

# DNA Based Methodologies for Shark and Stingray Conservation and Field Testing

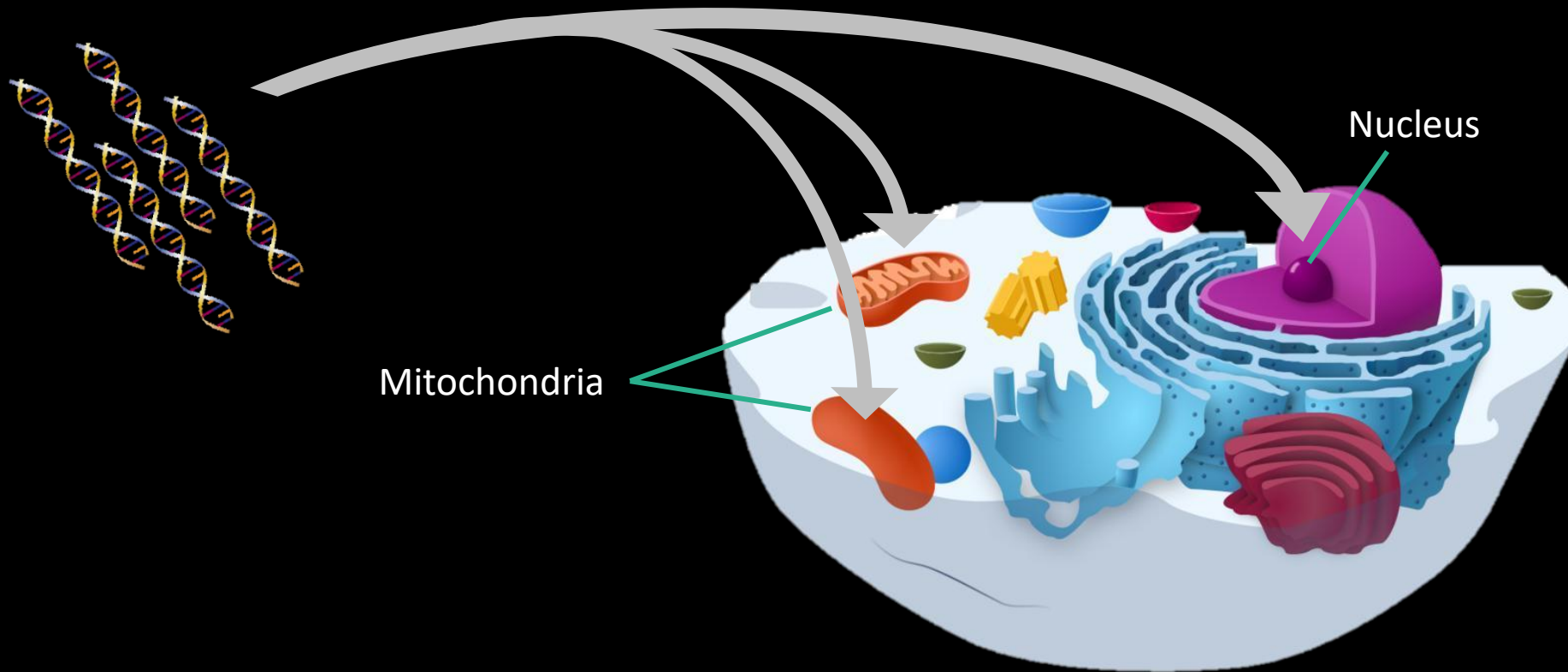
CPTPP: Second Workshop on Combatting Illegal Wildlife Trade

Sharks and Stingrays

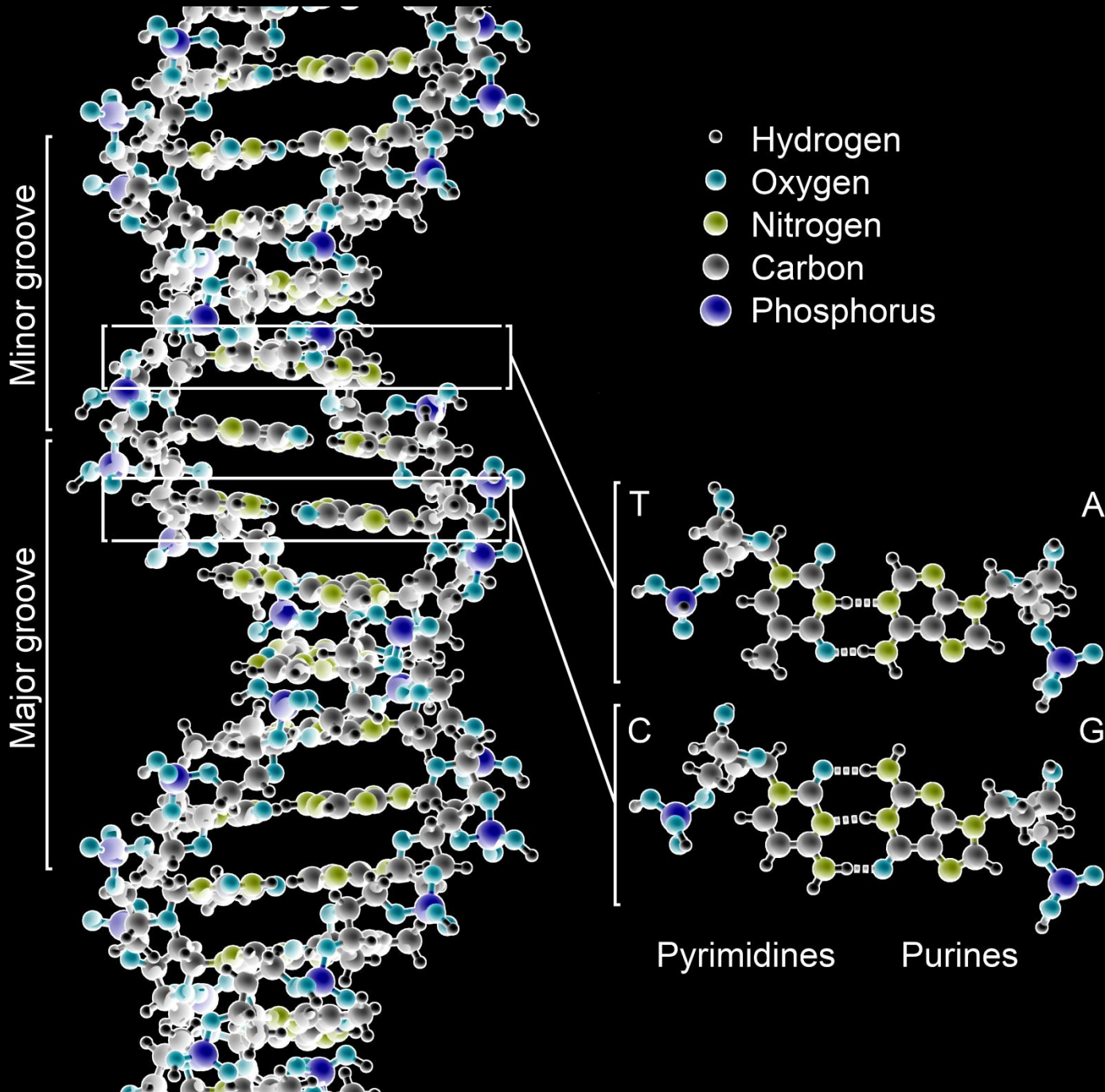
Dr. Christoph M. Deeg

[chrdeeg@gmail.com](mailto:chrdeeg@gmail.com)

