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Biological Analysis and Interpretation For Improved Research Outcomes By Dr Douglas Bassett Spring In the last few years, technological advancements in the life sciences have changed many ways in which we think about research. Next-generation sequencing, qPCR and microRNA offer new avenues to ask and answer research questions in more detail and in less time. However, much of the effort today centres around data gathering, and many researchers are realising that collecting massive quantities of data is not the same as biological discovery. Thought leaders in the life sciences industry are actively discussing data analysis approaches necessary for actionable biological insights 1,2 , but the challenge of what to do once data is generated is still often not given as much of our collective research mindshare as it merits. With the increasingly broad adoption of technologies that produce large quantities of data, and more research groups leveraging multiple types of data, there is a critical need to implement solutions that enable researchers to effectively sort through and better understand their data outputs to translate data into actionable information and insights. In academia, few are more knowledgeable about a molecular process or disease under study than the graduate students and their PIs who are at the forefront of the research. However, when large datasets are being interpreted such as whole-genome or exome resequencing studies or RNA-seq experiments, important potential causal relationships can easily be overlooked if they are not intimately tied to known players. Given the breadth and volume of data involved, manually assembling related connections to genes, diseases and other interesting biology is not only time-consuming, it is difficult to do thoroughly, given the vast quantities of published literature and information now available. Many freeware tools also only cover one aspect of analysis â€" pathways, or molecular interactions, or biological processes and partial annotations â€" rather than truly providing an integrated view of overall biology. It can be extraordinarily time-consuming to integrate and interpret the results from various independent pieces of software. Also, it is difficult to find research tools that are built on the foundation of a high-quality, comprehensive knowledge base of findings curated by experts from the biomedical literature â€" critical to provide the optimal context for biological interpretation of these large, integrated studies. The results from individual tools are difficult to reconcile and interpret collectively, often resulting in overlooked insights that could have been discovered had all factors been evaluated together and manually attempting to integrate multiple tools is error prone. On the other hand, commercial organisations frequently use a combination of in-house solutions to analyse data. This approach also presents challenges. Bioinformatics teams sometimes do not have the capacity to leverage the vast body of published biological knowledge in their tools. Instead, much like freeware tools, these solutions may focus on a particular subject matter or facet of biology. This makes it more difficult for researchers to obtain a systems-level understanding of the impacted biology. In either scenario, researchers face a lengthy, time-consuming process that results in limited information from disparate sources, along with a significant risk of missing novel connections or crucial insights from the data. And most importantly, the data analysis often falls short of effective biological analysis, so scientists are frequently left with unanswered questions about the larger biological context of their results, and the full potential value of the extensive dataset that has been generated remains unrealised. This article will discuss how biological analysis addresses these challenges and provides an efficient way to get actionable biological meaning from large datasets. It will provide a definition of biological analysis, discuss the benefits of incorporating biological analysis into a research workflow, and examine what factors are necessary for efficient and accurate biological analysis. The importance of biological analysis Biological analysis is a scientific approach that combines analytical tools and biological content in one place, so researchers can obtain a fundamentally deeper and broader understanding of biological relationships and processes known to be connected to experimental observations, and the translation of that understanding to actionable insights and concrete hypotheses. Biological analysis can transform basic data analysis results into useful research outcomes, so researchers can leverage what they

