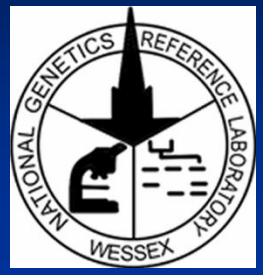


Batching for high throughput PCR setup

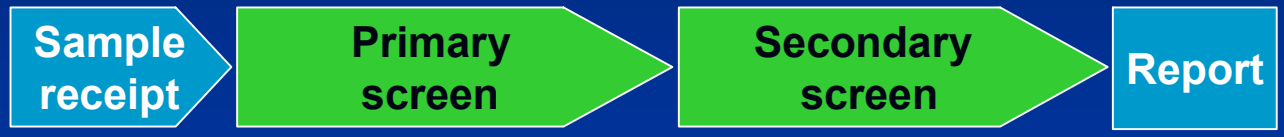
Chris Mattocks – NGRL (Wessex)

Batching Schemes

- The primary purpose of a batching scheme is to enable the timely processing of samples.
- Types of batching
  - Standard batching
  - Variable batching
  - Flexible batching
- Use of robotics and work lists

Variables

- Gene screens – screening a sample for multiple fragments e.g. BRCA, HNPCC
- Low volume tests



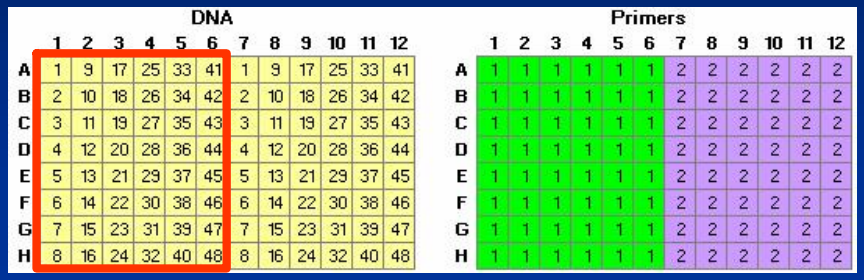
Many samples each for the same no. of fragments

Many samples for varying no. of fragments

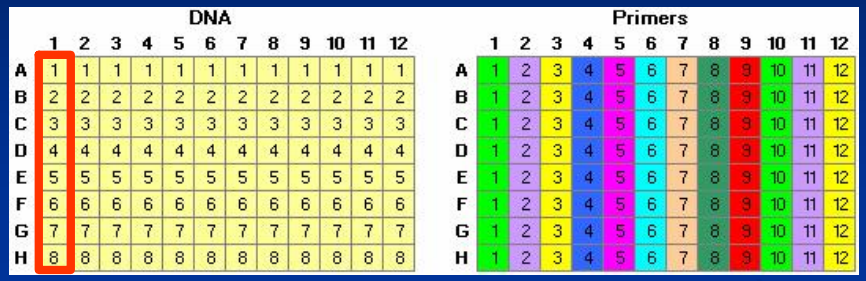
	Turn around time	Sample input rate
Routine diagnostics	fixed	variable
Backlogs	Fixed / variable	fixed

- Chosen batching scheme may affect
  - Efficiency >> cost & TAT
  - Implementation requirement (e.g. no of robotic protocols)

