A novel PCR-DGGE-based method for identifying plankton 16s rDNA for the diagnosis of drowning

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Drowning signs

- Fine froth at the mouth or nostrils
- Emphysema aquosum and impress of ribs on lungs

"Drowning signs were destroyed"

Diagnostic tool in forensic practice

- Diatom test
  - Disorganization with strong acids
  - Enzymatic digestion with proteinase K
  - Solubilization with Soluene-350

Cause of diatoms absent

- Absent in the drowning medium
  - Medium itself, seasonal variations, pollution, etc.
- Do not penetrate the alveoli-capillary barrier
- Destroyed during the sample preparation process
  - Strong acids, enzyme, chemical reagent

Denaturing gradient gel electrophoresis (DGGE)

- Denaturant concentration
- Temperature
- G-C concentration

Experimental methods

Drowning Group

Postmortem submersion Group

Control Group

Drowning Group

Submerge
(depth 30 cm,1min)

Take out
(30 s)

Re-submerge
(until rabbits dead)

Postmortem submersion Group

Sacrificed by closed brain injury

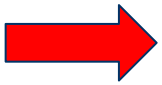
Submerge (depth 30 cm,6 hrs)

Control Group

Sacrificed by
Closed brain injury
With out postmortem

Tissue preparation

Washed with tap water

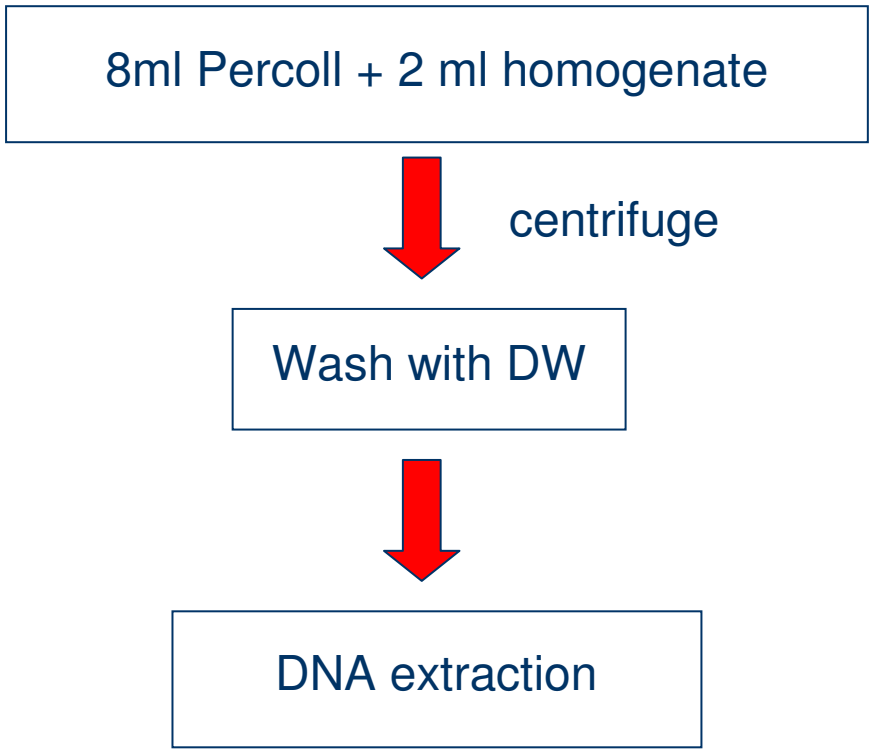


Open the thoracic cavity & abdominal cavity

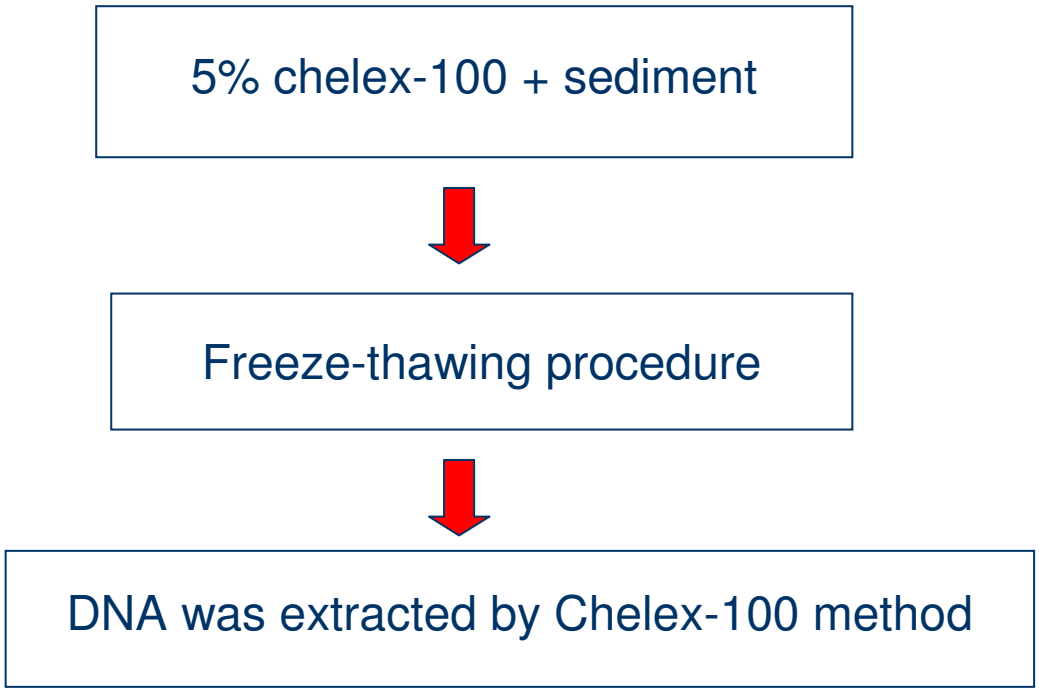
- Heart blood
- Lung
- Liver
- Kidney
- Brain tissue