have discovered to make informed decisions, generate well-formed, testable hypotheses, design follow-up experiments, and provide compelling biological and mechanistic evidence for results see Figure 1. Biological analysis represents a very natural but extraordinarily powerful extension to improve traditional data analysis and interpretation approaches. It connects molecular information coming out of various experimental platforms to help researchers understand whether the genes from their experiment work together as molecular modules, assess their impact on higher level biological processes and phenotypes, and determine whether or not those collections of events also impact diseases. For example, if the goal is to identify molecular mechanisms that link a genotype to a phenotype, biological analysis is the crucial approach that links gene expression changes in cancer cells to the observed cellular phenotype or related disease phenotype. Biological analysis can rapidly identify relationships already known to be involved in experimental changes. These capabilities help researchers by providing a broader biological picture when they analyse experimental results. For example, by examining a gene of interest in the context of a pathway, it becomes easier to get a sense of what is happening in an experimental model. What are the key players? What are the known interactions? What are the top pathways involved in the data set? Asking these kinds of questions and relating experimental data back to the larger biological picture is a key part of biological analysis. In addition to providing a more relatable, high level biological picture, biological analysis can identify key findings and novel discoveries from large amounts of data. Using biological analysis, a researcher could begin to narrow down and prioritise that list, using questions like: Which are known biomarkers? These advantages all demonstrate another key benefit of biological analysis, which is that it significantly decreases the time it takes to obtain a novel discovery. The integration of a wide variety of structured biological content in one place, in combination with analysis tools that let researchers effectively use that content to narrow in on a targeted set of experimental findings or explore outward from their findings to other biological relationships, saves an immense amount of time over manual, piecemeal or overly specific tools and approaches. Biological analysis also speeds the process of creating a validated and testable hypotheses, either at the end of an experiment using insights gained from experimental results, or prior to beginning a new experiment. Generating a hypothesis that can be interrogated and vetted against published research provides added confidence that wet lab testing makes sense. With biological analysis tools, researchers can challenge their hypothesis and examine it in the context of additional layers of biological and chemical knowledge before investing in the physical experiment. By informing decisions throughout the experimental cycle, biological analysis decreases the time it takes to get from instrument to insight, and improves the ability to complete that process without dead ends, mistaken directions and other research obstacles see Figure 2. Considerations necessary for strong biological analysis What are the critical enablers for biological analysis? The most crucial requirement is the tight integration of powerful analysis tools with an associated high-quality content database. This is a powerful resource for searching for relevant, validated knowledge, and for interpreting experimental results in the context of larger biological systems. Given current technology and the amount of available information, it can be highly inefficient to research discoveries or genes individually and even harder to put them into the context of an existing dataset with expression changes. A more streamlined research workflow occurs when the most relevant scientific findings can be summoned at the precise time they are needed. Combining content with analytics is not enough, however. The content must also be of sufficient breadth, quality and detail to enable sophisticated and accurate biological analysis. An effective biological analysis tool must make it easy for researchers to connect their data with that biological information through powerful analytics and an intuitive interface that encourages exploration and the generation of novel insights. The following sections explore these further. Key analysis capabilities Data analysis means different things to different people. Often, statistical analysis is considered the first step. Or, researchers turn to analysis software to indicate a few relevant pathways or diseases. But comprehensive biological analysis is a deeper dive. Researchers need to filter down their large amounts of initial data to focus on the most interesting and relevant information from their experimental results. They then need tools that allow them to explore outward to identify interesting connections and gather supporting evidence. Ultimately they need to understand if their experimental data holds together in a cohesive and supported biological story based on the known body of biological knowledge.