# DNA



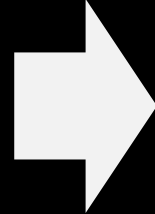
# DNA



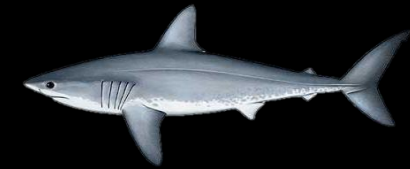
## Bases:

- Adenosine
- Guanine
- Cytosine
- Thymidine

# Species have unique DNA sequences



GTAATGTT**TC**GTAAT**CAG**CT**AT**CGGGAT**AC**GTGCTACG



GTAATGTT**TC**GTAAT**CAG**CT**AT**CGGGAT**CC**GTGCTACG



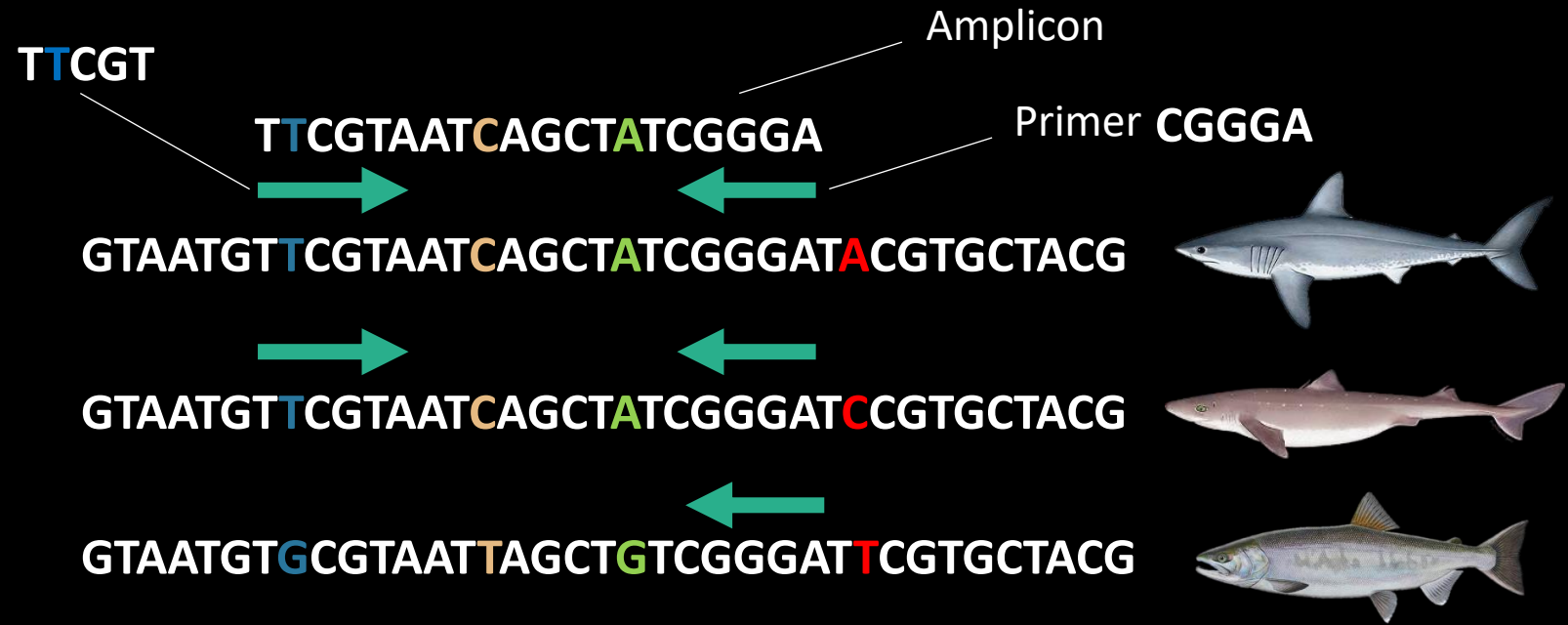
GTAATGT**G**CGTAAT**TA**GCT**G**TCGGGAT**T**CGTGCTACG



Species ID by sequence!

# Polymerase Chain reaction (PCR)

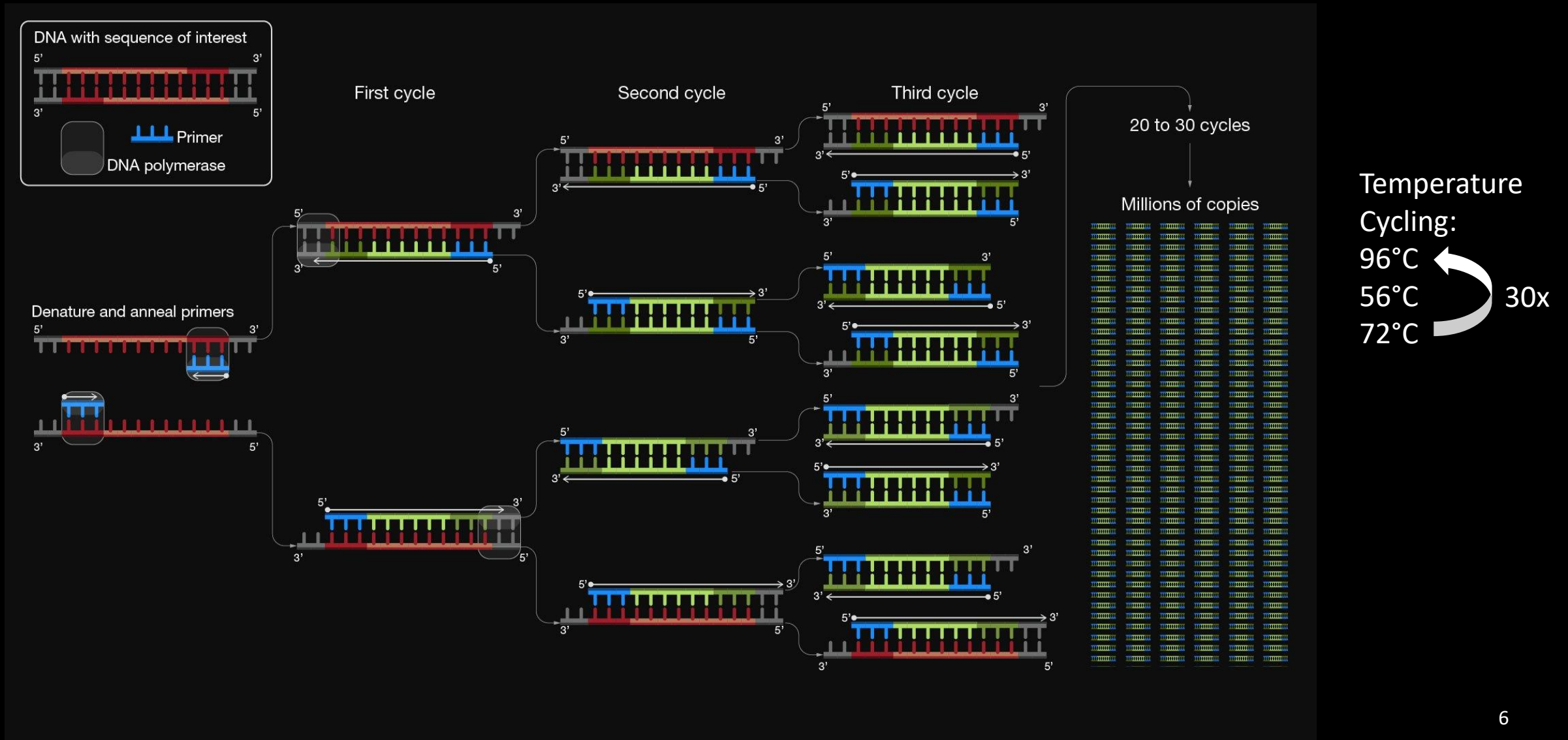
- “Make more DNA”
- Cellular replication machinery
- Design short DNA stretches (**primers**)
- Amplify specific DNA stretch (**amplicon**)