Standard batching



Requires 40 plates

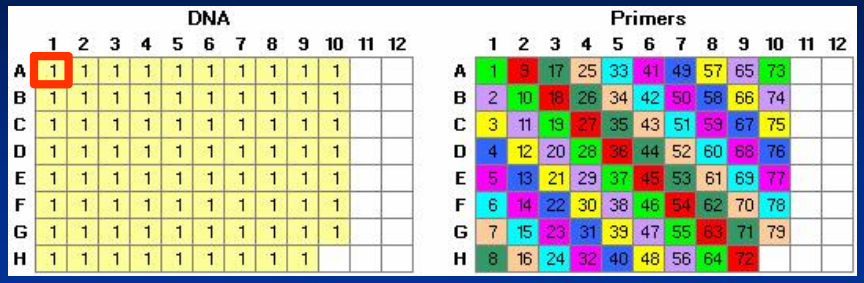


Requires 7 plates

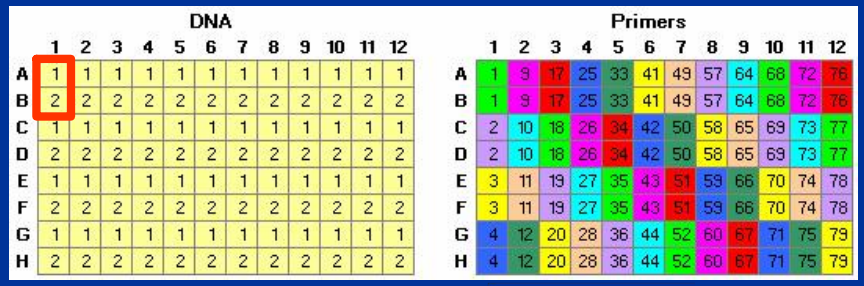
- **One robot protocol but:**
  - Different diseases may require different batching
  - Different diseases require different numbers of fragments
  - Even within disease referral is not regular

Is likely to require different protocols for different diseases / situations

One Sample per batch



No controls - Requires 1 plate - 100%



1 control - Requires 2 plates - 50%

Number of samples in batch	Number of controls				
	0	1	2	3	4
1	100	50	33	25	20
2	100	67	50	40	33
4	100	80	67	57	50
8	100	89	80	73	67
12	100	92	86	80	75
24	100	96	92	89	86
32	100	97	94	91	89
48	100	98	96	94	92
96	100	99	98	97	96

- If one standard batching scheme to be used throughout lab ‘one sample per batch’ is the most useful
- Least efficient scheme – efficiency depends on number of controls used

One plate per fragment

DNA

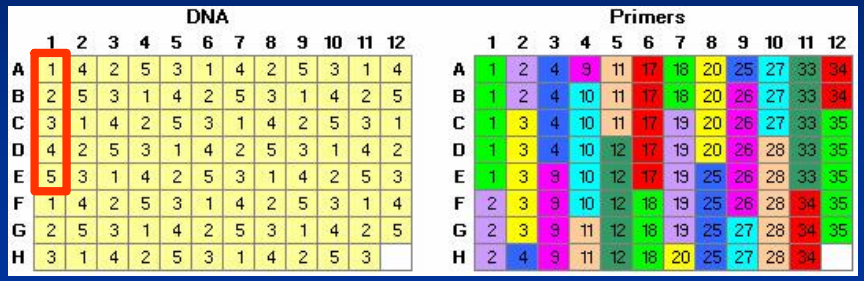
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

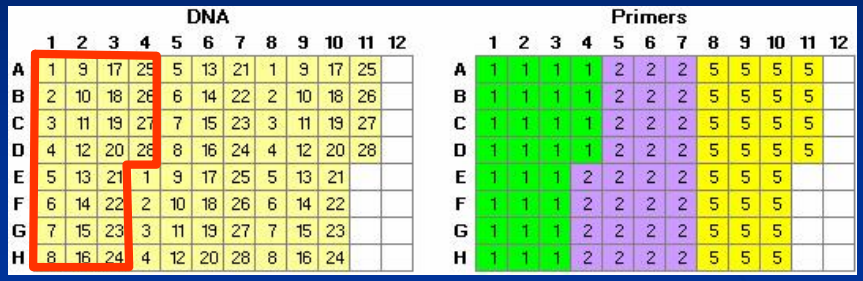
GS primer

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	1	1	1	1

- Does not require PCRs to be standardised
- Only suitable for large numbers of samples e.g. backlogs

Variable batching

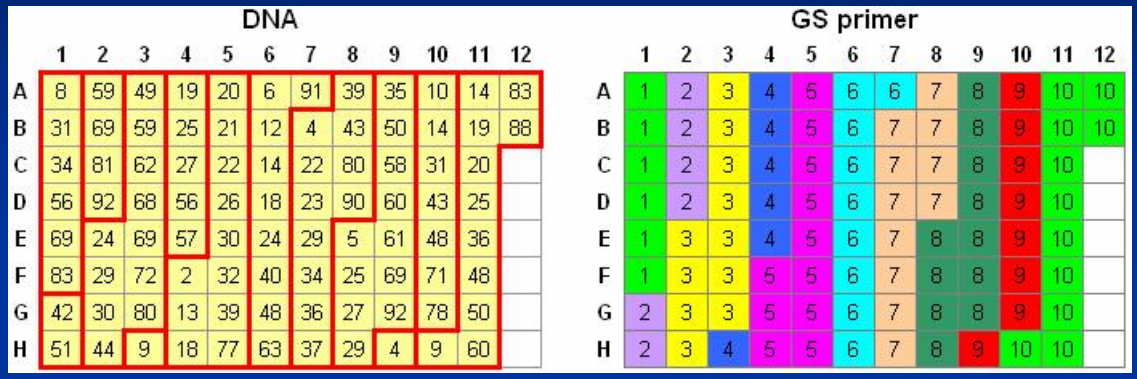




- Can vary batch size according to referral rate
- Maximises efficiency
- Allows use of scheduled testing to meet TAT
- Requires use of worklists to control robotics

