Agilent Yeast ChIP-on-chip Analysis

Protocol

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Research Use Only. Not for Diagnostic Procedures.

Agilent Technologies

Notices

Edition

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Contents

1 Sample Preparation

The steps in this protocol and the required amount of time are listed in Table 1 on page 6.

Table 1 Overview and time requirements.

Step	Time Requirement
Formaldehyde cross-linking of cells	1.5 hr
Binding of antibody to magnetic beads	0.5 hr, then overnight
Cell sonication	1 hr
Chromatin immunoprecipitation	0.5 hr, then overnight
Wash, elution, and cross-link reversal	2 hr, then overnight
Digestion of cellular protein and RNA	4 hr
T4 polymerase fill-in and blunt-end ligation	2 hr, then overnight
DNA amplification using ligation-mediated PCR (LM-PCR)	4 hr
Cy3/Cy5 labeling of IP and WCE material	4 hr
Microarray hybridization	1 hr, then 40 hr
Microarray washing	1 hr

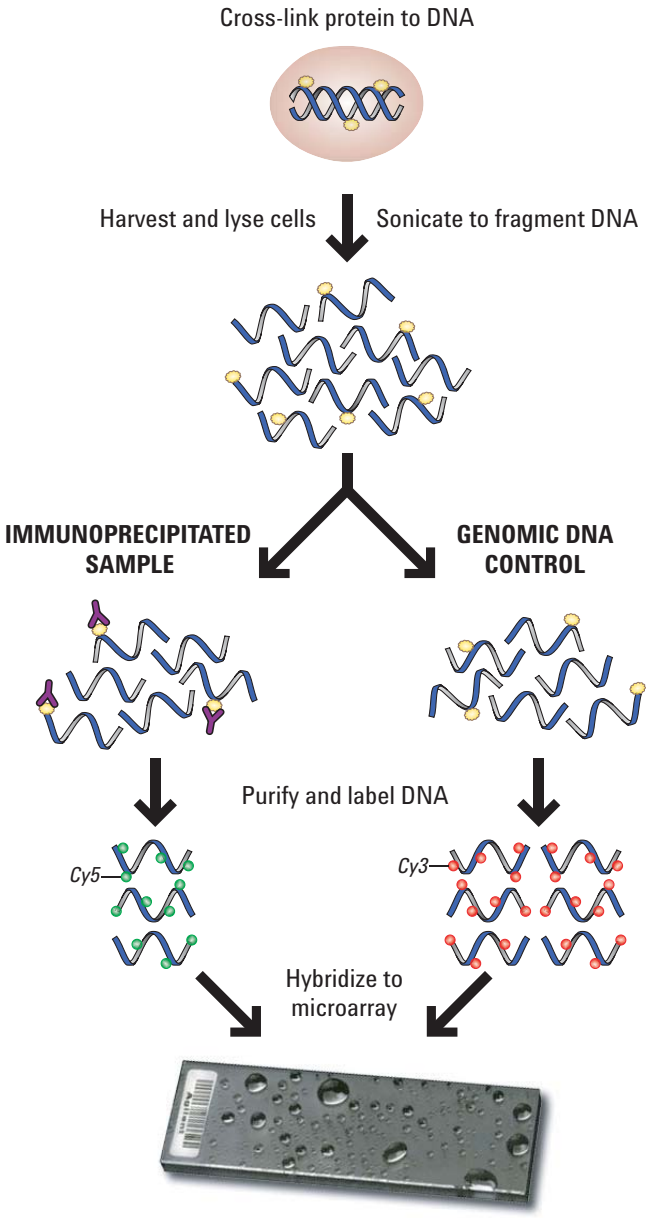


Figure 1 ChIP-on-chip overview

Step 1. Prepare cells and cross-link proteins to DNA

1 Inoculate 50 mL fresh media to OD600 = 0.1 from an overnight culture and allow yeast to grow to OD600 = 0.6 to 1.0.

2 Transfer each culture to separate 50 mL Falcon tubes (Cat. #352070), and add 1.4 mL of Formaldehyde (37% Formaldehyde stock, final concentration 1%, J.T.Baker Cat. #2106-01).

CAUTION Use a liquid dispenser for the formaldehyde and work in a fume hood.

3 Incubate the formaldehyde-treated cells for 20 minutes at room temperature on a rotating platform, and transfer to a rotating platform overnight at 4°C.

To achieve best results, some proteins may require optimization of the incubation duration with formaldehyde at room temperature.

If you plan to do the immunoprecipitation the following day, begin preparation of the magnetic beads (see "Step 5. Prepare magnetic beads" on page 12). Otherwise, make sure to prepare the beads the day prior to performing the immunoprecipitation step.

Step 2. Wash and store cells

1 Spin the 50 mL Falcon tubes containing the formaldehyde-treated cells for 5 minutes at 3000 rpm and 4°C in a table-top centrifuge to harvest the cells.

CAUTION Do not pour the supernatant (medium and formaldehyde) down the sink. Treat the supernatant as hazardous waste.

2 Pour off the supernatant and wash the cells 3 times in 1X TBS:

- **a** Add 40 mL of cold TBS (see Table 17 on page 50).
- **b** Mix by inversion until the cells are resuspended.
- **c** Spin at 3000 rpm and 4°C in a table-top centrifuge.
- **d** Pour off the supernatant.

3 After the last wash, resuspend the cell pellets using the remaining liquid (add a very small amount of 1X TBS, if necessary) and transfer to a 1.5 mL Eppendorf tube (Eppendorf Cat. #2236320-4).

4 Spin for 1 minute at 14,000 rpm and 4°C, and remove the remaining supernatant with a pipette.

5 If you are not using the cells immediately, snap-freeze them in liquid nitrogen and store at -80°C.

Step 3. Lyse the cells

1. Place the cell pellets on ice. (If the pellets were previously snap-frozen, thaw them on ice.)
2. Resuspend the cell pellets using 700 µL Lysis Buffer (see Table 18 on page 50).
3. Add the equivalent of a 0.5 mL PCR tube (USA/Scientific Cat.#1405-4400) of glass beads (425-600 µm, Sigma Aldrich Cat. #G-8772).
4. Lyse the cells using a Vibrax VXR (or equivalent) at a setting of 1800 for 2 hours at 4°C.
5. Pierce the bottom of the 1.5 mL tube with a needle (use Becton Dickinson Precision Glide 18G 1½).

NOTE Carefully push the needle into the tube wall just until liquid is drawn into the needle. Do not push more than this, or you may create a hole large enough in the tube for the beads to escape in the next step.

6. Carefully place the 1.5 mL tube into a 2 mL screw-cap tube.
7. Spin the nested tubes briefly to transfer the lysate to the 2 mL tube through the pierced hole, while the beads remain in the 1.5 mL tube.
8. Resuspend the lysate with a pipette and add lysis buffer to bring the volume up to 700 µL, as necessary (as smaller volumes may splash out during sonication).

