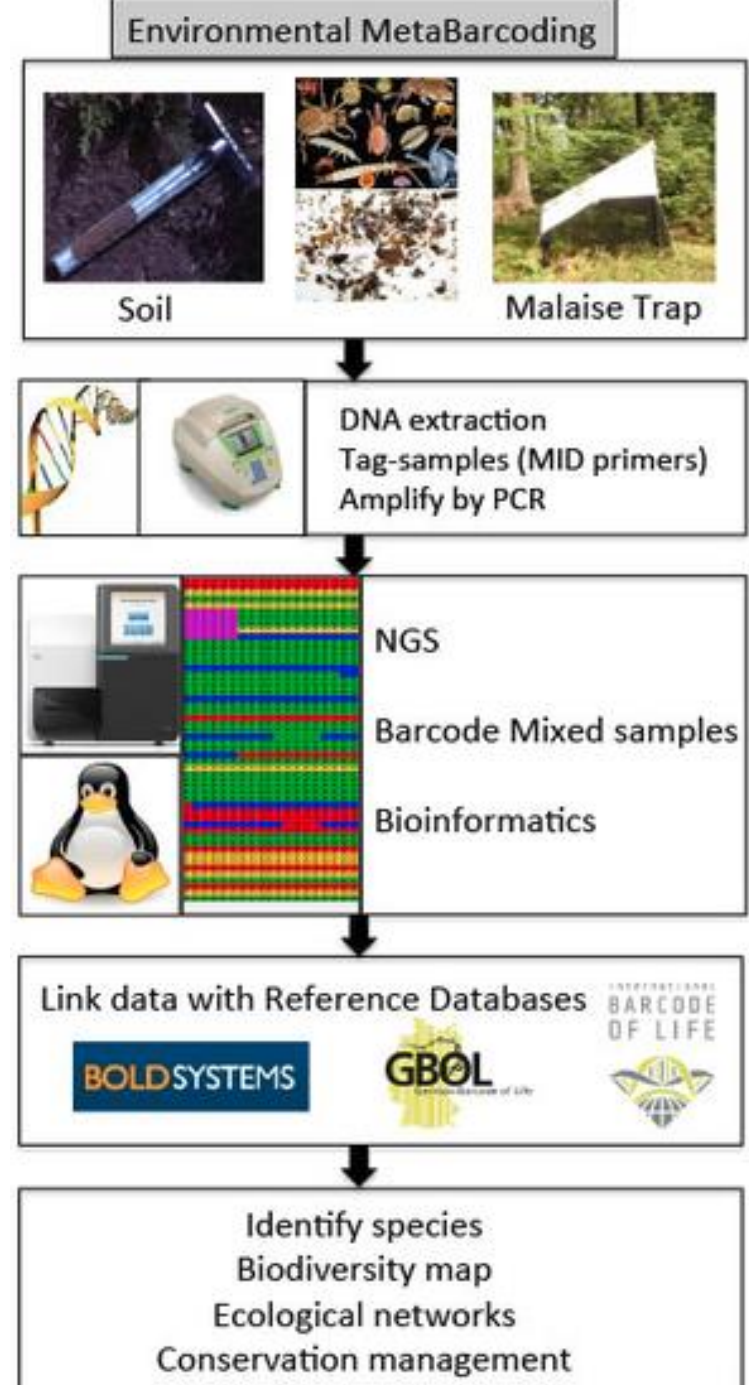
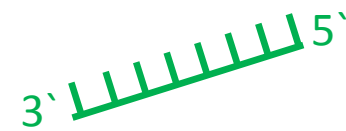
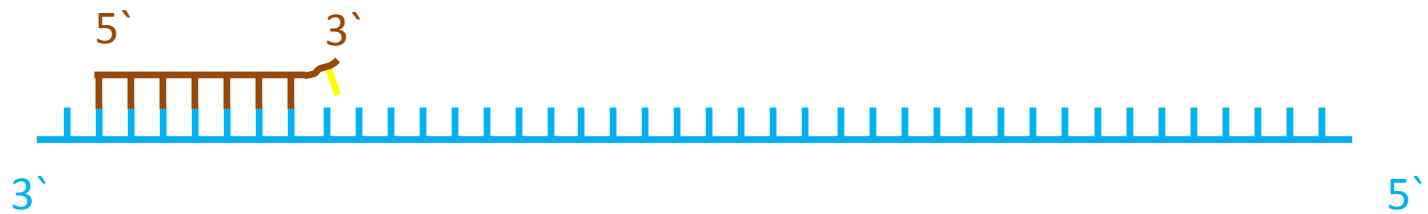
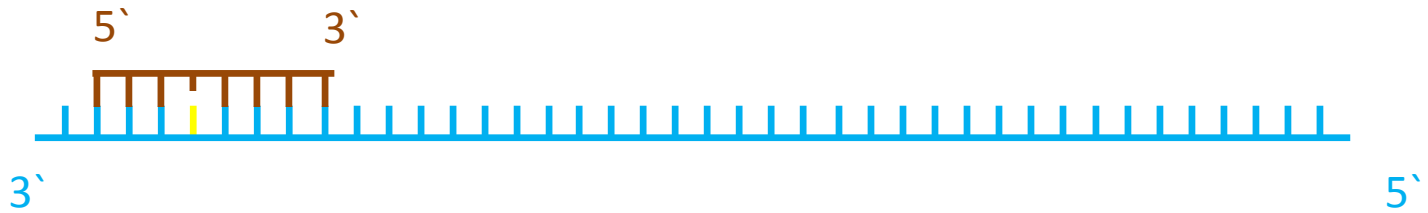
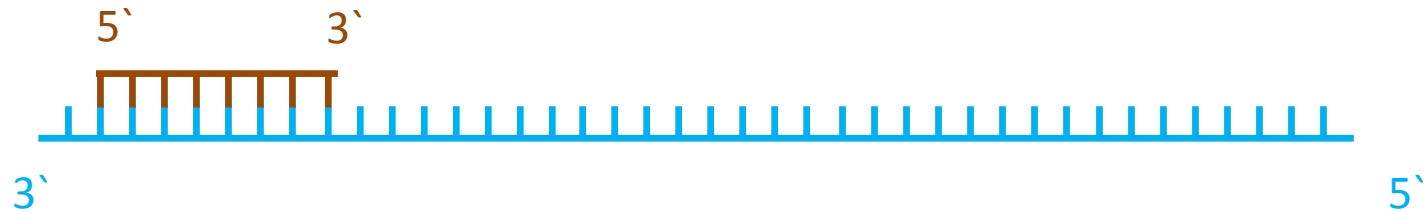


