

Integration of Sigma® TransPlex® WTA with the NimbleGen™ Expression Microarray Workflow

The TransPlex® WTA amplification product is suitable as a microarray target for expression analysis on the NimbleGen™ platform, and can be readily integrated into existing NimbleGen workflows. NimbleGen recommends the use of TransPlex WTA for amplification of total RNA for microarray applications.¹ See FAQ's

- If your total RNA sample quantity is less than 1 microgram, amplify the experimental and control (input) samples using the Sigma TransPlex Complete WTA2 Kit (**Cat. No. WTA2-50RXN**) before labeling.
- The TransPlex WTA amplification product ranges from ~200 to 1000 base pairs in size, averaging ~300 base pairs, making fragmentation unnecessary. This size is sufficiently large for NimbleGen microarray target.
- Purify samples using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**).

Preparation of TransPlex WTA Amplification Product for Labeling

1. Perform TransPlex WTA amplification as described in the product bulletin, found on the Sigma-Aldrich website (**TransPlex WTA Kits**).
2. Purify the amplification product using the GenElute PCR Cleanup kit (**Cat. No. NA1020**) eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

Note 1. Thirty microliters is the absolute minimum elution volume.

Note 2. The absolute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a **single** TransPlex WTA amplification reaction.

3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.

Entry into NimbleGen Workflow

Enter NimbleGen Arrays User's Guide: Gene Expression Analysis v3.2, Chapter 2.

Step 7. Spectrophotometric QC of cDNA

1. Quantitate each cDNA sample according to the following formula:

$$\text{cDNA Concentration } (\mu\text{g/ml}) = A_{260} \times 50 \times \text{Dilution Factor}$$

2. Verify that all samples meet the following requirements:

- Concentration $\geq 100\text{ng}/\mu\text{l}$
- $A_{260}/A_{280} \geq 1.8$
- $A_{260}/A_{230} \geq 1.8$

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Step 8. Bioanalyzer/Gel QC of cDNA

1. Transfer 250ng cDNA to a sterile microcentrifuge tube. Store the remainder of your sample on ice or at -20°C.
2. Analyze the samples using the Agilent Bioanalyzer.
3. Compare the Bioanalyzer traces to the traces displayed below. Verify that all samples meet the following requirement for acceptance:

- Median size ≥ 400 bp when compared to a DNA ladder.
- Looks similar to the examples of good cDNA sample traces displayed below.

Note: If using an agarose gel, compare the gel images to the Bioanalyzer's electropherogram images.

Samples exhibiting degradation should not be carried through labeling and hybridization because there is an unacceptable risk of poor results.

NimbleGen Arrays User's Guide: Gene Expression Analysis v3.2

| 17

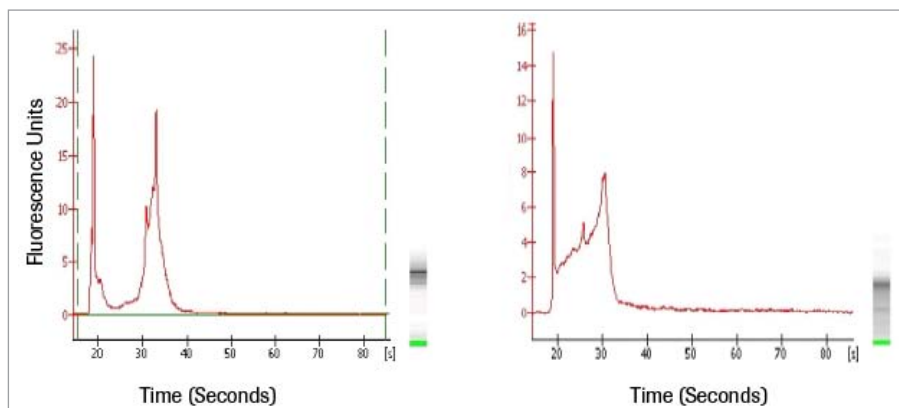


Figure 4: Example Traces Showing Good cDNA from a Eukaryotic Organism

Note: Your traces could look different than these examples. Compare your traces to reference traces for the organism being researched.

References

1. <http://www.nimblegen.com/products/exp/custom.html> (see "FAQs")

Special acknowledgement and thanks to Simon Melov, PhD, Associate Professor and Director of the Genomics Core, and Krysta Felkey, BS, Research Scientist, the Buck Institute for Age Research, Novato, CA, for their assistance in the preparation of this procedure.