NGRL (Wessex) DNA Extraction Evaluation: Chemistries

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Background

- Autogen NA 3000: Automated nucleic acid isolation system
  - Dec 2002
  - Full evaluation completed and presented at BSHG York 2003
  - Report yet to be published
- Subsequent trials:
  - Chemagen Chemagic Module I
  - ABI 6100 Nucleic Acid PrepStation
  - Qiagen EZ1 BioRobot

  Integrated systems combining robotic platform and chemistry
  - DRI ChargeSwitch Technology
  - Machery-Nagel Nucleospin 96
  - Promega Magnesil

  Individual chemistries set up on a liquid handling robotic platform:
  - **Tecan EVO 150**

Outline

- Tecan EVO Freedom 150
- How we evaluated the gDNAs
    - DNA quality
    - DNA quantification
    - PCRs
- Results / comments
    - DRI chemistry
    - Macherey Nagel chemistry
    - Promega chemistry
- General conclusions on robot-based chemistries
- Further testing

Tecan Freedom EVO 150

System tubing

Hotel
(extra plate storage)

LiHa
(Liquid Handling arm)

ROMA
(RObotic Manipulating Arm)

Fixed pipettes
(also location for DITI heads)

Tecan GENios Plus
(DNA quantifier)

Buffer reservoirs

Vacuum Manifold
(as used in Machery Nagel Nucleospin 96 chemistry)

Shaker
(used in Promega and DRI chemistries)

Tecan cont…

- Liquid handling robotic platform used to assess all robotic based chemistries
- Flexible platform suitable for many different applications
- Potential to evolve platform to reflect changing needs
- User friendly software

- Accessories and add-ons
  - Vacuum Manifold – MN NS96
  - Shaker – Promega, DRI
  - GENios Plus

Genomic DNA Quality

- Theoretical 120ng gDNA loaded on 0.6% agarose gel (assuming an expected “extraction efficiency” of ~30ug DNA per ml of blood)
- Each sample run against:
  - Lambda (λ) / *Hind III* ladder
  - 50, 100 and 200ng λ DNA

DNA Quantification

- Two methods used:
  - PicoGreen assay (fluorescence based)
  - Absorbance
- Compatible plates for DNA quantification:

Luminescence (requires white, FB plates)

Fluorescence (requires black, opaque flat bottomed (FB) plates – e.g. Greiner, Corning)

Absorbance (requires optically clear, UV transparent, FB plates – e.g. Greiner, Corning)

Tecan GENios Plus

Excitation filter slide (contains 4 different filters for 4 wavelength readings)

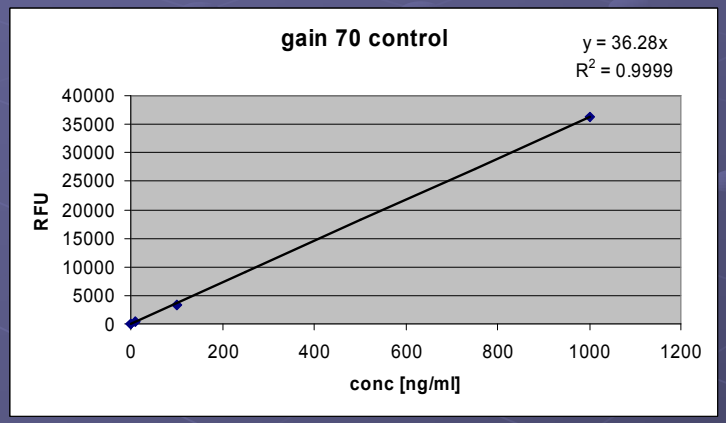
Sample plate

Emission filter slide (contains 4 different filters for 4 wavelength readings – used for fluorescence and luminescence quantification)

- Fluorescence, Absorbance, Luminescence
- Handles assays in 6 – 384 microplates, PCR tubes and cuvettes
- Multilabel reader
- Top or bottom reading capability