Batch	Week							
	1	2	3	4	5	6	7	8
1	Sample collection		Testing					
2			Sample collection		Testing			
3					Sample collection		Testing	

Flexible batching



DNA

	1	2	3	4	5	6	7	8	9	10	11	12
A	8	59	49	19	20	6	91	39	35	10	14	83
B	31	69	59	25	21	12	4	43	50	14	19	88
C	34	81	62	27	22	14	22	80	58	31	20	
D	56	92	68	56	26	18	23	90	60	43	25	
E	69	24	69	57	30	24	29	5	61	48	36	
F	83	29	72	2	32	40	34	25	69	71	48	
G	42	30	80	13	39	48	36	27	92	78	50	
H	51	44	9	18	77	63	37	29	4	9	60	

GS primer

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	6	7	8	9	10	10
B	1	2	3	4	5	6	7	7	8	9	10	10
C	1	2	3	4	5	6	7	7	8	9	10	
D	1	2	3	4	5	6	7	7	8	9	10	
E	1	3	3	4	5	6	7	8	8	9	10	
F	1	3	3	5	5	6	7	8	8	9	10	
G	2	3	3	5	5	6	7	8	8	9	10	
H	2	3	4	5	5	6	7	8	9	10	10	

- Some form of flexible batching is required by all labs e.g. to setup confirmatory sequencing reactions
- Virtually impossible to automate without the use of worklists
- Can be used to batch different ‘small screens’ together

Automation: Control by Robot Protocol

Aspirate from primer plate
  Well A01
Dispense to PCR plate
  Well A01
  Well B01
  Well C01
  Well D01
  Well E01
  Well F01
  Well G01
  Well H01
Aspirate from primer plate
  Well B01
Dispense to PCR plate
  Well A02
    etc…

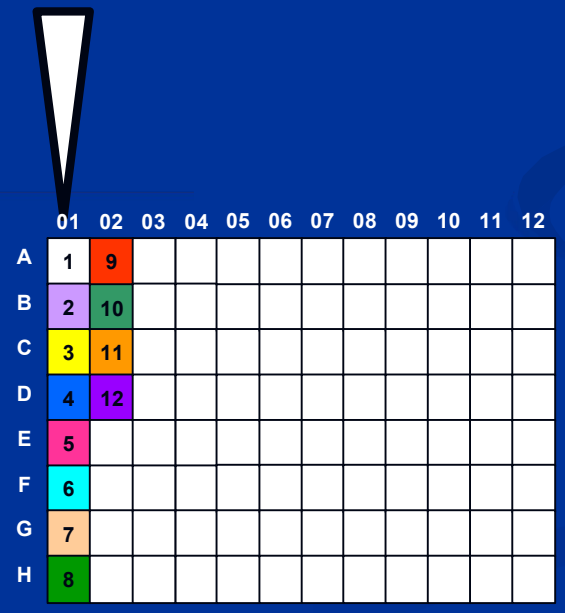
Primer plate

	01	02	03	04	05	06	07	08	09	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	1	2	3	4	5	6	7	8	9	10	11	12
C	1	2	3	4	5	6	7	8	9	10	11	12
D	1	2	3	4	5	6	7	8	9	10	11	12
E	1	2	3	4	5	6	7	8	9	10	11	12
F	1	2	3	4	5	6	7	8	9	10	11	12
G	1	2	3	4	5	6	7	8	9	10	11	12
H	1	2	3	4	5	6	7	8	9	10	11	12

