Fish, crayfish & mussels in environmental DNA

Naturhistoriska riksmuseet

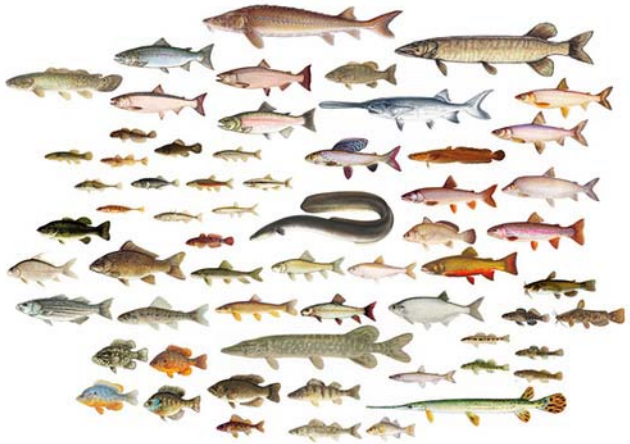
Laboratory analyses & results.

Rodrigo Esparza-Salas
Centre for Genetic Identification/Swedish Museum of Natural History
December 2014

Objectives

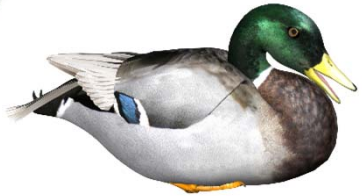
Naturhistoriska riksmuseet

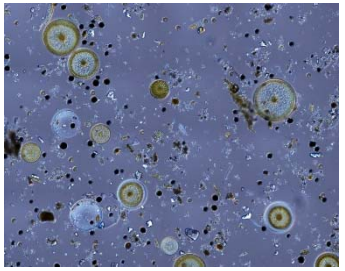
- Optimize sampling protocols
- Use available NGS methods for Fish DNA barcoding
- Develop simple methods for the detection of crayfish
- Develop methods for the detection of large mussel species in parallel (NGS)
- Develop NGS protocols for the correct identification of cyprinid fish



Environmental DNA

Naturhistoriska riksmuseet





Environmental DNA

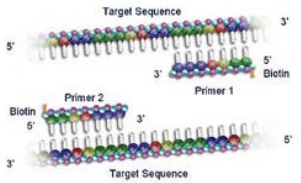
Naturhistoriska riksmuseet

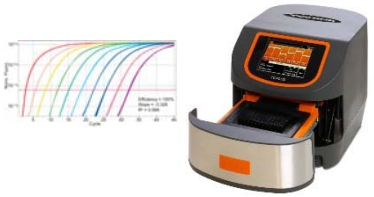
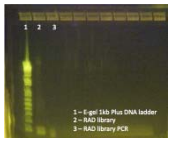


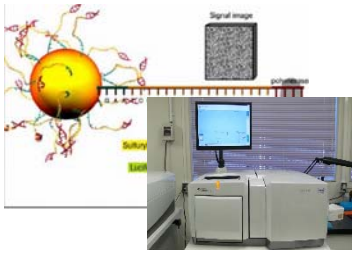
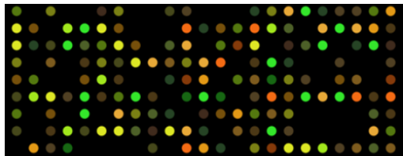




