High-Throughput Sequencing

Batching and Scheduling

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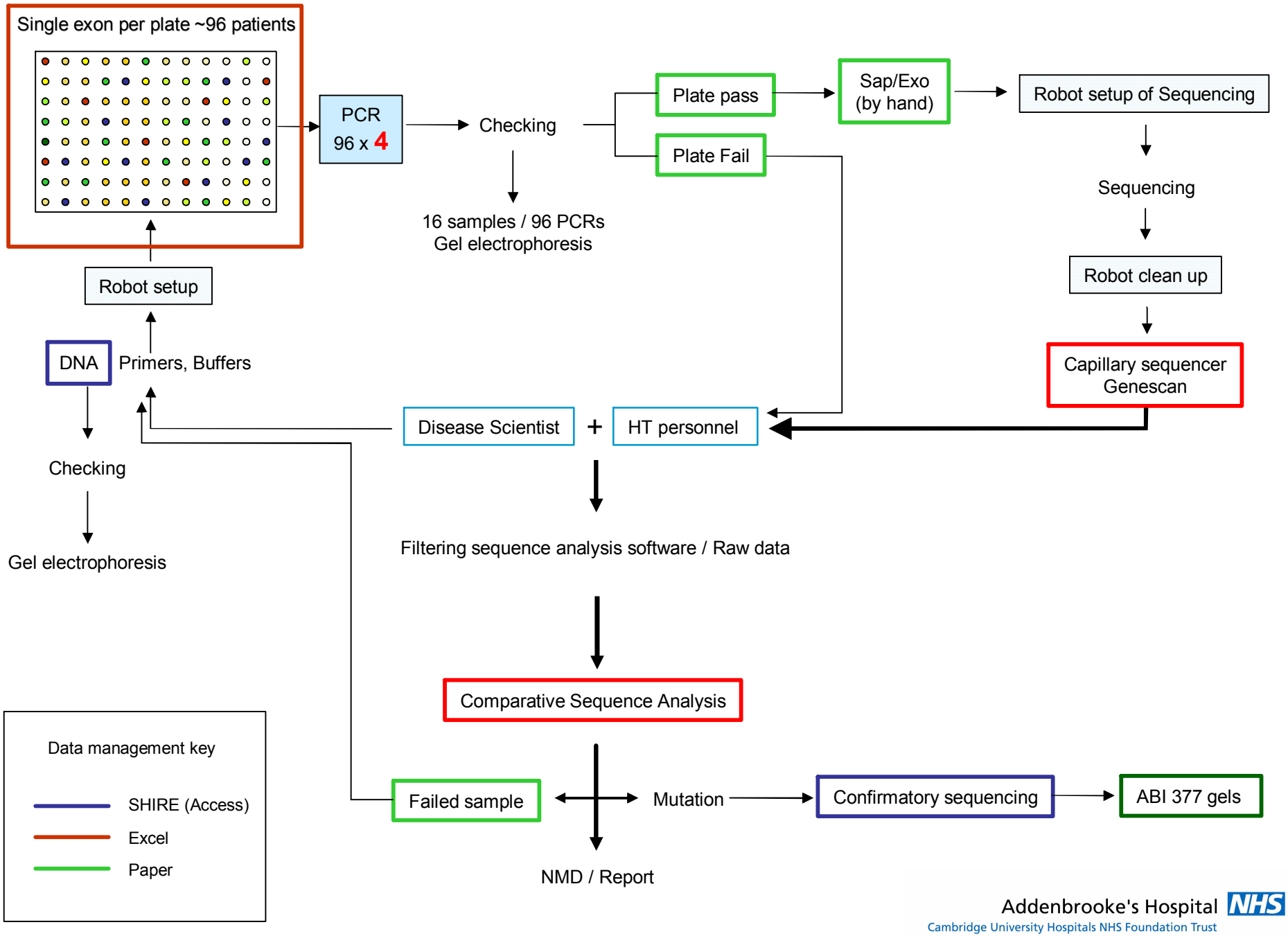
Single exon per plate ~96 patients
PCR
96 x 4
Checking
16 samples / 96 PCRs
Gel electrophoresis
Plate pass
Plate Fail
Sap/Exo
(by hand)
Robot setup of Sequencing
Sequencing
Robot clean up
Capillary sequencer
Genescan
Robot setup
DNA
Primers, Buffers
Checking
Gel electrophoresis
Disease Scientist
+
HT personnel
Filtering sequence analysis software / Raw data
Comparative Sequence Analysis
Failed sample
Mutation
Confirmatory sequencing
ABI 377 gels
NMD / Report
Data management key
SHIRE (Access)
Excel
Paper
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HT sequencing - 96 patients per plate

√ One exon per plate at same temperature

√ simple robot programmes

√ Easy sample tracking

√ Readily change exon conditions

X Slow throughput until plate assembled

X Compression of costs

X Large volume of sequence data

Genetics White Paper, June 2003

"Our inheritance, our future - realising the power of genetics in the NHS"

…….by 2006 genetic test results should be available to the following standards:

within three days where the result is needed urgently (e.g. for prenatal diagnosis)

within two weeks where the potential genetic mutation is already known (eg. because another family member has already been tested)

within eight weeks for unknown mutations in a large gene.

