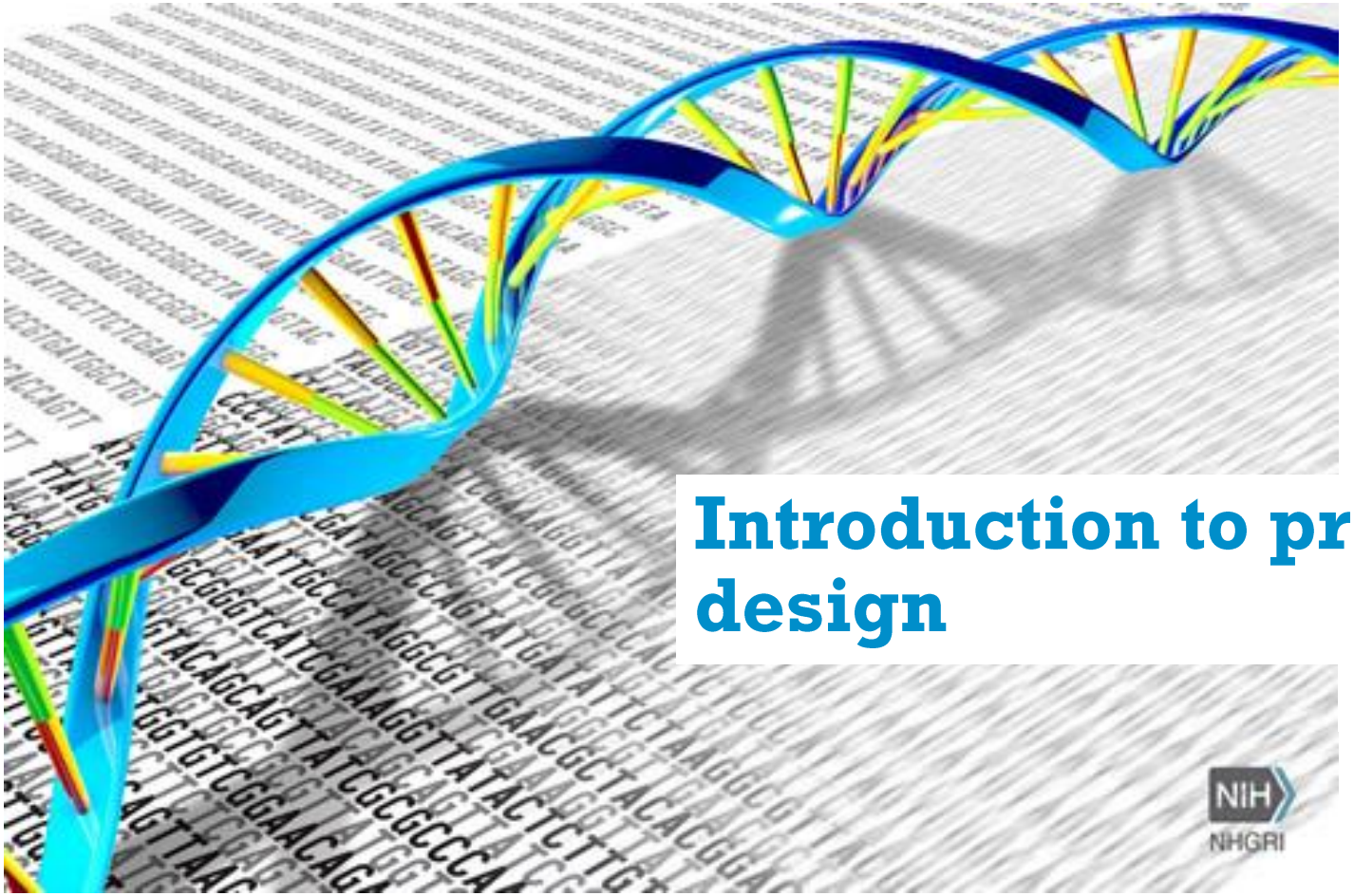
ZMT LEIBNIZ CENTRE for Tropical Marine Research