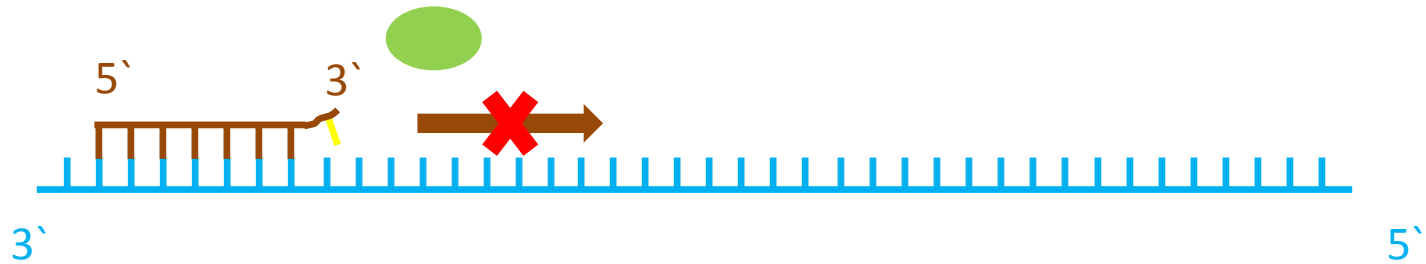
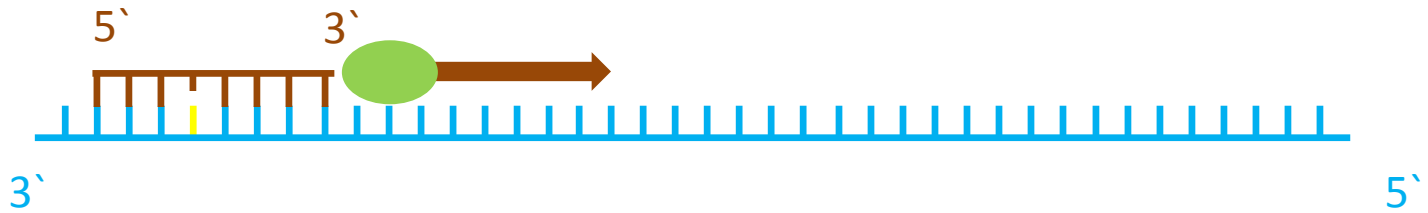
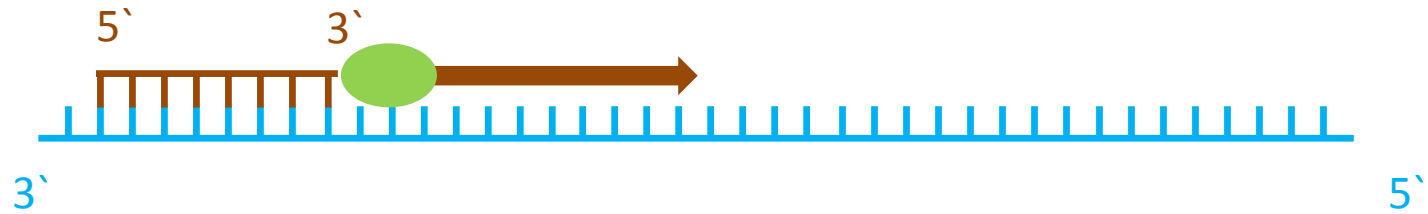
Introduction to primer design

