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

\[
N_T \left( t \rightarrow \infty \right) = \frac{k_T N_0 \rho_T}{k_T \rho_T + 1} \Rightarrow N_0
\]

\[
k_T \equiv \frac{k_F}{k_R} \rightarrow \infty \quad \text{Full absorption}
\]

Competitive binding at steady state

\[
N \left( t \rightarrow \infty \right) = N_T + N'_T + N_{Geom} = \frac{k_T N_0 \rho_T}{k_T \rho_T + 1} + \frac{k'_T N_0 \rho'_T}{k'_T \rho'_T + 1} + \frac{k_p N_p \rho_p}{k_p \rho_p + 1}.
\]

At steady-state

\[
D \nabla^2 \rho = 0
\]
Increase the signal

Competitive binding at steady state

\[ N(t \to \infty) = N_T + N_T' + N_{\text{Geom}} \]

\[ = \frac{k_T N_0 \rho_T}{k_T \rho_T + 1} + \frac{k_T' N_0 \rho_T'}{k_T' \rho_T' + 1} + \frac{k_p N_p \rho_p}{k_p \rho_p + 1}. \]

\[ \alpha = \frac{N_T}{N_T + N_T' + N_{\text{Geom}}}. \]

\[ \beta = \frac{N_T' + N_{\text{Geom}}}{N_T + N_T' + N_{\text{Geom}}}. \]

\[ \begin{pmatrix} \alpha + \beta \\ \beta \\ \beta \\ \alpha + \beta \end{pmatrix} = \begin{pmatrix} \alpha & \beta & \beta & \beta \\ \beta & \alpha & \beta & \beta \\ \beta & \beta & \alpha & \beta \\ \beta & \beta & \beta & \alpha \end{pmatrix} \begin{pmatrix} 1 \\ 0 \\ 0 \\ 1 \end{pmatrix} \]
Selectivity: A problem of Information theory?

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

Alam, Principles of Nanobiosensors, 2013
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Centrifuge improves SNR

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

Centrifuge differentiates components by mass
Centrifuge isolates components by mass: Svedberg equation

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

\[ F_{down} = \frac{m' \nu_d}{\tau} = \frac{q \nu_d}{q \tau / m'} \]
\[ = \frac{q \nu_d}{\mu} = \nu_d \frac{kT}{D} \]

Small, but persistent drift

\[ S \equiv \frac{\nu_d}{g} = \frac{m' D}{kT} \]
The final step of differentiation

Differential Centrifugation

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, a portion called the supernatant.

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Idealized Polymerase Chain Reaction (PCR)

Template DNA

1st cycle

2nd cycle

3rd cycle

4th cycle

4 copies

8 copies

16 copies

32 copies

68M copies!!

Exponentially ....

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
   92°C

2. Annealing
   50-65°C

3. Extension
   72°C

Alam, Principles of Nanobiosensors, 2013
Modeling of PCR reaction:

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

\[
\frac{\partial u_2}{\partial t} = D_2 \nabla^2 u_2 - k_e u_2 + k_d u_1
\]

\[
\frac{\partial u_1}{\partial t} = D_1 \nabla^2 u_1 - k_a u_1 + 2k_d u_3
\]
\[ k_d = 1, k_a = 1, k_e = 1/2 \]

\[ 2^5 = 32 \]

\[ t_d = t_a = t_e = 6; \]

\[ t_d = 1, t_a = t_e = 6 \]

\[ t_d = 6, t_a = 1, t_e = 6 \]
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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

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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=u2JSiyolnwo

• UTAH virtual labs (VIRTUAL LABS tab):
  http://learn.genetics.utah.edu/