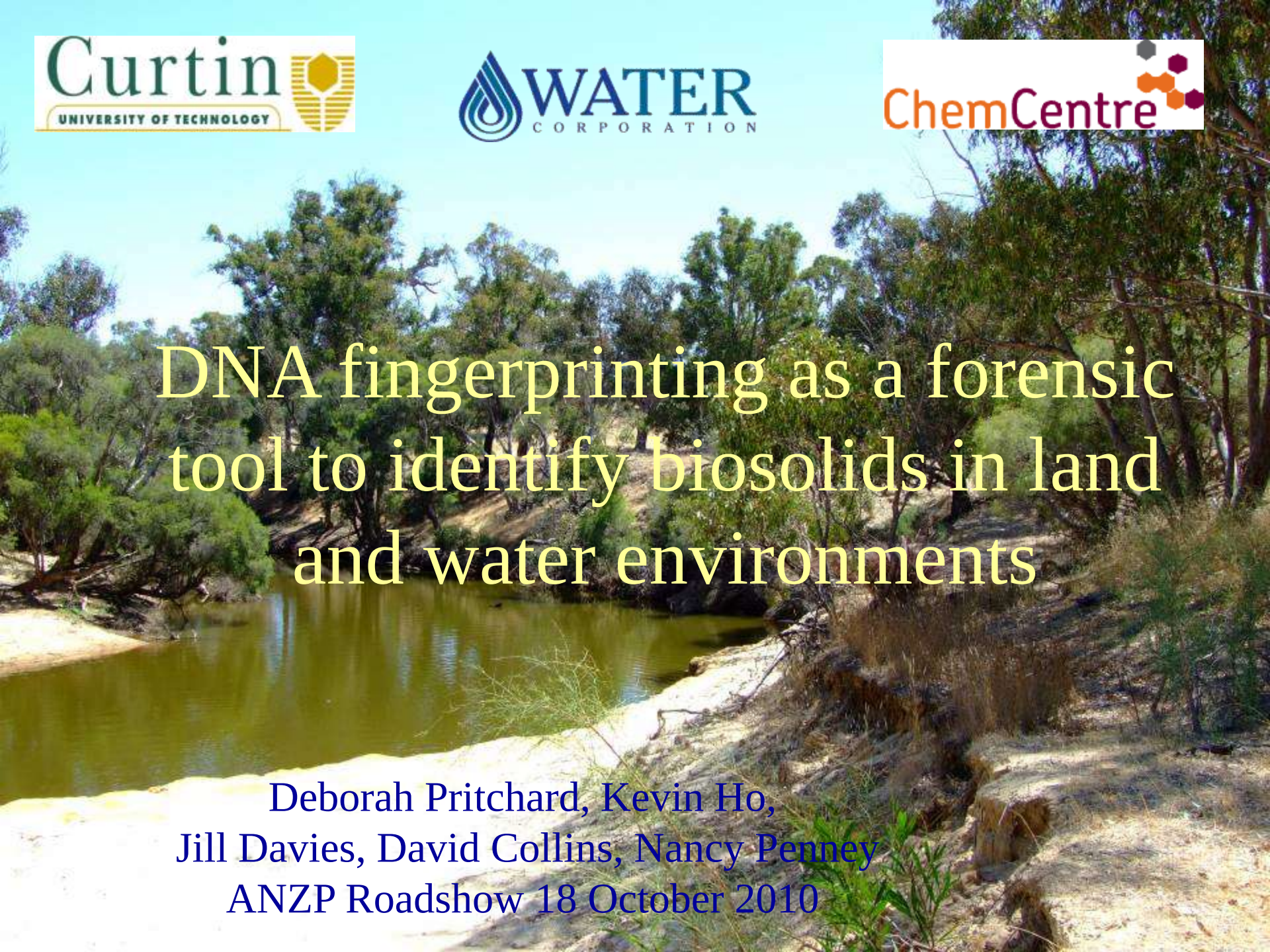

DNA fingerprinting as a forensic tool to identify biosolids in land and water environments

