The winning of the Western

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I recently attended the PhD student symposium of the Flanders Institute of Biosciences (VIB). Although there was no formal theme to the meeting, an eclectic roster of speakers presented personal views of some invention or technology, which may impact future society. We were also asked to participate in armchair panel discussions, akin to the format of breakfast-time television. In my session, the moderator asked us to reflect on the era when we would most like to have been active in science, and why. If we could reset the clock, where would we aim the compass of our personal time machine? Here is the gist of my answer.

Some speakers in the symposium had begun their careers in the pioneer age of molecular biology and recalled how they had extrapolated the details of gene anatomy and function without the aid of restriction enzymes, cloning, DNA sequencing, Southern (or any other kind of) blots, PCR or even gel electrophoresis as we know it. How they achieved anything at all is a miracle, but in spite of the technical limitations of the day, they laid the sturdy foundations of the subject. I landed somewhere in the latter phase of this period and, happily, soon had easy access to most of those tools.

Nevertheless, looking back, I am sure I could have achieved far more if only PCR had been developed 15 or 20 years earlier. I wouldn't lightly have missed my undergraduate education, where I was exposed not just to the ideas developed by the founders of the field, but to many of them in person. But in other respects, I would quite like to have been fast-forwarded to 1995, to take advantage of the speed, specificity and reliability of amplification methods to analyse nucleic acids in exquisite detail. Indeed, the

second plank of this technology, the fast and simple way to alter genomic DNA in virtually any organism, has come even later. In its absence, many of us wasted further years laboriously creating transgenic fly or mouse strains, or working with organisms whose genomes could hardly be manipulated at all. But at least PCR gave us a tool to analyse, if not edit to order.

At this point in time, I would nevertheless dearly love to parachute further into the future, when an analogous toolbox has been perfected to analyse proteins. For, amidst all of the buzz surrounding nanotechnology, synthetic biology and personalized medicine, and the advances in DNA sequencing and biocomputing that are bringing us rapidly towards the "10 dollar genome", we are still stuck with the Western blot practised pretty much like it was in 1980.

In theory, the antibodies upon which Westerns rely have high affinity for their targets and a commensurate degree of specificity. In practice, they often have neither. Virtually everyone in molecular biology has experienced that irritatingly stubborn cross-reaction to bands of the "wrong size", flagrant inconsistencies with the results of immunocytochemistry, signals that totally vanish after loading just half the amount of protein, or horrendous, inexplicable background mess that is the nightmare of PhD students and their supervisors. Often, there is little advice to give, beyond starting again with fresh reagents, troubleshooting every detail of the protocol or adding a further bunch of onerous controls.

I could fill ten editorials from my own laboratory's experiences. Just now we are struggling with an antibody that gives the expected signal when our favourite protein

is expressed in flies or mice, but in material from its native organism (*Ciona intestinalis*), the same antibody detects a band four times the predicted size, accompanied by a massive high-molecular-weight background that hints at a novel post-translational modification or a really annoying artefact. In another project, where we know that proteolytic enzymes are massively induced in the material we want to study, every protease-inhibitor cocktail we have tested protects the GAPDH or tubulin control, but not the proteins we are actually interested in. In a third case, we ordered a peptide antibody that reveals a beautiful band of the right size, but whose level is unaffected by overexpression or RNAi-based knockdown. Most likely, we waited months and paid thousands for a reagent that is useless, but a PhD student will now need to waste a few more months of his time to confirm it.

Even monoclonal antibodies, which have been around since near the dawn of Western civilization, rarely work any better. Shotgun proteomics offers some relief, but as a routine procedure to detect a single protein, it is a proverbial sledgehammer, and is about as quantitatively reliable as those Westerns where the overloaded protein appears as an anti-band in the fog.

Human invention depends on ingenuity and chance. Need is a spur, but on its own, it isn't enough. Even fictional time machines have a habit of landing somewhere else than their inventors intended. Similarly, we would be hard-pressed to predict exactly when we should pilot into the future, if we want to arrive at a time when a cheap, reliable technology for protein analysis has already been developed. Hopefully, we won't have to wait for time travel to be invented first.