Take two assays and don’t call me in the morning

Andrew D. Ellington
Fraser Professor of Biochemistry
Center for Systems and Synthetic Biology
University of Texas at Austin
While "the rabbit died" became a common way to announce a pregnancy after being popularized by Lucille Ball on a 1952 episode of "I Love Lucy", injecting a rabbit with a pregnant woman's urine will not kill the rabbit. Researchers did inject female rabbits and other animals with pregnant women's urine throughout the 20th century in an effort to discern pregnancy. They theorized that a chemical in a pregnant woman's urine, known as human chorionic gonadotropin (hCG), would stimulate the rabbit's ovaries. Unfortunately for the rabbit, the quickest way to see whether or not her ovaries were affected by the urine was to kill and dissect her.

Read more: Homemade Pregnancy Tests | eHow.com http://www.ehow.com/about_5390948_homemade-pregnancy-tests.html#ixzz1tQjG8weS
Of course, what the rabbits were really measuring was human chorionic gondotropin, a hormone induced early in pregnancy.
And if rabbits could measure it, surely we could, too? If only someone could figure out ... how?

Monoclonal antibody technology

Materials science

This is where Dr. Ian Richards, amongst others, comes in. Ian in the end made a very complex task ... very simple.

Social forces
What Ian did was to take a laboratory assay for hCG that was cumbersome, and make a complex device that was simple in design and use.
And this is the device, updated, that is still used today.
And now, we are finally to the point of the talk:

If we can measure hCG easily, why can’t we, and why don’t we, measure lots of other things in a similar manner?

Drugs of abuse

Bacteria of nastiness

Well, we do, sort of. It’s possible, but it hasn’t really caught on.
What Ian reminded me of, was that there was a social revolution going on in parallel with the scientific one.

We go from women not being trusted to make decisions that a doctor ‘should’ make, to ....

And here’s where my research comes in .....
Multidrug-resistant tuberculosis
There are nearly half a million new MDR-TB cases a year worldwide.

MDR-TB among new TB cases 1990-2007

- Less than 3%
- 3%-6%
- Greater than 6%
- No data

Highest Azerbaijan 22%

© 2009 MCT
Source: World Health Organization
A different woman, a different social revolution: monitoring drug resistant tuberculosis in Afghanistan

- Collect slide material
- Boil, centrifuge, collect supernatant
  \textit{---Phenol chloroform extraction}
- Nested PCR of \textit{rpoB}
- Sequencing
- Analysis

- Gain a picture of the extent of rifampin resistance in primary tuberculosis isolates.
- Identify relative frequency of resistance-conferring mutations to set up interpretation of new resistance tests.
- Determine geographical distribution of resistance.
- Evaluate the feasibility of molecular mutation detection for the Afghanistan National Tuberculosis Program, utilizing the existing infrastructure as an alternative to phenotypic susceptibility testing.
Sputum from Afghanistan

Clean, number, and select slides
Sequencing to identify drug resistance

<table>
<thead>
<tr>
<th>Afghan Cases</th>
<th>Slides Received</th>
<th>Slides Chosen</th>
<th>Samples Amplified</th>
<th>Resistant Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>~5000</td>
<td>1120</td>
<td>511</td>
<td>150</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Number(Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCG531 TTG</td>
<td>13(76)</td>
</tr>
<tr>
<td>TCG531 TGG</td>
<td>2(11.8)</td>
</tr>
<tr>
<td>CAC526 CGC</td>
<td>1(5.9)</td>
</tr>
<tr>
<td>TCG522 TTG</td>
<td>1(5.9)</td>
</tr>
</tbody>
</table>

So, we’ve gone from pregnancy tests of convenience, to life-or-death decisions where medical care is sparse ... but the story gets weirder (this is Austin, after all): ...
How to make computers with carbon, rather than silicon

Erik Winfree

Niles Pierce

Peng Yin

Zhang et al. (2007) Science 318:1121
Xi Chen, brilliant and driven graduate student