PicoGreen Assay

- Theoretical 50ng gDNA (assuming expected extraction efficiency of 30µg DNA per ml of blood) made up to 100µl with 1x TE
- 100µl PicoGreen (diluted 1/200 with 1x TE)
- Standard curve created using a range of λ control DNA from 0 - 1µg/ml



Highly correlated line of best fit (R^2=0.9999)

Relationship: $\text{Conc.} = \frac{\text{corrected RFU}}{36.28}$

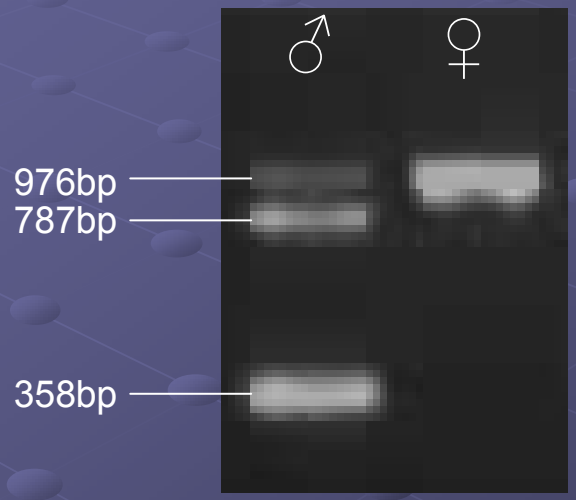
- Blank value = 49 RFU
- Data used to determine:
  - DNA conc. of sample (ng/µl = µg/ml)
  - Extraction efficiency (µg DNA per ml of blood)

Absorbance

- Optically clear, UV transparent FB plates
- Tecan GENios Plus
- Theoretical 2µg DNA (except DRI – 200ng)
    - made up to 200µl
- Readings taken @ 260nm, 280nm and 320nm:
    - 260 blank value: 0.050
    - 280 blank value: 0.033
    - 320 blank value: 0.026
- Light path = 6mm
    - → OD of 1.0 @ 260nm = 83.33ng/µl
- 260/280 purity ratio (1.8 ± 0.1)
- 320 (particulate contamination)

Downstream tests: PCR

- Theoretical 30ng DNA added to each PCR
- Duplex PCR:
  - AMXY F/R: amelogenin (Xp and Yp11.1)
  - SRY F/R: SRY gene



- Tests range of amplification up to 1kb

Tecan based chemistries

Chemistry	Machery Nagel NucleoSpin 96	Promega MagneSil	DRI ChargeSwitch Tecnology (CST)
Extraction method	SPE Vacuum based, Silica Membrane	MagneSil Paramagnetic Particle technology	pH based magnetic beads
Blood volume	200µl	5ml	20µl
Elution volume	200µl	~1000µl*	100µl
Number of Samples per run	96** (2x 36 blood, 2x 12 blank)	8 (6 blood, 2 blank)	48 (36 blood, 12 blank)
Total number of Runs	3 (288 samples total)	24 (192 samples total)	9 (432 samples total)
Total Blanks tested	48	48	84
Total Bloods tested	240	144	348

*Exact elution volume calculated by software based on actual volume of blood. All elutions were approximately 1ml

**Each run consists of two duplicate extractions of 48 blood samples. Run 1 contained no blanks, whilst runs 2 and 3 contained blanks

DRI ChargeSwitch™ Technology (*CST*®)