Leibniz Association



Introduction to primer design

Achim Meyer

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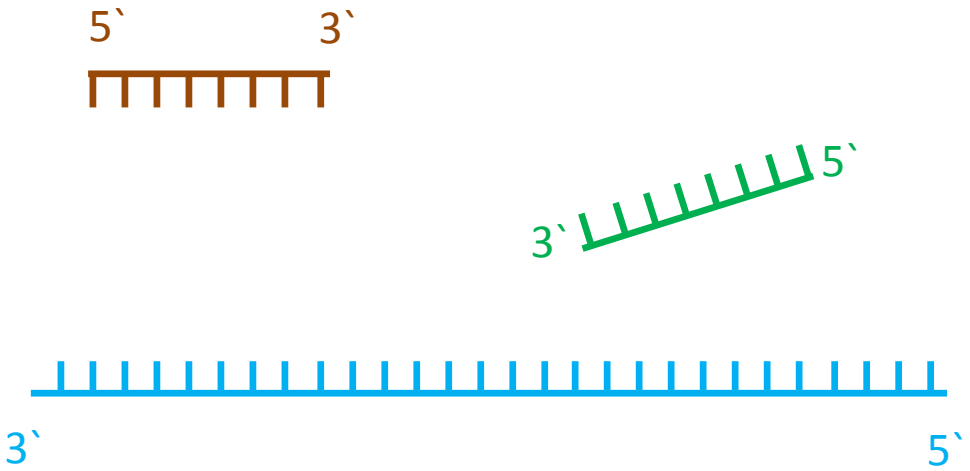
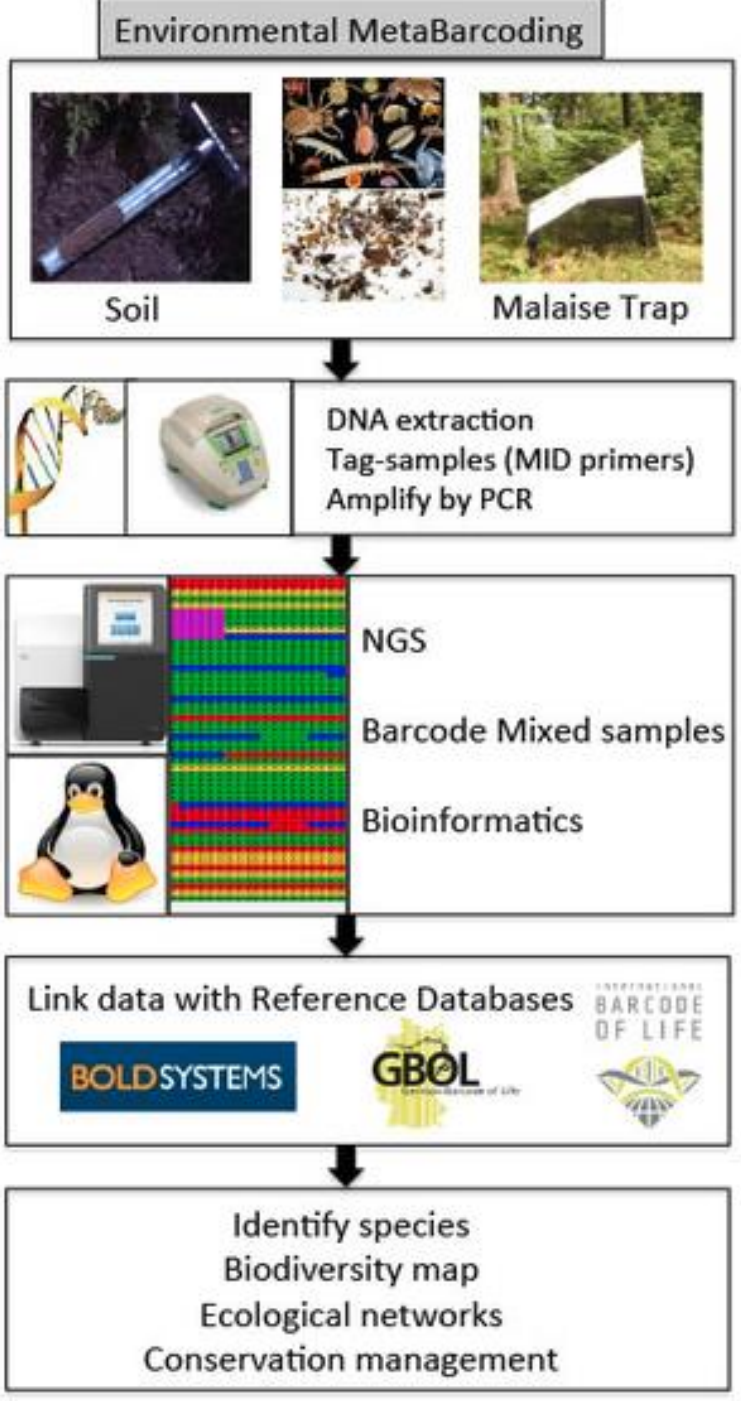
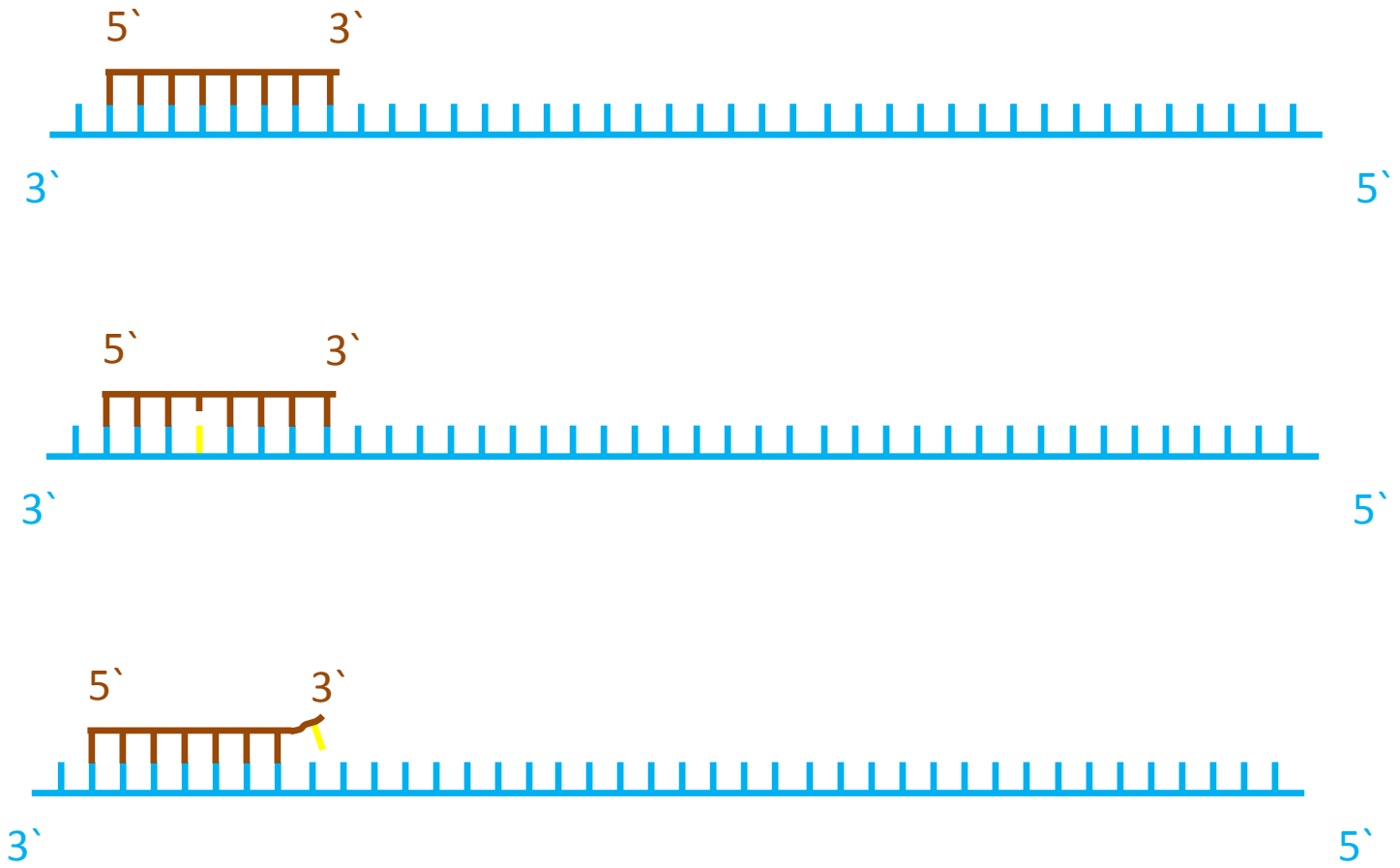
Selected primer are of crucial importance






5`
3`
3`
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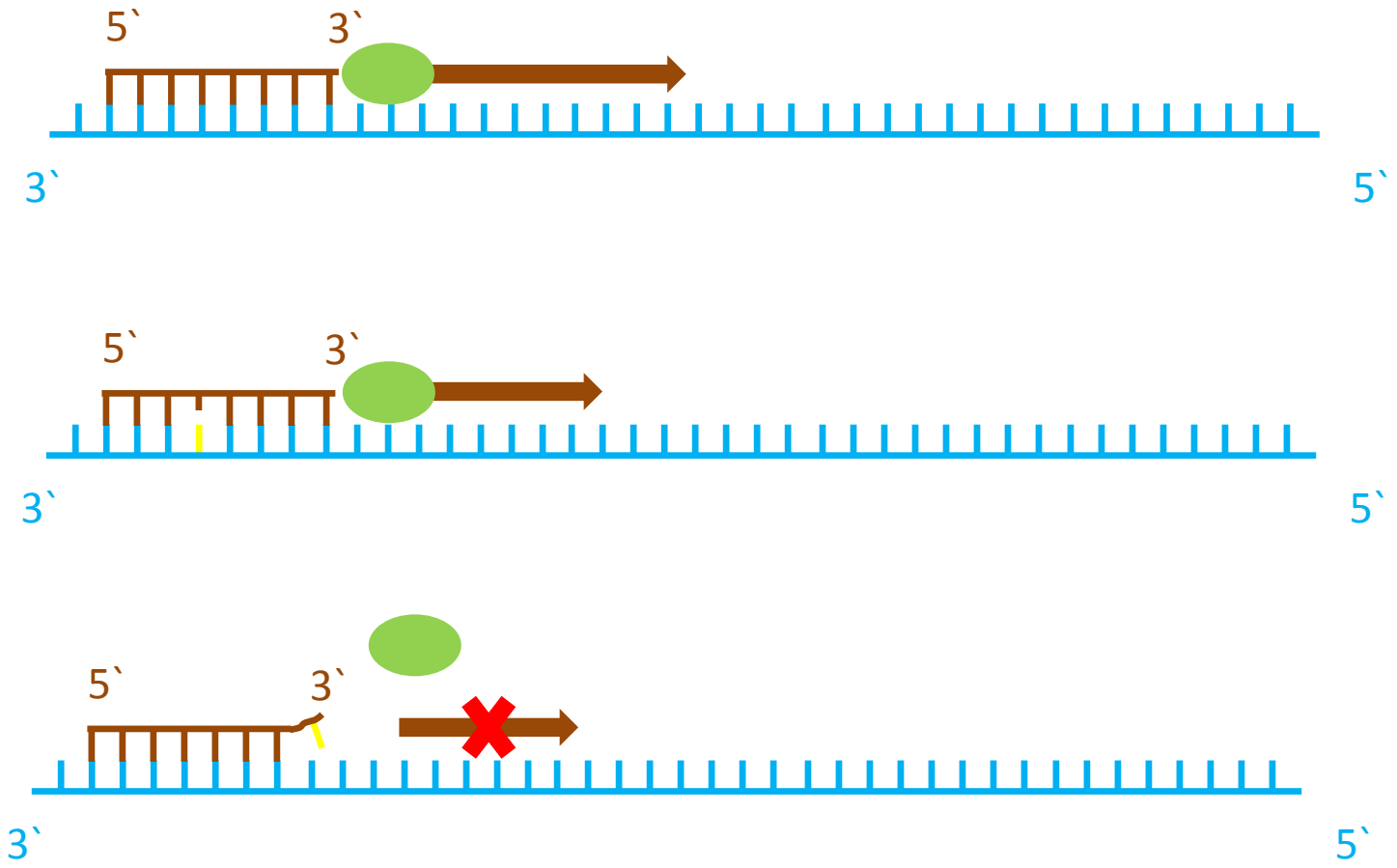
5`