Alternative batch sizes to consider

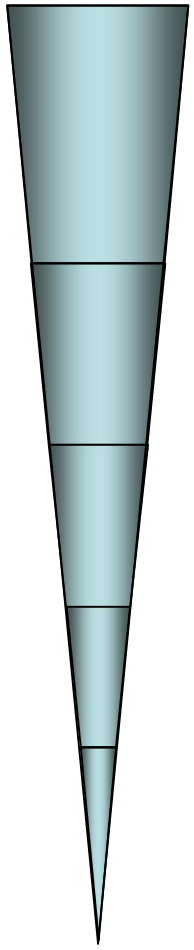
96 (successful but slow)

48 > 96

SCOBEC rationalisation approach

(n)8 approach (16 patients = 6epp)

1 patient = 1 assay = 1plate



TAT Complexity

TAT Complexity

TAT Complexity

TAT Complexity

TAT Complexity

Maximal HT sequencing - one patient per plate

- √ Fast throughput of samples
- √ No compression of costs
- √ Manageable volumes of sequence data
- X Complex robot programmes
- X Maximising use of the plate
- X Many exons and genes per plate at same temperature
- X Managing outlying PCRs and repeats
- X Complex sample tracking

Changes to one exon per plate (1epp) sequencing progressing towards 1ppp sequencing

- Capillary sequencer re-programming (**Sequence** v Genescan)
- Alternative sample analysis (**Sequence** v Genescan)
- New sequence analysis software - evaluate / implement - **Mutation Surveyor**
- Sequencing software issues (data manipulation / “contamination”)
- Robot programme changes (tip washing / DNA mixing)
- Re-optimisation of PCRs (PE 9600 vs MJR Tetrad)

Requirements from robotics for 1ppp sequencing

Variable PCR setup programmes (variable genes & disease sharing)

Flexibility with current genes and future genes

Manage incomplete 96 well plates / maximise use of a plate

Manage sample movement from 96 to 384 format

Manage large number of PCR reaction variables

Requirements from PCRs

Ideal scenario

all exons amplify at

same temperature
in same buffer
same efficiency
sequence with same primers

Reality

Settle for same temperature
despite PCR setup complexity

Exon	PCR	Sequence
TSC1 exon 3	F	F
TSC1 exon 4	E	F
TSC1 exon 5	F	F
TSC1 exon 6	F	F
TSC1 exon 7	F	R
TSC1 exon 8	F	F
TSC1 exon 9	F	F
TSC1 exon 10	E	R
TSC1 exon 11	H	F
TSC1 exon 12	H	F
TSC1 exon 13	F	F
TSC1 exon 14	F	F
TSC1 exon 15a	F	R
TSC1 exon 15b	F	F
TSC1 exon 16	C	F
TSC1 exon 17	H	R
TSC1 exon 18	F	R
TSC1 exon 19	E	F
TSC1 exon 20	F	F
TSC1 exon 21	C	R
TSC1 exon 22	E	F
TSC1 exon 23a	F	F
TSC1 exon 23b	D	R

Exon	PCR	Sequence
TSC2 exon 1	G	F
TSC2 exon 2	D	F
TSC2 exon 3	G	R
TSC2 exon 4	D	F
TSC2 exon 5	E	R
TSC2 exon 6	G	F
TSC2 exon 7	G	F
TSC2 exon 8	G	R
TSC2 exon 9	J	R
TSC2 exon 10	E	F
TSC2 exon 11	D	F
TSC2 exon 12	E	R
TSC2 exon 13	G	F
TSC2 exon 14	E	F
TSC2 exon 15	E	F
TSC2 exon 16	G	R
TSC2 exon 17	G	R
TSC2 exon 18	J	R
TSC2 exon 19	D	F
TSC2 exon 20	G	R
TSC2 exon 21	D	R
TSC2 exon 22	D	R
TSC2 exon 23	E	R

Exon	PCR	Sequence
TSC2 exon 24	H	R
TSC2 exon 25	G	R
TSC2 exon 26	D	R
TSC2 exon 27	E	R
TSC2 exon 28	J	F
TSC2 exon 29	D	R
TSC2 exon 30	D	R
TSC2 exon 31	G	R
TSC2 exon 32	D	R
TSC2 exon 33a	D	R
TSC2 exon 33b	J	R
TSC2 exon 34	H	R
TSC2 exon 35	D	F
TSC2 exon 36	J	R
TSC2 exon 37	H	F
TSC2 exon 38	E	R
TSC2 exon 39	E	R
TSC2 exon 40	E	R
TSC2 exon 41	G	F