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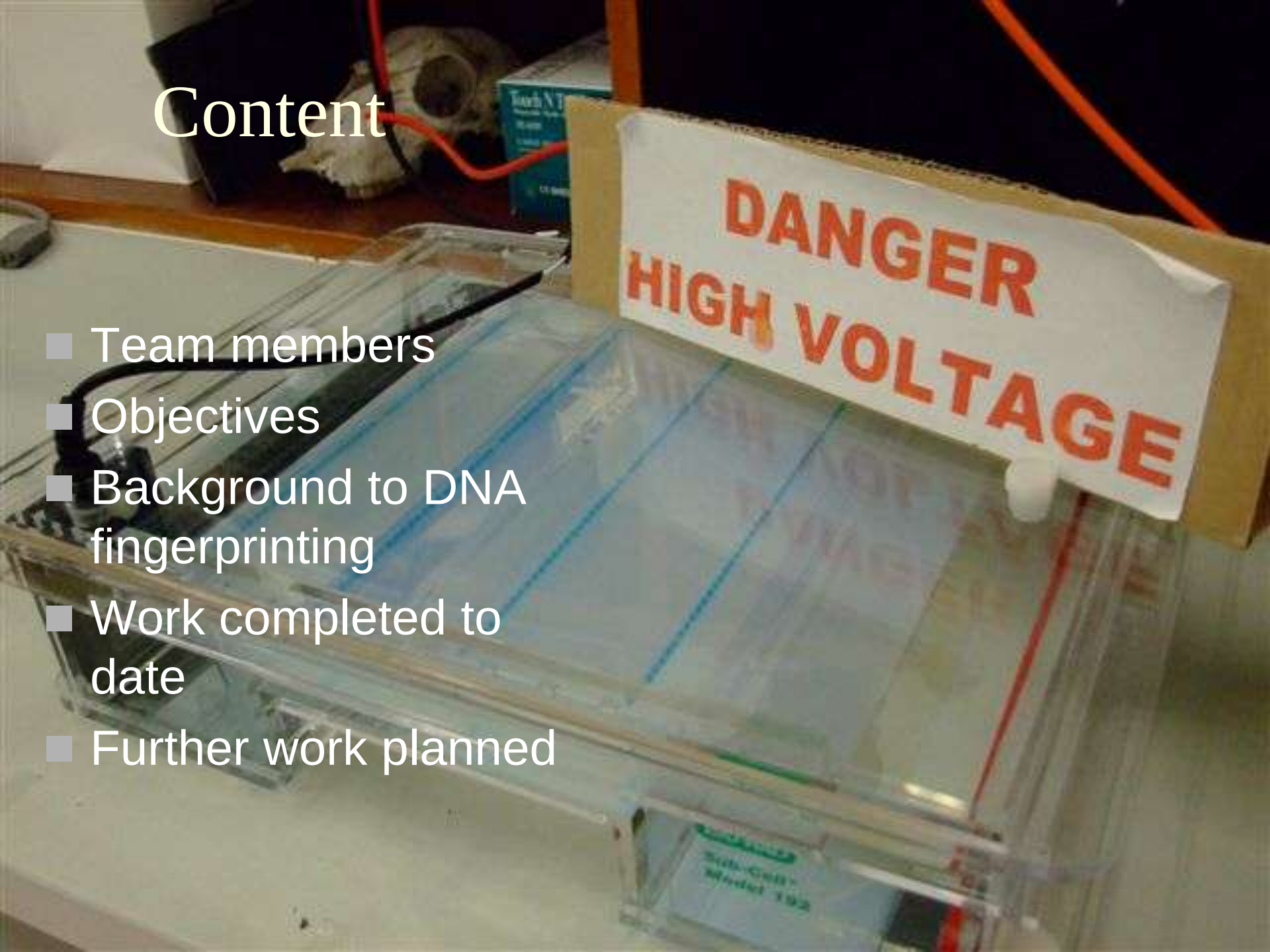
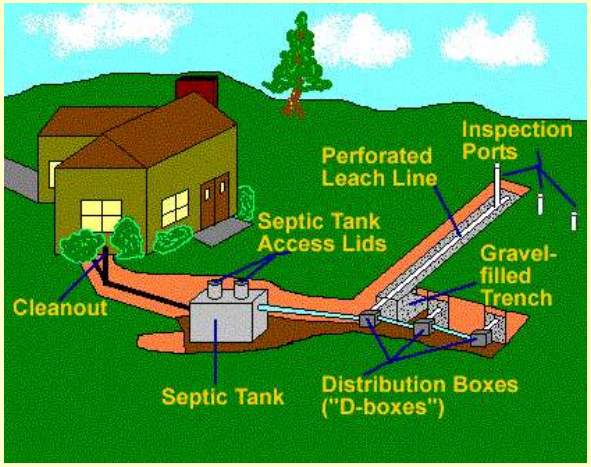
ANZP Roadshow 18 October 2010

