sequencing (NGS), multiplex PCR, qPCR, and droplet digital PCR (ddPCR). For example, DNA from whole blood is used as a matched-control for solid tumor somatic mutation profiling and for the detection of clinically-relevant variants in hematological malignancies, such as leukemia and myelodysplastic syndrome. An increasing number of clinical studies demonstrate the value of detecting disease-specific biomarkers from blood for early detection, diagnosis, monitoring treatment efficacy, and cohort study recruitment. Scaling down fresh blood volumes while scaling up processing capabilities is desirable to maximize laboratory throughput. At present, most DNA extraction methods require high volumes of blood and are challenging to automate because centrifugation or vacuum equipment are necessary. Additionally, conventional column and magnetic-based workflows need larger volumes for wash and elution steps, which require the use of deep well plates and other specialized consumables. Larger volumes are also needed to avoid excessive viscosity of the lysate, which can interfere with magnetic bead separation. To circumvent sample and process-related challenges, Covaris has adapted the oneTUBE-10 Plate for streamlining high-throughput nucleic acid extractions from whole blood using Adaptive Focused Acoustics (AFA) technology. Here, we performed 12 DNA extractions from healthy donors to evaluate recoveries, sample quality, and purity. Our results show recoveries in the range between 376 to 809 ng with an average fragment size of >1 kb. We also show that the extracted and purified DNA is devoid of any detectable PCR inhibiting contaminants. Taken together, this less than 90-minute AFA-enabled workflow significantly improves blood cell lysis and reduces hands-on time. The workflow can be performed on a liquid handler using the integrated Covaris R230 Focused-ultrasonicator, or off-deck using the LE220-plus Focused-ultrasonicator.

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Highly Sensitive Direct Quantification of Cf-miRNAs for Biomarker Profiling by Next Generation Sequencing (NGS)

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Cell-free circulating microRNAs (cf-miRNAs) are promising diagnostic and prognostic biomarkers for cancer and other diseases. cf-miRNAs are highly stable in biofluids and are therefore attractive as potential biomarkers via NGS-based detection compared to circulating DNA and protein markers. However, NGS detection of cf-miRNAs suffers from a high level of incorporation bias when sequencing libraries are prepared using the most widely used commercial kits. SomaGenics RealSeq library prep platform addresses this issue, with proven best-in-class accuracy of detection. The RealSeq platform includes a kit designed to profile miRNAs from biofluids (RealSeq-Biofluids) that typically detects four times as many cf-miRNAs as commercially available technologies. The latest kit in the RealSeq platform is RealSeq-T, the first targeted sequencing approach to detect panels of cf-miRNAs (up to 1,200) directly from plasma, with no organic extraction needed. Nearly 95% of sequenced reads from RealSeq-T libraries mapped to the miRNA panel. With these new capabilities, the RealSeq platform should advance the prospects of cf-miRNAs as clinical biomarkers.

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Imaging in Developmental Biology: An Essential Tool with No Instructions

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Imaging is a fundamental tool in biomedical disciplines. A critical aspect in the acquisition and evaluation of imaging data is a detailed and accurate description of the technology used in the literature. In our work at a major imaging core, we are often met with the situation in which the experiments our clients want to recreate are poorly described, making analysis and replication of the published literature difficult.

In order to evaluate the extent and severity of this problem, we have analyzed Developmental Biology publications. Research articles in three leading journals were analyzed for the importance of imaging (fraction of figure panels that contained original images) and compared with the detail given to the experimental specifics of image acquisition (fraction of the materials and methods section devoted to image acquisition and analysis). Finally, the quality of the imaging information given was evaluated for its completeness with a simple pass/fail grade.

Results indicate that imaging is an essential tool in Developmental Biology, with over 80% of the figures being images, largely microscopy. However, less than 5% of the text in the methods section of the analyzed articles is devoted to experimental details of image acquisition and analysis (on average 57 words). Furthermore, the overall quality of the information provided is dismal, with a large majority of publications obtaining a failing grade (83%), and many examples containing no usable information (10%).

The lack of information on the imaging methodologies used in published articles makes it impossible to accurately replicate the reported data. This is a serious problem that requires immediate attention. Imaging shared resources have a key role to play in ensuring accurate reporting of critical imaging parameters. This role includes providing off the shelf descriptions for the methods section of manuscripts and client education on the importance of reporting that information.