5`
3`
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3`
3`
5`
5`
3`
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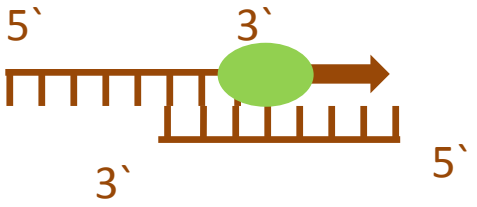
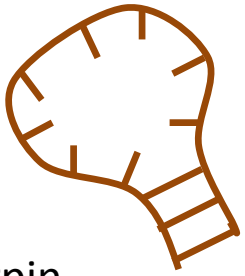
5`

Algorithm to predict hybridization behavior consider for example

- Melting temperature
- Homodimer
- Heterodimer
- Hairpin
- …



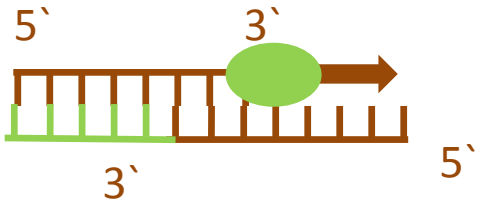
Homo/Heterodimer



Hairpin

Algorithm to predict hybridization behavior consider for example

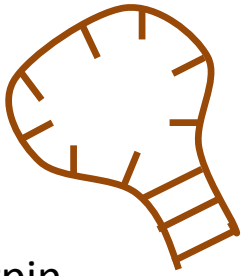
- Melting temperature
- Homodimer
- Heterodimer
- Hairpin
- …



Homo/Heterodimer

Implemented for example in

- Primer3
- OLIGO 7
- PrimerSelect
- Primer Express
- Primer Premier



Hairpin

Primer design: Base pairing

A=T

- 2 Hydrogen bonds
- Specific binding
- Low binding force

C≡G

- 3 Hydrogen bonds
- Less specific binding
- Strong binding force

3` 5`

$$Tm = 2°C * (A + T) + 4°C * (C + G)$$

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Primer design: Primer3

Primer3web version 4.1.0 - Pick primers from a DNA sequence.

disclaimer | code | cautions

Select the Task for primer selection generic

Template masking before primer design (available species)

Select species Example: Mus musculus

Primer failure rate cutoff < 0.1

Nucleotides to mask in 5' direction 1

Nucleotides to mask in 3' direction 0

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library) NONE

☑ Pick left primer, or use left primer below | ☐ Pick hybridization probe (internal oligo), or use oligo below | ☑ Pick right primer, or use right primer below (5' to 3' on opposite strand)

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Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

Reset page | Save search parameters | Retrieve recent results | Publication | Tips for finding specific primers

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range

	From	To
Forward primer		
Reverse primer		

Clear

Or, upload FASTA file Browse... No file selected.

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	70	1000

# of primers to return 10

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span No preference

Exon junction match

Exon at 5' side	Exon at 3' side
7	4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

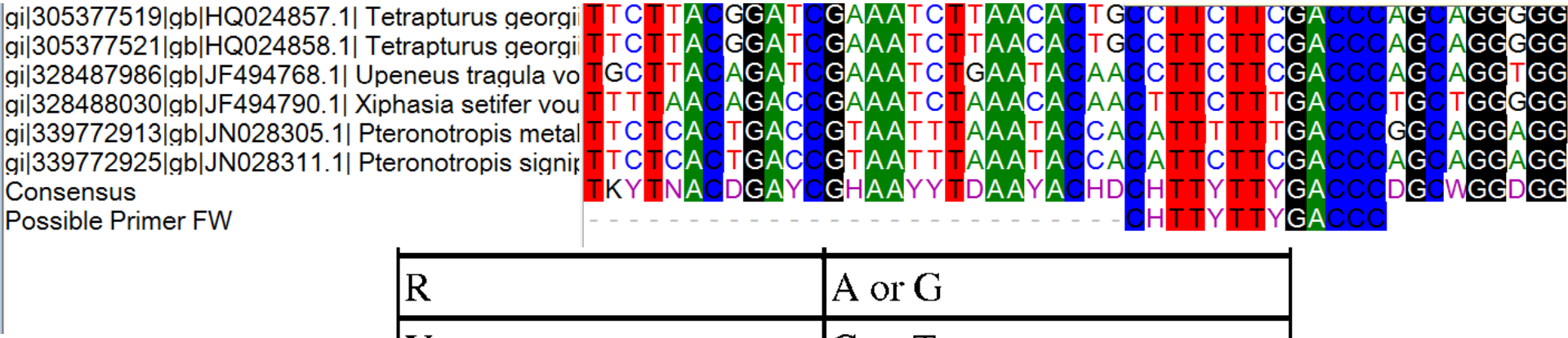
Min	Max
1000	1000000

Primer Pair Specificity Checking Parameters

Specificity check Enable search for primer pairs specific to the intended PCR template

Search mode Automatic

Manual primer design

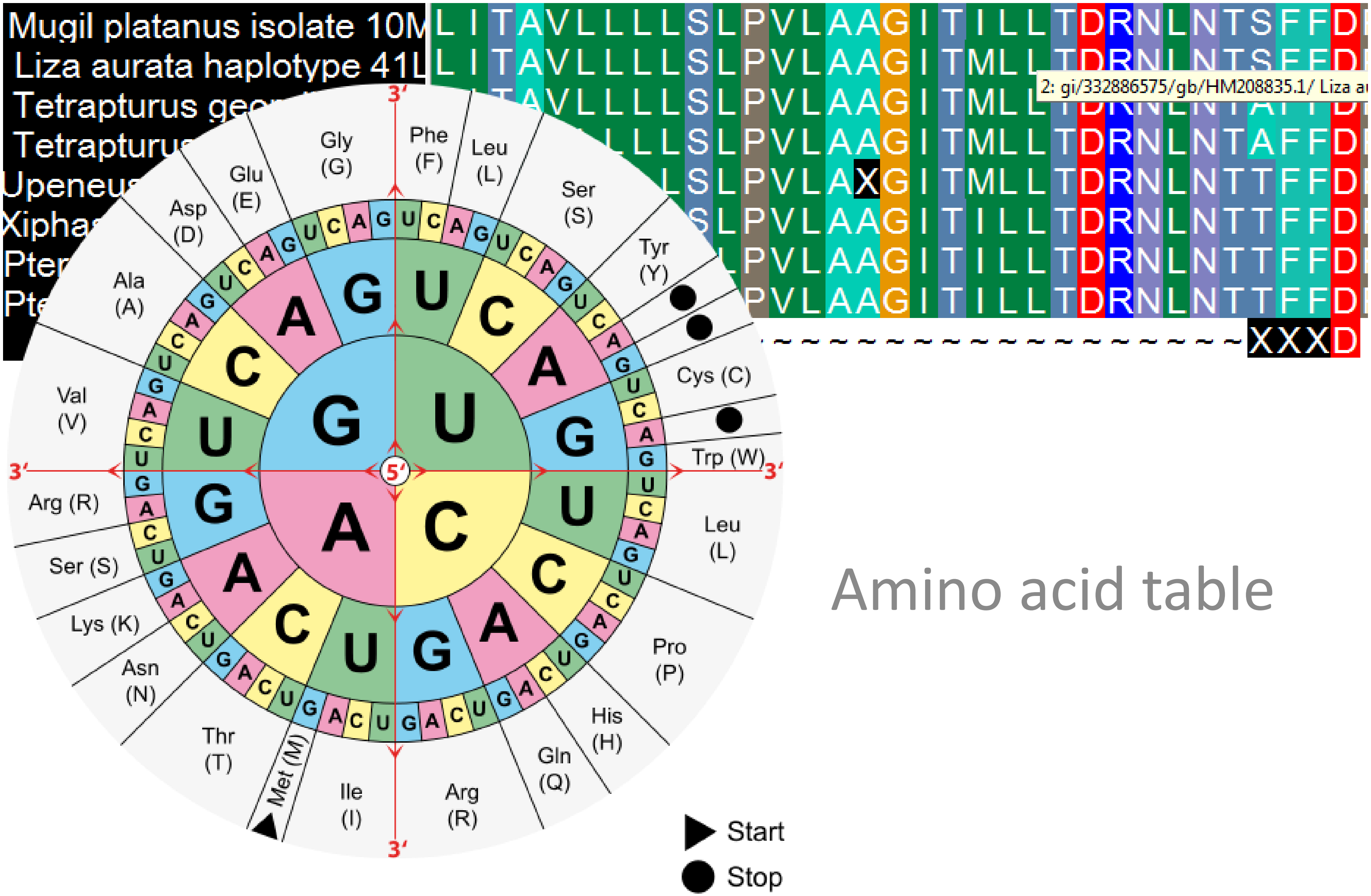


IUPAC code

R	A or G
Y	C or T
S	G or C
W	A or T
K	G or T
M	A or C
B	C or G or T
D	A or G or T
H	A or C or T
V	A or C or G
N	any base

