Implementation of the SCOBEC High Throughput Screening Facility

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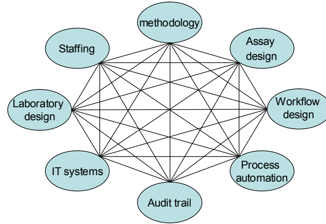
Introduction

The SCOBEC High Throughput Screening Facility has been designed and built to model and develop future methods of working for genetic diagnostics. In particular the workflow has been designed to meet the eight week turnaround for testing of large genes specified in the 2003 Genetics White Paper. However the combination of methodology and workflow move the application of automation beyond just the 'large genes' to any test that requires simple PCR based mutation scanning. The project has been characterised by a complex interaction of many areas of expertise (illustrated right). This poster describes the key elements of this implementation.

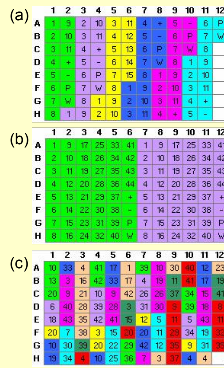


Batching

To cope with unpredictable referral rates the system has been designed to setup any size batch for any number of samples and controls - variable batching (a and b). This means a fixed cycle of sample collection and testing can be employed.

Batch	Week 1	2	3	4	5	6
1	Sample collection		1°screen / 2°screen + report			
2			Sample collection		1°screen / 2°screen + report	
3					Sample collection	
4						

'Cherry picking' for sequencing requires that the system can also set up plates with different numbers of samples for each fragment – flexible batching (c). This utility can also be used to mix different tests together on one plate expanding the utility of the system to include any PCR setup regardless of numbers of samples or fragments provided that suitable primer sets are available



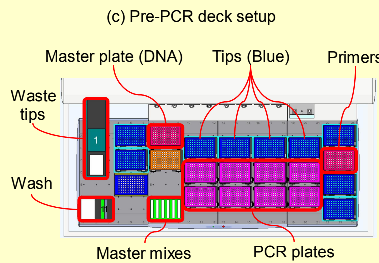
Automation

Liquid handling is carried out on two Perkin Elmer Mutiprobe II robots dedicated to pre and post PCR processes. A smalll set of generic programmes are used in conjunction with batch specific worklists generated by LIMS. The programmes simply tell the robot to pick tips, transfer liquid and then dispose tips (a). All information regarding location of aspiration and dispensing and how many times to repeat is provided by the worklists (b). This enables the system to setup any batch or plate configuration.

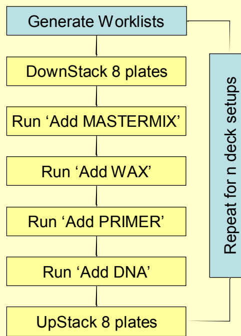
Example: Pre-PCR

One deck setup (c) is capable of setting up 8 plates of PCR using 6 simple programmes. To increase the 'walk-away' capacity of the system the robot is fed empty plates via a stacker system. Worklists are generaterd and the running order of the programmes is scheduled by the LIMS (d).