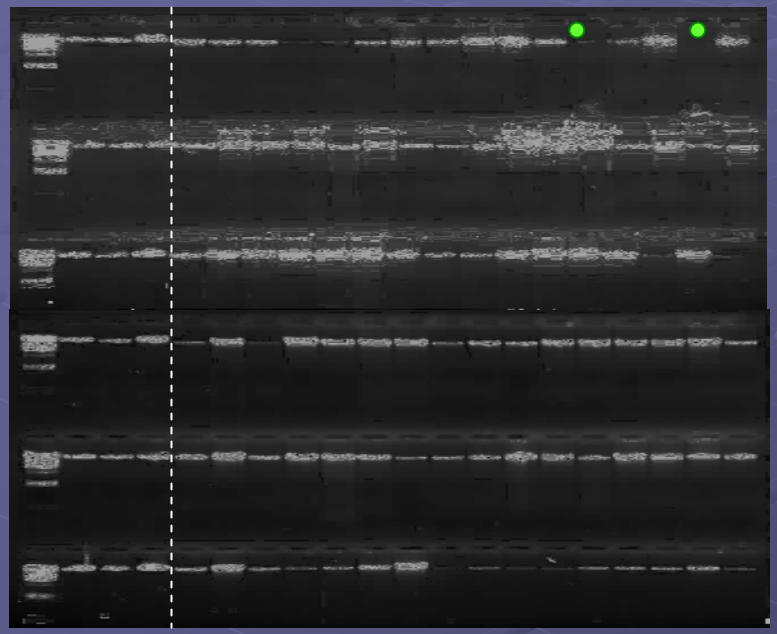
Overview

- Automated partnership with Tecan UK
- CST magnetic beads
- Switchable surface charge based on pH of surrounding buffer
    - ↓pH → +ve charge → binds DNA but not protein/contaminants
    - Impurites washed away in aqueous wash buffer
    - ↑pH (8.5) → -ve charge → elution of DNA into low salt elution buffer
- No enzyme inhibiting reagents (e.g. Alcohol, chaotropic salts, organic solvents)
- Range of extraction protocols
    - 10-20µl blood (manual and automated) - 100µl elution volume
    - 50-100µl blood (manual and automated)
    - Buccal cells (manual only)
- 84 blanks, 348 bloods

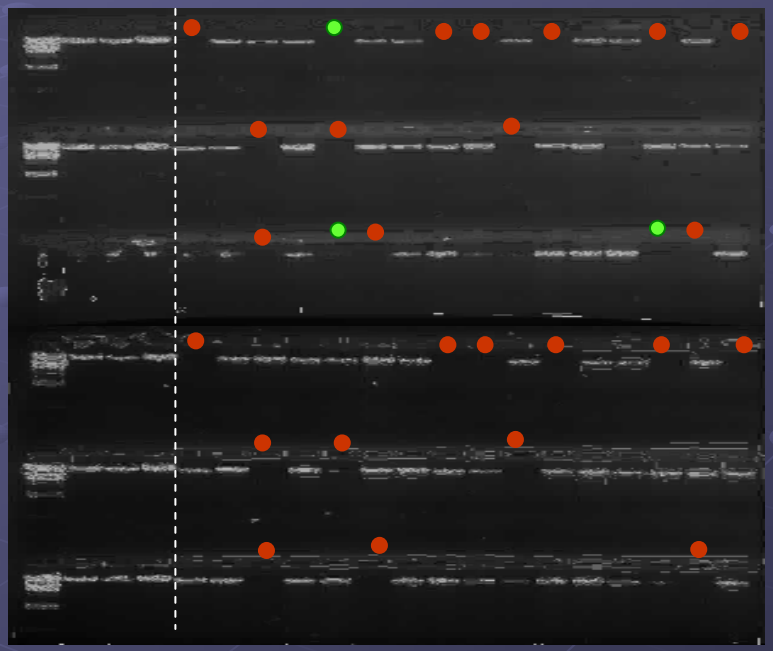
DRI ChargeSwitch™ Technology (*CST*®)

Sample Genomic DNAs

Trial Run



Run 3



● = Fail
● = Blank

TOTALS: BLANKS = 86

FAILS = 18/348 (5.2%)

DRI ChargeSwitch™ Technology (*CST*®)

