

Transparency: The ultimate validation of antibody

- Understanding how the market forces created antibody chaos

2015 is a stormy year in Antibody world

Finding the right antibody for the job

Vivien Marx

As new research applications for antibody-based assays emerge, the quest for quality intensifies in a crowded marketplace.

Antibodies are part of the body's defense system. They latch onto intruders with specific aims, marking them for destruction. This specificity makes antibodies a versatile lab tool. Unless it's Tuesday or there's a full moon, antibody-based assays fail on too many lab benches and often no clear-cut reasons.

Scientists interviewed by Nature Methods frequently involve returning the antibody to the supplier. Antibody companies tend to be untroubled about this practice. Money back guarantees and no questions asked exchanges of credits for buyers are common in this competitive marketplace.

As they address the expanding needs of researchers, antibody companies are refining how they help researchers find the right antibody for their purposes, increasing interactions with customers and adding validation methods. Separately, scientists who have learned hard truths about antibody shoppers.

Variability factor: biology
Monoclonal antibodies are produced from cell lines, whereas polyclonal antibodies are harvested from animal serum, usually from rabbits. The animals produce antibodies in response to an injected substance, an immune reaction. Variability is almost "the nature of the business," says Jeff Xu, who works on antibodies at EMD Millipore. Antibodies from different rabbits reacting to the same immunogen have to be compared. Biological variability can explain a simultaneous absence of an antibody from the company catalog, says Roisin Kerns of

Abcam, which sells antibodies and other proteomics tools. The animal origin causes variability in several other ways. What might seem to be the same antibody with the same identifier number in a catalog is not always the same antibody. During a rabbit's lifetime, the antibody quality can change, says Mike Bennett, president of the antibody consulting firm PhosphoSolutions. Variability slips into the production process, too, when companies do not test each batch or when antibodies are purified from insufficient starting material. As Claudia Molloy from EMD Millipore explains, she and her colleagues purify large batches and use serum when the animal is producing maximum antibody litter. But when there are months between litters, the antibodies can vary slightly, he says.

The synthetic nature of monoclonal antibodies can address variability. Abcam bought the firm Epitomics last year, which sells rabbit monoclonal antibodies produced with proprietary technology. Abcam hopes these batch variations "seen with polyclonal antibodies" can reduce issues of "batch-to-batch variation," says Danica Miller, who manages the company's operations. Monoclonal antibodies are produced by immortalized cells cultured, produce anti-animal sera, theoretically, produce antibodies indefinitely, says Anna Rudnikowicz, a researcher at the University of California in San Diego. "It is so much easier when the antibodies 'are indeed exactly the same' from year to year and can explain the variability with monoclonal antibodies happens more frequently than the scientific community first assumed, says Arizona

State University researcher Barbara Koski, who recommends whole-antibody lot numbers to help track reagent reproducibility of some of their larger-scale research projects. For all human proteins, Alice Volynsky, a performance of monoclonal antibodies, says quantum antibodies accurately produce the test results. The test of quality control, says Arizona

Antibody companies use rabbits to produce antibodies.

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BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

BY MONYA BAKER



In 2006, things were looking pretty good for David Rimm, a pathologist at Yale University in New Haven, Connecticut. He had developed a test to guide effective treatment of the skin cancer melanoma, and it promised to save lives. It relied on antibodies — large, Y-shaped proteins that bind to specific molecules and can be used to flag their presence in a sample. Rimm had found a combination of antibodies that, when used to stain tumour biopsies, produced a pattern that indicated whether the patient would need to take certain harsh drugs to prevent a relapse after surgery. He had secured more than US\$2 million in funding to move the test towards the clinic.

But in 2009, everything started to fall apart. When Rimm ordered a fresh set of antibodies,

his team could not reproduce the original results. The antibodies were sold by the same companies as the original batches, and were supposed to be identical — but they did not yield the same staining patterns, even on the same tumours. Rimm was forced to give up his work on the melanoma antibody set. "We learned our lesson: we shouldn't have been dependent on them," he says. "That was a very sad lab meeting."

Antibodies are among the most commonly used tools in the biological sciences — put to work in many experiments to identify and isolate other molecules. But it is now clear that they are among the most common causes of problems, too. The batch-to-batch variability that Rimm experienced can

produce dramatically more problematic results, says Edward S. Ruthazer, a protein scientist at the University of California, San Diego. "It's a little bit risky because it takes longer to come to the answers." But scientists now take character. "Some will not be fully reproducible in the long run, without question."

Creating choices
More choice also means more time spent choosing: selecting one reagent over another or even modifying protocols.

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Calling the next generation of affinity reagents

As complements to antibodies, new reagents to target proteins invite broad types of experiments.

Scientists in two large research projects and some companies, too, are looking for new ideas. They are developing new reagents and high-throughput ways to generate them.

