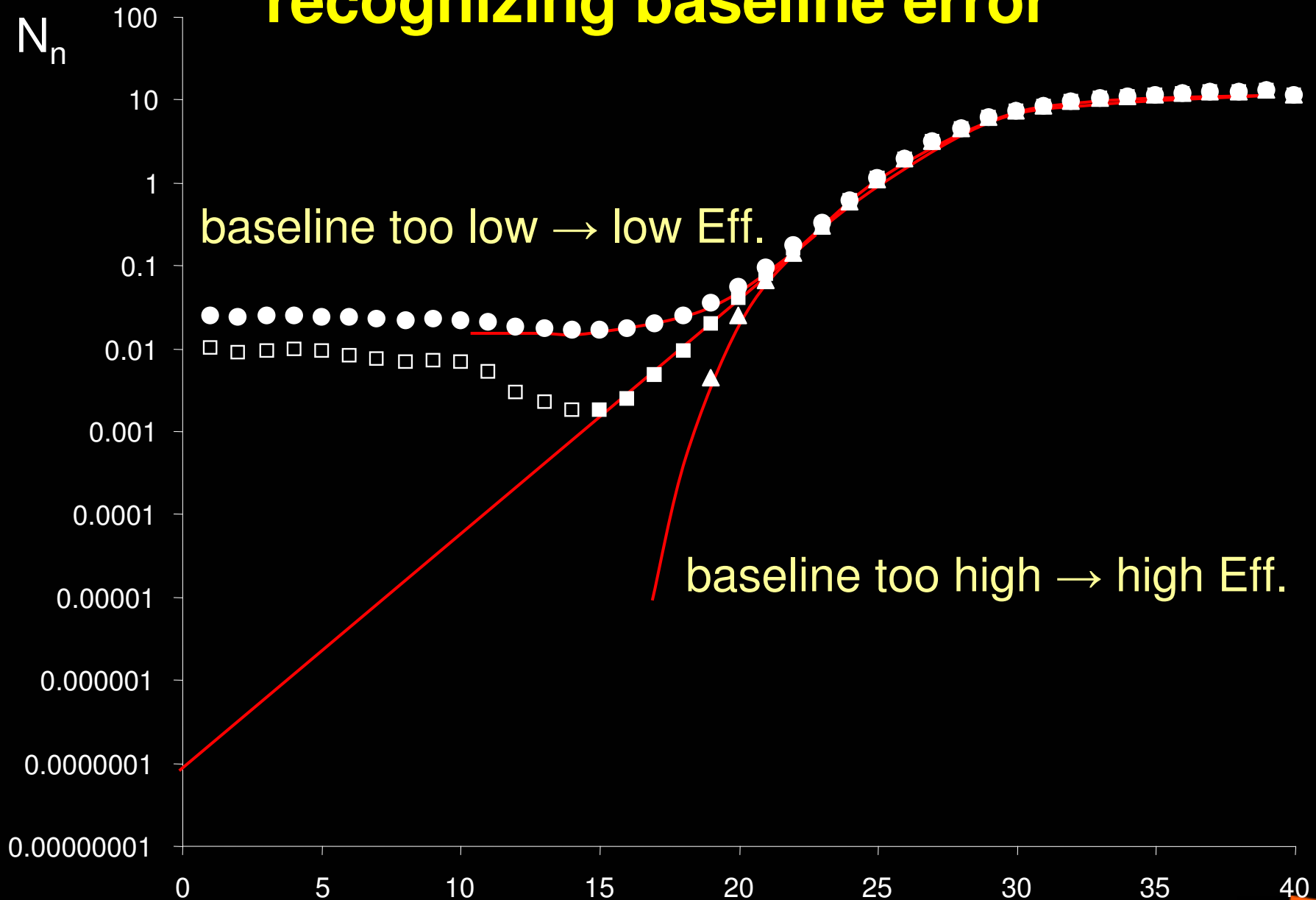


$$Eff = \frac{N_{c+1}}{N_c}$$

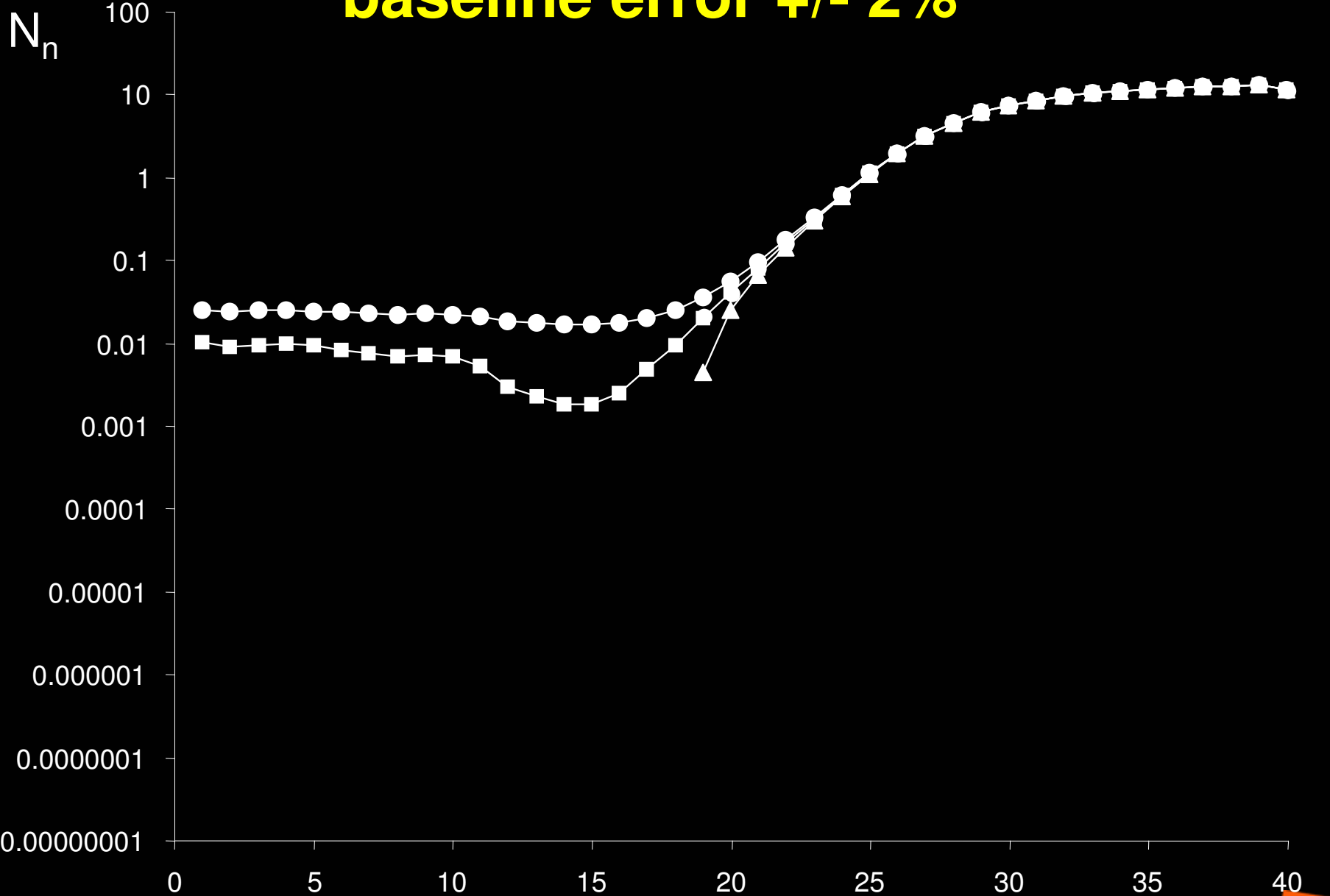
baseline too high:

