The eXpress profiling process for gene expression analysis
Simplified for multiplex capability

Beckman Coulter’s GenomeLab GeXP Genetic Analysis System uses a simplified two-step multiplex PCR* process for multiplexing biomarkers in gene expression assays. Each multiplex integrates biological controls in the same well as target genes and reference (housekeeping) genes. This approach not only reduces reagent consumption, but also eliminates pipetting variation and minimizes the need for technical replicates.

The true flexibility of the system is the ease and specificity of panel generation. By using the eXpress Designer module within the software, scientists can design research-specific panels using accession numbers or proprietary sequences. GeXP protocols accept any desalted, deprotected, unlabeled oligonucleotides. Users can define their targets, or use commercially available multiplexes from Beckman Coulter for analysis.

### Isolate
- Prepare RNA
- **Reverse transcription reaction from total RNA uses gene-specific reverse primers that add a flanking universal reverse sequence to resulting cDNAs.**

### Prepare (Step 1)
- Multiplex Reverse Transcription
- **Pre-designed panels can be used to expedite target screening.**
  - GenomeLab GeXP Human MetastasisPlex
  - GenomeLab GeXP Human Breast CancerPlex
  - GenomeLab GeXP Rat MultitoxPlex
  - GenomeLab GeXP Human ReferencePlex
  - To find out more about these panels, visit [www.CELeader.com](http://www.CELeader.com).

### Prepare (Step 2)
- Multiplex PCR
- **Universal tag forward sequence**
- **Gene specific forward sequence**
- **Universal tag reverse sequence**
- **Gene specific reverse sequence**

### Analyze and Evaluate
- Separate and Analyze
- **The multiplex reaction contains the cDNA for all genes of choice tagged with a universal sequence at the 5’ end. Two types of primers are present in the reaction:**
  1. Chimeric primers contain a gene-specific sequence with a universal tag at the 5’ end. They are used to synthesize a double-stranded DNA template.
  2. Universal primers have the same sequence as the universal tags in the chimeric primers. The forward universal primer is covalently labeled with a fluorescent dye for detection during capillary electrophoresis.

In the first two cycles, the PCR reaction is driven by the gene-specific sequence of the chimeric primers to produce amplicons that have universal tags at both ends. The universal primers take over during the third cycle and drive the remaining PCR reactions, due to their 60:1 molar excess relative to the chimeric primers. At this point, all of the templates are amplified with identical universal primers and any sequence bias is minimized.

The result is a pool of amplicons corresponding to the genes of interest. Each amplicon is designed to have a discrete length, and each is labeled with a WellRED fluorescent dye for detection.