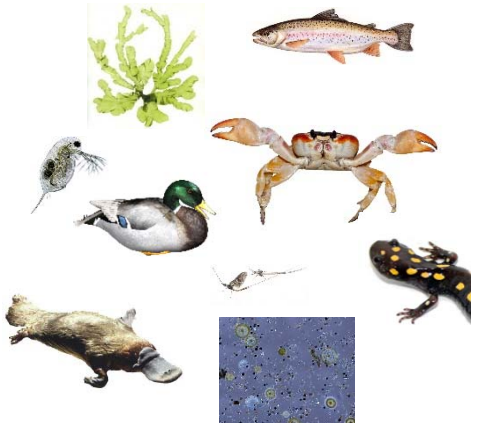






DNA extraction

Naturhistoriska riksmuseet

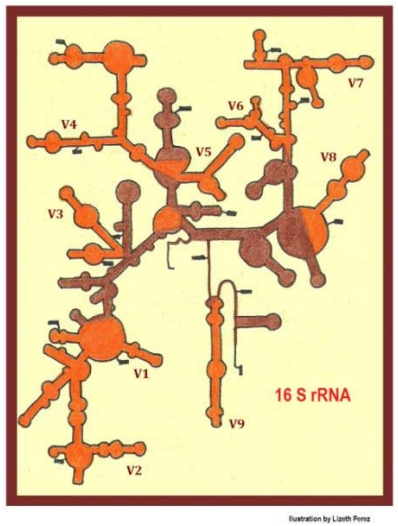
- Potential problems
  - Amount of recovered DNA
  - Inhibiting compounds in DNA sample (minerals, salts, proteins)
  - Sample cross-contamination

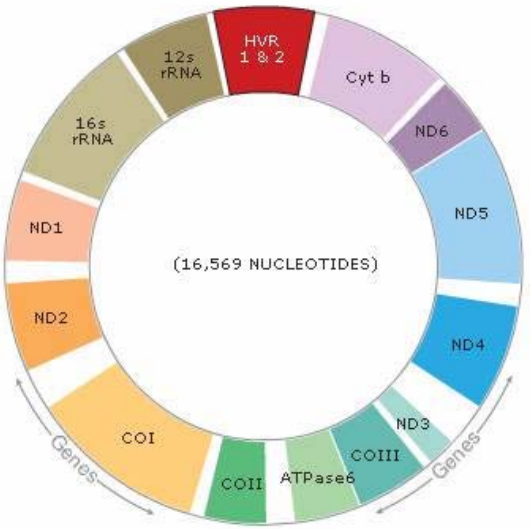
MoBio Power Water DNA isolation kit

Barcoding genes

Naturhistoriska riksmuseet

- Mitochondrial DNA
  - 16s
  - Cytochrome Oxidase I (COI)
  - Cytochrome B
  - ND2, etc.
- Nuclear DNA
  - 18s
  - ITS
  - Etc

