Overcoming RNA-seq challenges in a non-model organism

From data to insight with QIAGEN® Discovery Bioinformatics Services

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Introduction

Are you working with a non-classic model organism? Is there no reference genome available to support your genomics research? Do you find it too time consuming to scan the scientific literature to place your results into a biological context? These were some challenges that Philip James, Senior Researcher from Nofima (Norwegian Institute of Food, Fisheries and Aquaculture Research) was confronted with when researching the effect of diet on gene regulation in sea urchins. Nofima needed data processing and pathway analysis of RNA-seq data for samples collected from sea urchins, access to a knowledge base, analytic tools and expertise to analyze a non-model organism, the sea urchin. He also needed to run RNA-seq data processing and pathway analysis to put the results into a biological context.

Nofima found partnering with QIAGEN Bioinformatics Discovery Services to be a reliable and convenient way to extend their in-house resources. The team comprises highly skilled experts offering tailored bioinformatic services that ensure high-quality results.

The QIAGEN Discovery Bioinformatics Services experts work with researchers to help them reach their goals, whether they are exploring biological pathways, identifying causal variants, exploring large-scale next-generation sequencing (NGS) population studies or working with a clinical research organization (CRO) to deliver large-scale data analysis. No matter the size or scale of the data, QIAGEN Discovery Bioinformatics Services can be a trusted partner for any research team looking for bioinformatics support.

Inspirational and groundbreaking science is often unorthodox, out of the norm and tricky – even within bioinformatics research. Read on to follow the journey from initial contact, to delivery of the data analysis and submission of the study for scientific publication, by partnering with QIAGEN Discovery Bioinformatics Services.

Solving data to insight analysis bottlenecks for sea urchin RNA-seq

In one of the most northern parts of Norway, on a small island, lies Tromsø, the home of the Nofima main office. Close to the elements, it's a fitting location for Nofima as a leading institute for applied research within the fields of fisheries, aquaculture and general food research. Their AquaVitae project, led by Dr. Philip James, aims to develop solutions for increasing the cultivation of low-trophic species, such as sea urchins, as environmentally-friendly food sources for human consumption.

Though spiky and initially uninviting, crack open a sea urchin and it is regarded as a delicacy throughout the world. Eaten mostly in Japan, but also around the globe, sea urchins have been called the "foie gras of the ocean". However, all is not well at the bottom of the sea: Sea urchins can rapidly multiply and eat vast amounts of kelp forests, leaving a barren wasteland behind them. The kelp forests are not only crucial for marine life, but they are also essential carbon sinks and critical to the fight against climate change.

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So, what can we do? We could help control the population by eating more sea urchins.

It's the gonads of sea urchins that are consumed, with the gonad size increasing if the sea urchins are well-nourished. So, a well-fed sea urchin gives you more gonad for your buck. Therefore, nutrition and feeding are naturally a big part of the research of sea urchin aquaculture. One of Nofima's projects involves the capture of wild sea urchins to study the effect of diet on gonad size. They aim to create the optimal diet for cultivating wild sea urchins into a well-nourished delicacy, producing sustainable food, and combating climate change simultaneously.

To study the effect of diet on gene regulation in sea urchins, Nofima needed data processing and analysis of RNA-seq data for samples collected from sea urchins captured and cultured on an improved diet to enhance health. Expertise was required to build non-model organism genome models and run RNA-seq data processing and analysis, and finally to put the results into biological context using up-to-date curated databases and pathway analysis tools.

After a briefing meeting to discuss the project and its aims, the QIAGEN Discovery Bioinformatics Services team went to work. Figure 1 generally summarizes the entire process, from initial contact to service delivery.

The goal of this project was to compare RNA-seq data from the two groups of green sea urchin to elucidate the molecular effect of diet.

