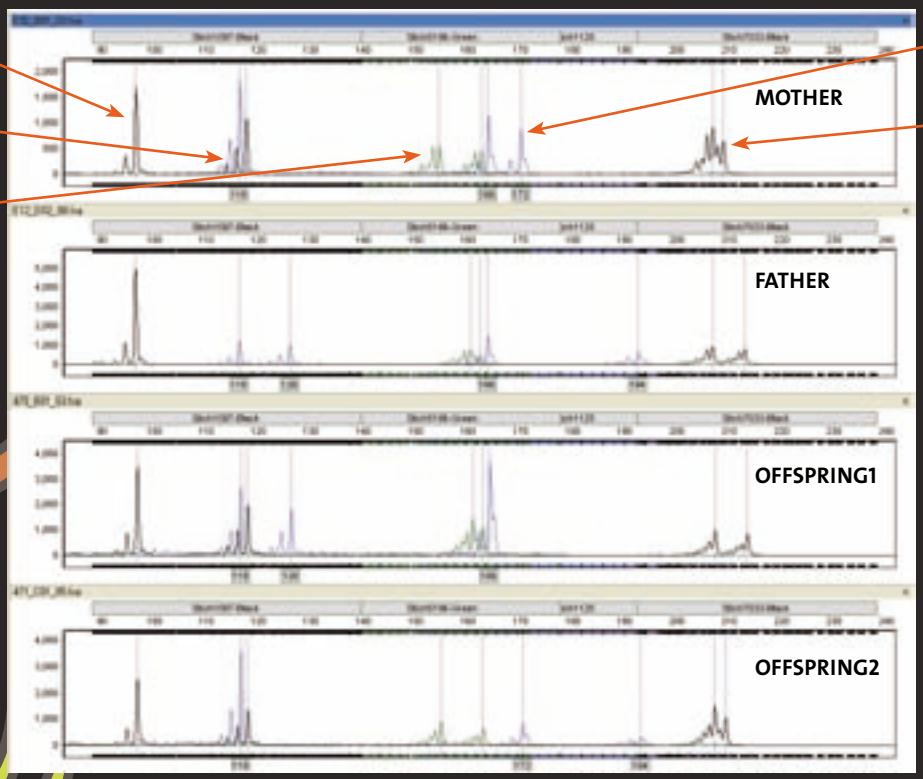
Automated DNA preparation pushes forward evolutionary biology research

Reliable high throughput genotyping on the Tecan Freedom EVO® 200 platform has helped research in parenthood analysis and population genetics of the three-spined stickleback at the Max Planck Institute for Evolutionary Biology, Plön, Germany.

In evolutionary biology research, progress in molecular techniques has allowed the studies of individual lifetime reproductive success and how it is affected by environmental and genetic factors. The three-spined stickleback (*Gasterosteus aculeatus)* is an ideal model organism for these investigations (Gibson, 2005) because it is amenable to laboratory-based monitoring and its reproduction, immunology and evolution have been well studied.

Three-spined sticklebacks have a complex mating system based on fitness-related traits and genes of the major histocompatibility complex (MHC), which encode for molecules that bind pathogen-derived peptides to trigger an adaptive and specific immune response. Studies have demonstrated that variation in MHC genes in the sticklebacks is ecologically relevant, through changes in resistance to parasite attack, suggesting a trade-off between parasite resistance and cost of immunity (Wegner *et al*., 2003a, 2003b). Dr Christophe Eizaguirre, Evolutionary Biologist at the Leibniz Institute of Marine Sciences at the Christian-Albrechts Universität zu Kiel (IFM-GEOMAR), and previously at the Max Planck Institute for Evolutionary Biology, Plön, Schleswig-Holstein, Germany, has spent several years studying the population genetics of three-spined sticklebacks. He explained: "Our research requires extraction of high quality DNA from hundreds of sticklebacks for MHC genotyping. Yearly, we run a large experiment during the sticklebacks' breeding season and we needed an automated, easy-to-handle method to increase productivity; in 2008 for instance, we collected almost 10,000 eggs to analyze in three months. Our Freedom EVO 200 platform from Tecan, equipped with an 8-channel liquid handling arm, meets our requirement for high throughput purification of genomic DNA from tissue and eggs. We use the Invisorb® DNA tissue HTS 96 Kit from Invitek GmbH, and it was very easy to establish an automated protocol."

For tissue DNA extraction, small dorsal spine samples are incubated overnight with continuous shaking at 52 °C in 96-well plates for lysis and protein digestion. The plates are then transferred to the Freedom EVO 200 platform for automated processing, including the addition of binding buffer, incubation, washing to remove protein, nucleases, PCR inhibitors and ethanol contamination, and elution of the purified DNA. For parenthood analysis, to determine the number of offspring produced by each individual (see figure), DNA is extracted from eggs, which is technically challenging because these eggs have an extremely tough shell. The shells are disrupted overnight with continuous shaking at 52 °C and, as with DNA extraction from tissue, subsequent steps are carried out on the Freedom EVO platform. Multiplex PCRs are performed on extracted DNA using 18 microsatellite loci and amplified products are analyzed by sequencing. However, the MHC Class II B loci are genotyped by capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) or reference strand-mediated conformation analysis (RSCA), and DNA quality is critical for the success of these methods.



Christophe continued: "The main advantage of using this instrument is its autonomy; basically, we switch it on and walk away and, for two hours, we can do something else. Also, because the Freedom EVO system is capable of normalization of DNA concentration, the instrument is useful for other tasks. We do a lot of work with cloning, so we also use it for automated plasmid DNA extraction and DNA normalization."

"Automating high throughput DNA extractions on the Freedom EVO platform efficiently purifies high quality genomic DNA from small pieces of tissues or early-developed stickleback eggs with minimal hands-on time. We can now address important questions in evolutionary biology such as individual reproductive success and ecological relationships between recently diverged species. Automated DNA sample preparation is saving us time and offering higher production rates, reliability and high quality DNA, which are all essential for sensitive downstream studies such as SSCP/RSCA analysis," Christophe concluded.

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Three-spined stickleback (Gasterosteus aculeatus)

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