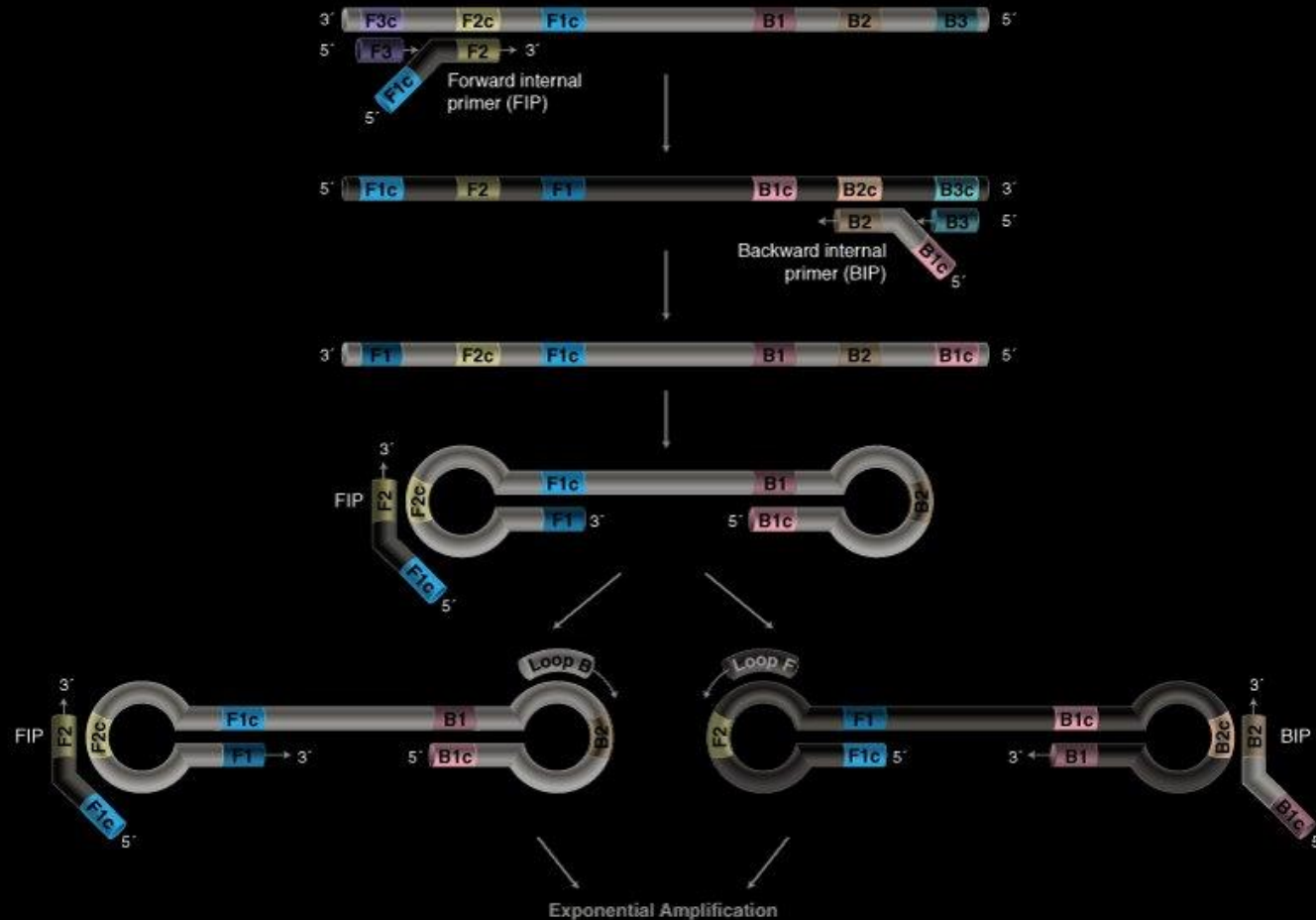
Shark specific PCR



# Polymerase Chain reaction (PCR)



# PCR alternative: Loop-Mediated Isothermal Amplification (LAMP)

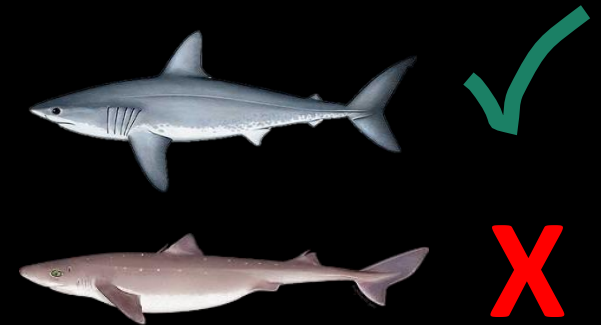
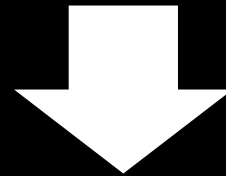
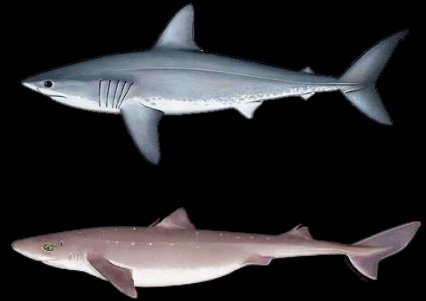
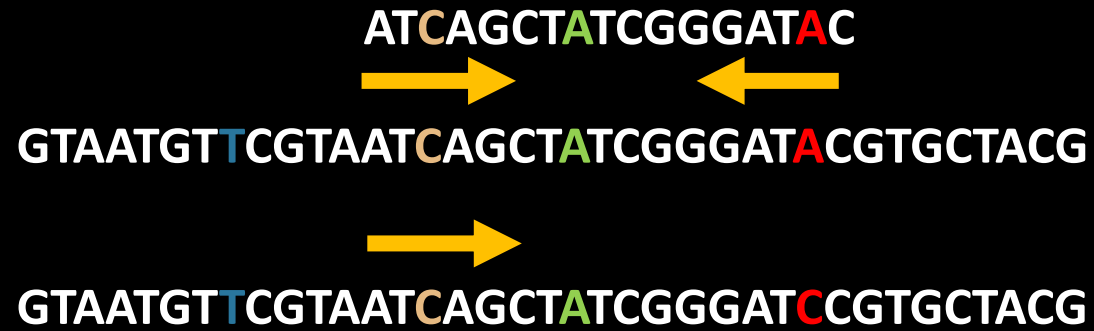