Grace Eckhoff, brilliant and compassionate undergraduate

(And this, my friends, is why you go to the University of Texas at Austin, rather than the University of Online)
Only one huge problem: not nearly sensitive enough.
A brief scientific digression, so you know what I’m talking about:

Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly
Catalyzed hairpin assembly

H1

1 2 3 4 3* 2* 5

H2

4 2* 3* 4* 3

1* 2* 3*
Catalyzed hairpin assembly

Overall reaction
Adaptation of catalytic hairpin assembly to detection

- Multiple different ways to look at the same reaction.
- Modularity of adaptation to platforms.
- 100-fold amplification (still not good enough!)
- A little slow (1/min turnover)
If one reaction isn’t good enough, maybe we can stack them, just like electronic circuits?
Problem: background leakage from misformed hairpins ➔
Get rid of the mis-formed material!

(Credit: Neima Briggs)

(Credit: Jeremy McLain)
Results – 1,000x amplification achieved with extensively purified DNA


Output
- 100 nM
- 5 nM (@5pM catalyst)
- 0 nM

(Credit: Neima Briggs)
Eliminate impurities with arge-scale (>1 nmole), enzymatic oligonucleotide synthesis

Table 1. Estimated cost for each batch of DNA hairpin

<table>
<thead>
<tr>
<th>Step</th>
<th>Major costly materials</th>
<th>Amount</th>
<th>Cost</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>DNA polymerase (Pfu-Sso7d or Vent)</td>
<td>0.003 mg</td>
<td>$0.03</td>
<td>home-made¹</td>
</tr>
<tr>
<td></td>
<td>dNTPs</td>
<td>1 mg each nucleotide</td>
<td>$2</td>
<td>Chem-Implex Intl.</td>
</tr>
<tr>
<td>Restriction digestion</td>
<td>EcoRV-HF or PvuII-HF</td>
<td>0.001 mg</td>
<td>$0.01</td>
<td>home-made¹</td>
</tr>
<tr>
<td>Nicking</td>
<td>Nt.BstNBI</td>
<td>0.001 mg</td>
<td>$0.01</td>
<td>home-made¹</td>
</tr>
<tr>
<td>Strand displacement</td>
<td>Vent</td>
<td>0.01 mg</td>
<td>$0.1</td>
<td>home-made¹</td>
</tr>
<tr>
<td>Other cost</td>
<td>Buffers, tubes, depreciation of equipments, etc</td>
<td>N/A</td>
<td>~$2</td>
<td>N/A</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>~$4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ We assume 1 L E.coli culture can yield at least 1 mg of protein of interest. Major costly materials in protein purification include LB broth (~$2 for 20 g), Ni-NTA beads (~$2.5 for 1 mL, considering re-use for at least 4 times), and other chemicals including antibiotics, IPTG, BME, imidazole (~$5 total). These add up to $10. Therefore we use a standard rate of $10 per mg protein of interest when estimating cost.

Cost / reaction = 5 cents!
Unprecedented purity = unprecedented amplification

Update: two-stage, 10^6-fold amplification!

Leakage is much more manageable.
Brief intermission:

- Great idea, non-enzymatic amplifiers that recognize sequence. Except no amplification.

- So, make a different mousetrap, 100-fold amplification.

- And stack the mousetraps (painful analogy?), 1,000-fold amplification.

- And optimize preparation, 10,000-fold amplification.

- And stage the background, 1,000,000-fold amplification.

- You know what comes next.

STILL NOT GOOD ENOUGH

- But only because it’s too slow, sigh. Back to the drawing board.
When life gives you lemons ... the heck with lemonade, cheat

- **Use enzymes, but in a convenient format, LAMP**
  Amplification with a DNA polymerase with strand displacement activity at a constant temperature (about 65°C).

