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# The Manchester FastScan Sequencing Pipeline

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New and Developing Technologies for Genetic Diagnostics
*NGRL (Wessex) 17-18 July 2006*

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# FastScan

- Dedicated ‘high-throughput’ section
- Initial focus: bi-directional mutation scanning
- Automation
  - 2 x Biomek NX Span8 (pre-PCR)
  - 1 x Biomek NX MC (post PCR)
  - 1 x 3730
- 1xMTO (lab), 1xCS, 2xMTOs (data analysis/lab), 1xProject Manager

| ID | Task Name | Start | Finish |
|---|---|---|---|
| 1 | Project Manager | 17/01/2005 | 16/03/2007 |
| 2 | MTO | 01/02/2005 | 28/02/2006 |
| 3 | MTO | 04/04/2005 | 30/12/2005 |
| 4 | Data Analysts MTOs | 24/06/2005 | 16/03/2007 |
| 5 | Clinical Scientist | 01/09/2005 | 16/03/2007 |
| 6 | MTO | 16/01/2006 | 16/03/2007 |

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# Initial Aims

- Enable high-throughput bi-directional mutation scanning by DNA sequencing
- Initial task: address BRCA backlog
  - Develop an ‘automated’ service using existing Meta PCR chemistry
  - Run backlog samples during development of service (no continuation of existing service provision)
  - Increase throughput six-fold
  - Reduce turnaround time eight-fold
  - Complete within 18 months
- June 2006 deadline to clear March 2005 backlog (333 scans)
- Staff new to BRCA and new to automation

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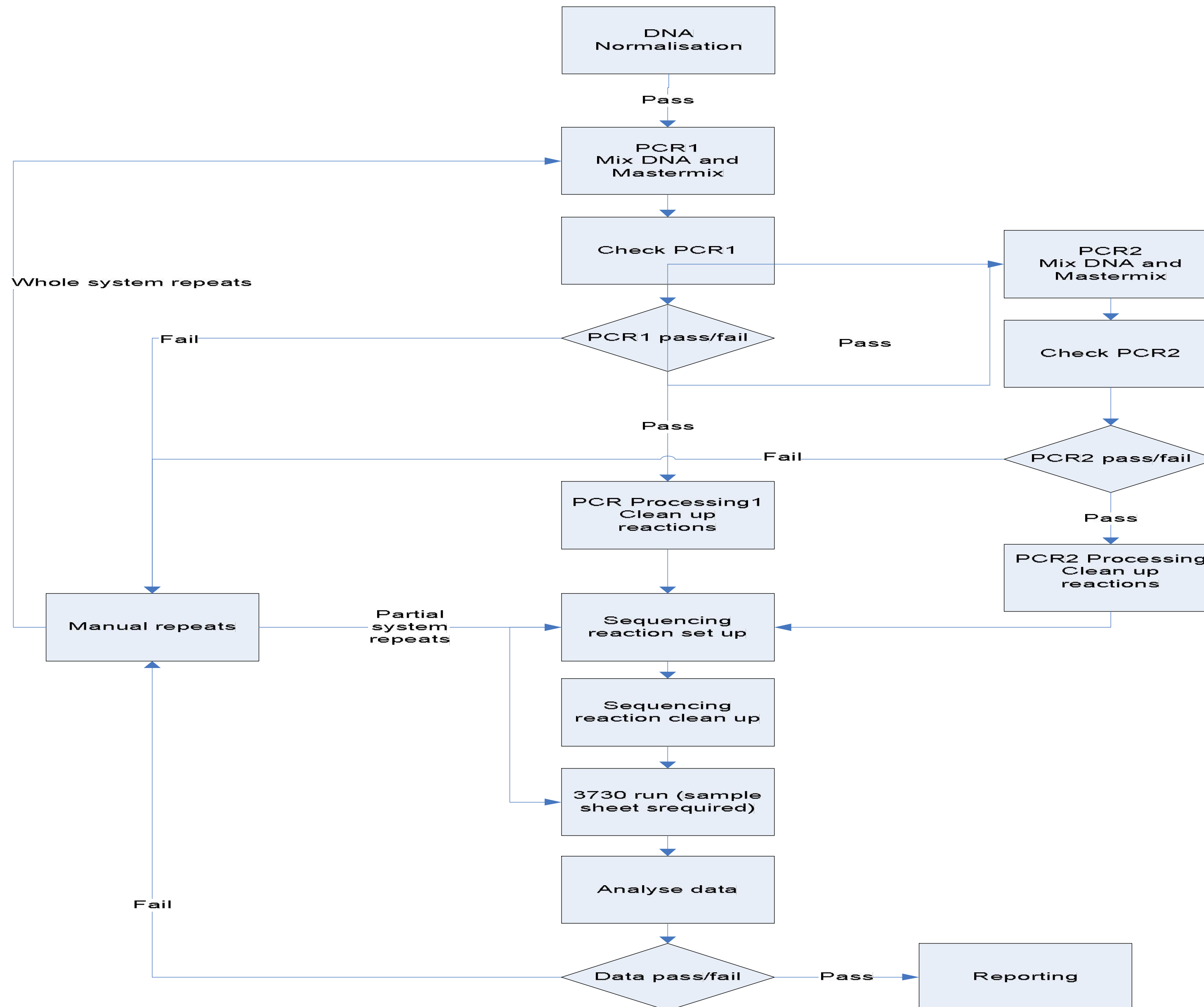
# FastScan Process (Meta-PCR)



