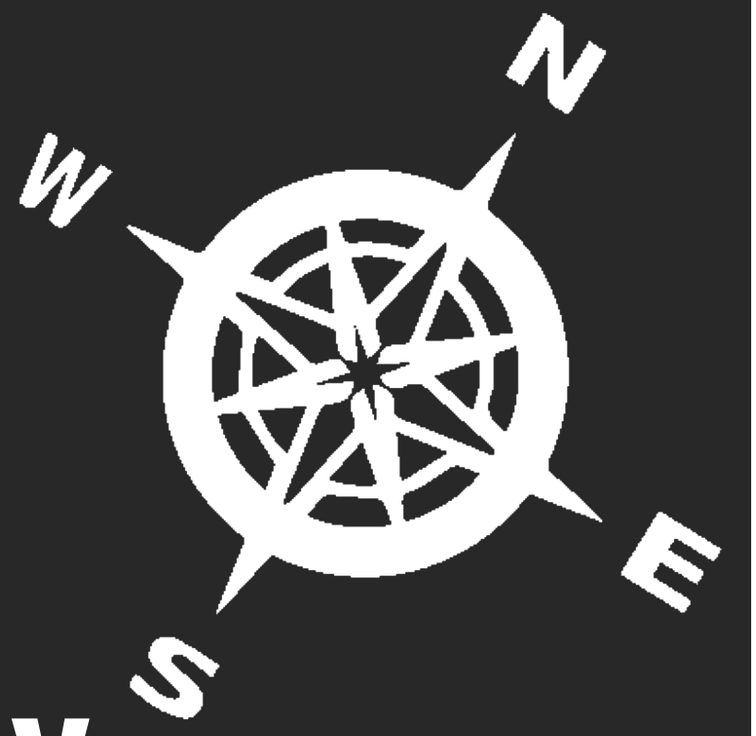
Curious Tales From The History of Biology



Part 1

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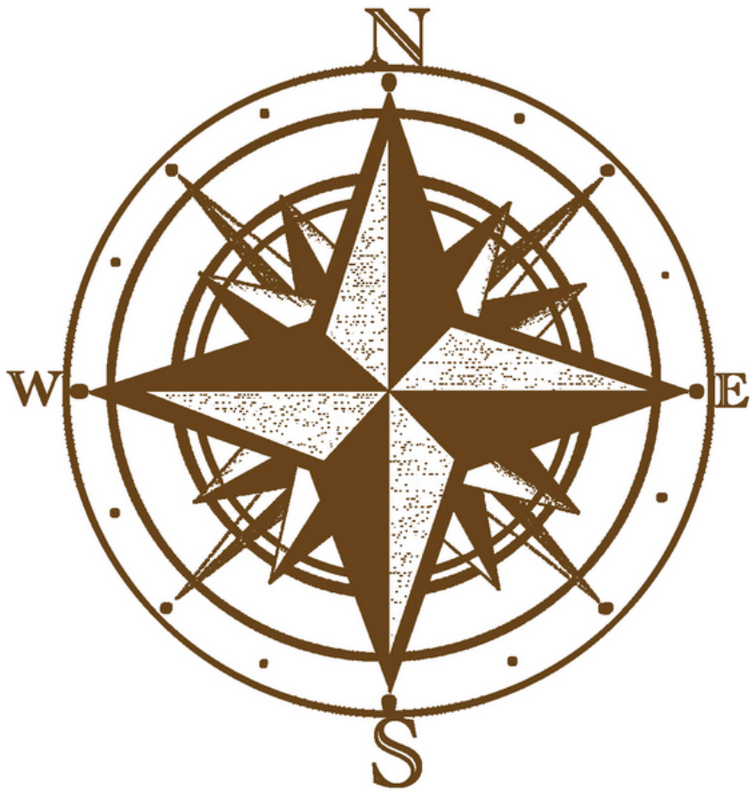
a BitesizeBio.com eBook

Southern, northern, western and eastern



“It’s official, biologists do have a sense of humor...”

Section 01

By Nick Oswald

It's official – biologists DO have a sense of humor, well some of them at least.

This is the story of how one of the most famous and quirky naming conventions in biology came into being. It's a story of discovery, comedy and the triumph of people power over the establishment.

This is the story of how the Southern, northern and western (etc) blots got their names.

