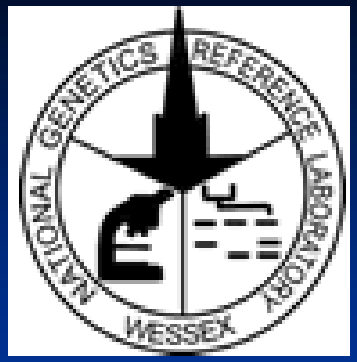
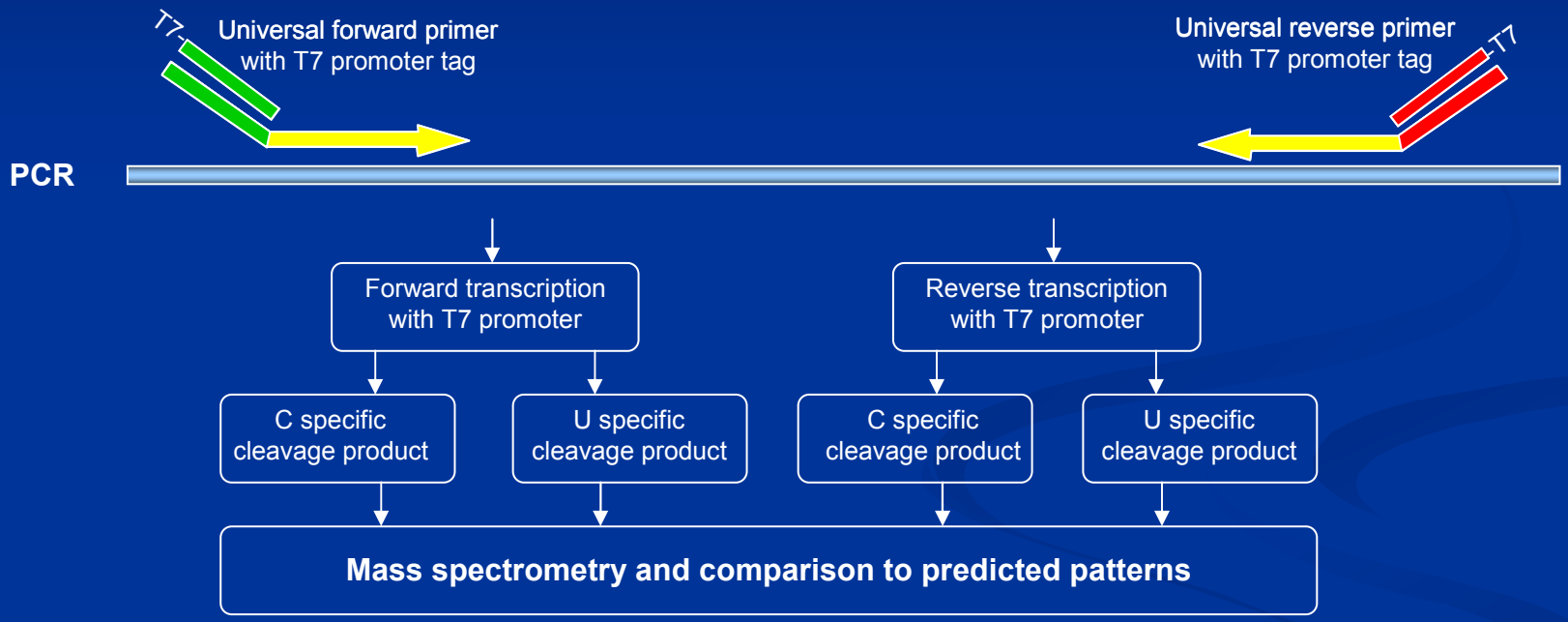


Evaluation of MassCleave™ Technology For Diagnostic Mutation Detection

Chris Mattocks

MassCleave™ chemistry



Ref: A strategy for the rapid discovery of disease markers using the MassARRAY system.
Rodi CP, Darnhofer-Patel B, Stanssens P, Zabeau M, van den Boom D. Biotechniques. 2002 Jun;Suppl:62-6, 68-9.

