



NGRL (Wessex) DNA Extraction Evaluation: Integrated Systems

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NHS Trust

Integrated Systems

Robotic Platform	Chemagen Chemagic Magnetic Separation Module I			ABI PRISM™ 6100 Nucleic Acid PrepStation	Qiagen BioRobot EZ1	
Chemistry	M-PVA Magnetic Bead Technology			Integrated vacuum system	Silica/Magnetic based particles	
Blood volume	200µl	1ml	5ml	200µl	200µl	350µl
Elution volume	200µl	500µl	1000µl	200µl	200µl	200µl
Number of Samples per run	96 (92 Bloods, 4 Blanks)	12 (9 Bloods, 3 Blanks)	12 (9 Bloods, 3 Blanks)	96 (No Blanks)	6 (No Blanks)	6 (No Blanks)
Total number of Runs	1 (96 samples total)	23 (276 samples total)	23 (276 samples total)	Half a run (48 samples total)	16 (96 samples total)	16 (94 samples total)
Total Blanks tested	4	69	69	0	0	0
Total Bloods tested	92	207	207	48	96	94

Chemagen Chemagic Magnetic Separation Module I

Chemagen/Liquid Handling Robot Integration



Chemagen Chemagic Magnetic Separation Module I

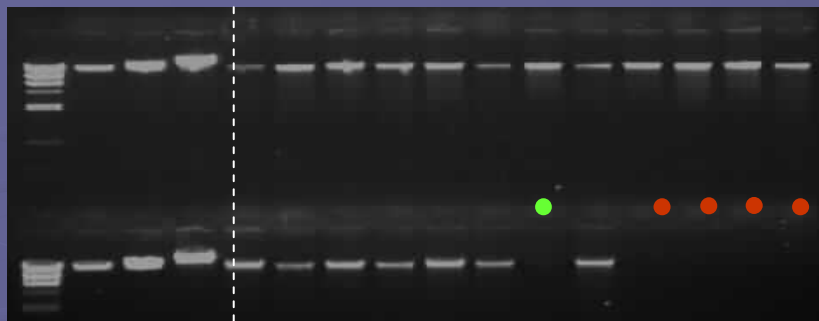
Overview

- M-PVA Magnetic Bead Technology
- PC attached integrated system
- Manual protease incubation (“semi” automated, but can be fully automated)
[see next slide]
- Inter-changable 12 / 96 magnetic rod heads
- Run time:
 - 12 – 40 mins
 - 96 – 20 mins
- Blood volumes: 200 μ l (96 head), 1 & 5ml (12 head)
- Elution volume: 200 μ l 500 μ l 1000 μ l
- Bloods / Blanks tested:
 - 200 μ l – 92 / 4
 - 1ml – 207 / 69
 - 5ml – 207 / 69

Chemagen Chemagic Magnetic Separation Module I

Sample Genomic DNAs

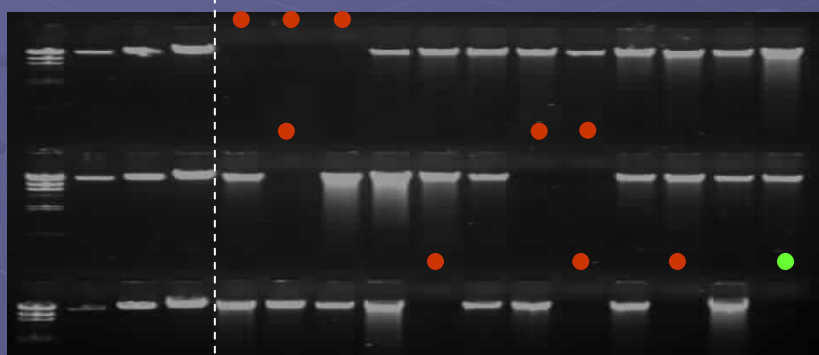
TOTALS:



200µl

BLANKS = 4

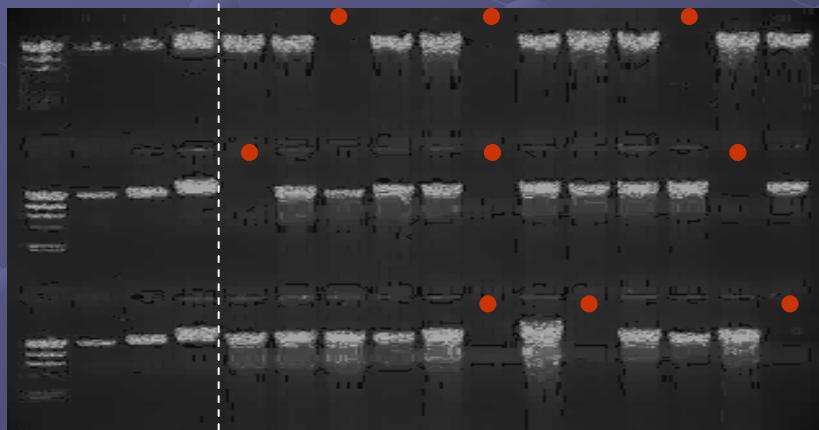
FAILS = 7



1ml

BLANKS = 69

FAILS = 1



5ml

BLANKS = 69

NO FAILS

Chemagen Chemagic Magnetic Separation Module I

PicoGreen / Absorbance Data - 200µl protocol

	PicoGreen		Absorbance			
	Elution Conc. (ng/µl)	Extraction Rate (µg/ml blood)	260/280	320	Elution Conc. (ng/µl)	Extraction Rate (µg/ml blood)
n	92	92	92	92	92	92
Min	0.1	0.1	1.02	0.001	2.0	2.0
Max	48.6	48.6	1.78	0.536	31.9	31.9
Average	17.1	17.1	1.53	0.017	15.5	15.5
St Dev	9.5	9.5	0.17	0.056	5.5	5.5
No. Fails	11		5			
% Fails	12.0%		5.4%			

“FAIL” =
<5ug/ml
extraction
rate

Chemagen Chemagic Magnetic Separation Module I

PicoGreen / Absorbance Data – 1ml protocol

	PicoGreen		Absorbance			
	Elution Conc. (ng/μl)	Extraction Rate (μg/ml blood)	260/280	320	Elution Conc. (ng/μl)	Extraction Rate (μg/ml blood)
n	207	207	207	207	207	207
Min	4.5	0.9	1.07	0.002	8.1	4.0
Max	482.4	96.5	1.84	0.079	255.9	128.0
Average	322.6	64.5	1.61	0.018	86.4	43.2
St Dev	101.6	20.3	0.10	0.009	39.7	19.9
No. Fails	5		1			
% Fails	2.4%		0.5%			

“FAIL” =
 <5ug/ml
 extraction
 rate

Chemagen Chemagic Magnetic Separation Module I

PicoGreen / Absorbance Data – 5ml protocol

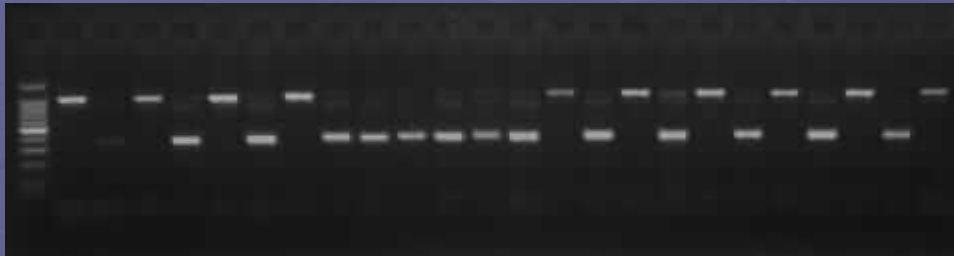
	PicoGreen		Absorbance			
	Elution Conc. (ng/μl)	Extraction Rate (μg/ml blood)	260/280	320	Elution Conc. (ng/μl)	Extraction Rate (μg/ml blood)
n	207	207	207	207	207	207
Min	488.6	19.5	0.44	0.003	64.4	12.9
Max	986.2	39.4	1.80	0.111	364.2	72.8
Average	723.7	28.9	1.39	0.012	192.9	38.6
St Dev	83.1	3.3	0.21	0.008	57.3	11.5
No. Fails	0		0			
% Fails	0%		0%			