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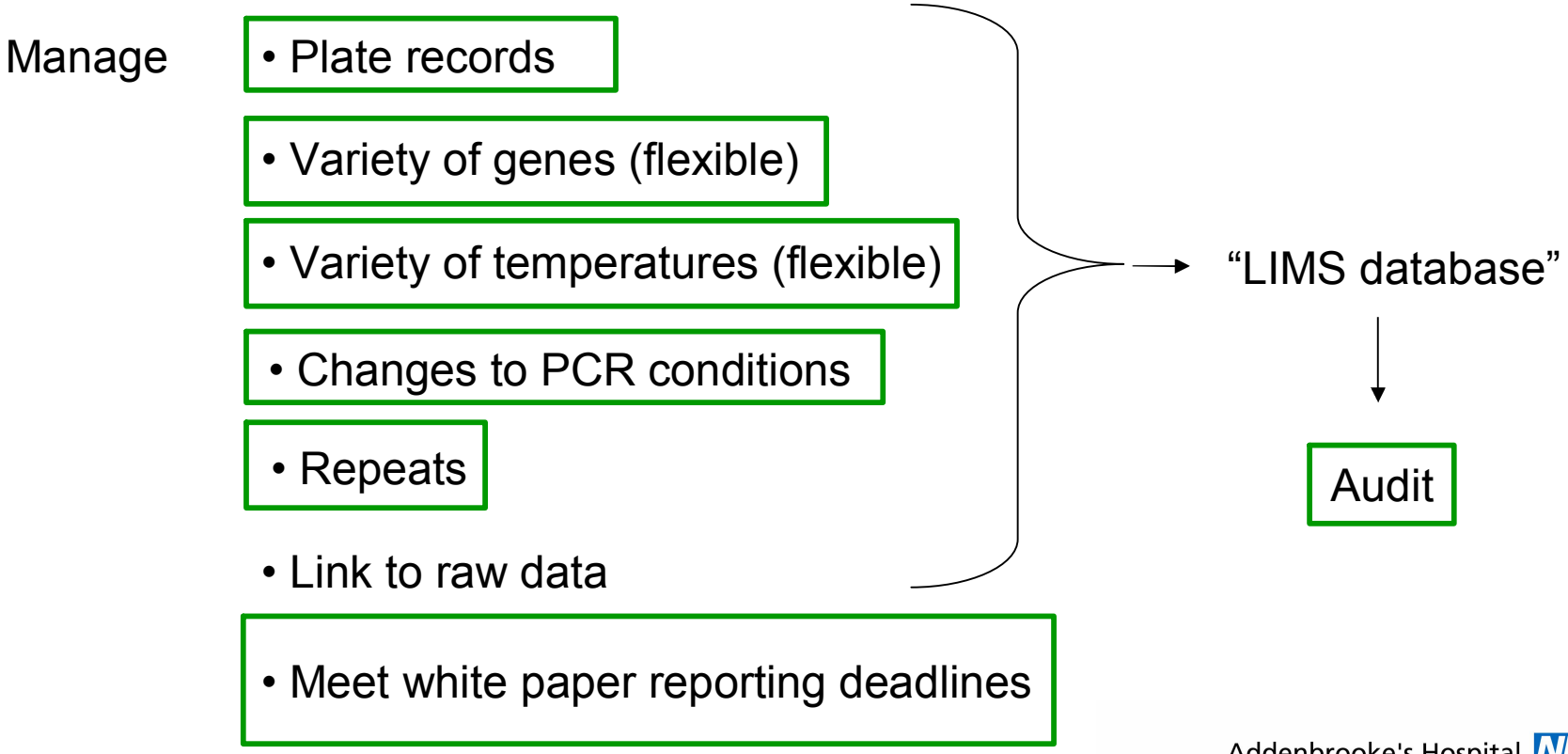