$$Eff_H = \frac{N_{c+1} - A}{N_c - A} > Eff$$

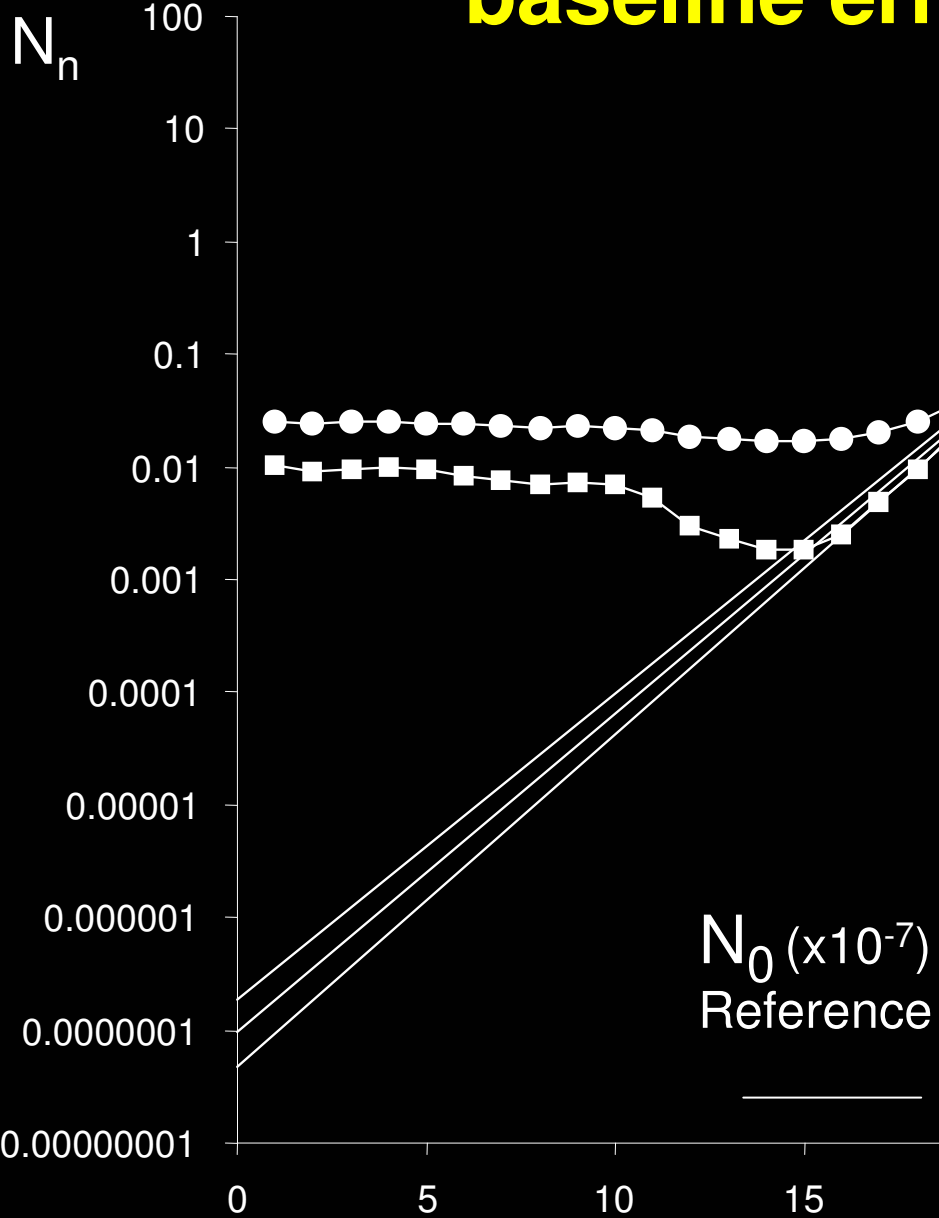
recognizing baseline error



baseline error +/- 2%



baseline error +/- 2%



Baseline (%error)	PCR	Value
● -2	1.87	0.46