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# FastScan

- BRCA1 & BRCA2 screened serially
- Initial set-up: 6 samples, 2 controls (cell line & no DNA)
- Changed to 14 samples, 2 controls
- Added random known positive samples to check analysis
- April ’05 - September ’05
  - Tested & optimised robotics for automated set up of PCRs & sequencing reactions
  - Simultaneous service provision & development work on ‘live’ samples
  - Began receiving Merseyside/Cheshire backlog samples June ‘05
- Issues:
  - Sample & Reagent quality
  - Instrument failures (Robotics, PCR)
  - Chemistry optimisation
  - Information tracking not covered by lab db

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# FastScan

- October ’05 – Current
  - Optimised chemistry for BRCA1 & 2
    - Reduced repeat numbers
  - Delayed introduction of automation until Jan ’06
  - Altered data analysis process
  - Further established systems for tracking information to suit our requirements
  - Completed previous 192 screens, began work on 182 new screens, now 90% complete
  - June ’06 shifted focus to 40wd TAT
  - Now run 3 batches per month (42 screens)

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# Data Analysis

- Very stringent data analysis – MTO 1st check, CS 2nd check, full bi-directional analysis in Staden
- April ’05 – June ’05
  - Primary analysis by lab or dedicated MTOs
  - All data second checked in Staden & reported by 3xCS
  - ~2hrs per screen for 2nd checking
- July ’05 - current
  - Introduced two dedicated MTO 1st checkers
  - Data QC and primary analysis in Staden & Mutation Surveyor & track all sample related info
- Feb ’06: began distributing 2nd checking to all Clinical Scientists in lab
  - Major changes to lab database
  - Required minimal training (Staden)
  - Spread marking workload across lab
  - Reporting centralised