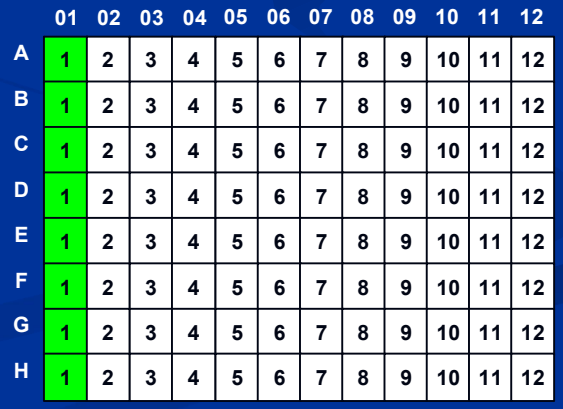
PCR plate

Automation: Control by Robot Protocol

Aspirate from primer plate
  Well A01
Dispense to PCR plate
  Well A01
  Well B01
  Well C01
  Well D01
  Well E01
  Well F01
  Well G01
  Well H01
Aspirate from primer plate
  Well B01
Dispense to PCR plate
  Well A02
  etc…



Primer plate

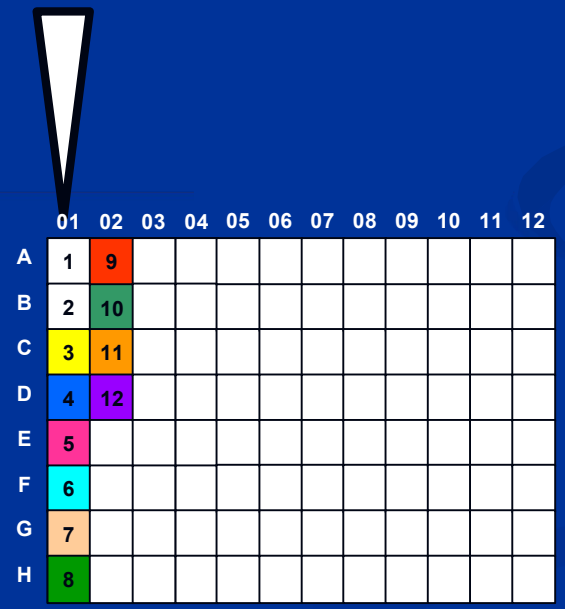


PCR plate

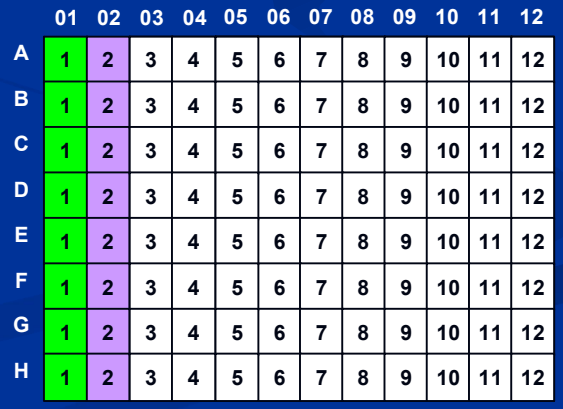
Automation: Control by Robot Protocol

- 1 programme for *each* plate layout
- Long and cumbersome to write
- Limited to standard batches only
- 1 test per batch

Aspirate from primer plate
Well A01
Dispense to PCR plate
Well A01
Well B01
Well C01
Well D01
Well E01
Well F01
Well G01
Well H01
Aspirate from primer plate
Well B01
Dispense to PCR plate
Well A02
etc…



Primer plate



PCR plate

Automation: Control by Worklist

- 1 programme for *any* plate layout
- Worklists generated by LIMS or simple spreadsheet
- Multiple tests per batch
- One off tests feasible
- Requires primer sets to be standardised across tests

(n)

Source plate	Dest. plate	Aspirate (a)	Disp 1 (b)	Disp 2 (b)	Disp 3 (b)	Disp 4 (b)	Disp 5 (b)	Disp 6 (b)	Disp 7 (b)	Disp 8 (b)
primer	PCR	1	1	2	3	4	0	0	0	0
primer	PCR	2	5	6	7	8	9	0	0	0

Repeat **n** times

Aspirate from primer plate
Well **a**
Dispense to PCR plate
Well **b**

Loop

	01	02	03	04	05	06	07	08	09	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5											
F	6											
G	7											
H	8											

Primer plate

	01	02	03	04	05	06	07	08	09	10	11	12
A	1	2	4	7	9	14	18	20	20	23	29	
B	1	3	5	7	10	15	18	20	20	24	30	
C	1	3	63	7	11	15	18	20	20	25	31	
D	1	3	7	8	11	16	18	20	20	26	32	
E	2	3	7	8	12	16	19	20	20	26	33	
F	2	4	7	8	12	16	19	20	20	27	34	
G	2	4	7	9	13	17	19	20	21	28	35	
H	2	4	7	9	14	18	20	20	22	29	35	