■ 0	1.92	0.94
▲ +2	1.97	1.80

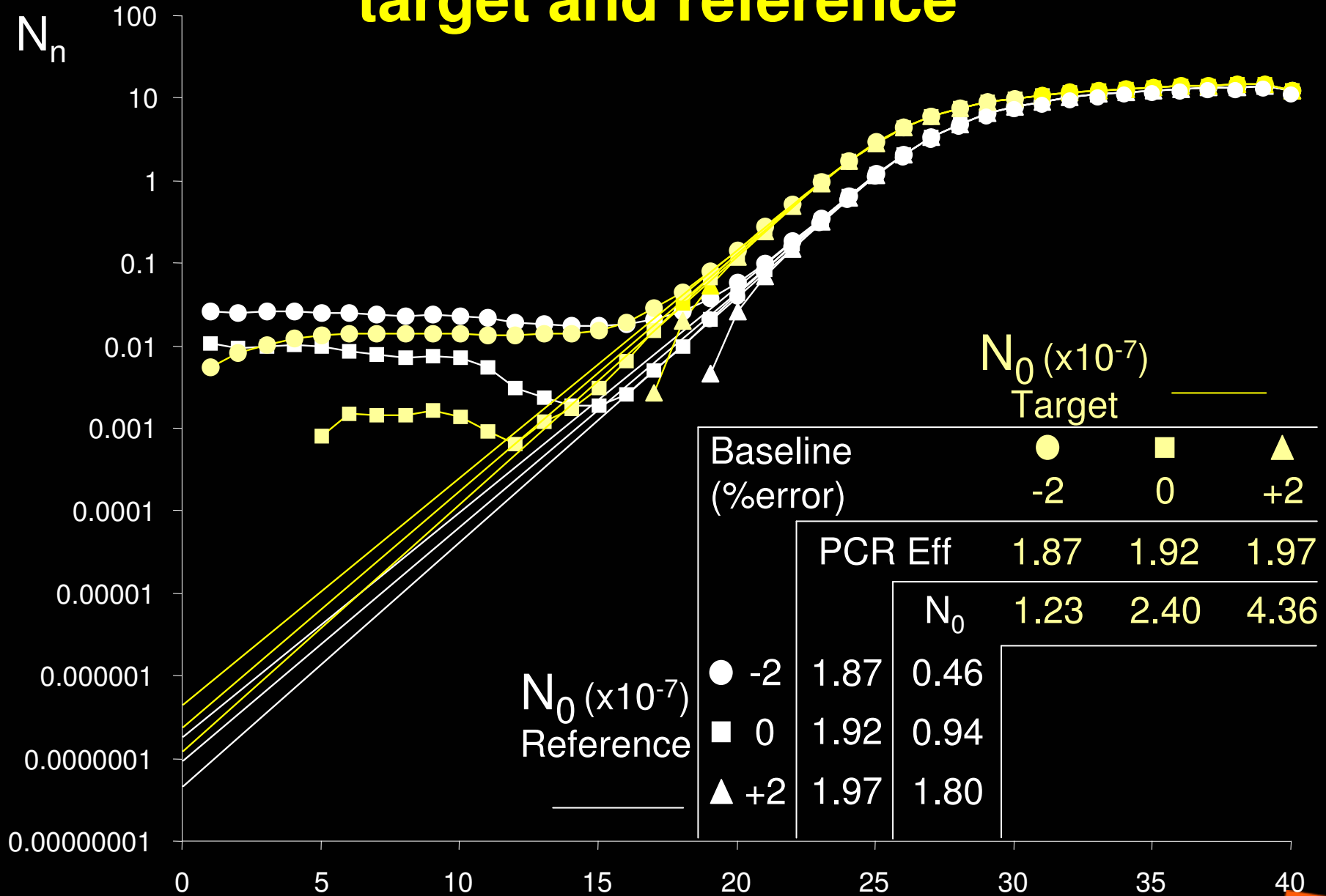
VEILIG | LENEN



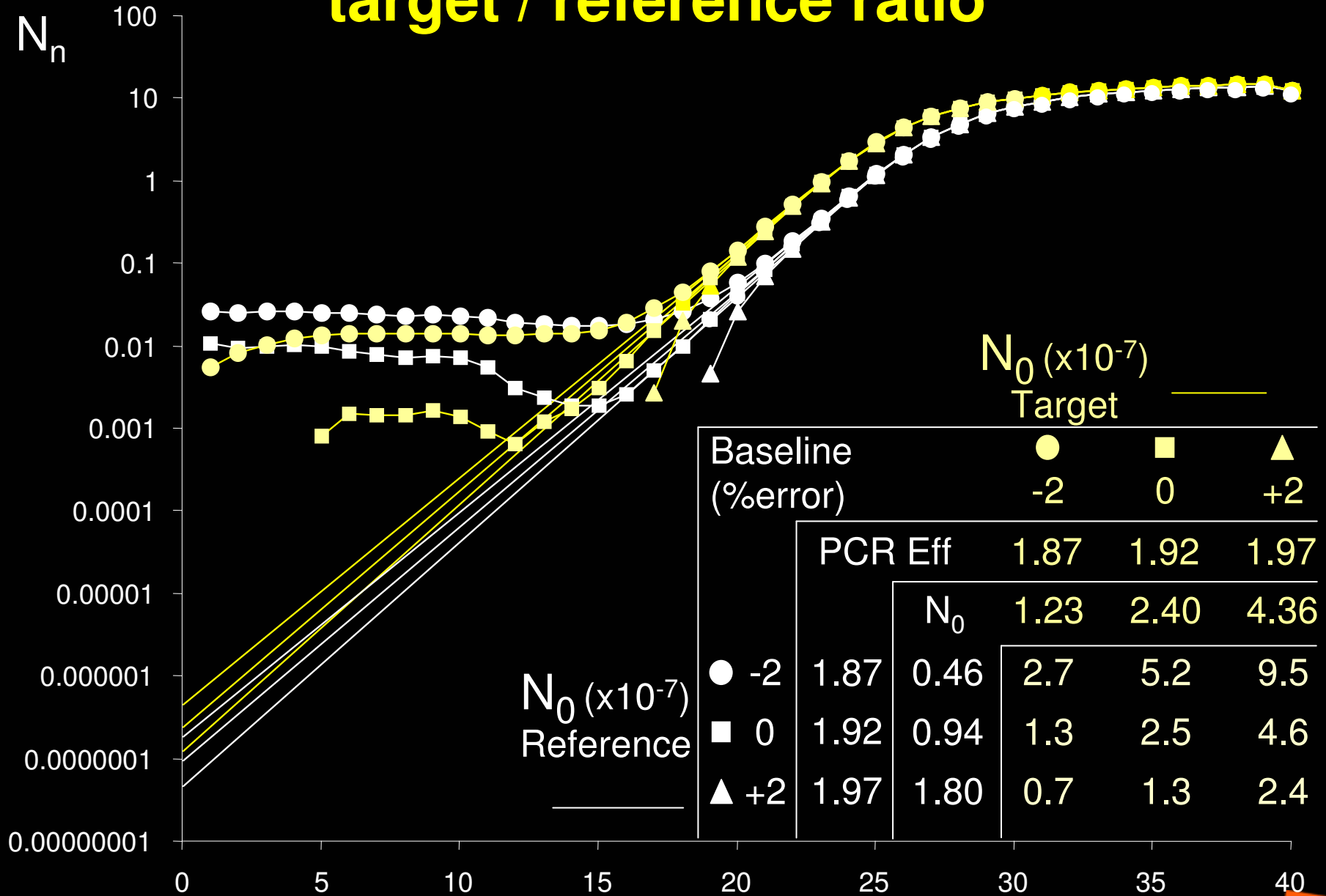
Rente: 3 tot 5%



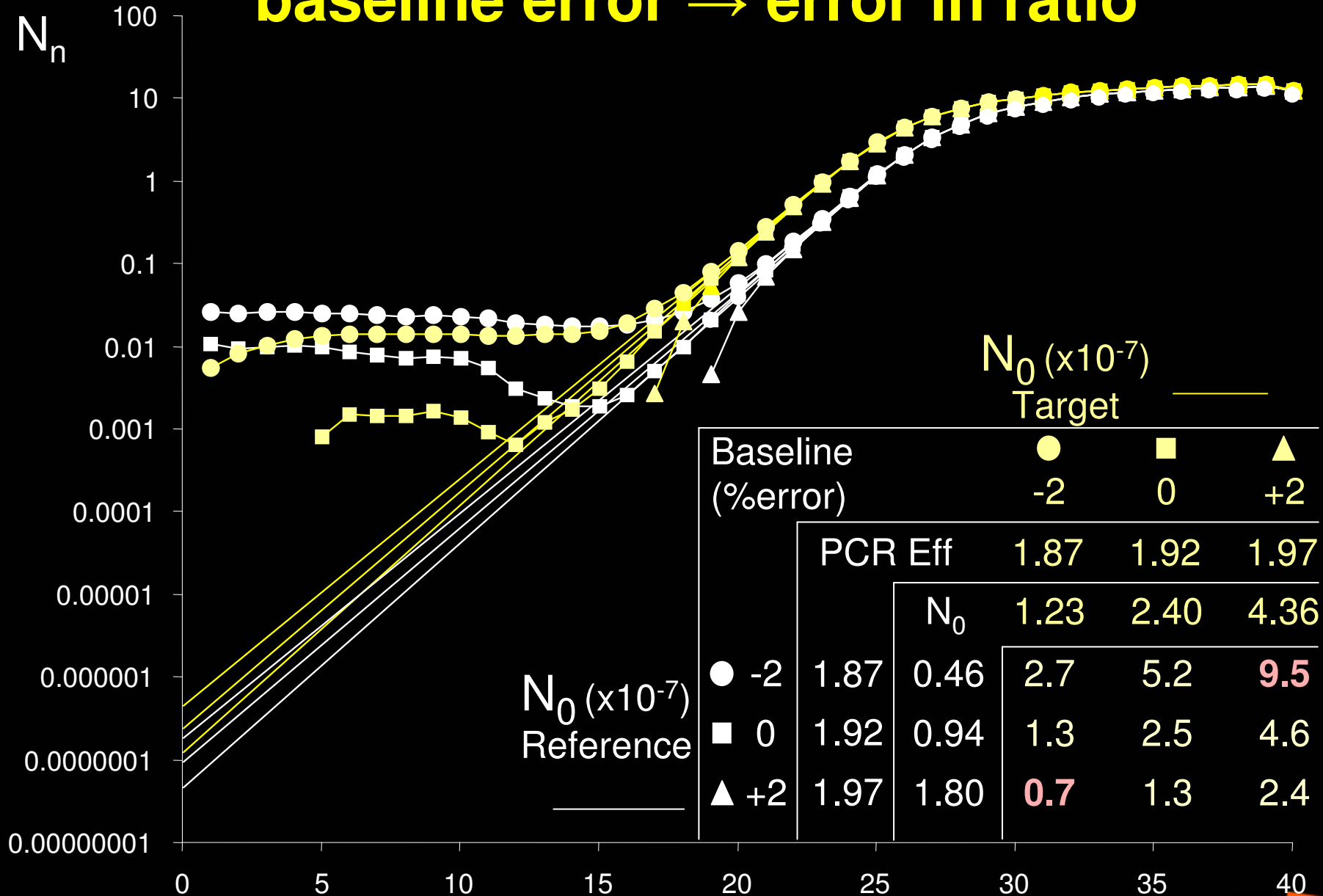
target and reference



