

Integration of Sigma® TransPlex® WTA with the Affymetrix Microarray Workflow

TransPlex WTA amplification product is suitable as microarray target for expression analysis on the Affymetrix platform, and can be incorporated into existing Affymetrix workflows.

A modified Affymetrix WT Terminal Labeling and Hybridization procedure¹ is followed for fragmentation, labeling, and hybridization, followed by specified washing and analysis, as defined for the expression microchip used.

The TransPlex WTA amplification product is double-stranded cDNA. Fragmentation and labeling is accomplished using the following Affymetrix products.

- GeneChip Fragmentation Reagent (Affymetrix product # 901010)
- 10X Fragmentation Buffer (Affymetrix product # 900422).
- Terminal Deoxynucleotidyl Transferase (rTdT), Recombinant, 500 units, with buffer (Affymetrix product # 72033 500 UN)
- Biotin-11-dXTP Analog (DNA Labeling Reagent, DLR), 250 nmol, 10 mM (Affymetrix product # 79015 250 NM)

Preparation of TransPlex WTA Amplification Product for Fragmentation and Labeling

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

Note 1. Elute with **30 - 50 µl nuclease-free water**. Thirty microliters is the absolute minimum elution volume, for highest potential concentration. Elute in 50 µL for maximum yield.

Note 2. The 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 necessary, adjust the concentration of the amplification product to > 0.36 µg/µL, using vacuum-centrifugation to avoid loss of amplified product. Determine DNA concentration using Nanodrop spectrophotometry.

Entry into Affymetrix Workflow

TransPlex WTA2 RNA Amplification and Pico Profiling

Pico Profiling^{2,3} is a complete process for isolation and purification of total RNA from single or very small numbers of cells, followed by amplification with TransPlex WTA2 and analysis on Affymetrix expression microarrays. However, the procedural steps subsequent to RNA isolation and purification steps can be used for **any input quantity of RNA**.

When working with small samples, follow the Pico Profiling protocol in its entirety; otherwise enter the protocol at the step, "cDNA Fragmentation".

TransPlex WTA2 is specifically designed to representatively amplify RNA of marginal quality, for example, degraded RNA extracted from formalin-fixed paraffin-embedded (FFPE) samples or small quantities of RNA damaged by laser capture. It is, however, equally suitable for good quality RNA template.

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Enter the Pico Profiling protocol, version 02/11, at “Step D. cDNA Fragmentation”

Follow procedure presented on the Institute for Research in Biomedicine (IRB, Barcelona) Functional Genomics Core Facility website as described.³

Note the following points:

1. Reaction volumes should be scaled to accommodate requirements for the specific microchip used. Use 8-10 µg cDNA target regardless of hybridization volume.
2. The fragmentation step has been rigorously optimized by Affymetrix. However it is critical that the Fragmentation Reagent is diluted correctly. Fragmentation should produce an Agilent Bioanalyzer peak of 40 - 70 base-pair fragments, with an overall range of 20 to 200 base pairs.
3. Terminal transferase labeling should be qualitatively assayed before proceeding to hybridization, utilizing the “Gel Shift” procedure described in Appendix B of the Affymetrix WT Terminal Labeling and Hybridization procedure.
4. For hybridization, follow the procedure described, a modification of the Genechip® Whole Transcript (WT) Double-Stranded Target Assay Manual.⁴

References

1. *Affymetrix GeneChip® WT Terminal Labeling and Hybridization User Manual*, P/N 702808 Rev. **4**, © 2009-2010 Affymetrix Inc.
2. Eva Gonzalez-Roca, Xabier Garcia-Albéniz, Silvia Rodriguez-Mulero, Roger R. Gomis, Karl Kornacker, Herbert Auer. 2010. *Accurate Expression Profiling of Very Small Cell Populations*. *PLoS One* **5** (12): e14418.
3. Pico Profiling, v. Feb, 2011. Institute for Research in Biomedicine (Barcelona) Functional Genomics Core Facility website: <http://www.dnaarrays.org>. Downloads: http://www.dnaarrays.org/D_FileS1Feb8.pdf.
4. *Affymetrix Genechip® Whole Transcript (WT) Double-Stranded Target Assay Manual*, P/N 702179 Rev. **3**, ©2005-2006 Affymetrix Inc.