PCR plate

Work lists

plate no	α	#	well ref	DNA	GS primer
1	A	1	1	1	1
1	B	1	2	2	1
1	C	1	3	3	1
1	D	1	4	4	1
1	E	1	5	5	1
1	F	1	6	1	2
1	G	1	7	2	2
1	H	1	8	3	2
1	A	2	9	4	2
1	B	2	10	5	2
1	C	2	11	1	3
1	D	2	12	2	3
1	E	2	13	3	3
1	F	2	14	4	3
1	G	2	15	5	3
1	H	2	16	1	4
1	A	3	17	2	4
1	B	3	18	3	4
1	C	3	19	4	4
1	D	3	20	5	4
1	E	3	21	1	9
1	F	3	22	2	9
1	G	3	23	3	9
1	H	3	24	4	9
1	A	4	25	5	9
1	B	4	26	1	10
1	C	4	27	2	10

DNA

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	4	2	5	3	1	4	2	5	3	1	4
B	2	5	3	1	4	2	5	3	1	4	2	5
C	3	1	4	2	5	3	1	4	2	5	3	1
D	4	2	5	3	1	4	2	5	3	1	4	2
E	5	3	1	4	2	5	3	1	4	2	5	3
F	1	4	2	5	3	1	4	2	5	3	1	4
G	2	5	3	1	4	2	5	3	1	4	2	5
H	3	1	4	2	5	3	1	4	2	5	3	

Primers

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	4	9	11	17	18	20	25	27	33	34
B	1	2	4	10	11	17	18	20	26	27	33	34
C	1	3	4	10	11	17	19	20	26	27	33	35
D	1	3	4	10	12	17	19	20	26	28	33	35
E	1	3	9	10	12	17	19	25	26	28	33	35
F	2	3	9	10	12	18	19	25	26	28	34	35
G	2	3	9	11	12	18	19	25	27	28	34	35
H	2	4	9	11	12	18	20	25	27	28	34	

1	9	17	25	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

Add DNA

1st 8 tips

2nd 8 tips

SP	SR	R1	R2	R3	R4	R5	R6	R7	R8	Rn	P1	P2	P3	P4	P5	P6	P7	P8
1	MP	1	2	3	4	0	0	0	0	5	1	6	11	16	21	26	31	36
2	MP	1	2	3	4	0	0	0	0	5	2	7	12	17	22	27	32	37
3	MP	1	2	3	4	0	0	0	0	5	3	8	13	18	23	28	33	38
4	MP	1	2	3	4	0	0	0	0	5	4	9	14	19	24	29	34	39
5	MP	1	2	3	4	0	0	0	0	5	5	10	15	20	25	30	35	40
1	MP	1	2	3	4	0	0	0	0	0	41	46	51	56	61	66	71	76
2	MP	1	2	3	4	0	0	0	0	0	42	47	52	57	62	67	72	77
3	MP	1	2	3	4	0	0	0	0	0	43	48	53	58	63	68	73	78
4	MP	1	2	3	4	0	0	0	0	0	44	49	54	59	64	69	74	79
5	MP	1	2	3	4	0	0	0	0	0	45	50	55	60	65	70	75	80
1	MP	1	2	3	4	0	0	0	0	0	81	86	91	0	0	0	0	0
2	MP	1	2	3	4	0	0	0	0	0	82	87	92	0	0	0	0	0
3	MP	1	2	3	4	0	0	0	0	0	83	88	93	0	0	0	0	0
4	MP	1	2	3	4	0	0	0	0	0	84	89	94	0	0	0	0	0
5	MP	1	2	3	4	0	0	0	0	0	85	90	95	0	0	0	0	0

DNA

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	4	2	5	3	1	4	2	5	3	1	4
B	2	5	3	1	4	2	5	3	1	4	2	5
C	3	1	4	2	5	3	1	4	2	5	3	1
D	4	2	5	3	1	4	2	5	3	1	4	2
E	5	3	1	4	2	5	3	1	4	2	5	3
F	1	4	2	5	3	1	4	2	5	3	1	4
G	2	5	3	1	4	2	5	3	1	4	2	5
H	3	1	4	2	5	3	1	4	2	5	3	

