



Hybridisation

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Hybridisation of processed biological materials to a microarray:

- Hybridisation of labelled material to cDNA microarrays using a Genomic Solutions hybridisation station with Ambion Hybridisation Buffer #1 ([jump](#))
- Hybridisation to amino-modified long oligonucleotide microarrays using a Genomic Solutions hybridisation station with the Biosolutions hybridisation buffer ([jump](#))
- Hybridisation to unmodified gDNA microarrays using a Genomic Solutions hybridisation station ([jump](#))

Hybridisation of labelled material to cDNA microarrays using a Genomic Solutions Hybridisation Station with Ambion Hybridisation Buffer #1

Overview

Samples or samples and controls have been labelled with Cy3-dCTP or Cy5-dCTP and then mixed together with a blocking agent. We now need to add the hybridisation buffer and hybridise the labelled biological material to the cDNA microarrays. This protocol was based on a method from Genomic Solutions (<http://www.genomicsolutions.com>).

Equipment and reagents

- SlideHyb Glass Array Hybridisation Buffer #1 (Ambion; Cat. No. AM8861)
- 1 x SSC + 0.03% SDS
- 0.23 x SSC
- 0.06 x SSC
- 20 x SSC, pH 7.0
- Grant QBT2 hot-block
- Kenair air duster
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)

Procedure

How to set up the hybridisation cassettes:

1. Ensure slide covers are clean. If necessary, dab gently with a tissue soaked in 70% EtOH. Remove any dust by spraying with canned air and make sure the channels are free of liquid. Check that the red O-rings are in position.
2. Ensure that the metal plate is lying flat. If not, loosen screws with thumbnail and re-tighten with the plate in its proper position.
3. Fit black O-rings to slide cover. Do not stretch and avoid dust.
4. Spray slides with canned air and place slides on the black slide holder. Ensure slides are in the correct orientation.
5. Whilst holding the slide holder pointing slightly downwards and to the right, ensure that the slides are correctly positioned
6. Place the slide cover on top of the holder, without dislodging the slides.
7. Hold this 'slide cassette' together and place on the hybridisation station
8. Lower the clamp mechanism into position and screw until finger tight.

Starting the hybridisation:

9. Switch the hybridisation station on and when it has finished booting up insert the floppy disc with the hybridisation protocol.
10. Ensure that the wash solutions are full and the waste is empty and then connect each to the corresponding wash bottle
11. Press Start a run on the LCD field and select 'Floppy disk'. Scroll down to and then load the cDNA hybridisation protocol.
12. Select the slide positions for the run and press continue
13. The protocol will start automatically and prompt you to add the samples after 15 minutes
14. In the mean time prepare the samples that need to be loaded:
 - ◆ Preheat the SlideHyb Glass Array Hybridisation Buffer #1 to 65 °C for 15 to 30 minutes
 - ◆ Heat the labelled sample at 100 °C for 2 minutes on a hot-block
 - ◆ Add 140 µl SlideHyb Buffer to the sample
 - ◆ Centrifuge for 1 minute at 13,000 rpm
 - ◆ Add 135 µl of the labelled sample to the microarray, avoiding any precipitate

Hybridisation

- The instrument will tell you which sample it wants. To add it select the slide position and press probe - a tick should appear and the valve opens.
- Add sample by pipetting slowly so as to avoid any bubbles. Put a plastic plug in the hole and press finish for this slide. The valve will then close.
- Repeat procedure with second slide of the block, when finished press finished at the bottom of screen. The hybridisation station will now perform the hybridisation and washes. This will take 16 hours plus 20 minutes per block for the washes.

Hybridisation protocol

Step	Solution	Temperature	Duration	Agitation	Flow	Hold	Cycles
Hybridisation	Sample in SlideHyb	65 °C	16 hours	Yes	-	-	1

After the hybridisation:

- Unscrew the clamp mechanism and remove the white plugs. Hold the cassette tightly together and then remove it from the hybridisation station.
- Invert the sandwich and remove the slides by holding them by one edge and lifting away from the slide cover.
- Now perform a manual wash as described below.

