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CGH Protocols
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Genomic DNA Isolation (Day 1-approximately 1.5 hours in addition to the 12-16 hours required to grow yeast overnight)

Steps 1-3 are adapted from Zymo YeaStar Genomic DNA kit (catalogue # D2202). Kit need not be purchased, because necessary reagents can be obtained separately.

1. Each standard prep isolation yields approximately 5-15 μg of genomic DNA. Therefore, grow one swab of yeast in 3 mL of liquid media overnight at 28°C for each isolation necessary. Once the yeast have grown up, tap/swirl the tube(s) of yeast in liquid media to mix. If performing multiple isolations of DNA from the same strain of yeast, combine all vials of yeast in liquid media to attain a standard concentration of cells.
2. Spin 1.5 mL of fresh yeast cells down briefly in a 1.5 mL microcentrifuge tube at 5,000 rpm for 2 minutes. Remove supernatant completely. Repeat this procedure two additional times so that there is a total of 4.5 mL spun-down liquid yeast culture in one 1.5 mL microcentrifuge tube and no supernatant remains.
3. Add 120 μL of YD Digestion Buffer (Zymo catalogue# D2002-1) and 5 μL of R-Zymolyase (Zymo catalogue# E1003) to the yeast cells. Resuspend the pellet by vortexing and incubate at 37°C for 12-16 hours.

Steps 4-11 are adapted from the Zymo ZR Genomic DNA Kit Whole Blood protocol (catalogue #D3004)

4. Ensure that the Zymobeads are fully resuspended by vortexing for a few seconds. Add 750 μL of Genomic Lysis Buffer and 30 μL of Zymobeads to ~125 μL of digested yeast cells from step 3. Mix by inversion several times. Let stand at room temperature for 2 minutes. Centrifuge at 5,000 rpm for 1 minute. Discard supernatant.
5. Add 750 μL of Genomic Lysis Buffer to the pellet by vortexing or break up the pellet using a pipette tip. It is very important to completely resuspend and break up the pellet. Otherwise, impurity may be trapped in the aggregate and contaminate the recovered DNA. Centrifuge at 5,000 rpm for 1 minute. Discard supernatant.
6. Add 1.5 mL of g-DNA Wash Buffer to the Zymobeads/DNA complex. Resuspend pellet as in step 5. Centrifuge at 5,000 rpm for 1 minute. Discard supernatant.
7. Add 1.5 mL of g-DNA Wash Buffer to the Zymobeads/DNA complex. Resuspend the pellet as in step 5. Discard the supernatant. Recentrifuge briefly and remove any residual wash buffer.
8. Add 105 μL of dH₂O, vortex or break up pellet by pipetting, and incubate at 65°C for 5 minutes. (I use a water bath)
9. Vortex briefly. Centrifuge at 14,000 rpm for 1 minute.
10. Collect the supernatant and quantify the solution using a Nanodrop or other device. The supernatant now contains purified genomic DNA and now can be used immediately or stored at -20°C for future use.

Concentration of Genomic DNA (Day 1-optional; approximately 1 hour)

Protocol adapted from How to “Clean” DNA in the Davidson College Molecular Biology lab manual Spring 2000:

11. Concentrate DNA, if necessary, using steps 12-16
12. Add 1/10 volume of 3M sodium acetate to DNA solution and mix (e.g.: 40 μ L of sodium acetate for 400 μ L DNA).
13. Add 2 volumes of 4°C 100% EtOH and vortex for 10 seconds (e.g. add 800 μ L of EtOH for 400 μ L of DNA). Put the tube in the -70°C freezer for 30 minutes or in a -20°C freezer overnight.
14. Spin in a microfuge tube for 10 minutes at 14,000 rpm. Pour out the EtOH but save the pellet!
15. Wash the pellet with 500 μ L of +4°C 70% EtOH, gently roll the tube, then dump the EtOH but keep the pellet, and speedvac the pellet. Speedvac until the moisture level does not recede, but do not over-dry the pellet. SAVE the pellet!
16. Resuspend the DNA in appropriate volume of water.
17. Quantify the concentration and quality of isolated DNA using Nanodrop or other device.
18. Store Eluded DNA at -20°C or +4°C. This is a possible stopping point.

N.B. This procedure can lead to supersaturated DNA solution if too small a volume of water is added at step 16. Make sure DNA solution is clear and OD₂₆₀ readings are <1.5 before proceeding with concentrated DNA.