Primers

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	4	9	11	17	18	20	25	27	33	34
B	1	2	4	10	11	17	18	20	26	27	33	34
C	1	3	4	10	11	17	19	20	26	27	33	35
D	1	3	4	10	12	17	19	20	26	28	33	35
E	1	3	9	10	12	17	19	25	26	28	33	35
F	2	3	9	10	12	18	19	25	26	28	34	35
G	2	3	9	11	12	18	19	25	27	28	34	35
H	2	4	9	11	12	18	20	25	27	28	34	

Add primer

SP	SR	DR	DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13	DP14	DP15	DP16	DP17	DP18	DP19	DP20	DP21	DP22	DP23	DP24
1	GS	1	1	2	3	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	GS	1	6	7	8	9	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	GS	1	11	12	13	14	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	GS	1	16	17	18	19	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	GS	2	1	2	3	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	GS	2	6	7	8	9	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

DNA

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	4	2	5	3	1	4	2	5	3	1	4
B	2	5	3	1	4	2	5	3	1	4	2	5
C	3	1	4	2	5	3	1	4	2	5	3	1
D	4	2	5	3	1	4	2	5	3	1	4	2
E	5	3	1	4	2	5	3	1	4	2	5	3
F	1	4	2	5	3	1	4	2	5	3	1	4
G	2	5	3	1	4	2	5	3	1	4	2	5
H	3	1	4	2	5	3	1	4	2	5	3	