“FAIL” =
 <5ug/ml
 extraction
 rate

Chemagen Chemagic Magnetic Separation Module I

Sample Sexing PCRS

TOTALS:



200µl

BLANKS = 4

FAILS = 7



1ml

BLANKS = 69

FAILS = 1



5ml

BLANKS = 69

FAILS = 1

Correspond to
gDNA fails

Chemagen Chemagic Magnetic Separation Module I

Summary / Comments

- Easy to use
- Plug-in and ready to go

Takes up a lot of space (requires corner for full automation with LH robot)

- Manual protease incubation at start adds ~45/35 mins (5ml/1ml) to run time
→ 85 / 55 mins total run time (5ml / 1ml)
- 200 μ l protocol run carried out manually – results not accurate reflection as this protocol should/would be carried out robotically to ensure consistent incubations between samples
- Full automation relatively expensive as it requires liquid-handling robot as well as Chemagen Module I
- 1ml / 5ml results consistent and acceptable (average extraction rate ~50 μ g/ml blood (1ml) and ~35 μ g/ml (5ml))
- 5ml DNAs appear to be better quality (0% fails vs 0.5-2.4% fail for 1ml (PG/Abs assays))
- 200 μ l protocol needs further evaluation under fully automated conditions

Qiagen BioRobot EZ1

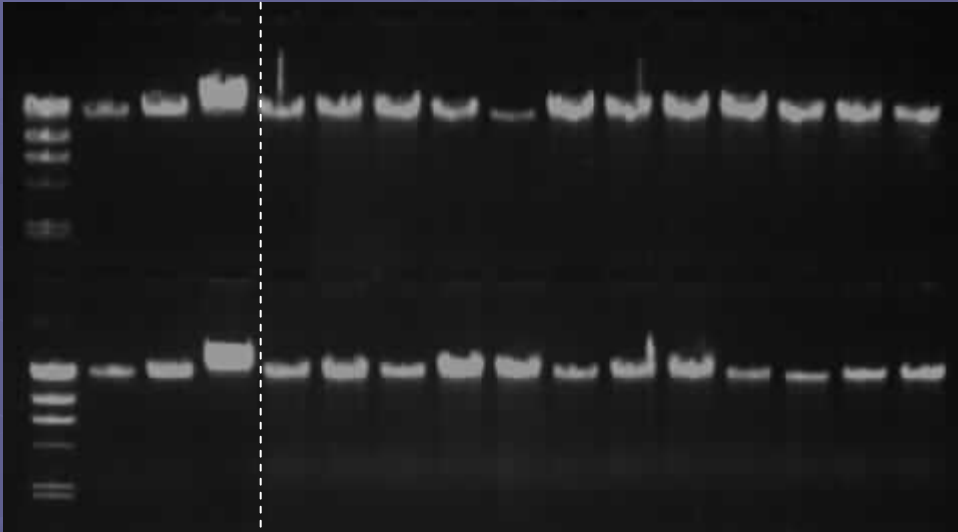
Overview

- EZ1 magnetic-particle technology
- Easy to use reagent cartridges
- Range of sample types (bloods, tissues, amnios)
- Plug-in cards for predefined protocols



- 1-6 samples in 15-20 mins
- Bloods tested:
 - 200 μ l: 96
 - 350 μ l: 94
 - No Blanks
- Elution volume: 50/100/200 μ l

