

Labeling and Purification of 96 Genomic DNA Samples Using the SciGene ArrayPrep® aCGH Genomic 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)
- 2x **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

- 3x **ArrayPrep® aCGH Genomic Labeling System*** (SciGene #2000-10-1)
- **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 or A29152/A29153)
- **100% Ethanol** (Sigma-Aldrich #E7023-500ML)
- **10 mM Tris-HCl**, pH 8.0, 1 mM EDTA (FisherScientific #BP2473-1)
- **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 22 µl TE.

- If DNA is in TE Buffer, add TE Buffer, to a final volume of 22 µl (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).
- If DNA is in water, add 4.4 µl of 5mM EDTA (pH 8.0) to the sample to bring the final volume to 22 µl.

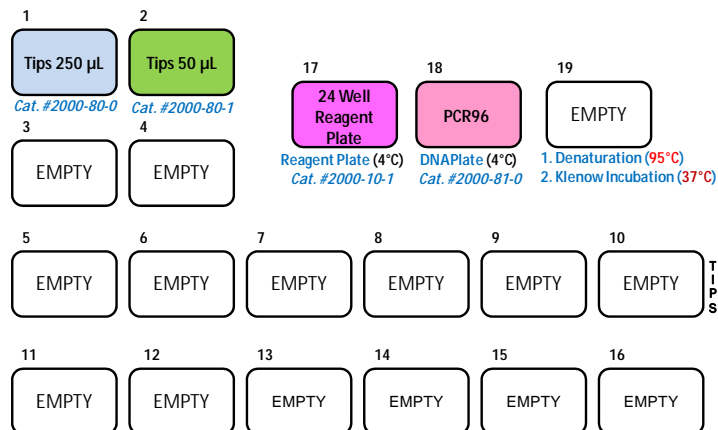


Figure 1. Deck layout for 96 labeling reactions.

Instrument Setup for Labeling

1. Ensure that all labware is placed correctly on the ArrayPrep® instrument (Figure 1). Place a container for used tips on the bench adjacent to position 10 (Figure 2).
2. Turn on main power to instrument.
3. Switch on the three temperature controlled blocks. Set deck positions 17 and 18 to 4 °C. Set position 19 to 95 °C.

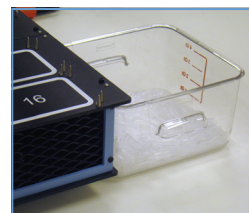


Figure 2. Used tips container.

4. Connect a lid to position 19 at least 10 minutes before incubating at 95 °C (Figure 3). Thirty minutes before starting the protocol, remove three **ArrayPrep® aCGH Genomic Labeling Systems** from the freezer and place them into the pre-cooled 4 °C block at position 17. Orient the plates immediately next to each other, on the left side of the block, with the pink A3 wells on the left (Figure 4).



Figure 3. Connect lid.

Set Up DNA Plate

1. Pipette DNA samples (1 µg / 22 µl TE) into each well of odd **columns 1, 3, 5, 7, 9 and 11** of the 96-well PCR plate to be labeled with **Alexa Fluor®-3 (A3)**.
2. Pipette DNA samples (1 µg / 22 µl TE) into each well of even **columns 2, 4, 6, 8, 10 and 12** of the 96-well PCR plate to be labeled with **Alexa Fluor®-5 (A5)**.
3. Place the DNA plate on block position 18 at 4 °C (Figure 5).



Figure 4. Add reagent kits.



Figure 5. Add sample plate.

*Manufactured and supplied to SciGene under license from Life Technologies Corporation

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ArrayPrep® Labeling and Purification of 96 Genomic DNA Samples

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” program. Press START.
2. The instrument will dispense 25 µl of A3 and A5 2x reaction mixes to the DNA plate and then pause. Seal the plate with PCR Plate Sealer (Figure 6) 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 preheated lid (Figure 7) to minimize condensation.
4. After denaturation, remove the heated lid and move the sample plate back to position 18 at 4 °C. Ensure that the foil is tightly sealing the plate. Cool samples for at least 5 minutes at 4 °C.
5. Lower the temperature of block at position 19 to 37 °C. Attach 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 3 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 7). Using a timer, incubate samples for 2 hours at 37 °C.
10. After the labeling reaction is complete, spin plate for 30 seconds.
11. Return sample plate to position 18 at 4 °C and unseal plate. The labeled DNA is ready for purification.

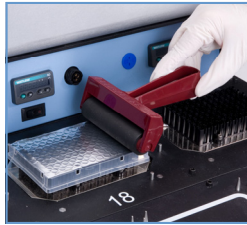


Figure 6. Roll film to ensure a good seal.



Figure 7. Place lid over block in position 19.

— End Labeling Protocol —

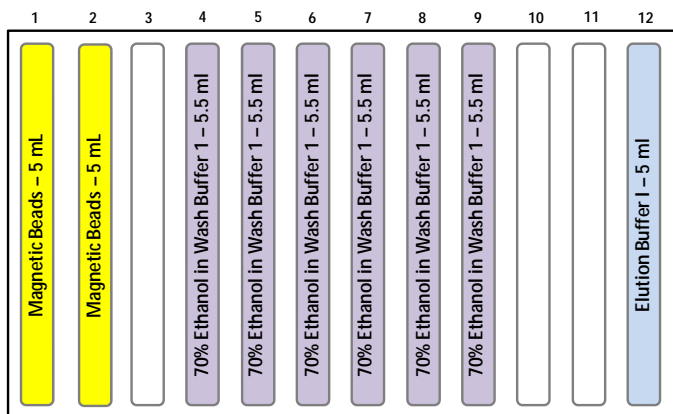


Figure 8. Magnetic Beads and Buffers for Purification.

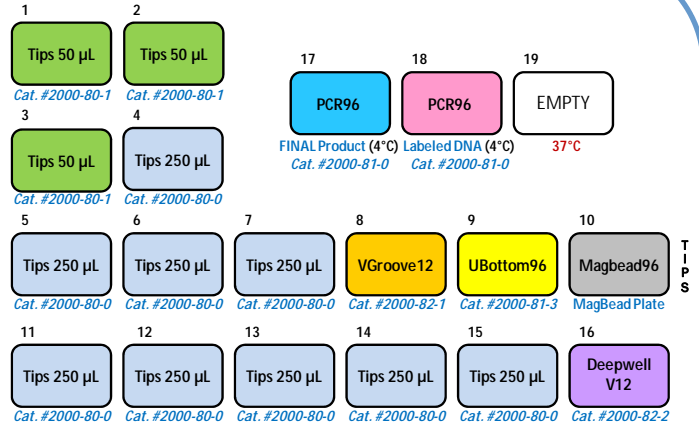


Figure 9. Deck layout for magnetic bead purification of 96 samples.

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 8).
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 9).
2. On the ArrayPrep® software, Select File | Run Protocol. Go to folder “Desktop/ArrayPrep Protocols” and select the “96 rxns Bead-based Purification” 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 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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