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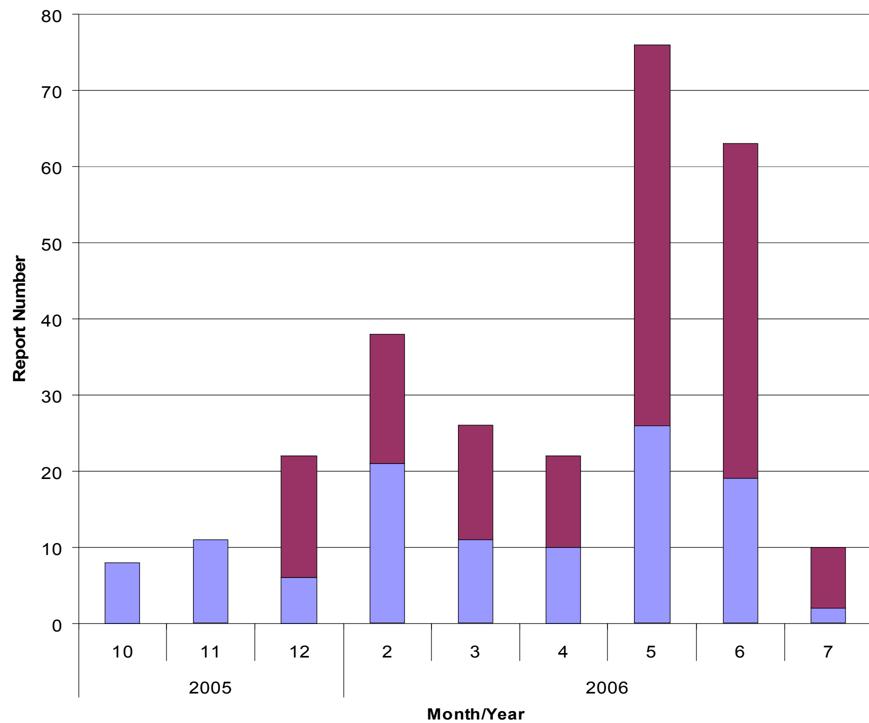
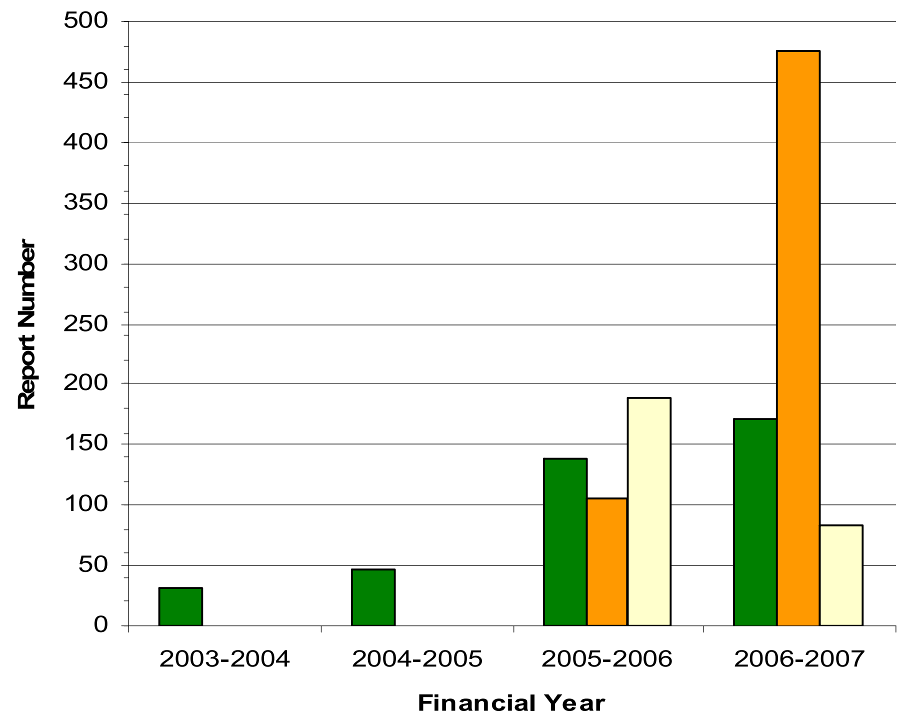
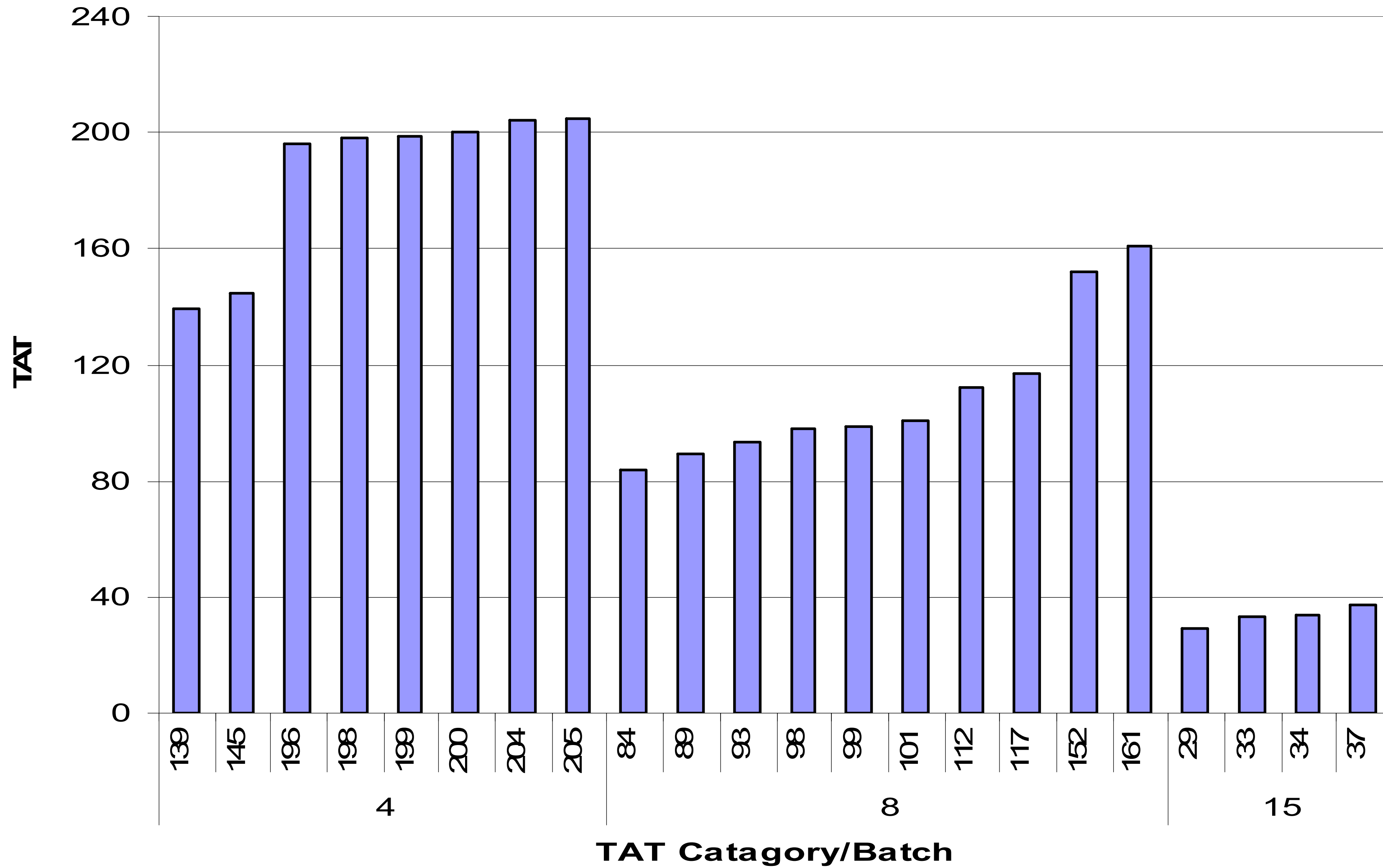
# Report Output