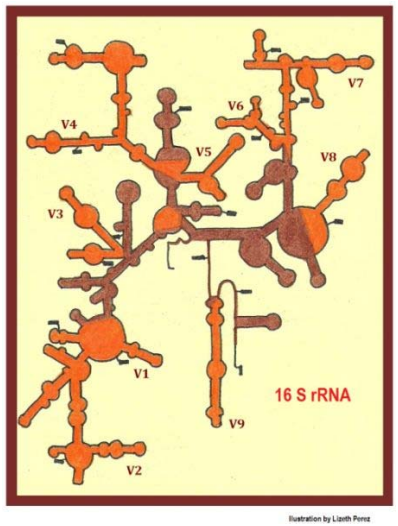


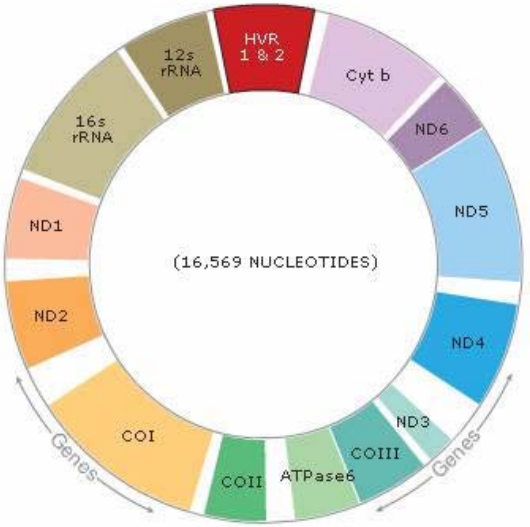


Choice of DNA markers

Naturhistoriska riksmuseet

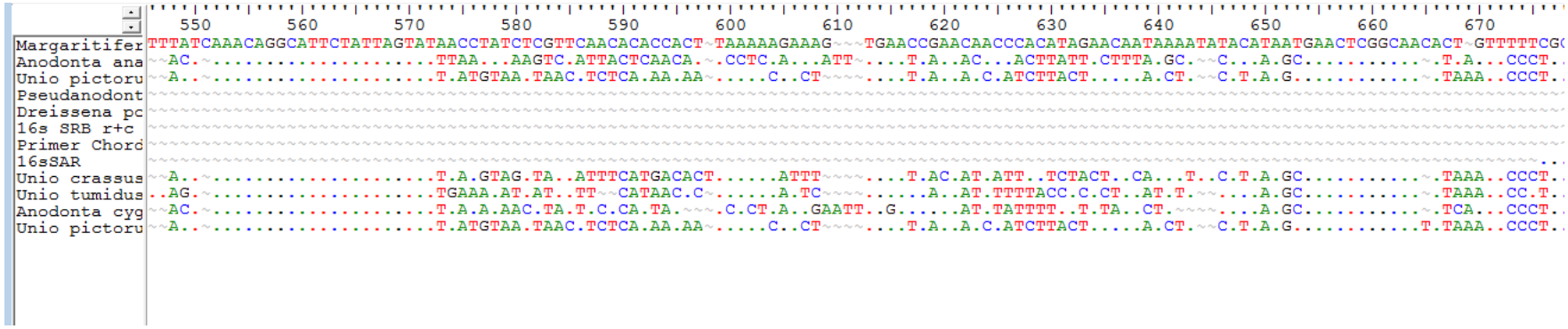
- Next-generation sequencing
- Fish
  - 300 base pair long section of 16s rDNA gene
- Mussels
  - 170 base pair section of 16s rDNA
- Crayfish
  - 170bp species-specific sectin of COI





Choice of DNA markers

Naturhistoriska riksmuseet



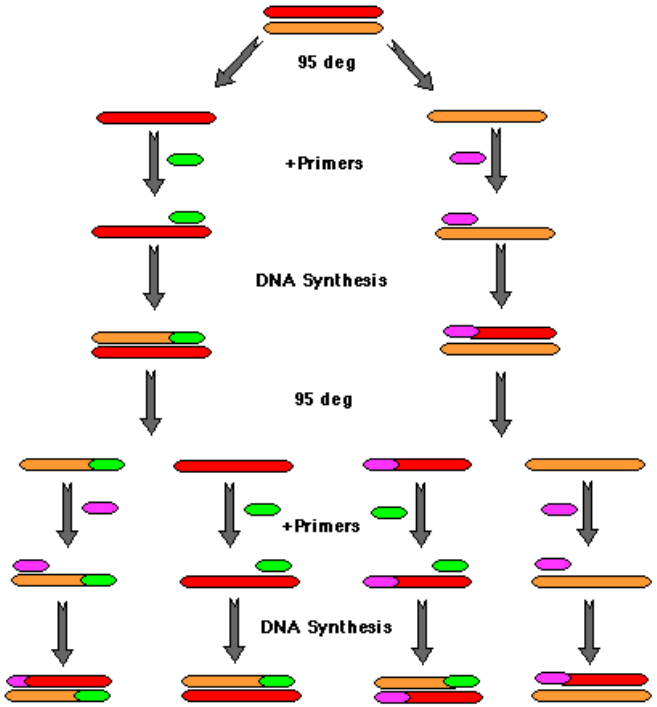
Conserved region (PCR primer)

Variable region (Target sequence)

Conserved region (PCR primer)

