

## Labeling and Purification of 96 Genomic DNA Samples Using the Agilent Genomic Enzymatic Labeling System

### Required Equipment

- **ArrayPrep® Target Preparation System**, 115v/230v. (SciGene #2000-00-1/2000-00-2)
- **Centrifuge** with swing out rotor for 96 well microplates
- P10, P20, P200, P1000 pipettes

### Labware Needed

- 4x **50 µl tips** (SciGene #2000-80-1)
- 10x **250 µl tips** (SciGene #2000-80-0)
- 3x **96-well PCR Plates** (SciGene #2000-81-0)
- 2x **V-Groove Reservoir** (SciGene #2000-82-1)
- **U-Bottom 96-well Plate** (SciGene #2000-81-3)
- **Deepwell Plate** (SciGene #2000-82-2)
- **ArrayPrep® PCR Plate Sealer** (SciGene #2000-81-1) – 2 films
- **ArrayPrep® Sealing Film Roller** (SciGene #2000-81-2)
- **96-well Magnetic Ring Stand** (Applied Biosystems #AM10050)
- **Plastic container** for used tips (FisherScientific #03-484-21) or similar

### Reagents Needed

- **Agilent Genomic Enzymatic Labeling System** (Agilent #5190-0449)
- **Magnetic Bead Binding Enhancer, 10x** (SciGene #2000-13-1) Prior to use, dilute entire 50 µl contents with 400 µl nuclease-free water and 50 µl 10x TE Buffer to a total volume of 500 µl. Aliquot 50 µl into 10 vials and store at -20 °C.
- **ArrayPrep® Magnetic Bead Wash Buffer I**, 50 ml (SciGene #2000-11-1)
- **ArrayPrep® Magnetic Bead Elution Buffer I**, 50 ml (SciGene #2000-12-1)
- **Agencourt® AMPure® Magnetic Beads** (Agencourt #A50850, A29152 or A29153)
- **100% Ethanol** (Sigma-Aldrich #E7023-500ML)
- **10 mM Tris-HCl, pH 8.0, 1 mM EDTA (1x TE)** (FisherScientific #BP2473-1)
- **100 mM Tris-HCl, pH 7.6, 10 mM EDTA (10x TE)** (FisherScientific #BP2475-1)
- **5mM EDTA, pH 8.0**, (FisherScientific #BP2482-100, 0.5M stock). Dilute 1:100 with nuclease-free water before use.
- **Nuclease-free water** (FisherScientific #BP2470-1)

### DNA Preparation

For each labeling reaction, use 1 µg of high quality genomic DNA fragmented to a size range of 500 to 2,000 bp in 23.5 µl TE.

- If DNA is in TE Buffer, add TE Buffer, to a final volume of 23.5 µl (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).
- If DNA is in water, add 4.7 µl of 5mM EDTA (pH 8.0) to the sample to bring the final volume to 23.5 µl.

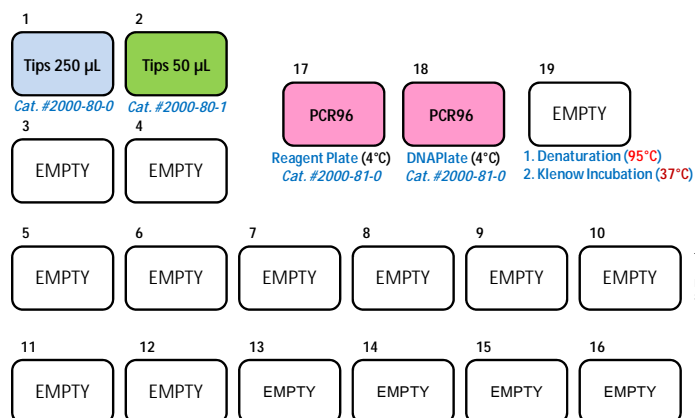


Figure 1. Deck layout for 96 labeling reactions.