} homogenize

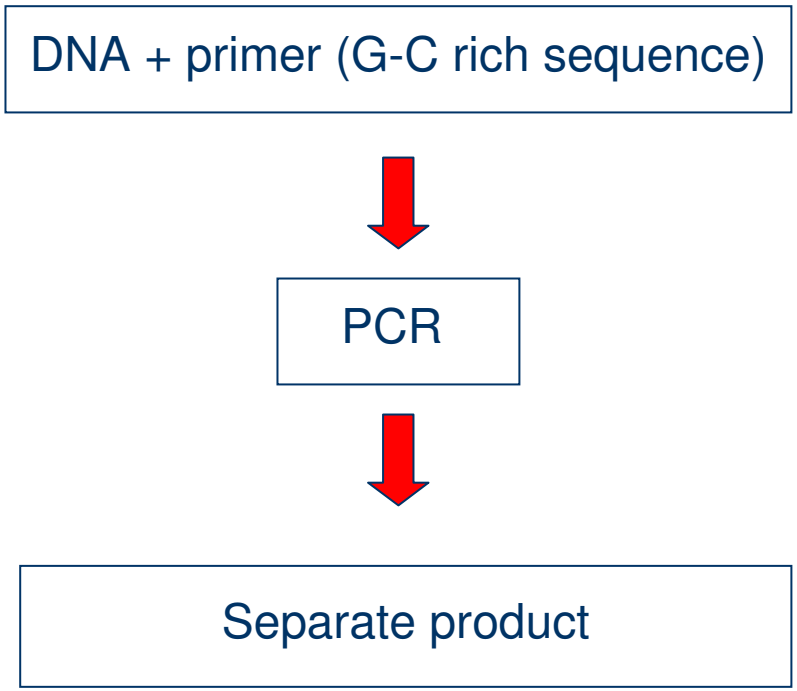
Plankton isolation



DNA extraction

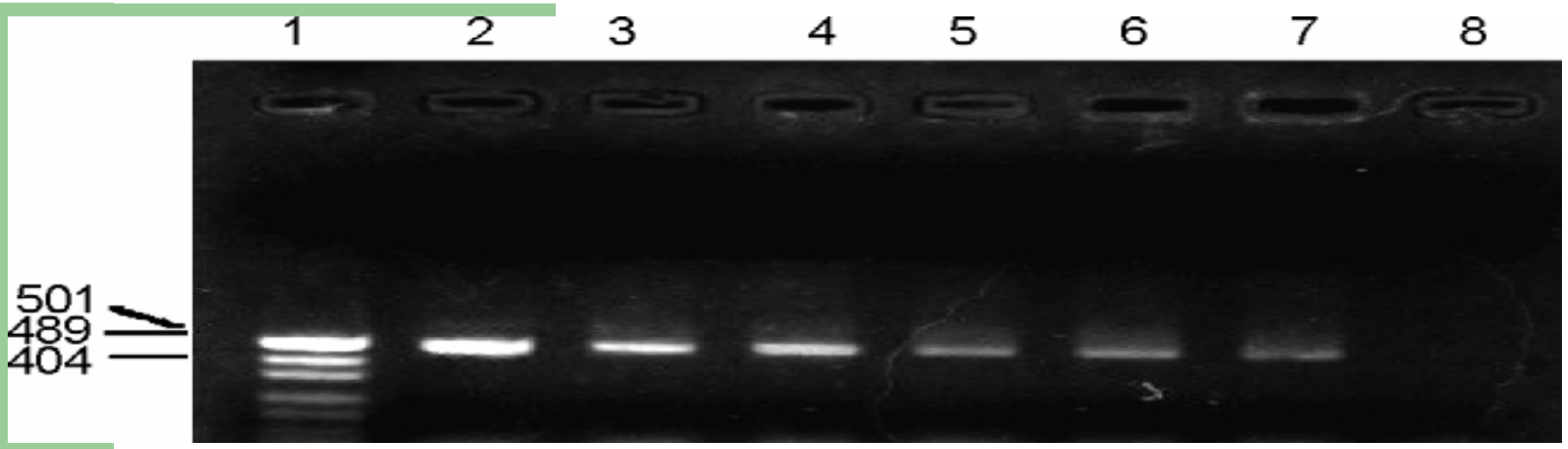


PCR amplification & product detection

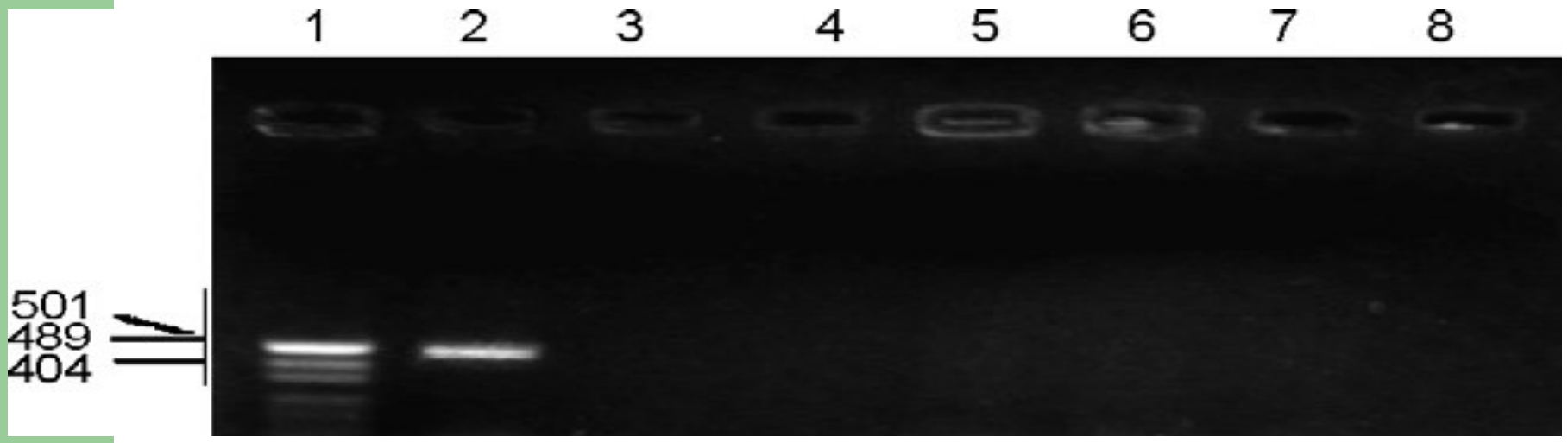


Result

Group	positive number and percentage				
	lung	liver	kidney	blood	brain