Step 4. Shear the DNA by sonication

1 Sonicate the cells to shear the chromatin.

Any sonicator may be used to shear DNA. The following steps and setting have been optimized and are for use with a Branson Sonifier 250:

a Rinse the tip of the sonifier with 100% EtOH.

b Set the settings to **Hold** and **Constant Power**.

c Immerse the tip of the sonifier in the sample.

d Turn the power on for 20 seconds at power 1.5.

e Turn off the power.

f Repeat step a through step e 3 more times.

The sheared DNA should average roughly 400 bp in size. (Fragment size may be verified on an agarose gel.)

CAUTION Keep samples on ice between each round of sonication.

2 After the final round of sonication, spin each sample for 5 minutes at 14,000 rpm and 4°C.

3 Transfer the supernatant to another tube on ice, and discard the cell debris pellet.

CAUTION Do not throw out the supernatant. The supernatant contains the yeast whole cell extract (yWCE).

Step 5. Prepare magnetic beads

Prepare the day before using the magnetic beads

1. Aliquot 50 µL of beads (Dynabeads, Pan Mouse IgG, Invitrogen Cat. # 110-41) for each sample to be immunoprecipitated into a single 15 mL Falcon tube and spin 1 minute at approximately 3000 rpm and 4°C in a table-top centrifuge (such as a Sorvall RT 6000 or equivalent).
2. Carefully remove the supernatant with a pipette and resuspend the beads in a 10 mL 1X PBS solution containing 5 mg/mL BSA (prepare the PBS/BSA solution immediately before use from Sigma BSA powder, Cat. #A-3350) and spin again for 1 minute at 3,000 rpm.
3. Wash again with fresh 10 mL of PBS/BSA solution.
4. Carefully remove the supernatant with a pipette and add 1 to 2 µL of anti-Myc 9E11 antibody + 250 µL PBS/BSA solution per 50 µL of beads used in step 1.

NOTE 1 to 2 µL of anti-Myc 9E11 antibody is recommended, although you may need to empirically determine the appropriate amount of antibody to saturate the beads.

5. Incubate 8 hours to overnight on a rotating platform at 4°C.

Day of use (prepare immediately before immunoprecipitation)

1. Spin for 1 minute at 3000 rpm and 4°C in a table-top centrifuge (such as a Sorvall RT 6000 or equivalent).
2. Carefully remove the supernatant with a pipette and resuspend the beads in a 10 mL 1X PBS solution containing 5mg/mL BSA (make immediately before use).
3. Wash again with fresh 10 mL of PBS/BSA solution.
4. Carefully remove the supernatant with a pipette and resuspend the beads in 30 µL of PBS/BSA solution per sample.

Step 6. Immunoprecipitate the protein-DNA complexes

1. Set up a new tube on ice for each supernatant sample collected in "Step 4. Shear the DNA by sonication" on page 11:
   a. Transfer 500 µL of yWCE to the new tube on ice.
   b. Add 30 µL of the suspension of previously prepared magnetic beads.
2. Transfer 5 µL of the remaining yWCE into a second tube on ice (this will be processed later and serve as a control). Store this and any remainder of the yWCE at -20°C.
3. Mix the bead and yWCE mixture well using a vortex mixer.
4. Incubate the mixture overnight on a rotating platform at 4°C to immunoprecipitate the DNA.

Step 7. Wash the beads

For best results, do the washes in a cold room.

1 Place tubes into a magnetic device, such as a Dynal MPC-S magnet (Invitrogen Cat. #120-20D) or equivalent.

2 Invert the tubes to mix and allow the magnet to collect the beads.

3 Open the tubes and carefully remove the supernatant with a vacuum (be sure to also remove any liquid left in the tube cap).

4 For each wash solution in Table 2 below:

   a Add the wash solution.

NOTE You do not need to add protease inhibitors to any of the lysis buffers for bead washes.

   b Close the tubes and transfer them back onto the rotating platform.

   c Rotate each wash until the beads are completely resuspended.

   d Transfer the tubes to the magnetic device, and invert to mix.

   e Allow the magnet to collect the beads and carefully remove the wash solution with a vacuum.

   f Repeat step a through step e for the number of repetitions at each wash in Table 2.

Table 2

Wash solution	Repetition	Comments
1 mL Lysis Buffer	2 x	
1 mL Lysis Buffer + an additional 360 mM NaCl	2 x	720 µL of 5 M NaCl in 10 mL Lysis Buffer has a final concentration of 500 mM NaCL.
1mL Wash Buffer	2 x	See Table 19 on page 50.
1 mL TE	1 x	see Table 21 on page 51.

5 After the last wash, spin the tubes for 3 minutes at 3000 rpm and 4°C; remove any remaining liquid with a pipette.

Step 8. Elute the protein-DNA complexes from beads and reverse cross links

You can do these steps at room temperature.

1. Add 50 µL elution buffer to the magnetic beads. (See Table 20 on page 51).
2. Briefly mix the beads mixture on a vortex mixer to resuspend the beads and incubate at 65°C for 10 minutes.
3. Mix briefly every 2 minutes during this incubation.
4. Spin for 30 seconds at 14,000 rpm and room temperature, and transfer 30 µL of the supernatant to a new tube. Discard the rest, including the beads.
5. Add 120 µL of TE/SDS (see Table 21 on page 51) to the supernatant in this new tube.
6. Add 95 µL of TE/SDS to the 5 µL of yWCE that was saved from "Step 6. Immunoprecipitate the protein-DNA complexes" on page 13 (prepare one yWCE sample for each immunoprecipitated sample).
7. Incubate both the bead mixtures and the yWCE control mixtures overnight at 65°C.

Step 9. Purify the DNA

1. Add 150 µL of "proteinase K mix" (see Table 22 on page 51) to each tube.
2. Incubate for 2 hours at 37°C.
3. Extract 2 times with phenol (Sigma Cat. #P-4557):
   a. Add 1 volume phenol and mix on a vortex mixer.
   b. Spin for 5 minutes at 14,000 rpm and room temperature.
   c. Transfer the aqueous layer to a new tube, and discard the organic layer.

CAUTION Phenol is a caustic organic solvent. It must be disposed of properly.

4. Extract once with chloroform/isoamyl alcohol (Sigma Cat. #C-0549):
   a. Add 1 volume chloroform/isoamyl alcohol to the extracted aqueous layer from step 3, and mix with a vortex mixer.
   b. Spin for 5 minutes at 14,000 rpm and room temperature.
   c. Transfer the aqueous layer to a new tube, and discard the organic layer.
   d. Add NaCl to a final concentration of 200 mM in each tube. (Use 8 µL of 5 M NaCL stock for each 200 µL of sample).

CAUTION Chloroform/isoamyl alcohol is a caustic organic solvent. It must be disposed of properly.