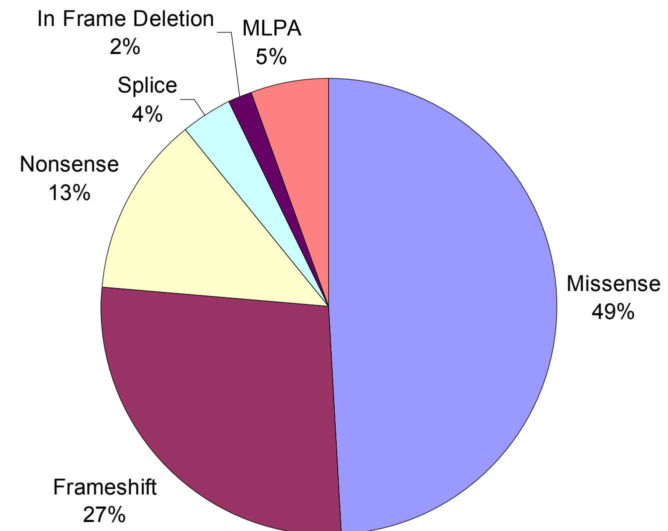
# TAT Improvement



# Reporting Summary

| | Total reports | Mutations Found (inc. MLPA) | UVs | Mutation Rate | Variation Rate (M+UV) |
|---|---|---|---|---|---|
| BRCA1 | 114 | 7 | 8 | 6.1% | 13.2% |
| BRCA2 | 162 | 16 | 24 | 9.9% | 24.7% |
| All | 276 | 23 | 32 | 8.3% | 19.9% |

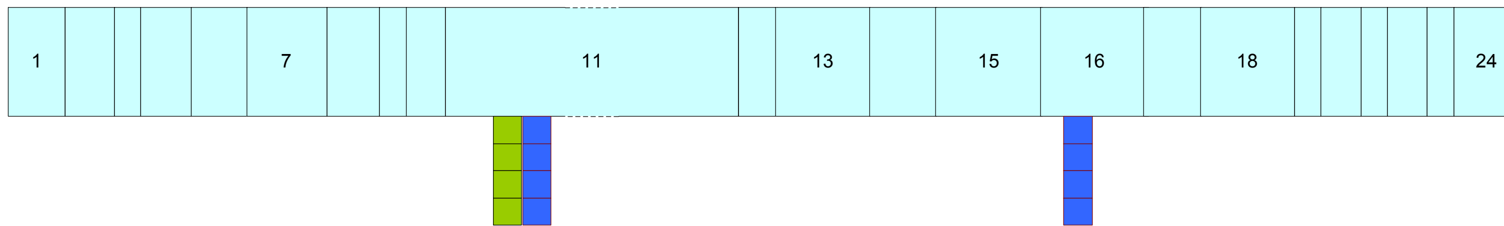
| Year | BRCA1 | BRCA2 | Total |
|---|---|---|---|
| Jan-Dec '05 Total | 25 | 16 | 41 |
| Jan-current '06 Total | 89 | 146 | 235 |
| Grand Total | 114 | 162 | 276 |