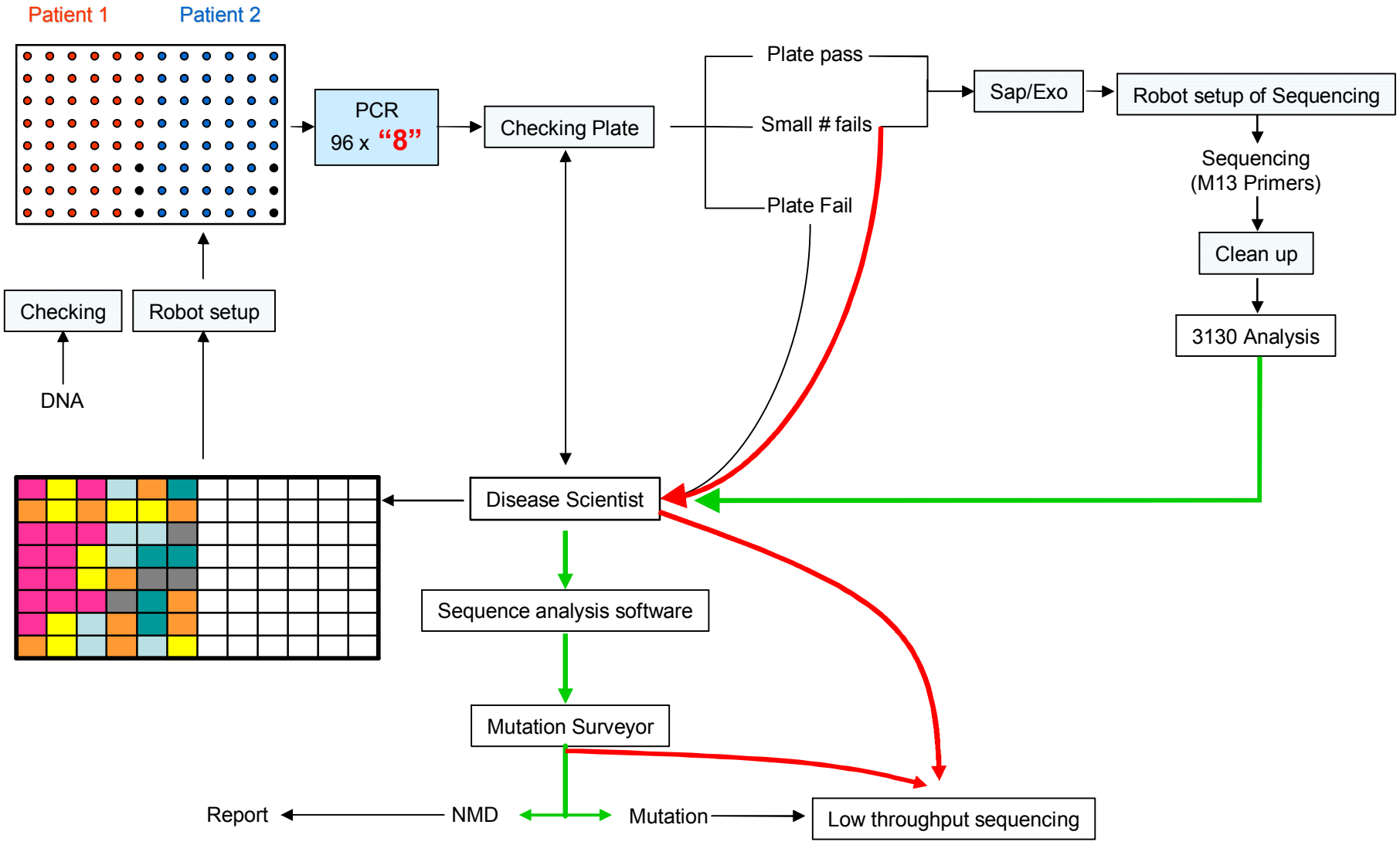
Requirements for managing 1ppp approach