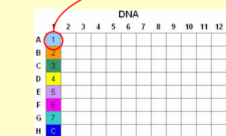
(c) Pre-PCR deck setup



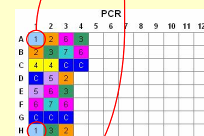
(d) PCR setup schedule



(a) Programme



(b) Worklist



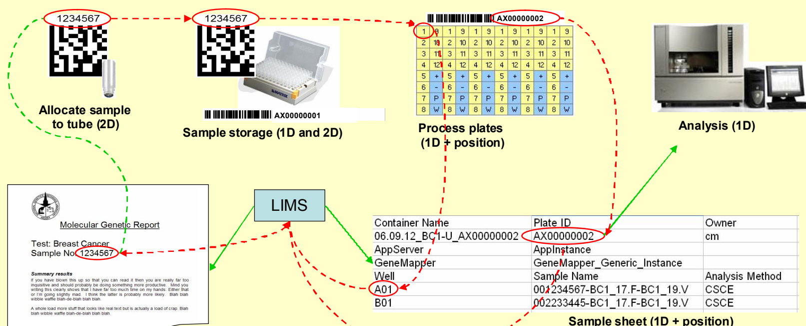
Source	Asp.	Destination	Disp1	Disp2	Disp3	Disp4	Disp5	Disp6
DNA	A1	PCR	A1	H1	-	-	-	-
DNA	B1	PCR	B1	A2	D3	H3	-	-
DNA	C1	PCR	B2	H2	E3	A4	-	-
DNA	D1	PCR	C1	C2	-	-	-	-
DNA	E1	PCR	E1	D2	-	-	-	-
DNA	F1	PCR	F1	E2	A3	F3	B4	-
DNA	G1	PCR	F2	B3	-	-	-	-
DNA	H1	PCR	D1	G1	G2	C3	G3	C4

DNA

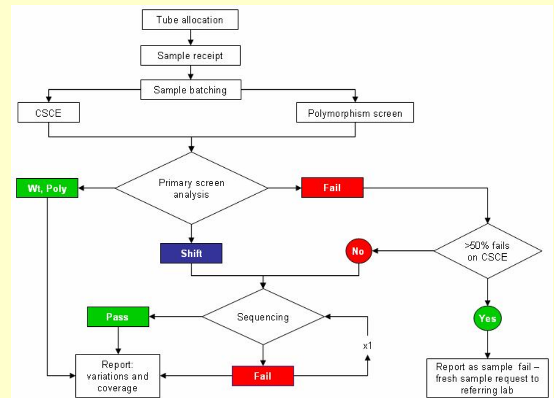
PCR

Sample tracking

A combination of 2D barcoded tubes and 1D barcoded plates are used to create a 'closed loop sample tracking system'

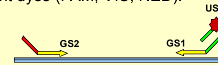


Workflow

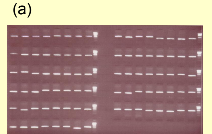


Assay design

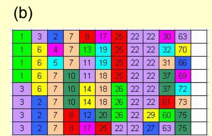
Fragments are amplified using a novel 3 primer system with two universally tagged gene specific primers (GS1 and GS2) and one fluorescently labelled universal primer (US1F). This allows any fragment to be labelled with any of three fluorescent dyes (FAM, VIC, NED).



(a)



(b)



All primer pairs have been designed to work at a single annealing temperature (61°C). The example shows an agorose gel image of 35 different fragments from the BRCA set amplified on a single plate using the flexible batching protocol.

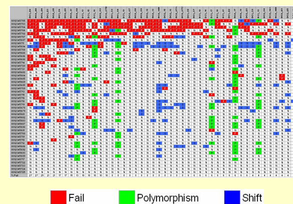
See poster 2.19 for more details

Process Analysis

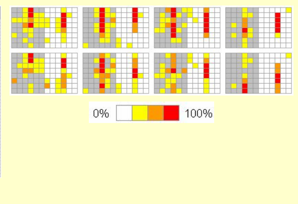
Each batch is analysed to give an overview of failures, shifts and polymorphisms (a). This is useful to determine how well particular assays are working, whether polymorphisms are occurring in the expected fragments at the expected frequencies and if there are any sample failures due to DNA quality.

To identify any potential problems with the process, an analysis of the failure frequency at each well position in the PCR deck setup is also carried out (b). The example given is a batch requiring 23 PCR plates each with two exons for 48 samples (Batch = 44 samples + 4 controls x 46 fragments). This batch required three deck setups and the failure rate at each well position is summarised as a 'thermal ideogram' (b). From this analysis it was simple to deduce that primer was not being delivered to particular locations on each plate and that there was a particular problem with the pipetting protocol.

(a) Batch analysis



(b) Summary deck-setup analysis



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