ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: GenIII spotter operating protocol		PAGE: 1 of 4
SOP #: MA001	REVISION LEVEL: .1	DATE: 19 August 2005
AUTHOR: D. Theron	REVIEWERS:	

1. PURPOSE

This procedure describes the preparation and operation of the GenIII Microarray Spotter from Molecular Dynamics for manufacturing spotted microarray slides.

2. SCOPE

The procedure outlined here applies to spotting microarray slides using any printing buffer (50% DMSO, 150mM sodium phosphate etc.) suitable for use with the GenIII Microarray Spotter system at the ACGT Microarray Facility.

3. MATERIALS

- Nitrogen gas UHP (Affrox; Cat# 340466)
- Purified water, Millipore Simplicity 185 system 18.2 Mohm/cm (Millipore; Cat# SIMS5V000)
- 100% pure ethanol, AR grade (Radchem; Cat# E330)
- 0.2M potassium hydroxide, AR grade (Saarchem; Cat# 5044420)
- GAPS II coated microarray slides, barcoded (Corning; Cat# 40005)
- Humidifier wicks (Bemis; Cat# 1040)
- Humidifier filter (Bemis; Cat# 1050)
- Easy Peel heat sealing foil (ABgene Cat# AB-0745)
- Adhesive plate seals (ABgene Cat# AB-0580)
- UV Cross linker (UVItec Cat# CL-508)

4. PREPARATION

- Ensure the washing solutions are full and that the waste containers are emptied.
  - Wash 1 – purified water
  - Wash 2 – 100% pure ethanol
  - Wash 3 – 0.2M KOH
  - DI water – purified water

  NOTE: It is easier to do this before applying vacuum and nitrogen gas pressure to the system.
- Check that nitrogen gas cylinder is full enough for the duration of the entire spotting run.

  NOTE: An average spotting run decreases the cylinder pressure by about 100 kPa.
- Turn on vacuum pump and nitrogen gas supply.
- Make sure the vacuum and nitrogen gas connections are secure and not leaking
- Set the temperature control of the spotter room to below 22ºC

- Open the spotter's front and top covers by clicking on the button that says “Load slides/Plates”. This is to allow the spotter to acclimatize to the ambient temperature and humidity. If the ambient humidity is above 55% (as reflected on the spotter control computer) set the temperature control for the room colder so the air can dry out more, normally only required on exceptionally humid days.
- Fill the humidifier with purified water to the level indicated. Replace the humidifier wicks and filter every 6 – 12 months.

5. OPERATION

NOTE: Under normal usage conditions the spotter instrument and the spotter computer can be left on. It is only necessary to turn the spotter and computer off for servicing purposes.

To switch the spotter system on or off, follow these instructions:

5.1 TURNING THE SPOTTER ON

ALWAYS INSTRUMENT FIRST

1. Check wash solutions and waste levels if not done already
2. Turn on vacuum pump and nitrogen gas supply
3. Turn on nitrogen valve on spotter if it was off
4. Turn on the spotter with the switch on the lower-right side panel
5. Turn on the spotter computer and start the GenIII software

5.2 TURNING THE SPOTTER OFF

ALWAYS COMPUTER FIRST

1. Make sure the penset is stored in its box
2. Remove slides
3. Close the software and shutdown computer if needed
4. Switch off the vacuum pump and close the nitrogen gas cylinder.
5. Switch off the spotter instrument.

5.3 OPERATING THE SPOTTER INSTRUMENT

- Complete a spotting log sheet for the intended spotting run. See Appendix A for a blank example.
- Load clean, blank microarray slides to be spotted on and fill the rest of the row on the slide tray with blank slides from the appropriate box.