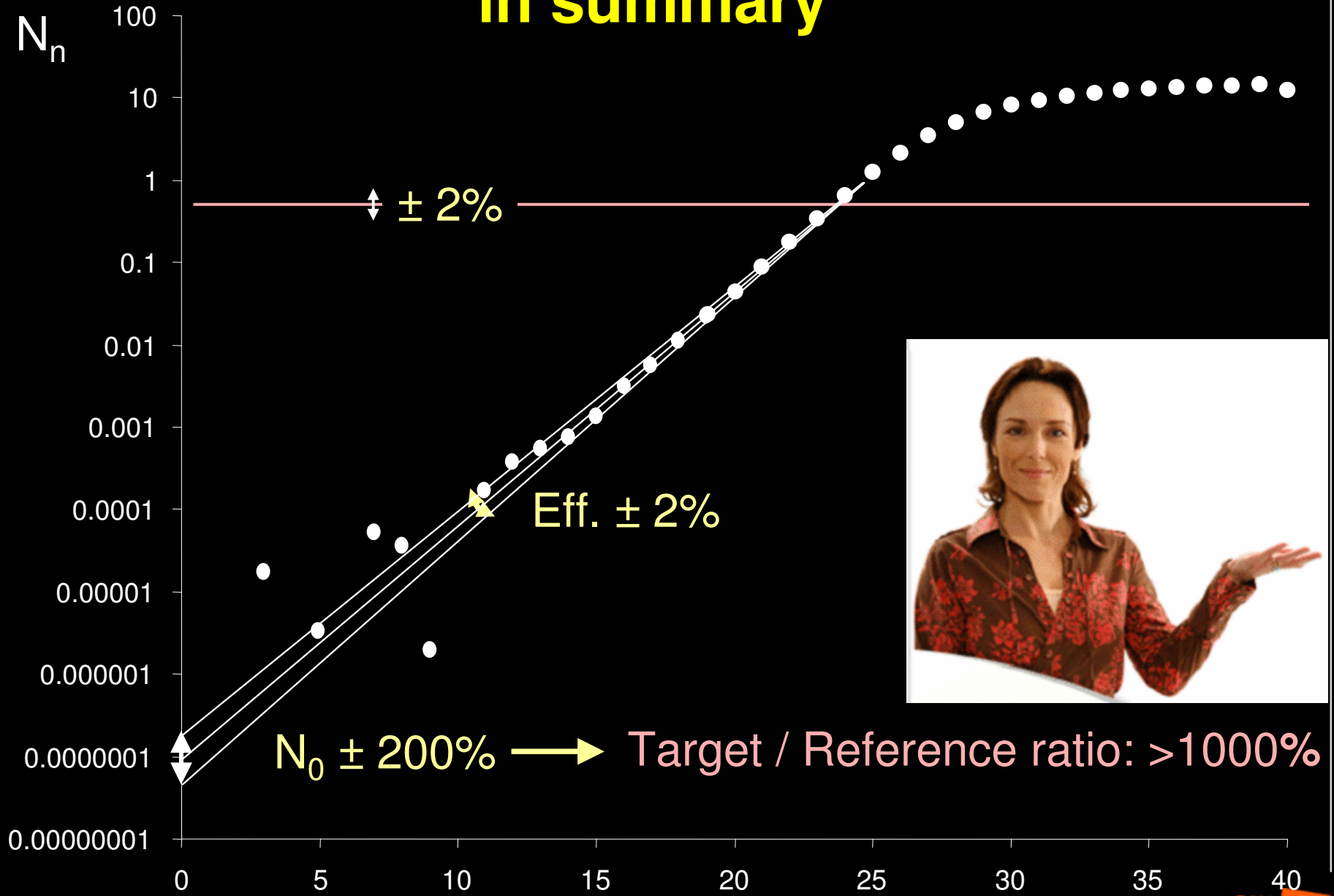
target / reference ratio



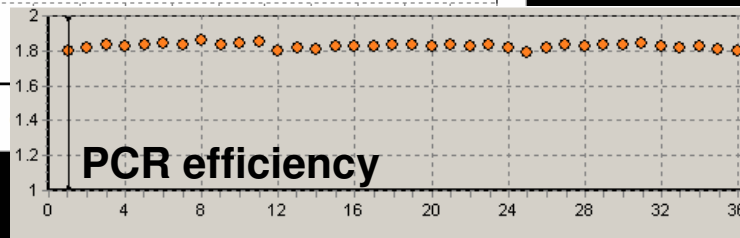
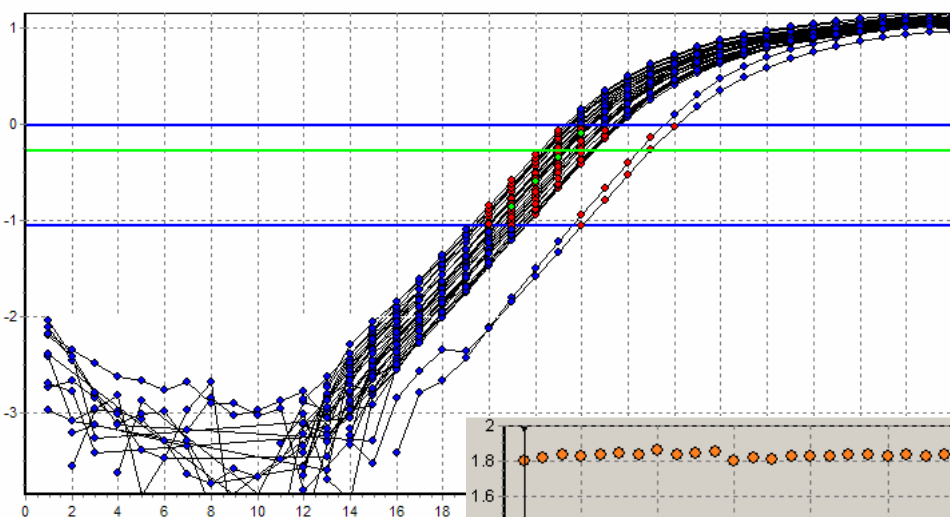
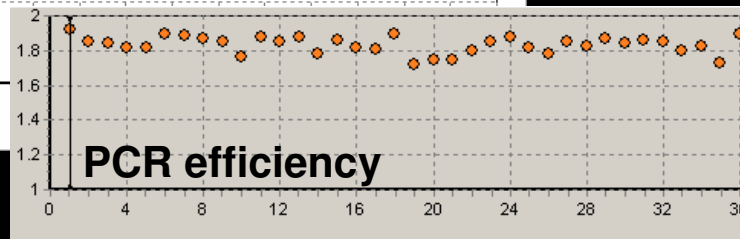