Polymerase chain reaction

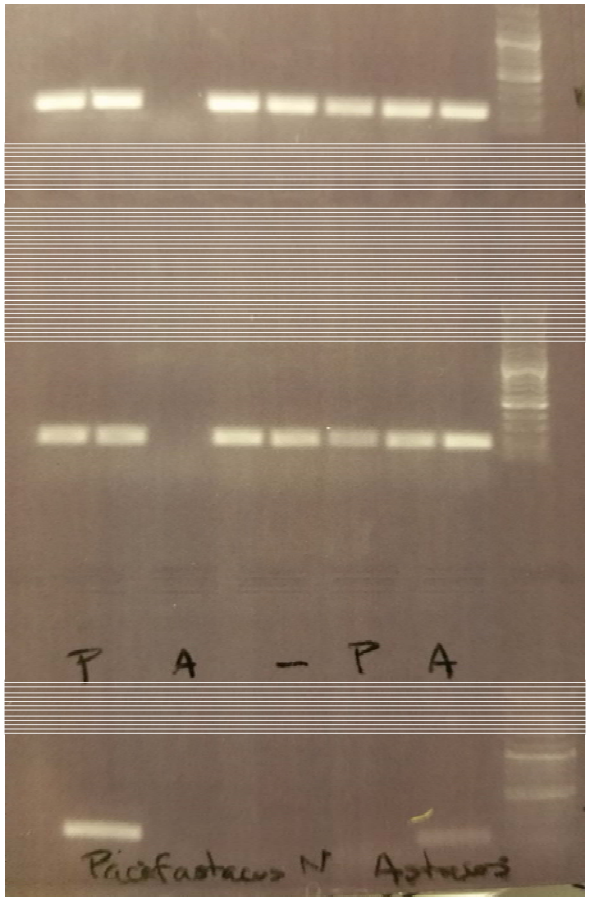
Naturhistoriska riksmuseet



40 PCR cycles → 2^{40} copies

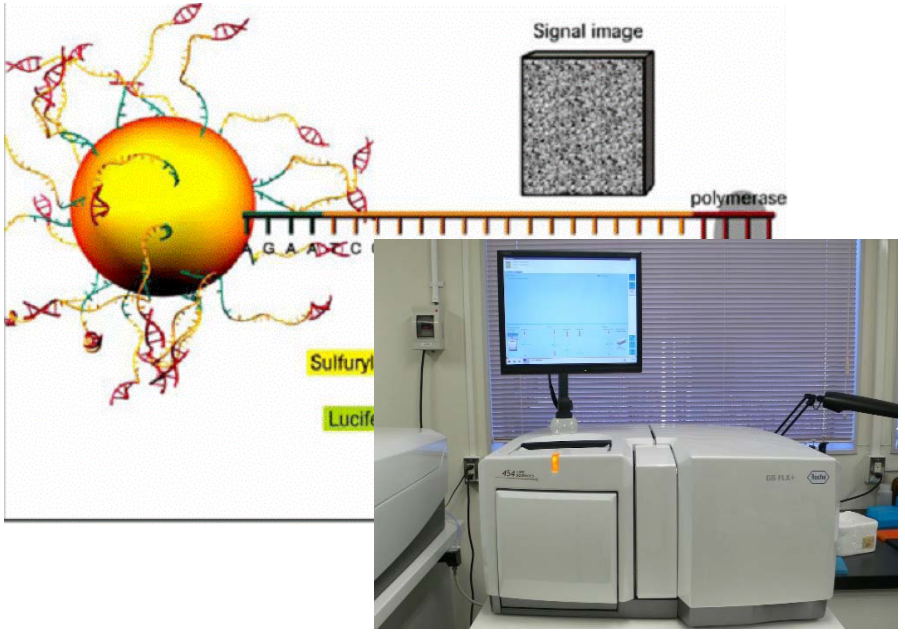
(2000000000000000000000000000000000000)

