Howard Hughes Medical Institute
Research Laboratories

Sean B. Carroll, Ph.D.
Investigator

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Yves Brun
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Dear Dr. Brun,

I am very pleased to offer my most enthusiastic recommendation in support of Dr. Barry Williams' application to join your faculty. Barry is the complete package – he is a creative and rigorous researcher, a superb teacher with a broad knowledge and deep grasp of biology, an excellent mentor, and a generous colleague and collaborator.

Barry came to my lab with an excellent background in conservation biology and genetics. His original intention was to study the evolution of morphological traits in drosophilid flies. Indeed, he and Trisha Wittkopp (then a graduate student) collaborated on the analysis of the genetics of pigmentation divergence among a trio of species in the *D. virilis* group (Wittkopp, Williams et al (2003 ) PNAS 100 : 1808). They showed that several loci contributed to differences in body pigmentation in this group, the *ebony* locus in particular and, surprisingly, not the *yellow* locus (which we have found to be involved in many other species differences).

Barry then took a dramatically different direction. His change of course was prompted by many discussions and debates about the fraction of amino acid replacements that are fixed by selection. Several papers in the literature at the time sparked some spirited criticism and reanalysis in the lab. All of the work to date has been based on statistical approaches that are very sensitive to parameter assumptions. What Barry wanted to do was to develop an experimental approach to studying protein evolution and selection that was quantitative and systematic. After extensive research and discussion, he settled on using yeast as a model platform. Despite that fact that my experience with yeast was limited to the residue in local microbrews, Barry boldly set out to get some training and to get the experiments up and running.

It took him very little time. He attended the Cold Spring Harbor Laboratory course and brought back many tools and contacts. His research evolved into a three-pronged approach. One of his first creative inspirations was catalyzed by his advance knowledge of the then-forthcoming sequencing of a handful of fungal genomes. Barry knew that he would use this immense database as a foundation for ortholog replacement studies, but he also knew that it presented a golden opportunity to tackle a long-standing question in phylogenetics – the relationship between gene sequence data set size and phylogenetic accuracy. Barry enlisted the help of two postdoc office-mates, Antonis Rokas (soon to be at the Broad Institute) and Nicole King (now an Assistant Professor at U.C.-Berkeley). Barry and Antonis led the effort to explore how the availability of genome-scale data sets could help overcome the incongruence of

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phylogenetics based a single or small sets of genes. The fruit of that work, of which Barry was co-first author (Rokas, Williams, King, and Carroll (2003) Nature 425 :798) quickly became one of our most cited papers, and the data set we assembled has been requested and analyzed by a large number of researchers in the phylogenetics community.

The phylogeny Barry et al. established and the genome-scale data sets were the foundation of the two still-ongoing studies that Barry tackled next. I want to focus on the ortholog replacement studies first. Barry has developed a novel experimental platform for studying the relative fitness of yeasts that differ by sets of discrete, engineered genetic differences. The experimental details are available in Barry's synopsis of his work. I want to emphasize a few points here. First, the platform required a whole lot more engineering and rigorous experimental investigation than we foresaw. Every detail, from vectorology, the site of gene replacement, marker protein usage, promoter usage, cell sorting technology, and clonal analysis required diagnosis of phenomena we did not understand at first, followed by some inspired troubleshooting, and yet more engineering. Barry now has a high-throughput system that can reliably detect selection coefficients on the order of 0.005. Second, this system has produced some surprising results including: *i*) some orthologs from other species actually increase fitness in *S. cerevisiae*; and *ii*) the sign of selection coefficients for the same replacement can vary depending upon the environment.

I believe that this work, which is not yet prepared for publication, well be of broad interest and impact. Furthermore, there is a host of interesting issues that Barry will explore using this platform such as the role of compensatory mutation and protein coevolution, the diversification of gene duplicates, and more. I do realize that it would have been preferable to have this work published in some form at this critical junction in Barry's job search. However, we have held back in order to make the story as strong as we can and I am very optimistic about Barry's chances for a high-impact paper in the near future. My message here is not to worry about the papers, but to seize the opportunity to get a very strong scientist into your department.

The third thrust of Barry's work was to undertake a systematic study to understand population structure and genetic variation in natural yeast strains, and to search for signs of selection among a large cohort of genes. Barry has all of the data – from the sequencing of 100 genes from 30-odd strains. I think that some of the most provocative results will be his comparison and contrast of the signs of selection uncovered using different methods. This work will also be readied for publication in the near future.

Barry's body of work reflects just some of his many outstanding attributes – creativity, rigor, breadth, and courage. Barry is also a very clear thinker. He is a voracious reader with the talent to boil information down to the essentials. His presentations are unusually lucid, and Barry has a great grasp of the challenging elements of phylogenetics, population genetics, and quantitative genetics. He is going to be a standout teacher without a doubt. Barry is also an excellent mentor and manager. For the past two years he has worked with my long-time technician Jane Selegue and she adores working with him.

Does this future All-Star have any weaknesses? I would say that Barry has shown a tendency to want to accumulate the maximum amount of data in his experimental plans. This is not a great sin, but the scale of both his initial ortholog replacement study and his genetic variation survey were very large. It could be argued that we might have scaled back and gone to press sooner. But Barry chose to cover a lot of bases and I have supported his approach. I am

keenly aware that two more publications on his yeast work would be welcome now, but I urge you to look past that matter to his significant body of published and soon to be polished work.

In summary, Barry is exactly the kind of scientist I want as a colleague. I have had the privilege and good fortune of having many talented students and postdocs in my group, Barry is among the best of them. I am absolutely confident that he will excel wherever he goes and will be an all-around outstanding contributor to the missions of any department.

I hope that you are able to invite Barry to campus and see what he has to offer. Please feel welcome to contact me to discuss Barry further.

Best wishes,

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