### Instrument Setup for Labeling

- Ensure that all labware is placed correctly on the ArrayPrep® System (Figure 1). Place a container for used tips next to position 10 (Figure 2).
- Turn on main power to instrument.
- Switch on the three temperature controlled blocks. Set positions 17 and 18 to 4 °C. Set position 19 to 95 °C and connect a lid (Figure 3).
- Allow temperature to stabilize.
- Take an **Agilent Genomic Enzymatic Labeling System** from the freezer and place vials on ice until thawed.
- Prepare Master Mix 1 (Cy3) and 2 (Cy5) according to Table 1.
- Pipette into a PCR plate as follows:
  - 160 µl of the **Master Mix 1** (Cy3) into each well of **column 1**.
  - 160 µl of the **Master Mix 2** (Cy5) into each well of **column 2**.
  - 20 µl **Exo-Klenow** into each well of **column 3**.
- Place the reagent plate into position 17, pre-cooled to 4 °C.

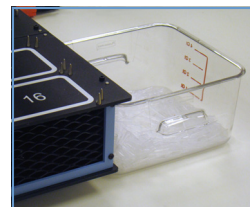


Figure 2. Used tips container.



Figure 3. Connect lid.

Table 1. Preparation for Labeling Master Mix 1 and 2

Master Mix 1		Volume	Master Mix 2		Volume
Random Primers		252 µl	Random Primers		252 µl
5x Buffer		504 µl	5x Buffer		504 µl
10x dNTP		252 µl	10x dNTP		252 µl
Magnetic Bead Binding Enhancer (1x)		126 µl	Magnetic Bead Binding Enhancer (1x)		126 µl
Cy3-dUTP		152 µl	Cy5-dUTP		152 µl
<b>Total: 1286 µl</b>			<b>Total: 1286 µl</b>		

### Set Up DNA Plate

- Pipette DNA samples (1 µg / 23.5 µl TE) into each well of **columns 1, 3, 5, 7, 9 and 11 (Cy3) and columns 2, 4, 6, 8, 10 and 12 (Cy5)** of the 96-well PCR plate.
- Place the DNA plate on block position 18 at 4 °C.

# Labeling and Purification of 96 Genomic DNA Samples for the Agilent System

## Run Labeling Protocol

1. Launch the ArrayPrep® software by clicking the “ArrayPrep” icon on the desktop. Select File | Run Protocol. Go to folder “Desktop/ArrayPrep Protocols” and select the “96 rxns Genomic DNA Labeling\_Agilent” program. Press START.
2. The instrument will dispense 25.5 µl of Master Mix 1 and 2 to the DNA plate and then pause. Seal the plate with PCR Plate Sealer (Figure 4) and place it in position 19 at 95 °C.
3. Using a timer, denature the DNA for 5 minutes at 95 °C. Cover the block with the pre-heated lid (Figure 5) to minimize condensation.
4. After denaturation, remove heated lid and move sample plate back to position 18 at 4 °C. Ensure foil is tightly sealing the plate. Cool samples for 5 minutes at 4 °C.
5. Lower the temperature of block at position 19 to 37 °C. Connect an unheated lid (Figure 3).
6. Remove the sample plate from position 18 and spin down any condensate for 30 seconds.
7. Return the sample plate to position 18 and unseal plate.
8. Press CONTINUE on the ArrayPrep® software to re-start the protocol. The instrument will dispense 1 µl of Exo-Klenow to all DNA samples.
9. Seal the sample plate again, place in position 19 and cover with a lid pre-heated to 37 °C (Figure 5). Incubate samples for 2 hours at 37 °C.
10. Recover remaining Exo-Klenow reagent and pipette into a fresh reaction tube for re-use. Store at -20 °C.
11. After the labeling reaction is complete, spin plate for 30 seconds.
12. Return sample plate to position 18 at 4 °C and unseal plate. The labeled DNA is ready for purification.