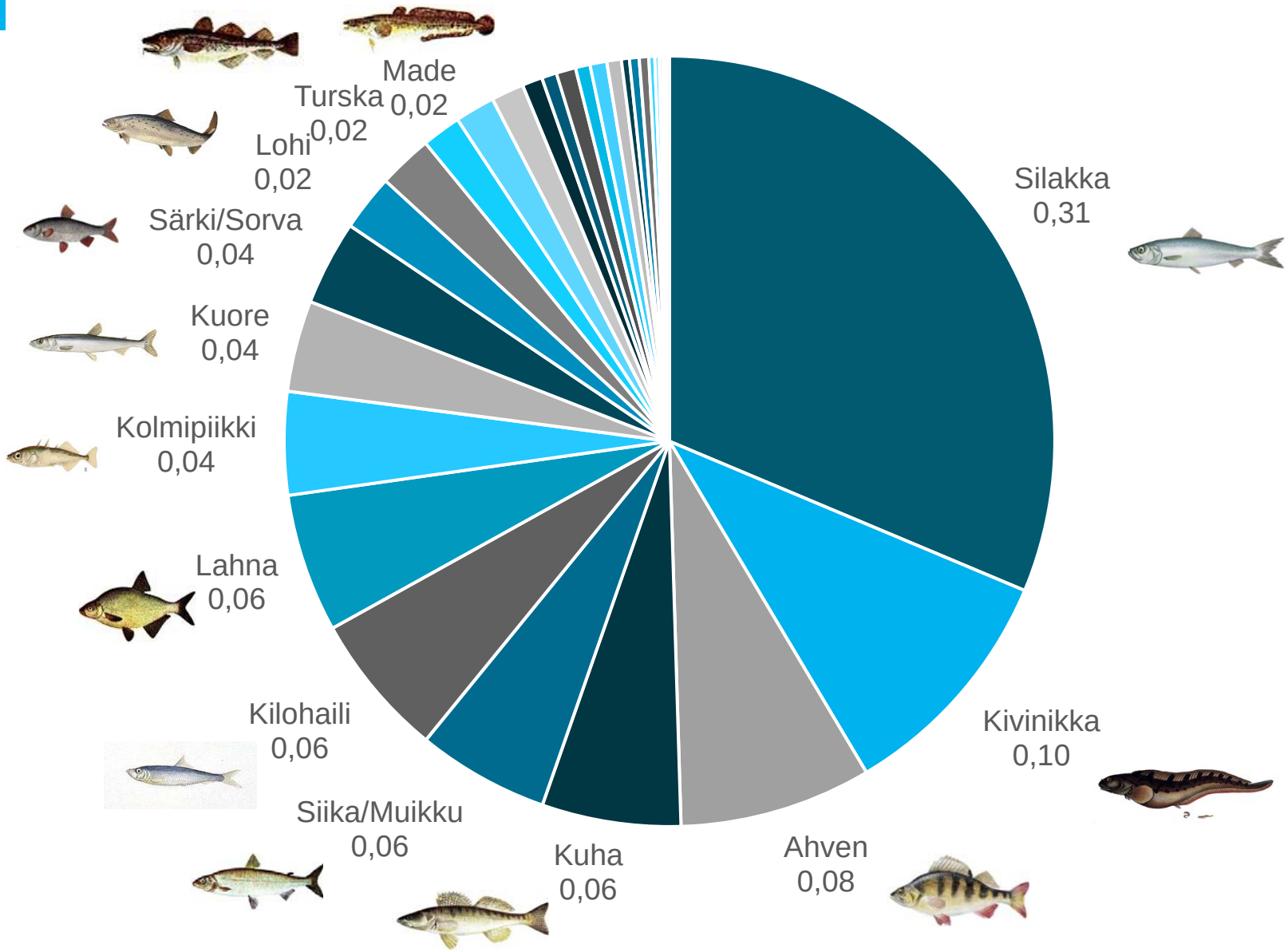
Naturhistoriska riksmuseet



Naturhistoriska riksmuseet

- Pyrosequencing for Fish and mussels
- PCR + gel electrophoresis for crayfish

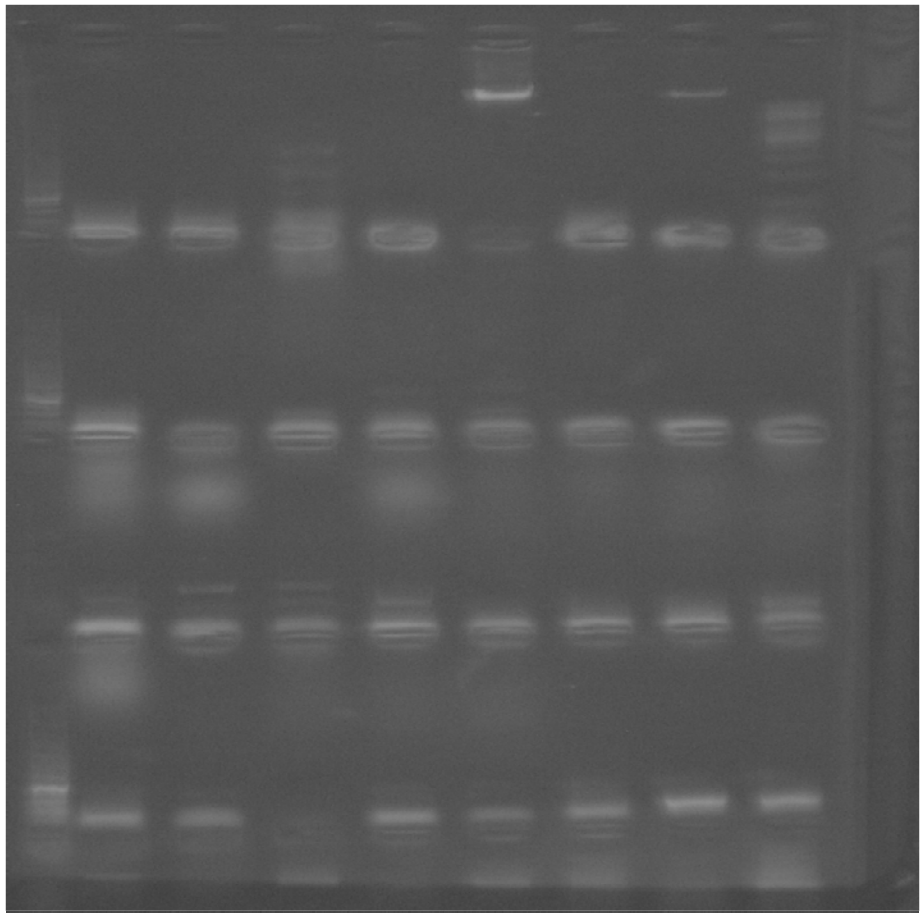



Made
0,02
Turska
0,02
Lohi
0,02
Särki/Sorva
0,04
Kuore
0,04
Kolmipiikki
0,04
Lahna
0,06
Kilohaili
0,06
Siika/Muikku
0,06
Kuha
0,06
Ahven
0,08
Kivinikka
0,10
Silakka
0,31

Some results

Naturhistoriska riksmuseet

- Fish DNA
  - Positive PCR product in 12/55 samples for first marker
  - Non-specific amplification for second marker
- Mussel DNA
  - Positive PCR products in 13/55 samples
- Crayfish DNA
  - Negative



Mussel PCR ~170 bp

Next-generation sequencing

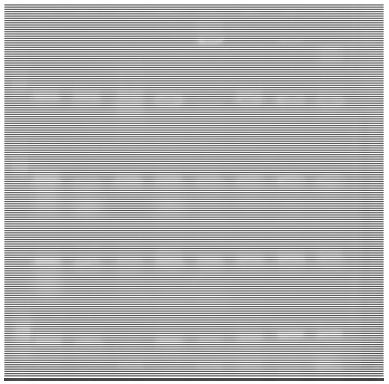
Naturhistoriska riksmuseet

- First attempt using fish and Mussel PCR products
  - ~84 000 total DNA sequences
  - 66% low quality reads
  - 30% adaptor-dimers (non-specific)
  - 0,06% target sequences
- Other non-target organism DNA
  - Bacteria, microalgae, protozoans, vertebrates, crustaceans, annelids,

Next steps (2014)

Naturhistoriska riksmuseet

- Re-sequencing with modified protocol for NGS
  - PCR reaction
  - Selection of target PCR products



- Develop a specific marker for Zebra mussel (Dreissena polymorpha)
  - test with field samples

Next steps (2015)

Naturhistoriska riksmuseet

	Illumina MiSeq	IonTorrent PGM	Roche GS Junior
Instrument price		x	
Read length			x
Throughput	x		
Run time		x	
Price/run		x	
Hands-on time	x		
Read quality	x		
Assembly: coverage			x
Assembly: fewest pieces			x
Assembly: gene space	x		