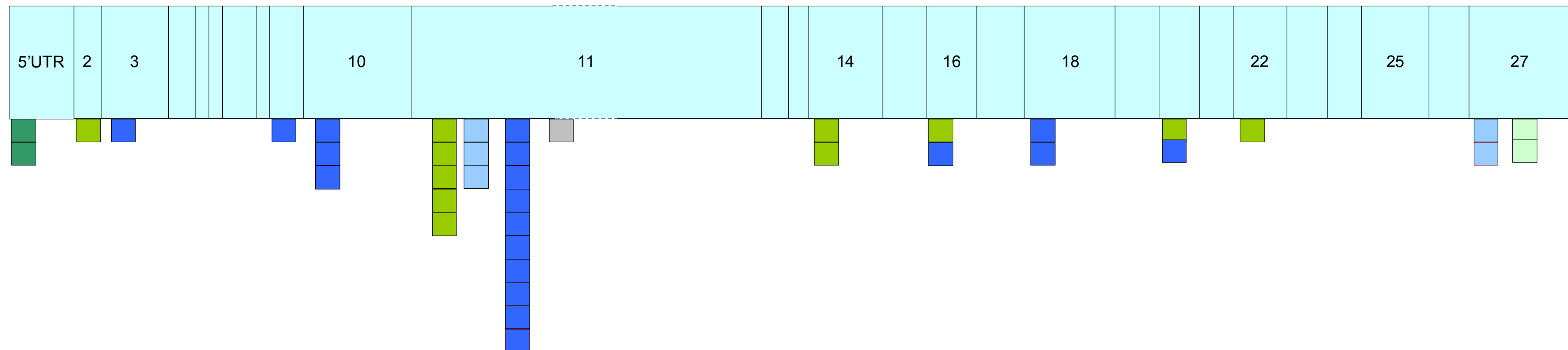
# BRCA Variant Spectrum

BRCA1 n=114 , BRCA2 n=162

BRCA1




BRCA2



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# FastScan Summary

- ✓ Produced 266 scans (80% of target) by June ‘06 deadline
- ✓ Most change has occurred in laboratory processes, not in laboratory automation; re-organisation has been key
- ✓ Repeats were a problem, now manageable
- ✓ Distributed data analysis has been a success, but…
  - ✗ Additional burden on some staff
  - ✗ Needs careful management
- ✓ 40wd turn around time is achievable with this procedure
- ✗ Meta PCR method is still manual
  - ✗ Gel cut outs still in operation
  - ✗ Sequencing fragments too large for automated clean-up
  - ✗ Requires experience to operate well
- ✗ Sample quality problems
- ✗ Development time in production environment