- Load the 384-well spotter sample plates in the hotel making sure that their orientation is correct. (well A1 must be on the far right hand corner when loaded in the hotel)
- Attach the penset to the spotter:
  - Take the utmost care not to touch the pens against anything.
  - Undo the two screws that hold the lid of the storage box using the tool provided
  - Carefully remove the lid and place the box containing the penset on the pedestal under the spotter arm.
  - Press down on the spotter arm and fasten the two screws until finger tight.
  - Remove the penset storage box from the pedestal, replace its lid and leave inside the spotter.
- Close the spotter instrument front and top covers.
- Activate the humidity control by clicking on the 'Humidity Control' switch and set it to 55%.
- Setup the spotter software according to the details on the log sheet regarding spotting mode, number of slides, number of plates etc.
- Make sure the spotting parameters (under the 'Options' menu) are set correctly to allow for the number of well sets that need to be spotted.
  NOTE: If the spotter had been switched off, it defaults to a spot diameter that only allows 25 well sets. In that case, change the spot diameter to 250 micron so it allows the full 32 well sets of the plate to be spotted
- Perform a manual wash three times and check the appropriate box on the spotter log sheet.
- Once the inside of the spotter instrument has reached the correct humidity (the humidity controller indicator will go from red to green when humidity is within 3% of the desired setting), start the spotting run by clicking on the 'Start' button and note the time on the spotter log sheet.
- Expect to see the following:
  - the pens will move to the wash station and go through a washing cycle
  - row for row, the slides will get lifted slightly by gas to float into position after which they get sucked down in position by the vacuum. A warning signal will appear if there is a vacuum leak on any of the slides. The operator must then open the spotting chamber and rectify the problem. Such a leak is normally due to a small grain of dust on the slide tray which must be removed.
  - The shovel will pull out sample plate number one from the hotel and position it for loading.
  - From the wash station, the pens will move toward the sample plate and dip into it to load.
  - This is followed by a 0.2 sec vacuum over the wash station to remove any drops that may be sticking to the outside of the pens.
  - From there the spotter proceeds to print the samples in duplicate on all the slides.

- Inspect the spotter throughout the spotting run to ensure everything is running smoothly.

- Upon completion of the spotting run, note the time of completion on the spotter log sheet.
- Give the spots sufficient time to dry inside the spotter before opening it up. When none of the spotted drops are visible on the slides anymore, open the spotter instrument front cover.
- Open the penset storage box and place the bottom part on the pedestal underneath the penset. Press down on the spotter arm to push the penset into the storage box and undo the two screws that holds the penset in position. Make sure the screws are completely loosened and carefully ease the pressure on the spotter arm, making sure the penset is free and stays behind in the storage box. Remove the storage box from the pedestal and replace the lid, making sure the storage box is full of purified water.
- Carefully remove the sample plates from the hotel and seal them for storage in the freezer.
  NOTE: For sealing the 384-well plates the heat sealer with Easy-peel sealing mats may be used although this is not ideal. The heat sealing process melts the tops of the wells and effectively diminishes the size of the well openings. The safer method would be to use adhesive plate seals making sure to apply pressure across the entire plate so the individual wells seal properly.
- Leave the slides inside the spotter for another few hours with the humidity control still switched on. This will allow the slides to dry slowly and help ensure good spot morphology.
- Once the slides have dried properly, open the spotter instrument and carefully put the slides into a dust-free tray. UV link the slides with 250mJ and store in slide mailers in a benchtop desiccator until used.
- Close the spotter instrument covers, switch off the humidity controller and vacuum pump and close the nitrogen gas supply.
- The spotter is now on standby until the next spotting run.

APPENDIX A

Date		**Project**		**Exp.#**	

AIM	

Spotting conditions

Temperature	OUT		IN	
Humidity (%)	OUT		IN	

Spotting mode	Normal	Single plate	Single plate single well	Repeat

Penset	#2	#4

Array design

Plates	Slides		Time begin
Well sets	Spots/dip		Time end

Slides

Cat No.	
Batch No.	

Slide numbers (indicate dummy slides with x)

384-well Plate numbers

Data files
Scans of slides saved to bioinformatics server milliways: /data/microarray/projects/

Comments & conclusions:

Manual washing x3 before starting run ☐
Manual washing x2 after spotting run ☐