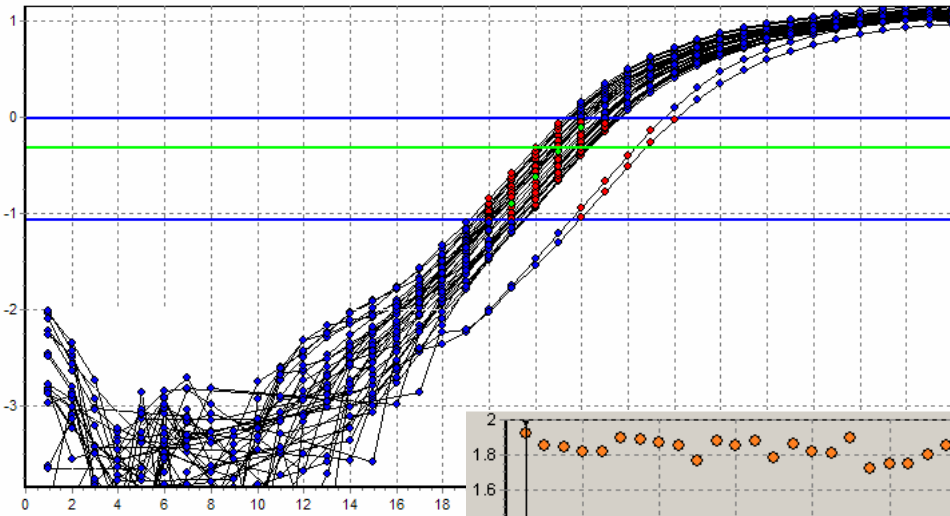
baseline error → error in ratio



in summary

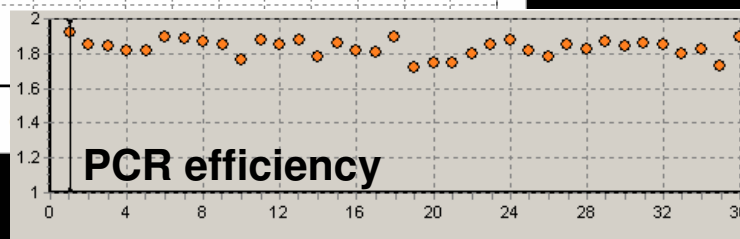
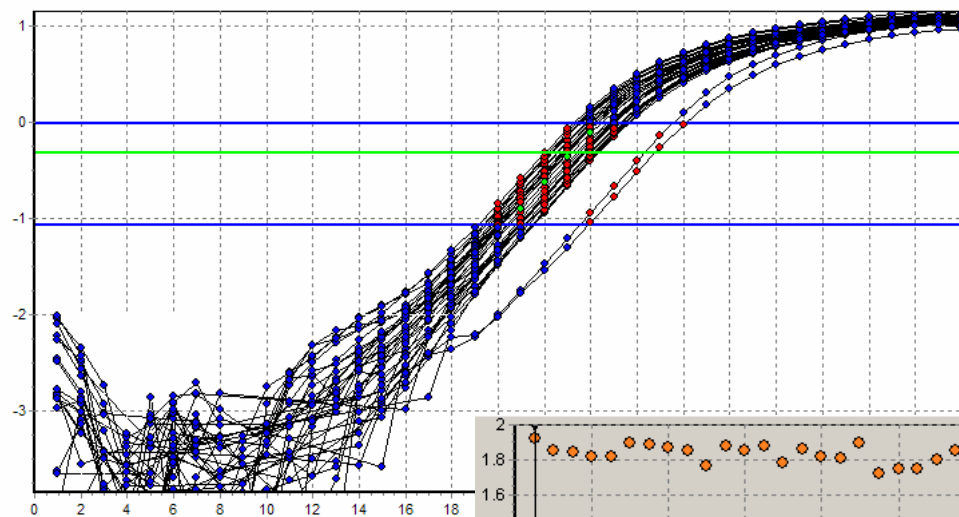


PCR system baseline (trend through cycle 3-10)