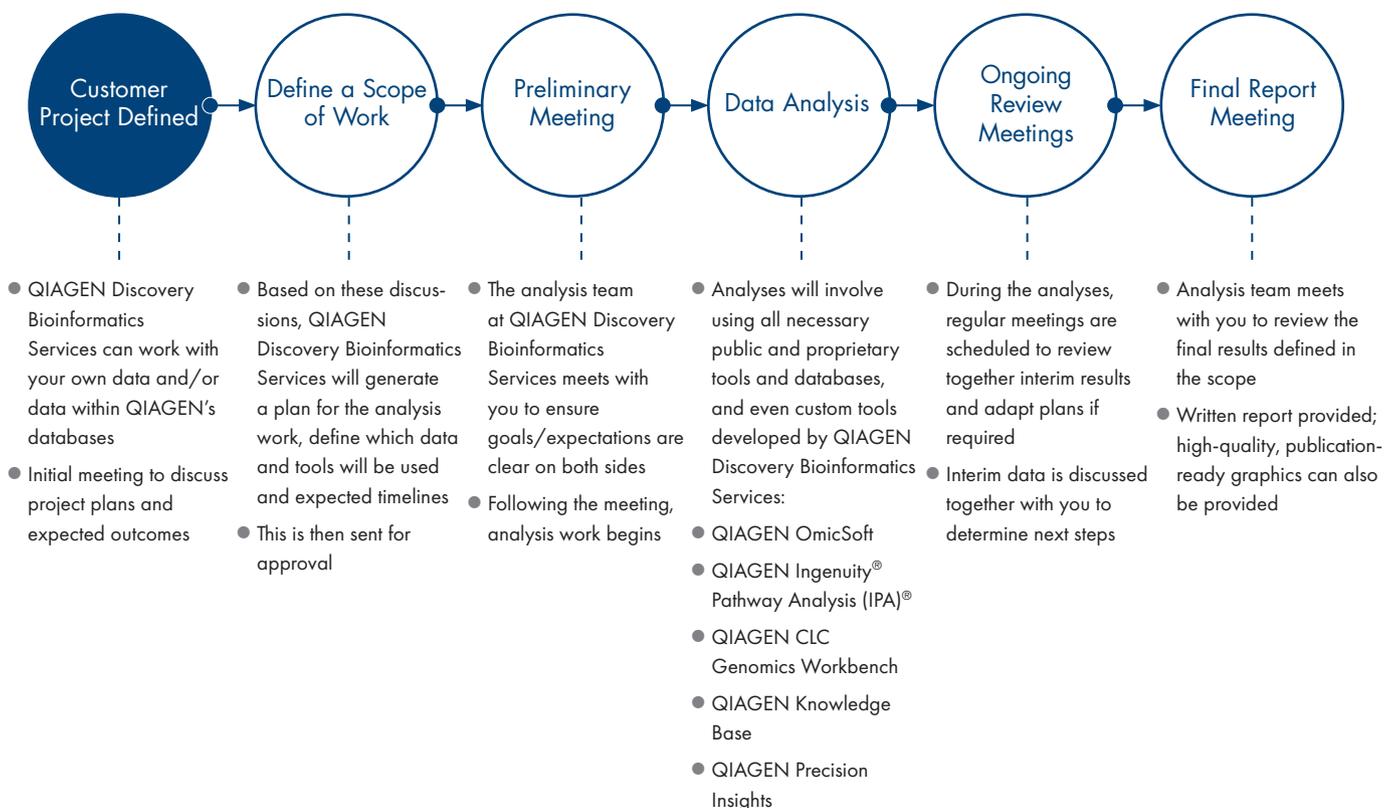## Data analysis and interpretation by QIAGEN Discovery Bioinformatics Services