PicoGreen/Absorbance Data

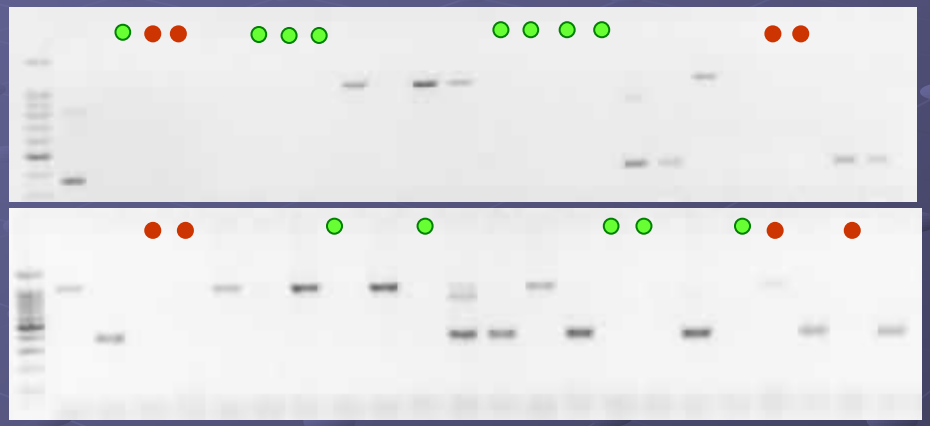
	PicoGreen		Absorbance			
	Elution Conc. (ng/µl)	Extraction Efficiency (µg DNA /ml blood)	260/280	320	Elution Conc. (ng/µl)	Extraction Efficiency (µg DNA /ml blood)
n	348	348	348	348	348	348
Min	0.0	0.0	0.44	0.005	3.1	15.3
Max	23.7	236.8	1.44	1.092	354.0	1770.1
Average	5.2	51.8	1.14	0.052	21.5	107.5
St Dev	5.0	49.8	0.01	0.119	37.7	188.4
No. Fails	34		0			
% Fails	9.8%		0%			

"FAIL" = <5ug/ml extraction rate

DRI ChargeSwitch™ Technology (*CST*®)

Sample Sexing PCRs

● = Fail
● = Blank



TOTALS: BLANKS = 84

FAILS = 195/348 (56.0%)

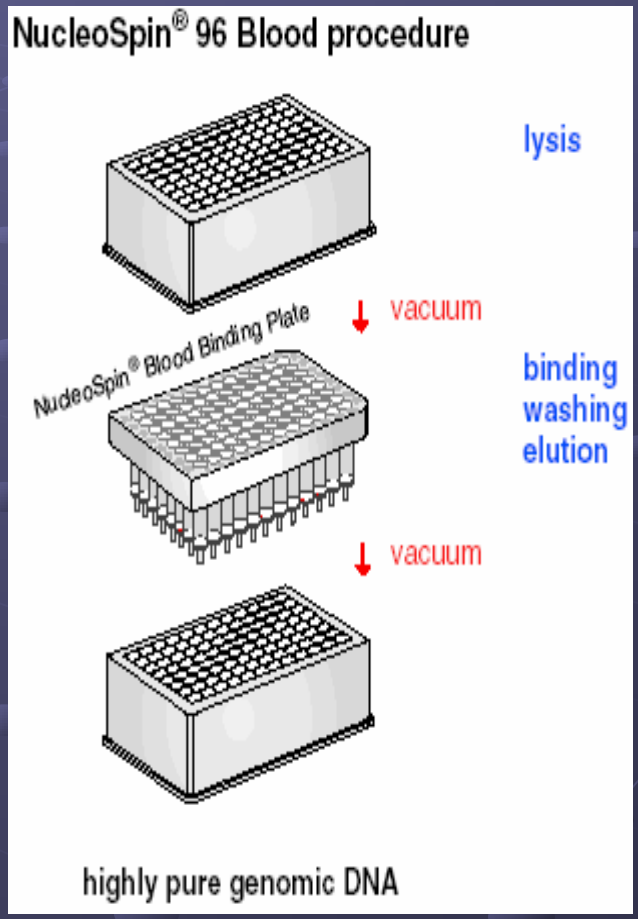
DRI ChargeSwitch™ Technology (*CST*®)