5. Add 2 volumes of ice-cold EtOH and mix briefly with a vortex mixer.
6. Incubate at -20°C for at least 30 minutes.
7. Spin for 15 minutes at 14,000 rpm and 4°C.
8. Pour off the supernatant, add 1 mL ice-cold 75% EtOH, briefly mix with a vortex mixer, and spin for 5 minutes at 14,000 rpm and 4°C.
9. Pour off the supernatant, spin briefly and remove the remaining liquid with a pipette.
10. Let the pellet air-dry briefly, and resuspend it in 30 µL TE containing 10 µg RNaseA.

To prepare this TE/RNaseA solution, add 33 µL of 10 mg/mL RNaseA to 1 mL of TE.

11 Incubate for 1 hour at 37°C.

12 Purify using the Qiagen PCR purification kit (Qiagen Cat. #28106). Elute with 50 µL of 10 mM Tris pH 8.0. (Qiagen Buffer EB)

13 If you plan to ligate the DNA immediately, place the samples on ice and continue to "Step 10. Prepare blunt-end DNA" on page 18. Otherwise, store samples at -20°C.

Step 10. Prepare blunt-end DNA

1. Transfer 40 µL of each immunoprecipitated DNA to a new tube on ice for each immunoprecipitation reaction. Add 1.5 µL of each yWCE DNA and 39 µL ddH_2O to a second new tube on ice for each corresponding yWCE reaction. Store the remaining DNA at -20°C.
2. Make the blunting mix according to Table 3 and add 70 µL to each tube. If are creating a master mix, add 10 percent extra.:

Table 3 Blunting Mix

Component	Amount
NEBuffer 2 (10x) (NEB Cat. #B7002S)	11.0 µL
BSA (10 mg/mL) (NEB Cat. #B9001S)	0.5 µL
dNTP mix (20 mM each)	0.5 µL
T4 DNA polymerase (3U/µL) (NEB Cat. #M0203S)	0.2 µL
ddH_2O	57.8 µL
Total Volume	**70.0 µL**

3. Use a pipette to mix each reaction. Incubate the reactions at 12°C for 20 minutes.
4. Make the mix according to the table below and add 12 µL to each tube. If you are creating a master mix, add 10 percent extra.

Table 4

Component	Amount
3M NaOAc (Sigma Cat. #S-7899)	11.5 µL
Glycogen (20 mg/mL) (Roche Diagnostics Cat. #901393)	0.5 µL
Total Volume	**12.0 µL**

5. Mix the solution with a vortex mixer, and add 120 µL of phenol/chloroform/isoamyl alcohol.

6 Mix the solution with a vortex mixer, and spin in a centrifuge for 5 minutes at 14,000 rpm and room temperature.

7 Transfer 110 µL of the aqueous phase to a new 1.5 mL Eppendorf tube, and add 230 µL cold 100% EtOH.

8 Mix the solution with a vortex mixer, and spin for 15 minutes at 14,000 rpm and 4°C.

9 Carefully pour off supernatant and wash pellet with 500 µL cold 75% EtOH.

10 Spin for 5 minutes at 14,000 rpm and 4°C.

11 Carefully pour off the supernatant, briefly spin in a centrifuge, and remove any remaining liquid with a pipette. Allow to air-dry briefly.

12 Resuspend pellet in 25 µL ddH_20 and place on ice.

Step 11. Ligate the blunt-end DNA to the linker

1 Add 25.2 µL of the cold ligase mixture to each tube:

Table 5 Cold Ligase Mixture

Component	Amount
ddH2O	8.0 µL
5X DNA ligase buffer (Invitrogen Cat. #46300-018)	10.0 µL
Annealed linkers (15 µM) (see "To prepare unidirectional linker" on page 44)	6.7 µL
T4 DNA ligase (NEB Cat. #M0202L)	0.5 µL
Total Volume	**25.2 µL**

2 Use a pipette to mix the reaction, and incubate overnight at 16°C.

Step 12. Amplify the IP and WCE samples

NOTE PCR methods and reagents may be covered by one or more third-party patents. It is the user's responsibility to obtain any necessary licenses and/or licensed PCR reagents for such patents.

NOTE This protocol enables large-scale amplification of IP and WCE samples. After 15 cycles of PCR-based amplification, the reaction is diluted and used as template for a second round of 25 cycles. Remaining template can be stored long-term at -20°C.

1. Add 6 µL of 3M NaOAc (pH 5.2) (Sigma Cat. #S-7899) to each overnight linker-ligated DNA reaction.
2. Use a vortex mixer to mix the solution, and add 130 µL cold EtOH.
3. Use a vortex mixer to mix the solution, and spin in a centrifuge for 15 minutes at 14,000 rpm and 4°C.
4. Pour off the supernatant and wash with 500 µL 75% EtOH.
5. Spin in a centrifuge for 5 minutes at 14,000 rpm and 4°C.
6. Pour off the supernatant, briefly spin in a centrifuge, and use a pipette to remove any remaining liquid.
7. Resuspend the DNA pellet in 25 µL ddH_20 and place the tube on ice.
8. Put 25 µL each of IP and WCE DNA into separate PCR tubes (0.2 to 0.5 mL).
9. Make two buffer mixes:

Table 6 Mix A

Stock	1x Mix	Final Concentration*
10X Thermopol buffer (NEB)	4.00 µL	1x
dNTP mix (2.5 mM each)	5.00 µL	250 µM
oligo JW102 (40 µM)	1.25 µL	1 µM
ddH_2O	4.75 µL	
Total	**15.00 µL**	

* The Final Concentration is the reagent concentration in the final reaction and *not* the master mix.

Table 7 Mix B

Stock	1x Mix	Final Concentration*
10X Thermopol buffer (NEB)	1.0 µL	1x
Taq polymerase (5U/µL)	0.5 µL	0.25 U
ddH_2O	8.5 µL	
Total	**10.0 µL**	

* The Final Concentration is the reagent concentration in the final reaction and *not* the master mix.

10 Add 15 µL of Mix A to each sample.

11 Run an LM-PCR program in a thermocycler:

a Start the program below.

b Midway through Step 1, pause the program.

c Add 10 µL Mix B to each tube to hot start the reactions.

Maintain the tubes at 55°C while adding Mix B.

d Continue the program.

Step 1:	55°C	4 minutes
Step 2:	72°C	3 minutes
Step 3:	95°C	2 minutes
Step 4:	95°C	30 seconds
Step 5:	55°C	30 seconds
Step 6:	72°C	1 minute
Step 7:	GO TO Step 4 x 14 times	
Step 8:	72°C	5 minutes
Step 9:	4°C	HOLD

12 Transfer the product to a 1.5 mL microfuge tube and add 475 µL ddH_20 (total volume approximately 525 µL).

13 Put 5 µL of the resulting PCR product into a PCR tube (0.2 to 0.5 mL) for a second expansion.

14 Make the PCR mixture:

Table 8 PCR Mixture

Stock	1x Mix	Final Concentration*
10x Thermopol buffer (NEB)	5.00 µL	1x
dNTP mix (2.5 mM each)	5.00 µL	250 µM
oligo oJW102 (40 µM)	1.25 µL	1 µM
Taq polymerase (5U/µL)	0.25 µL	1.25 U
ddH_2O	33.50 µL	
Total	45.00 µL	

* The Final Concentration is the reagent concentration in the final reaction and *not* the master mix.