Customer Project Defined	Define a Scope of Work	Preliminary Meeting	Data Analysis	Ongoing Review Meetings	Final Report Meeting
• QIAGEN Discovery Bioinformatics Services can work with your own data and/or data within QIAGEN's databases • Initial meeting to discuss project plans and expected outcomes	• Based on these discussions, QIAGEN Discovery Bioinformatics Services will generate a plan for the analysis work, define which data and tools will be used and expected timelines • This is then sent for approval	• The analysis team at QIAGEN Discovery Bioinformatics Services meets with you to ensure goals/expectations are clear on both sides • Following the meeting, analysis work begins	• Analyses will involve using all necessary public and proprietary tools and databases, and even custom tools developed by QIAGEN Discovery Bioinformatics Services: • QIAGEN OmicSoft • QIAGEN Ingenuity® Pathway Analysis (IPA)® • QIAGEN CLC Genomics Workbench • QIAGEN Knowledge Base • QIAGEN Precision Insights	• During the analyses, regular meetings are scheduled to review together interim results and adapt plans if required • Interim data is discussed together with you to determine next steps	• Analysis team meets with you to review the final results defined in the scope • Written report provided; high-quality, publication-ready graphics can also be provided

Figure 1. Outline of the process/project journey when working with QIAGEN Discovery Bioinformatics Services.

Data analysis and results

The study included a combination of differences in diet for both male and female green sea urchins. After initial quality control of the RNA-seq data, differential expression analyses were used to look at the effects of both diet and gender. FASTQ files were generated by Nofima from male and female green sea urchins either wild-caught or enhanced by diet. The files were rapidly analyzed by using cloud computing resources enabled through QIAGEN OmicSoft Suite (version 10.1.1.14), starting with alignment to the closely-related purple sea urchin genome, since the genome sequence for green sea urchin is unavailable.

After making certain customizations to the alignment algorithm to account for minor differences in the genomes of purple and green sea urchins to improve the alignment rate and obtain data for a maximum number of annotated genes, stringent quality control was applied and differential expression analysis controlling for gender biases was performed. Differential expression gene signatures were uploaded to QIAGEN Ingenuity Pathway Analysis (IPA), and gene symbols were mapped to the curated ontology in the QIAGEN IPA database. In the study, 255 genes were determined to be significantly up- or down-regulated.

Data analysis and interpretation by QIAGEN Discovery Bioinformatics Services – Nofima Project

Customer Project Defined
- Nofima provided information on the sea urchin study goals and RNA-seq data collected
- Initial meeting to discuss the project plans and expected outcomes

Define a Scope of Work
- QIAGEN Discovery Bioinformatics Services generated an initial plan for the analysis work, identified related genomic data and annotations, the tools to be used (QIAGEN OmicSoft and QIAGEN IPA) and expected timelines

Preliminary Meeting
- The analysis team at QIAGEN Discovery Bioinformatics Services meets with Nofima to ensure goals/expectations are clear on both sides
- Following the meeting, analysis work begins

Data Analysis
- Green sea urchin RNA-seq was mapped and annotated against the purple sea urchin genome as the closest available reference genome
- Differential expression analyses were carried out in QIAGEN OmicSoft, comparing diet and gender

Ongoing Review Meetings
- During the analyses, it was decided to orthologue-map the sea urchin data to human gene data, so the differentially expressed genes from the QIAGEN OmicSoft analyses could be mapped to biological pathways in QIAGEN IPA

