The Cytogenetics Copy Number assay protocol is optimized for processing 4 to 24 samples at a time to obtain copy number results. This protocol is not intended for genome-wide association studies. An assay protocol for processing 48 samples is described in the Affymetrix® Genome-Wide Human SNP Nsp/Sty 6.0 User Guide, P/N 702504.
Quick Reference Card
Cytogenetics Copy Number Assay
Stage 1 – Digestion

**DIGESTION MASTER MIX**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Per Sample</th>
<th>8 Samples Nsp MM</th>
<th>8 Samples Sty MM</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, AccuGENE</td>
<td>11.55 µL</td>
<td>106.3 µL</td>
<td>106.3 µL</td>
<td></td>
</tr>
<tr>
<td>NE Buffer 2 (Nsp MM only)</td>
<td>2 µL</td>
<td>18.4 µL</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>NE Buffer 3 (Sty MM only)</td>
<td>2 µL</td>
<td>—</td>
<td>18.4 µL</td>
<td></td>
</tr>
<tr>
<td>BSA (100X; 10 mg/mL)</td>
<td>0.2 µL</td>
<td>1.8 µL</td>
<td>1.8 µL</td>
<td></td>
</tr>
<tr>
<td>Nsp I (10 U/µL)</td>
<td>1 µL</td>
<td>9.2 µL</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Sty I (10 U/µL)</td>
<td>1 µL</td>
<td>—</td>
<td>9.2 µL</td>
<td></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>14.75 µL</strong></td>
<td><strong>135.7 µL</strong></td>
<td>—</td>
<td><strong>135.7 µL</strong></td>
</tr>
</tbody>
</table>

**DIGESTION MASTER MIX**

<table>
<thead>
<tr>
<th>Nsp Samples</th>
<th>Volume</th>
<th>Sty Samples</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDNA (50 ng/µL)</td>
<td>5.00 µL (250 ng)</td>
<td>gDNA (50 ng/µL)</td>
<td>5.00 µL (250 ng)</td>
</tr>
<tr>
<td>Nsp Master Mix</td>
<td>14.75 µL</td>
<td>Sty Master Mix</td>
<td>14.75 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>19.75 µL</strong></td>
<td><strong>Total Volume</strong></td>
<td><strong>19.75 µL</strong></td>
</tr>
</tbody>
</table>

**Digestion and Ligation Plate**

**Important Points**

- Aliquot genomic DNA (gDNA) to opposite ends of the plate to lessen the chance of pipetting errors.
- Add gDNA to wells marked 1 through 6 in the plate diagram above.
- Two digestion master mixes are prepared (Nsp and Sty).
  - Be sure to use the correct enzyme for each master mix (Nsp or Sty)
  - Leave Nsp and Sty enzymes at –20 °C until ready to use.
- Add 5 µL Ref103 DNA as positive control to wells marked +.
- Add 5 µL water (AccuGENE) as negative control to wells marked –.

1. Seal plate with adhesive film.
2. Vortex plate at high speed for 3 sec.
4. Ensure lid of thermal cycler is preheated.
5. Load plate onto thermal cycler and run the Cyto Digest program.

**Cyto Digest**

<table>
<thead>
<tr>
<th>Temp</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 °C</td>
<td>2 hr</td>
</tr>
<tr>
<td>65 °C</td>
<td>20 min</td>
</tr>
<tr>
<td>4 °C</td>
<td>Hold</td>
</tr>
</tbody>
</table>

For research use only. Not for use in diagnostic procedures.
Important Points

- Sample plate used for digestion and ligation.
- Two ligation master mixes are prepared (Nsp and Sty).
  - Be sure to use the correct adaptor for each master mix (Nsp or Sty)
  - Leave T4 DNA Ligase at –20 °C until ready to use.
  - Thaw T4 DNA Ligase Buffer on ice. Vortex to ensure any precipitate is resuspended and buffer is clear.
Cytogenetics Copy Number Assay
Stage 3a – PCR

1. Dilute ligated samples.
   - **Nsp Samples**
     - **Volume**: 25 μL
     - **Volume**: 25 μL
   - **Sty Samples**
     - **Volume**: 75 μL
     - **Volume**: 75 μL
   - **Total Volume**: 100 μL

2. Seal plate with adhesive film.
3. Vortex at high speed for 3 sec; spin down at 2000 rpm for 30 sec.

4. Transfer four 10 μL aliquots of each Nsp sample to the PCR plate.
5. Transfer three 10 μL aliquots of each Sty sample to the PCR plate.
6. Prepare the PCR Master Mix.

