Principles of Electronic Nanobiosensors

Unit 4: Selectivity

Lecture 4.3: When all else Fails, Tag, Filter and Amplify

By Muhammad A. Alam
Professor of Electrical and Computer Engineering
Purdue University
alam@purdue.edu

Outline

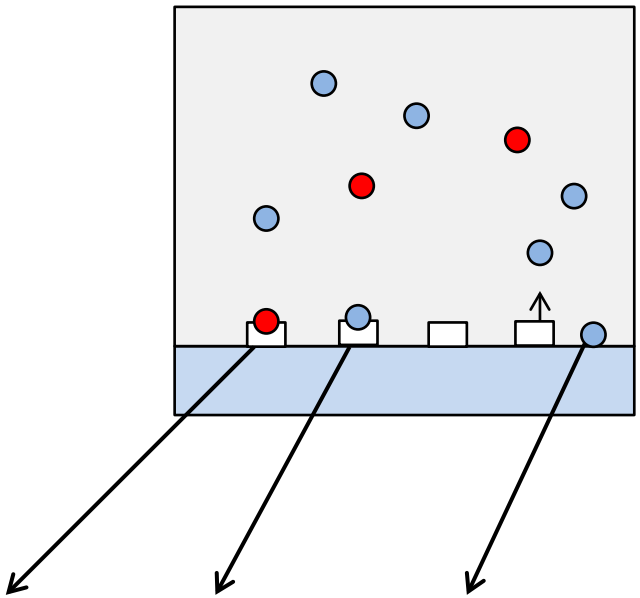
- 'Improve' the signal for improved selectivity
- Lysing the cell
- Mechanics of PCR amplification
- Reducing parasitic signal by tagging
- Conclusions

Classical approach to selectivity

Recall $$\frac{dN_T}{dt} = k_F\left(N_0 - N_T\right)\rho_s - k_R N_T,$$

At steady-state

$$D\nabla^2\rho = 0$$



$$N_T\left(t \to \infty\right) = \frac{k_T N_0 \rho_T}{k_T \rho_T + 1} \Rightarrow N_0$$

$$k_T \equiv \frac{k_F}{k_R} \to \infty$$ Full absorption

Competitive binding at steady state

$$N\left(t \to \infty\right) = N_T + N_T^{'} + N_{Geom} = \frac{k_T N_0 \rho_T}{k_T \rho_T + 1} + \frac{k_T^{'} N_0 \rho_T^{'}}{k_T^{'} \rho_T^{'} + 1} + \frac{k_p N_p \rho_p}{k_p \rho_p + 1}.$$

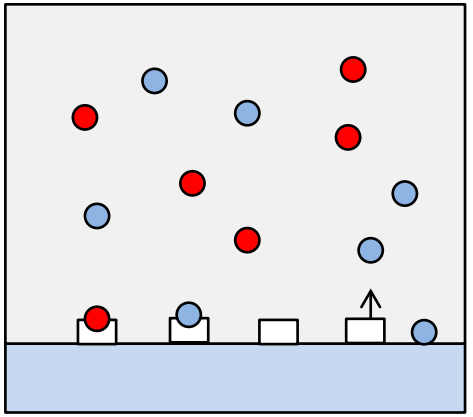
Increase the signal

Competitive binding at steady state

$$N\left(t \to \infty\right) = N_T + N_T^{'} + N_{Geom}$$

$$= \frac{k_T N_0 \rho_T}{k_T \rho_T + 1} + \frac{k_T^{'} N_0 \rho_T^{'}}{k_T^{'} \rho_T^{'} + 1} + \frac{k_p N_p \rho_p}{k_p \rho_p + 1}.$$

$$D\nabla^2\rho = 0$$



$$\alpha = \frac{N_T}{N_T + N_T^{'} + N_{Geom}}.$$

$$\beta = \frac{N_T^{'} + N_{Geom}}{N_T + N_T^{'} + N_{Geom}}.$$

$$\begin{pmatrix} \alpha+\beta \\ \beta \\ \beta \\ \alpha+\beta \end{pmatrix} = \begin{bmatrix} \alpha & \beta & \beta & \beta \\ \beta & \alpha & \beta & \beta \\ \beta & \beta & \alpha & \beta \\ \beta & \beta & \beta & \alpha \end{bmatrix} \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \end{pmatrix}$$