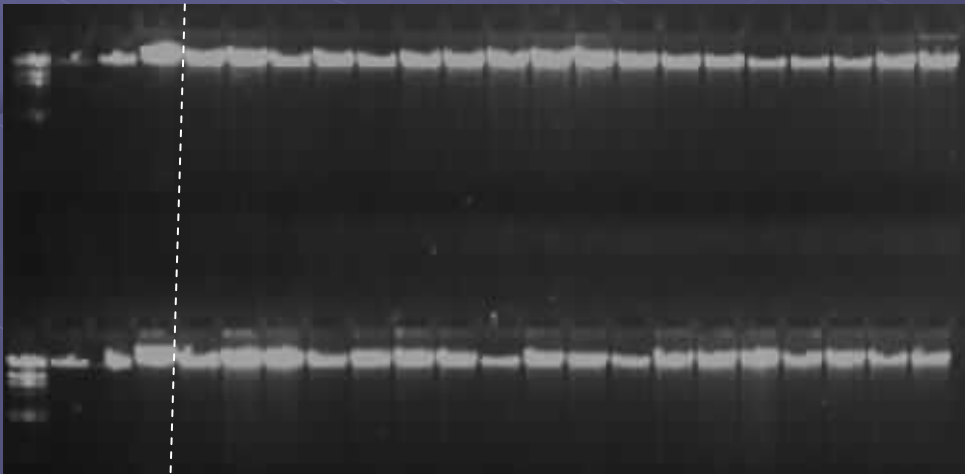
Qiagen BioRobot EZ1 Sample Genomic DNAs



200µl

NO BLANKS

FAILS = 3



350µl

NO BLANKS

NO FAILS

TOTALS:

Qiagen BioRobot EZ1

PicoGreen / Absorbance Data - 200µl protocol

	PicoGreen		Absorbance			
	Elution Conc. (ng/µl)	Extraction Rate (µg/ml blood)	260/280	320	Elution Conc. (ng/µl)	Extraction Rate (µg/ml blood)
n	96	96	96	96	96	96
Min	0.2	0.2	1.16	0.002	4.6	4.6
Max	165.0	165.0	1.87	0.025	86.2	86.2
Average	62.8	62.8	1.63	0.010	30.1	30.1
St Dev	24.5	24.5	0.10	0.004	10.5	10.5
No. Fails	3		1			
% Fails	3.1%		1.0%			

“FAIL” =
<5ug/ml
extraction
rate

Qiagen BioRobot EZ1

PicoGreen / Absorbance Data - 350µl protocol

	PicoGreen		Absorbance			
	Elution Conc. (ng/µl)	Extraction Rate (µg/ml blood)	260/280	320	Elution Conc. (ng/µl)	Extraction Rate (µg/ml blood)
n	94	94	94	94	94	94
Min	26.7	15.2	1.15	0.002	7.5	10.8
Max	198.1	113.2	1.87	0.033	49.6	61.8
Average	97.0	55.4	1.61	0.010	19.3	27.2
St Dev	36.0	20.6	0.11	0.006	7.6	9.9
No. Fails	0		0			
% Fails	0%		0%			

“FAIL” =
<5ug/ml
extraction
rate

Qiagen BioRobot EZ1

Sample Sexing PCRs

TOTALS:

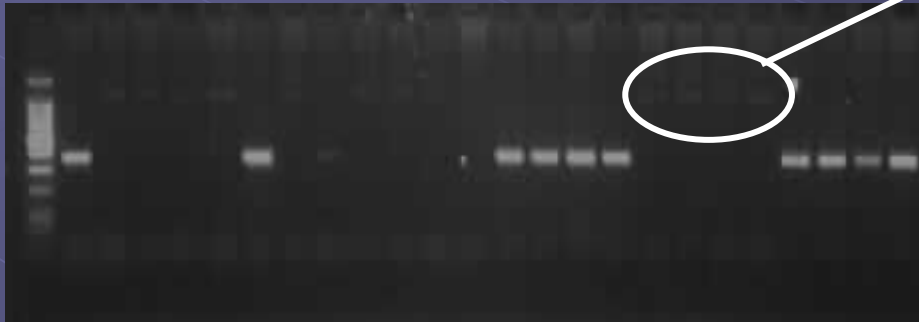


200µl

NO BLANKS

FAILS = 10

Female AMX bands
weak, but present



350µl

NO BLANKS

FAILS = 3

Qiagen BioRobot EZ1

Summary / Comments

- Very simple to operate and use
- Pre-packaged buffer cartridges easy, quick and reliable
- Plug-in and ready to go
- Very small and practical
- Very quick (both protocols setup in 5 mins and run in ~20 mins)
- Cheap (~£17K)
- Wide range of sample types each requiring a different card and buffer cartridge make the EZ1 excellent as an additional small scale extractor for samples that do not fit the exact criteria needed for the main automated DNA extraction platform that may be in use (e.g. amnios, Lith/Hep bloods)
- Consistent and acceptable gDNAs obtained
- Average extraction rates ~ 30-60 µg/ml blood for both protocols
- Further PCRs to be carried out to evaluate longer length fragment amplification (faint AMX bands on sexing PCRs~ 970bp)