7. Add PCR Master Mix to samples.
   - **Sample**: Nsp or Sty Sample
     - **Volume**: 10 μL
   - **PCR Master Mix**: 90 μL
   - **Total Volume**: 100 μL

8. Seal PCR plate, vortex at high speed for 3 sec, spin down at 2000 rpm for 30 sec.
9. Keep plate on ice and move to Post-PCR Room/Area.
10. Ensure thermal cycler lid is preheated.
11. Load plate onto thermal cycler and run the Cyto PCR program.
12. Hold overnight.

**PCR MASTER MIX**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Per Sample</th>
<th>8 Samples</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, AccuGENE</td>
<td>39.5 μL</td>
<td>2544 μL</td>
<td></td>
</tr>
<tr>
<td>TITANIUM™ Taq PCR Buffer (10X)</td>
<td>10.0 μL</td>
<td>644 μL</td>
<td>✓</td>
</tr>
<tr>
<td>GC-Melt (5M)</td>
<td>20.0 μL</td>
<td>1288 μL</td>
<td></td>
</tr>
<tr>
<td>dNTPs (2.5 mM each)</td>
<td>14.0 μL</td>
<td>902 μL</td>
<td></td>
</tr>
<tr>
<td>PCR Primer 002 (100 μM)</td>
<td>4.5 μL</td>
<td>290 μL</td>
<td></td>
</tr>
<tr>
<td>TITANIUM™ Taq Polymerase</td>
<td>2.0 μL</td>
<td>129 μL</td>
<td></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td>90.0 μL</td>
<td>5796 μL</td>
<td></td>
</tr>
</tbody>
</table>

**Diluted Ligated Samples**

- Nsp Samples: 25 μL
- Sty Samples: 75 μL
- Water, AccuGENE: 75 μL
- TITANIUM™ Taq PCR Buffer (10X): 10.0 μL
- GC-Melt (5M): 20.0 μL
- dNTPs (2.5 mM each): 14.0 μL
- PCR Primer 002 (100 μM): 4.5 μL
- TITANIUM™ Taq Polymerase: 2.0 μL
- **Total Volume**: 100 μL

**Cyto PCR – ABI 9700**

<table>
<thead>
<tr>
<th>Temp</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 °C</td>
<td>3 min</td>
<td>—</td>
</tr>
<tr>
<td>94 °C</td>
<td>30 sec</td>
<td>—</td>
</tr>
<tr>
<td>60 °C</td>
<td>45 sec</td>
<td>30</td>
</tr>
<tr>
<td>68 °C</td>
<td>15 sec</td>
<td>—</td>
</tr>
<tr>
<td>68 °C</td>
<td>7 min</td>
<td>—</td>
</tr>
<tr>
<td>4 °C</td>
<td>Hold</td>
<td>—</td>
</tr>
</tbody>
</table>

**Cyto PCR – MJ Tetrad PTC-225**

<table>
<thead>
<tr>
<th>Temp</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 °C</td>
<td>3 min</td>
<td>—</td>
</tr>
<tr>
<td>94 °C</td>
<td>30 sec</td>
<td>—</td>
</tr>
<tr>
<td>60 °C</td>
<td>30 sec</td>
<td>30</td>
</tr>
<tr>
<td>68 °C</td>
<td>15 sec</td>
<td>—</td>
</tr>
<tr>
<td>68 °C</td>
<td>7 min</td>
<td>—</td>
</tr>
<tr>
<td>4 °C</td>
<td>Hold</td>
<td>—</td>
</tr>
</tbody>
</table>
1. Aliquot 3 μL of 2X Gel Loading Dye to 16 wells of a new 96-well plate (the gel plate).
2. Transfer 3 μL of each reaction from one Nsp column to the corresponding wells of the gel plate.
3. Transfer 3 μL of each reaction from one Sty column to the corresponding wells of the gel plate.
4. Seal the gel plate.
5. Vortex on high speed for 3 sec; spin down at 2000 rpm for 30 sec.
6. Load reactions from the gel plate onto a 2% TBE gel, and run the gel.
7. While the gel is running, begin Stage 4 – PCR Purification.