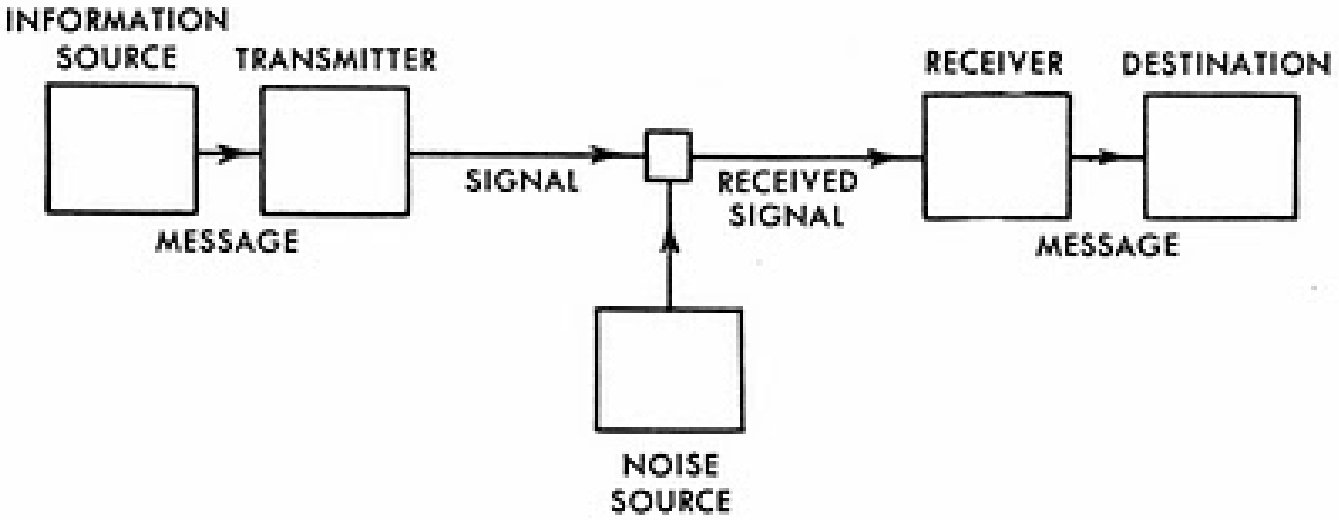
Selectivity: A problem of Information theory?

DNA sequence
[1001]

Parasitic molecules
Homopolymers
Sensor noise

Sensor output

$[\alpha+\beta, \beta, \beta, \alpha+\beta]$



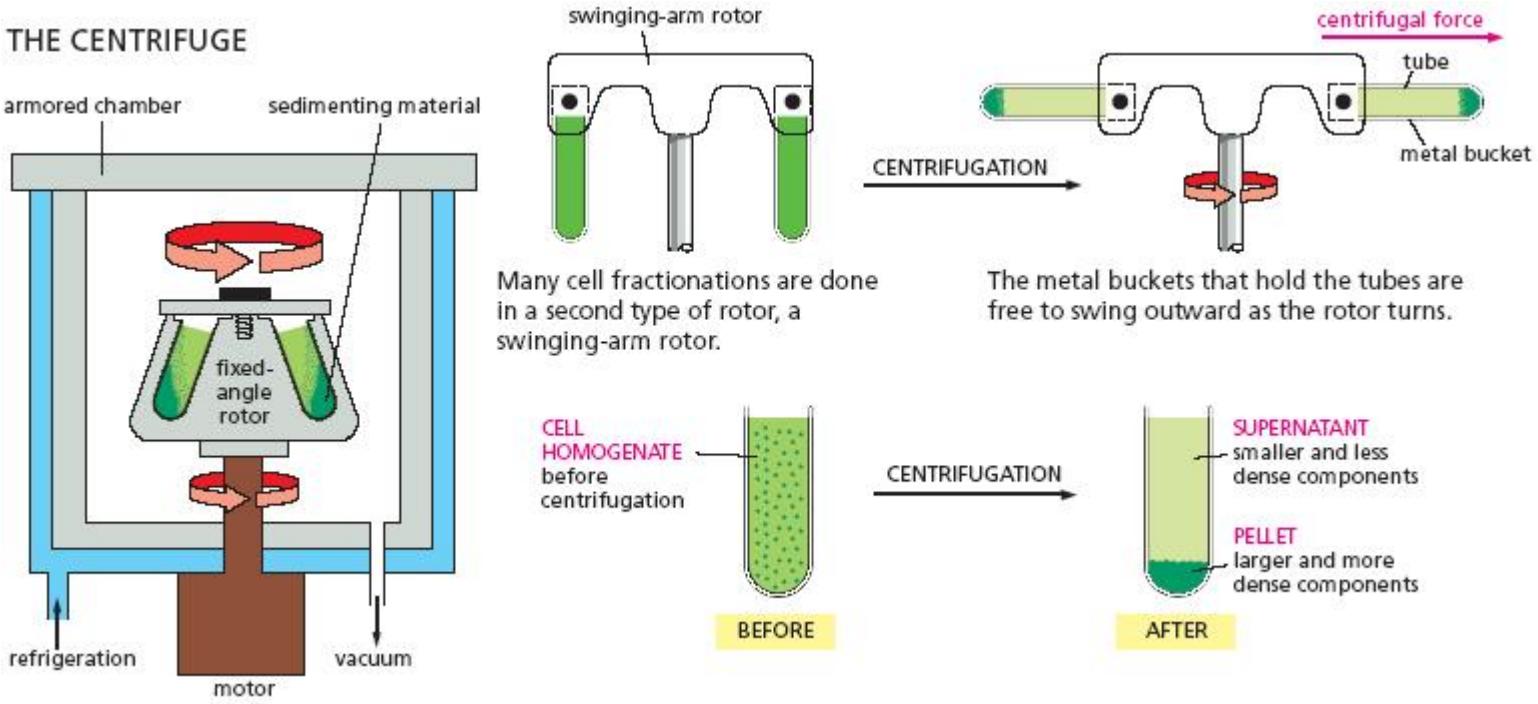
Better S/N ratio by increasing signal strength (PCR), resampling, or by suppressing the noise by tagging