Manual wash:

- Pre-heat wash solution 1 (0.2 x SSC; 0.2% SDS) and wash solution 2 (0.2 x SSC) to 55 °C using a water bath
- Gently remove the slides submerged in pre-heated wash solution 1
- Transfer the slides in a slide staining rack without letting the slides dry
- Place the rack into a slide staining trough containing pre-heated wash solution 1
- Mix on an orbital shaker at 50 rpm for 20 minutes at room temperature
- Transfer the slides to a staining trough containing pre-heated wash solution 2
- Gently dip the slides up and down for 3 seconds in wash solution 2
- Transfer the slides to a second staining trough containing fresh pre-heated wash solution 2
- Gently dip the slides up and down for 3 seconds in wash solution 2
- Rinse the slides 3 times with fresh deionized water at room temperature (dip the rack in MilliQ water for 3 seconds)
- Transfer the slides to a clean microscope slide box with tissue at the base and centrifuge at 1000 rpm for 5 minutes
- The slides are now ready to be scanned.

Cleaning the hybridisation station:

- Replace the hybridised slides with some blank slides and put the cassettes back on the hybridisation station
- Place all of the in-flow tubes in 55 °C MilliQ water (helps to remove salt deposits)
- Go to the main menu and select 'maintenance', followed by 'machine cleaning cycle'
- Select the appropriate slide positions and start the cleaning cycle
- Once the cleaning cycle has finished, remove the O-rings and place them in 200 ml boiling MilliQ water
- Place the cassettes (not the metallic back plates) in a plastic beaker with warm MilliQ water, soak for a couple of minutes
- Spray the cassettes with 70% ethanol and then leave them to dry at room temperature

B. Fischer (09-07-2008).

Hybridisation of labelled material to amino-modified long oligonucleotide microarrays using a Genomic Solutions Hybridisation Station with the Ocimum hybridisation buffer

Overview

Samples or samples and controls have been labelled with Cy3-dCTP or Cy5-dCTP and then mixed together with a blocking agent. We now need to add the hybridisation buffer and hybridise the labelled biological material to the amino-modified long oligonucleotide microarrays. This protocol was based on a method developed by FlyChip. The washes are based on a method recommended by Full Moon Biosystems (<http://www.fullmoonbio.com/>).

Equipment and reagents

- 10% SDS (Sigma; Cat. No. L4522)
- Ocimum hybridisation buffer (Biosolutions; Cat. No. 1180-200000)
- 20 x SSC, pH 7.0
- Grant QBT2 hot-block
- Kenair air duster
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)

Procedure

How to set up the hybridisation cassettes:

1. Ensure slide covers are clean. If necessary, dab gently with a tissue soaked in 70% EtOH. Remove any dust by spraying with canned air and make sure the channels are free of liquid. Check that the red O-rings are in position.
2. Ensure that the metal plate is lying flat. If not, loosen screws with thumbnail and re-tighten with the plate in its proper position.
3. Fit black O-rings to slide cover. Do not stretch and avoid dust.
4. Spray slides with canned air and place slides on the black slide holder. Ensure slides are in the correct orientation.
5. Whilst holding the slide holder pointing slightly downwards and to the right, ensure that the slides are correctly positioned
6. Place the slide cover on top of the holder, without dislodging the slides.
7. Hold this 'slide cassette' together and place on the hybridisation station
8. Lower the clamp mechanism into position and screw until finger tight.

Starting the hybridisation:

9. Switch the hybridisation station on and when it has finished booting up insert the floppy disc with the hybridisation protocol.
10. Press Start a run on the LCD field and select 'Floppy disk'. Scroll down to and then load the oligo hybridisation protocol.
11. Select the slide positions for the run and press continue
12. The protocol will start automatically and prompt you to add the samples after 15 minutes
13. In the mean time prepare the samples that need to be loaded:
 - ◆ Add 140 µl of Ocimum hybridisation buffer to the labelled mixture
 - ◆ Heat at 100 °C for 2 minutes on a hot-block
 - ◆ Centrifuge for 3 minutes at 13,000 rpm
 - ◆ Add 135 µl of the labelled sample to the microarray, avoiding any precipitate
14. The instrument will tell you which sample it wants. To add it select the slide position and press probe - a tick should appear and the valve opens.
15. Add sample by pipetting slowly so as to avoid any bubbles. Put a plastic plug in the hole and press finish for this slide. The valve will then close.