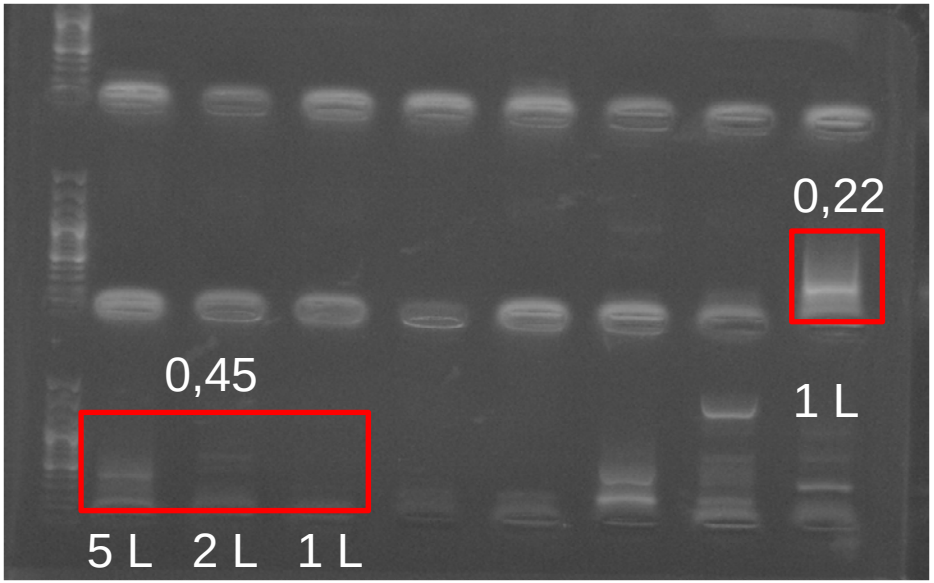
Reads	24 M	100 000

Sampling recommendations

Naturhistoriska riksmuseet

- Double filtration protocol

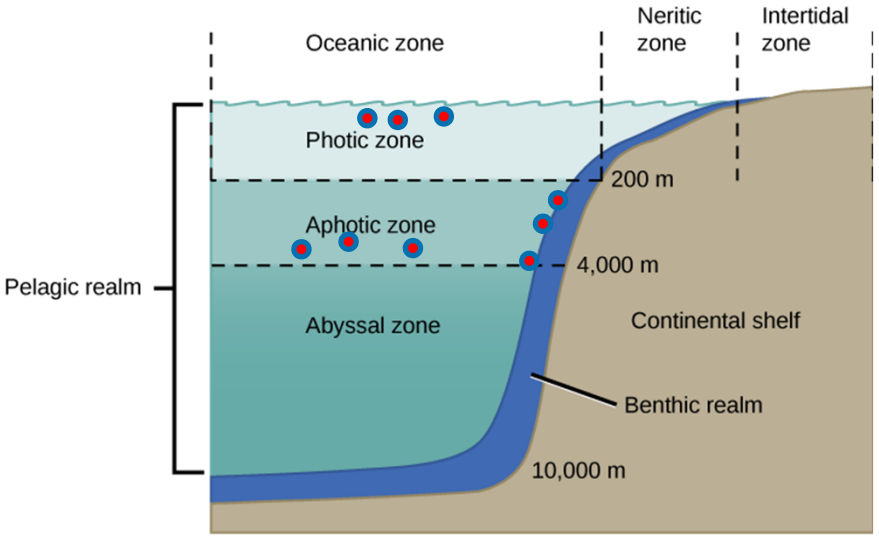
  - Pre-filtration (e.g. glass-fiber filter
  - Second filtration with 0,22 micrometer membrane



Sampling recommendations

Naturhistoriska riksmuseet

Targeted sampling to maximise DNA recovery



Objectives

Naturhistoriska riksmuseet

- Optimize sampling protocols
- Use available NGS methods for Fish DNA barcoding
- Develop simple methods for the detection of crayfish
- Develop methods for the detection of large mussel species in parallel (NGS)
- Develop NGS protocols for the correct identification of cyprinid fish