Outline

- 'Improve' the signal for improved selectivity
- Origin of signal and noise: Lysing the cell
- Mechanics of PCR amplification
- Reducing parasitic signal by tagging
- Conclusions

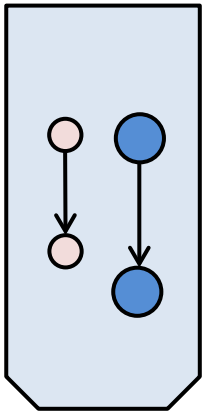
Centrifuge improves SNR

http://cellbiologyolm.stevegallik.org/node/74



Centrifuge differentiates components by mass

Centrifuge isolates components by mass: Svedberg equation



Small, but persistent drift

$$F_{down} = m'g \equiv (m - V\rho)g$$

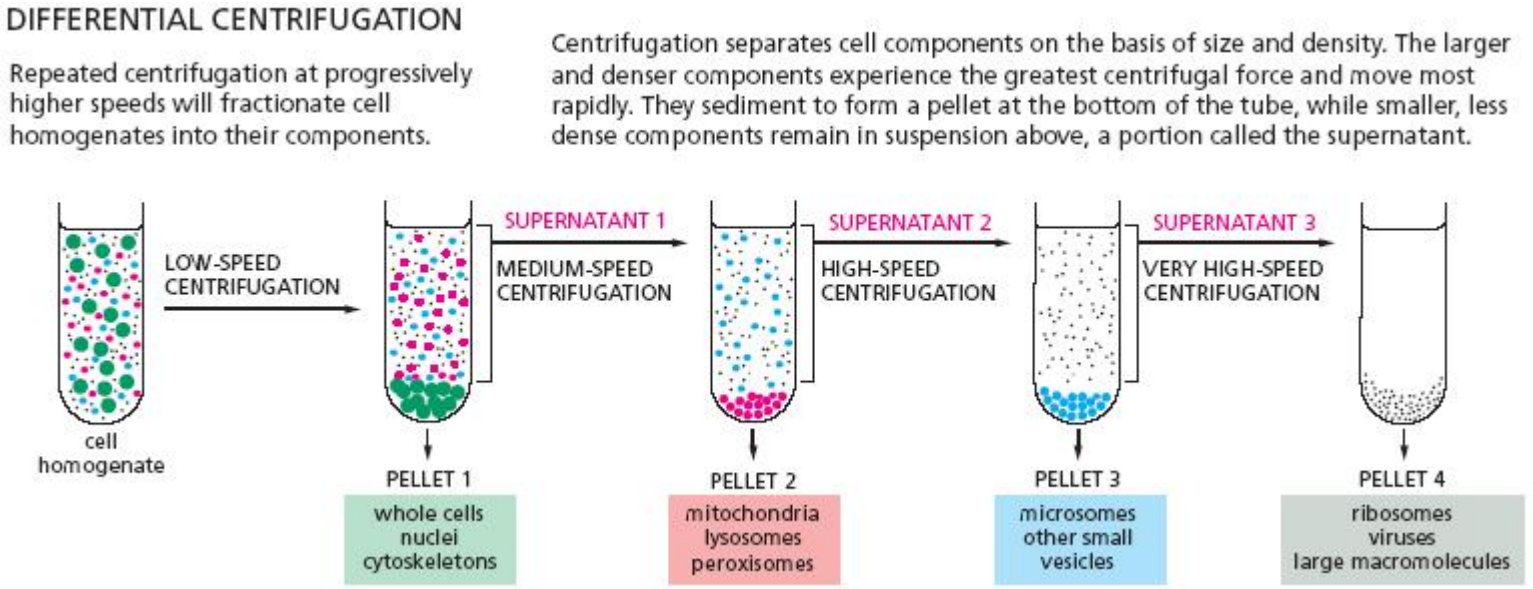
$$F_{down} = \frac{m'\upsilon_d}{\tau} = \frac{q\upsilon_d}{q\tau/m'}$$