Money is one reason. A lab can obtain a set of bodies from a service provider by supply or over 1,000 proteins to investigate 500 proteins, that approach will break the bank in most labs, says US National Institutes of Health (NIH) Common Fund's \$54 million Protein Capture Reagents program, now in its second year.

Two NIH reagent projects, now in its targeting — 1,500 human transcription factors, many of which have a role in disease. At Sechi explains, no reagent in disease reagents exist for some of these proteins. One project, at Johns Hopkins University, is building a pipeline for monoclonal antibodies, and the other, at the University of Chicago, is focused on generating recombinant antibodies. Both groups are addressing some underestimated production bottlenecks, Sechi says. The production of the antigens is definitely one of the major challenges in this field. "The production of antigens need to be properly added, a requirement that is both technically and financially challenging to meet. The two centers will generate validated reagents for human transcription factors

to be made available through community resources such as the University of Iowa's Developmental Studies Hybridoma Bank and DNASU at Arizona State University. The four US technology hubs developing high-throughput approaches to generate new reagents are at Los Alamos National Laboratory, Arizona State University, the University of Illinois at Santa Barbara, and the University of California, San Diego. These centers have cooperative arrangements with the NIH that involve close interaction with the traditional funding mechanisms and include ongoing evaluation by external panels, Sechi says.

In the EU affinity reagents project, technology development and reagent generation are combined. "We've concentrated on establishing the pipeline, whereas NIH also has these technology centers which are looking very much to the future," says Michael Tassiss of the UK Biotechnology Institute, who coordinates the £20-million EU program with 20 teams across Europe. Affinomics is the third phase of the multi-year, EU-financed program focused on new reagents for signaling

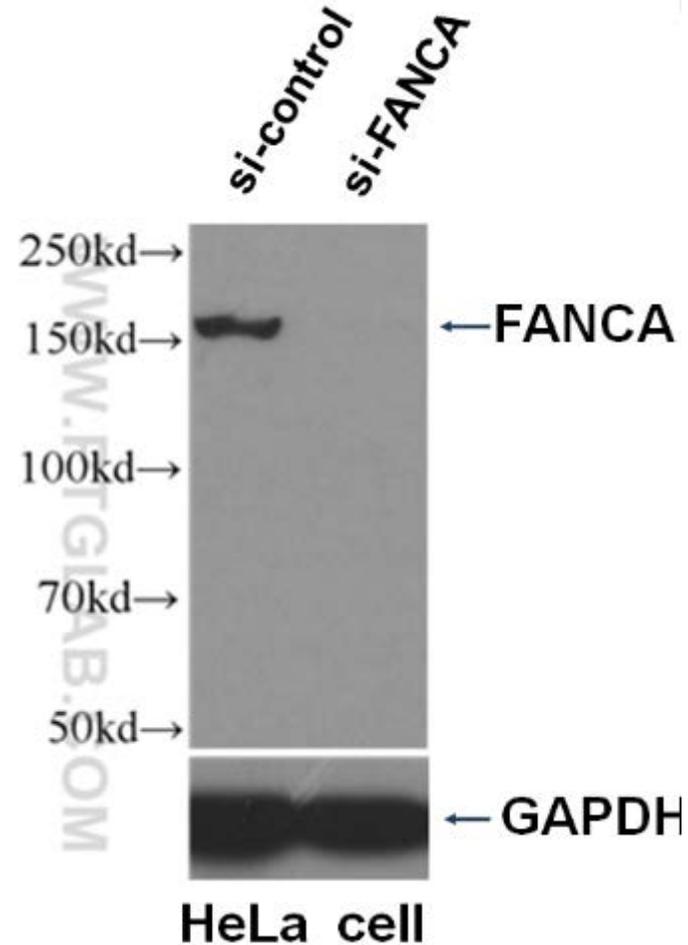
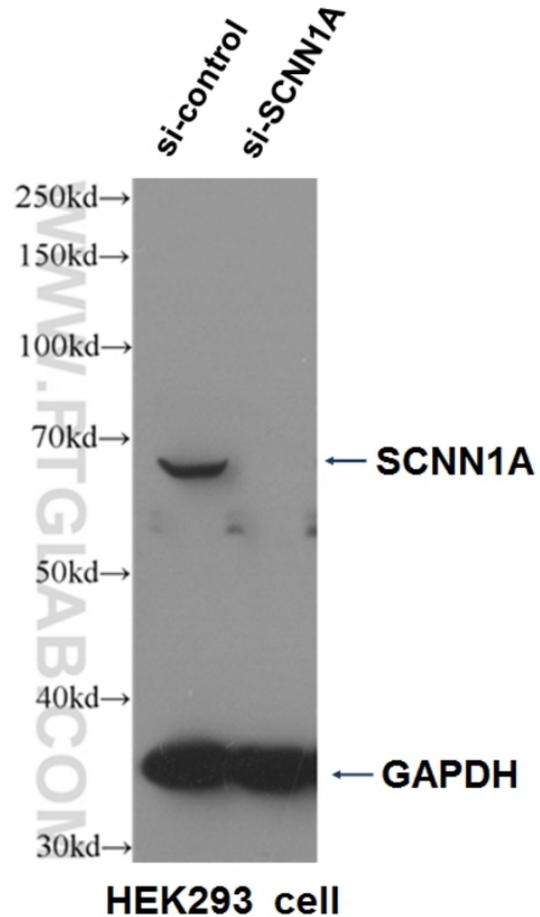
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New reagent types are on the horizon.