15 Put 45 µL of PCR mix to individual PCR tubes.

16 Run the LM-PCR program below in a thermocycler:

Step 1:	95°C	2 minutes
Step 2:	95°C	30 seconds
Step 5:	55°C	30 seconds
Step 6:	72°C	1 minute
Step 7:	GO TO Step 2 x 24 times	
Step 8:	72°C	5 minutes
Step 9:	4°C	HOLD

17 Make the precipitation mix:

Table 9 Precipitation Mix

Stock	1x Mix	Final Concentration*
7.5 M Ammonium acetate	25.0 µL	625 mM
Ethanol	225.0 µL	75%
Total	**250.0 µL**	

* The Final Concentration is the reagent concentration in the final reaction and *not* the master mix.

18 Transfer the product to a 1.5 mL microfuge tube.

19 Add 250 µL precipitation mix to each tube.

20 Cool for 30 minutes at -80°C.

21 Spin at 20,000 x g for 10 minutes at 4°C to pellet DNA.

22 Wash the pellets with 500 µL of 70% EtOH.

23 Dry the pellets for 10 minutes with a vacuum desiccator, such as a Savant Speed Vac, and resuspend each pellet in 50 µL H_2O.

24 Measure DNA concentration with NanoDrop (NanoDrop Technologies) (use 10-fold dilutions, if necessary) and normalize all samples to 100 ng/µL.

Step 13. Label the IP and WCE

In this step, you use the random-primed, Klenow-based extension protocol that is used with Invitrogen's CGH Labeling kit. This protocol varies from the instructions provided by Invitrogen in both reaction volume and reagent concentrations (yielding 20 reactions per "30 reaction" Invitrogen kit, p/n 18095-011). A pair of reactions, one for each dye, yields enough material for 1 to 2 hybridizations. To scale up for more arrays, increase the number, not the volume, of individual reactions.

1 Open the required number of Invitrogen CGH kits and consolidate the stocks of 2.5x Random Primer Solution, 10x dUTP Nucleotide Mix, Klenow, and Stop Buffer.

2 Put the LM-PCR product into a PCR tube (0.2 to 0.5 mL) and add random primer solution and water as follows:

Table 10

Stock	1x Mix	Final Concentration*
LM-PCR product (100 ng/µL)	20.0 µL	2 µg
2.5x random primer solution	35.0 µL	1x
ddH_2O	20.0 µL	
Total	75.0 µL	

* The Final Concentration is the reagent concentration in the final reaction and *not* the master mix.

3 Mix on a vortex mixer for 30 seconds.

4 Place the tubes in a thermal cycler preheated to 95°C and incubate for 5 minutes.

5 Immediately transfer the tubes to an ice-water bath and cool for 5 minutes.

6 While the reactions are cooling, create the Labeling Mix (see Table 11).

Typically Cy5 mix is used for IP DNA and Cy3 for WCE DNA.

Table 11 Labeling Mix

Stock	1x Mix	Final Concentration
10X dUTP Nucleotide Mix	8.2 µL	112/56 nM
Cy5- or Cy3-dUTP (1 mM)	1.5 µL	17 µM
Klenow (40 U/µL)	1.5 µL	60 U
ddH_2O	1.8 µL	
Total	**13.0 µL**	

Keep the reactions in dark as much as possible to minimize degradation of Cy dyes.

7 Mix the mixture on a vortex mixer for 30 seconds.

8 Put 13 µL of the label mix in each tube. Mix by pipetting up and down multiple times.

9 Incubate for 3 hours at 37°C. Keep the samples in the dark.

10 Add 9 µL stop buffer to each tube and mix.

11 Transfer each sample to a 1.5 mL microfuge tube.

12 Clean up the samples using Invitrogen's CGH column as follows:

- **a** Add 0.4 mL of Purification Buffer A to each tube and mix with a vortex mixer for 30 seconds.
- **b** Place the Purification Column into a 2 mL collection tube.
- **c** Use a pipette to transfer the sample to the Purification Column.
- **d** Spin the column in a centrifuge at 8,000 × g for 1 minute at room temperature.
- **e** Add 0.6 mL of Purification Buffer B to the column (make sure Ethanol has been added to Buffer B).
- **f** Spin the sample in a centrifuge at 8,000 × g for 1 minute at room temperature. Discard the flow-through from the collection tube, and place the column back in the tube.
- **g** Add 0.2 mL of Purification Buffer B to the column.

h Spin the sample in a centrifuge at 8,000 × g for 1 minute at room temperature. Discard the flow-through.

i Place the Purification Column in a new, sterile 1.5-mL collection tube.

j Add 50 µL of sterile water and incubate at room temperature for 1 minute.

k Spin the sample in a centrifuge at 8,000 × g for 1 minute at room temperature. The flow-through contains the purified labeled DNA sample.

13 Measure Cy label and total DNA yield using the NanoDrop.

Expect >3.5 pmol/µL Cy3 label incorporation, >2.5 pmol/µL Cy5 label incorporation, and >5 µg total DNA per reaction (approximately 100 ng/µL).

NOTE If you plan to hybridize using a multi-pack format, you may need to speed-vac your samples. Speed-vac to dryness and resuspend in the appropriate volume. See on page .

2
Hybridization, Wash and Scan

In this section, you hybridize, wash, scan and extract data from ChIP-on-chip microarrays.

Hybridization and Wash

This section contains information to help you hybridize and wash your microarray.

Step 1. Hybridize the microarray

1 Prepare the 10X Blocking Agent:

   a Add 1350 µL of nuclease-free water to the vial containing lyophilized 10X Blocking Agent (supplied with Agilent Oligo aCGH Hybridization Kit).

   b Leave at room temperature for 60 minutes to reconstitute sample before use or storage.

   The 10X Blocking Agent can be prepared in advance and stored at -20°C.

NOTE Use foil and amber tubes to keep samples in the dark as much as possible.

The next steps are an abbreviated version of the Agilent aCGH array hybridization protocol. Please refer to aCGH protocol for details, notes, and warnings.

2 Equilibrate water baths or heat blocks to 95°C and 37°C.

3 Combine Cy5- and Cy3-labeled samples with ddH_2O in a 1.5 mL microfuge tube for a total volume as indicated in Table 12.

Table 12

	8x15K Array Format	4x44K Array Format	2x105K Array Format	1x44K or 1x244K Array format
Cy5-labeled Samples	1.25 to 2.5 µg	2.5 to 5.0 µg	4 to 5 µg	5 µg
Cy3-labeled Samples	1.25 to 2.5ug	2.5 to 5.0 µg	4 to 5 µg	5 µg
Total Volume with ddH_20	16 µL	39 µL	79 µL	158 µL

NOTE The input mass should be balanced for each channel, e.g. 2.5 µg of Cy3 labeled DNA and 2.5 µg of Cy5 labeled DNA.