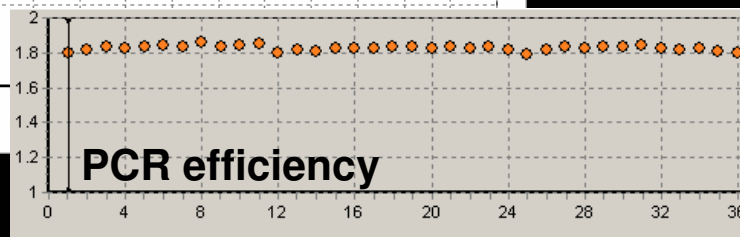
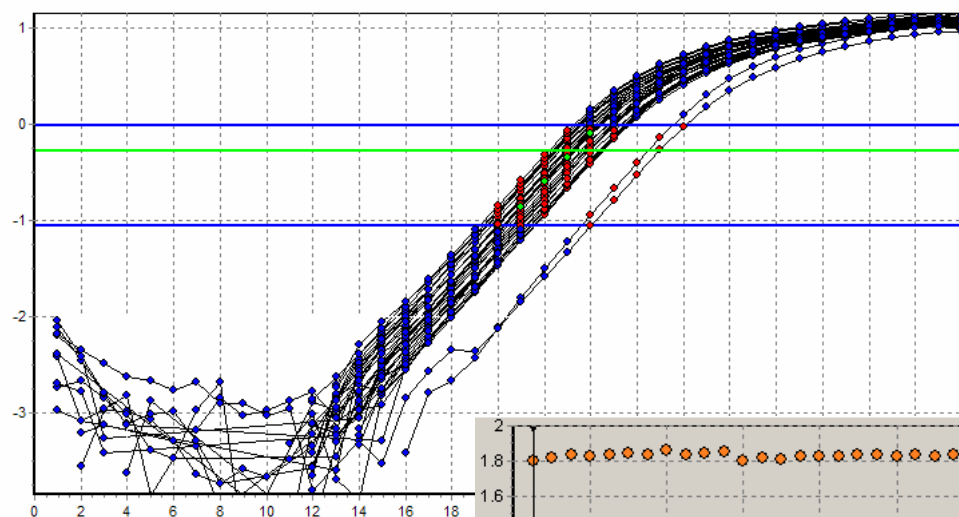
$E_{mean, amplicon}$

PCR system baseline (trend through cycle 3-10)

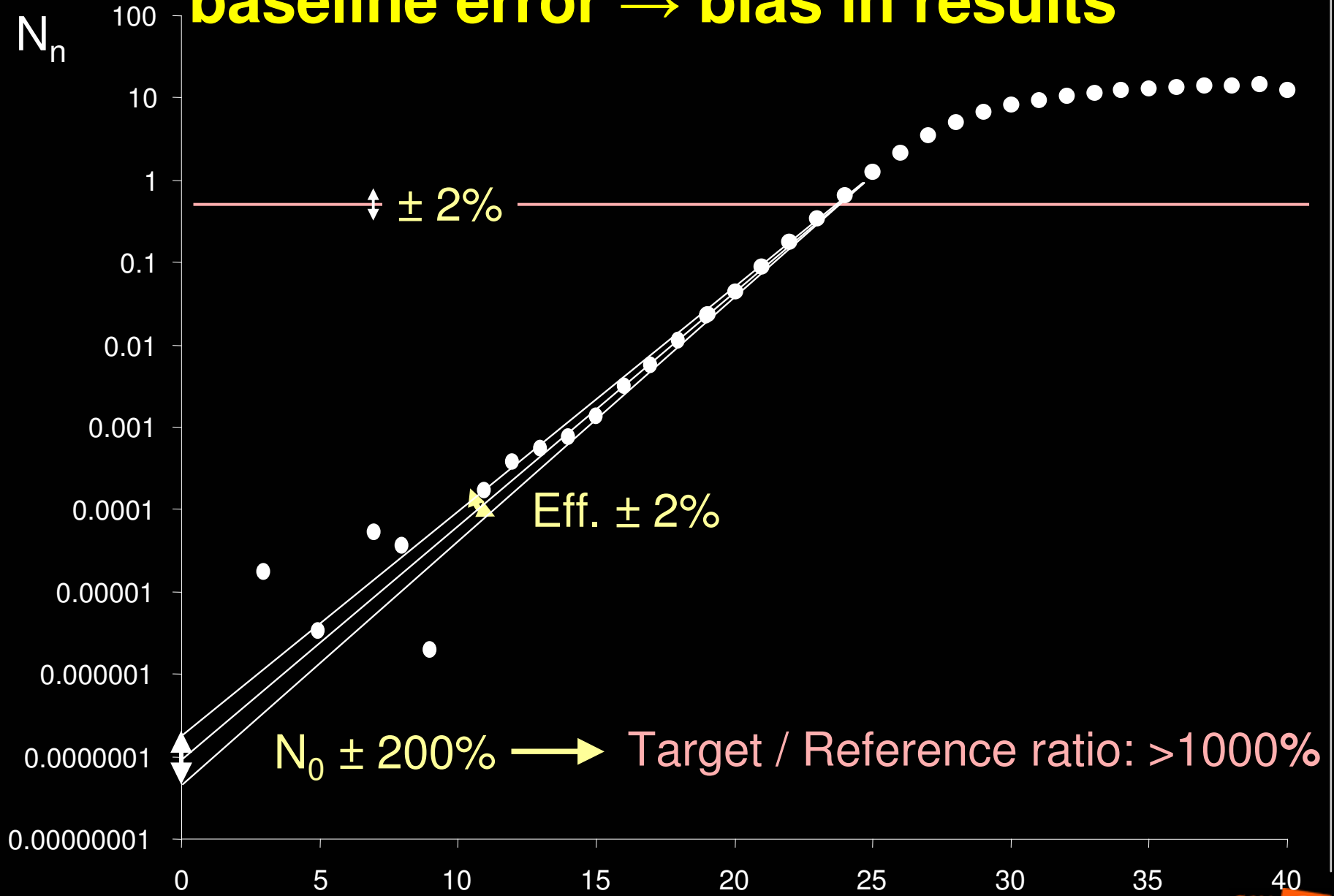


LinRegPCR 10.x (Nucleic Acids Research, 2009)