Achim Meyer

Selected primer are of crucial importance

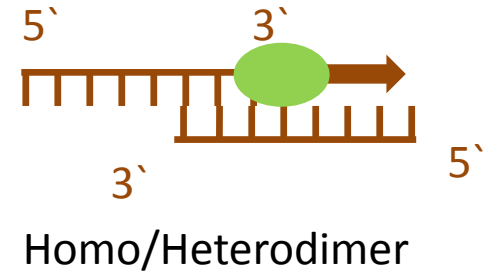






Algorithm to predict hybridization behavior
consider for example

- Melting temperature
- Homodimer
- Heterodimer
- Hairpin
- ...

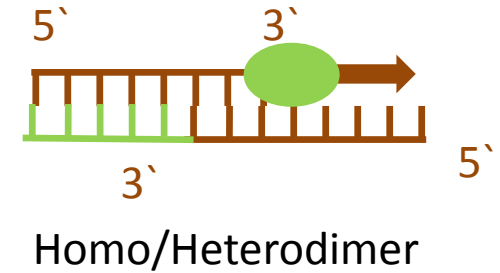


Algorithm to predict hybridization behavior consider for example

- Melting temperature
- Homodimer
- Heterodimer
- Hairpin
- ...

Implemented for example in

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




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' $T_m = 2^{\circ}\text{C} * (A + T) + 4^{\circ}\text{C} * (C + G)$ 5'

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	Nucleotides to mask in 5' direction 1	
Primer failure rate cutoff < 0.1	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

<input checked="" type="checkbox"/> Pick left primer, or use left primer below	<input type="checkbox"/> Pick hybridization probe (internal oligo), or use oligo below	<input checked="" type="checkbox"/> Pick right primer, or use right primer below (5' to 3' on opposite strand)

Primer-BLAST

A tool for finding specific primers

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

PCR Template

[Reset page](#)[Save search parameters](#)[Retrieve recent results](#)[Publication](#)[Tips for finding specific primers](#)

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

[Clear](#)

Or, upload FASTA file

No file selected.

Range

Forward primer

From

To

Reverse primer

[Clear](#)

Primer Parameters

Use my own forward primer
(5'→3' on plus strand)

[Clear](#)

Use my own reverse primer
(5'→3' on minus strand)

[Clear](#)

PCR product size

Min

Max

of primers to return

Primer melting temperatures
(T_m)

Min

Opt

Max

Max T_m difference

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

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

Primer Pair Specificity Checking Parameters

Specificity check

☒

Enable search for primer pairs specific to the intended PCR template ⓘ

Search mode

Automatic

Manual primer design

gi|305377519|gb|HQ024857.1| Tetrapturus georgii
 gi|305377521|gb|HQ024858.1| Tetrapturus georgii
 gi|328487986|gb|JF494768.1| Upeneus tragula vo
 gi|328488030|gb|JF494790.1| Xiphasia setifer vou
 gi|339772913|gb|JN028305.1| Pteronotropis metal
 gi|339772925|gb|JN028311.1| Pteronotropis signip
 Consensus
 Possible Primer FW