Three key scientific capabilities can enable this approach: These capabilities enable researchers to approach a single biological problem from multiple angles and result in substantially cleaner, more relevant, more accurate and more verifiable data. Data filtering One of the most powerful and time-saving aspects of biological analysis is its ability to help researchers rapidly narrow in on what is most relevant in those experimental results and identify a small body of information, such as a subset of relevant genes, for follow-up and investigation. As an example, a set of significant genes is an interesting initial result from a data analysis, but the real power of biological analysis comes when you consider the utility of narrowing that down to only 30 cancer-pathway specific genes, and from there identifying four known to be biomarkers of a particular form of cancer. Or, from those same significant genes, you could identify the ones known to be highly upregulated in your experimental sample, and filter down to those known to be downstream of a particular target molecule, or those known to be present in liver tissue. A good example of this is a paper published in the Journal of the American Medical Association, where researchers integrated different types of molecular data to generate a network model of genes interacting in the promotion of gliomagenesis. From their data, they identified genes affected by dosage effects in the glioma genome that were connected by protein-protein or functional interactions. From this, they organised genes and proteins into functional networks and examined genes with high connectivity as a proxy for good potential therapeutic targets. They filtered down to 11 highly connected hub genes with tumour-promoting functions and several of these had a known biological role in gliomagenesis. Using a tool that allowed them to quickly filter based on biological information, such as functional role or level of connectivity, they identified a compelling subset of genes for further investigation. Data exploration Producing novel insights depends on an exploratory approach to data which can synthesise multiple levels of biology in a unified, efficient approach. Biological analysis approaches produce comprehensive, high-level summaries of the biology most significantly affected in an experiment. These can include molecular networks, disease processes and biological pathways. However, just as statistical analysis is not enough, a limited biological analysis is not enough. Discovering implicated processes can only take the researcher so far. With an exploratory approach, researchers can investigate interesting neighbouring molecular interactions, related biology and possible avenues of connection. When exploration is applied to a small set of interesting genes that are the result of a filtering exercise, the effort spent is minimal and the amount of relevant knowledge returned is maximised. For example, biological analysis can be applied to understand and identify the key mechanistic differences between drugs see Figure 3. Celebrex celecoxib is on the market today; however, Vioxx rofecoxib has been withdrawn due to cardiovascular side-effects. Using biological analysis to explore outward from their target molecule can help identify where these drugs differ in terms of the pathways they impact, the cellular and toxicity phenotypes they play a role in and the downstream genes they impact. The resulting comparisons can be used to generate testable hypotheses around drug mechanism of action and mechanism of toxicity. Data visualisation Visualisation tools, such as interactive pathways or molecular network building tools, help researchers intuitively understand their data at a glance. Researchers can easily redefine the unit of study from a single gene to a more functionally comprehensible pathway, cellular function or phenotype. The visualisations highlight relevant information, and bring multiple levels of biology together within a single view. Visualisation has been widely recognised for its benefits within the field of biological research. Pathways provide a wealth of information about molecular interactions, including upstream and downstream effects, and allow researchers to make biologically-informed decisions about next steps. However, with an exploratory approach to biological analysis, a pathway becomes more than a visual representation of fixed information. The most critical aspect is the dynamic nature of these explorations. Unlike static pathways used for reference, dynamic pathways backed by strong biological knowledge bases help researchers quickly associate their results with relevant biology. When combined with filtering tools, researchers can exclude irrelevant information, include additional information most relevant to their models and verify facts. This exploration amounts to enormous time-savings in data analysis and results in more meaningful research outcomes. A single tool that provides an intuitive interface and advanced visualisation techniques does more than speed up research productivity. It actually improves research efficacy by facilitating better insights that might otherwise be missed. Using a knowledge base Strong data filtering,

exploration and analysis are made possible by leveraging a detailed knowledge base. For example, it is not possible to narrow down genes in a dataset known to be present in liver tissue, unless reference is made to a knowledge base that includes information about which tissues your genes are located in. The more detail a reference knowledge base contains, the more powerful filtering and exploration tools can be. In other words, biological analysis can only be as good as the content being leveraged. Content quality is about more than just accuracy or breadth. Biological analysis must use a comprehensive, accurate and up-to-date knowledge base in order for researchers to accurately interpret biological data within the context of molecular mechanisms, and relate a wide variety of molecular events to higher-order cellular and disease processes, organismal physiology and pathophysiology. Comprehensive To relate more specific molecular events to largerscale biological processes and diseases, the knowledge base must include information about a wide range of biological objects and their relationships including proteins, genes, metabolites, protein complexes, cells, cellular components, biomarkers, tissues, organs, small molecules, cellular phenotypes, pathways and disease processes. Missing any one of these components will result in the inability for the researcher to fluidly infer novel biological connections and benefit from related biological discoveries that might be relevant but which occur on a slightly different biological level see Figure 5.

Chapter 2 : The Analysis of Biological Data (2nd ed.) | Michael Whitlock & Dolph Schluter