6-8 Primers  
65°C

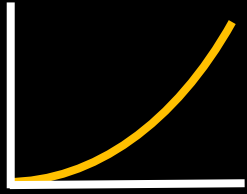


# LAMP (PCR)

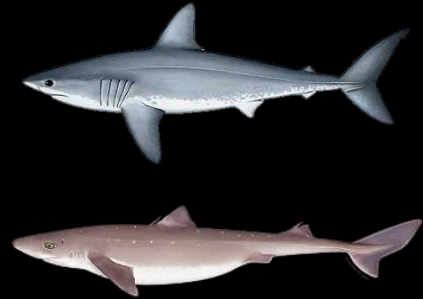
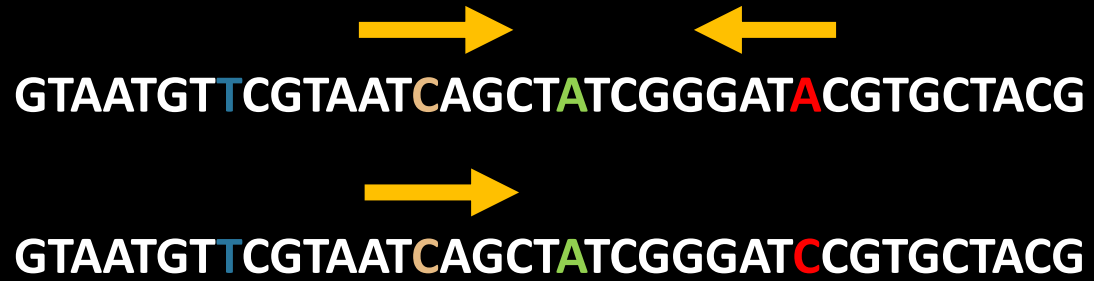
- Specific primer design
- Amplify DNA  
Detect  
amplification  
(Y/N)



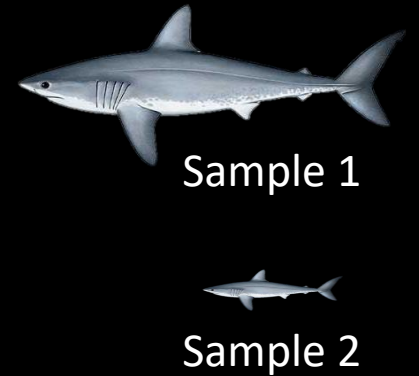
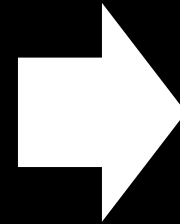
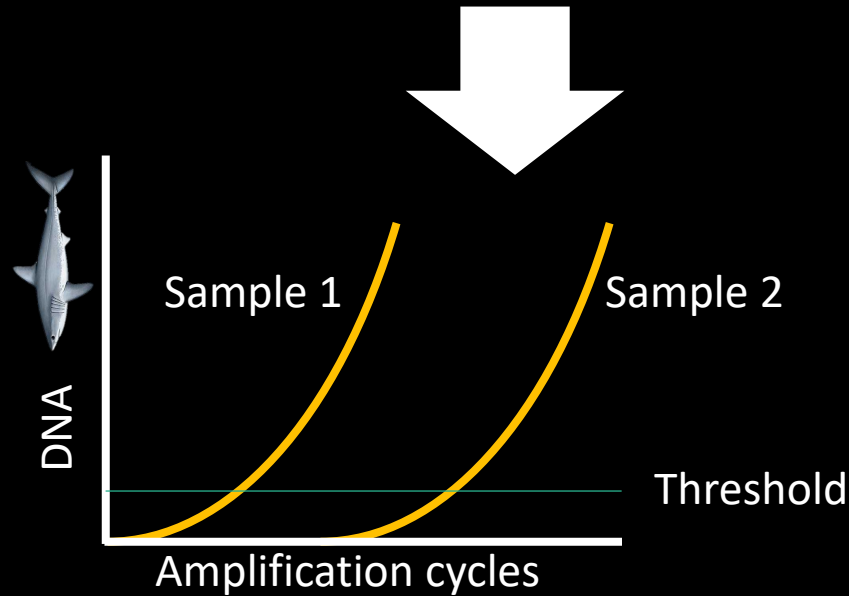




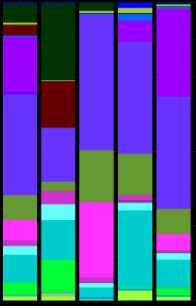
# quantitative (q)PCR



- Measure DNA amplification
- Cycle threshold (Ct) reported

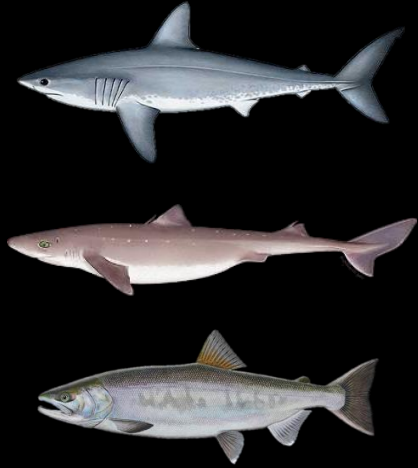
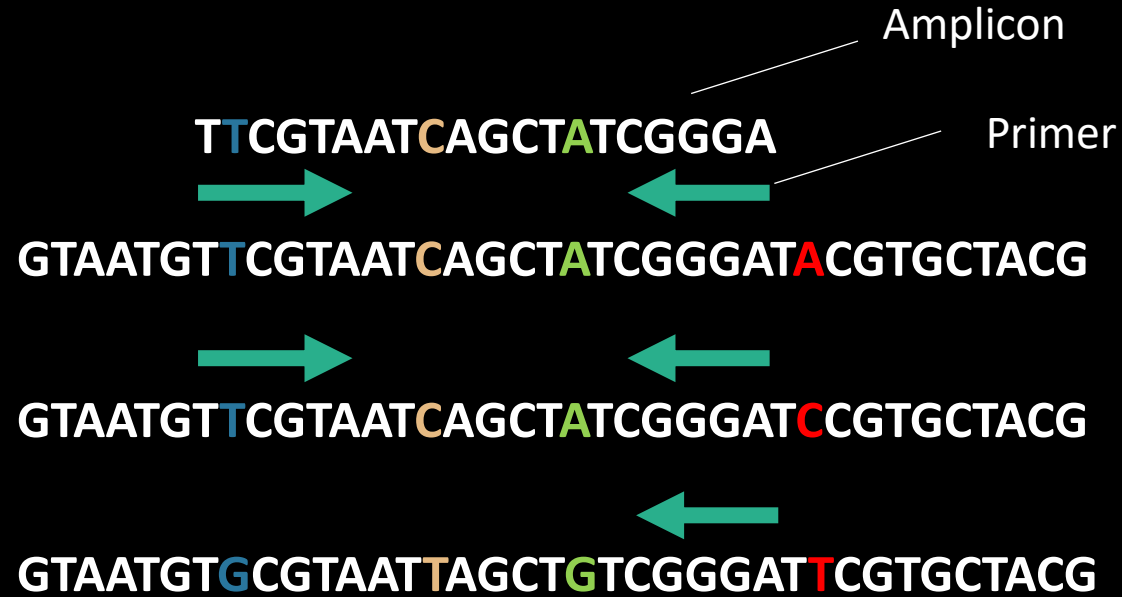


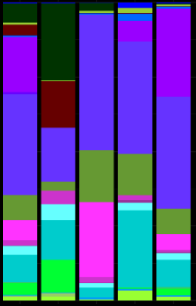
Salmon shark specific qPCR



# DNA Metabarcoding

- Amplify DNA with PCR
- Sequence (“read”) the DNA amplicon
- Compare to database



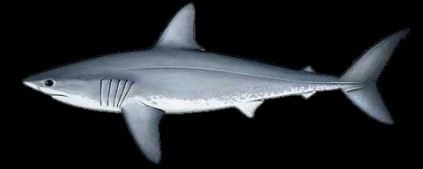


# Metabarcoding amplicon selection

- Technical size limit to amplicon
- Sequence divergence
- Target amplicon to question