4 Add the following in the order indicated to the microfuge tube:

Table 13

Stock	8x15K Array Format	4x44K Array Format	2x105K Array Format	1x44K or 1x244K Array format
Human Cot-1 DNA (1.0 mg/mL)	2	5	25	50
Agilent Blocking Agent (10x)*	4.5	11	26	52
Agilent Hybrdization Buffer (2x)*	22.5	55	130	260

* Supplied in the Agilent Oligo aCGH/ChIP-on-chip Hybridization Kit

5 Mix contents and quick spin to collect.

6 Heat samples for 3 minutes at 95°C.

7 Immediately transfer the sample tubes to a circulating water bath or heat block at 37°C and incubate for 30 minutes.

8 Spin at 17,900 × g for 1 minute at room temperature to collect the sample.

9 Load a clean gasket slide into the Agilent SureHyb chamber base with the label facing up and aligned with the rectangular section of the chamber base. Ensure that the gasket slide is flush with the chamber base and is not misaligned.

Refer to the *Agilent Microarray Hybridization Chamber User Guide* (G2534-90001) for in-depth instructions on how to load slides, assembly and disassembly of chambers, as well as other helpful tips. This user guide is available with the Agilent Microarray Hybridization Chamber Kit (G2534A) and can also be downloaded from the Agilent Web site at www.agilent.com/chem/dnamanuals-protocols.

10 Slowly dispense hybridization sample onto the gasket well in a “drag and dispense” manner in this amount:

8x15K format	40 µL/array (8 individual samples of 40 µL each)
4x44K format	100 µL/array (4 individual samples of 100 µL each)
2x105K format	250 µL (2 individual samples of 240 µL each)
1x244K format	490 µL
1x44K format	490 µL

NOTE Multi-pack users: When you form the sandwich pair, keep the arrays parallel to the gasket as you lower it so that the gasket surfaces contact the active side of the array evenly.

11 Place a microarray “active side” down onto the SureHyb gasket slide, so the numeric barcode side is facing up and the “Agilent” barcode is facing down. Verify that the sandwich-pair is properly aligned.

12 Place the SureHyb chamber cover onto the sandwiched slides and slide on the clamp assembly. Hand-tighten the clamp onto the chamber.

13 Vertically rotate the assembled chamber to wet the gasket and assess the mobility of the bubbles. Tap the assembly on a hard surface if necessary to move stationary bubbles.

14 Place assembled slide chamber in rotisserie hybridization oven set to 65°C. Hybridize as follows:

4x44K or 8x15K formats	Hybridize at 20 RPM for 24 hours
1x244K or 1x44K formats	Hybridize at 20RPM for 40 hours

Step 2. Prewarm Oligo aCGH/ChIP-on-chip Wash Buffer 2

Warm the **Oligo aCGH/ChIP-on-chip Wash Buffer 2** to 31°C as follows:

1. Dispense 1000 mL of Oligo aCGH/ChIP-on-chip Wash Buffer 2 directly into a sterile 1000-mL bottle. Repeat until you have enough prewarmed Wash Buffer 2 solution for your experiment.
2. Tightly cap the 1000-mL bottle and place in a 31°C water bath the night before washing arrays. Alternatively, remove the plastic cubitainer from the box and place it in a 31°C water bath the night before washing the arrays.
3. Put a slide-staining dish into a 1.5 L glass dish three-fourths filled with water. Warm to 31°C by storing overnight in an incubator set to 31°C.

Step 3. Prepare the equipment

Always use clean equipment when doing the hybridization and wash steps. Designate and dedicate dishes to two-color experiments. The acetonitrile wash is only necessary if the staining dishes, racks and stir bars were used in previous experiments with the Agilent Stabilization and Drying Solution. Otherwise proceed to "Milli-Q water wash" on page 32.

Acetonitrile wash

Wash staining dishes, racks and stir bars that were used in previous experiments with the Agilent Stabilization and Drying Solution with acetonitrile to remove any remaining residue.

WARNING **Conduct acetonitrile washes in a vented fume hood.**

1. Add the slide rack and stir bar to the staining dish.
2. Transfer the staining dish with the slide rack and stir bar to a magnetic stir plate.
3. Fill the staining dish with 100% acetonitrile.
4. Turn on the magnetic stir plate and adjust the speed to a setting of 4 (medium speed).
5. Wash for 5 minutes.

6 Discard the acetonitrile as is appropriate for your site.

7 Repeat steps step 1 through step 6.

8 Air dry the staining dish in the vented fume hood.

9 Proceed to "Milli-Q water wash" below.

Milli-Q water wash

Wash all dishes, racks, and stir bars with Milli-Q water.

1 Run copious amounts of Milli-Q water through the staining dish.

2 Empty out the water collected in the dish.

3 Repeat steps 1 and 2 at least 5 times, as it is necessary to remove any traces of contaminating material.

4 Discard the Milli-Q water.

CAUTION Some detergents may leave fluorescent residue on the dishes. Do not use any detergent in the washing of the staining dishes. If detergent is used, all traces must be removed by copiously rinsing with Milli-Q water.

Step 4. Wash the microarray slides

NOTE Cyanine 5 has been shown to be sensitive to ozone degradation. Ozone levels as low as 5 ppb (approximately 10 µg/m^3) can affect cyanine 5 signal and compromise microarray results. The Agilent Stabilization and Drying Solution is designed to protect against ozone-induced degradation of cyanine dyes. Use this solution when working with Agilent oligo-based microarrays in high ozone environments.

NOTE When setting up the apparatus for the washes, be sure to do so near the water bath containing the pre-warmed Wash 2 solutions.

Table 14 lists the wash conditions for the wash procedure.

Table 14 Wash conditions

	Dish	Wash Buffer	Temperature	Time
Disassembly	1	Oligo aCGH/ChIP-on-chip	Room temperature	
1st wash	2	Oligo aCGH/ChIP-on-chip	Room temperature	5 minute
2nd wash	3	Oligo aCGH/ChIP-on-chip	31°C	5 minute

1. Completely fill slide-staining dish #1 with Oligo aCGH/ChIP-on-chip Wash Buffer 1 at room temperature.
2. Place a slide rack into slide-staining dish #2. Add a magnetic stir bar. Fill slide-staining dish #2 with enough Oligo aCGH/ChIP-on-chip Wash Buffer 1 at room temperature to cover the slide rack. Place this dish on a magnetic stir plate.
3. Remove one hybridization chamber from incubator and record time. Record whether bubbles formed during hybridization and if all bubbles are rotating freely.
4. Prepare the hybridization chamber disassembly.
   a. Place the hybridization chamber assembly on a flat surface and loosen the thumbscrew, turning counterclockwise.
   b. Slide off the clamp assembly and remove the chamber cover.
   c. With gloved fingers, remove the array-gasket sandwich from the chamber base by grabbing the slides from their ends. Keep the microarray slide

numeric barcode facing up as you quickly transfer the sandwich to slide-staining dish #1.

d Without letting go of the slides, submerge the array-gasket sandwich into slide-staining dish #1 containing Oligo aCGH/ChIP-on-chip Wash Buffer 1.