Chapter 9 Part A: Many of the documents listed on this site are PDF files. Part A this file , and Part B States are faced with the challenge of not only developing tools that are both appropriate and cost-effective Barbour , but also the ability to translate scientific data for making sound management decisions regarding the water resource. The approach to analysis of biological and other ecological data should be straightforward to facilitate a translation for management application. In fact, biological monitoring should combine biological insight with statistical power Karr Karr and Chu state that a knowledge of regional biology and natural history not a search for statistical relationships and significance should drive both sampling design and analytical protocol. A framework for bioassessment can be either an a priori or a posteriori approach to classifying sites and establishing reference condition. To provide a broad comparison of the 2 approaches, it is assumed that candidate reference sites are available from a wide distribution of streams. In the first stage, data collection is conducted at a range of reference sites and non-reference or test sites regardless of the approach. The differentiation of site classes into more homogeneous groups or classes may be based initially on a priori physicochemical or biogeographical attributes, or solely on a posteriori analysis of biology Stage 2 as illustrated in Figure Analysts who use multimetric indices tend to use a priori classification; and analysts who use one of the multivariate approaches tend to use a posteriori, multivariate classification. However, there is no reason a priori classification could not be used with multivariate assessments, and vice-versa. Two data analysis strategies have been debated in scientific circles Norris , Gerritsen over the past few years -- the multimetric approach as implemented by most water resource agencies in the United States Davis et al. The contrast and similarity of these 2 approaches are illustrated by Figure in a 5-stage generic process of bioassessment development. The development of the reference condition from the range of reference sites Figure , Stage 4 , is formulated by a suite of biological metrics in the multimetric approach whereas the species composition data are the basis for models used in the multivariate approach. However, both multivariate techniques differ in their probability models. Once the reference condition is established, which serves as a benchmark for assessment, the final stage becomes the basis for the assessment and monitoring program. In this fifth and final stage Figure , the multimetric approach uses established percentiles of the population distribution of the reference sites for the metrics to discriminate between impaired and minimally impaired conditions. The BEAST multivariate technique uses a probability model based on taxa ordination space and the "best fit" of the test site s to the probability ellipses constructed around the reference site classes Reynoldson et al. Comparison of the developmental process for the multimetric and multivariate approaches to biological data analysis patterned after ideas based on Reynoldson, Rosenberg, and Resh, unpublished data. The bioassessment program in Maine is an example of a state that uses a multivariate analysis in the form of discriminant function models and applies these models to a variety of metrics. Decisions are made with regard to attainment or non-attainment of designated aquatic life uses. The approach used by Maine is based on characteristics of both the multivariate and multimetric approach. In this chapter, only the multimetric approach to biological data analysis is discussed in detail. The first phase is a developmental process and is only necessary as biological programs are being implemented. This process is essentially the characterizing of reference conditions that will form the basis for assessment. It is well-documented Davis and Simon , Gibson et al. Developing the framework for reference conditions i. The actual assessment of biological condition is ongoing and becomes cost-effective once Phase 1 has been completed, and the thresholds for determining attainment or non-attainment impairment have been established. The establishment of reference conditions through actual sites or other means is crucial to the determination of metric and index thresholds. These thresholds are essential elements in performing the assessment. It is possible that reference conditions and resultant thresholds will need to be established on a seasonal basis to accommodate year-round sampling and

assessment. The 2 phases in data analysis for the multimetric approach are discussed separately in the following section. The reader is referred to supporting documentation cited throughout for more in-depth discussion of the concepts of multimetric assessment. However, once this process has been completed and the various technical issues have been addressed, continued monitoring becomes cost-effective. The conceptual process for proceeding from measurements to indicators to assessment of condition is illustrated in Figure Paulsen et al. Index development outlined in this section requires a stream classification framework to partition natural variability and in which metrics are evaluated for scientific validity. The core metrics representing various attributes of the targeted aquatic assemblage can be either aggregated into an index or retained as individual measures.

Chapter 3 : The Analysis of Biological Data - Michael Whitlock, Dolph Schluter - Google Books

The Analysis of Biological Data is the most widely adopted introductory biological statistics textbook. It is now used at well over schools and on every continent. It is now used at well over schools and on every continent.