$$= \frac{q\upsilon_d}{\mu} = \upsilon_d \frac{kT}{D}$$

$$F_{down} = m'g \equiv \upsilon_d \frac{kT}{D}$$

$$S \equiv \frac{\upsilon_d}{g} = \frac{m'D}{kT}$$

The final step of differentiation

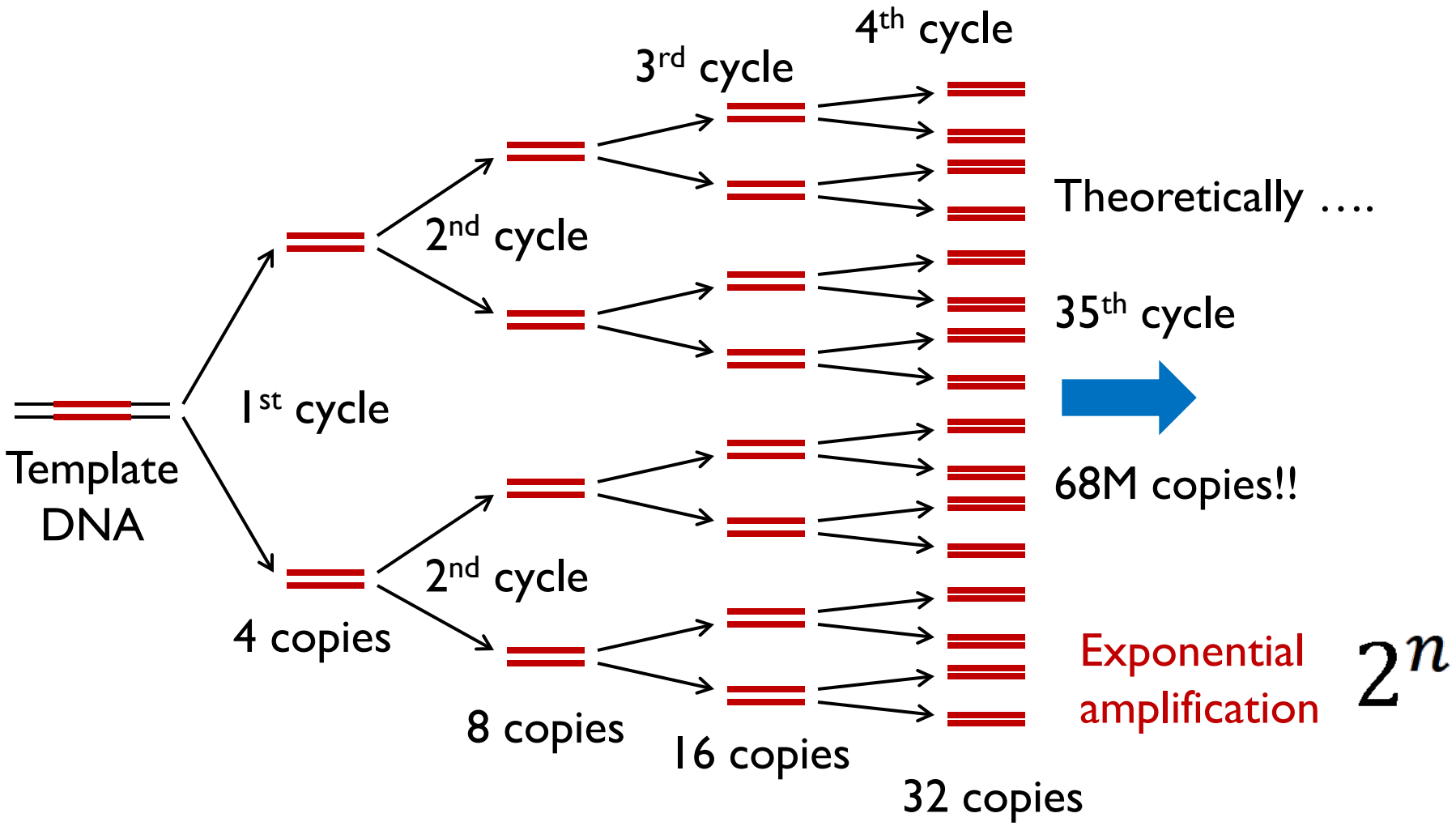


http://cellbiologyolm.stevegallik.org/node/74

Outline

- 'Improve' the signal for improved selectivity
- Origin of signal and noise: Lysing the cell
- Mechanics of PCR amplification
- Reducing parasitic signal by tagging
- Conclusions

Idealized Polymerase Chain Reaction (PCR)

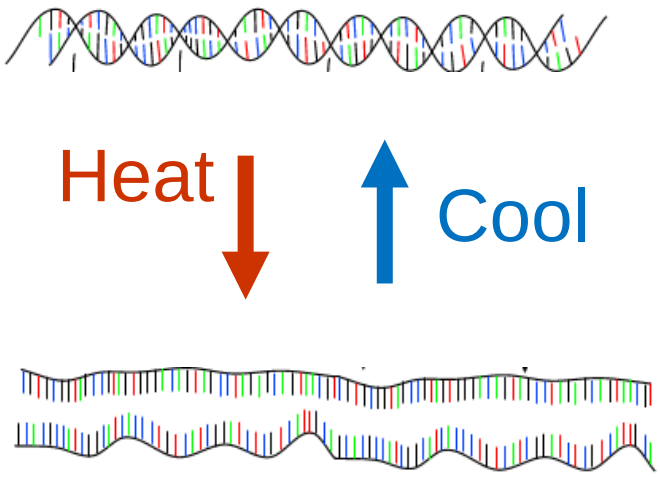


Andy Vierstraete, 1999

How PCR Doubles DNA Count

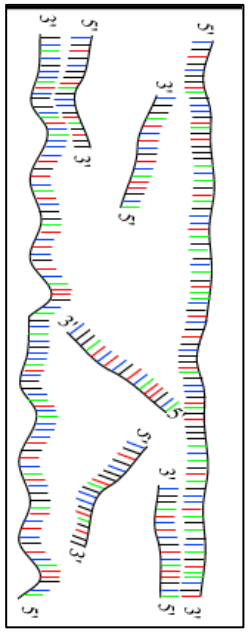
Denaturation (T~92C)

Separation of ds-DNA
2 ss-DNA's



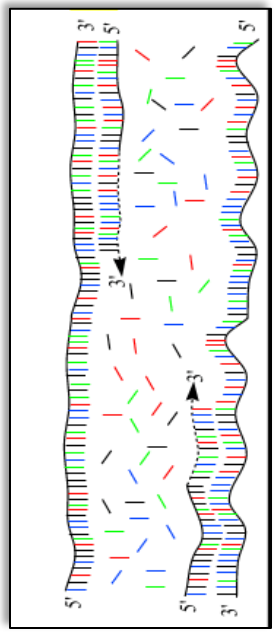
Annealing (50-65C)