Summary Comments

- Very low volume of blood (20µl) – probably too low to ever be used diagnostically (100-200µl protocol available)
- CST bead residue (inert?)
- Unacceptable failure rate (9.8%)
- Average extraction efficiency ~ 50-100ug/ml blood (PG v Abs?)
- 56.0% PCR failure rate more reflective of problems with initially setting up the protocol on the Tecan Evo 150, not necessarily protocol itself
- Fine tuning required to reduce alcohol/wash buffer contamination (→ downsteam failure ?)

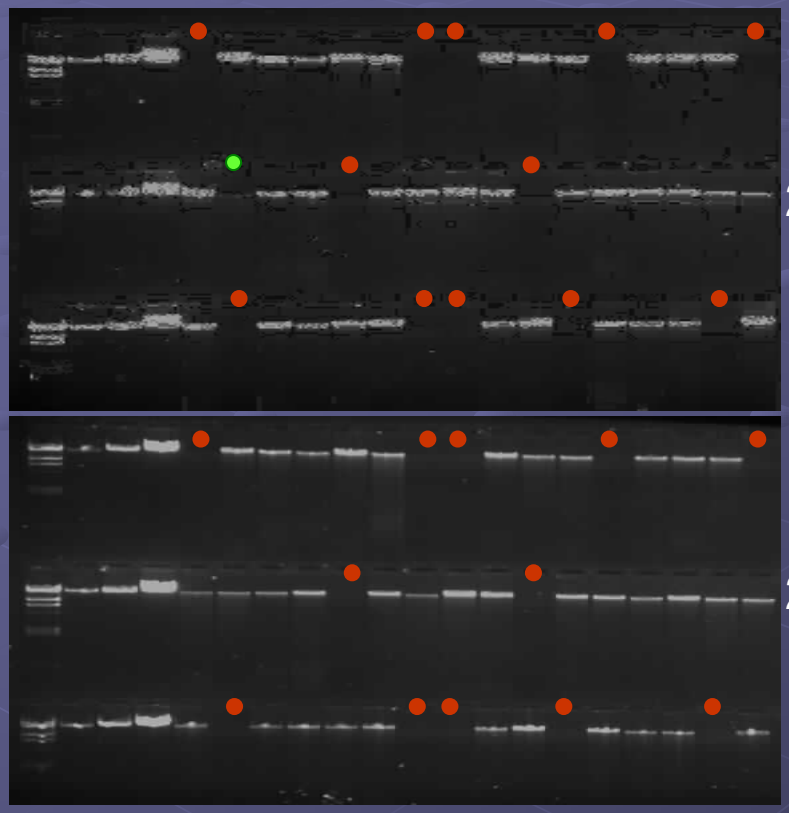
Macherey Nagel NucleoSpin® 96 Blood
Overview

- Silica membrane technology:
  - Blood cells lysed
  - gDNA selectively bound to membrane whilst impurities are washed away via vacuum pull through
  - Elution into 96-well plate for storage
- Vacuum based
- 200ul blood
- 100ul elution volume
- 48 blanks
- 240 bloods



Macherey Nagel NucleoSpin® 96 Blood

Sample Genomic DNAs



2A

2B

Duplicate runs

TOTALS: BLANKS = 48

FAILS = 2/240 (0.8%)

● = Blank

● = Fail

Macherey Nagel NucleoSpin® 96 Blood

PicoGreen / Absorbance Data

	PicoGreen		Absorbance			
	Elution Conc. (ng/µl)	Extraction Efficiency (µg DNA /ml blood)	260/280	320	Elution Conc. (ng/µl)	Extraction Efficiency (µg DNA /ml blood)
n	240	240	240	240	240	240
Min	1.1	1.1	1.20	0.003	7.1	7.1
Max	46.1	46.1	1.91	0.031	48.7	48.7
Average	19.6	19.6	1.55	0.012	24.1	24.1
St Dev	8.3	8.3	0.11	0.004	6.9	6.9
No. Fails	6		0			
% Fails	2.5%		0%			