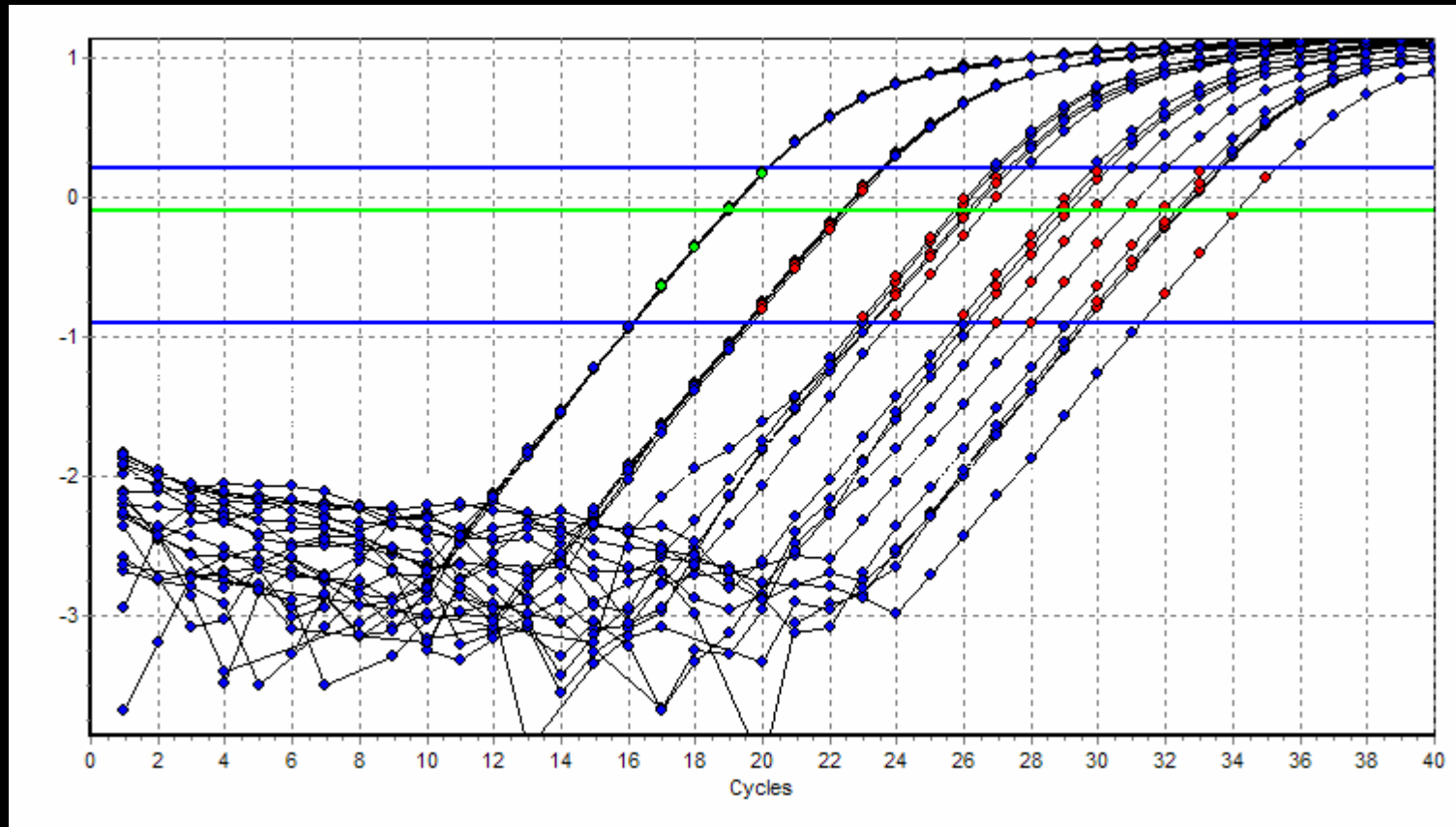
$$N_0 = N_t / E_{mean, amplicon}^{C_{t, sample}}$$



