



Genomics

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

The TransPlex® WTA amplification product is suitable as a microarray target for expression analysis on the Agilent platform, and can be readily integrated into existing Agilent workflows.¹ The following modifications are required:

- **The TransPlex WTA amplification product is double-stranded cDNA.** Labeling, hybridization, and wash procedures are performed using the workflow outlined in the **Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis ULS Labeling for Blood, Cells, Tissues or FFPE (with a High Throughput option)** (Version 3.1, August 2009).
- Fragmentation of the amplification product is not required.
- The **Agilent Genomic DNA ULS™ (Universal Linkage System) Labeling Kit** procedure (Agilent Cat. No. 5190-0419), allows for direct labeling of the microarray target, **preferable** to enzymatic incorporation of modified nucleotides during or following amplification.

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 capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a **single** Transplex WTA amplification reaction.

Note 3. Divalent cation contamination (e.g. Mg²⁺) will negatively affect ULS labeling efficiency. The GenElute PCR Cleanup kit adequately removes divalent cation contaminants and chaotropic salt contamination.

3. Determine concentration of purified amplification product using Nanodrop spectrophotometry. Use the following table (adapted from the Agilent procedure) to assure you have the appropriate DNA concentration for target labeling for the respective Agilent array formats.

Microarray format *	DNA input amount [ng]†	Volume of DNA [µL]	Minimum DNA concentration [ng/µL]
1x microarray (non-FFPE samples)	1500	16.5	91
1x microarray (FFPE samples)	2000	16	125
2x microarray	1000	17	59
4x microarray	500	8	62.5
8x microarray	250	8	32

* Input DNA requirements and volumes are the same for both FFPE and non-FFPE samples for the 2x, 4x, and 8x arrays.

† You can use more DNA, but you will also need to use more ULS dye. Always use a ratio of 1 µL ULS dye per microgram DNA (see procedure below).

Note 4. The DNA volume shown is required for subsequent steps in the Agilent workflow. Add nuclease-free water to bring to volume if necessary.

4. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.
5. Fragmentation of Transplex WTA amplification product is unnecessary.

Entry into Agilent Workflow

1. Enter **Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis ULS Labeling for Blood, Cells, Tissues or FFPE** at Step 3. "ULS Labeling", page 38.
2. **Proceed without deviation** from the Agilent procedure, through the following steps:
 - Step 3. ULS Labeling**
 - Step 4. Removal of non-reacted Cy-ULS**
Proceed through Yield, Degree of Labeling/Specific Activity Determination
3. **Proceed with modifications to the Agilent procedure**, at Step 5. "Preparation of Labeled Genomic DNA for Hybridization":
 - Modification 1.** Reference: procedural step 3 and Tables 25-28", describes the preparation of the Hybridization Master Mix:

Do not add Cot-1 DNA to the Hybridization Master Mix (**replace with nuclease-free water**). Cot-1 DNA blocking is unnecessary since transcribed sequence lacks the highly repetitive sequences found in genomic DNA.

Table 26 Preparation of Hybridization Master Mix for 1x microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	x 8 rxns (µL) (including excess)	x 24 rxns (µL) (including excess)	x 48 rxns (µL) (including excess)
Nuclease-free water	37.8	321.3	945	1,890
Cot-1 DNA (1.0 mg/mL)*	50	425	1,250	2,500
Agilent 100X Blocking Agent†	5.2	44.2	130	260
Agilent 2X Hi-RPM Hybridization Buffer†	260	2,210	6,500	13,000
Final Volume of Hybridization Master Mix	353	3,000.5	8,825	17,650

Replace with Water

* Use Cot-1 DNA from the appropriate species.

† Supplied with Agilent Oligo aCGH Hybridization Kit

Table 27 Preparation of Hybridization Master Mix for 4x microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	x 8 rxns (µL) (including excess)	x 24 rxns (µL) (including excess)	x 48 rxns (µL) (including excess)
Cot-1 DNA (1.0 mg/mL)*	5	42.5	125	250
Agilent 100X Blocking Agent†	1	8.5	25	50
Agilent 2X Hi-RPM Hybridization Buffer†	55	467.5	1,375	2,750
Final Volume of Hybridization Master Mix	61	518.5	1,525	3,050

Replace with Water

* Use Cot-1 DNA from the appropriate species.

† Supplied with Agilent Oligo aCGH Hybridization Kit

Table 28 Preparation of Hybridization Master Mix for 8x microarray, non-FFPE and FFPE samples

Component	Volume (µL) per hybridization	x 8 rxns (µL) (including excess)	x 24 rxns (µL) (including excess)	x 48 rxns (µL) (including excess)
Cot-1 DNA (1.0 mg/mL)*	2	17	50	100
Agilent 100X Blocking Agent†	0.5	4.25	12.5	25
Agilent 2X Hi-RPM Hybridization Buffer†	22.5	191.25	562.5	1,125
Final Volume of Hybridization Master Mix	25	212.5	625	1,250

Replace with Water

* Use Cot-1 DNA from the appropriate species.

† Supplied with Agilent Oligo aCGH Hybridization Kit

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Modification 2. Reference: procedural step 6 and Tables 30¹, describes the **30-minute, 37° C incubation:**

In accordance with Modification 1 above, **omit the 37° C, 30-minute incubation.** (This step allows the Cot-1 DNA to hybridize to highly-repeated *genomic* target sequences before applying to the array, for the purpose of reducing non-specific target-probe interaction. Since Cot-1 DNA is not added, omit this step.)

3 DNA Labeling

Step 5. Preparation of Labeled Genomic DNA for Hybridization

6 Incubate the samples:

a Transfer sample tubes to a circulating water bath or heat block at 95°C. Incubate at 95°C for 3 minutes.

Omit

b Immediately transfer sample tubes to a circulating water bath or heat block at 37°C. Incubate at 37°C for 30 minutes.

or

Transfer sample tubes to a thermocycler. Program the thermocycler according to the following table and run the program:

Table 30

Step	Temperature	Time
Step I	95 °C	3 minutes
Step II	37 °C	30 minutes

Omit

Following step 1 in Table 30: 95 °C for 3 minutes, place samples immediately on ice until applied to the array.

Note 5. Reference: Step 1. **Microarray Hybridization**, procedural step 8, page 53, describes the **hybridization incubation times:**
Forty hours of 65° C hybridization incubation is recommended in all cases.

4 Microarray Processing and Feature Extraction

Step 1. Microarray Hybridization

- Put a microarray slide “active side” down onto the gasket slide, so the numeric barcode side is facing up and the “Agilent”-labeled barcode is facing down. Assess that the sandwich-pair is properly aligned.
- Put the SureHyb chamber cover onto the sandwiched slides and slide the clamp assembly onto both pieces.
- Hand-tighten the clamp onto the chamber.
- Vertically rotate the assembled chamber to wet the slides and assess the mobility of the bubbles. Tap the assembly on a hard surface if necessary to move stationary bubbles.
- Put assembled slide chamber in the rotator rack in a hybridization oven set to 65°C. Set your hybridization rotator to rotate at 20 rpm.
- Hybridize at 65°C:
 - 24 hours for blood, cell and tissue samples (4x and 8x microarrays)
 - 40 hours for blood, cell and tissue samples (1x and 2x microarrays)
 - 40 hours for FFPE samples (1x, 2x, 4x and 8x microarrays)

References

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