  
 GTAATGTT**TC**GTAAT**CAG**CTATCGGGAT**AC**GTGCTACG






  
 GTAATGTT**TC**GTAAT**CAG**CTATCGGGAT**CC**GTGCTACG







  
 GTAATGT**G**CGTAAT**TA**GCT**G**TCGGGAT**T**CGTGCTACG



 Can't differentiate between sharks

 Can't amplify salmon

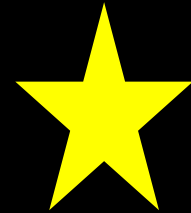
GCCATAT**G**CGACTAT**TA**GCACTGGGATGTT**TC**GTC**G**CGTACG



# DNA analysis approaches

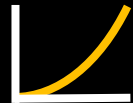
## LAMP (PCR)

- Cheap and fast
- Measure accumulation
- Presence of specific species (Y/N answer)



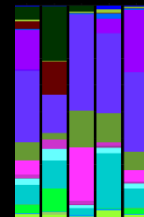
## qPCR

- Moderately expensive and fast
- Quantitative Polymerase Chain Reaction
- Presence and quantity of specific species or group



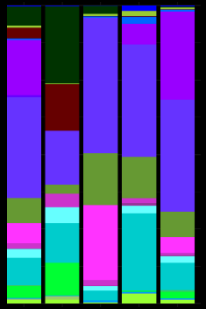
## Metabarcoding

- Expensive
- Sequence (“Read”) the amplified DNA and assign to species
- Presence and “relative abundance of all species”



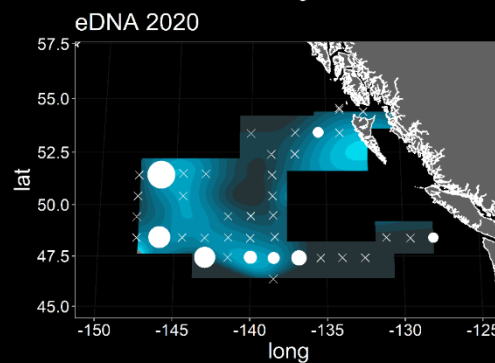
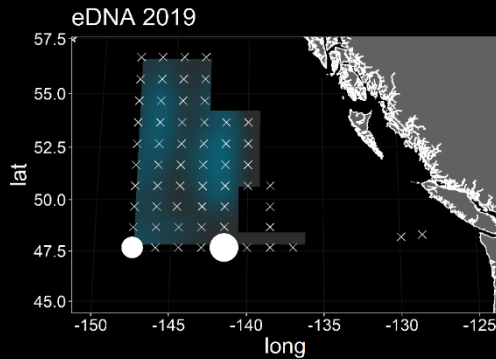
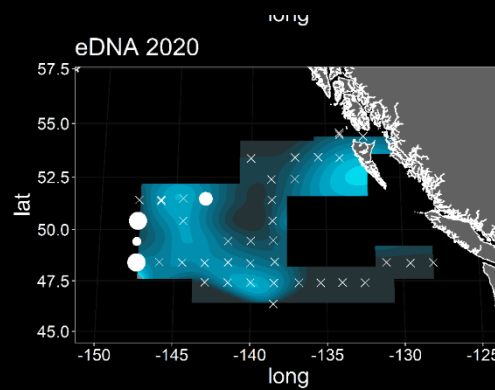
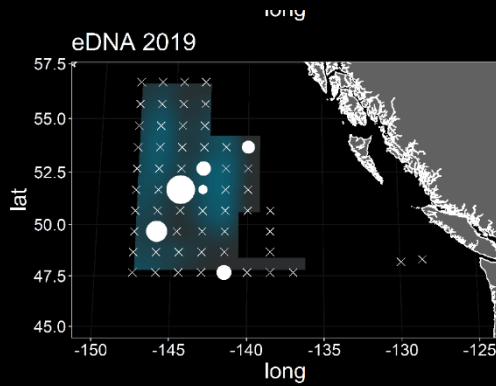
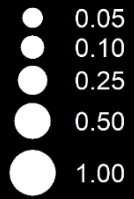


# Case study1: IYS Gulf of Alaska eDNA: Sharks



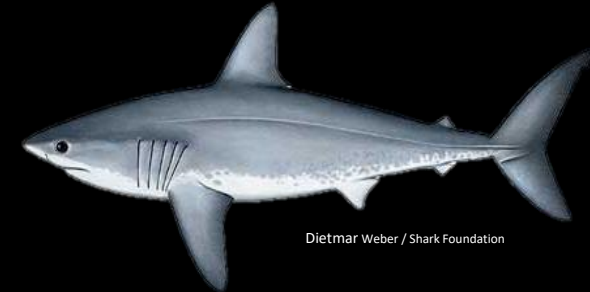
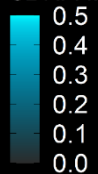
Shark

eDNA Index



Oncorhynchus  
spp.

eDNA Index



Dietmar Weber / Shark Foundation

**Salmon shark (*Lamna ditropis*)**



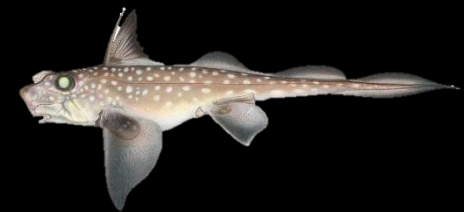
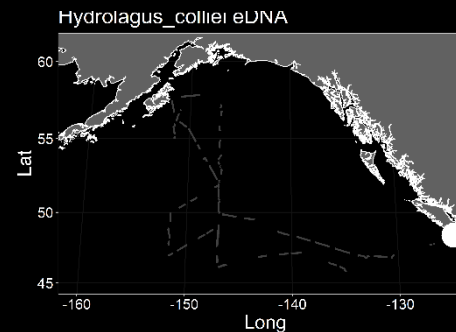
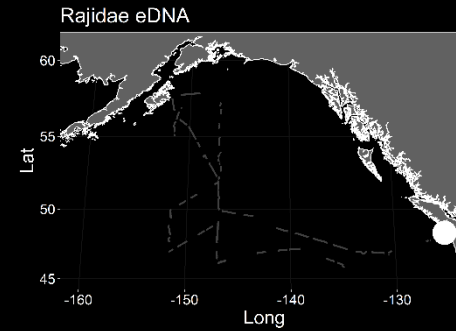
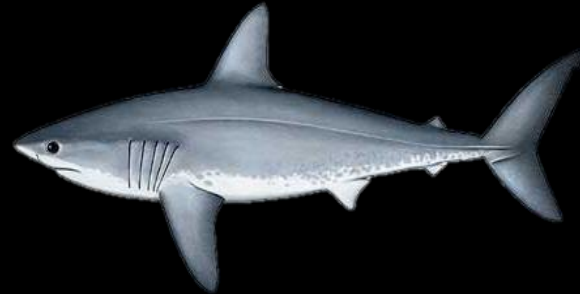
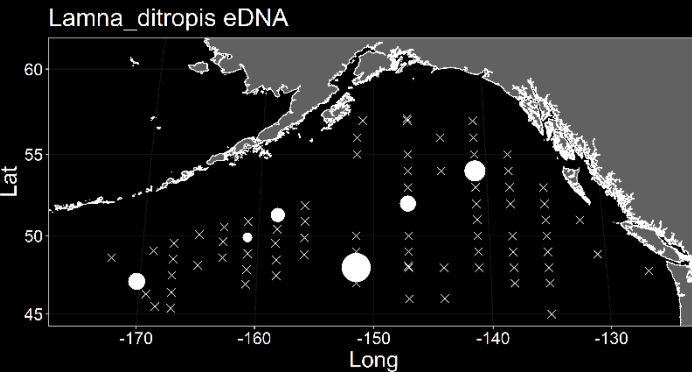
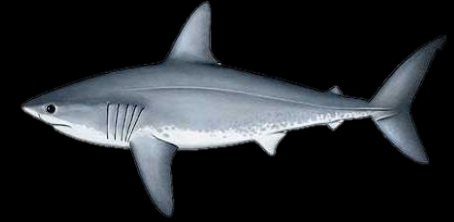
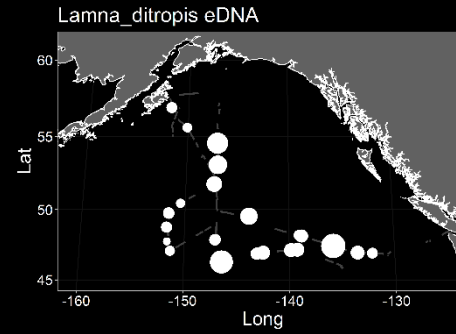
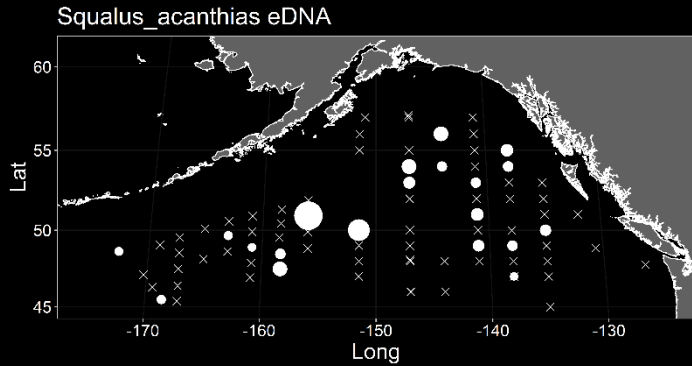
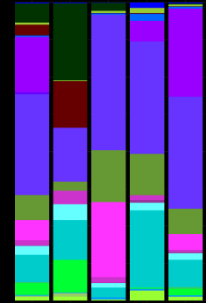
fishwatch.govpphido