Example of PCR products run on a 2% TBE gel at 120 volts for 1 hour. Average product size is between 200 and 1100 bp.
1. Pool the PCR products for each sample by transferring all 7 aliquots to the appropriately marked 2.0 mL round bottom tube.

2. Examine the PCR plate to ensure that the total volume from each well has been transferred.

3. Thoroughly mix the magnetic beads (AmpPURE) by vigorously shaking the bottle until the mixture is homogeneous.

4. Add 1 mL of magnetic beads to each pooled sample.

5. Securely cap each tube and mix well by inverting 10 times.

6. Incubate at room temperature for 10 min.

7. Centrifuge the tubes – with hinges facing out – for 3 min at maximum speed (16,100 rcf).

8. Place the tubes on a magnetic stand.

9. Leaving the tubes in the stand, pipet off the supernatant without disturbing the bead pellet. Discard the supernatant.

10. Using a P1000 pipet, add 1.5 mL 75% ethanol (EtOH) to each tube.

11. Cap the tubes, load into the foam adapter, and vortex at 75% power for 2 min.

12. Centrifuge the tubes for 3 min at maximum speed.

13. Place the tubes back on the magnetic stand.

14. Leaving tubes in the stand, pipet off the supernatant without disturbing the bead pellet. Discard the supernatant.

15. Spin the tubes for 30 sec at maximum speed, then place back on the magnetic stand.

16. Using a P20 pipet, remove any drops of EtOH from the bottom of each tube.

17. Allow any remaining EtOH to evaporate by leaving the tubes uncapped at room temperature for 15 min.

18. Using a P200 pipet, add 55 μL of Buffer EB to each tube.

19. Cap the tubes, load into the foam adapter, and vortex at 75% power for 10 min to resuspend the beads.

20. If the beads are not fully resuspended, vortex an additional 2 min.

21. Centrifuge the tubes for 5 min at maximum speed.

22. Place the tubes on the magnetic stand for at least 5 min until all beads are pulled to the side.

23. Transfer 47 μL of eluted sample to the appropriate well of a fresh 96-well plate.
**Prepare the Quantitation Plate**
Thoroughly mix the samples and water using one of these methods:
- Seal the plate, vortex, and spin down.
- Pipet up and down 5 times.

**Plate Spectrophotometer**
1. Measure the OD of each PCR product at 260, 280 and 320 nm.
2. Determine the OD260 measurement for the water blank and average.
3. Calculate one OD reading for every sample:
   \[ \text{OD} = (\text{sample OD}) - (\text{average water blank OD}) \]
4. Calculate the undiluted concentration for each sample in μg/μL:
   \[ \text{OD} \times 0.05 \text{ ug/μL} \times 100 \]

**NanoDrop**
1. Blank the NanoDrop using water.
2. Take 2 μL of diluted sample and measure the OD of each PCR product at 260, 280 and 320 nm.
3. Calculate the undiluted concentration for each sample in μg/μL:
   \[ \text{OD reading} \times 10 \]

**Assess OD Readings**
- An acceptable OD should fall within the range of 0.9 to 1.4.
- DNA yield equivalent = 4.5 to 7.0 μg/μL.
- The OD260/OD280 ratio should be between 1.8 and 2.0.
- The OD320 measurement should be very close to zero (< 0.1).
- If metrics fall outside of these ranges, refer to the Affymetrix® Cytogenetics Copy Number Assay User Guide for more information.
Quick Reference Card
Cytogenetics Copy Number Assay
Stage 6 – Fragmentation