In the Beginning, there was Ed Southern

In 1975 when Ed Southern invented his method of using a radiolabeled DNA probe to detect a specific DNA sequence within a DNA sample (e.g. a fractionated genome) and named it after himself – the "Southern blot" – I'm sure that he had no idea about what he had started [1].

Two years later, J.C. Alwine, a biologist with a sense of humor, developed a technique analogous to the Southern blot, this time for the identification of a specific RNA within a complex RNA sample using a radio-labelled DNA probe [2].

Alwine couldn't resist the temptation to call his technique the "northern blot" in an allusion to Southern's technique, raising chuckles in labs everywhere.

Then W. Neal Burnette, a post-doc working in the Nowinski group at the Hutchinson Cancer Center in Seattle, started the real fun.

A scientist in 1975, just after reading Alwine's "northern blot" paper

Go west(ern)...

Burnette was searching for a way to combine the powers of radio immunoassay and SDS-PAGE electrophoresis so that he could pinpoint specific antigens in a complex protein mixture, such as a cell extract.

After some "laughably naive" (his own words – see [3] for a wonderful account by Burnette himself) attempts to visualize the interaction between antibodies and the separated proteins in the gels, he was inspired by Alwine's northern blot method (so indirectly by Southern) to make a solid phase replica of the gel.

So he developed the method of using electrophoresis to blot the protein onto nitrocellulose paper and after some further work, perfected the technique of blocking non-specific binding sites and visualizing the specific radioimmunolabelled antigens using an X-Ray film.

In a historic, but mostly forgotten conversation with his boss, Robert Nowinski, Burnette coined the name “western blot” for his technique.

What fun! Like northern blotting, “western blot” was an allusion to the Southern technique, but Burnette had upped the ante by throwing in a geographical reference to location of the Nowinski lab (Seattle, Western USA).

So if the Nowinski lab had been in New York, we would all be doing “eastern” blots.

Get one back on your boss...

A quick aside for the pedants among us. Note that amongst these techniques, only the Southern blot should be capitalized since it refers to Southern’s name. The others – northern, western etc – are not proper nouns, so should not be capitalized.

Try pulling your boss up on that one next time he is in mid-flow talking about a “Western blot” in a departmental presentation.

Even publishing the *original western blot paper* wasn’t easy!

Anyway, back to our story. Unfortunately for Burnette no sooner had he perfected his technique than a paper describing a very similar method, also inspired by northern blotting, was published by Towbin et al working at the Friedrich Miescher Institute in Switzerland (see [4] for the reference and [5] for Towbin’s account of events).

Burnette was dejected, but nonetheless, convinced that his methodology was sufficiently different to Towbin’s - and that the brand name of his technique was much cooler - he decided to submit a manuscript on his western blot method to the Analytical Biochemistry journal.

The reviewers hated it, they hated the name even more – obviously humor was not high on their agenda – and the manuscript was rejected.

But despite this, the popularization of Burnette’s technique, and particularly the name “western blot” still happened even without the assistance of the literary establishment.

It happened through the sense of humor of the researchers at the bench, through people power, and through a lot of help from Xerox power.

It happened because researchers, besides being interested in the technique itself, were tickled enough by its quirky name to make copies and send it to their friends. In Burnette’s words…

> ***“…the few preprints I had sent to colleagues seemed to have undergone logarithmic Xerox multiplication. I began receiving phone calls from researchers unable to read the umpteenth photocopied generation of the pre-print, a sort of technical samizdat that I had to endlessly interpret”***

A few years later, Burnette eventually coaxed Analytical Biochemistry into accepting his paper and it was published in 1981 [6], but by then, word of mouth had already beaten them to it. The paper had already been "published" long before it ever hit the journal.

Ironically, considering the people power that was doubtless (at least partly) responsible for it's eventual publication, Burnette's paper is available only to Analytical Biochemistry subscribers. **end of open access rant**

And then it just got silly...

Bowen and colleagues continued the naming convention in 1981 with their publication of the southwestern blot, a technique for identifying DNA-binding proteins in nuclear protein extracts using specific oligonucleotide probes [7].

The "south" in the name refers to the use of DNA probes, while the "west" refers to the protein blot. (So it's not that silly actually, I take it back).

Interestingly, Bowen's paper alludes to Burnette's western blot even though it was published *before* Burnette's paper, which shows just how strongly word-of-mouth actually publicized the western blot.