Content



- Team members
- Objectives
- Background to DNA fingerprinting
- Work completed to date
- Further work planned

Pro-active Approach to Assure the Public that Biosolids are not a Risk to the Environment



Project Objective 1

Use molecular source tracking (DNA fingerprinting) to conclusively identify biosolids from animal faecal samples

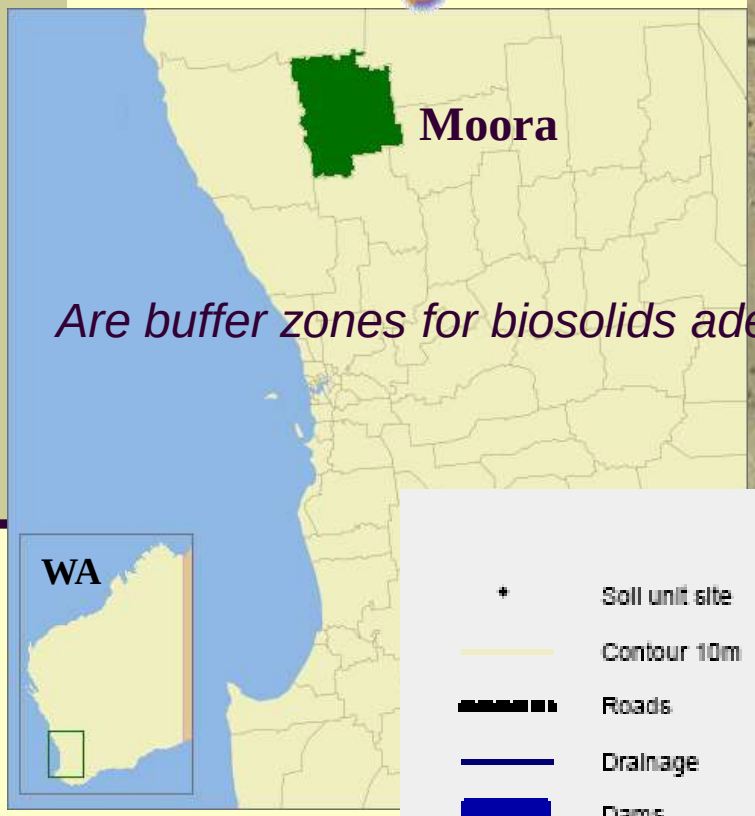
‘*Biosolids type’ substance found on a road by rate-payers who claimed there was a biosolids contamination issue*