Key question: does this antibody really bind what it supposed to?



Responses from Scientific Community

1. Calling for a national registry of antibodies
2. Calling for “golden standards” such as knock-down (siRNA), knockout (traditional or current Cas9-CRISP) or direct identification of antibody-bound antigen by Mass Spectrum.
3. Calling for users be aware.

These are all necessary and essential corrections. However, none of these are new technologies. They've existed for some time now, and been proposed over and over, so we must ask ourselves: *why aren't they already in place?*

The good old days, or not?

Stage 1: The market emerges

In the pre-genomic era, scientists made their own antibodies. Colleagues in the same field often shared antibodies, but slow and labor-intensive.

Stage 2: Specialty manufacturers enter

Facing the widespread need to study novel proteins revealed by Human Genome Project, companies began to create antibodies in anticipation of future market demand. However, antibody usage is specialized and demand varies. As such, manufacturers produced a wide range of antibodies, relying on the success of one or two products to fund continuing R&D.

Stage 3: Super IT Distributors enter

By offering antibodies from a variety of manufacturers, distributors provided scientists with a single hub that met their antibody needs. At the same time, manufacturers were free to devote more resources to production, rather than marketing. In an ideal scenario, these partnerships would benefit all three parties.

The 4 forces working in the antibody market

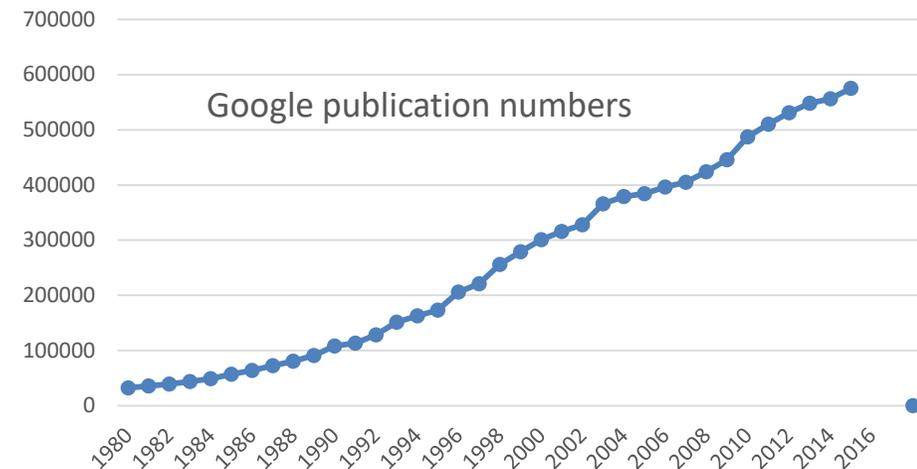
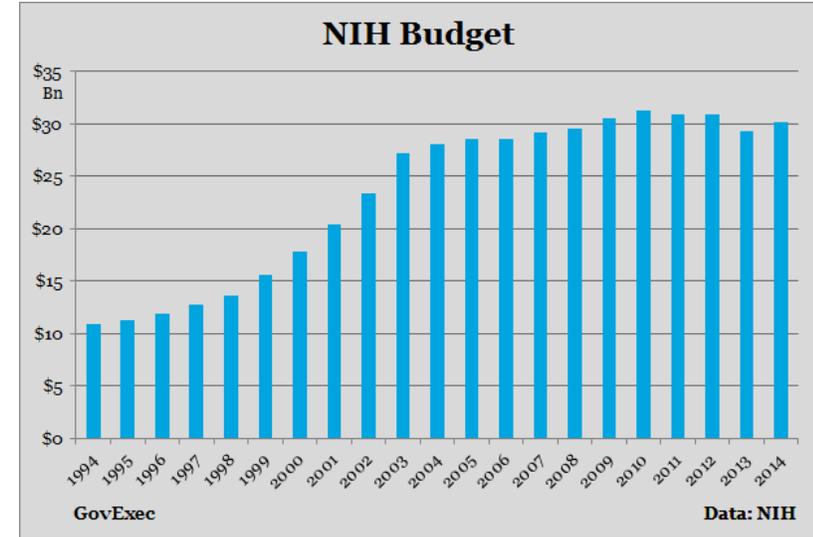
1. The Scientists (consumers who uses antibodies)
2. Manufacturers (the people who makes antibodies)
3. The distributors (re-branders)
4. The capital investments (the people who.....)

