PRECISE MULTIPLEXED MEASUREMETET OF GENE EXPRESSION: QUANITATIVE NUCLEASE PROTECTION ASSAY (qNPA™)

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ABSTRACT

qNPA is a 21st century gene expression technology that only requires the lysis of samples (no extraction), is multiplexed (16 genes/sample), fast and simple, and has single gene copy sensitivity, but most importantly provides precision associated in the past only with protein assays. Whole assay CV’s of cells, tissues and plants are on average ≤10%, which means that changes in gene expression ≤1.2-fold are significant. The assay is repeatable between labs and day-to-day, where fold changes can be measured reliably with an average CV of 2%. The precision enables precise EC50 data to be generated useful to scientists and for clustering of genes into mechanistic signatures. Whole plants or plant parts can be lysed, prepped by a Genogrinder, and tested. This expanded Abstract will discuss (1) how HTG Inc.’s qNPA™ and ArrayPlate Technology performs, (2) the use of qNPA™ in an Arabidopsis study used to observe differential gene expression when challenged with NaCl, and (3) the use of qNPA™ in the Dose-response of Arabidopsis transcripts to NaCl treatment.

HTG Inc.’s qNPA™

The quantitative nuclease protection assay (qNPA™) allows researchers to quickly and accurately measure the gene expression levels in a wide range of mammalian, plant and bacterial cell types with a precision not previously possible. The fast, automated protocol requires NO RNA EXTRACTION, NO RNA AMPLIFICATION AND NO RNA LABELING. Samples are treated in a 96-well plate with HTG, Inc.’s proprietary lysis buffer, thereby releasing total RNA for analysis by qNPA™ in a multiplexed assay.

Nuclease Protection is the first phase of the qNPA™ Assay. Nuclease Protection converts labile mRNA to stoichiometric amounts of stable DNA Probe (Fig 1).
Sixteen Nuclease Protection Probes with uniform characteristics, target-specific for 16 different genes are added in large excess to the cell lysate for solution-phase hybridization. This is followed by S1 Nuclease Digestion where unprotected probes are destroyed. The protected undigested probes (Processed Probes) allows for stoichiometric conversion of mRNA levels when they are captured, detected and quantified on the ArrayPlate™.

**HTG Inc.’s ArrayPlate**

Processed Nuclease Probes are detected at specific array elements on the ArrayPlate through a series of hybridization steps (Fig 2).
Programming Linker is hybridized to one of the 16 anchors on a well; processed nuclease protection probe is added and will hybridize to the appropriate Program Linker; Detection Linker is added which will hybridize to a portion of the Processed Nuclease Protection Probe; Detection Probe is added which will hybridize to the detection linker. Luminescent signal is emitted from the “hybridized sandwich” and picked up by HTG Inc.’s Omix ™ detector.

**Arabidopsis Study**

Collaborators at High Throughput Genomics, Inc., NuvoGen Research, LLC, and The Department of Plant Sciences, University of Arizona, all in Tucson Arizona used qNPA™ to study Salt tolerance in *Arabidopsis*. Excerpts from that study follow, however the full manuscript is available both online and in print: Richard Martin Kris, Stephen Felder, Michael Dehyolos, Georgina M. Lambert, James Hinton, Ilhab Botros, Ralph Martel, Bruce Seligmann, and David W. Galbraith. 2007. “High-Throughput, High Sensitivity Analysis of Gene Expression in Arabidopsis.” Plant Physiol. 144: 1256-1266.

One aspect of the *Arabidopsis* study to be discussed is the test the use of qNPA™ to observe differential gene expression when challenged with NaCl. Another aspect to be discussed is the Dose-response of *Arabidopsis* transcripts to NaCl treatment using the qNPA™ platform.

For the differential gene expression study, *Arabidopsis* seedlings were grown in 96-well plates. Ten to 14 day old seedlings were treated with 150 mM NaCl for 4 hours. The seedlings were then homogenized and qNPA™ was performed. Salt-stressed seedlings were compared to control seedlings dosed with water. As expected, up-regulation was observed in genes known to be induced by osmotic stress, including NaCl treatment; GST, COR47, KIN1, KIN2, RD29A, RD29B, ERD14 and PAL1. Signal was not observed for the Human β-thromboglobulin gene, which served as a negative control (Fig 3).
Layout of the microarrays within the ArrayPlate wells after programming to detect 16 specific targets. (A) Actin, S-19, βTub8 are Housekeeping genes; HMG17 is a negative control; all others are test genes. (B) Image of Chemiluminescence emission captured at the positions of the array elements. Analysis was done of transcripts in homogenates from plants following treatment with 150 mM NaCl or the corresponding water control. Differential gene expression, was as expected, with induction of transcript levels observed for GST, COR47, KIN1, KIN2, RD29A, RD29B, ERD14 and PAL1.

For NaCl dose-response analysis, Arabidopsis seedlings were grown from seed in 96-well plates for 10 days, then treated for 4 hours with different amounts of NaCl. Tissue was harvested and analyzed using the qNPA™ technology. In this manner, the EC50 was established for KIN1, KIN2 and COR47 (Fig 4).
Arabidopsis plants, grown from seed for 10 days in 96-well plates, treated with the indicated amounts of NaCl for 4 hours and then harvested. (A) Data are from quadruplicate biological samples and SEs are shown by error bars. Data are normalized to the average of the transcript levels of actin and S-19. Note that two genes (KIN1 and KIN2) exhibit an EC50 of approximately 112 mM, whereas a third, COR47, exhibits an EC50 >175 mM. (B) Scale of the ordinate is expanded to illustrate the sensitivity of the assay without loss of reproducibility.

CONCLUSIONS

Using HTG Inc.’s qNPA platform, Arabidopsis osmotic stress genes (GST, COR47, KIN1, KIN2, RD29A, RD29B, ERD14 and PAL1) were shown to be up-regulated upon exposure to NaCl. This platform was observed to be highly sensitive in a dose-response curve where individual targets were up-regulated at different salt concentrations.

Furthermore, experimental results indicate that HTG Inc.’s qNPA platform is a highly sensitive, accurate method to measure levels of specific transcripts without requiring RNA extraction, cDNA synthesis or RNA amplification from plant material. Eliminating a RNA extraction step removes a time-consuming and costly bottleneck in high-throughput screening, and reduces a potential sample variation. Eliminating the need for cDNA synthesis removes another source of potential sample variation. qNPA™ greatly simplifies the experimental process by using standard DNA oligonucleotides and S1 nuclease, rather than RNA amplification.