MassCleave™ Evaluation

- Existing PCR designs
- 12 fragments x 15 samples each + H_2O control
- Plate 1:
  - BRCA1 exon 11 - 6 fragments (B,C,G,J,K,L)
- Plate 2:
  - hMLH1 – 5 fragments (exons 2,4,12,13 & 16)
  - MSH2 – exon 15 only
- Sequenom provided primers
- Amplifications carried out in NGRL
- Analysis carried out by Sequenom

BRCA1 Results

Mutation type	Seq. results	Mutations identified with MassCLEAVE (SNP discovery software aided analysis)					
		flagged	missed	False +ve	position	nature	Zygosity hom/het
Insertion	5	4	1*	7	0	2	3
Deletion	32	26	6*	1	15	7	26
Het. point	34	34	0	0	20	34	34
Hom. point	60	60	0	0	45	60	60
Total	131	126	7*	8+1#	80	103	123

*retrospective inspection of spectra clearly displays the mutation identifying signals
#one unclassified variant was called in a normal sample.

hMLH1 &hMSH2 Results

Mutation type	Seq. results	Mutations identified with MassCLEAVE (SNP discovery software aided analysis)					
		flagged	missed	False +ve	position	nature	Zygosity hom/het
Insertion	5	4	0	0	4	4	5
Deletion	12	10	2*	0	1	1	8
Het. point	25+1#	25+1#	0	0	25+1#	25	25+1#
Hom. point	4	4	0	0	4	4	3
Total	46+1#	44+1#	2*	0	34+1#	34	41+1#

*retrospective inspection of spectra clearly displays the mutation identifying signals.
#two base pair substitution, AA>GC

Theoretical cleavage

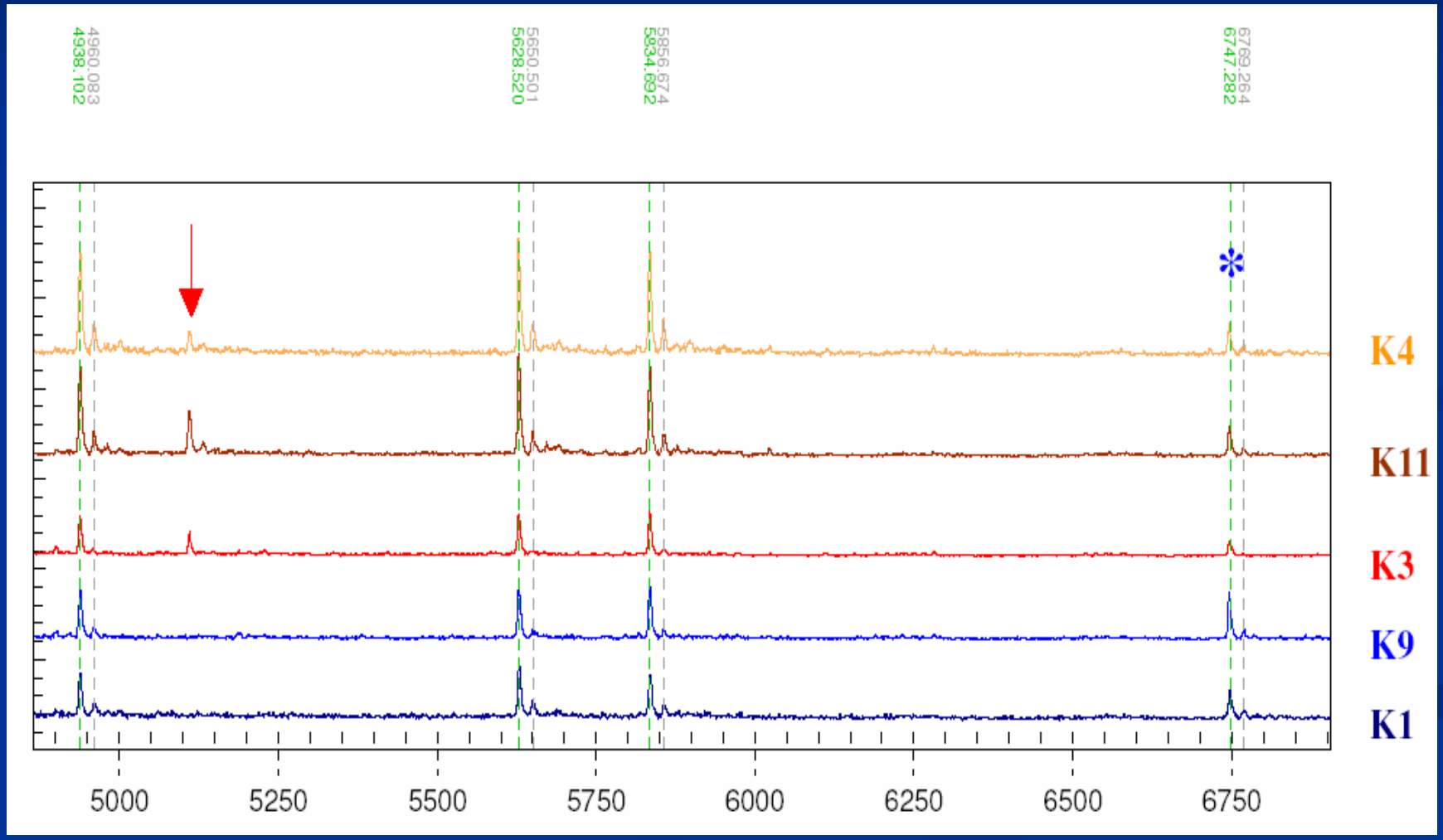
Rxn	mass	Relative frequency in normal	Relative frequency in mutant	Sequence
TR	4918	1	0.5	GAAAACGGAGCAAAT
TR	5247	0	0.5	GAAAACAGGAGCAAAT
CR	1689	1	0.5	GGAGC
CR	2018	0	0.5	AGGAGC