5 With the sandwich completely submerged in Oligo aCGH/ChIP-on-chip Wash Buffer 1, pry the sandwich open from the barcode end only:

a Slip one of the blunt ends of the forceps between the slides.

b Gently turn the forceps upwards or downwards to separate the slides.

c Let the gasket slide drop to the bottom of the staining dish.

d Remove the microarray slide and place into slide rack in the slide-staining dish #2 containing Oligo aCGH/ChIP-on-chip Wash Buffer 1 at room temperature. Minimize exposure of the slide to air. *Touch only the barcode portion of the microarray slide or its edges!*

More effort is needed to separate the multi-pack than the single-pack sandwiched slides.

6 Repeat steps step 3 through step 5 for up to seven additional slides in the group. For uniform washing, do up to a maximum of eight disassembly procedures yielding eight microarray slides.

7 When all slides in the group are placed into the slide rack in slide-staining dish #2, stir using setting 4 for 5 minute.

8 During this wash step, remove Oligo aCGH/ChIP-on-chip Wash Buffer 2 from the 31°C water bath and pour into the slide-staining dish #3.

9 Approximately 1 min before the end of Wash1, put the prewarmed 1.5 L glass dish filled with water and containing slide-staining dish #3 on a magnetic stir plate with heating element. Fill the slide-staining dish #3 approximately three-fourths full with Oligo aCGH Wash Buffer 2 (warmed to 31°C). Add a magnetic stir bar. Turn on the heating element and maintain temperature of Oligo aCGH Wash Buffer 2 at 31°C. Monitor using a thermometer.

10 Slowly remove the slide rack minimizing droplets on the slides. It should take 5 to 10 seconds to remove the slide rack.

11 Discard used Oligo aCGH/ChIP-on-chip Wash Buffer 1 and Oligo aCGH/ChIP-on-chip Wash Buffer 2.

12 Repeat step 1 through step 11 for the next group of eight slides using fresh Oligo aCGH/ChIP-on-chip Wash Buffer 1 and Oligo aCGH/ChIP-on-chip Wash Buffer 2 pre-warmed to 31°C.

13 Scan slides immediately to minimize the impact of environmental oxidants on signal intensities. If necessary, store slides in orange slide boxes in a N_2 purge box, in the dark.

NOTE Use fresh Oligo aCGH/ChIP-on-chip Wash Buffer 1 and 2 for each wash group (up to 8 slides).

Scanning and Feature Extraction

Step 1. Scan the slides

Agilent Scanner Settings

1 Assemble the slides into an appropriate slide holder, either version B or A. Place the slides into the slide holder such that the numeric barcode side is visible (*not* the "Agilent"-labeled barcode side). Refer to "General Microarray Layout and Orientation" on page 56.

2 Place assembled slide holders into scanner carousel.

3 Verify scan settings for two-color scans.

Table 15 Scan Settings

	For 1x22K, 1x44K Formats	For 1x244K, 2x105K, 4x44K and 8x15K Formats
Scan region	Scan Area (61 x 21.6 mm)	Scan Area (61 x 21.6 mm)
Scan resolution (µm)	10	5
Dye channel	Red&Green	Red&Green
Green PMT	100%	100%
Red PMT	100%	100%

To change any settings, click **Settings > Modify Default Settings**. A window pops up from which you can change the settings.

4 Select settings for the automatic file naming

- **Prefix 1** is set to **Instrument Serial Number**
- **Prefix 2** is set to **Array Barcode**

5 Verify that the Scanner status in the main window says **Scanner Ready**.

6 Click **Scan Slot *m*-*n*** on the Scan Control main window where the letter ***m*** represents the Start slot where the first slide is located and the letter ***n*** represents the End slot where the last slide is located.

Gene Pix scanner settings

Only GenePix 4000A and 4000B scanners are supported for scanning Agilent microarrays.

Refer to the manufacturer's user guide for appropriate scanner settings.

Refer to "General Microarray Layout and Orientation" on page 56 for appropriate slide layout and orientation in GenePix scanner.

NOTE

Agilent 1x244K, 2x105K, 4x44K and 8x15K microarrays require 5 µm scan resolution, which is not supported by the GenePix 4000A.

Step 2. Extract data using Agilent Feature Extraction Software

Feature Extraction is the process by which information from probe features is extracted from microarray scan data, allowing researchers to measure gene expression in their experiments. To get the most recent Feature Extraction software for gene expression, go to the Agilent Web site at www.agilent.com/chem/fe.

Feature Extraction (FE) 9.5.1 supports extraction of two-color .tif images of Agilent microarrays scanned on Agilent Scanner or GenePix (Axon/Molecular Devices) scanner.

After generating the microarray scan images, extract .tif images using the Feature Extraction software.

1 Open the Agilent Feature Extraction (FE) software version 9.5.1.

   To get the most recent Feature Extraction protocols for gene expression, go to the Agilent Web site at www.agilent.com/chem/FEprotocols.

2 Add the images (.tif) to be extracted to the FE Project.

   a Click **Add New Extraction Set(s)** icon on the toolbar or right-click the **Project Explorer** and select **Add Extraction...**

   b Browse to the location of the .tif files, select the .tif file(s) and click **Open**. To select multiple files, use the Shift or Ctrl key when selecting.

   The FE program automatically assigns a default grid template and protocol for each extraction set, if the following conditions are met:

   - For auto assignment of the grid template, the image must be generated from an Agilent scanner or GenePix (4000A or 4000B) and have an Agilent barcode.
   - For auto assignment of the ChIP-on-Chip FE protocol, the **default ChIP-on-chip protocol** must be specified in the FE Grid Template properties.

     To access the FE Grid Template properties, double-click on the grid template in the Grid Template Browser.

3 Set FE Project Properties.

a Select the **Project Properties** tab.

b In the **General** section, enter your name in the **Operator** text box.

c In the **Input** section, verify that at least the following default settings as shown in Figure 2 below are selected.

For outputs that can be imported into Rosetta Resolver, select MAGE and JPEG.

⊟ General	
Operator	Unknown
⊟ Input	
Number of Extraction Sets Included	0
⊟ Output and Data Transfer	
⊟ Outputs	
⊞ MAGE	None
JPEG	None
⊞ TEXT	Local file only
Visual Results	Local file only
Grid	Local file only
QC Report	Local file only
FTP Send Tiff File	False
⊟ Local File Folder	
Same As Image	True
Results Folder	
⊞ FTP Setting	
⊟ Automatic Protocol Assignment	
Highest Priority Default Protocol	Grid Template Default
Project Default Protocol	
⊟ Automatic Grid Template Assignment	
Use Grid file if available	True
⊟ Other	
QC Metric Set	
External DyeNorm List File	
Overwrite Previous Results	False

Figure 2 Default settings

4 Check the Extraction Set Configuration.

a Select the **Extraction Set Configuration** tab.

b Verify that the correct grid template is assigned to each extraction set in the **Grid Name** column. To assign a different grid template to an extraction set, select one from the pull down menu.