Two primers comp. to the two ends attach to ss-DNA



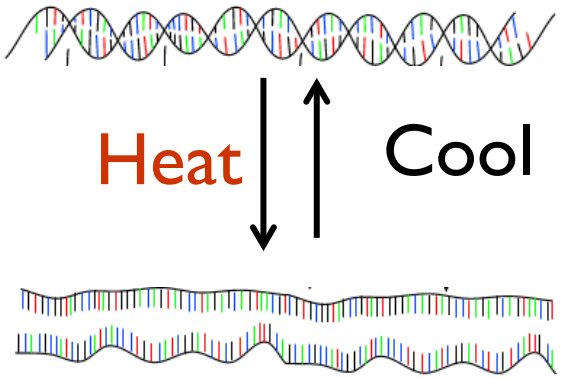
Extension (72C)

DNA polymerase extends the primer attached DNA to form 2 double stranded DNA



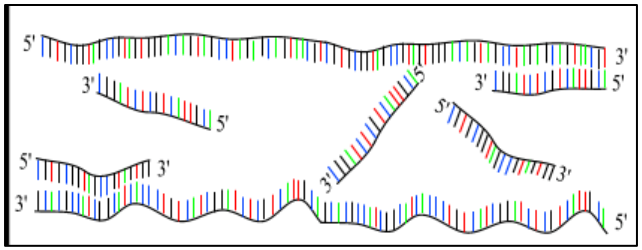
1. Denaturation

92C



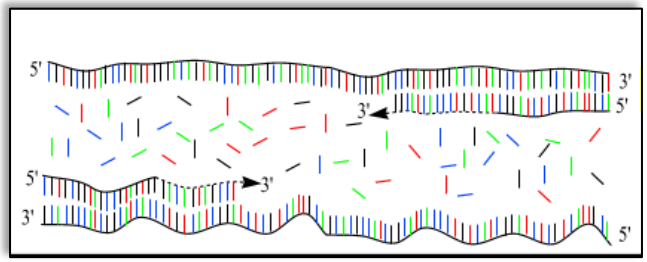
2. Annealing

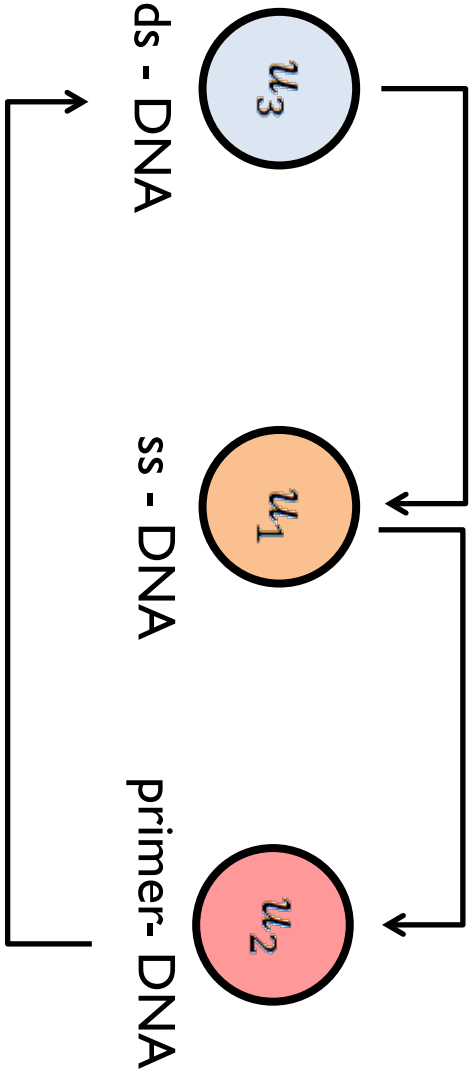
50-65C



3. Extension

72C





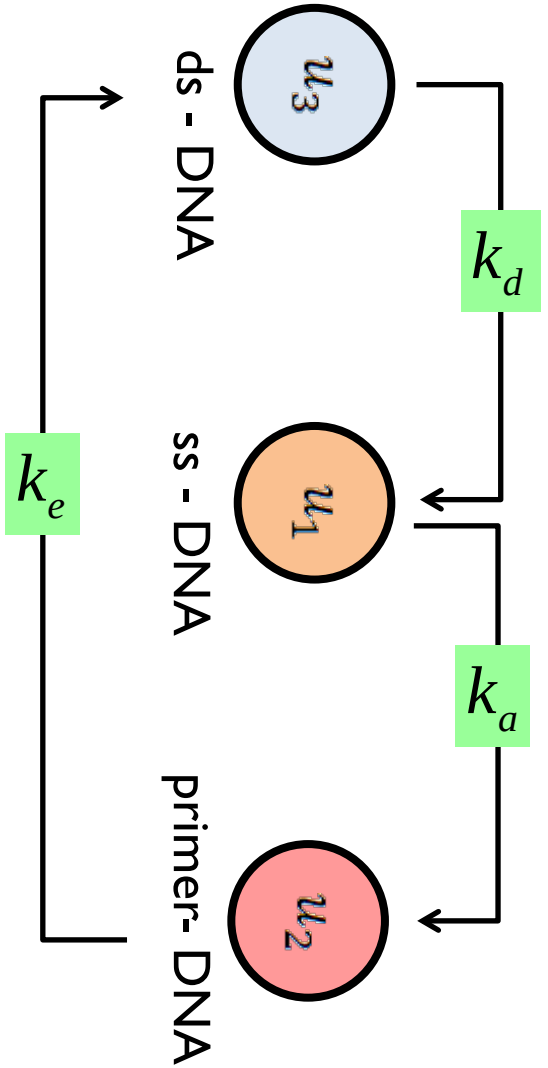
Alam, Principles of Nanobiosensors, 2013