Force #1, The Consumer

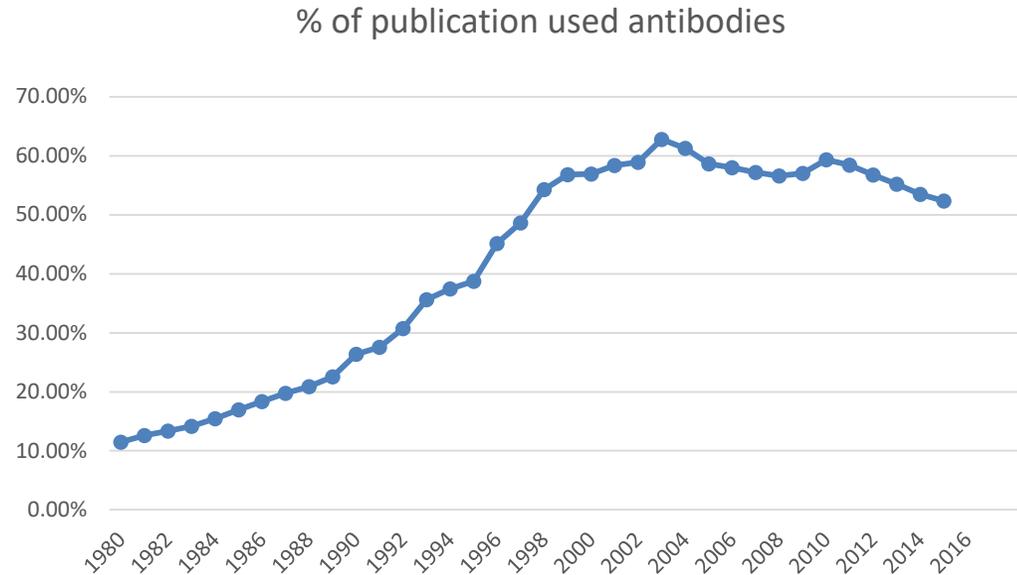
A quote from Nature Editorial:

Next milestone of human genomics is to decode the structure and function of proteins, the end product of the genome, i.e. the human proteomics.

With the start of Proteomic projects, antibody demand went up dramatically, yet, the funding did not.



The force #2 Commercial antibody Manufacturers



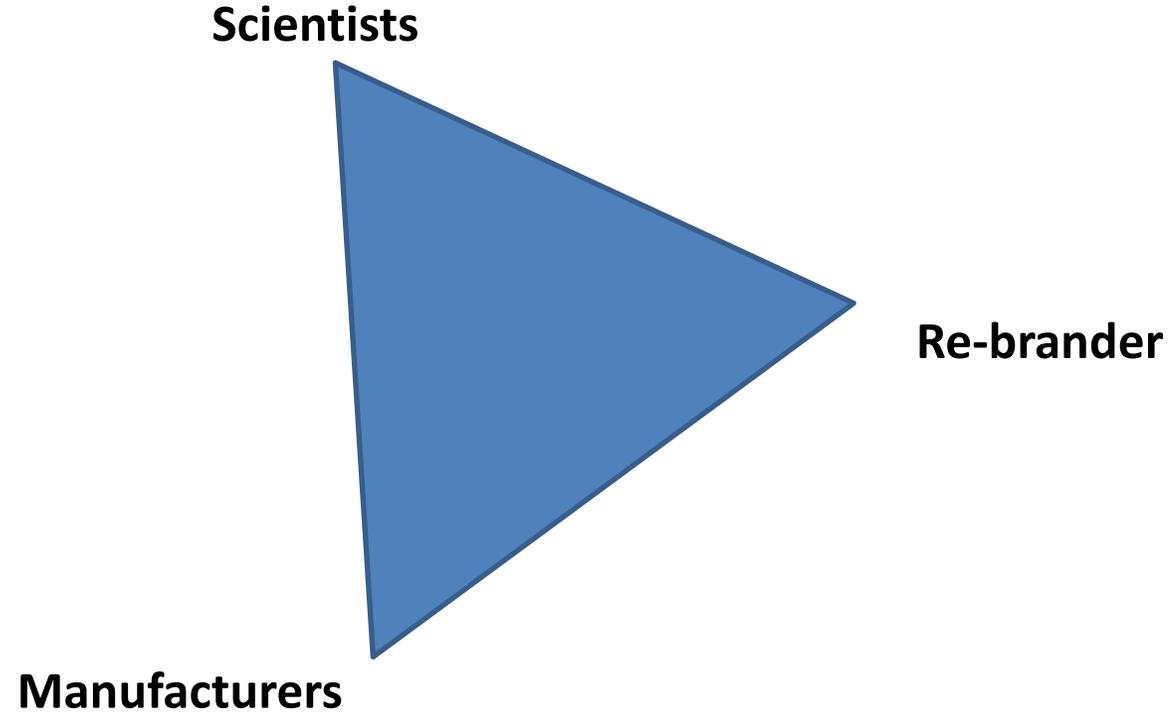
Is commercial antibody necessary? The answer is yes.