If a grid template is not available to select from the pull down menu, you must add it to the Grid Template Browser. To add, right-click inside the Grid Template Browser, select **Add**. Browse for the design file (.xml) and click **Open** to load grid template into the FE database.

To update to the latest grid templates via Online Update, right-click **Grid Template Browser** and select **Online Update**. You can also download the latest grid templates from Agilent Web site at www.agilent.com/chem/downloaddesignfiles. After downloading, you must add the grid templates to the Grid Template Browser.

After a new grid template is added to the Grid Template Browser, remember to specify the default protocol for the new grid template if you want the Feature Extraction program to automatically assign a FE protocol to an extraction set.

c Verify that the protocol **ChIP-v1_95_May07** is assigned to each extraction set in the **Protocol Name** column. To assign a different protocol to an extraction set, select one from the pull down menu.

The protocols automatically distinguish the formats for processing the data.

If a protocol is not available to select from the pull down menu, you must import it to the FE Protocol Browser. To import, right-click **FE Protocol Browser**, select **Import**. Browse for the FE protocol (.xml) and click **Open** to load the protocol into the FE database. Visit the Agilent Web site at www.agilent.com/chem/FEprotocols to download the latest protocols.

NOTE These FE Protocols were optimized using data from Agilent catalog arrays, which have many replicated probes and validated Negative Control probes. If custom arrays without enough replicated probes are used, or arrays with custom probes designated as Negative Control probes are used, the default FE Protocols may not be optimal.

5 Save the FE Project (.fep) by selecting **File > Save As** and browse for desired location.

6 Verify that the icons for the image files in the FE Project Window no longer have a red X through them. A red X through the icon indicates that an extraction protocol was not selected. If needed, reselect the extraction protocol for that image file.

7 Select **Project > Start Extracting**.

8 After the extraction is completed successfully, view the QC report for each extraction set by double-clicking the QC Report link in the **Summary**

Report tab. Determine whether the grid has been properly placed by inspecting Spot Finding at the Four Corners of the Array.

Refer to the application note on *Use of Agilent Feature Extraction Software (v8.1) QC Report to Evaluate Microarray Performance* (publication 5989-3056EN) for more details on quality assessment and troubleshooting with the Feature Extraction QC Report. This technical note can be downloaded from the Agilent Web site at www.agilent.com/chem/dnaapplications.

3 Supplementary Protocols

This section contains tasks that you may have to do as part of the protocol.

To prepare unidirectional linker

1 Mix the following:

250 µL	Tris-HCl (1 M) pH 7.9
375 µL	oligo oJW102 (40 µmol stock)
375 µL	oligo oJW103 (40 µmol stock)
1000 µL	total

oJW102: 5'-GCGGTGACCCGGGAGATCTGAATTC-3'

oJW103: 5'-GAATTCAGATC-3'

Order these oligos in desiccated form, then resuspend in dd$H_2$0.

2 Make 50 or 100 µL aliquots in Eppendorf tubes.

3 Place in a 95°C heat block for 5 minutes.

4 Transfer samples to a 70°C water-filled heat block.

5 Transfer the entire block to room temperature and allow it to cool to 25°C.

6 Transfer the block to 4°C and allow to stand overnight.

7 Store at -20°C.

To do a gene-specific PCR for Chromatin IP reactions

Use 1 µL of IP DNA and series of dilutions of the input DNA (yWCE) 2µL, 1µL, 1µL (1:2) and 1 µL (1:4) as template for the PCR.

You need to do multiple PCR reactions containing:

- One control primer pair (for a gene that should not be bound or for which the binding should not vary during the experiment)
- 1 to 3 primer pairs for genes to be tested (this can include a positive control if such a gene is known). If you have more then 3 genes to test, use multiple primer mixes (Protocol B). Too many primers in a PCR reaction can interfere with each other.

Protocol A - One primer mix

1 Prepare the following master mix for n+2 samples:

0.05 µL	^{32}P- dATP
0.35 µL	10 mmol dNTPs
1.5 µL	10X reaction buffer (Perkin Elmer)
1.5 µL	$MgCl_2$ (Perkin Elmer)
0.15 µL each	Primers
0.15 µL	Taq polymerase (Perkin Elmer)
Complete to 14 µL	ddH_2O

2 Use a pipette to add DNA into PCR tubes (as mentioned above) and distribute 14 µL of master mix to each. Use a pipette to mix and place in thermocycler.

3 Run the following program.

Table 16

Step	Temperature/ Instruction	Time
1	95°C	1 min 30 sec
2	95°C	40 sec
3	50°C	40 sec
4	72°C	40 sec
5	Repeat steps 2 through 4, 20X	
6	4°C	hold

4 During the PCR reaction, prepare a 6% acrylamide gel (add APS and TEMED just prior to pouring):

3.75 mL	40% acrylamide (30:1) solution (Sigma cat #A-9926)
5 mL	5X TBE
Complete to 25 mL	ddH2O
250 µL	10% APS
25 µL	TEMED (Sigma cat #T-7024)

5 Mix and pour gel slowly.

6 After PCR, spin briefly to collect sample at the bottom of the tube.

7 Add tracking dye to 1X.

8 Set pipet to 10 µL, mix by pipetting and load 10 µL onto gel.

9 Store the remaining reaction at 4°C in a beta-secure container.

Protocol B - Multiple primer mixes

1 Prepare the following master mix for 'n+2+number of mixes' samples:

0.05 µL	^{32}P- dATP
0.35 µL	10 mmol dNTPs
1.5 µL	10X reaction buffer (ABI)
1.5 µL	$MgCl_2$ (ABI)
0.15 µL	Taq polymerase (ABI)
Complete to 14 µL	ddH_2O

2 Prepare the following primer mixes:

0.15 µL each	Primers
14 µL	Master mix

3 Use a pipette to add DNA into PCR tubes (as mentioned above) and distribute the appropriate amount of primer mix to each tube. Use a pipette to mix and place in the thermocycler.

4 Follow the remainder of Protocol A from step 3.

4 Reference

This section contains reference information for this protocol.

