# Customer Case Study:
# Hy-Line International

## Why poultry?

Between 2000 and 2010, the global egg output expanded by more than 25% from 51 million tonnes to 63.8 million tonnes. World egg production will likely reach a record 65.5 million tonnes in 2013[1].

In order to increase the ability of the hens to produce these eggs, and assure that these eggs are of the highest quality, research regarding their genetic make-up is required to make sure the best individuals are identified and used to breed the next generation to meet the growing production requirements.

Poultry breeding uses a combination of experience, expertise and science that requires long term investments of both time and resources. Certain traits (genetically determined characteristics) such as feed consumption, egg shell quality and rate of lay can be measured and analysed by geneticists involved with poultry breeding.

Each bird is evaluated for the full range of useful characteristics and compared against others; elite birds which exhibit these characteristics of better performance are then used to breed and produce the next generation of egg-laying hens.

These superior birds have naturally occurring changes (variation) within their DNA that impacts their performance. One type of DNA sequence variation commonly used for this type of analysis is single nucleotide polymorphisms or SNPs; recent research has identified over 39 million novel variations that exist within the DNA code of chickens.

Detection of SNPs by genotyping is an important tool for identifying variation in important trait genes and understanding how this variation can affect the traits. Such techniques can help poultry breeding research scientists to identify and measure the genetic variation of traits which are important to commercial egg production and bird welfare.

## Industry challenge:

It is estimated that there are between 5 and 5.5 billion egg-laying hens in the world.

Today's laying hens are each capable of producing over 300 eggs per year. The world's human population is growing rapidly and the high-quality protein and nutrient-rich egg is an inexpensive and transportable source of nutrition for this growing population.

The challenge for the poultry industry is to produce this food source in an efficient way. A decreased input is needed to produce each egg, to ensure that each egg can reach the consumer while at the same time decreasing the carbon footprint of the laying hen.

Hy-Line International strive to create the highest standard of commercial egg-laying hen with good liveability, feed efficiency, nesting behaviour and persistency of lay that also produces a good quality shell and subsequently the best egg to get to market.

Hy-Line International uses genetic research and testing to combat these challenges. By identifying variation within the DNA of the egg-laying bird, they can decrease the amount of feed needed to produce each egg. Variation within the DNA that affects the proteins in the egg shell can be identified to improve the quality of the shells so that more eggs can reach the consumer. The carbon footprint caused by producing these eggs can also be addressed indirectly by improving production parameters.

## Technical challenge

The Hy-Line International research program has been operating since 1936. Within the program, Hy-Line measures over 30 different traits of importance to commercial egg production and bird welfare. Information from thousands of birds within both the research farms and the commercial field test programs is fed into extensive databases and subjected to extensive statistical analyses. The challenge is to integrate information from molecular genetics into the existing breeding program system.

The company has developed the world's most extensive DNA archive in the poultry industry consisting of samples from over 15 generations of birds from multiple lines. Each of these samples is associated with trait information as well as information on all family members. The Hy-Line breeding program involves identification of multiple SNPs within genetic regions of interest and the subsequent genotyping of large number of individuals within multiple lines. Application of this information into the breeding program requires that the results be provided rapidly to enable selection of the next generation of breeding birds. Dr Janet Fulton, Molecular Geneticist at Hy-Line International comments,

"When the Molecular Genetics program was initiated in 1996, the state-of-the-art technology at the time allowed us to study 100 different DNA variations (or markers). Bird-to-bird variation was identified in 5,000 samples per week. However, the requirements of our current research meant that we needed to expand the number of samples tested without expanding the costs. With the increased numbers of samples we also needed to ensure that every sample and every result was correctly identified and tracked".

The scientists at Hy-Line sought a technology that could analyse hundreds of thousands of samples quickly and that could do so without large financial resources or disruption to their current analysis procedure. The scientists depend upon the results in order to complete the breeding cycle. This means that very accurate data must be delivered within a very short time.

In a recent study[2], researchers at Hy-Line identified *ovocalyxin-32* (*OCX32*), as a candidate gene for selection for eggshell traits in commercial poultry populations. The OCX32 protein is a component of the avian uterine milieu present during formation of the eggshell. Although the specific function of *OCX32* in eggshell formation and structure is unknown, the presence of the protein as a component of the outer layer of the eggshell suggests it has an important role within the avian eggshell. SNP variation in *OCX32* gene has been reported in a number of poultry breeds and researchers at Hy-Line wanted to identify polymorphic sites within the *OCX32* gene in their breeding stock. Initially, SNP variation was detected using allele-specific PCR primers that result in a different amplicon size for each SNP followed by gel based detection. However, this method was costly, labour intensive and prone to sample tracking errors.

## The solution

When identifying variations in the *OCX32* gene, SNP detection was changed to a more rapid and less expensive method based on KASP™ genotyping chemistry from LGC. Hy-Line implemented a high-throughput genotyping method using KASP assays to help detect SNP variations. Hy-Line found that KASP genotyping allowed them to do rapid in-house detection of SNP variations and that the technology permitted them to carry out individual testing of thousands of samples, for hundreds of carefully selected SNPs, at a rate of over 100,000 tests per day.

"The KASP technology provided the best and most rapid identification of genetic variation within our populations."