1. Antibodies become immediately available, saves time
2. Antibodies evenly distributed to scientific communities no monopoly
3. Cost dramatically down so scientists can do more with the grant.

Force #3, The super IT global distributors (Re-branders)

Benefit:

1. find those hard to find antibody
2. Cross custom barrier between countries.
3. Supposedly to decrease marketing and distribution cost



This fundamentally untenable business situation has been masked by a flood of entry of small antibody producers relying on capitals from investors, but the core issue remains: revenues have not risen, costs have not fallen, but the market has a new middleman taking a cut.

Something has to give, unfortunately it is the Quality

When in doubt, ask Carl Marx

The conflicts between of the **productivity** and **relations of production**



manufacturers

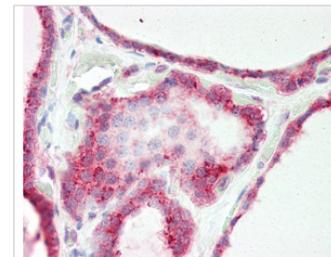
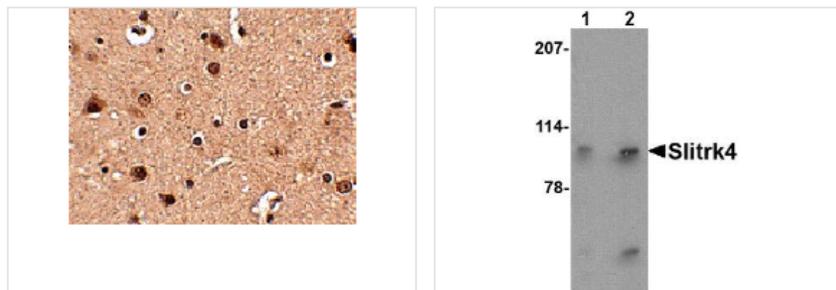
Re-Brander

Carl Marx, [Preface to the Critique of Political Economy \(1859\)](#)

At a certain stage of development, the material productive forces of society come into conflict with the existing relations of production or – this merely expresses the same thing in legal terms – with the property relations within the framework of which they have operated hitherto. From forms of development of the productive forces these relations turn into their fetters. Then begins an era of social **revolution**.

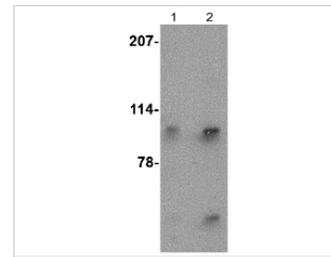
Distributors (Re-branders)'s Headache

Re-Branding created a chaos in antibody identity, effectively stripe any manufacturers responsibility. Not only the same antibody are sold by multiple companies without telling customers, some companies even are selling the same antibody under multiple catalog number not realizing they are the same.



ab115690 at 5ug/ml staining SLITRK4 in Human thyroid tissue by immunohistochemistry (FFPE). Following primary incubation slides were incubated with biotinylated goat anti-rabbit IgG secondary antibody, alkaline phosphatase-streptavidin and chromogen.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SLITRK4 antibody (ab115690)



Lane 1 : Anti-SLITRK4 antibody (ab115690) at 0.5 µg/ml
Lane 2 : Anti-SLITRK4 antibody (ab115690) at 1 µg/ml

Lane 1 : Mouse brain tissue lysate
Lane 2 : Mouse brain tissue lysate

Predicted band size : 94 kDa

Western blot - Anti-SLITRK4 antibody (ab115690)

Datasheet	Specific References	Protocols
Overview		Visit the Product Wall for Abreviews and Q&A
Product name	Anti-SLITRK4 antibody See all SLITRK4 primary antibodies ...	
Description	Rabbit polyclonal to SLITRK4	
Specificity	ab67308 is predicted to have no cross-reactivity to other SLITRK proteins.	
Tested applications	ICC/IF, WB, IHC-P ▶ more details	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	A 14 amino acid synthetic peptide from near the amino terminus of human SLITRK4.	

References for Anti-SLITRK4 antibody (ab115690)

ab115690 has not yet been referenced specifically in any publications.