drowning group (n=12)	12 (100%)	10 (83%)	9 (75%)	10 (83%)	5 (42%)
postmortem submersion group (n=12)	2 (16.7%)	0 (-)	0 (-)	0 (-)	0 (-)
control group (n=6)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)

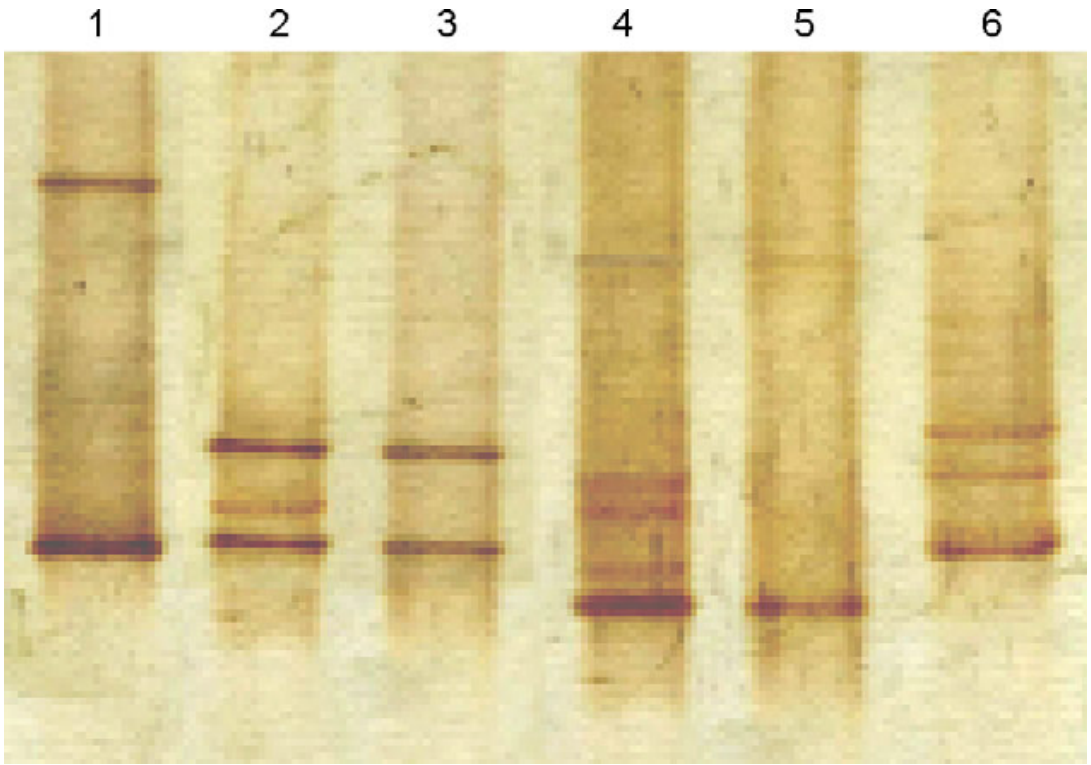


Agarose gel patterns of drowning group



Agarose gel patterns of control group

DGGE



Any Question