Final Report Meeting
- QIAGEN Discovery Bioinformatics Services reviewed the final results with Nofima to talk through the written report summarizing the work and providing figures of the key findings and tables of the differentially expressed genes identified
- Figures were selected for generation of high-quality publication-ready graphics
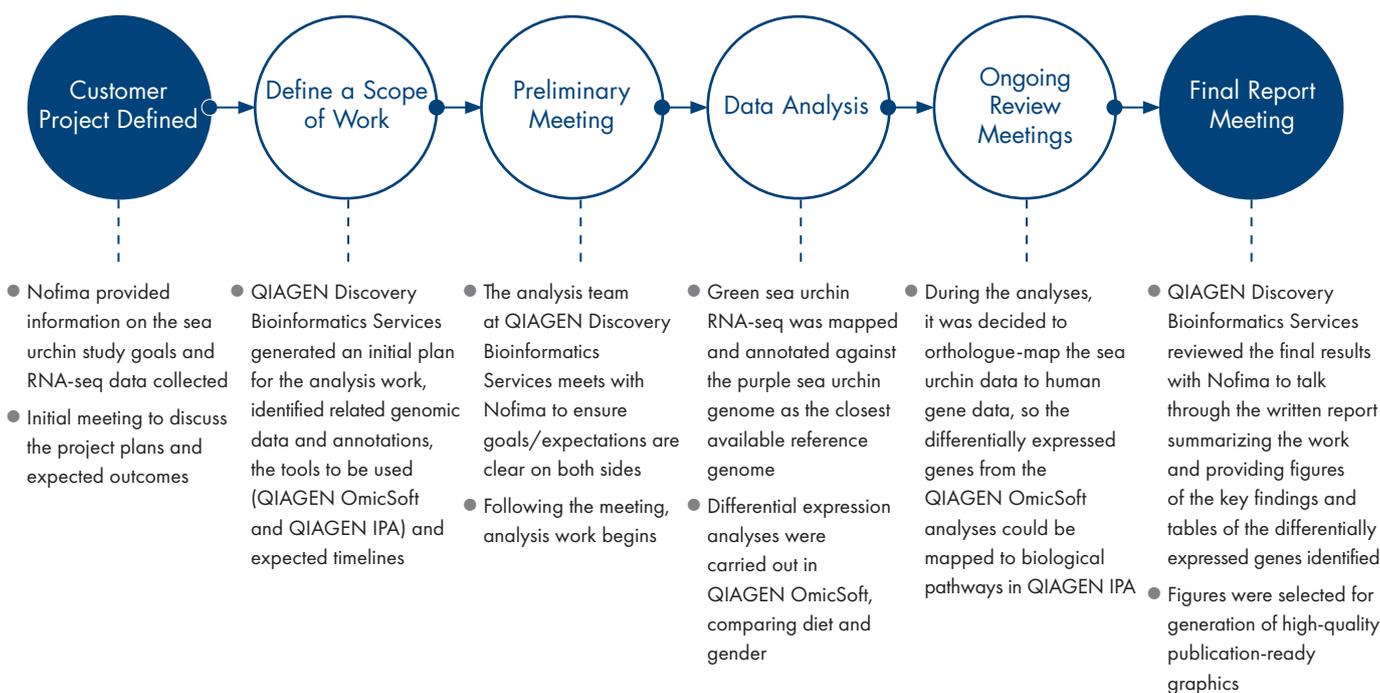
Figure 2. The bioinformatics workflow followed by Nofima and QIAGEN Discovery Bioinformatics Services.

After differential expression analysis, the genes were subject to Core Analysis in QIAGEN IPA to discover the canonical pathways and upstream regulators affected by differential expression. The predicted pathways that were activated are related to metabolism (e.g., cholesterol biosynthesis and ethanol degradation). Examination of the upstream regulators showed factors predicted to be highly activated. QIAGEN IPA with Analysis Match supported the evidence for activation of metabolic pathways and down-regulation of pathways associated with mortality.

Bringing in the full curated findings of these networks and their downstream functions and diseases, metabolism is predicted to be activated in the "enhanced" urchins, compared to the "wild", consistent with a healthier sea urchin population after enhancement.

Conclusions

Nofima experienced multiple benefits from working with QIAGEN Discovery Bioinformatics Services, having saved time by letting the QIAGEN team build the foundation for analysis and then analyze these RNA-seq data, completing their project in just 10 weeks. The main driver for this project was the rapid analysis performed by the QIAGEN Discovery Bioinformatics Services team, thus enabling Nofima to quickly advance their research and prepare to publish their results. The result of Nofima's collaboration with QIAGEN Discovery Services enabled:

- Fast turnaround time and reduced burden for project delivery
- Rapid and novel insights into key systems and regulators

''In this study, QIAGEN offered us a cost effective, timely and high-quality solution to our analysis requirements and we were very happy with the outcome.''

Philip James, Ph.D., Senior Researcher, Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima)

Contact QIAGEN Discovery Bioinformatic Services for more information about how we can help you on your journey from Sample to Insight.

Learn more at **digitalinsights.qiagen.com/services-overview/qiagen-discovery-bioinformatics-services/**

Discover more about Nofima and their sea urchin case study at **https://aquavitaeproject.eu/**

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