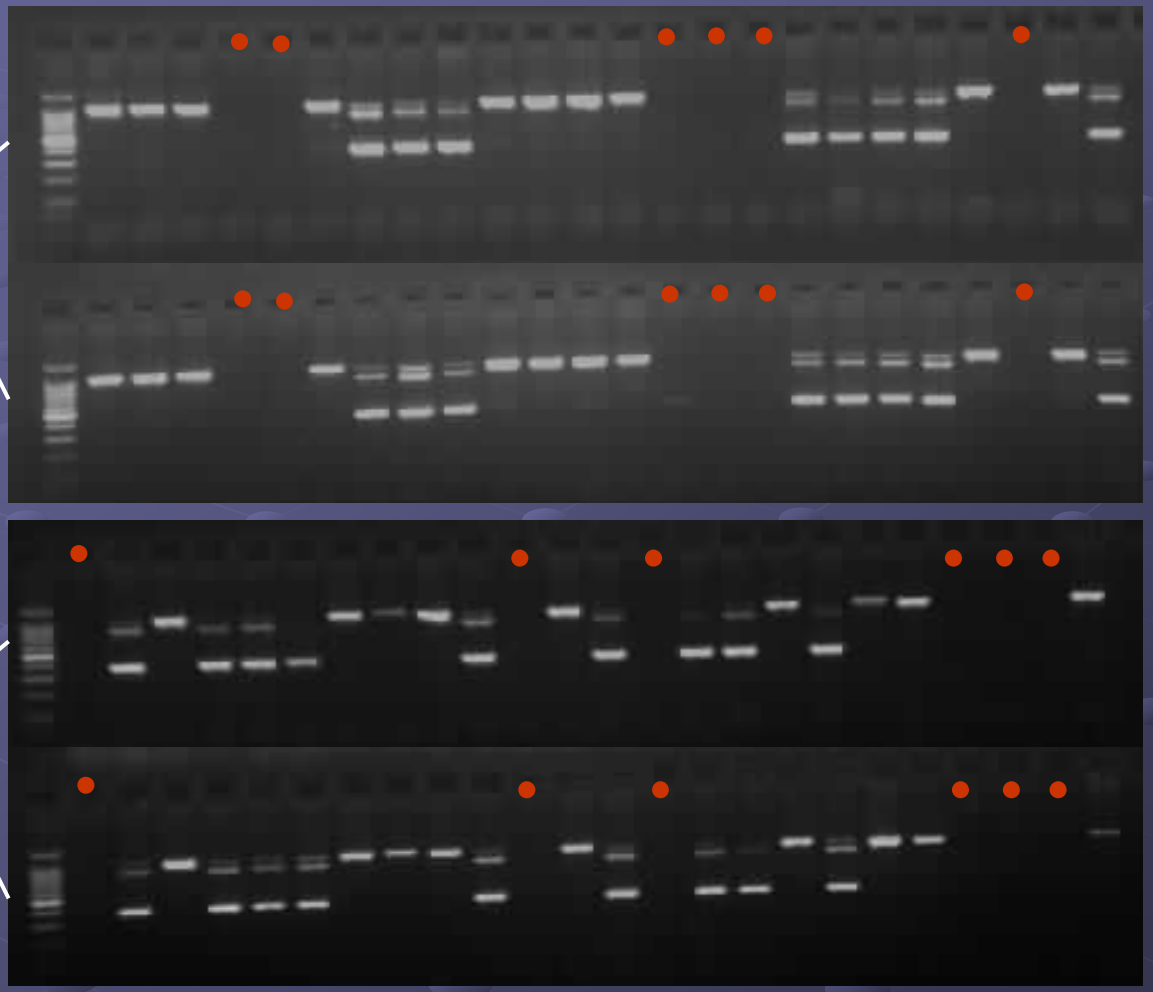
"FAIL" = <5ug/ml extraction rate

Macherey Nagel NucleoSpin® 96 Blood

Sample Sexing PCRs

Duplicate runs

Duplicate runs



TOTALS: BLANKS = 48 FAILS = 2/240 (0.8%)