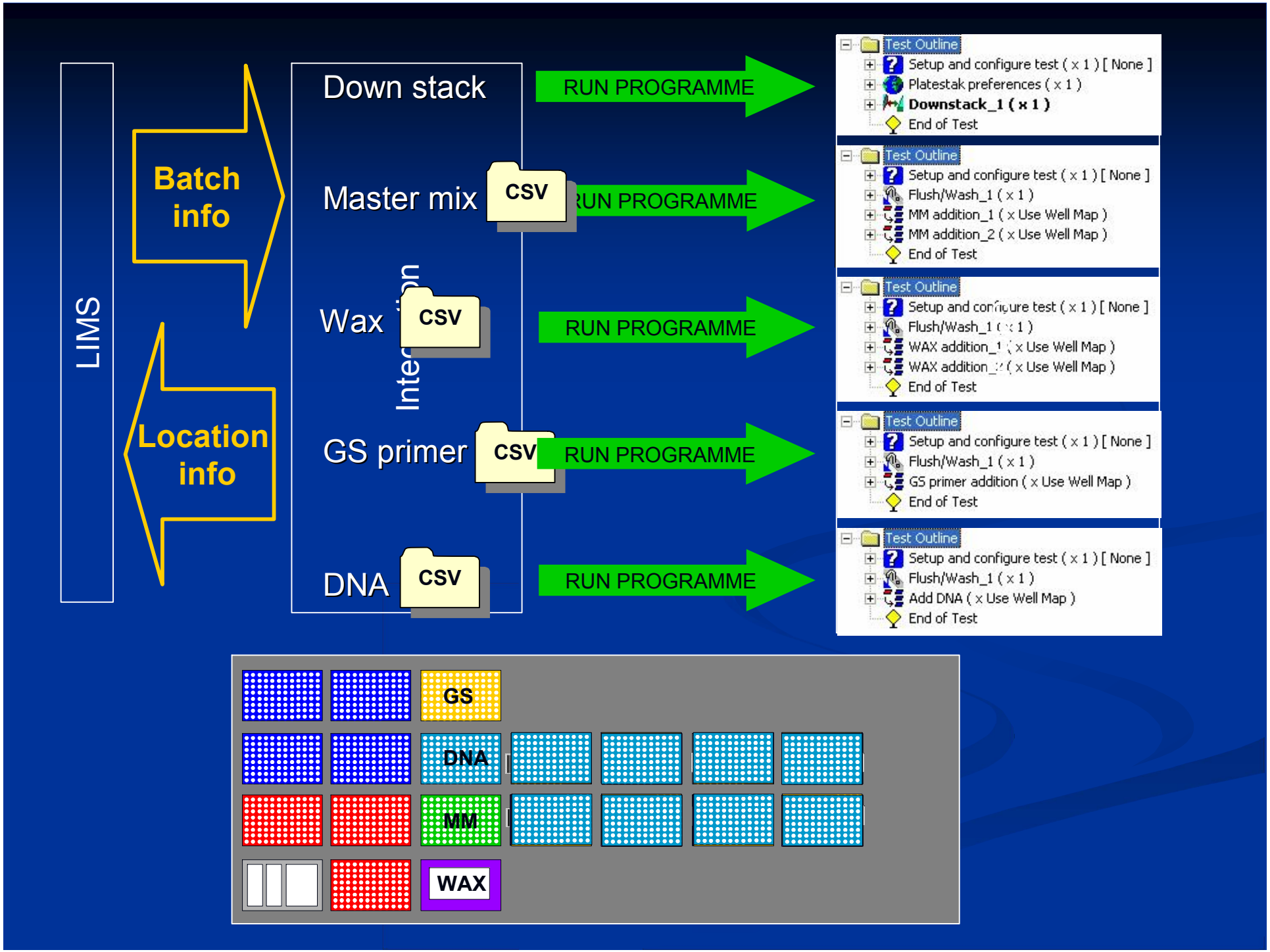
Primers

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	4	9	11	17	18	20	25	27	33	34
B	1	2	4	10	11	17	18	20	26	27	33	34
C	1	3	4	10	11	17	19	20	26	27	33	35
D	1	3	4	10	12	17	19	20	26	28	33	35
E	1	3	9	10	12	17	19	25	26	28	33	35
F	2	3	9	10	12	18	19	25	26	28	34	35
G	2	3	9	11	12	18	19	25	27	28	34	35
H	2	4	9	11	12	18	20	25	27	28	34	

Cherry picking – flexible batching

plate no	α	#	well ref	DNA	GS primer
1	A	1	1	1	1
1	B	1	2	2	1
1	C	1	3	3	1
1	D	1	4	4	1
1	E	1	5	5	1
1	F	1	6	1	2
1	G	1	7	2	2
1	H	1	8	3	2
1	A	2	9	4	2
1	B	2	10	5	2
1	C	2	11	1	3
1	D	2	12	2	3
1	E	2	13	3	3
1	F	2	14	4	3
1	G	2	15	5	3
1	H	2	16	1	4
1	A	3	17	2	4
1	B	3	18	3	4
1	C	3	19	4	4
1	D	3	20	5	4
1	E	3	21	1	9
1	F	3	22	2	9
1	G	3	23	3	9
1	H	3	24	4	9
1	A	4	25	5	9
1	B	4	26	1	10
1	C	4	27	2	10

plate no	α	#	well ref	DNA	GS primer
1	B	1	2	2	1
1	G	1	7	2	2
1	F	2	14	4	3
1	G	2	15	5	3
1	D	3	20	5	4


LIMS
Batch info
Location info
Down stack
RUN PROGRAMME
Master mix
CSV
RUN PROGRAMME
Wax
CSV
RUN PROGRAMME
GS primer
CSV
RUN PROGRAMME
DNA
CSV
RUN PROGRAMME
Test Outline
Setup and configure test (x 1) [None]
Platestak preferences (x 1)
Downstack_1 (x 1)
End of Test
Test Outline
Setup and configure test (x 1) [None]
Flush/Wash_1 (x 1)
MM addition_1 (x Use Well Map)
MM addition_2 (x Use Well Map)
End of Test
Test Outline
Setup and configure test (x 1) [None]
Flush/Wash_1 (x 1)
WAX addition_1 (x Use Well Map)
WAX addition_2 (x Use Well Map)
End of Test
Test Outline
Setup and configure test (x 1) [None]
Flush/Wash_1 (x 1)
GS primer addition (x Use Well Map)
End of Test
Test Outline
Setup and configure test (x 1) [None]
Flush/Wash_1 (x 1)
Add DNA (x Use Well Map)
End of Test
GS
DNA
MM
WAX

Summary

	For	Advantages	Drawbacks
Standard	Primary	Simple direct robot programming	Inflexible wrt referral patterns Many robot protocols required
1 sample / batch	Primary	Copes with all referral patterns	Potentially inefficient
1 fragment / plate	Primary	No PCR standardisation required	Only good for backlogs
Variable	Primary	Copes with all referral patterns Maximises efficiency Only one robot protocol required LIMS automation and audit trail	Requires initial effort to setup worklist operation
Flexible	Primary & Secondary	Will cope with any PCR setup Only one robot protocol required Different tests may be batched together > basis for general PCR automation	Requires initial effort to setup worklist operation

- Key requirements / recommendations
  - Standardised PCR
  - Control of robotics by worklist