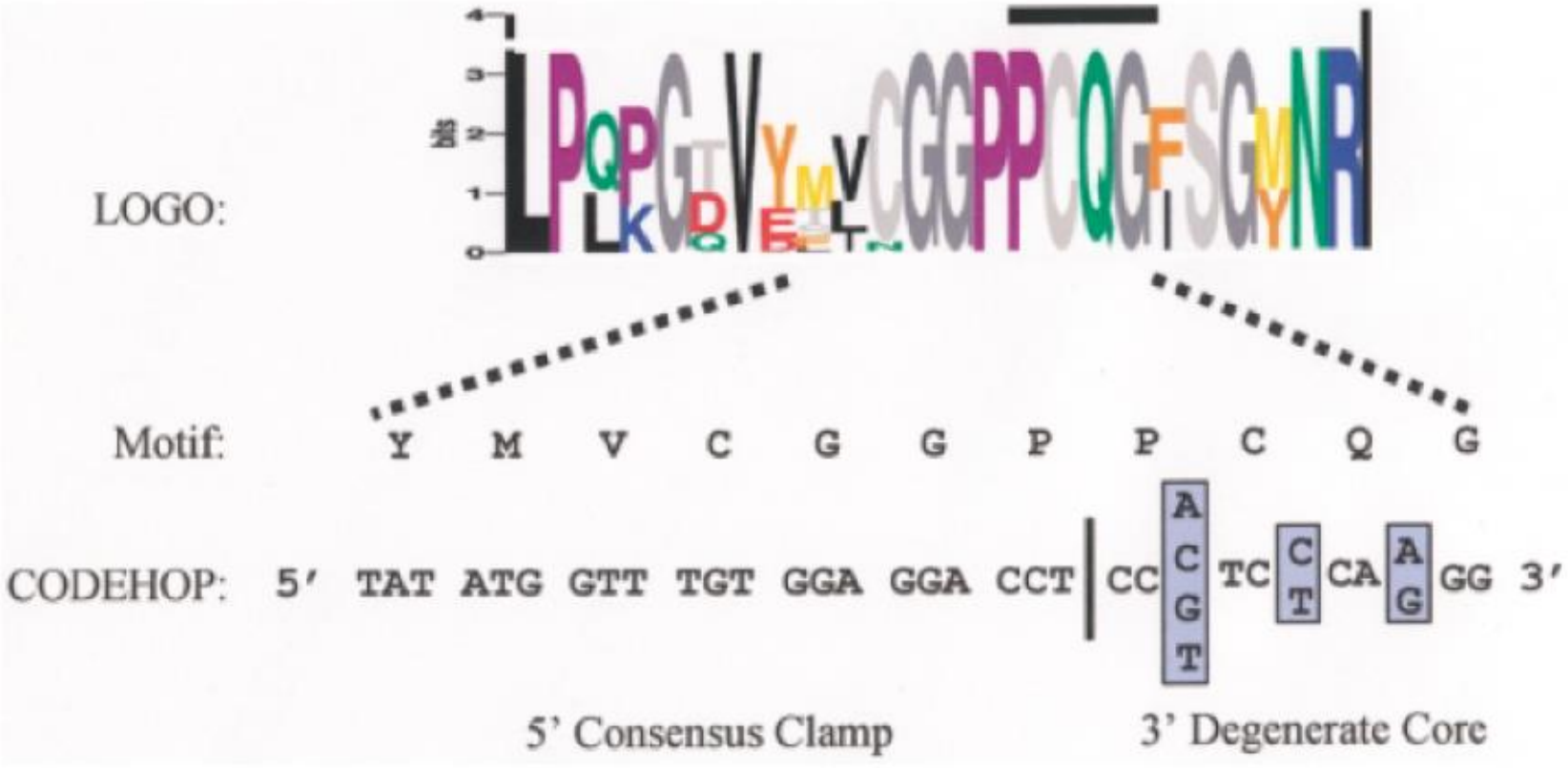
Bases

Manual primer design



Amino acid table

J-CODEHOP (Boyce R. et al. 2009)



Example 2: http://primaclade.org/cgi-bin/primaclade.cgi

Updates to the FDA Single Laboratory Validated Method for DNA Barcoding for the Species Identification of Fish

Michelle M. Moore[1]*, Sara M. Handy[2], Christopher J. Haney[3†], Gabrielle S. Pires[1], Lynda L. Perry[1], Jonathan R. Deeds[2], and Haile F. Yancy[4]

3.2 Reagents for both PCR master mixes

A. M13-tailed CO1 primers:

1. **FISHCO1LBC_ts**:
   CACGACGTTGTAAAACGACTCAACYAATCAYAAAGATATYGGCAC
2. **FISHCO1HBC_ts**:
   GGATAACAATTTCACACAGGACTTCYGGGTGRCCRAARAATCA

Note: M13 tails are used to stream-line the sequencing process and allow for longer reads in the CO1 gene (7).

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   GGATAACAATTTCACACAGGACTTCYGGGTGRCCRAARAATCA

Note: M13 tails are used to stream-line the sequencing process and allow for longer reads in the CO1 gene (7).