Macherey Nagel NucleoSpin® 96 Blood
Summary Comments

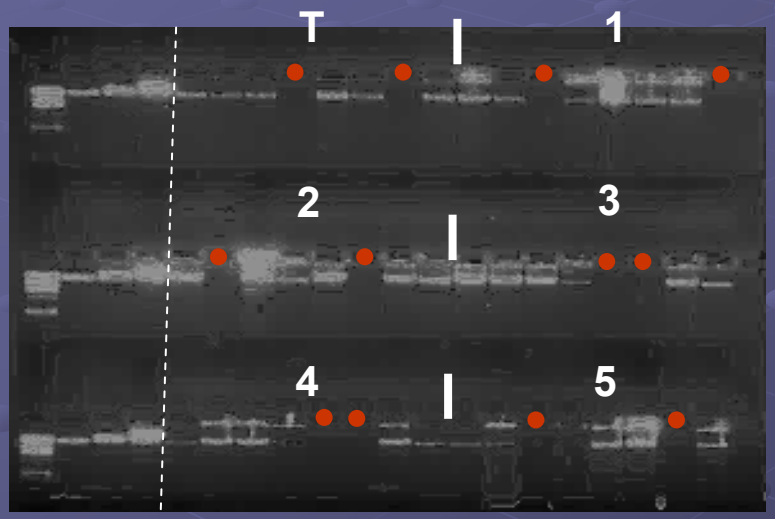
- Vacuum manifold *very* noisy!
- Vacuum manifold required manual pressure to ensure an acceptable suction (manual removal of blocked wells also required to prevent overflow contamination)
- Liquid sensing step after each suction to ensure total pull through
- 2 failed samples (0.8%) due to blocked wells (no DNA expected)
- average extraction efficiency ~ 20ug/ml blood
- Good quality gDNA (high PCR success rate indicates no contamination – no wash/removal step as all wash buffer is pulled though)
- Vacuum steps need to function in a fully automated way

Promega MagneSil™ Blood Genomic
Overview

- MagneSil™ paramagnetic particles
  - Lysis buffer added
  - Magnetic beads added
  - Lysis buffer / alcohol wash
  - Elution
- Additional equipment:
  - Shaker
  - Elution incubator
  - Promega tube and magnet rack (4x2 holding 50ml falcon tubes)
- 5ml blood
- ~1ml elution volume
- 48 blanks
- 144 bloods

Promega MagneSil™ Blood Genomic

Sample Genomic DNAs



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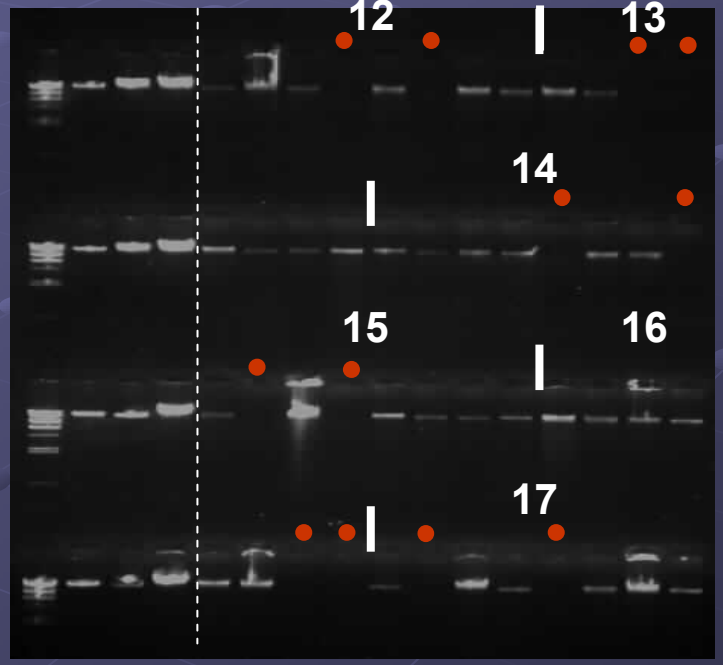