Solutions

Table 17 TBS (store at 4°C)

1X	5X	for 1L of 5X
20 mM Tris-HCl pH7.5	100 mM Tris-HCl pH 7.5	100 mL of 1M
150 mM NaCl	750 mM NaCl	150 mL of 5M

Table 18 Lysis Buffer (add protease inhibitors just prior to use, store stock solution at 4°C)

1X	for 150 mL	for 5 mL
50 mM HEPES-KOH pH7.5	7.5 mL of 1 M	250 µL of 1 M
140 mM NaCl	4.2 mL of 5 M	140 µL of 5 M
1 mM EDTA	300 µL of 500 mM	10 µL of 500 mM
1% Triton X-100	15 mL of 10%	500 µL of 10%
0.1% Na-deoxycholate	3 mL of 5%	100 µL of 5%
1 mM PMSF, 1 mM Benzamidine	1.5 mL of 100X	50 µL of 100X
10 µg/mL Aprotinin, 1 µg/mL Leupeptin	1.5 mL of 100X	50 µL of 100X
1µg/mL Pepstatin	1.5 mL of 100X	50 µL of 100X

Table 19 Wash Buffer (store at 4°C)

1X	for 500 mL
10 mM Tris-HCl pH 8.0	5 mL of 1 M
250 mM LiCl	25 mL of 5 M
0.5% NP40	2.5 mL of 100%
0.5% Na-deoxycholate	25 mL of 10%
1 mM EDTA	1 mL of 500 mM

Table 20 Elution buffer (make with ddH_2O, store at room temperature)

1X	For 100 mL
50 mM Tris-HCl pH8.0	5 mL of 1 M Tris HCl pH 8.0
10 mM EDTA	2 mL of 500 mM EDTA
1% SDS	10 mL of 10% SDS

Table 21 TE/SDS (make with ddH_2O, store at room temperature)

1X	For 500 mL
10 mM Tris HCl pH8.0	5 mL of 1 M
1 mM EDTA	1 mL of 500 mM
1% SDS	50 mL of 10% SDS

Table 22 Proteinase K mix (make fresh)

For each sample
140 µL of TE
3 µL of glycogen (Roche Diagnostics Cat# 901393)
7.5 µL of proteinase K (20 mg/mL stock) (Invitrogen 25530-049)

Table 23 PMSF/Benzamidine mix 100X stock (aliquot and store at -20°C)

1X	For 10 mL of 100X
1 mM PMSF	0.1742 g
1 mM Benzamidine	0.1566 g
EtOH	Bring to a volume of 10 mL

Table 24 Aprotinin/Leupeptinin mix 100X stock (aliquot and store at -20°C)

1X	For 10 mL of 100X
10 µg/mL Aprotinin	0.01 g
1 µg/mL Leupeptin	0.001 g
ddH_2O	Bring to a volume of 10 mL

Table 25 Pepstatin mix 100X (aliquot and store at-20°C)

1X	For 10 mL of 100X
1 µg/mL Pepstatin	0.001 g
DMSO	Bring to a volume of 10 mL

Supplies list

Table 26 Crosslinking, cell lysis, immunoprecipitation, and DNA purification

Component	Manufacturer	Catalog Number	Amount
37% formaldehyde	J.T. Baker	2106-01	500 mL
Glass beads	Sigma	G-8772	1 kg
Pan mouse IgG Magnetic beads	Invitrogen	110-41	5 mL
c-myc 9.00E+11 antibody	Biosource	AHO0052	100 µg
BSA	Sigma	A-7906	50 g
Proteinase K	Invitrogen	25530-049	5 x 1 mL
Glycogen	Roche Diagnostics	901393	1 mL
Phenol	Sigma	P-4557	100 mL
Chloroform / isoamyl alcohol	Sigma	C-0549	100 mL
3M NaOAc	Sigma	S-7899	100 mL
Phenol / chloroform / isoamyl alcohol (25:24:1)	Sigma	P-3803	1 pint
QIAquick PCR Purification Kit	Qiagen	28106	250 spin columns
Ethanol	Amresco	E193-500ML	500 mL
Nuclease-free distilled water	Invitrogen	10977-015	500 mL

Table 27 Blunting, Ligation, Amplification and Labeling

Component	Manufacturer	Catalog number	Amount
Cyanine-3-dUTP	Perkin Elmer	NEL578	>95%, 25 nmol
Cyanine-5-dUTP	Perkin Elmer	NEL579	>95%, 25 nmol
CGH Labeling Kit	Invitrogen	18095-011	30 reactions
NEBuffer 2	NE Biolabs	B7002S	6 mL
BSA (10 mg/mL)	NE Biolabs	B9001S	25 mg
T4 DNA polymerase (3U/µL)	NE Biolabs	M0203S	150 units
3M NaOAc	Sigma	S-7899	100 mL
T4 DNA ligase buffer (5X)	Invitrogen	46300-018	2 x 1 mL
T4 DNA ligase	NE Biolabs	M0202L	100,000 units
10X ThermoPol reaction buffer	NE Biolabs	B9004S	6 x 1 mL
AmpliTaq polymerase (5 U/µL)	Applied Biosystems	N8080161	250 units
Chloroform/isoamyl alcohol	Sigma	C-0549	1 pint

Table 28 Microarray Hybridization and Washing

Component	Manufacturer	Catalog Number	Amount
Oligo aCGH/ChIP-on-chip Hybridization Kit	Agilent	5188-5220	25 reactions
Oligo aCGH/ChIP-on-chip Wash Buffer 1	Agilent	5188-5221	4 L
Oligo aCGH/ChIP-on-chip Wash Buffer 2	Agilent	5188-5222	4 L
Acetonitrile	JT Baker	9017-02	1 L

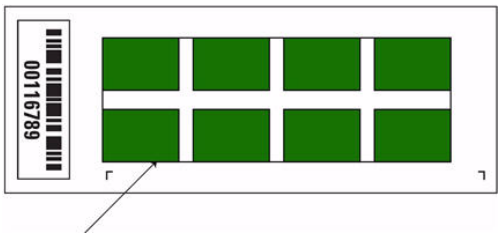
Table 28 Microarray Hybridization and Washing

Component	Manufacturer	Catalog Number	Amount
Stabilization and Drying Solution	Agilent	5185-5979	500 mL
Human Cot-1 DNA	Invitrogen	15279-5979	500 µg

General Microarray Layout and Orientation

Agilent oligo microarray (8 microarray/slide format) as imaged on the Agilent microarray scanner (G2565BA)

Microarrays are printed on the side of the glass labeled with the "Agilent" bar code (also called the "active side" or "front side").



Agilent Microarray Scanner scans through the glass. (Back side scanning.)

Agilent microarray slide holder

Figure 3 Agilent microarray slide and slide holder

Agilent oligo microarray formats and the resulting "microarray design files" are based on how the Agilent microarray scanner images 1-inch × 3-inch glass slides. Agilent designed its microarray scanner to scan through the glass slide (back side scanning). The glass slide is securely placed in an Agilent microarray slide holder with the "Agilent"-labeled barcode facing down. In this orientation, the "active side" containing the microarray is protected from potential damage by fingerprints and other elements. Once securely placed, the numeric barcode, non-active side of the slide, is visible.

Figure 3 depicts how the Agilent microarray scanner reads the microarrays and how this relates to the "microarray design files" that Agilent generates during the manufacturing process of its *in situ*-synthesized oligonucleotide microarrays.

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Agilent Technologies