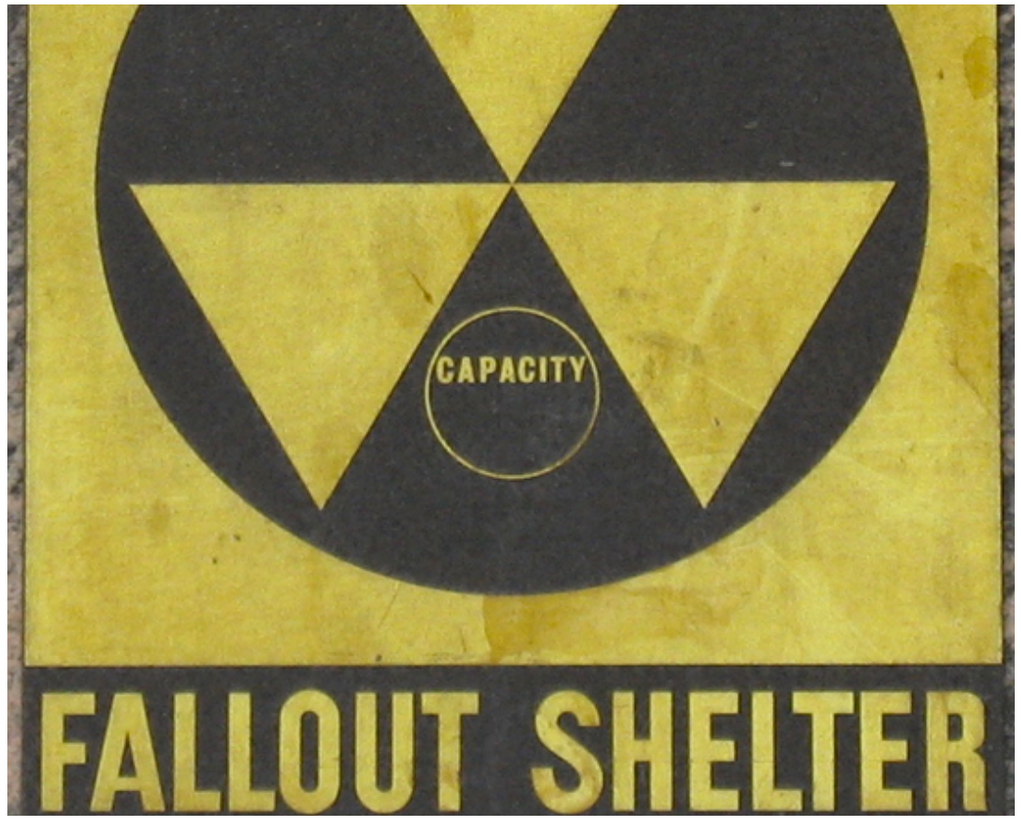
And in 1998, Ishikawa and Taki published their far-eastern blotting method [8], no doubt a reference to their geographical location, for the analysis of lipids by TLC separation followed by blotting onto a PDVF membrane.

Finally, there is one more blot technique that deserves mention. Legend has it (well, the legend of the bio.net forum at least) that Ethan Signer coined the phrase "eastern blot" for the tantric practice of willing a failed gel into show bands [9].

Apparently, you take your blank gel, meditate, repeat the mantra, and the bands appear…

…if only!

How Plasmids Became Embroiled In the Cold War



“This... led to proponents of cytoplasmic inheritance being viewed suspiciously, as anti-communist hysteria swept the USA”

Section 02

By Nick Oswald

The humble plasmid. We now know it so well, but as little as 70 years ago the field of extra-chromosomal heredity was decidedly murky.

Not only was it the subject of great debate, conflict and friction within the scientific community, it was even used as a politico-religious tool during the Cold War.

The origin of the term “plasmid”

But first, what is a plasmid anyway?

The term “plasmid” was coined in 1952 by Joshua Lederburg [10]. It is a hybrid of the terms “cyto**plasm**” (or possibly “**plasm**agene”; a term put forward by Darlington in 1944 to define “self-reproducing cytoplasmic particles”) and the “id”, the latin for “it”, as in plastid or chromatid.

For Lederburg, the plasmid was a generic term for any extra-chromosomal genetic particle.

