

# Integration of Sigma® TransPlex® WTA with the Illumina Microarray Workflow

The TransPlex WTA amplification product is suitable as microarray target for expression analysis on Illumina microarrays, and can be incorporated into existing Illumina workflows.

## Preparation of TransPlex WTA Amplification Product for 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's. [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 using vacuum-centrifugation to avoid loss of amplified product. Determine DNA concentration using Nanodrop spectrophotometry.

4. **Fragmentation of the amplification product is not required.**

## Labeling Procedure

The TransPlex WTA amplification product is double-stranded cDNA. Labeling is accomplished using ARP ((N-amonoxyacetyl)-N'-(D-biotinoyl) hydrazine, trifluoro-acetic acid salt) in the presence of phosphate buffered acetic acid. ARP is used for detection of nucleic acid abasic sites in neutral solutions.<sup>1</sup>

A previous report describes the use of ARP for DNA labeling, in conjunction with dUTP incorporation during amplification and subsequent UNG digestion.<sup>2</sup> It has been shown, however, that the acidic conditions prescribed in "Illumina Solution, Application Note 2" generates sufficient abasic sites in WTA-amplified cDNA for effective labeling with ARP.<sup>2,3</sup>

### Labeling reagents:

- Labeling buffer [0.952M acetic acid (Sigma Cat. No. [A6283](#)) and 28 mM MgCl<sub>2</sub> (Sigma Cat. No. [M1028](#))]
- ARP solution [11.3 mg/mL ARP (N-amonoxyacetyl)-N'-(D-biotinoyl) hydrazine, trifluoroacetic acid salt (Molecular Probes, Cat. No. A10550) in 22.4mM Phosphate (K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O), Sigma Cat. No. [P9666](#)]

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## Labeling:

1. Add 3-5 µg of TransPlex WTA2-amplified cDNA to labeling mix comprised of
  - i. 5 µL Labeling buffer [0.952M acetic acid and 28mM MgCl<sub>2</sub>]
  - ii. 5 µL ARP solution [(N-amonooxyacetyl)-N'-(D-biotinoyl) hydrazine, trifluoroacetic acid salt in 22.4mM Phosphate buffer (K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O)]
2. Bring reaction volume to 45 µL with DNase/RNase-free water.
3. Incubate at 50 °C for 1 hour
4. Purify reaction using GenElute PCR Cleanup kit as prescribed.
5. Adjust concentrations to the following concentrations, determined by the beadchip type:

BeadChip Type	cDNA Mass	cDNA Volume
6-Sample	1.5 µg	10 µl
8-Sample	750 ng	5 µl
12-Sample	750 ng	5 µl

## Entry into Illumina Microarray Workflow

Enter the Illumina Whole-Genome Gene Expression Direct Hybridization Assay<sup>4</sup> at CHAPTER 3 “Direct Hybridization Assay Protocols”, “Hybridize BeadChip” and proceed through the remainder of the procedure as prescribed, with the following modification.

**Modification:** At Step 5 of “Hybridize Beadchips”, incubate BeadChips at 48 °C instead of 58 °C.

## References:

1. Kubo K, Ide H, Wallace SS, Kow YW. *A novel, sensitive, and specific assay for abasic sites, the most commonly produced DNA lesion.* 1992. *Biochemistry* **31**(14): 3703-8.
2. Illumina Solution, Application Note 2. Joe Don Heath, Donald Cox, Tom Goralski, Jacques Retief. *Nugen's WT-Ovation™ FFPE System Coupled with a Novel Labeling Protocol Enables Genome-wide Gene Expression Profiling of FFPE Samples on the Illumina Beadarray Platform.* M01069\_FFPE\_IllumApp Note2\_10/07a. © Copyright 2007, NuGEN Technologies Inc.
3. Personal communications: Neil Weingarden Head of Operations, UHN Microarray Centre, T. Robert Beamish Family Convergence Centre for Medical Discovery, Toronto, ON; and Julie Tsao, UHN Microarray Centre Illumina Service Coordinator.
4. Ken Heuermann, Sigma Aldrich. Unpublished data.
5. Illumina Whole-Genome Gene Expression Direct Hybridization Assay, Catalog # BD-901-1002 Part # 11322355 Rev. A, © Copyright 2006–2010 Illumina, Inc.