Fragmentation (Day 1 or 2-as time allows; approximately 1.25 hours)

Protocol adapted from Genisphere 3DNA Array 900DNA kit (catalog #: RDNA184):

Preparation for Restriction Enzyme Digest

19. Thaw genomic DNA if necessary.
20. Prepare Vial 10 as directed: Thaw at room temperature, vortex, and briefly microfuge for 1 minute at 14,000 rpm.
21. Prepare Vial 2 as directed: Thaw at room temperature, vortex, and briefly microfuge for 1 minute at 14,000 rpm.
22. Locate Alu I restriction enzyme. (Promega, catalog #: R6281)

Alu I Restriction Enzyme Digest:

23. Prepare the following digestion reaction (must be in duplicate if using both red and green dyes):
 - a. Place 1-17 μ L yeast genomic DNA (aim for 3-5 μ g DNA per digestion) into a 600 μ L microfuge tube.
 - b. Add nuclease free water (Vial 10) for a total volume of 17 μ L.
24. Heat tube to 55°C for 10 minutes using thermocycler and immediately transfer to ice for 2 minutes.
25. Add the following components to each tube for a total volume of 20 μ L.
 - a. 2 μ L 10X Tailing Buffer (Vial 2)
 - b. 1 μ L Alu I Restriction Enzyme, 10 U/ μ L (not in kit; purchased separately)
26. Incubate at 37°C for 1 hour.
27. Proceed to TdT Tailing Reaction below. This is a possible stopping point.

Tagging (Day 2- approximately 2.5 hours)

Protocol adapted from Genisphere 3DNA Array 900DNA kit (catalog #: RDNA184)

Preparation

28. Prepare Vials 3, 4, and 12 from freezer: Thaw on ice, briefly microfuge for 1 minute at 14,000 rpm, and KEEP ON ICE AT ALL TIMES. DO NOT VORTEX.
29. Prepare Vials 11 red top and 11 blue top: Thaw on ice, vortex, and briefly microfuge. No need to store on ice.
30. Locate or prepare 0.5 M EDTA and TE buffer (not in kit; To make 1X TE Buffer: 10mM Tris-HCl, pH 8.0/1mM EDTA).
31. Test pH of dH₂O from a local source. Water must be >5.0 for Zymo DNA Clean & Concentration protocol. Bring water to 70°C (I used a thermocycler).

Terminal Deoxynucleotidyl (TdT) Tailing Reaction:

32. Boil the tubes of 20 μ L of Digested DNA for 10 minutes in boiling water and immediately transfer to ice 2 minutes.
33. Add the following components to each tube for a total volume of 26 μ L:
 - a. 4 μ L 10 mM dTTP (Vial 3)
 - b. 2 μ L Terminal Deoxynucleotidyl Transferase, 15 U/ μ L (Vial 4)
34. Incubate at 37°C for 30 minutes.
35. Immediately proceed to Ligation to Capture Sequence (below). This is not a good stopping place.

Ligation to Capture Sequence (Preparation of “Tagged” DNA)

36. Boil the 26 μ L of Tailed DNA for 10 minutes in boiling water and immediately transfer to ice for 2 minutes.
37. Add the following components for a total volume of 33.5 μ L:
 - a. 5 μ L 6X Ligation Mix (Either Vial 11: Cy3/Alexa546 or Vial 11: Cy5/Alexa647, as appropriate)
 - b. 2.5 μ L T4 DNA Ligase, 1 U/ μ L (Vial 12)
38. Mix gently, and incubate at room temperature for 30 minutes.
39. To stop ligation reaction, add 3.5 μ L of 0.5M EDTA (must be made separately; not in kit) and vortex thoroughly for 5 seconds.
40. Add 63 μ L of TE buffer for a total volume of 100 μ L. TE Buffer is not provided in the kit, must be made as per directions above.
41. Proceed to Purification of Tagged DNA (below). This is a possible stopping place.

Purification of Tagged DNA

Protocol adapted from Zymo DNA Clean and Concentration kit (catalog #: D4013):

42. Combine into a single tube the Cy3/Alexa546- and Cy5/Alexa647-tagged DNA for a total volume of 200 μ L. You can do this by pipetting the Cy3/Alexa546 into the Cy5/Alexa647 tube, and then transferring the mixture to a clean 1.5 mL tube.
43. Add 400 μ L of DNA Binding Buffer to the 200 μ L of combined DNA in the 1.5 mL tube. Mix briefly by vortexing.

44. Label the Zymo-Spin Column appropriately, and transfer mixture to the appropriately labeled Zymo-Spin Column plus a collection tube. Make sure to use a micro-centrifuge that will not rub on the tops of these columns.
45. Micro-centrifuge the sample on the column + collection tube at 14,000 rpm for 30 seconds. Discard the flow-through and place the column in the same collection tube.
46. Add 200 μ L Wash Buffer to the column. Centrifuge at 14,000 rpm for 30 seconds. Repeat this wash step #46 a second time.
47. Add 10 μ L of 60°C water directly to the column matrix. Be careful to place the water in the middle of the filter and avoid the sides as best you can. Discard the collection tube and transfer the column to a clean and appropriately labeled 1.5 mL micro-centrifuge tube. Wait for 1 minute and then centrifuge at 14,000 rpm for 15-30 seconds to elute the DNA. It is important to let the filter incubate in the water but the column can be stored vertically or in the micro-centrifuge rotor.
48. Store clean DNA at 4°C. This is a possible stopping place.