**Spiny dogfish (*Squalus acanthias*)**





# Case study1: IYS Gulf of Alaska eDNA: Sharks





# Case study 2: DFO's Conservation & Protection -IUU Fishing - North Pacific Offshore Fisheries Inspections



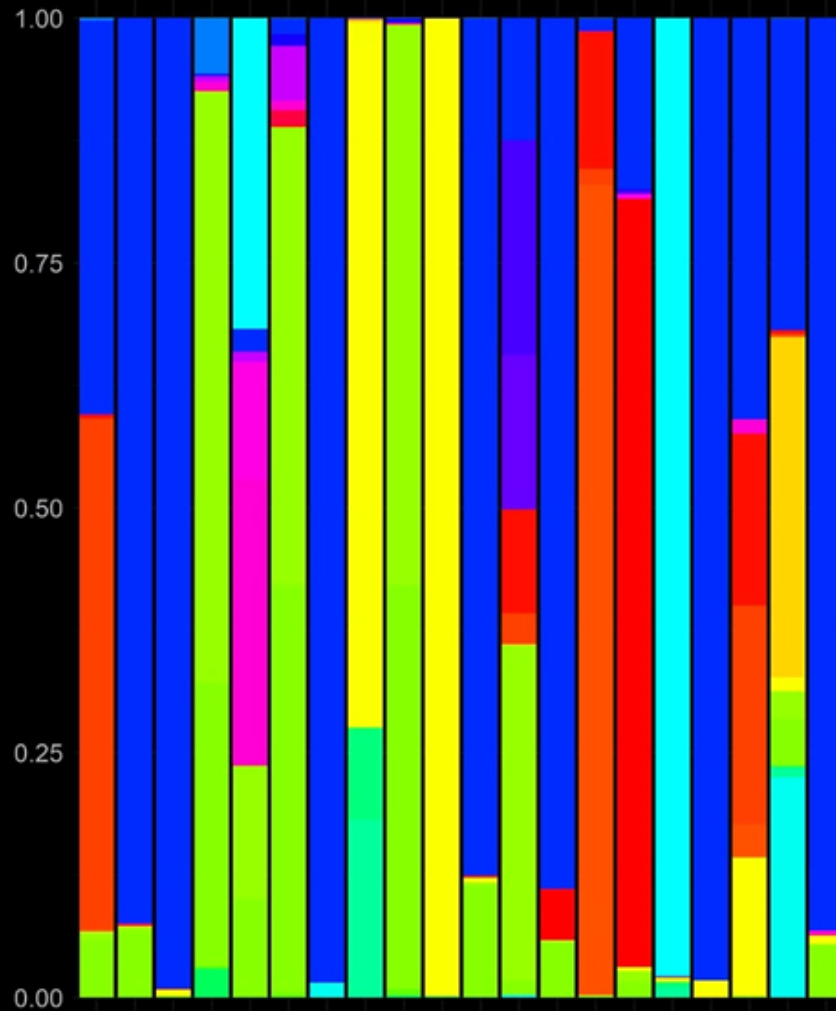




# Case study 2: IUU Fishing - North Pacific Offshore Fisheries Inspections

Catch composition identified by metabarcoding

- Longliners
- Squid boats
- Purse-seiner



Species composition by vessel

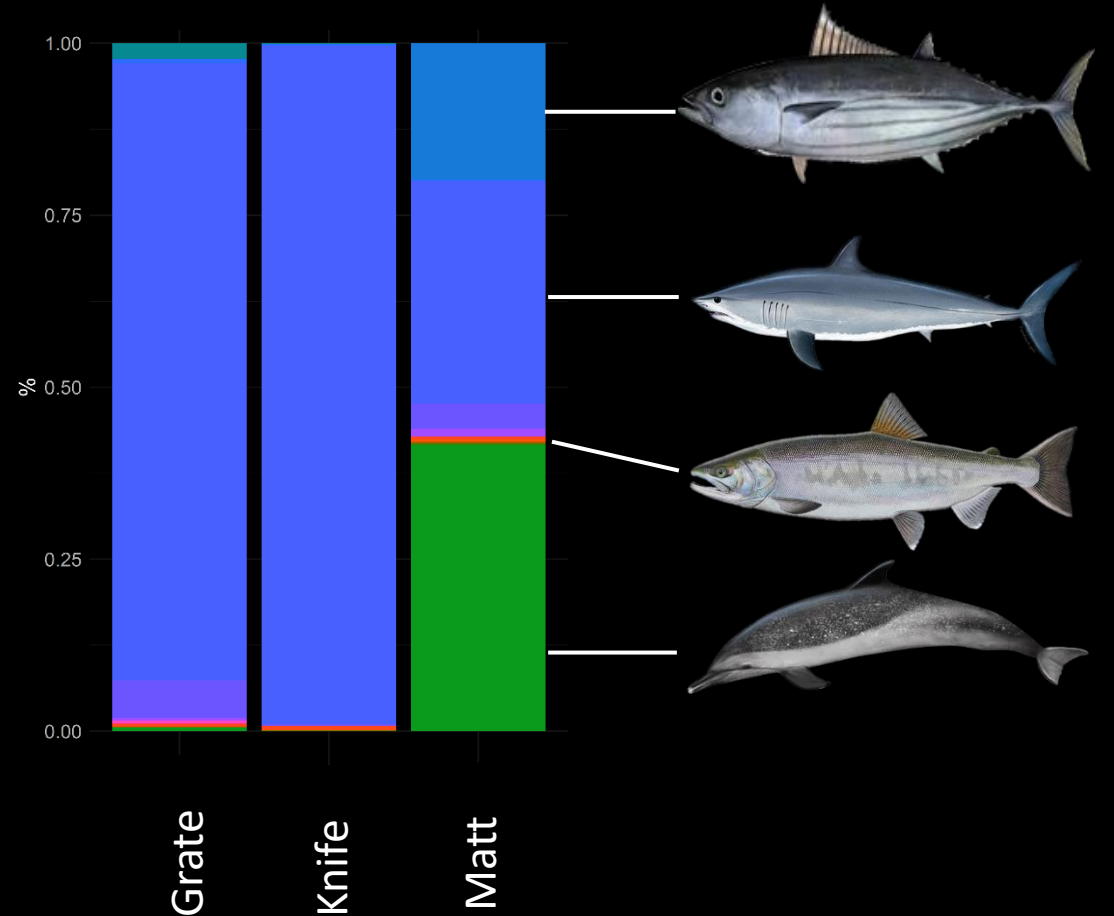


Species

Acanthocybium solandri	Gadus morhua	Ommastrephes bartramii	Prionace glauca	Thunnus
Brama	Hyperoglyphe japonica	Oncorhynchus	Sardinops	Thunnus tonggol
Cololabis saira	Lamna	Oncorhynchus keta	Scomber	Xiphias gladius
Engraulis	Lamna ditropis	Oncorhynchus kisutch	Scomber japonicus	
Gadus	Mola mola	Percomorphaceae	Teuthida	



# Case study 2: IUU Fishing - North Pacific Offshore Fisheries Inspections





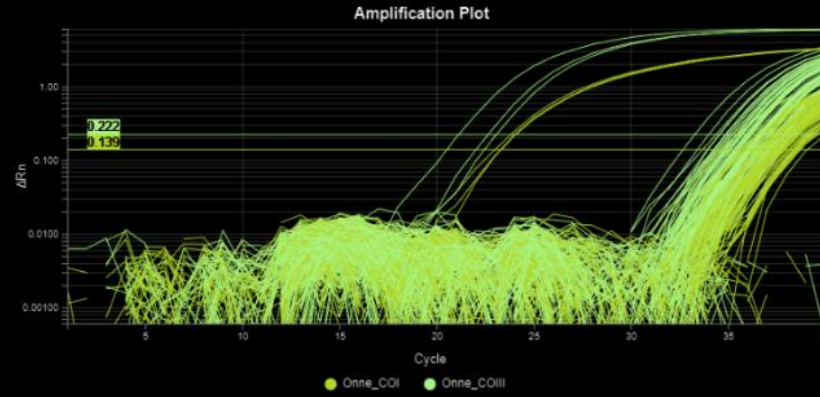
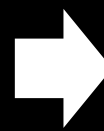


# Case study 2: IUU Fishing - North Pacific Offshore Fisheries Inspections



comb

Trans	L	o	DNA	e	GL	lo	DNA	mean	CT	mean	CT	Til	mean	D	N	comb	Dens	Dens	Dens	N_Jar	Dens
8.600	8.410			8.505	8.324	8.350			7.1684	29400			26200							32200	
1	273	772	0.14	522	093	448	7.07	8.33727	21	279	00	3765	00	3353	0	0	0	0	0	0	412
	7.282	8.266		7.774	7.347	8.436			11	15.66	89300		83900								
2	177	712	3.42	445	02	964	8.28	7.891992	7	644	0	978	0	919	0	0	0	0	0	53600	59
	7.812	8.478		8.145	7.374	8.370			9	16.01	37100		35400								
3	862	596	6.02	729	596	828	3.8	7.872712	82	844	0	297	0	283	0	0	0	0	0	17700	14
				7.752	9.007				12	8.379	30500		26700								