FRAGMENTATION MASTER MIX

<table>
<thead>
<tr>
<th>Reagent</th>
<th>2 U/μL</th>
<th>2.25 U/μL</th>
<th>2.50 U/μL</th>
<th>2.75 U/μL</th>
<th>3.0 U/μL</th>
<th>✓</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (AccuGENE)</td>
<td>85.00 μL</td>
<td>96.25 μL</td>
<td>107.50 μL</td>
<td>118.75 μL</td>
<td>130.00 μL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10X Fragmentation Buffer</td>
<td>10.00 μL</td>
<td>11.25 μL</td>
<td>12.50 μL</td>
<td>13.75 μL</td>
<td>15.00 μL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragmentation Reagent</td>
<td>5.00 μL</td>
<td>5.00 μL</td>
<td>5.00 μL</td>
<td>5.00 μL</td>
<td>5.00 μL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>100.00 μL</td>
<td>112.50 μL</td>
<td>125.00 μL</td>
<td>137.50 μL</td>
<td>150.00 μL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Important Points – Fragmentation Master Mix Preparation
- Check concentration of Fragmentation Reagent (enzyme; varies between 2 and 3 U/μL).
- Leave Fragmentation Reagent (enzyme) at −20 °C until ready to use.
- Thaw 10X Fragmentation Buffer on ice.
- Keep all reagents, including water, on ice. Perform all additions on ice.
- Preheat thermal cycler block to 37 °C.

1. Add 5 μL of 10X Fragmentation Buffer to each sample.
2. Prepare the Fragmentation Master Mix.
3. Aliquot the master mix equally to one set of strip tubes.
4. Using a multi-channel pipet, add 5 μL of Fragmentation Master Mix to each sample.
5. Seal the plate with adhesive film.
6. Vortex at high speed for 3 sec.
7. Spin down at 2000 rpm for 30 sec.
8. Ensure the thermal cycler block is preheated.
9. Load plate onto thermal cycler and run the Cyto Fragment program.

Proceed immediately to Labeling.
Labeling

<table>
<thead>
<tr>
<th>LABELING MASTER MIX</th>
<th>Per Sample</th>
<th>8 Samples</th>
<th>✓</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdT Buffer (5X)</td>
<td>14.0 µL</td>
<td>128.8 µL</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>DNA Labeling Reagent (30 mM)</td>
<td>2.0 µL</td>
<td>18.4 µL</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>TdT Enzyme (30 U/µL)</td>
<td>3.5 µL</td>
<td>32.2 µL</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>19.5 µL</td>
<td>179.4 µL</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

1. Transfer 2 µL of each fragmented sample to the corresponding well of a fresh 96-well plate (the Fragmentation QC Gel Plate).
2. Prepare the Labeling Master Mix.
3. Add 19.5 µL of master mix to each sample.
4. Tightly seal the plate, and vortex at high speed for 3 sec.
5. Spin down at 2000 rpm for 30 sec.
6. Load plate onto thermal cycler and run the Cyto Label program.

Important Points

- Leave the TdT enzyme at –20 °C until ready to use.
- Thaw the 5X TdT Buffer and DNA Labeling Reagent on ice.
- Ensure the plate is tightly sealed to avoid evaporation while on the thermal cycler.

Example of fragmented samples run on a 4% TBE gel at 120 volts for 1 hr. Average fragment size is < 180 bp.

Fragmentation QC Gel Plate

<table>
<thead>
<tr>
<th>Temp</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 °C</td>
<td>4 hr</td>
</tr>
<tr>
<td>95 °C</td>
<td>15 min</td>
</tr>
<tr>
<td>4 °C</td>
<td>Hold</td>
</tr>
</tbody>
</table>

If possible, store the Label plate overnight at –20 °C. Otherwise, OK to hold at 4 °C overnight.
HYBRIDIZATION MASTER MIX

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Per Sample</th>
<th>8 Samples</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES (12X; 1.25 M)</td>
<td>12 μL</td>
<td>110.4 μL</td>
<td></td>
</tr>
<tr>
<td>Denhardt’s Solution (50X)</td>
<td>13 μL</td>
<td>119.6 μL</td>
<td></td>
</tr>
<tr>
<td>EDTA (0.5 M)</td>
<td>3 μL</td>
<td>276 μL</td>
<td></td>
</tr>
<tr>
<td>Herring Sperm DNA (10 mg/mL)</td>
<td>3 μL</td>
<td>276 μL</td>
<td></td>
</tr>
<tr>
<td>Oligo Control Reagent, 0100</td>
<td>2 μL</td>
<td>18.4 μL</td>
<td></td>
</tr>
<tr>
<td>Human Cot-1 DNA (1 mg/mL)</td>
<td>3 μL</td>
<td>276 μL</td>
<td></td>
</tr>
<tr>
<td>Tween-20 (3%)</td>
<td>1 μL</td>
<td>9.2 μL</td>
<td></td>
</tr>
<tr>
<td>DMSO (100%)</td>
<td>13 μL</td>
<td>119.6 μL</td>
<td></td>
</tr>
<tr>
<td>TMACL (5 M)</td>
<td>140 μL</td>
<td>1288.0 μL</td>
<td></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>190 μL</strong></td>
<td><strong>1748.0 μL</strong></td>
<td></td>
</tr>
</tbody>
</table>