Hybridisation

- Repeat procedure with second slide of the block, when finished press finished at the bottom of screen. The hybridisation station will now perform the hybridisation, which will take 16 hours.

Hybridisation protocol

Microarray	Step	Solution	Temperature	Duration	Agitation	Flow	Hold	Cycles
<u>FL001</u>	Hybridisation	Sample in Hybridisation buffer	50 °C	16 hours	Yes	-	-	1
<u>FL002</u>	Hybridisation	Sample in Hybridisation buffer	51 °C	16 hours	Yes	-	-	1

After the hybridisation:

- Unscrew the clamp mechanism and remove the white plugs. Hold the cassette tightly together and then remove it from the hybridisation station.
- Invert the sandwich and remove the slides by holding them by one edge and lifting away from the slide cover
- Now perform a manual wash as described below.

Manual wash:

- Pre-heat wash solution 1 (0.2 x SSC; 0.2% SDS) and wash solution 2 (0.2 x SSC) to 55 °C using a water bath
- Place the slides in a slide staining rack without letting the slides dry
- Place the rack into a slide staining trough containing pre-heated wash solution 1
- Mix on an orbital shaker at 50 rpm for 20 minutes at room temperature
- Transfer the slides to a staining trough containing pre-heated wash solution 2
- Gently dip the slides up and down for 1 minute in wash solution 2
- Repeat steps 24 to 25 twice with fresh wash solution 2
- Rinse the slides 3 times with fresh deionized water at room temperature (dip the rack in MilliQ water for 3 seconds)
- Transfer the slides to a clean microscope slide box with tissue at the base and centrifuge at 1000 rpm for 5 minutes
- The slides are now ready to be scanned.

Cleaning the hybridisation station:

- Rinse the cassettes with running reverse osmosis water, to remove the salt deposits
- Replace the hybridised slides with some blank slides and put the cassettes back on the hybridisation station
- Place all of the in-flow tubes in 55 °C MilliQ water (helps to remove salt deposits)
- Got to the main menu and select 'maintenance', followed by 'machine cleaning cycle'
- Select the appropriate slide positions and start the cleaning cycle
- Once the cleaning cycle has finished, remove the O-rings and place them in 200 ml boiling MilliQ water
- Place the cassettes (not the metallic back plates) in a plastic beaker with MilliQ water, soak for a couple of minutes
- Spray the cassettes with 70% ethanol and then leave them to dry at room temperature

R. Auburn (26-07-2006).

Hybridisation of labelled material to genomic DNA (gDNA) microarrays using a Genomic Solutions Hybridisation Station

Overview

Samples or samples and controls have been labelled with Cy3-dCTP or Cy5-dCTP and then mixed together with a blocking agent. We now need to add the hybridisation buffer and hybridise the labelled biological material to the gDNA microarrays. This protocol was based on a method from Genomic Solutions (<http://www.genomicsolutions.com>).

Equipment and reagents

- ArrayHyb hybridisation buffer (Sigma; Cat. No. A7718)
- 1 x SSC + 0.03% SDS
- 0.23 x SSC
- 0.06 x SSC
- 20 x SSC, pH 7.0
- Grant QBT2 hot-block
- Kenair air duster
- Hettich Rotina 35 microtitre plate centrifuge
- Microscope slide box (Merck EuroLab; Cat. No. 406/0286/00)
- Slide staining rack (Philip Harris; Cat. No. B52651)
- Slide staining trough (Philip Harris; Cat. No. B52649)

Procedure

How to set up the hybridisation cassettes:

1. Ensure slide covers are clean. If necessary, dab gently with a tissue soaked in 70% EtOH. Remove any dust by spraying with canned air and make sure the channels are free of liquid. Check that the red O-rings are in position.
2. Ensure that the metal plate is lying flat. If not, loosen screws with thumbnail and re-tighten with the plate in its proper position.
3. Fit black O-rings to slide cover. Do not stretch and avoid dust.
4. Spray slides with canned air and place slides on the black slide holder. Ensure slides are in the correct orientation.
5. Whilst holding the slide holder pointing slightly downwards and to the right, ensure that the slides are correctly positioned
6. Place the slide cover on top of the holder, without dislodging the slides.
7. Hold this 'slide cassette' together and place on the hybridisation station
8. Lower the clamp mechanism into position and screw until finger tight.