Frameshifts not flagged

- 3819 del GTAAA base 154 to 158 fragment K - 3/3 missed
  - Only 1 additional signal (within normal analysis range)
  - V. Low potential score
  - Software not currently designed to detect 5 bp deletions

Rxn	mass	Relative frequency in normal	Relative frequency in mutant	Sequence
TF	1658	1	0.5	AAAGT
CF	6747	1	0.5	TTGTTATTTGGTAAAGTAAAC
CF	5110	0	0.5	TTGTTATTTGGTAAAC
CR	1566	2	1.5	TTTAC

3819 del GTAAA C-froward



Frameshifts not flagged

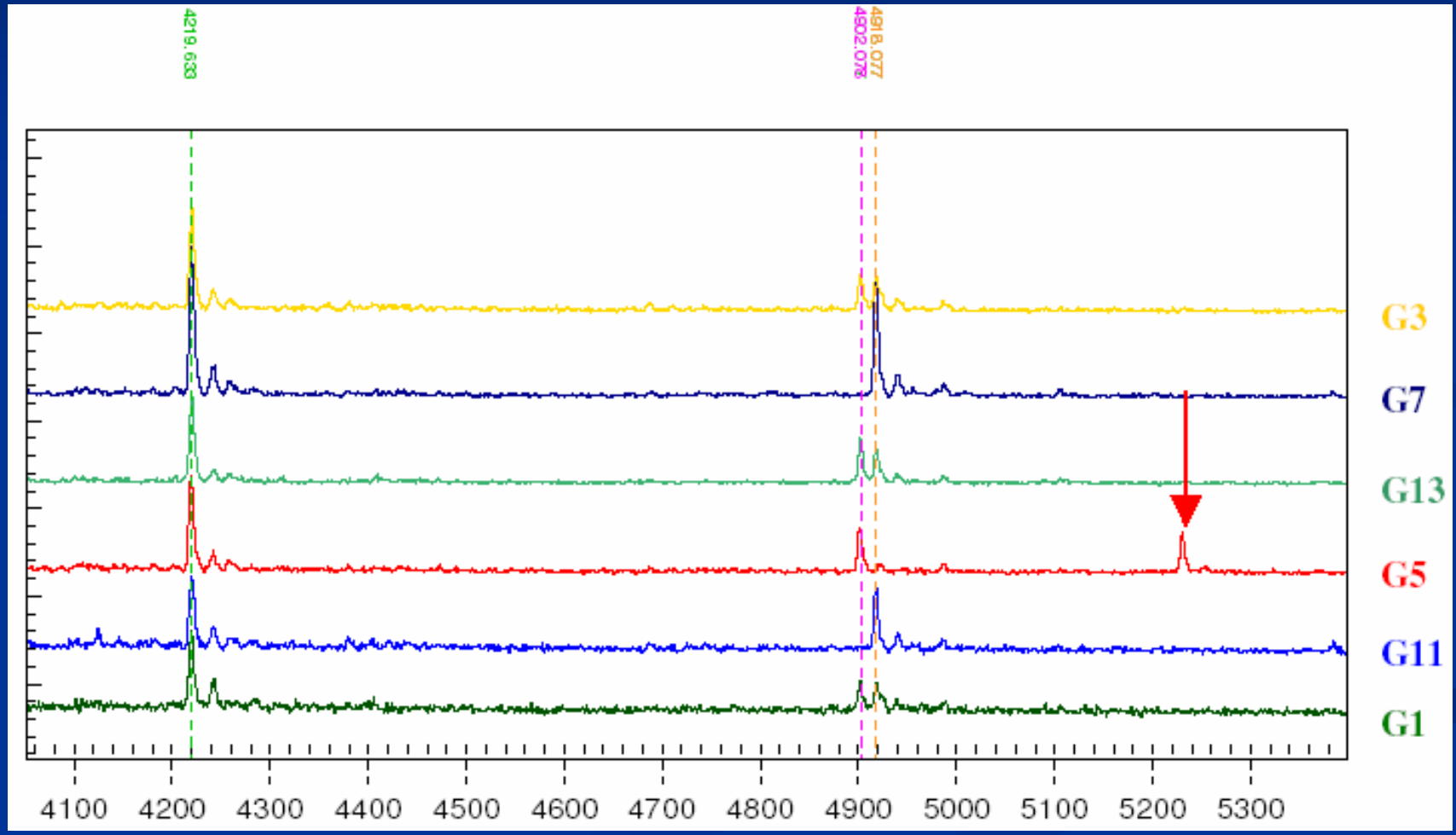
- 4184 del TCAA base 231-234 fragment L – 2/9 missed
  - Close proximity of 2nd mutation
  - Only T-forward gives unambiguous signals
  - Both amplicons failed/poor quality in T-forward
  - T rich fragment in C-reverse