Dr Fulton adds,

"The volumes and complexity of data generated by this technology is unimaginable. The dedicated Kraken™ software system is used to analyse and track the SNP data and the results are then rapidly integrated into the existing databases that contain the thousands of collected data points gathered from the research farms and global field tests."

Utilising newly developed statistical methods, KASP is used to associate SNP variations with performance. Both the speed and accuracy of the selection process is improved with genomic selection made possible with KASP genotyping. Not only will the right birds now be more accurately identified, but their genetic potential is identified earlier in the birds' life.

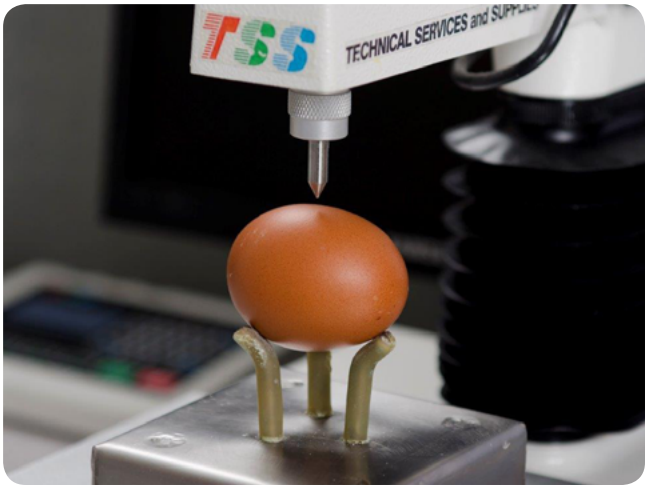
## Results

KASP genotyping perfectly fits the requirements of very large numbers of samples to be tested over a large number of individual SNP assays; the *OCX32* gene is a perfect example of the use of KASP genotyping. Janet Fulton commends KASP's versatility and accuracy, explaining that,

"You have so much control over what you can do in terms of designing the primers to detect your specific variants. You can detect this variation with different equipment but using the same materials and you'll get the same results. This makes it easy to adjust assay volumes when moving between low and high numbers of samples and manual to semi-automated systems."

## Why KASP?

The KASP genotyping assay utilises a unique form of competitive allele-specific PCR that delivers high levels of assay and accuracy, whilst ensuring cost savings. The technology enables highly accurate bi-allelic scoring of SNPs and InDels at specific loci across a wide range of genomic DNA samples. The benefit for Hy-Line's research was KASP's ability for rapid SNP testing while still producing highly accurate results needed for input into a commercial breeding program.




## Future

Hy-Line's research will affect the egg industry on a larger scale than simply improving the quality of egg shells. With this genetic program, the key performance traits needed to be a successful layer are identified and improved across multiple lines. The genome variation which influences the quality of the shell are found and improved across generations. Hy-Line's research continues to improve hens through their laying rate, their feed efficiency and their liveability. KASP genotyping facilitates the selection process for those birds that will produce the next generation of elite breeding stock.

## References

1. The Poultry Site, Jan 2013, http://www.thepoultrysite.com/articles/2653/global-poultry-trends-world-egg-production-sets-a-record-despite-slower-growth.
2. Fulton, J. E., Soller, M., Lund, A. R., Arango, J., & Lipkin, E. (2012). Variation in the ovocalyxin - 32 gene in commercial egg - laying chickens and its relationship with egg production and egg quality traits. Animal genetics, 43(s1), 102-113

## About Hy-Line International

Hy-Line International is a world leader in poultry genetics with a rich history of innovation. Founded in 1936, by Henry A. Wallace, Hy-Line International was the first of the modern egg layer (hen) genetics companies to incorporate hybridisation and the potential of hybrid vigor into its breeding program on a commercial scale. The program was used alongside time tested methods of genetic selection coupled with scientific statistical analysis to develop and improve one of the world's most extensive gene pools. Today, Hy-Line International continues to be a pioneer as the first company with its own in-house molecular genetics team. It is leading the industry in the application of DNA-based technology to its breeding and genetics program.

The company has several commercial products across the global marketplace and access to one of the world's most extensive gene pools. Hy-Line International produces and sells both brown and white egg stock to more than 120 countries worldwide and breeds hens known for their superior liveability, feed efficiency and egg quality.

Research is the key component in the genetic development process and Hy-Line geneticists use exacting research procedures to preserve the unique genetic balance while making continuous improvements to the performance of all Hy-Line stock. Hy-Line uses only established and universally accepted methods to detect naturally occurring genetic variation, for evaluation and subsequent selection in their birds. There is no use of genetic modification or cloning anywhere within its breeding program.

## About LGC

LGC is an international science-based company and market leader in the laboratory services, measurement standards, genomics, reference materials and proficiency testing marketplaces. LGC operates in a variety of markets – including, but not confined to, Food & Agriculture, Government, Pharmaceuticals and Biopharmaceuticals and Sports – which underpin the safety, health and security of the public and the regulation of industry, for both private and public sector clients.

With headquarters in Teddington, South West London, LGC employs over 2,000 staff, operating out of 22 countries worldwide. Its operations are extensively accredited to international quality standards such as ISO/ IEC 17025.

Set up in 1842 as the Laboratory of the Government Chemist, for more than 100 years LGC has held the unique function of the Government Chemist in the UK. LGC was privatised in 1996 and is now majority-owned by funds managed by Bridgepoint.

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