APPLICATION NOTE

# QuantiGene Plex Assays

# Gene expression and DNA copy number analysis in plants

### **QuantiGene Plex Assays**

Invitrogen<sup>™</sup> QuantiGene<sup>™</sup> Plex Assays combine branched DNA (bDNA) signal amplification and Luminex<sup>®</sup> xMAP<sup>®</sup> (multianalyte profiling) technologies to enable simultaneous direct quantification of multiple RNA or DNA targets from a variety of sample types. The branched DNA (bDNA) technology utilizes sandwich nucleic acid hybridization for a unique approach to RNA and DNA quantification by amplifying the reporter signal rather than the template (Figure 1). By measuring the RNA or DNA at the sample source, the assay avoids biases and variability inherent to extraction techniques.



Figure 1. QuantiGene Plex Assay overview. The assay uses Luminex xMAP technology to simultaneously measure as many as 80 genes in a single well.



### **Features and benefits**

- Any gene, any species—customers have utilized our QuantiGene assays to measure gene expression and DNA copy number variation from wheat, corn, soybean, rice, tobacco, lettuce, potato, tomato, cherry tomato, *Arabidopsis*, and many others
- Fast customization—if a particular target is not found, we can create your custom panel within 2 weeks
- **True multiplexing**—measure up to 80 genes of interest and housekeeping genes in the same well with no crossreactivity; 96- or 384-well plate formats are available, offering results in as little as 1.5 days
- Standardized platform—96-well plate format compatible with Luminex<sup>®</sup> 100<sup>™</sup>, 200<sup>™</sup>, MAGPIX<sup>®</sup>, and FLEXMAP 3D<sup>®</sup> systems
- Simple workflow—ELISA-like workflow for direct hybridization of transcripts to beads and transcript labeling

### Example data

QuantiGene Plex Assays can be used for the quantitative analysis of gene expression and DNA copy number variation (Figures 2 and 3). Sample types include:

- Plant tissues, roots, stem branches, leaves, and seeds
- Protoplasts
- Cultured cells
- Isolated DNA or RNA
- Blood, tissues, bacteria, and more

Applications include:

- Plant breeding
- Analysis of transgenic plants, gene knockouts, siRNA knockdown
- Zygosity studies
- GMO studies
- Viral, fungal, and bacterial assays



**Figure 2. Gene expression time course in soybean.** Expression of *AtBBX32* in soybean affects the transcript abundance of central clock components near dawn, or zeitgeber time (ZT) 0. Levels of both central clock components (**A**) *GmLCL2* and (**B**) *GmTOC1* were analyzed by QuantiGene Plex RNA assay from V2-stage leaf tissue harvested from soybean plants grown in a controlled environment. The growth chamber experiment was performed in a 14:10 hour photoperiod (light:dark) with 650 mE of light. *P* values are based on the difference between both transgenic lines and wild type control (\**P* ≤ 0.05). Error bars are not visible where they are smaller than the data points. Reprinted from Preuss SB et al. (2012) Expression of the *Arabidopsis thaliana BBX32* gene in soybean increases grain yield. *PLoS One* 7(2):e30717.



Figure 3. DNA copy number variation in lettuce. Results of the QuantiGene Plex DNA assay with different sample sources are shown, including purified genomic DNA (gDNA) as well as lysates from seeds and leaves of a transgenic lettuce plant. Data are normalized to gene 10 to indicate the relative DNA ratio.

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### **Specifications**

Gene expression	
Limit of detection	≤1,000–2,000 transcripts/assay well
Limit of quantitation	≤2,000–4,000 transcripts/assay well
Linear dynamic range	≥3.0 logarithmic units
Assay CV	≤15% intra-assay; ≤20% inter-assay
Compatible sample types	Cultured cells, plant tissue, protoplasts, purified RNA
Assay format	96- or 384-well plate
Targets/well	3–80 for both gene expression and DNA copy

DNA copy number	
Limit of detection	≤10,000 copies/assay well
Limit of quantitation	≤20,000 copies/assay well
Linear dynamic range	≥2.5 logarithmic units
Assay CV	≤5% intra-assay; ≤20% inter-assay
Compatible sample types	Cultured cells, plant tissue, protoplasts, purified DNA
Assay format	96- or 384-well plate
Targets/well	3–80 genes

### **Custom panels**

Custom panel sets are available for any gene and any species. Custom panels ranging from 3- to 80-plex can be designed and shipped within 2 weeks. Customers simply provide a gene list, RefSeq IDs, or a DNA sequence.

#### References

- 1. Preuss SB et al. (2012) Expression of the Arabidopsis thaliana BBX32 Gene in Soybean Increases Grain Yield. *PLoS One* 2: e30717.
- Armstrong TA et al. (2013) Quantification of Transgene-Derived Double-Stranded RNA in Plants Using the QuantiGene Nucleic Acid Detection Platform. J Agric Food Chem Dec 61: 12557–12564.
- Cui C et al. Determination of Target Gene Copy Number in Plants Using QuantiGene Branched DNA Technology. ASA-CSSA-SSSA International Annual Meeting, Nov 14 2006, #162-1.

## Find out more and order your panel at thermofisher.com/quantigene



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