baseline error → bias in results

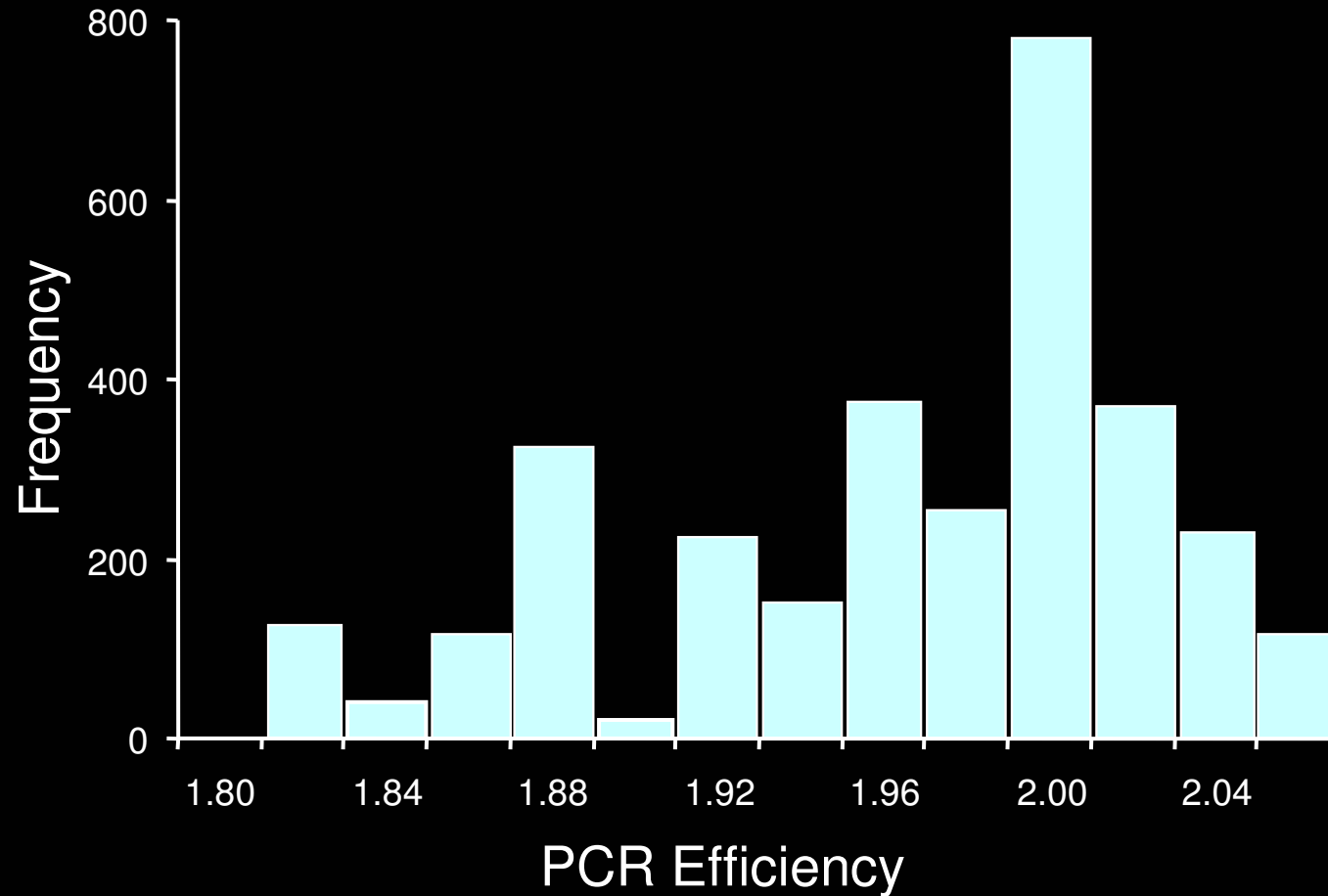


efficiency derived from a standard curve



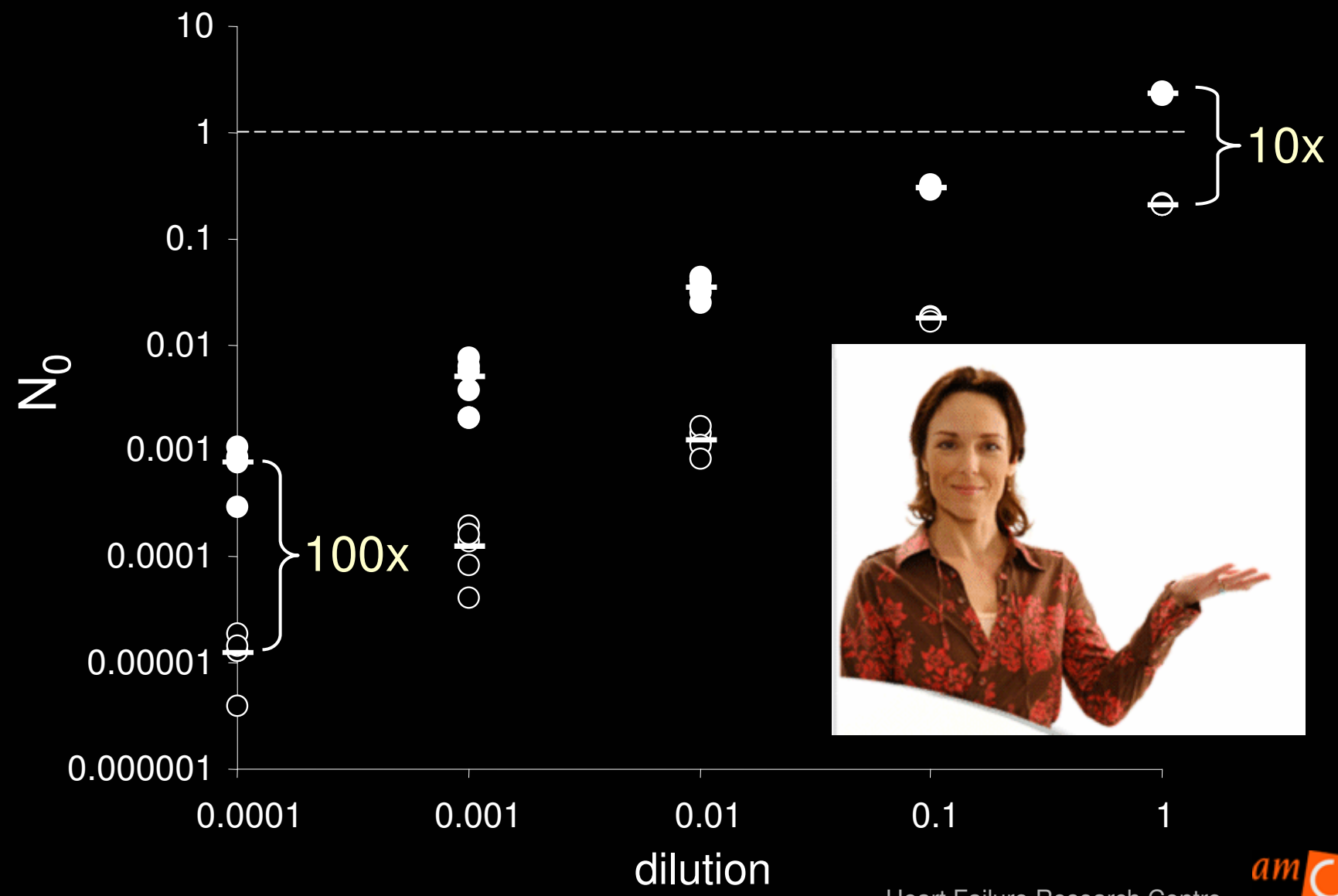
5 dilutions
5 replicates per dilution } 3125 standard curves

efficiencies derived from a standard curve

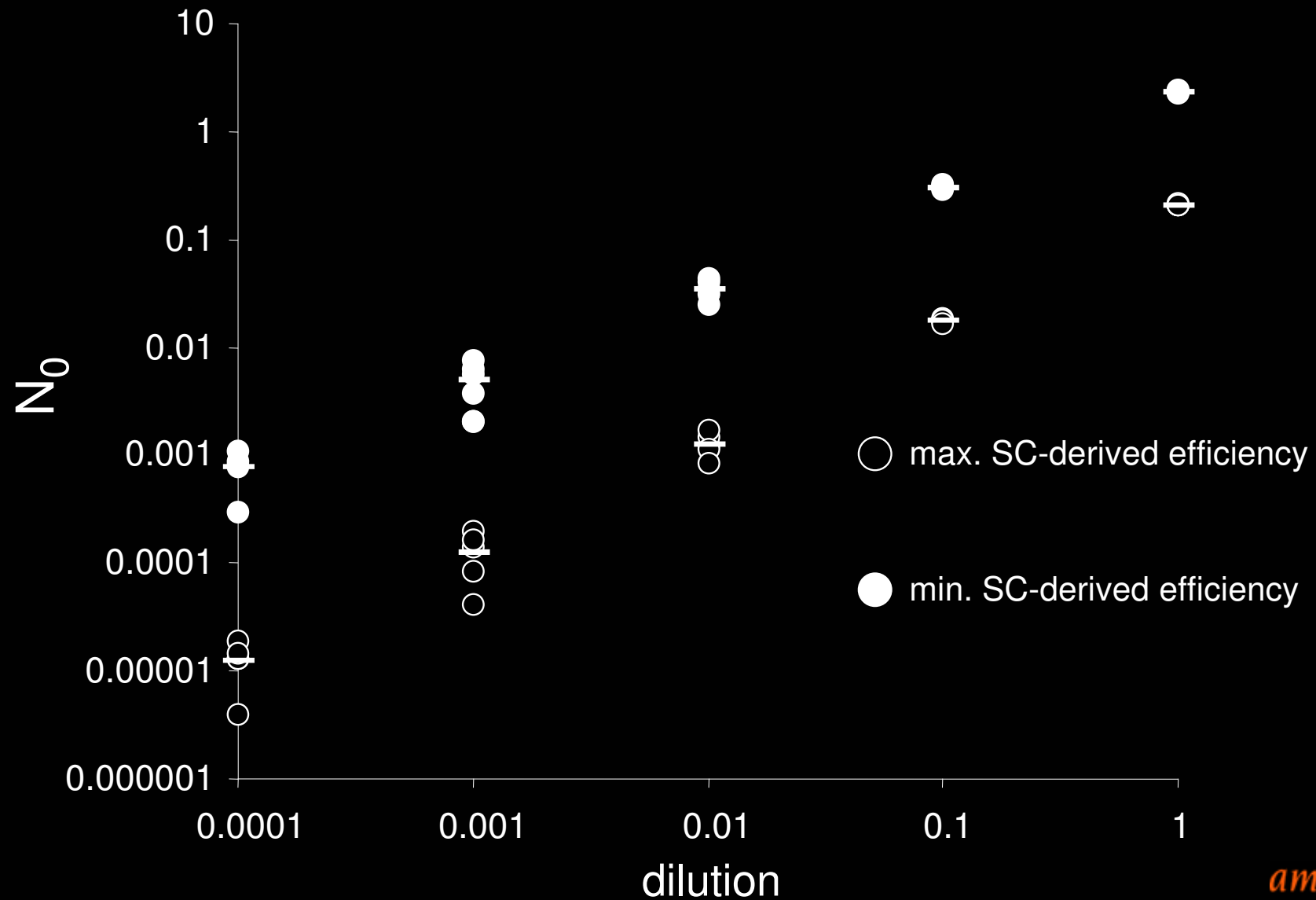


5 dilutions
5 replicates per dilution } 3125 standard curves

bias from using the wrong efficiency



bias from using the wrong efficiency



unbiased N_0 with mean efficiency