4	NA	NA	NA	NA	093	586	12.4	8.379839	4	839	00	1968	00	1723	91000	59	0	0	0	186	
				8.039	4.210				6	6.125	14500		12600								
5	NA	NA	NA	NA	713	673	6.32	6.125193	32	193	000	7626	000	6601	0	225	00	800			
				3.769	7.383	3.900			4	9.411	62000		57100		19500						
6	582	0	45	291	192	236	10.49	5.641714	9	005	00	1950	00	1795	0	61	0	0	0	94	
				4.046	8.334				5	6.190	19000		18000								
7	NA	NA	NA	NA	026	374	5.78	6.190278	2	00	727	00	689	0	0	0	0	0	0	100E	38
				7.902	8.002				8	7.952	41100		36600								
8	NA	NA	NA	NA	571	652	8.09	7.952611	09	611	0	340	0	303	0	0	0	0	0	44600	37
				8.126	8.926				16	17.21	40100		36600								
9	093	019	6.94	056	055	234	9.13	8.685644	7	17	0	327	0	298	0	0	0	0	0	35100	29
				7.355	8.960				9	8.158	48800		45500								
10	NA	NA	NA	NA	538	627	12.94	8.158082	4	082	0	458	0	427	0	0	0	0	0	32700	31
				7.391	7.487				8	7.439	33100		29200								
11	NA	NA	NA	NA	016	171	8.25	7.439093	25	093	00	1167	00	1030	99800	35	0	0	0	102	
				7.705	8.213				4	15.60	22500		20500								
12	348	955	45	651	877	259	6.45	7.650068	5	972	00	1539	00	1403	70000	48	0	0	0	88	
				8.285	7.863				14	4	8.074	26300		22200							
13	NA	NA	NA	NA	012	391	14.45	8.074201	5	201	00	1848	00	1557	75700	53	0	0	0	238	
				8.024	9.033				10	8.534	18400		15400								
14	NA	NA	NA	NA	788	502	10.05	8.534145	5	145	00	1112	00	933	52600	32	0	0	0	148	
				4.075	4.075				4	2.037	21700		19200								
15	NA	NA	NA	NA	49	0	4.41	2.037745	41	745	00	1357	00	1200	65700	41	0	0	0	116	
				7.838	8.848				5	8.343	26400		23600								
16	NA	NA	NA	NA	275	501	5.69	8.343388	69	388	00	1871	00	1670	80600	57	0	0	0	143	