Here I describe how you should determine the best way to analyze your biological experiment. How to determine the appropriate statistical test I find that a systematic, step-by-step approach is the best way to decide how to analyze biological data. I recommend that you follow these steps: Specify the biological question you are asking. Put the question in the form of a biological null hypothesis and alternate hypothesis. Put the question in the form of a statistical null hypothesis and alternate hypothesis. Determine which variables are relevant to the question. Determine what kind of variable each one is. Design an experiment that controls or randomizes the confounding variables. Based on the number of variables, the kinds of variables, the expected fit to the parametric assumptions, and the hypothesis to be tested, choose the best statistical test to use. If possible, do a power analysis to determine a good sample size for the experiment. Examine the data to see if it meets the assumptions of the statistical test you chose primarily normality and homoscedasticity for tests of measurement variables. Apply the statistical test you chose, and interpret the results. Communicate your results effectively, usually with a graph or table. One important point for you to remember: You should do a lot of thinking, planning, and decision-making before you do an experiment. Verrelli and Eanes measured glycogen content in Drosophila melanogaster individuals. The flies were polymorphic at the genetic locus that codes for the enzyme phosphoglucomutase PGM. At site 52 in the PGM protein sequence, flies had either a valine or an alanine. At site , they had either a valine or a leucine. One biological question is "Do the amino acid polymorphisms at the Pgm locus have an effect on glycogen content? The biological null hypothesis is "Different amino acid sequences do not affect the biochemical properties of PGM, so glycogen content is not affected by PGM sequence. The statistical null hypothesis is "Flies with different sequences of the PGM enzyme have the same average glycogen content. Testing your statistical null hypothesis is the main subject of this handbook, and it should give you a clear answer; you will either reject or accept that statistical null. Whether rejecting a statistical null hypothesis is enough evidence to answer your biological question can be a more difficult, more subjective decision; there may be other possible explanations for your results, and you as an expert in your specialized area of biology will have to consider how plausible they are. The two relevant variables in the Verrelli and Eanes experiment are glycogen content and PGM sequence. Glycogen content is a measurement variable , something that you record as a number that could have many possible values. Other variables that might be important, such as age and where in a vial the fly pupated, were either controlled flies of all the same age were used or randomized flies were taken randomly from the vials without regard to where they pupated. It also would have been possible to observe the confounding variables; for example, Verrelli and Eanes could have used flies of different ages, and then used a statistical technique that adjusted for the age. This would have made the analysis more complicated to perform and more difficult to explain, and while it might have turned up something interesting about age and glycogen content, it would not have helped address the main biological question about PGM genotype and glycogen content. Because the goal is to compare the means of one measurement variable among groups classified by one nominal variable, and there are more than two categories, the appropriate statistical test is a one-way anova. A power analysis would have required an estimate of the standard deviation of glycogen content, which probably could have been found in the published literature, and a number for the effect size the variation in glycogen content among genotypes that the experimenters wanted to detect. In this experiment, any difference in glycogen content among genotypes would be interesting, so the experimenters just used as many flies as was practical in the time available. The experiment was done: The anova assumes that the measurement variable, glycogen content, is normal the distribution fits the bell-shaped normal curve and homoscedastic the variances in glycogen content of the different PGM sequences are equal , and inspecting histograms of the data shows that the data fit these assumptions. The one-way anova was done, using a spreadsheet, web page, or computer program, and the result of the anova is a P value less than 0. The interpretation is that flies with some PGM sequences have

different average glycogen content than flies with other sequences of PGM. The results could be summarized in a table, but a more effective way to communicate them is with a graph: Glycogen content in Drosophila melanogaster. Each bar represents the mean glycogen content in micrograms per fly of 12 flies with the indicated PGM haplotype. References Picture of Drosophila melanogaster from Farkleberries. The functional impact of PGM amino acid polymorphism on glycogen content in Drosophila melanogaster. They were interested in interactions among the individual amino acid polymorphisms, so they used a two-way anova. Table of Contents This page was last revised December 4, Its address is http: It may be cited as McDonald, J. Handbook of Biological Statistics 3rd ed. Sparky House Publishing, Baltimore, Maryland. This web page contains the content of pages in the printed version. You can probably do what you want with this content; see the permissions page for details.

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Step-by-step analysis of biological data I find that a systematic, step-by-step approach is the best way to analyze biological data. The statistical analysis of a biological experiment may be broken down into the.

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If possible, do a power analysis to determine a good sample size for the experiment. Do the experiment. Examine the data to see if it meets the assumptions of the statistical test you chose (primarily normality and homoscedasticity for tests of measurement variables). If it doesn't, choose a more appropriate test.

Chapter 9 : Biological analysis and interpretation for improved research outcomes

Biological analysis must use a comprehensive, accurate and up-to-date knowledge base in order for researchers to accurately interpret biological data within the context of molecular mechanisms, and relate a wide variety of molecular events to higher-order cellular and disease processes, organismal physiology and pathophysiology.