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Immuno-Biotechnology and Bioinformatics in Community Colleges

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Immuno-biotechnology is one of the fastest growing areas in the field of biotechnology. Digital World Biology's

Biotech-Careers.org database of biotechnology employers (>6800) has nearly 700 organizations that are involved with immunology in some way. With the advent of next generation DNA sequencing, and other technologies, immuno-biotechnology has significantly increased the use of computing technologies to decipher the meaning of large datasets and predict interactions between immune receptors (antibodies / T-Cell receptors / MHC) and their targets.

The use of new technologies like immune-profiling - where large numbers of immune receptors are sequenced en masse - and targeted cancer therapies - where researchers create, engineer, and grow modified T cells to attack tumors - are leading to job growth and demands for new skills and knowledge in biomanufacturing, quality systems, immuno-bioinformatics, and cancer biology. In response to these new demands, Shoreline Community College (Shoreline, WA) has begun developing an immuno-biotechnology certificate. Part of this certificate includes a five-week course (30 hours hands-on computer lab) on immuno-bioinformatics.

The immuno-bioinformatics course includes exercises in immune profiling, vaccine development, and operating bioinformatics programs using a command line interface. In immune profiling, students explore T-cell receptor datasets from early stage breast cancer samples using Adaptive Biotechnologies (Seattle, WA) immunoSEQ Analyzer public server to learn how T-cells differ between normal tissue, blood, and tumors. Next, they use the IEDB (Immune Epitope Database) in conjunction with Molecule World (Digital World Biology) to predict antigens from sequences and verify the results to learn the differences between continuous and discontinuous epitopes that are recognized by T-cell receptors and antibodies. Finally, to get hands-on experience with bioinformatics programs, students will use cloud computing (CyVerse) and IgBLAST (NCBI) to explore data from an immune profiling experiment.

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Impact of Enrichment Strategy on Observed Expression in Fresh Frozen Tissues

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Human tissues obtained during clinical and surgical procedures are an invaluable resource for determining diagnosis, treatment response, and disease progression. In many cases, these biological specimens are preserved for future analyses, most commonly by formaldehyde fixation and paraffin embedding (FFPE). FFPE has the advantage that such specimens are stable at room temperature for years, but introduces additional complexity in terms of sample preparation, protein modifications or degradation. Frozen tissues are costly to store, but are preferred for post translational modification analyses even though they suffer some degradation on long timescales. Significant interest remains in optimizing recovery from both storage and sample processing conditions for these precious specimens. Here, we compare fresh frozen samples of different biological origin and complexity, such as heart, brain and lung tissues, and assess the differences between peptides enriched by hydrophilic interaction chromatography (HILIC) bound to MagReSyn polymer particles as compared to standard reversed phase C18 enrichment. For example, we lysed and extracted proteins from ~18mg of frozen normal autopsy heart tissue, digested with Trypsin/LysC and analyzed them using Orbitrap mass spectrometry. From a 500ng injection, we observed 1,749 proteins from C18 prep and 1,678 proteins from HILIC prep with 20k peptide spectrum matches in both cases. The proteins identified from both preparations are broadly similar in terms of protein families and function. But with more detailed analysis of the particular proteins and pathways observed, such as through tools like GeneTrail2, we observe each preparation prefers particular pathway members. Here, HILIC preparation favors identification of proteins involved in glycolysis while C18 preparation identified proteins involved in GTP hydrolysis and ribosomal assembly. We will further analyze additional tissues, including lung tissue with adenocarcinoma and brain tissue with glioblastoma, with an eye to understanding the advantages or disadvantages of particular preparations based on the biological hypothesis being tested.

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Impact of Fecal Microbiome Extraction Technique on Relative Abundance of Genera within Expected and Unexpected Communities

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Microbiome analysis has gained significant interest as sequencing technology has improved, allowing for the examination of microbial communities often believed to play a key role in human health and disease. 16S rRNA sequencing analysis, utilizing various publicly available bioinformatic tools, allows the study of diversity in microbial communities. It has previously been shown that extraction techniques are impactful for the analysis, however, these studies have often been performed with a single cultured sample, or with evenly distributed mock communities, failing to provide variability often seen with human source samples and excluding the impact of amplification and/or sequencing related bias.

Within this study, we analyzed six different extraction protocols, utilizing commercially available reagents, with a naturally collected fecal sample, two mock communities, all of uneven species distribution, and a manufactured extraction control, of known species distribution. In addition, six sequencing controls, already extracted DNA of variable concentration and percentage of species distributed within