1. Unpackage the arrays and allow to equalibrate to room temperature prior to use.
2. Preheat the hybridization ovens for at least 1 hr at 50 °C with the rotation turned on.
3. Prepare the Hybridization Master Mix.
4. Add 190 μL of master mix to each sample.
5. Tightly seal the plate, vortex at high speed for 30 sec, and spin down at 2000 rpm for 30 sec.
6. Load plate onto thermal cycler and run the Cyto Hyb program.
7. Leaving the samples on the thermal cycler, load 200 μL of sample onto each array using a single-channel P200 pipet.
8. Clean any excess fluid from around the septa.
9. Apply Tough-Spots to the septa and press firmly.
10. Load arrays into the hybridization oven four at a time.

Hyb arrays 16 to 18 hr at 50 °C

**Important Points**
- Samples must remain on the thermal cycler while loading the arrays.
- To avoid damaging the septa, use a single-channel P200 pipet to load the arrays.
- Shake arrays a few times to ensure bubbles are not visible through the window.
- When 4 arrays are loaded, immediately place them into the hybridization oven.
Washing and Staining Arrays

1. Remove the hybridization solution from each array.
2. Fill the arrays with 270 μL 1X Array Holding Buffer.
3. Load arrays onto the Fluidics Station.
4. Using GCOS or AGCC, run the SNP6_450 protocol.

Before Scanning

1. Ensure no bubbles are visible through the window.
2. Cover the septa with Tough-Spots; then load onto the scanner.

Important Points

- The hybridization solution removed from the arrays can be stored long term at –80 °C.
- The 12X MES Stock Buffer, SAPE Solution, and Array Holding Buffer are light sensitive and must be stored at 4 °C.
- If necessary, the array can be stored in Array Holding Buffer at 4 °C for up to 3 hr before washing and staining.
**Wash A: Non-Stringent Wash Buffer**  
(6X SSPE, 0.01% Tween-20)  

For 1000 mL:  
- 300 mL of 20X SSPE  
- 1.0 mL of 10% Tween-20  
- 699 mL of molecular biology grade water  
Filter through a 0.2 μm filter.  
Store at room temperature.

**Wash B: Stringent Wash Buffer**  
(0.6X SSPE, 0.01% Tween 20)  

For 1000 mL:  
- 30 mL of 20X SSPE  
- 1.0 mL of 10% Tween-20  
- 969 mL of molecular biology grade water  
Filter through a 0.2 μm filter.  
The pH should be 8.  
Store at room temperature.  
Tightly seal container to avoid changes in salt concentration due to evaporation.  
Prepare in smaller quantities to avoid long-term storage.

**0.5 mg/mL Anti-Streptavidin Antibody**  
Resuspend 0.5 mg in 1 mL of molecular biology grade water.  
Store at 4 °C.

**12X MES Stock Buffer**  
(1.25 M MES, 0.89 M [Na⁺])  

For 1000 mL:  
- 70.4 g of MES hydrate  
- 193.3 g of MES sodium salt  
- 800 mL molecular biology grade water  
Mix and adjust the volume to 1000 mL.  
The pH should be between 6.5 and 6.7.  
Filter through a 0.2 μm filter.  
Do not autoclave.  
Store at 2 °C to 8 °C. Shield from light.

**1X Array Holding Buffer**  
(Final 1X concentration is 100 mM MES, 1M [Na⁺], 0.01% Tween-20)  

For 100 mL:  
- 8.3 mL of 12X MES stock buffer  
- 18.5 mL of 5 M NaCl  
- 0.1 mL of 10% Tween-20  
- 73.1 mL molecular biology grade water  
Store at 2 °C to 8 °C. Shield from light.