TOTALS: BLANKS = 48

NO FAILS (0%)

Promega MagneSil™ Blood Genomic

PicoGreen / Absorbance Data

	PicoGreen		Absorbance			
	Elution Conc. (ng/µl)	Extraction Efficiency (µg DNA /ml blood)	260/280	320	Elution Conc. (ng/µl)	Extraction Efficency (µg DNA /ml blood)
n	144	144	144	144	144	144
Min	1.2	0.1	0.52	0.001	6.8	1.5
Max	2764.4	125.7	5.27	0.321	1218	267.2
Average	779.3	35.4	1.38	0.018	78.8	20.8
St Dev	553.2	24.9	0.52	0.032	150.2	35.9
No. Fails	5		5			
% Fails	3.5%		3.5%			

"FAIL" = <5ug/ml extraction rate

Promega MagneSil™ Blood Genomic

Sample Sexing PCRs

TOTALS: BLANKS = 48 FAILS = 96/144 (66.7%)

Promega MagneSil™ Blood Genomic
Summary / Comments

- 0 - 3.5% failure rates for gDNA gels and both quantification assays suggests generally good DNA extraction, but…
- 66.7% PCR failure rate almost certainly due to buffer/alcohol contamination due to frequent failure to remove all the waste between washes (LiHa alignment in 50ml tubes when removing waste)
- Average extraction rate ~ 20-35 µg/ml blood
- Further evaluation of protocol required (in addition to Macherey Nagel and DRI) with more time to fine tune the robot-chemistry integration

PicoGreen Results

(Bloods only)

	Promega MagneSil		Machery Nagel NucleoSpin 96		DRI CS*T*	
	Elution Conc. / ng/µl	Extraction rate / µg/ml blood	Elution Conc. / ng/µl	Extraction rate / µg/ml blood	Elution Conc. / ng/µl	Extraction rate / µg/ml blood
n	144	144	240	240	348	348
Min	1.2	0.1	1.1	1.1	0.0	0.0
Max	2764.4	125.7	46.1	46.1	23.7	236.8
Average	779.3	35.4	19.6	19.6	5.2	51.8
SD	553.2	24.9	8.3	8.3	5.0	49.8
No. Fails (<5µg/ml blood)		1		6		34
% Fails		0.7%		2.5%		9.8%

Summary of Fails

	Promega MagneSil	Machery Nagel NucleoSpin 96	DRI *CST*
gDNAs (no visable band)	0%	0.8%	5.2%
PicoGreen (<5µg DNA /ml)	3.5%	2.5%	9.8%
Absorbance (<5µg DNA /ml)	3.5%	0%	0%
PCRs (no visable band/s)	66.7%	0.8%	56.0%

General robot-based chemistry conclusions

- Setup time
  - Chemistries not ready to go
  - Adjustments / development
  - Months rather than days / weeks
- Unsuitable diagnostic setup
  - System tubing (Promega or any large volume chemistry)
  - Open blood tubes
  - Lab specific requirements
- DNA quantification
  - Not straight forward
  - Varying results between techniques
  - Further evaluation required

Given time we are confident that all three chemistries could be successfully, reliably and efficiently integrated into a diagnostic laboratory environment

Future Testing

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

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