— End Labeling Protocol —

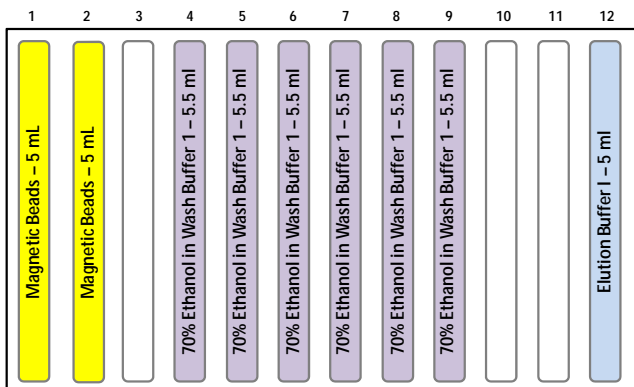


Figure 6. Magnetic Beads and Buffers for Purification.

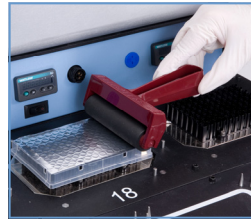


Figure 4. Roll film to ensure a good seal.

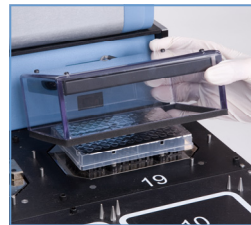


Figure 5. Place lid over block in position 19.

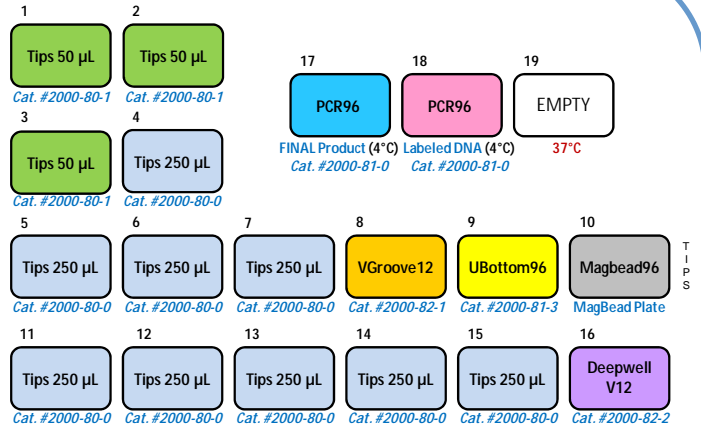


Figure 7. Deck layout for magnetic bead purification of 96 samples.

## Reagent Preparation for Purification

1. Allow magnetic beads stored at 4 °C to equilibrate to room temperature for at least 1 hour before starting the protocol.
2. Thoroughly shake the bottle with magnetic beads. Pipette 5 ml into columns 1 & 2 of the V-Groove Reservoir (Figure 6).
3. Make a 70% Ethanol solution in Wash Buffer I. Mix 24.5 ml of fresh 100% EtOH with 10.5 ml of Wash Buffer I. Add 5.5 ml into columns 4-9 of the V-Groove Reservoir.
4. Add 5 ml Elution Buffer I into column 12 of the V-Groove Reservoir.

## Run Purification Protocol

1. Ensure that all labware is placed on the correct positions on the ArrayPrep® instrument (see Figure 7).
2. On the ArrayPrep® software, Select File | Run Protocol. Go to folder “Desktop/ArrayPrep Protocols” and select the “96 rxns Bead-based Purification\_Agilent” program. Press START.
3. Set a timer and after 90 minutes, prepare a second V-Groove Reservoir containing beads and buffers as described above.
4. The instrument automatically purifies samples for columns 1-6 and then pauses. Immediately replace the V-Groove Reservoir in position 8 with the second freshly prepared reservoir. To avoid piling up of used tips, empty the container by position 10 as needed.
5. Press CONTINUE on the ArrayPrep® software to resume the protocol. The instrument automatically purifies samples for columns 7-12.
6. Samples are now ready for measuring yield, purity and dye incorporation. For details, refer to Quality Metrics in the *ArrayPrep® aCGH Genomic Labeling System User Manual*.

— End Purification Protocol —

### IMPORTANT POINTS:

- Ensure correct placement of reagent plate on cooling block position 17.
- Use a pre-heated lid during incubation.
- Equilibrate magnetic beads to RT before use.
- Always prepare 70% Ethanol in Wash Buffer I fresh. Discard after use.

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Automating Array Workflows

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