- **High amplification efficiency**
  $10^9$-$10^{10}$-fold in 15-60 minutes

- **High specificity**
  Single mismatch discrimination ability
Only one problem: it’s too good; high background, many false positives

Specific, single-stranded, loop

Detection of true versus spurious LAMP amplicons. (A) 2% agarose gel electrophoretic analysis of a LAMP reaction without betaine after 90 min. The reactions in lanes a and b were seeded with 0 and $10^5$ copies of M13Mp18, respectively. (B) CHA kinetic curves of LAMP products.
The return of paper!

Peter Allen
And finally ... good enough. That’ll do, enzyme. That’ll do.
Getting reagents to the enzymes ... flow sets the staging

Order of reagent delivery

Red binds

Most sites occupied by red

Red, Green

Green, Red

Green

Red

Null
Except ... we have to detect multiple mutations in parallel

Overlap PCR with mutated primers will be used to generate the following mutated alleles:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>Mutated codon (mutant base shown in red)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>rpoB (RNA polymerase beta subunit)</td>
<td>S531L (TCG→T[T]G)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H526Y (CAC→T[T]C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q513L (CAA→CTA)</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>katG (catalase-peroxidase)</td>
<td>S315T (AGC→A[CC])</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>rpsL (ribosomal protein S12)</td>
<td>K43R (AAG→A[GG])</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB (indolylacetylinositol arabinosyltransferase)</td>
<td>M306V (ATG→G[TG])</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA (DNA gyrase subunit A)</td>
<td>D94G (GAC→G[GC])</td>
</tr>
</tbody>
</table>

- Isoniazid, rifampicin, streptomycin and ethambutol are essential first-line antituberculosis drugs.
- Ethambutol and streptomycin are also included in the group 1 and 2 drugs for treatment of MDR-TB while fluoroquinolones are part of the reserve second-line drugs.
George Whitesides at Harvard is a very clever fellow who can make paper do many things. But Dick Crooks at Texas ... had a better idea.
Our assays on their paper

Karen Scida

A: \[ \text{(Catalyst)} \]

\[ K_{\text{cat}} > 1 \text{ min} \]

B: Adding C1

Pre-dry H1

Pre-dry H2, and Reporter duplex

C: Released Q

Reporter duplex

Folding

Sealing

Adding sample

Reacting

Opening
Improvements
Photolithography is eliminated in favor of wax printing using a $700 office printer
Device is laminated to prevent evaporation during assays and to prevent contamination
A voltmeter is used for read-out, which is both sensitive and quantitative
o-PAD 2 Fabrication and Fluidics

(a) A sheet of paper printed using an office printer (6 devices per sheet of paper)
(b) The folded and laminated o-PAD
(c) A corner is snipped off to admit the analyte solution
(d) The analyte is introduced into the fluidics
(e) The unfolded device showing the results of 3-D fluidic penetration
India has some 545 million cell phones, enough to serve about 45 per cent of the population, but only about 366 million people or 31 per cent of the population had access to improved sanitation in 2008.

If we can deliver paper, we can deliver health care.
But it’s bigger than that, and impacts us here at home, too.

Complex system diagnostics: then

Crud, it doesn’t work

And now
Complex system diagnostics: then

And now
What if ... we could just logon to get our health status?

The Traitwise Technology

- We crowd-source information by having users answer personalized questions & ask questions of their own
- By using gaming paradigms we optimize every panel to engage the user.
- New questions emerge at the top of the page to create user anticipation and eliminate page navigation
- Traitwise becomes a dialog to create a personalized experiences

Fun survey interface designed by game developers

[Full disclosure: I own equity that is worth almost $10.52]
The Traitwise Technology (continued)

- Traitwise provides instant feedback and survey results.
- The average number of questions answered using Traitwise is over 50.
- Nearly 20% of participants answer over 100 questions with a tail of distribution going out to over 2,000.

Fun and addictive!

How many people answer how many questions?