ABI PRISM™ 6100 Nucleic Acid PrepStation

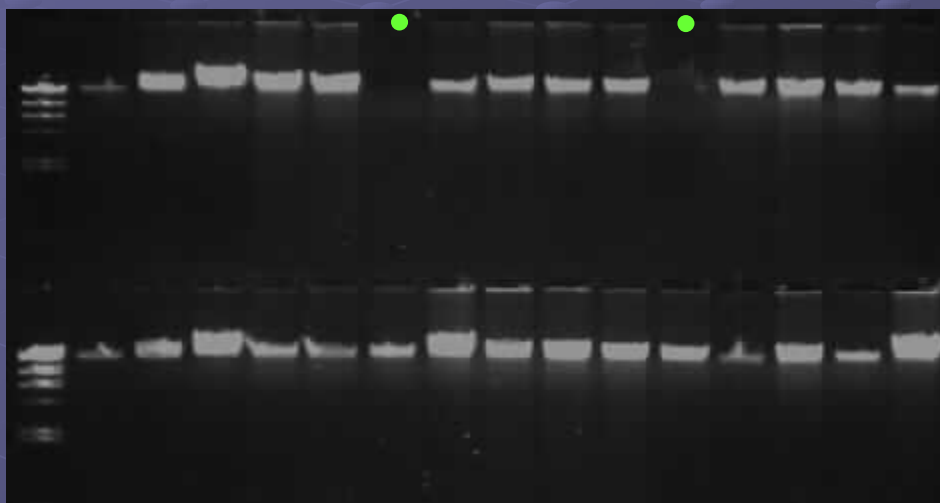
Overview

- Fully integrated vacuum based system
- Range of sample types
- 96-well plate format
- Small, desktop
- Manual reagent addition required
- Can create/edit custom purification routines
- Blood volume: 200µl
- Elution volume: 200µl
- 48 bloods, (no blanks)



ABI PRISM™ 6100 Nucleic Acid PrepStation

Sample Genomic DNAs



TOTALS:

NO BLANKS

FAILS = 3

ABI PRISM™ 6100 Nucleic Acid PrepStation

PicoGreen / Absorbance Data

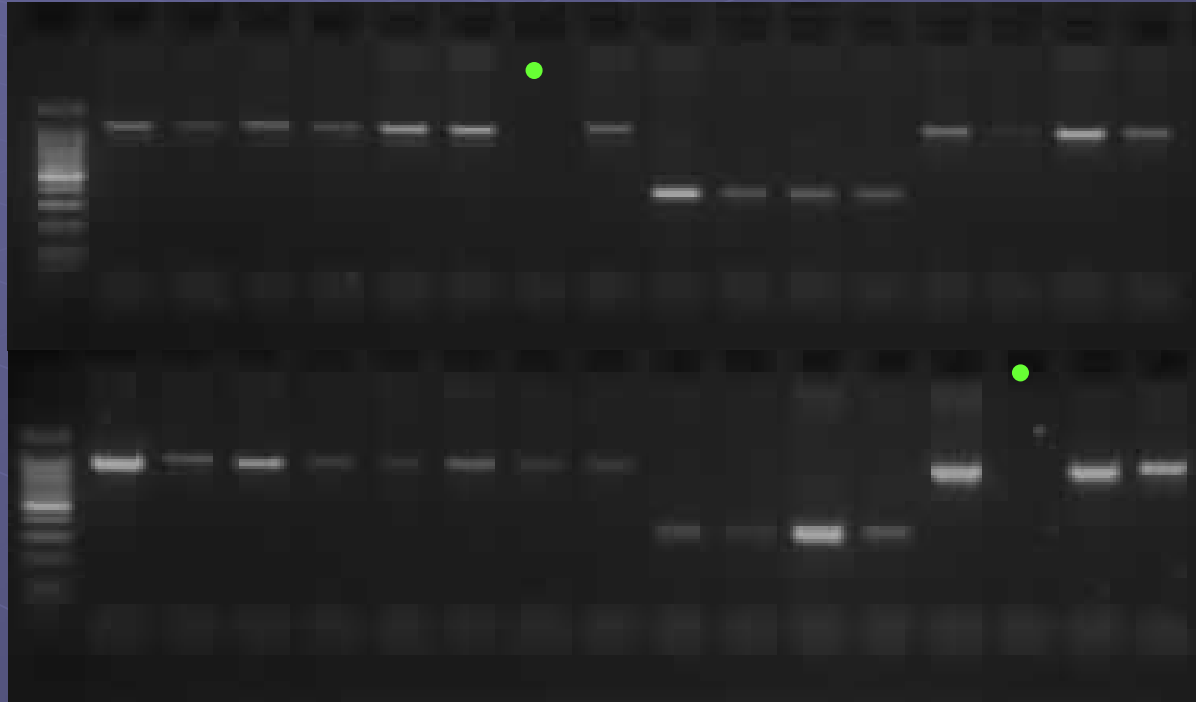
	PicoGreen		Absorbance			
	Elution Conc. (ng/μl)	Extraction Rate (μg/ml blood)	260/280	320	Elution Conc. (ng/μl)	Extraction Rate (μg/ml blood)
n	48	48	48	48	48	48
Min	0.1	0.1	0.91	0.001	0.8	0.8
Max	128.2	128.2	2.38	0.017	16.2	16.2
Average	51.4	51.4	1.34	0.009	7.5	7.5
St Dev	33.3	33.3	0.25	0.003	3.9	3.9
No. Fails	3		15			
% Fails	6.3%		31.3%			