Tagged DNA Hybridization (Day 3, approximately 1 hour; must return 16 hours after completion)

Protocol adapted from Genisphere 3DNA Array 900DNA kit (catalogue#: RDNA184) unless otherwise noted.

Preparation of:

Solutions

49. Thaw and resuspend the 2X Hybridization Buffers (Vial 7) by heating to 70°C in water bath for at least 10 minutes or until completely resuspended. Vortex to ensure that the components are resuspended evenly. You want to look carefully for any precipitates in the solution. If necessary, repeat heating and vortexing until all the material has been resuspended. Micro-centrifuge at 14,000 rpm for 1 minute.
50. Also, prepare nuclease free water and LNA dT Blocker (Vials 10 and 9) by thawing at room temperature, vortexing, and briefly microfuging at 14,000 rpm for 1 minute.
51. Locate and thaw 266 ng/μL calf thymus DNA (not in kit, alternative sources of blocking DNA are acceptable).

DNA Microarrays

52. Start flame. Set beaker of distilled water to boil. Wave microarray slide, DNA side down, over the boiling water, making sure no large droplets are deposited on the slide. Immediately, place the glass slide on the heated heating block DNA side up to snap dry. The steaming and snap drying should be repeated once, leaving the slide on the heating block about a minute the second time.

Cover slips (from ISB_hybe_august_2005)

53. Blow dust off microarray slide if necessary.
54. Prepare 22x22 mm glass cover slip by dipping in 100% isopropanol and blow off liquid with compressed CO₂. You want to blow liquid off analogous to a leaf blower rather than drying like a hair drier.

Hybridization Mixture

(proceed only when ready to begin the hybridization immediately after step 56)

55. For each microarray slide, prepare the following Tagged DNA Hybridization Mix in 600 μL microfuge tubes for a total solution volume of 40 μL (glass cover slip size 22 x 22 mm):
 - a. 10 μL Tagged DNA
 - b. 2 μL LNA dT Blocker (Vial 9)
 - c. 2 μL Calf Thymus DNA (266 ng/μL)
(not in kit, alternative sources of blocking DNA are acceptable)
 - d. 6 μL Nuclease Free Water (Vial 10)
 - e. 20 μL 2X Hybridization Buffer (Vial 7)
40 μL Total Volume
56. Gently vortex and briefly micro-centrifuge the Tagged DNA hybridization Mixes at 14,000 rpm for 1 minute. Incubate the Tagged DNA Hybridization Mix at 95-100°C for 10 minutes. Immediately proceed to hybridization described below.

Hybridization

57. While the DNA is still hot, place the Tagged DNA Hybridization Mix (from steps 55-56 above) to the prepared microarrays (from steps 53-54 above), taking care to leave behind any precipitate at the bottom of the tube. Use the following method (from ISB-hybe_august_2005):
 - a. Pipette 20 μ L of sample onto one end of the array (for 22 x 22 mm cover slip). Place one end of the cover slip onto the end with the sample. Use a fine gauge needle (e.g. 18 gauge) to lower the other end (between 70% and 95% of the spots should be wetted) of the cover slip but raise it back up before the solution makes its way over the entire area. This serves to mix the sample while it is applied to the array.
 - b. Repeat step a for duplicate meta-array on the glass microarray slide.
 - c. Be sure to hydrate and seal hybridization chamber.
58. Incubate overnight for 16 hours in a dark humidified chamber at the appropriate hybridization temperature (52°C for Vial 7 buffer)
59. Prewarm the 2X SSC, 0.2% SDS wash to 42°C in a glass coplin jar for oligonucleotide spotted arrays. Prevent evaporation.

Post Hybridization Wash (Day 4, approximately 1 hour)

Protocol adapted from Genisphere 3DNA Array 900DNA kit (catalogue #: RDNA184)

60. Remove the cover slips from microarray slide by submerging the glass slide in prewarmed 42°C 2X SSC, 0.2% SDS on a bench top for 3 minutes or until the cover slip falls off.
61. Wash for an additional 12 minutes in same prewarmed 42°C 2X SSC, 0.2% SDS on the bench top.
62. Wash for 15 minutes in fresh 2X SSC at room temperature.
63. Wash for 15 minutes in fresh 0.2X SSC at room temperature.
64. Blow off any moisture (analogous to a leaf blower rather than a hair drier) on the microarray slide(s) using the compressed CO₂ tank.