Modeling of PCR reaction:

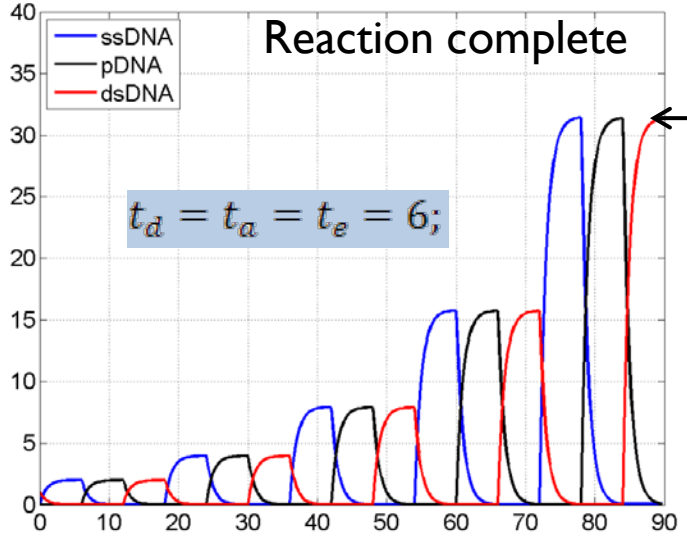
$$\frac{\partial u_3}{\partial t} = D_3 \nabla^2 u_3 - k_d u_3 + 2k_e u_2$$

$$\frac{\partial u_2}{\partial t} = D_2 \nabla^2 u_2 - k_e u_2 + k_a u_1$$

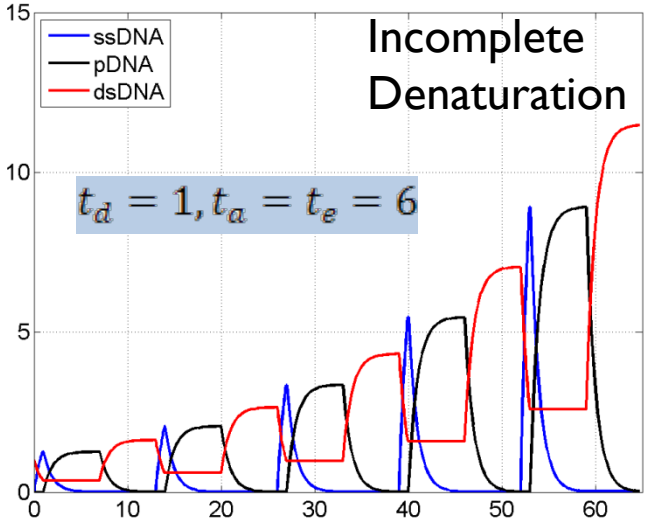
$$\frac{\partial u_1}{\partial t} = D_1 \nabla^2 u_1 - k_a u_1 + 2k_d u_3$$