Sample collection

qPCR detection of salmon

Species identification



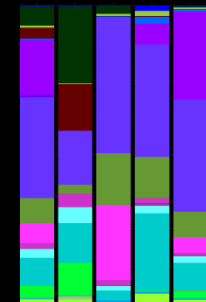
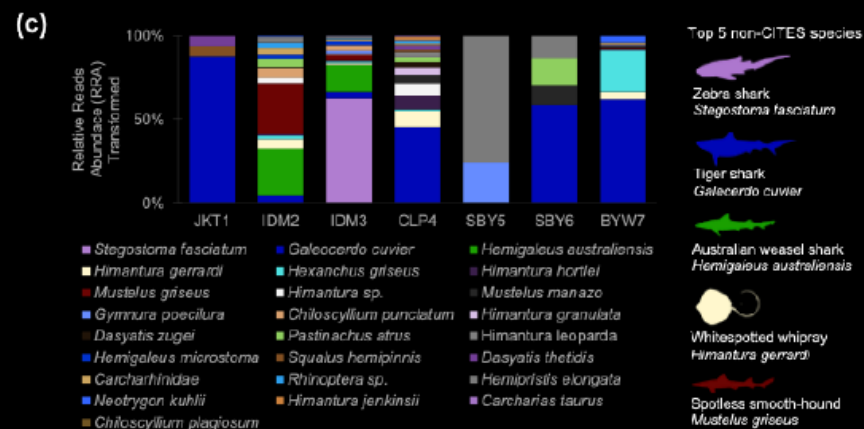
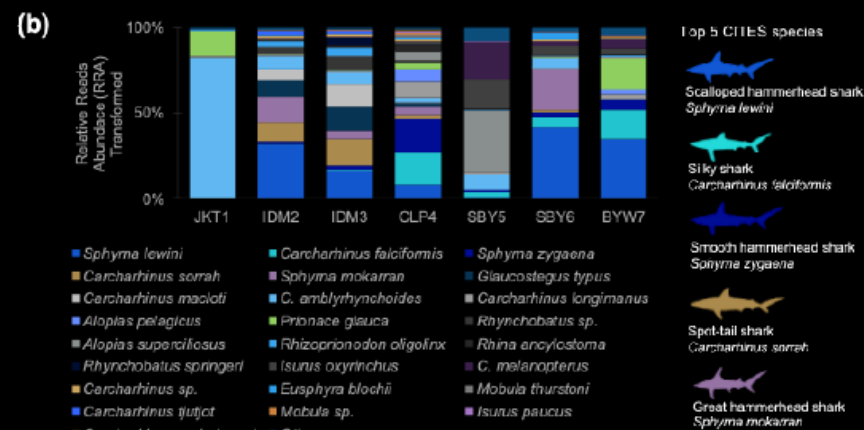
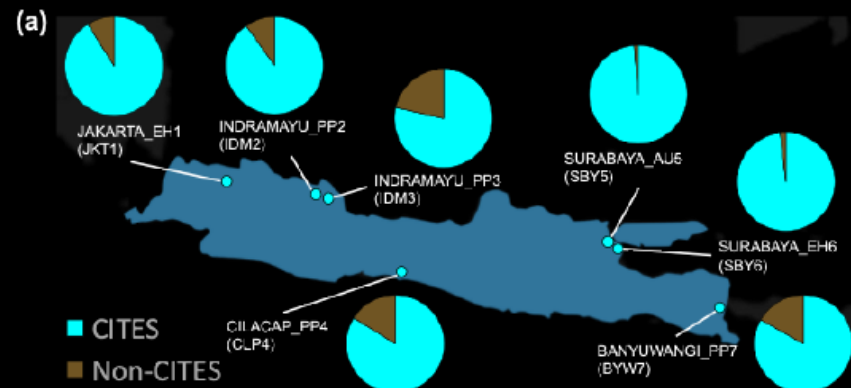
# Shark-dust: High-throughput DNA sequencing of processing residues unveils widespread trade in threatened sharks and rays

Andhika P. Prasetyo,<sup>1,2,3\*</sup> Joanna M. Murray,<sup>4</sup> Muh. Firdaus A. K. Kurniawan,<sup>5</sup> Naiara G. Sales,<sup>1</sup> Allan D. McDevitt,<sup>1,6†</sup> Stefano Mariani<sup>7†\*</sup>



Metabarcoding of dust with elasmobranch specific amplicon:

- 54 shark and ray taxa detected
- Outperformed all other detection and identification methods



# Assay development (qPCR / LAMP)

Received: 3 December 2020 | Revised: 4 April 2021 | Accepted: 14 April 2021  
DOI: 10.1002/edn3.202

**ORIGINAL ARTICLE**

**Environmental DNA**  
Dedicated to the study and use of environmental DNA for basic and applied sciences

**WILEY**

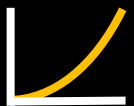
## Development of an environmental DNA assay for detecting multiple shark species involved in human–shark conflicts in Australia

Anthony van Rooyen<sup>1</sup> | Adam D. Miller<sup>2,3</sup> | Zach Clark<sup>2</sup> | Craig D. H. Sherman<sup>2,3</sup> | Paul A. Butcher<sup>4</sup> | Justin R. Rizzari<sup>2</sup> | Andrew R. Weeks<sup>1,5</sup> 

**SCIENTIFIC REPORTS**  
nature research

**OPEN** **Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use**

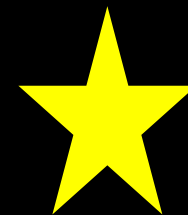
Grace Wing-Chiu But<sup>1</sup>, Hoi-Yan Wu<sup>2,3</sup>, Kwang-Tsao Shao<sup>4</sup> & Pang-Chui Shaw<sup>1,2,3\*</sup>



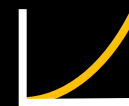
Bull shark  
Great white shark  
Tiger shark

**Resource for validated assays:**

<https://labs.wsu.edu/edna/edna-assays/>



Pelagic thresher shark  
Bigeye thresher shark  
Common thresher shark  
Great white shark  
Silky shark  
Oceanic whitetip shark  
Basking shark  
Porbeagle shark  
Whale shark  
Scalloped hammerhead shark  
Great hammerhead shark  
Smooth hammerhead shark

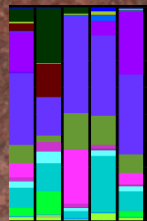





# Mobile platforms



MinION:  
Metabarcoding



Biomeme:  
qPCR 



Biomeme:  
DNA extraction kit

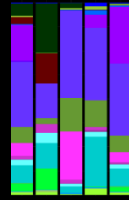


LAMP assay  
(COVID-19)

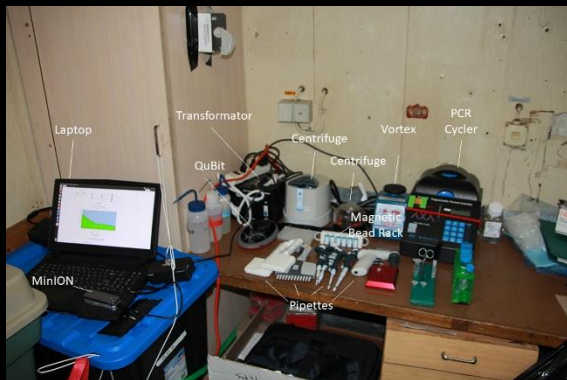


# Field application options

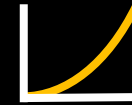
## MinION Metabarcoding



- ~ \$25k equipment
- \$1.5k/run
  - Up to 96 samples
  - Infinite targets
- 1-2 day turnaround



## Biomeme qPCR



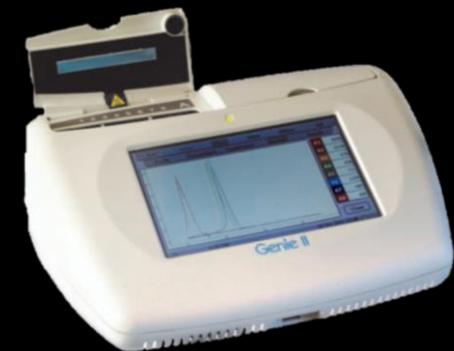
- ~ \$20k equipment
- \$250/run
  - Up to 9 samples
  - 3 targets (27 assays)
- 2-3h turnaround



## Genie II LAMP



- ~\$20k equipment
- \$200/run
  - Up to 9 samples
  - 2 targets (18 assays)
- 2-3h turnaround



# Potential application

- Species identification
  - Elasmobranch samples
  - Elasmobranch containing products
  - Fishery catches
- Detection of elasmobranch DNA
  - Food
  - Livestock/pet food
  - Cosmetics
  - Pills / potions / remedies
- Identification
  - IUU fishing vessels
  - Processing facilities
  - Transportation







# Thank you!

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