- Import, organise and prioritise patient samples
- Assemble into 96 well plates and generate text lists
- Assemble 384 well plates and generate text lists





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Patient input and prioritising

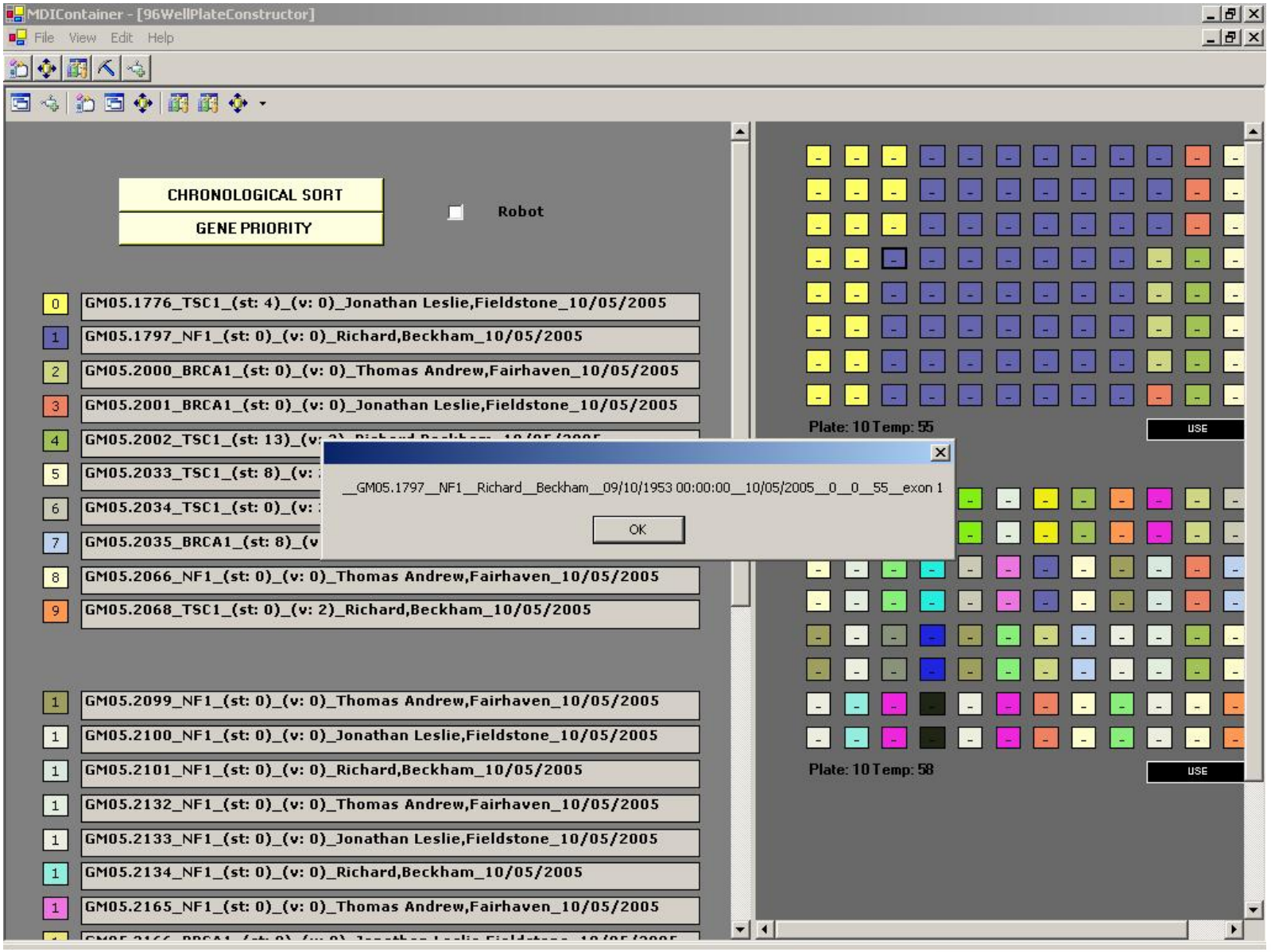
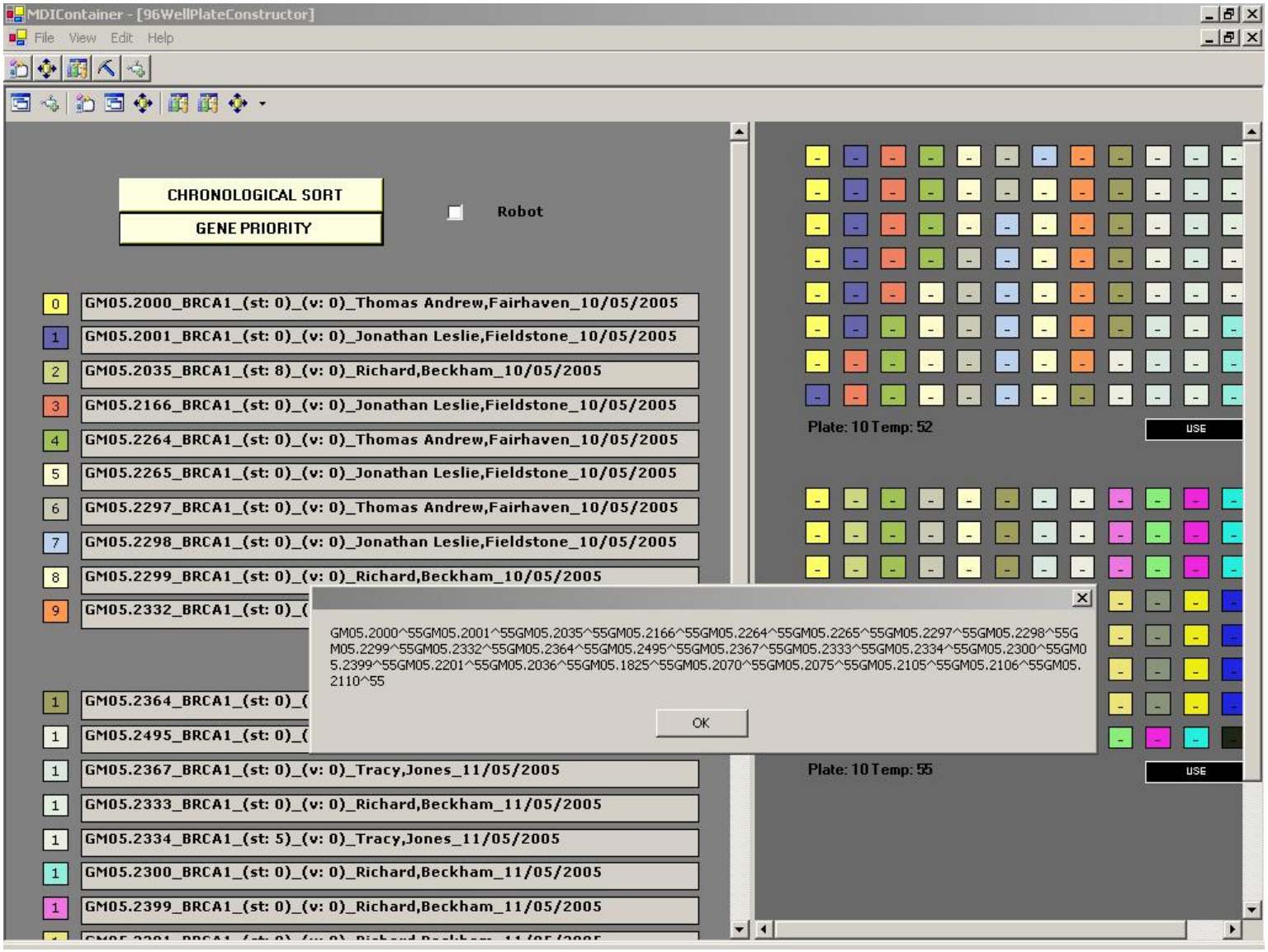
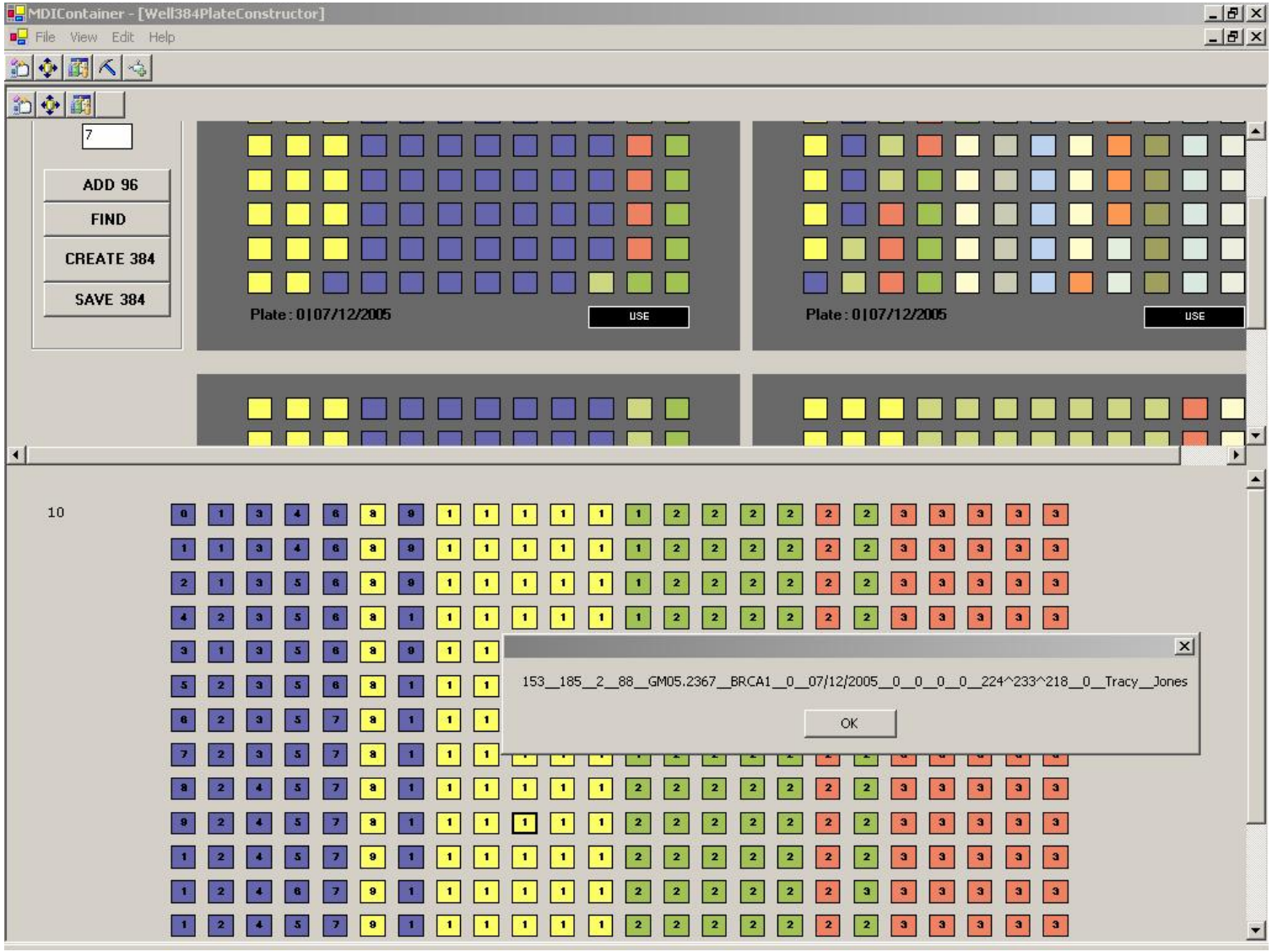