Starting the hybridisation:

9. Switch the hybridisation station on and when it has finished booting up insert the floppy disc with the hybridisation protocol.
10. Ensure that the wash solutions are full and the waste is empty and then connect each to the corresponding wash bottle
11. Press Start a run on the LCD field and select 'Floppy disk'. Scroll down to and then load the gDNA hybridisation protocol.
12. Select the slide positions for the run and press continue
13. The protocol will start automatically and prompt you to add the samples after 15 minutes
14. In the mean time prepare the samples that need to be loaded:
 - ◆ Add 140 µl ArrayHyb hybridisation solution to the labelled mixture
 - ◆ Heat at 100 °C for 2 minutes on a hot-block
 - ◆ Centrifuge for 1 minute at 13,000 rpm
 - ◆ Add 135 µl of the labelled sample to the microarray, avoiding any precipitate
15. The instrument will tell you which sample it wants. To add it select the slide position and press probe - a tick should appear and the valve opens.

Hybridisation

16. Add sample by pipetting slowly so as to avoid any bubbles. Put a plastic plug in the hole and press finish for this slide. The valve will then close.
17. Repeat procedure with second slide of the block, when finished press finished at the bottom of screen. The hybridisation station will now perform the hybridisation and washes. This will take 16 hours plus 20 minutes per block for the washes.

Hybridisation / wash protocol

Step	Solution	Temperature	Duration	Agitation	Flow	Hold	Cycles
Hybridisation	Sample in ArrayHyb	65 °C	16 hours	Yes	-	-	1
Wash 1	1 x SSC + 0.03% SDS	55 °C	-	-	20	40	5
Wash 2	0.23 x SSC	40 °C	-	-	20	40	5
Wash 3	0.06 x SSC	25 °C	-	-	20	40	5

After the hybridisation:

18. After the run has finished make sure that the washes have been performed correctly. If not, a manual rescue wash will need to be performed. This is outlined at the end of this protocol.
19. Unscrew the clamp mechanism and remove the white plugs. Hold the cassette tightly together and then remove it from the hybridisation station.
20. Invert the sandwich and remove the slides by holding them by one edge and lifting away from the slide cover.
21. Place slides in a microscope slide boxes with fresh lint free tissue at the base and centrifuge at 650 rpm for 10 minutes in a microtitre plate centrifuge. The slides are now ready to be scanned.

Manual (rescue) wash:

Manual washes are only required when the hybridisation station fails to wash the slides correctly. If the slides have already been scanned and been found to have a high background further manual washes may not help.

22. Fill a black slide staining trough with 1 x SSC + 0.03% SDS and another with 0.06 x SSC
23. Put the slides into a slide staining rack without letting the slides to dry. Slide drying at this stage will lead to a high background.
24. Place the rack into the first staining trough and move the rack gently up and down 10 times
25. Remove the rack and blot off excess wash solution on a tissue
26. Place the rack into the second staining trough and move the rack gently up and down 10 times
27. Remove the rack and blot off excess wash solution on a tissue
28. Place slides in a microscope slide boxes with fresh lint free tissue at the base and centrifuge at 650 rpm for 10 minutes in a microtitre plate centrifuge. The slides are now ready to be scanned.

Cleaning the hybridisation station:

29. Replace the hybridised slides with some blank slides and put the cassettes back on the hybridisation station
30. Place all of the in-flow tubes in 55 °C MilliQ water (helps to remove salt deposits)
31. Got to the main menu and select 'maintenance', followed by 'machine cleaning cycle'
32. Select the appropriate slide positions and start the cleaning cycle
33. Once the cleaning cycle has finished, remove the O-rings and place them in 200 ml boiling MilliQ water
34. Place the cassettes (not the metallic back plates) in a plastic beaker with MilliQ water, soak for a couple of minutes
35. Spray the cassettes with 70% ethanol and then leave them to dry at room temperature

R. Auburn (26-07-2006).