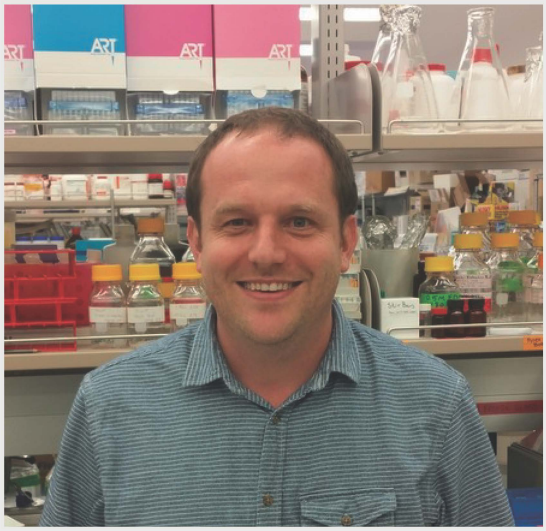
PARAGON GENOMICS

Scientist Insider

Targeted genomic datasets provide unprecedented resolution into phylogenetic relationships



Scott is a Research Entomologist at the USDA-ARS U.S. Pacific Basin Agricultural Research Center in Hilo, Hawaii. He utilizes genomic techniques to study tephritid fruit flies, specifically to develop novel tools for quarantine and eradication of pest species from the mainland United States. This includes developing diagnostic tools for species identification and source determination, as well as applying genomics towards improving sterile insect technique programs.

"We are looking for a 'future proof method', a better way to do high throughput screening at a species level diagnosis."

Which key issues do you face with your research?

With the rapid influx of pests coming into the US, mandates are needed to identify the origin of these insects. We work specifically with tephritid fruit flies, which are pests of fruits and vegetables worldwide. We need to develop methods for rapid species identification, as well as population genomic tools for source determination. The systematics of this group is complicated and cannot be resolved with traditional DNA barcoding methods, which provide limited resolution.

There is a growing interest to build robust, rapid, cost-effective, phylogenomics methods for species determination in these flies, and we want to create it in a system that is simple yet straightforward. Additionally, we are looking for ways to make procedures

more routine and consistent.

Ideally, we would like to look at many array-based designs for oligos to help reduce costs of high multiplexing PCR assays and design but this does not seem possible. Currently, limitations are around the cost of synthesizing large numbers of multiplexing assays. Huge capital is required for >20,000 oligos per experiment.

Which technologies are currently available to further your research?

We used capture based/hybridization technologies and TaqMan Assays previously. Both options work well, but often only interrogates a particular single nucleotide polymorphism (SNP) or can be cost inhibitive. There is a need to test more SNPs, more markers, and more flies. A newer technique using multiplex PCR technology enables higher density testing coupled with Next Generation Sequencing machines. Paragon Genomics has developed a rapid 2.5-hour library preparation allowing multi-gene methodology, letting us look at lots of unique loci across multiple genomes.

"It offers a 'one and done' approach instead of many different, disconnected experiments."

What are the drawbacks of other technologies?

With capture based technologies, even though there are a lot of probes, coverage across a region can be variable. Capture technologies also require a lot of high quality DNA which needs to be first made into an NGS library. This incurs additional costs for equipment or the need to send the sample out to a Core Lab (who has that expensive equipment) which also increases turnaround time.

Where does Paragon Genomics fit in?

USDA has a pipeline developed to identify orthologous regions in a broad range of insects. Other technologies have a fixed generic content whereas Paragon Genomics can look at multiple organisms across many different species. They can compare highly conserved regions yet use degenerate primers to capture highly diverse sequence variation. With capture based technologies, even though there are many probes, at times evenness of coverage can be an issue. With Paragon Genomics, the loci are recovered from start to end and the 200bp amplicon design is flexible if amplicons are longer. The technology can also recover the whole amplicon and allows an alignment-free consensus caller.

What will CleanPlex® technology enable you to do today?

Some of the samples we have obtained are curated from museums and are extremely compromised in nature—i.e. we don't get a lot of DNA from them. It's great to have a protocol that is flexible and costs less for some of these low-quality samples. CleanPlex® technology enables us to use questionable quality DNA of very small volumes.

"You don't need to have a lot of capital to do the things you want. All you need is a thermal cycler."

It would be interesting to explore the limitations of the amplicon sizes. Paragon Genomics originally set a limit between 150-250bp, but our original design was 400bp, and it worked great. We used the MiSeq, with CleanPlex® technology and recovered the entire sequence so we would like to understand how this can evolve with other sequencing a few hours versus a few days on current sequencers. We would also like to look

at the VolTRAX System and then run the Nanopore using the CleanPlex technology.

Are there any other applications you envision CleanPlex® technology being applied?

Turnaround time is fast so there is a huge application for diagnostic utility. CleanPlex® technology can be easily run in the field and has a fast turnaround time. This technology would be ideal for the Centers for Disease Control (CDC), Homeland Security and Infectious Diseases. Anyone screening for diseases, viruses or good versus bad bacteria detection could use this technology as a diagnostic.

What are some recent findings?

Previously, we used TaqMan Assays to look at 100s of SNPs. Now with our vast pipeline, combined with Paragon Genomics multiplex PCR, we can investigate 1000s of regions with the flexibility to look at a single panel to discriminate species. Additionally, amplicons can be applied for certain species to help organize population structure. This will help us determine the fly species and fly origin in a single assay. Previously, this work would take a minimum of one week and was a 2-step process.

"Now we can analyze data in multiple ways. We have the ability to identify and genotype more species."

How will your research be applied?

Field offices, which are Port of Entry sites, have basic molecular biology functionality and generally use TaqMan technology to run minimal assays. With the presence of low cost benchtop sequencers providing rapid sequencing, robust diagnostic tools could be developed for running in the common laboratory.

If you had one wish?

That this work can help create a momentum to change people's perception of how to do these types of panels. At times we need to continue to generate the standard data (COI sequencing, SSRs, etc.), but want to expand further. With the huge number of targets that can be assayed with multiplex PCR, you can include the "old stuff" (SSR regions, COI, etc.), and at the same time expand your assay with new markers, regions, etc., without having an increase in cost to perform the assay.

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