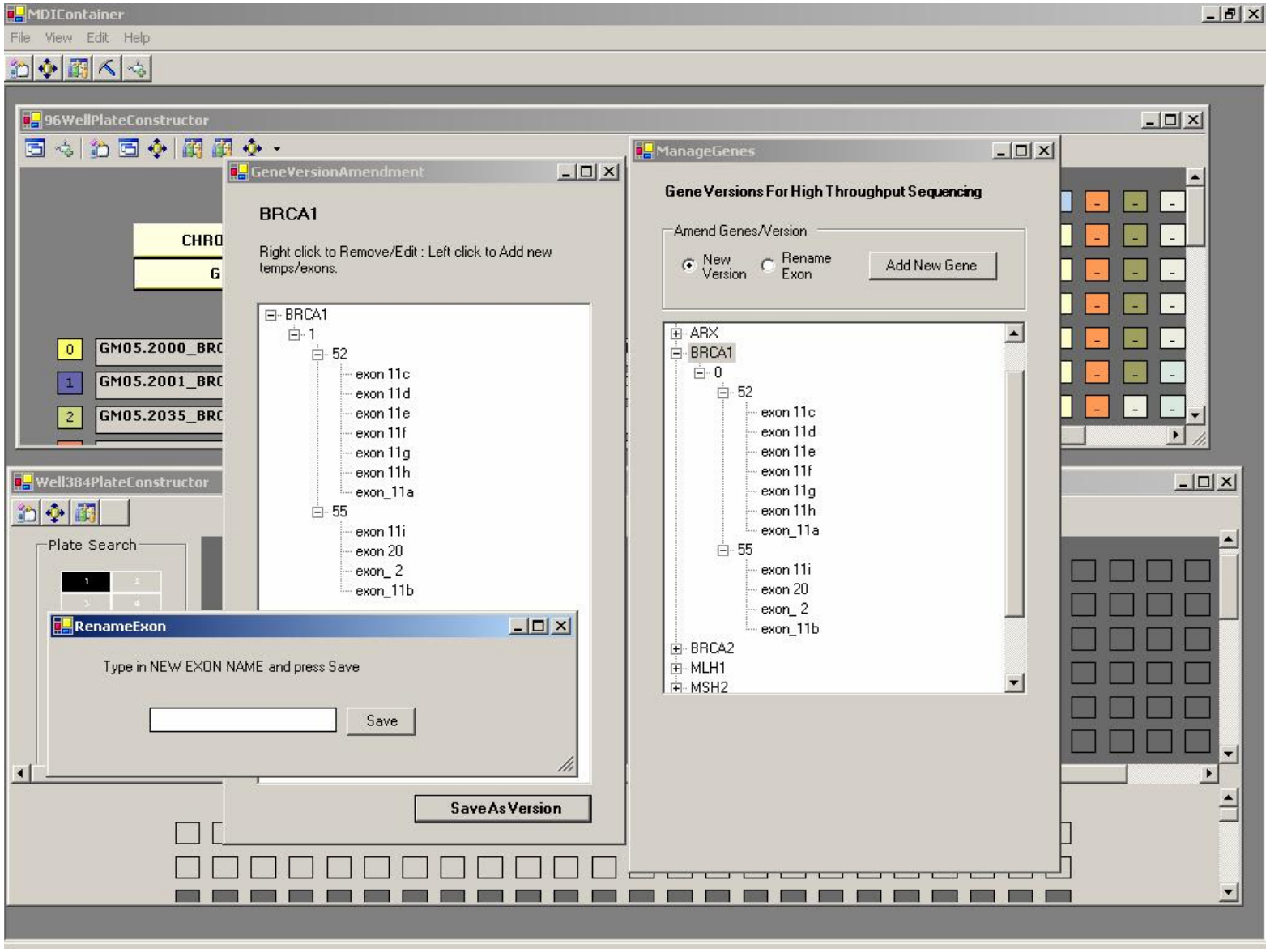
Plate assembly – managing outliers



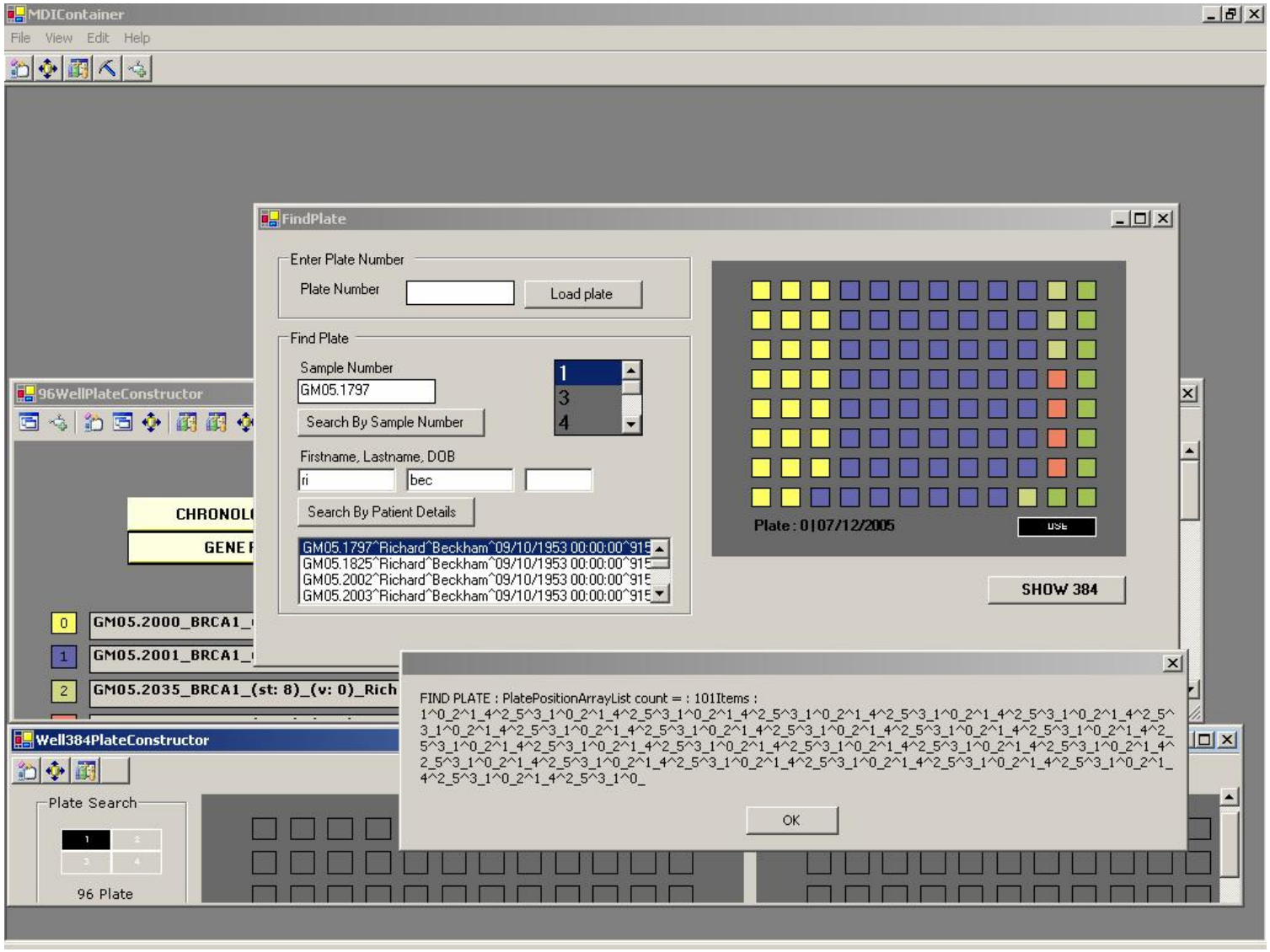
384-well plate assembly



Gene management and archiving



Archive management



Summary

- Have achieved a single PCR setup programme on Biomek 3000 that assembles all possible variations of patient and disease gene on a single plate
- Chosen an approach to maximise throughput whilst minimising wastage by mixing and matching patients and their disease genes on each plate
- Plate assembly approach applicable to genes of any size
- Developed a strategy allowing workflow management without large LIMS database
- Successfully trialled the system and achieved a TAT below 8 weeks

Acknowledgements

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