Project Objective 2

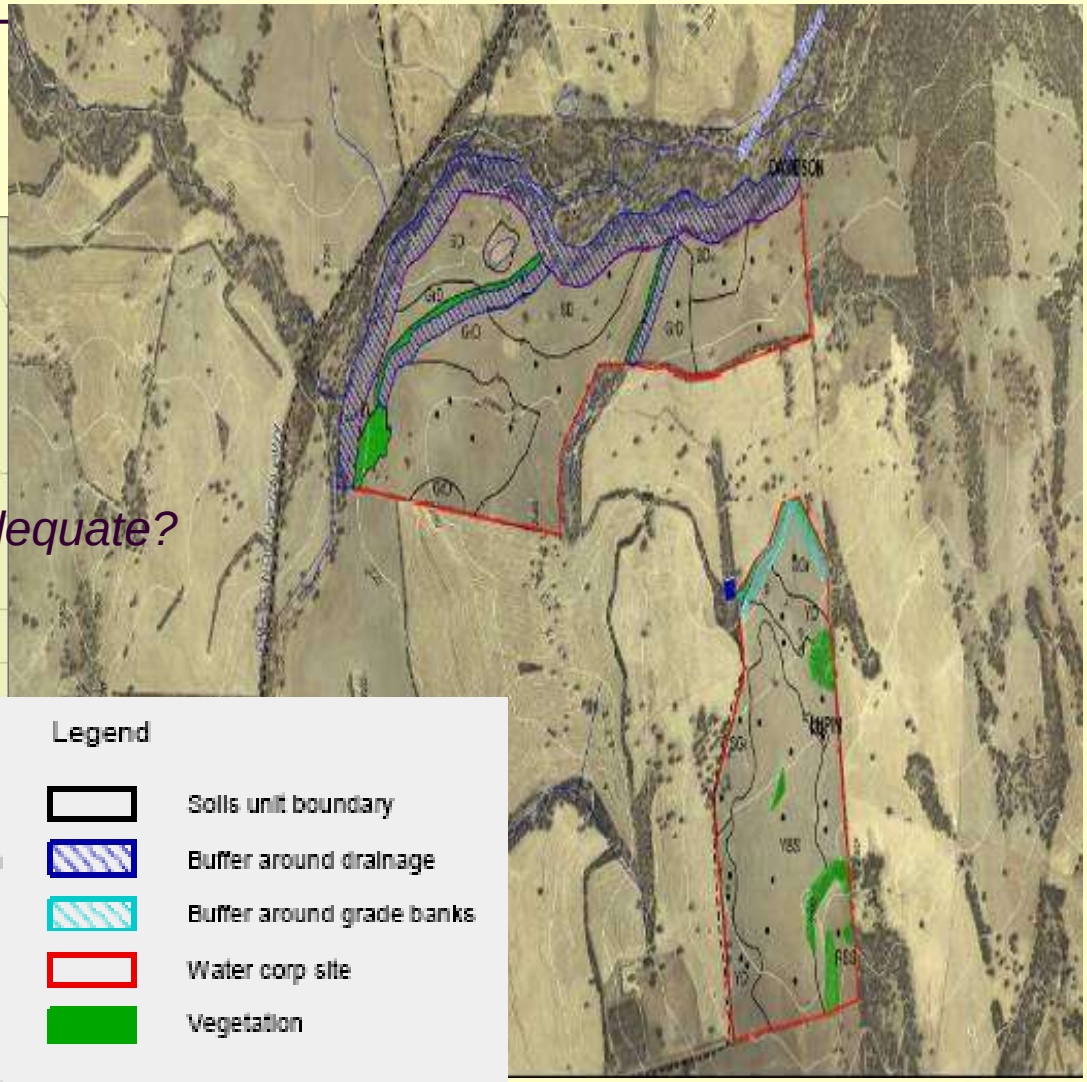
- Improve the limit of detection of faecal contamination in water samples collected from the field containing sediment (i.e. creek, dam, river) using molecular source tracking

Nutrient enrichment of waterways with elevated levels of nitrogen & phosphorus reduces water quality. Can we identify the source of pollution? Possibly organic pollutants, endocrine disruptors, pathogens etc.

Are Waterways at Risk of Contamination?



Are buffer zones for biosolids adequate?



Legend

•	Soil unit site		Soils unit boundary
	Contour 10m		Buffer around drainage
	Roads		Buffer around grade banks
	Drainage		Water corp site
	Dams		Vegetation
	Grade banks		

Background to DNA fingerprinting

- Anaerobic gastrointestinal bacteria unique to animals are discharged with faeces (e.g. *Bifobacterium* spp. & *Bacteroides* spp.
- Different species of animals have distinctive gastrointestinal populations
- Amplify target DNA across the 16S ribosomal RNA gene sequence by PCR to increase numbers of bacteria sufficient for detection
- Distinguish the strains of microorganisms specific to host species by their distinctive DNA pattern as identified by gel electrophoresis
- Provides forensic evidence that has many applications

Preliminary Work to date (2008-2009)

Faecal samples collected from animals in 2 major agricultural regions & 2 wildlife parks for:

- Cattle
- Pig (swine)
- Sheep
- Kangaroo
- Chicken
- Horse

Samples of biosolids collected from Woodman Point and Beenyup WWTP

Aim to develop a reference sample database

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PCR Amplification

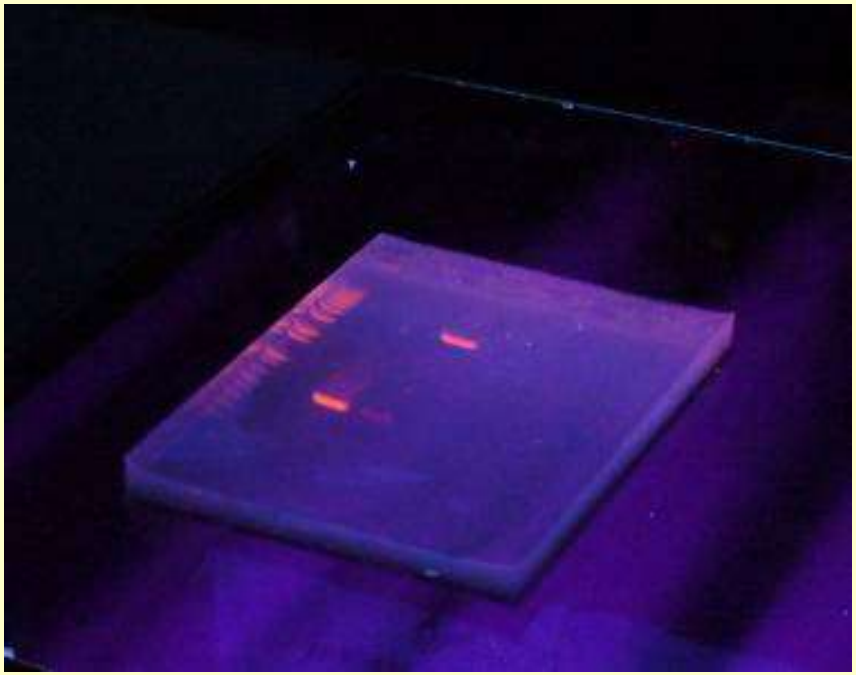
DNA isolated from 200mg various animal faecal samples

PCR Thermal cycler

31 primer pairs tested

Typical amplification conditions: e.g. 95^{o}C 2 min, 35 cycles 95^{o}C 15s, 50^{o}C 30s & 72^{o}C 1min & 72^{o}C 10 min

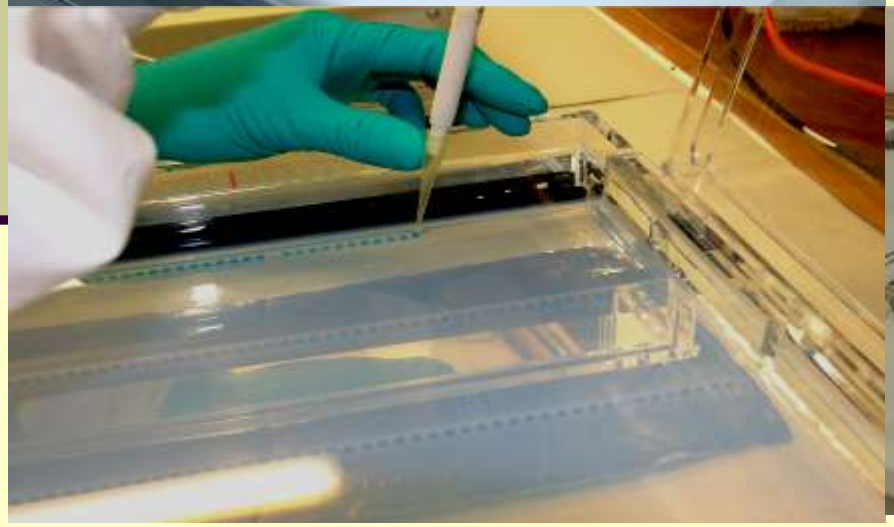
Restriction Enzyme Digestion



- ~ 24 different restriction enzymes were screened & sized against a DNA weight ladder VI