Consistent with gDNA and PCR failure rate

“FAIL” = <5ug/ml extraction rate

ABI PRISM™ 6100 Nucleic Acid PrepStation

Sample Sexing PCRs



TOTALS:

NO BLANKS

FAILS = 3

ABI PRISM™ 6100 Nucleic Acid PrepStation

Summary / Comments

- Low scale evaluation (1 day, 48 samples)
- Semi-automated (lots of manual intervention (e.g. adding wash buffers) and no significant amount of “walk away” time)
- 3 fails (for gDNA gels, PCRs and PG assay) – all of which resulted from blocked wells early in process and therefore no DNA was expected to be obtained
- High “failure” (31.3%) according to Absorbance data clearly incorrect and further analysis of samples required to establish the cause of the low 260nm readings
- Average extraction rate ~50 µg/ml blood (PG) – (~7µg/ml for Abs assay ?)
- gDNA quality acceptable if blocked wells can be consistently avoided during the vacuum process
- Not suitable as automated DNA extraction platform – too many manual steps
(Similar role as Qiagen EZ1 as “back up” extractor ?)

Summary of Fails

	Chemagen Chemagic Magnetic Separation Module I			ABI PRISM™ 6100 Nucleic Acid PrepStation	Qiagen BioRobot EZ1	
	200µl	1ml	5ml	200µl	200µl	350µl
gDNAs (no visible band)	7.6%	0.5%	0%	6.3%	3.1%	0%
PicoGreen (Extraction Rate <5µg/ml)	12.0%	2.4%	0%	6.3%	3.1%	0%
Absorbance (Extraction Rate <5µg/ml)	5.4%	0.5%	0%	31.3%	1.0%	0%
PCRs (no visible band/s)	7.6%	0.5%	0.5%	6.3%	10.4%	3.2%

Future Testing

- Further analysis of Absorbance v PicoGreen data
- Downstream:
 - Further PCRs
 - Multiplex Ligation Probe Amplification (MLPA)
 - Southern blotting

...Whatman FTA

- FTA card based extraction
- <120µl blood blotting on card and left to dry
- 1mm punch taken, washed and added to PCR



Summary

- Chemagen system worked well for large scale extractions.
- Qiagen EZ1 low cost, simple to use and flexible for small scale extractions. Probably suitable as secondary protocol if large scale extraction required.
- ABI 6100 worked well but only semi-automated (extensive manual intervention required).
- Whatman FTA system looks promising for simple PCR based testing but further work needed. Automation options available.

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