- 0-10 (Just looking)
- 10-30 (engaged)
- 30-100 (very engaged)
- 100-2,000 (addicted)
Research Using Patient Reported Outcomes

- Patient reported outcomes are being used to reduce health care costs by comparative effectiveness research
  - By a combination of screening and patient reported outcomes, researchers are identifying the treatment effectiveness
  - Patent reported research also “reveals acquired behaviors and individual responses to health programs” necessary for improved health (1)

What is the Freshman Research Initiative?

- An innovative, ground breaking, faculty initiated program
- **Developed to**
  - Tap the resources of the research university – ideas, expertise, mentorship, facilities, etc.
  - To benefit of the education of our undergraduate students.
- **Provides a research experience and all its benefits**
  - Excitement and engagement, retention, relationships, etc.
  - To students early in their careers
- **Operates at scale** – hundreds of students each year.
  - Over 7 years > 3,000 new UT students have participated
  - Again in 2012, ~ 700 students will start FRI, ~33% of the incoming Natural Sciences class
**FRI Student Timeline**

### Freshman
- **Fall**
  - Research Methods
- **Spring**
  - Stream Selection
  - Intro to lab techniques
  - Begin Research

### Sophomore
- **Fall**
  - Fellowships/Summer School
  - Head start on Fall Research
  - Independent Research
  - Mentor Research Methods
- **Spring**
  - Publish or Present
  - Peer Mentor
  - Join faculty labs, REUs, internships

---

Research Stream
Improves retention and Increases overall STEM graduation rates

- 35% more students graduate with a science or math degree if they participated in FRI.

It works!

4 Year Retention Percentages (students graduated or on track to graduate)

* 38.6% = National STEM 6-year graduation rate
### FRI student-authored publications

<table>
<thead>
<tr>
<th></th>
<th>Number of papers</th>
<th>Papers on FRI stream research</th>
<th>Number of student authors</th>
<th>Number of Risk student authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In preparation</strong></td>
<td>21</td>
<td>16</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td><strong>Submitted</strong></td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td><strong>Published or in press</strong></td>
<td>115</td>
<td>96</td>
<td>84</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>143</strong></td>
<td><strong>120</strong></td>
<td><strong>132</strong></td>
<td><strong>34</strong></td>
</tr>
</tbody>
</table>

Undergraduate student authorship is not tracked at the university. A survey of 15 faculty recognized for their undergraduate research track-records, gives a generous estimate of 2% of Chem/Biochem majors become coauthors each year. 11% of the FRI06 cohort and 9% of the FRI07 cohorts are published authors.
Re-gifting the Gates Computer Science Building ....

D.I.Y. Disease Diagnostics Stream

An extremely generous gift of Bob and Cathy O’Rear

Pradeep Ravikumar, Computer Science

POC Diagnostics!

Social Networking!

Robots!

Peter Stone, Computer Science
The future of health care?

I'm sorry, Dave, I can't let you do that. Come with me if you want to live.

Would you like to play a game?
So, what does the future look like?

- Complex diagnostics, made cheap and uploadable (is that a word?)

- Complex analyses, easy and online.

- People taking control of their own healthcare.
The folks responsible for the rainbows and unicorns:

<table>
<thead>
<tr>
<th>Grace Eckhoff, Marshall Scholar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xi Chen, Harvard Fellow</td>
</tr>
<tr>
<td>Bingling Li</td>
</tr>
<tr>
<td>Sanchita Bhadra</td>
</tr>
<tr>
<td>Peter Allen</td>
</tr>
<tr>
<td>Zack Simpson</td>
</tr>
<tr>
<td>Matt Winkler and Asuragen ERI</td>
</tr>
<tr>
<td>Jeff Taylor, John Jacob, Oscar Ayala</td>
</tr>
<tr>
<td>Dick Crooks, Karen Scida</td>
</tr>
<tr>
<td>Peter Stone, Pradeep Ravikumar</td>
</tr>
</tbody>
</table>

NIH
DARPA
Gates Foundation

The great state of Texas
(I am a state employee, something I seldom forget)

The Freshman Research Initiative!
Gwen Stovall, Research Educator;
Sarah Simmons, The Awesome!