3DNA (dendrimer) Hybridization (Day 4, approximately 1 hour, can be completed simultaneously while arrays are washing in steps 60-63 above; must return 4 hours after completion of step 75)

Protocol adapted from Genisphere 3DNA Array 900DNA kit (catalogue #: RDNA184)

Preparation

65. Prepare the 3DNA (dendrimer) Array 900DNA Capture Reagents (Vial 1 red top and Vial 1 blue top).
 - a. Thaw the 3DNA Array 900DNA Capture Reagents in the dark at room temperature for 20 minutes.
 - b. Vortex at hard for 3 seconds and micro-centrifuge briefly for 1 minute at 14,000 rpm.
 - c. Incubate at 52.5°C for 10 minutes. Protect from light as much as possible.
 - d. Vortex hard for 3 seconds.
 - e. Micro-centrifuge the tube briefly at 14,000 rpm for 1 minute to collect the contents at the bottom. No need to store vials on ice.
66. Prepare nuclease free water (Vial 10) as per instructions: Thaw at room temperature, vortex and briefly microfuge for 1 minute at 14,000 rpm. No need to store on ice.
67. Prepare Anti-Fade Reagent (Vial 8) as per instructions: Thaw on ice, vortex, and briefly microfuge for 1 minute at 14,000 rpm. Store on ice.
68. Thaw and resuspend the 2X Hybridization Buffer (Vial 7) by heating to 70°C in a water bath for at least 10 minutes or until completely resuspended. Vortex to ensure that the components are resuspended evenly. Microfuge at 14,000 rpm for 1 minute. Store on ice.
69. Label a clean 600 µL microfuge tube. Prepare a master mix solution by combining 1 µL of Anti-fade (Vial 8) to 100 µL of 2X Hybridization Buffer (Vial 7). Return vials 8 and 7 to freezer. [Store any unused hybridization buffer at -20°C for possible use within two weeks.] Refreeze the Anti-Fade Reagent after use. Return vials 8 and 7 to -20°C freezer.
70. For each microarray slide, prepare the following 3DNA Hybridization Mix for a total solution volume of 40 µL (glass cover slip size 22 x 22 mm):
 - a. 2.5 µL 3DNA Capture Reagent (Vial 1, blue top)

- b. 2.5 μ L 3DNA Capture Reagent (Vial 1, red top)
 - c. 15 μ L Nuclease Free Water (Vial 10)
 - d. 20 μ L 2X Hybridization Buffer with Anti-Fade added (Step 69 above)
71. Gently vortex and briefly microfuge the 3DNA Hybridization Mix. Incubate the 3DNA Hybridization Mix first at 80°C for 10 minutes. Prewarm the microarrays on a hotplate to approximate hybridization temperature.

Hybridization

72. Gently vortex and briefly microfuge the 3DNA Hybridization Mix. Pipet 20 μ L of 3DNA Hybridization Mix to the appropriate portion of the pre-warmed microarray, taking care to leave behind any precipitate at the bottom of the tube.
73. Apply glass cover slips to the array as described before.
74. Repeat steps 72 and 73 for the other array on the slide.
75. Incubate the slides for 4 hours in dark, humidified water baths at 52.5°C for Vial 7 Buffer.
76. Prewarm a fresh glass coplin jar of 2X SSC, 0.2% SDS wash to 65°C.

Post 3DNA Hybridization Wash (Day 4, approximately 1 hour + scanning time)

Protocol adapted from Genisphere 3DNA Array 900DNA kit (catalogue #: RDNA184)

Perform these washes in the dark to avoid photobleaching/fading of fluorescent dyes.

77. Remove the cover slips by washing the microarray slide(s) in the 65°C prewarmed 2X SSC, 0.2% SDS for 3 minutes on a bench top until the cover slip falls off.
78. Wash for an additional 12 minutes in the same 65°C 2X SSC, 0.2% SDS on a bench top.
79. Wash for 12 minutes in a fresh coplin jar of 2X SSC at room temperature.
80. Wash for 12 minutes in a fresh coplin jar of 0.2X SSC at room temperature.
81. Blow off the microarray slide(s) with the compressed CO₂ (analogous to a leaf blower rather than a hair drier). Wipe off non-DNA side of the slide with a lint-free cloth.
82. Store the slides in the dark until scanned. Protect from ozone if possible. Summers are more prone to ozone spikes. (see <<http://www.wunderground.com/health/ozone.asp>>)
83. Scan as soon as possible. It takes approximately 20 minutes to scan each array.