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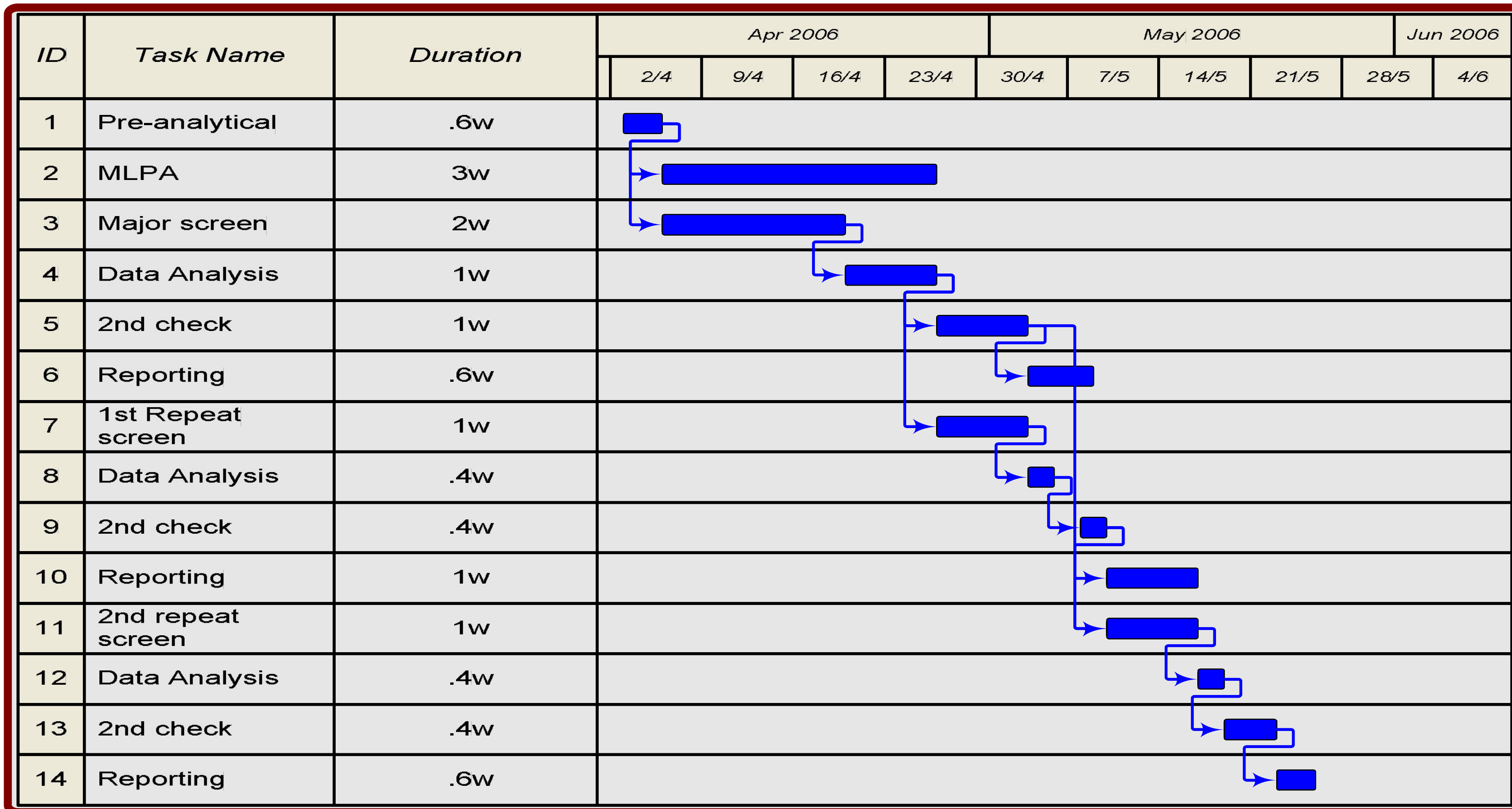
# 40wd BRCA Gene Screen Gantt



| ID | Task Name | Duration |
|---|---|---|
| 1 | Pre-analytical | .6w |
| 2 | MLPA | 3w |
| 3 | Major screen | 2w |
| 4 | Data Analysis | 1w |
| 5 | 2nd check | 1w |
| 6 | Reporting | .6w |
| 7 | 1st Repeat screen | 1w |
| 8 | Data Analysis | .4w |
| 9 | 2nd check | .4w |
| 10 | Reporting | 1w |
| 11 | 2nd repeat screen | 1w |
| 12 | Data Analysis | .4w |
| 13 | 2nd check | .4w |
| 14 | Reporting | .6w |

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# Future Work

- BRCA
  - Aim: 100% automation of laboratory work (non meta PCR); maximise efficiency of process (work in progress)
  - Refine data analysis procedures
    - Investigate sole use of Mutation Surveyor
    - Need to closely define expected timeframes in distributed system
  - Develop in-house db of UV/poly information to reduce confirmation numbers
- Overall
  - Construct and develop model for simultaneously screening multiple disease genes with variable sample numbers
  - Aim: the sample is the batch
  - Global design & optimisation strategy
    - SOPs should work ‘out of the box’ for all users
    - ? Batch testing of reagents, how to use controls
- Continual assessment of strategy & resources
- Use of ‘best practise’ and lessons from other laboratories
- Introduction of STARLIMS

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# Acknowledgements


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**Autumn Workshop**

Identifying Issues For Best Practise In High-Throughput Systems

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