Of course, that wide definition could describe viruses, mitochondria or many other things, but over the years this was honed down to reserve the term for double-stranded, extra-chromosomal, self-replicating DNA.

A microbiologist embracing the idea of extra-chromosomal inheritance

The debate on extra-chromosomal inheritance

Early experiments seemed to show that certain genes could be infectively transmitted from cell to cell but the idea of “cytoplasmic inheritance” was dismissed by much of the scientific community who argued that the chromosome was the sole heritable unit and that the observed inheritance was due to infection of the cells by parasites.

In fact, the split in opinion went straight (and simply) down the lines of who studied what: Microbiologists embraced the idea of extra-chromosomal inheritance, while those who worked on Drosophila dismissed it.

With the perspective of hindsight, it seems quite obvious why this would be the case, but at the time it was a very hot and contentious debate.

Plasmids In The Cold War

In those politically charged times, this scientific argument even became embroiled in clash between the communist and capitalist ideologies.

This was because followers of the Soviet Lysenkist doctrine [11], which criminalized the teaching of Mendelian genetics in the USSR, held up cytoplasmic inheritance as evidence that Mendelian genetic theory was wrong.

This in turn led to proponents of cytoplasmic inheritance being viewed suspiciously, as anticommunist hysteria swept the USA.

The Genetics Society of America even came close to dismissing the theory based purely on political grounds. Now that would have been something to talk about!

This is all impressive work for a small piece of circular DNA, but given the revolution plasmids sparked in bioscience in recent decades, perhaps we shouldn't be surprised.

For further perspective on the early days of plasmid research, Lederburg's 1998 reflective essay on the topic is a must-read [12].

The Invention of PCR

“While driving his Honda Civic on Highway 128 from San Francisco to Mendocino, Mullis made an intellectual leap.”

Section 03

By Nick Oswald

Few technical breakthroughs have changed the face of their field like the Polymerase Chain Reaction (PCR).

Gene cloning, sequencing of complex genomes, DNA fingerprinting and DNA-based diagnostics are just some of the techniques that were either inefficient, crude or plain impossible before PCR.

PCR has revolutionized biological research and biotechnology to such an extent that it can be considered as one of the major reasons for the boom that the field has experienced over the last 20-30 years.

Where it all began

Kary Mullis is generally credited with inventing PCR in 1983 while working for Cetus Corporation in Emeryville, California.

Mullis' role at Cetus was to synthesize oligonucleotides for groups working on, amongst other things, methods to detect point mutations in human genes.

Mullis was hatching an idea to detect the point mutations using Sanger-type DNA sequencing, employing DNA polymerase in the presence of an oligonucleotide primer and ddNTPs.

The problem was that sequencing a single copy gene within the expanses of the human genome was impossible; the primer would bind in too many places.

A 1983 Honda civic. Just like the one in which the intellectual leap was made.

What he needed was a way to increase the concentration of the specific gene of interest.

Eureka on Highway 128

While driving his Honda Civic on Highway 128 from San Francisco to Mendocino, Mullis made an intellectual leap.

He reasoned that by using two opposed primers, one complementary to the upper strand and the other to the lower, then performing multiple cycles of denaturation, annealing and polymerization he could exponentially amplify the piece of DNA between the primers.

The idea of PCR was born, but the technique was still very much in it's infancy.

Mr Cycle and Taq

The *E.coli* DNA polymerase used in the early days was not heat stable and therefore was destroyed during the denaturation step. This meant it had to be replenished after every cycle.

Cetus workers quickly developed the first thermal cycler named “Mr Cycle”, which automatically added new polymerase after each heating step.

In 1985, Mullis came up with the idea of using polymerase isolated from the extremophilic bacterium *Thermophilus aquaticus*.

The polymerase, known as Taq polymerase, has optimal activity at 72°C and can withstand the 94°C required for denaturation of the DNA, meaning that many reaction cycles could be performed without the need to replenish the enzyme. More information on the development of Taq can be found in the next section of this ebook “The Taq behind PCR”.

This breakthrough, together with advances in oligonucleotide synthesis made PCR both cost effective and convenient and it quickly entered mainstream research.

PCR goes exponential

Researchers have literally flocked to PCR. As such, they have brought their variations and improvements to the technique. Consequently, there are now hundreds of PCR-based applications used in many fields of biology.