Agarose Gel

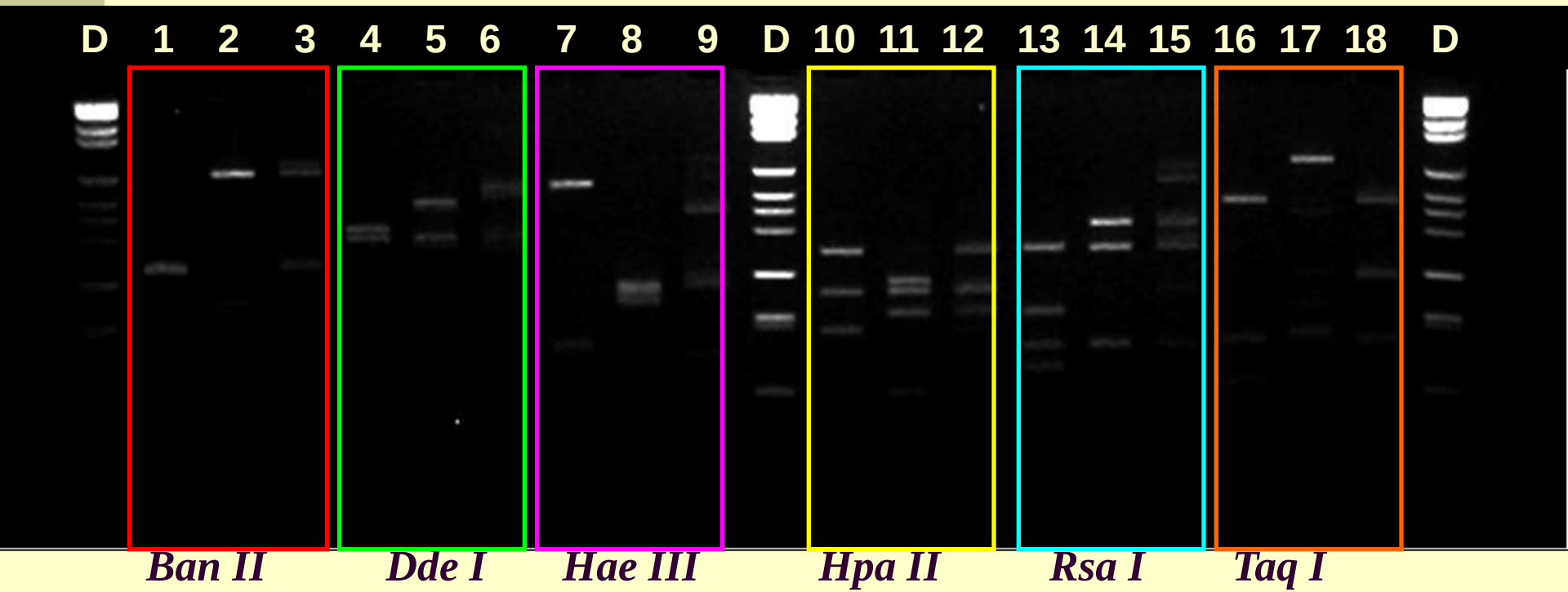
❖ Digests were electrophoresed on a 4% agarose gel



DNA ladder (example)

Reference Standards Lanes 1 & 2 *B. fragilis & B. vulgatus*
Bifidobacteria Lanes 3-6: Biosolids Lane 8: Faecal samples Cow & Sheep
Lanes 7 & 10 respectively: Kangaroo faecal sample Lane 9:
Lane D : DNA molecular weight ladder VI

Restriction Enzyme Digestion of PCR Amplified DNA



Cow faecal material: Lanes 1, 4, 7, 10, 13 & 16
Biosolids: Lanes 2, 5, 8, 11, 14 & 17
Sheep faecal material: Lanes 3,6, 9, 12, 15 & 18
Lane D: DNA molecular weight ladder VI

Results to date

- Bacteroides-Prevotella (Bac32F/Bac 708R) - greatest host range over 31 published bacterial primer pairs tested for biosolids & animal faeces
- Biosolids - greatest potential to be differentiated from animal faeces when subject to digestion by restriction enzymes (Bam H1, Nde II & Pst 1) of the 24 tested

Limit of Detection in Water Samples

- Bacterial markers can persist in aquatic environments & thus positively identify faecal contamination
- Water samples collected from WWTPs, Perth waterways, creeks & dams from biosolids land application sites at Wongan Hills
- Level of detection of biosolids possible at 5-log dilution in spiked samples of fresh water and work is ongoing

Work to be completed

1. Validate results for Bac32F/Bac 708R amplified gene fragments of Bacteroides-Prevotella - identify the best restriction enzymes and PCR amplification
2. Increase our current DNA fingerprint database (genotype library) – increase the sample size and accuracy
3. Determine factors that affect survival times of Bacteroides-Prevotella for samples under typical field conditions

Work to be completed

4. Investigate if DNA fingerprinting can be used to identify biosolids from different WWTPs (or raw sewage?)

5. Investigate if the DNA fingerprint from WWTP's samples vary over the year

Work to be completed

6. Improve the limit of detection for PCR amplified DNA of Bacteroides-Prevotella recovered in water samples containing suspended clay
7. Conduct on-site testing of water samples in paddocks to which biosolids have been applied (i.e. holding dam at centralised BSF)

Deliverables

- Develop a DNA fingerprint database comprising a range of biosolids and animal species
- Improve the exisiting methodology to improve DNA recovery in field and water samples
- Identify any evidence of contamination of water resources from biosolids for typical land application sites

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Acknowledgements

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