6.2 Cycle Sequencing Reaction Recipe

A. M13 primers

1. **M13F (-29):** CACGACGTTGTAAAACGAC
2. **M13R:** GGATAACAATTTCACACAGG

CCDB

Canadian Centre for DNA Barcoding

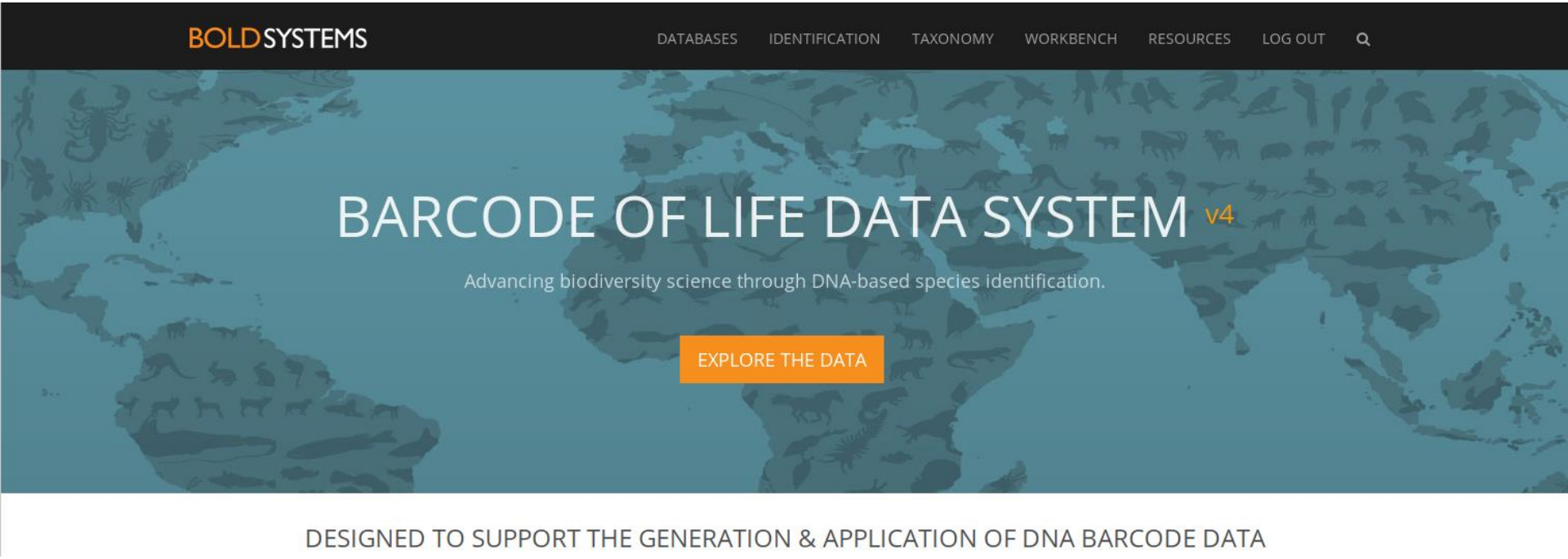
Protocols

Primer Sets

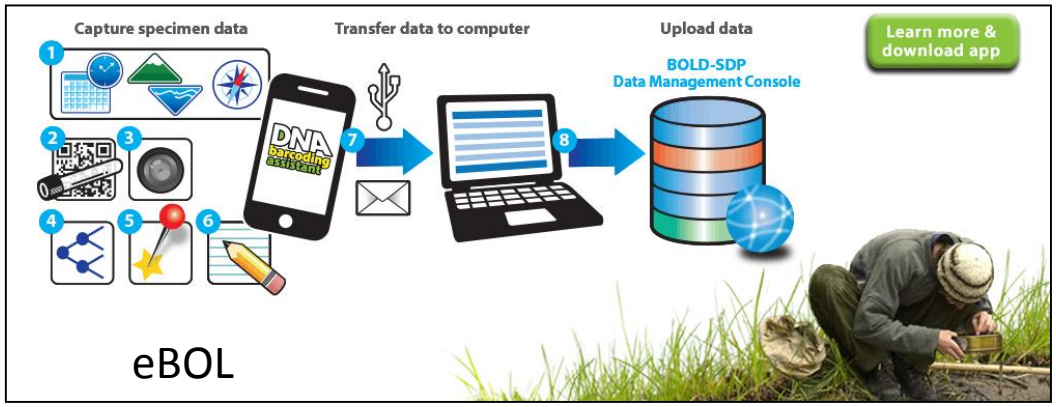
Primer sets comprising major analytical pipelines at CCDB

Name	Ratio	Cocktail name / Primer sequence 5'-3'	Taxonomic Groups	Reference
Regular primer pairs				
		Folmer primers	various phyla	Folmer et al. 1994
LCO1490		GGTCAACAAATCATAAAGATATTGG		
HCO2198		TAAACTTCAGGGTGACCAAAAAATCA		
		Lepidoptera primers	insects, amphibians	
LepF1		ATTCAACCAATCATAAAGATATTGG		Hebert et. al. 2004a
LepR1		TAAACTTCTGGATGTCCAAAAAATCA		Hebert et. al. 2004a
MLepF1		GCTTTCCCACGAATAAATAATA (use with LepR1)		Hajibabaei et al. 2006
MLepR1		CCTGTTCCAGCTCCATTTTC (use with LepF1)		Hajibabaei et al. 2006
		Bird primers	birds	Hebert et al. 2004b
BirdF1		TTCTCCAACCACAAAGACATTGGCAC		
BirdR1		ACGTGGGAGATAATTCCAAATCCTGG		
M13-tailed cocktails				
		C_VF1LFt1 – C_VR1LRt1 (Mammal cocktail)	mammals, reptiles, fish, amphibians	Ivanova et al. 2007

www.boldsystems.org



DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA



eBOL

Primer Database

A comprehensive registry of primers used in the generation of barcode sequences. The registry is maintained by users of BOLD.

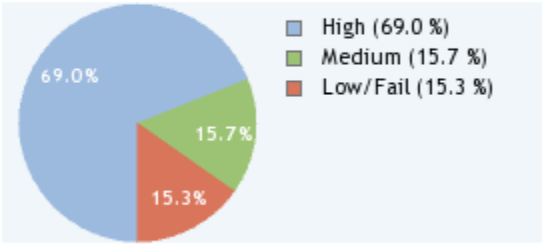
www.boldsystems.org

Primer Database

A comprehensive registry of primers used in the generation of barcode sequences. The registry is maintained by users of BOLD.

Primer Stats

Number of Sequencing Runs: 25231 samples



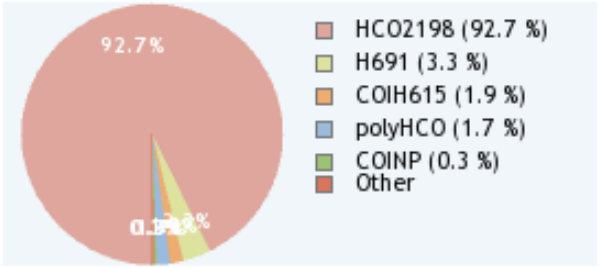
Paired Primer Stats

Paired Primers Summary

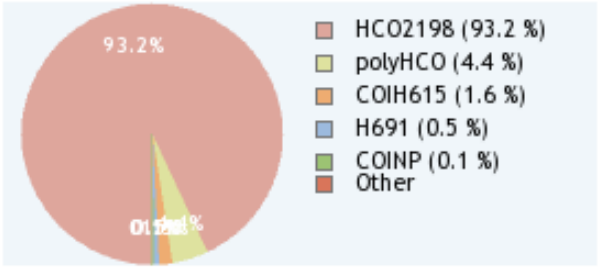
Primer	High	Medium	Low/Failed
HCO2198	16159	4275	4019
H691	580	24	15
COIH615	326	75	39
polyHCO	297	204	421
COINP	51	4	7
CRYPCOIR	18	2	0
C1-N-2191	8	0	0
MLepR1	1	4	28
LepR1	0	0	5