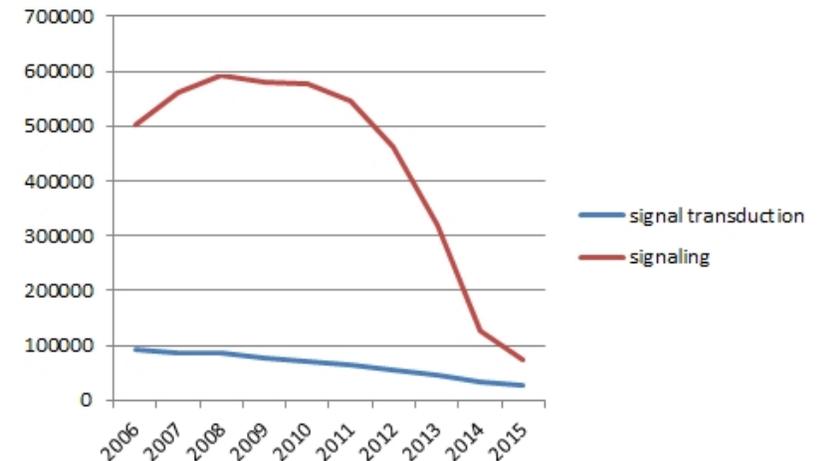
Manufacturer's Dilemma

Fact is, the Validation cost of an antibody counts a minimum 2/3 of entire antibody cost in the case of peptide antibody production. Burden of Inventories are completely on the shoulders of manufacturers.

The dilemma that the manufacturers face is: Should the limited capital go toward validating successful antibodies or toward making more not-so-well validated antibodies which may become hot sellers?

With distributors relabeling their products, the incentives of brand loyalty are eliminated, and some manufacturers choose to sacrifice quality.

Total number these words appeared in articles



Cost Structures of Re-brander and Manufacturers

	Millions (British Pounds)	As of:	Y2011	Y2012	Y2013	Y2014	
A Re-Brander	Total Revenues		83.3	97.8	122.2	144	29.5%
	Cost of Goods Sold		27.2	30.3	35.5	42.5	70.5%
	Gross Profit		56	67.6	86.7	101.5	
	Operating Expenses		23.8	29.9	42.2	55.4	
	Operating Income		32.3	37.6	44.6	46.1	

	Millions (Taiwan Dollars)	As of:	y2011	Y2012	y2013	y2014	
A Manufacturer	Total Revenue		472.6	463.8	463.7	438.1	48.9%
	Cost of Goods Sold		204	208.9	227.5	214.4	51.1%
	Gross Profit		268.5	254.9	236.2	223.8	
	Operating Expenses		146.7	160.5	156.9	161.3	
	Operating Income		121.8	94.3	79.3	62.5	

Market force #4: Capital investment, a blessing and a curse



Let's be clear about something: None of the top biotech investors invested in Theranos. Five of the best-regarded biotech firms—Venrock, Third Rock Ventures, Polaris, Deerfield, Versant—either didn't meet with Theranos at all or passed on the pitch. The Silicon Valley firms with health care practices, like Google Ventures and Bessemer Venture Partners, weren't swayed, either. <https://www.fastcompany.com/3059230/the-theranos-scandal-is-just-the-beginning>

Hundreds of newly started small antibody companies in recent years fueled mainly by money from real estate over spill and governmental start-up funding. It is simply because the entry barrier is low so fund seeker can easily demonstrate a prototype – a rabbit in the back yard. But the market barrier is so high that some thing had to give.

Example A: a company raised 5 million USD produced 6,000 peptide generated antibodies. We randomly tested 40 of them, all failed.

Example B: Elisa Kit company – see reference on GenomeWeb - Faulty Antibodies Continue to Enter US and European Markets