Stephen Scharf, Mullis’ former colleague at Cetus, put it quite nicely:

> ***“One of PCR’s distinctive characteristics is unquestionably its extraordinary versatility. That versatility is more than its “applicability” to many different situations. PCR is a tool that has the power to create new situations for its use and those required to use it.”***

Perhaps the most influential of all techniques enabled by PCR is massive-scale genomic sequencing, which itself has transformed the biological and biotechnological research arena.

And the rewards for the inventor of this ground-breaking technique? Well, Mullis received the Nobel Prize in 1993.

He also received a $10,000 bonus from his employers, Cetus, who later sold the patent rights to Hoffmann La-Roche for a cool $300,000,000. Seems to me there’s a lesson in there somewhere…

For further reading on this topic, check out the original *Nature* paper in which PCR was described [13], *The Polymerase Chain Reaction* book by Mullis *et al* [14] and Mullis’ 1993 Nobel Prize lecture transcript [15].

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The Taq Behind PCR

“At the time, scientists believed that bacteria optimally lived at about 55°C and that nothing lived above 73°C.”

Section 04

By Andrew Porterfield

Nobel Laureate Kary Mullis is generally credited with inventing the polymerase chain reaction, but his discovery owes a lot to a microbiologist who loved to travel, some refuted assumptions of what can live in hot springs, and a now-closed field station in Yellowstone National Park.

Here's the story

In the 1960s, Thomas Brock was a biologist at Indiana University whose interest was shifting toward microbial ecology; he began studying microorganisms in intertidal pools, freshwater lakes, cold springs, and finally, geysers and hot springs.

Brock had a travel bug, and was increasingly interested in doing field ecology. He started a field research station in Yellowstone National Park, though he says at first he wasn't interested in geysers.

At the time, scientists believed that bacteria optimally lived at about 55°C, and that nothing lived above 73°C. So, he assumed there was nothing to find.

But, he soon found pink bacterial filaments living in the Octopus Hot Spring at temperatures above 80°C. The bacteria: *Thermus aquaticus*, which contains the DNA polymerase that ultimately became the backbone of PCR.

The key to PCR, published in 1969

In a Journal of Bacteriology paper with an honors undergraduate Hudson Freeze (now head of glycoprotein research at the Burnham Institute in La Jolla, Calif.), Brock introduced the new species in 1969 [16].

The key to making PCR work is a heat-tolerant enzyme that can endure conditions in the chain reaction. Since *Thermus aquaticus* was the first organism known to exist (and reproduce) at these high temperatures, it naturally became the focus point of Mullis' later invention.

Taq is all over....

T. aquaticus, it turned out, is ubiquitous at high temperatures. Brock found the bacteria in hot springs in California, soil in a tropical-temperature greenhouse, and even the hot water supply at Indiana University!

The bacteria are also in hot springs worldwide, including Japan, New Zealand, and Iceland. But Yellowstone was the easiest to study; being a national park, habitats were not destroyed or developed into spas and resorts, which made international research challenging.

A hot spring, the easiest place to find T. aquaticus, apparently.

Ironically, Brock at first had no interest in Yellowstone, because of the park's reputation in the 1950s and 60s as more of an amusement park than natural habitat.

Brock continued his work on extreme thermophiles, but closed the Yellowstone research station in 1975.

Only several years later, when PCR technology was announced, did interest in his work rebound.

PCR Benefits the National Parks...

Scientific and biotechnology interest in Yellowstone has rebounded, too. The National Park Service, wizened to the fact that it had, free of charge, owned the wellspring of a $300 million industry, now manages biology, chemistry and biotechnology projects at the park.

Researchers worldwide have now been issued permits to perform microbe research, and part of that permit initially involved sharing benefits of the research with the Park Service. As one Park Service manager said, "When you see the money that's being made, that's hard for a starving bureaucrat to overlook."

However, the Park Service was sued over the benefit-sharing arrangement, and those arrangements have been on hold since 1999. The Park Service says it is now conducting an environmental impact study to determine the overall impact of benefit-sharing agreements with biotechnology companies.

But hey, it's for a good cause.

For more information on this topic, check out Thomas Brock's website, Life at High Temperatures [17], his 1972 paper. "The Upper Temperature Limit for Eukaryotic Organisms" [18] and the US National Parks Service's view on bioprospecting [19].

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