Paired Primer Stats

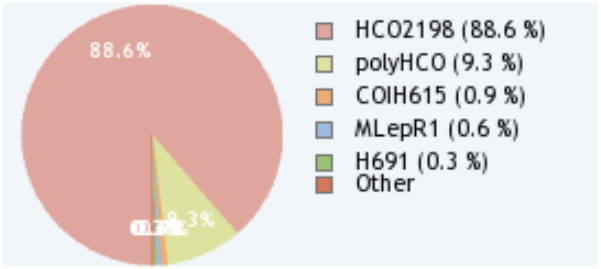
High Quality Runs: 17440 samples



Medium Quality Runs: 4588 samples

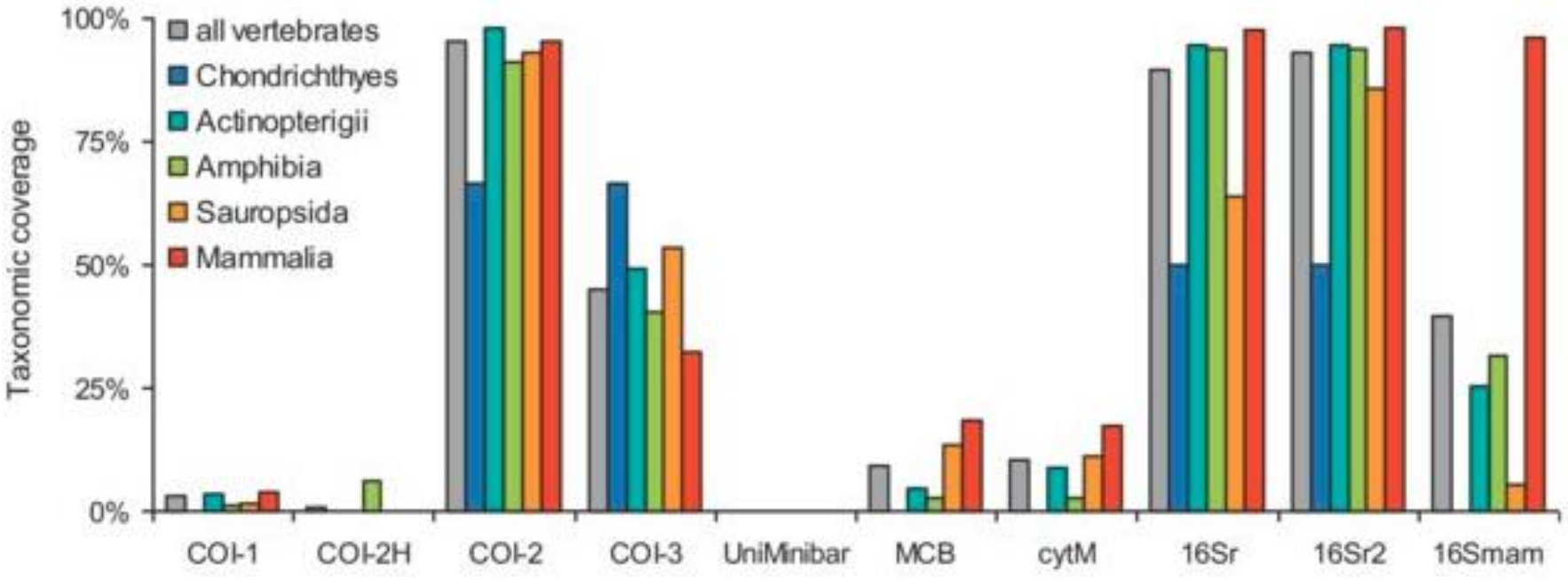


Low Quality/Failed Runs: 4534 samples

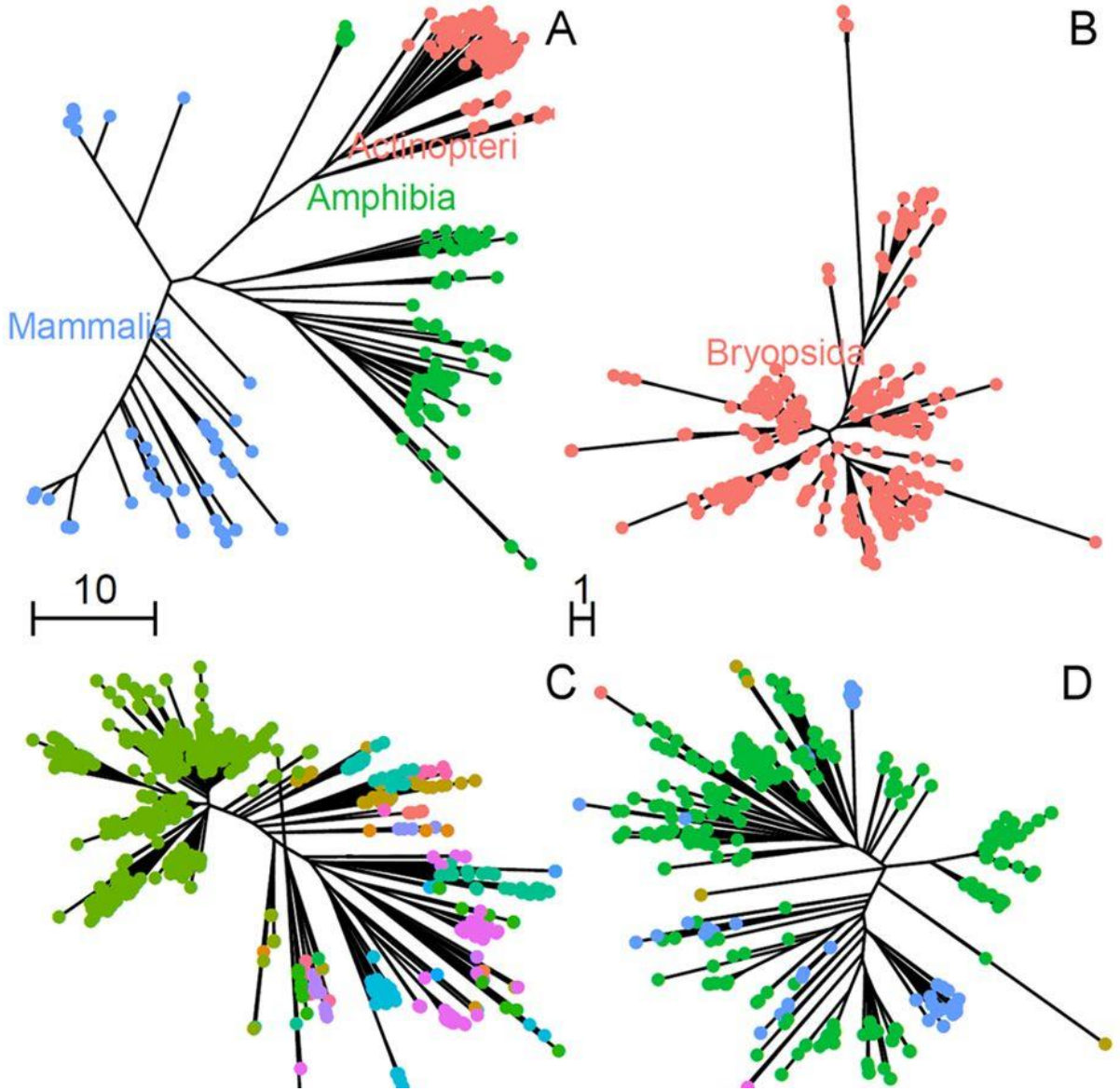


In silico comparison of primers for DNA barcoding

(Ficetola et al. 2010)