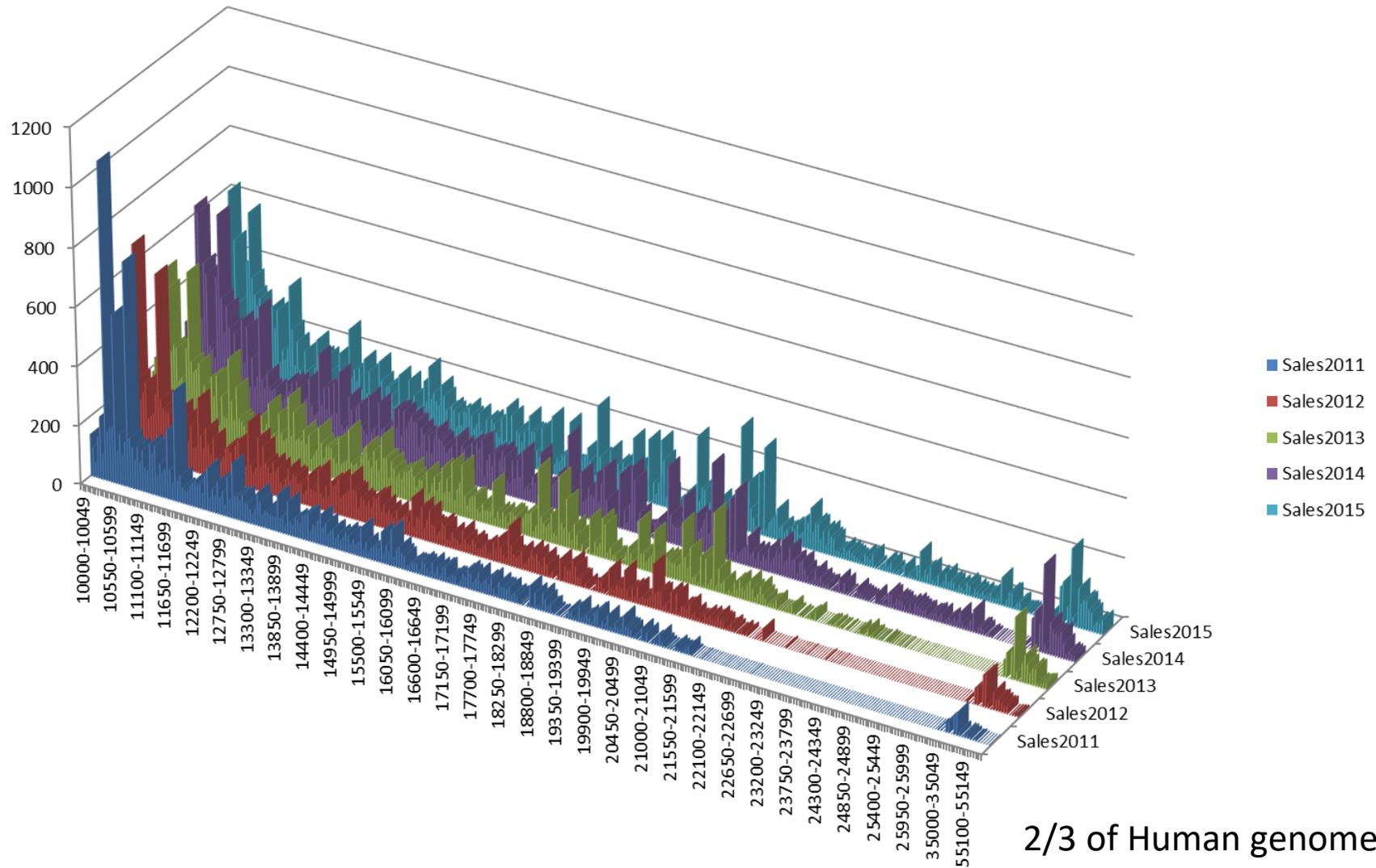
Cheating sheets for dumb money

year 1	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 2		\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 3			\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 4				\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 5					\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 6						\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 7							\$2,800,000	\$2,800,000	\$2,800,000	\$2,800,000
year 8								\$2,800,000	\$2,800,000	\$2,800,000
year 9									\$2,800,000	\$2,800,000
year 10										\$2,800,000
subtotal	\$2,800,000	\$5,600,000	\$8,400,000	\$11,200,000	\$14,000,000	\$16,800,000	\$19,600,000	\$22,400,000	\$25,200,000	\$28,000,000
									Total	\$154,000,000

The kids are alright

All these are just growing pain. The antibody market eventually will have a vertical integration to reach the equilibrium:

1. Either Distributors integrate manufacturing, and it is happening, or
2. The manufacturers increase their target coverage to 100% either by merging with each other or self manufacturing. It is happening too.