Rxn	mass	Relative frequency in normal	Relative frequency in mutant	Sequence
TF	5520	1	0.5	CAAGAAGAACAAAGCAT
TF	5247	0	0.5	AAGAAGAACAAAGCAT
CF	2990	1	0.5	AAGAAGAAC
CF	5210	1	0.5	TTGGAAGAAAATAATC
CF	6913*	0	0.5	TTGGAAGAAAATAAGAAGAAC
CR	3761	1	0.5	TTGATTATTTTC
CR	2479	0	0.5	TTATTTTC

Frameshifts Misscalled

- **2731 ins T base 294 fragment G – miss-called as 2731 C>T hom**
  - Can only be discriminated by T-reverse and C-reverse
  - Presence second mutation
  - De-convoluted in retrospect

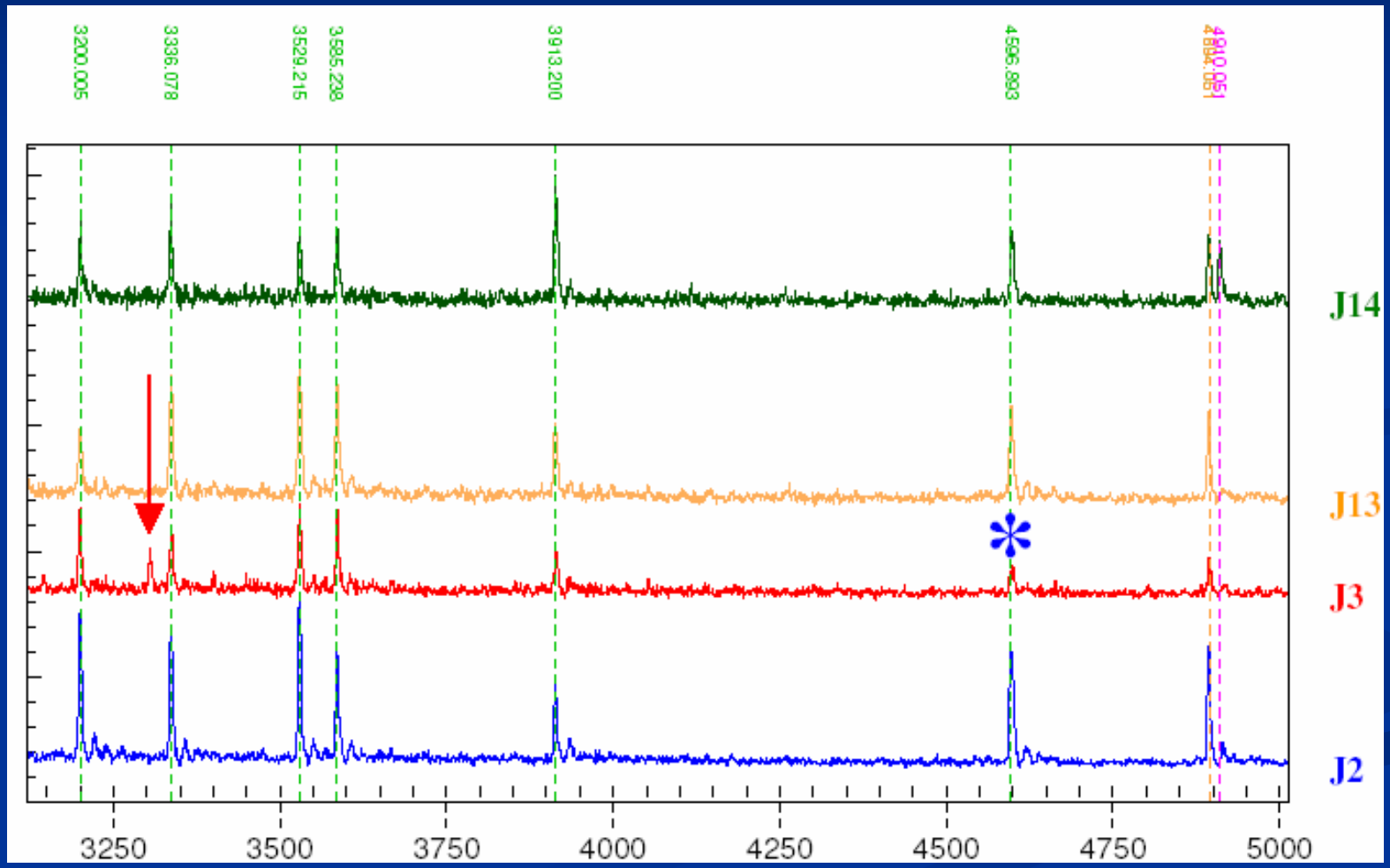
2731 ins T T-reverse



Frameshifts Misscalled

- **3450 del CAAG base 154 to 157 fragment J**
  - Only T-forward informative
  - Confounding noise (also seen in another sample)
  - Indicator seen in retrospect

3450 del CAAG T-forward



Point mutations miss-located

- 14 hets (3 unique)
  - 11 in fragments with 3 mutations
  - 2 in fragments with 2 mutations
  - 1 in fragment with 1 mutation
- 15 Hom (1 mutation – 15/15 cases)
  - One of calls correct

Summary

- Design non-optimal
- All point mutations flagged and called but position occasionally ambiguous
- 32/37 frameshifts flagged – only 2 unique mutations missed
- 2 frameshifts misscalled - would need confirmatory sequencing regardless

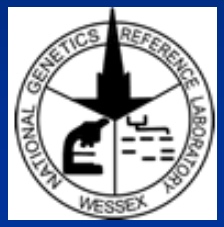
Conclusions

- MassCleave™ provides:
  - Fast sample turn around
  - Very low false positive rate