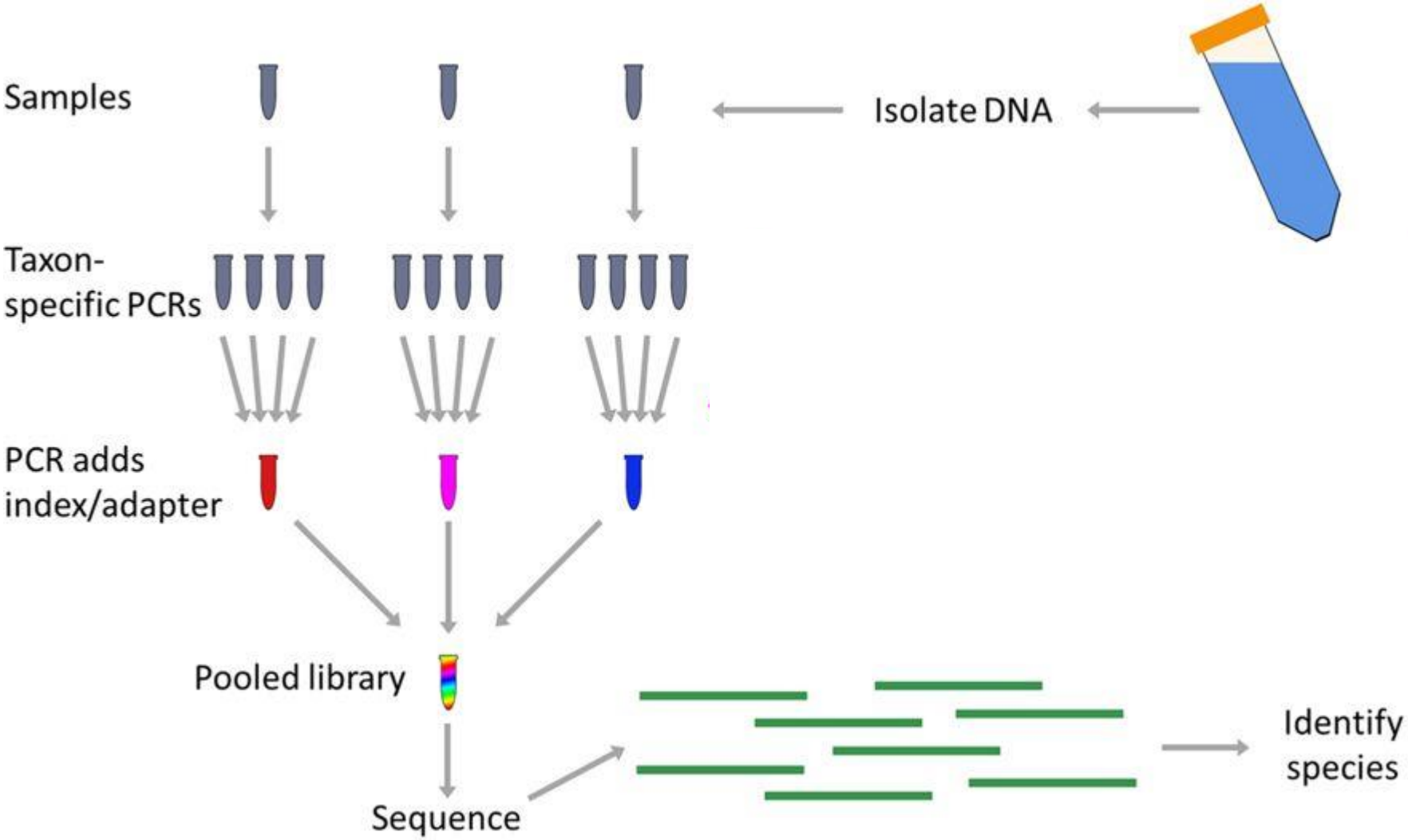
primerTree



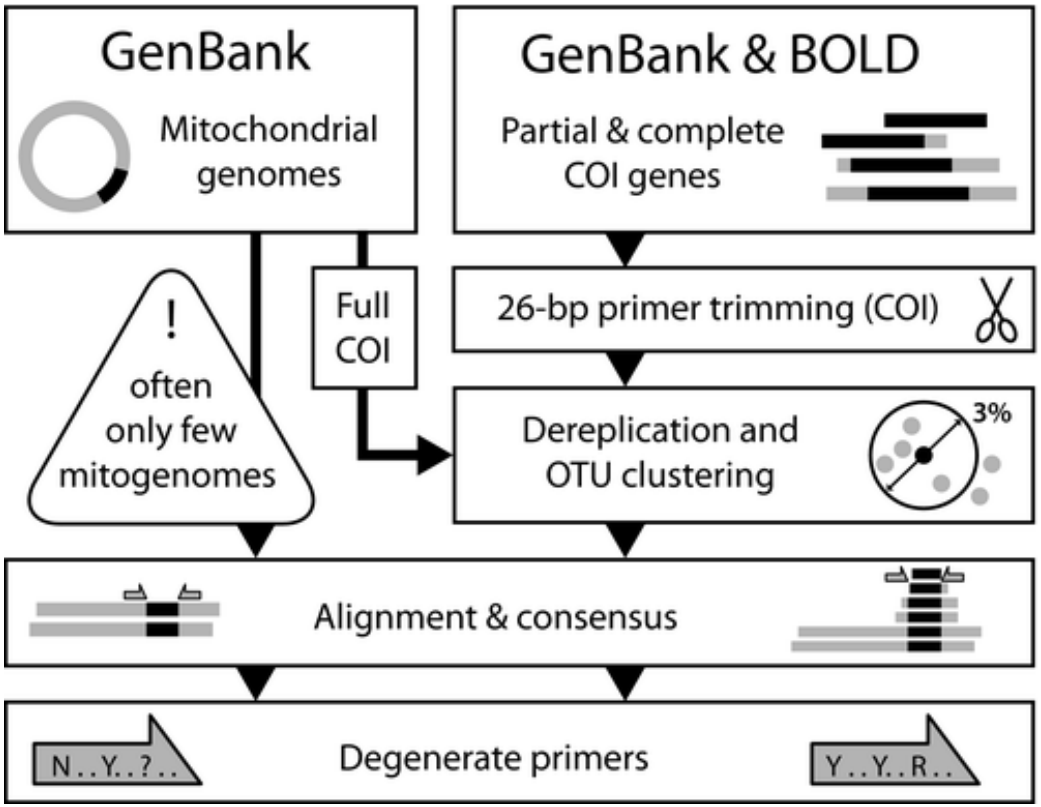
Description

`primerTree` has two main commands: `search_primer_pair` which takes a primer pair and returns an primerTree object of the search results `plot.primerTree` a S3 method for plotting the primerTree object obtained using `search_primer_pair`

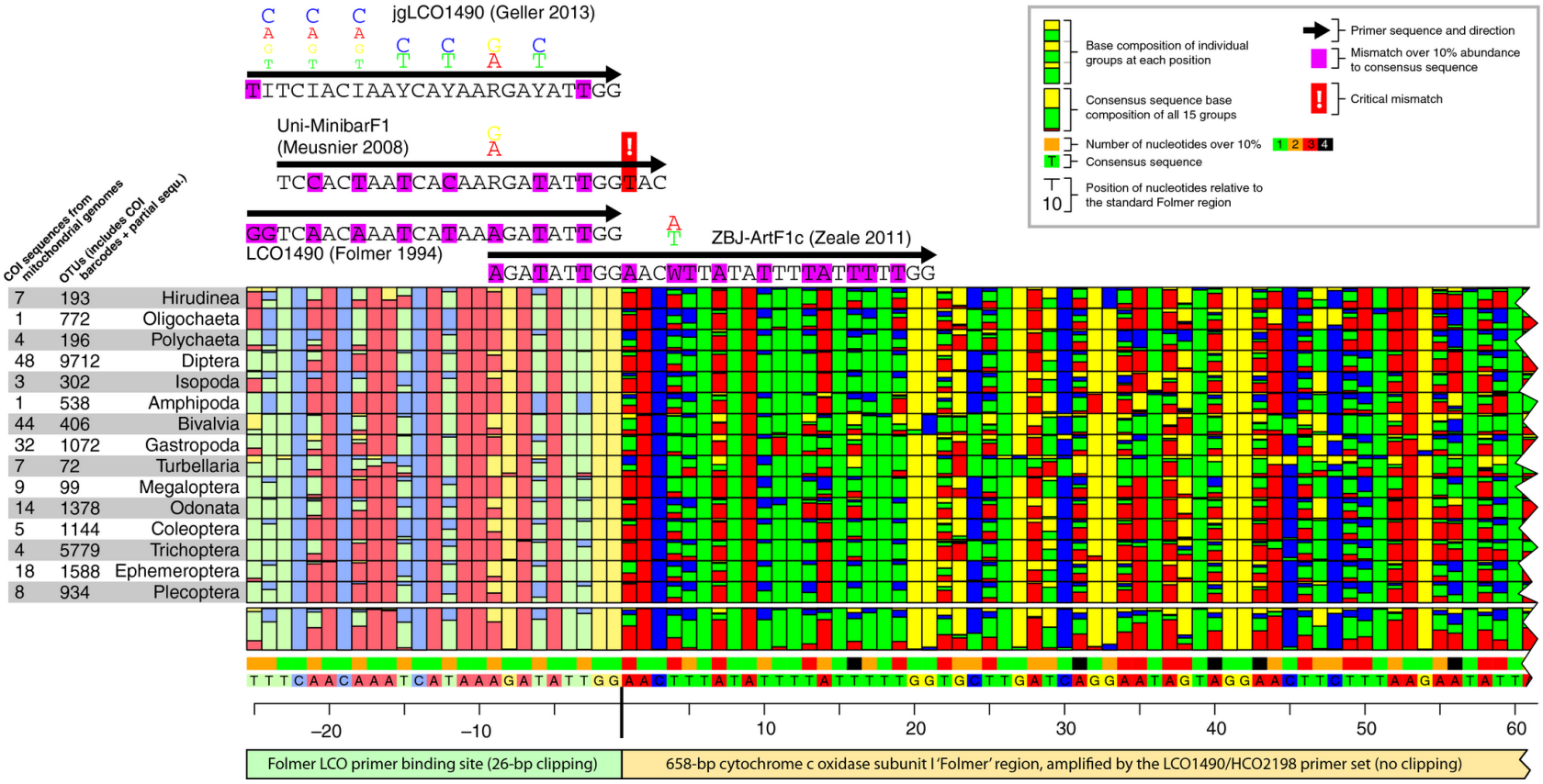
primerTree


Samples
Isolate DNA
Taxon-specific PCRs
PCR adds index/adapter
Pooled library
Sequence
Identify species