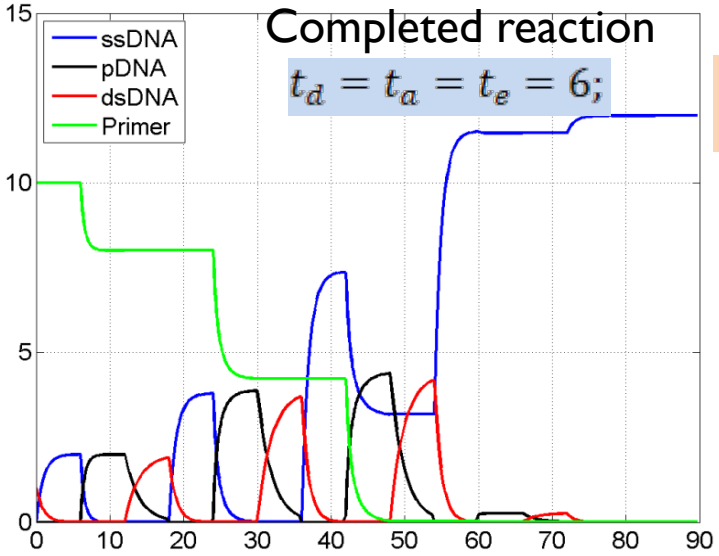
$k_d = 1\ , k_a = 1, k_e = 1/2$

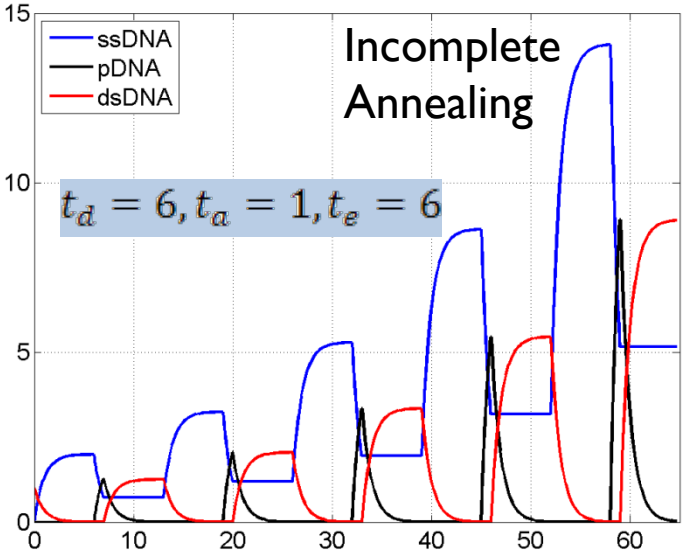


2^5=32



12





Outline

- 'Improve' the signal for improved selectivity
- Lysing the cell
- Mechanics of PCR amplification
- Reducing parasitic signal by tagging
- Conclusions

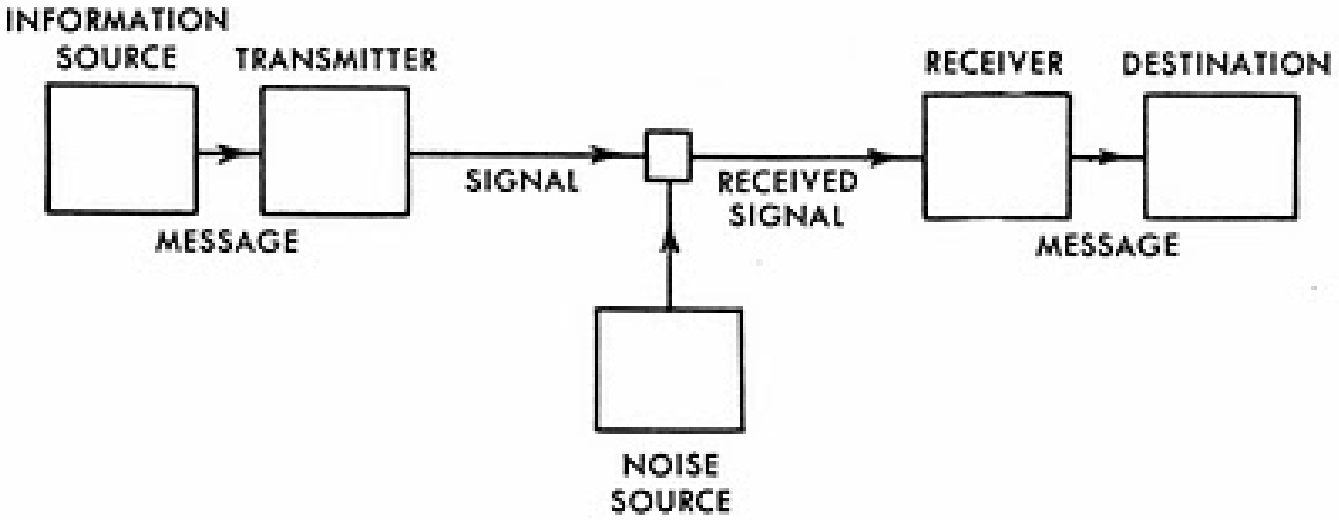
Selectivity: A problem of Information theory?

DNA sequence
[1001]