  - Very promising comparative re-sequencing method for diagnostic screening
- But:
  - More stringent (objective) quality criteria plus extension of data analysis range needed

Further work

- Larger study
  - Optimal design
  - Reference mutation controls
  - Defined quality criteria
  - Cost analysis
- Software development
  - Mulitple alignments?
  - Frameshifts?
  - Work in progress by Sequenom

Acknowledgements



NGRL (Wessex)
Helen White

SEQUENOM®
Niels Storm
Susan Muller

Preliminary report

available at http://www.ngrl.co.uk/Wessex/maldi_tof.htm

Preliminary Report

Evaluation of MassCLEAVE™ for Diagnostic Screening.

Prepared by Chris Mattocks, National Genetics Reference Laboratory (Wessex) in collaboration with SEQUENOM

Introduction

A number of the UK molecular genetics laboratories carry out routine [diagnostic] screens for diseases associated with multiple or large genes. To screen a single patient would typically require between 30 and 100 separate tests. Currently such screens are largely restricted to cancer genes such as BRCA1, BRCA2, hMLH1 and MSH2, but this list is likely to grow. This type of testing requires a high throughput approach in order to achieve adequate patient turnaround time. This has become particularly pertinent since the publication of the recent white paper on genetics (DOH 2003: *Our Inheritance,*