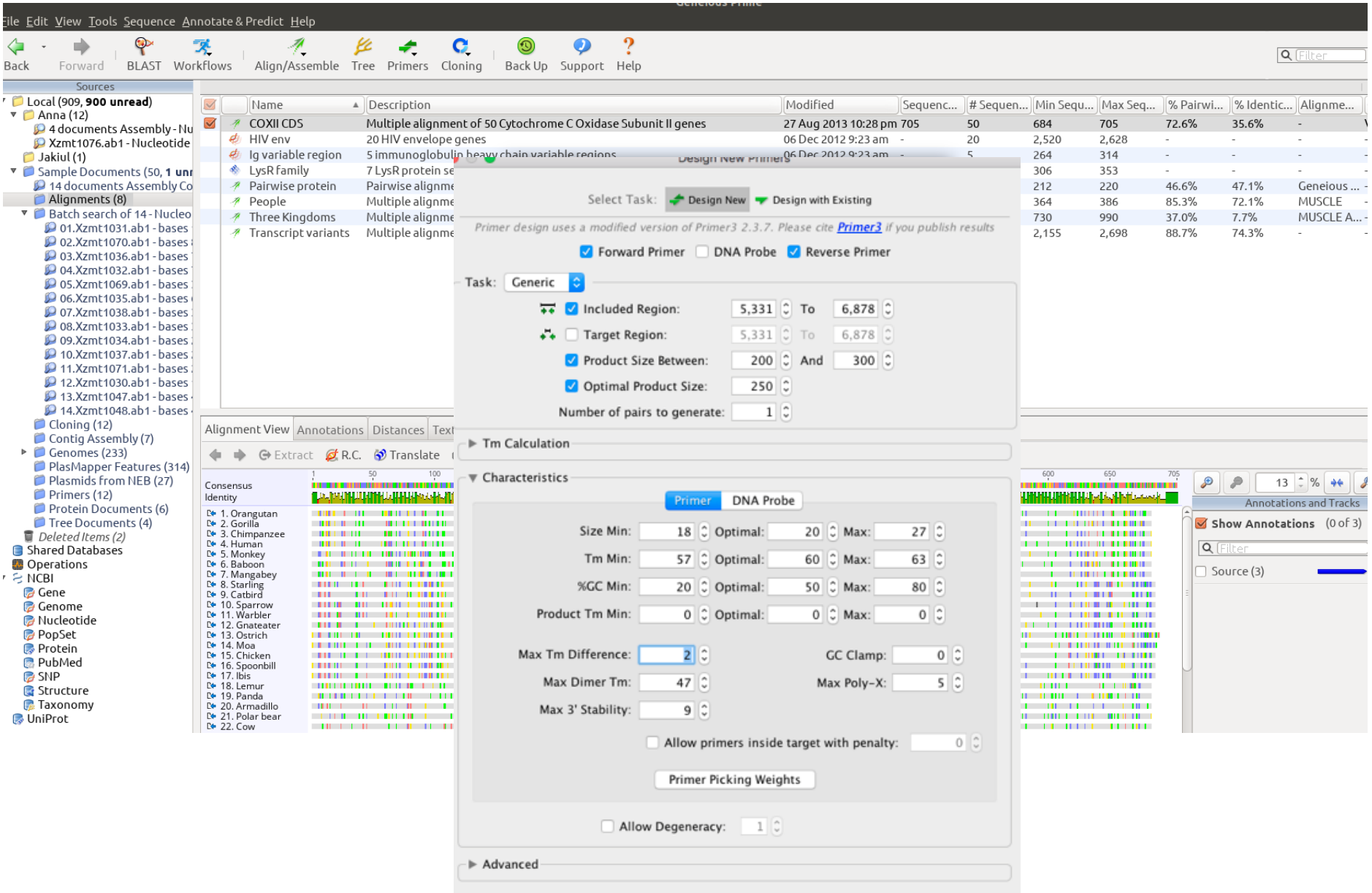
PrimerMiner: an R package for development and in silico validation of DNA metabarcoding primers



PrimerMiner: an R package for development and in silico validation of DNA metabarcoding primers



geneious prime



HIV sequence database

DATABASES SEARCH ALIGNMENTS TOOLS PUBLICATIONS GUIDES search sit Search

PrimerDesign-M

Purpose: To find primers for PCR, sequencing (including NGS), and other uses. Primers are based a multiple alignment and optimized to user-defined criteria. See PrimerDesign-M Explanation.

Alignment

Paste your alignment

[Sample Input]

Or upload alignment file Browse... No file selected.

Or use premade HIV or SIV sequence alignment

Gap stripping Remove columns having greater than 50 % gaps

Sequence, Adaptor, and Barcode Options

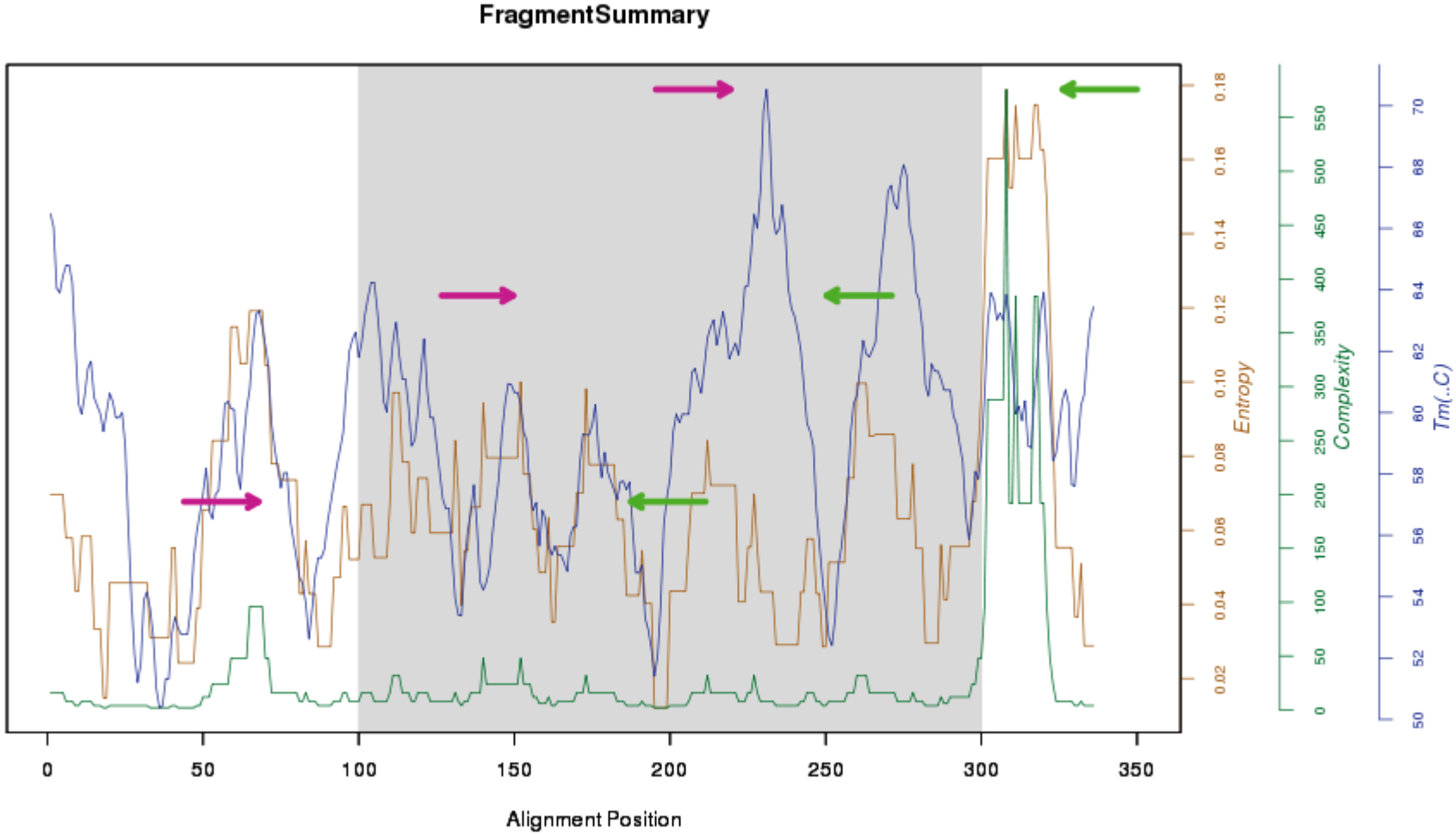
Region of Interest Start Stop

Multiple fragments Single fragment Multiple fragments

70 68 66 64 62 60 58 56 54 52 50

Tm(..C)

Alignment Position


FragmentSummary
0
50
100
150
200
250
300
350
Alignment Position
Entropy
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
Complexity
0
50
100
150
200
250
300
350
400
450
500
550
Tm(..C)
50
52
54
56
58
60
62
64
66
68
70

U.S. National Library of Medicine | NCBI National Center for Biotechnology Information

Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

Reset page | Save search parameters | Retrieve recent results | Publication | Tips for finding specific primers

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred) Clear

Range

	From	To
Forward primer		
Reverse primer		

Clear

Or, upload FASTA file Browse... No file selected.

Primer Parameters

Use my own forward primer (5'->3' on plus strand) Clear

Use my own reverse primer (5'->3' on minus strand) Clear

	Min	Max
PCR product size	70	1000

# of primers to return 10

	Min	Opt	Max	Max Tm difference
Primer melting temperatures (Tm)	57.0	60.0	63.0	3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span No preference

Exon junction match

Exon at 5' side	Exon at 3' side
7	4

Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range

Min	Max
1000	1000000

Primer Pair Specificity Checking Parameters

Specificity check Enable search for primer pairs specific to the intended PCR template

Search mode Automatic

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Primer design part II: Obitools

Veronique Helfer