Parasitic molecules
Homopolymers
Sensor noise

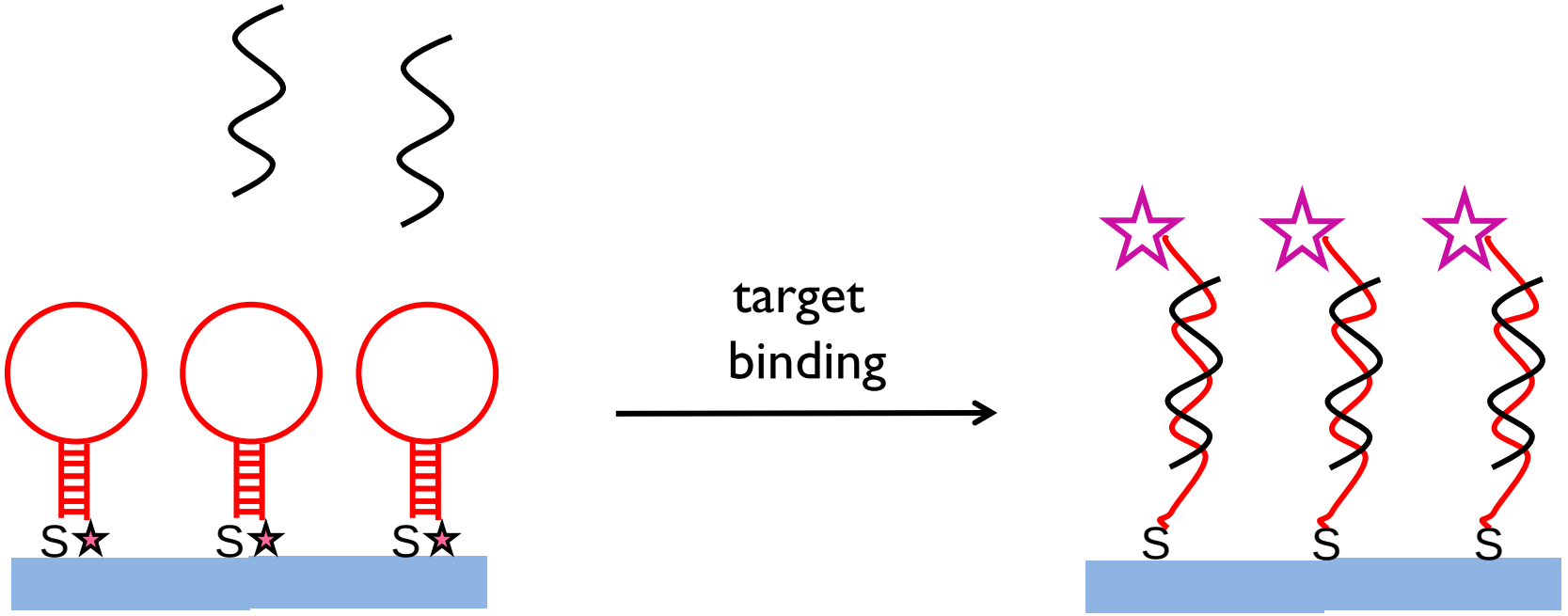
Sensor output

$[\alpha+\beta, \beta, \beta, \alpha+\beta]$

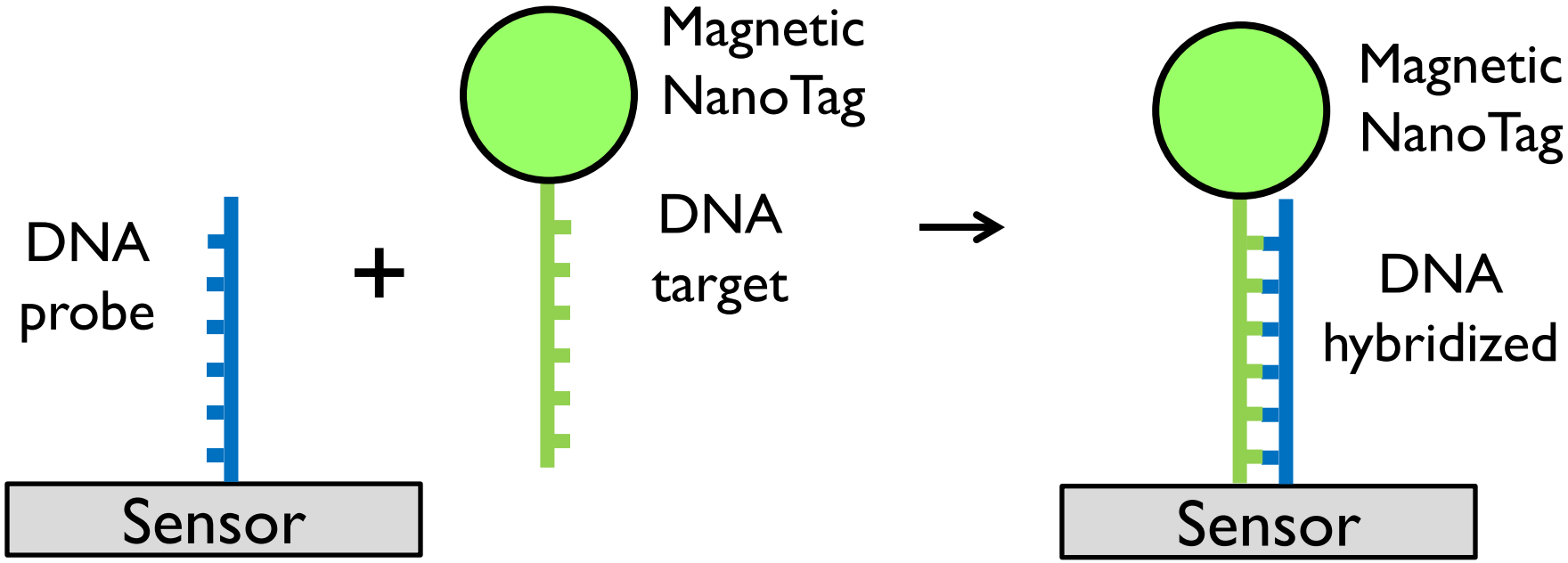


Better S/N ratio by increasing signal strength (PCR), resampling, or by suppressing the noise by tagging

Reduce noise: Optical tag-based sensing



Magnetic tag-based sensing



Most biomolecules do not carry magnetic moment

Conclusions

- The selectivity problem is best viewed as a problem of information communication and one can borrow techniques from information theory.
- SNR is improved by either amplifying the signal over noise, or by repeatedly sending the same message. PCR focus on the first approach, while resampling in genome sequencer follow the second approach.
- SNR can be also improved by suppressing noise. Optical and magnetic tagging use this approach – because most parasitic molecules do not luminesce nor carry a strong magnetic moment.

References

- DNA extraction: http://learn.genetics.utah.edu/content/labs/extraction/
- PCR: http://learn.genetics.utah.edu/content/labs/pcr/
- Emulsion PCR: http://www.youtube.com/watch?v=u2JSiyoInwo
- UTAH virtual labs(VIRTUAL LABS tab): http://learn.genetics.utah.edu/