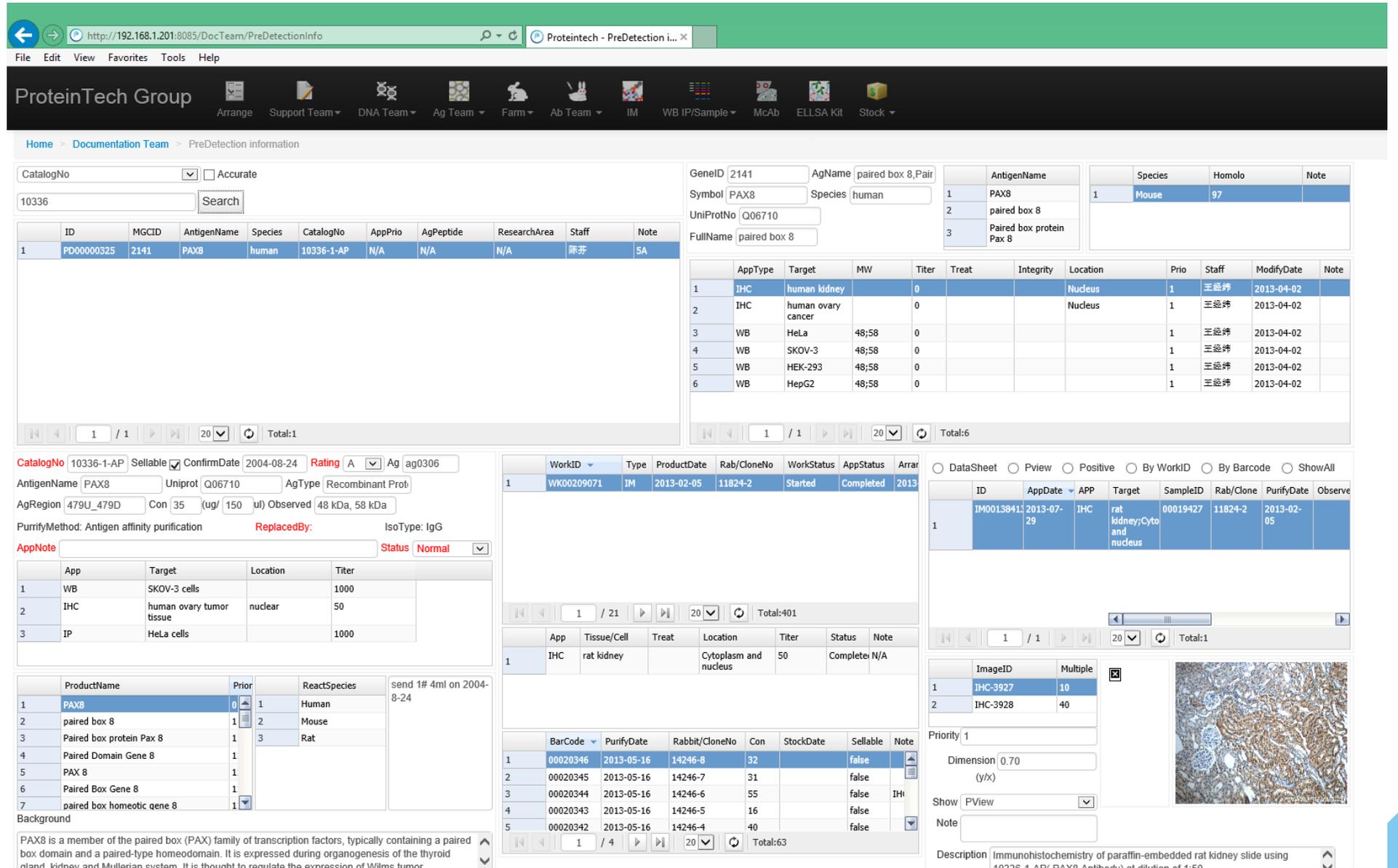
2/3 of Human genome

What now?

-- Do what scientists always do, ask questions and transparency.

1. Know who made the antibody. This is your collaborator, not your supermarket. Have the scholarly exchange of information as you always do. Since they are an continued research, scientists of the consumer side, so be in direct communication with scientists in manufacturer sides.
2. Know your antibodies. Antibody and antibody applications are ongoing research progress. So it is a progress report. understand the how the production (like experiment) was made, and more importantly understand what further need to be done.
3. Assign the responsibilities to the antibody manufacturers, as if they are carrying a part of your experiment, rather than a faceless shadow hidden on an supermarket shelf.

We offer the history of every antibody, from the inception to validation



The screenshot displays the ProteinTech web application interface, showing a comprehensive view of antibody history and validation data. The interface is organized into several key sections:

- Navigation and Search:** At the top, there is a search bar for the CatalogNo (10336) and a search button. The breadcrumb trail indicates the user is in the Documentation Team > PreDetection information section.
- Antibody Details:** A central table lists antibody entries with columns for ID, MGCID, AntigenName, Species, CatalogNo, AppPrio, AgPeptide, ResearchArea, Staff, and Note. The first entry (ID: PD00000325) is highlighted.
- Gene and Species Information:** A sidebar provides details for GeneID 2141 (Symbol: PAX8, Species: human) and UniProtNo Q06710 (FullName: paired box 8). It includes a table for AntigenName, Species, Homolo, and Note.
- Application History Table:** A table below the main list shows application details with columns: AppType, Target, MW, Titer, Treat, Integrity, Location, Prio, Staff, ModifyDate, and Note. Applications 1 through 6 are listed, including IHC and WB assays on various targets like human kidney and HeLa cells.
- Validation and Assay Data:** A section on the right shows a table for WorkID, Type, ProductDate, Rab/CloneNo, WorkStatus, AppStatus, and Arrar. Below this, a detailed table for application 1 (WK00209071) shows assay parameters like Tissue/Cell, Treat, Location, Titer, Status, and Note.
- Product and Reagent Information:** A section at the bottom left provides details for the product name (PAX8), priority, react species (Human, Mouse, Rat), and background information. The background text states: "PAX8 is a member of the paired box (PAX) family of transcription factors, typically containing a paired box domain and a paired-type homeodomain. It is expressed during organogenesis of the thyroid gland, kidney, and Mullerian system. It is thought to regulate the expression of Wilms tumor..."
- Image and Description:** A section on the right shows an image of a rat kidney slide with IHC staining. Below the image, there are fields for ImageID, Multiple, Priority, Dimension (y/x), Show (PView), Note, and Description: "Immunohistochemistry of paraffin-embedded rat kidney slide using 10336-1-AP/PAX8 Antibody at dilution of 1:50".