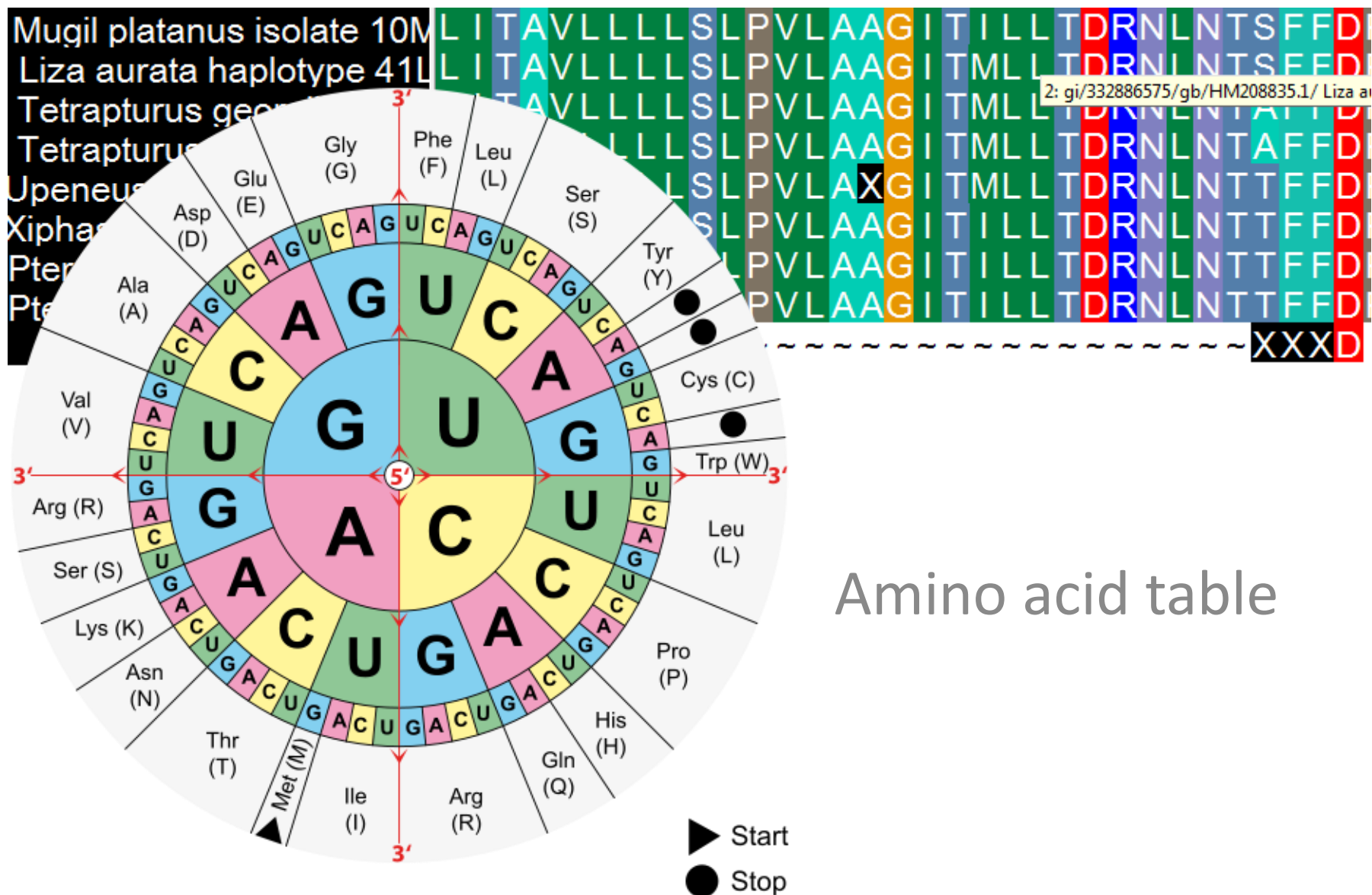
T T C T T A C G G A T C G A A A T C T T A A C A C T G C C T T C T T C G A C C C A G C A G G G G G
 T T C T T A C G G A T C G A A A T C T T A A C A C T G C C T T C T T C G A C C C A G C A G G G G G
 T G C T T A C A G A T C G A A A T C T G A A T A C A A C C T T C T T C G A C C C A G C A G G T G G
 T T T T A A C A G A C C G A A A T C T A A A C A C A A C T T T C T T T G A C C C T G C T G G G G G
 T T C T C A C T G A C C G T A A T T T A A A T A C C A C A T T T T T G A C C C G G C A G G A G G
 T T C T C A C T G A C C G T A A T T T A A A T A C C A C A T T C T T C G A C C C A G C A G G A G G
 T K Y T N A C D G A Y C G H A A Y Y T D A A Y A C H D C H T T Y T T Y G A C C C D G C W G G D G G
 ----- C H T T Y T T Y G A C C C

IUPAC code

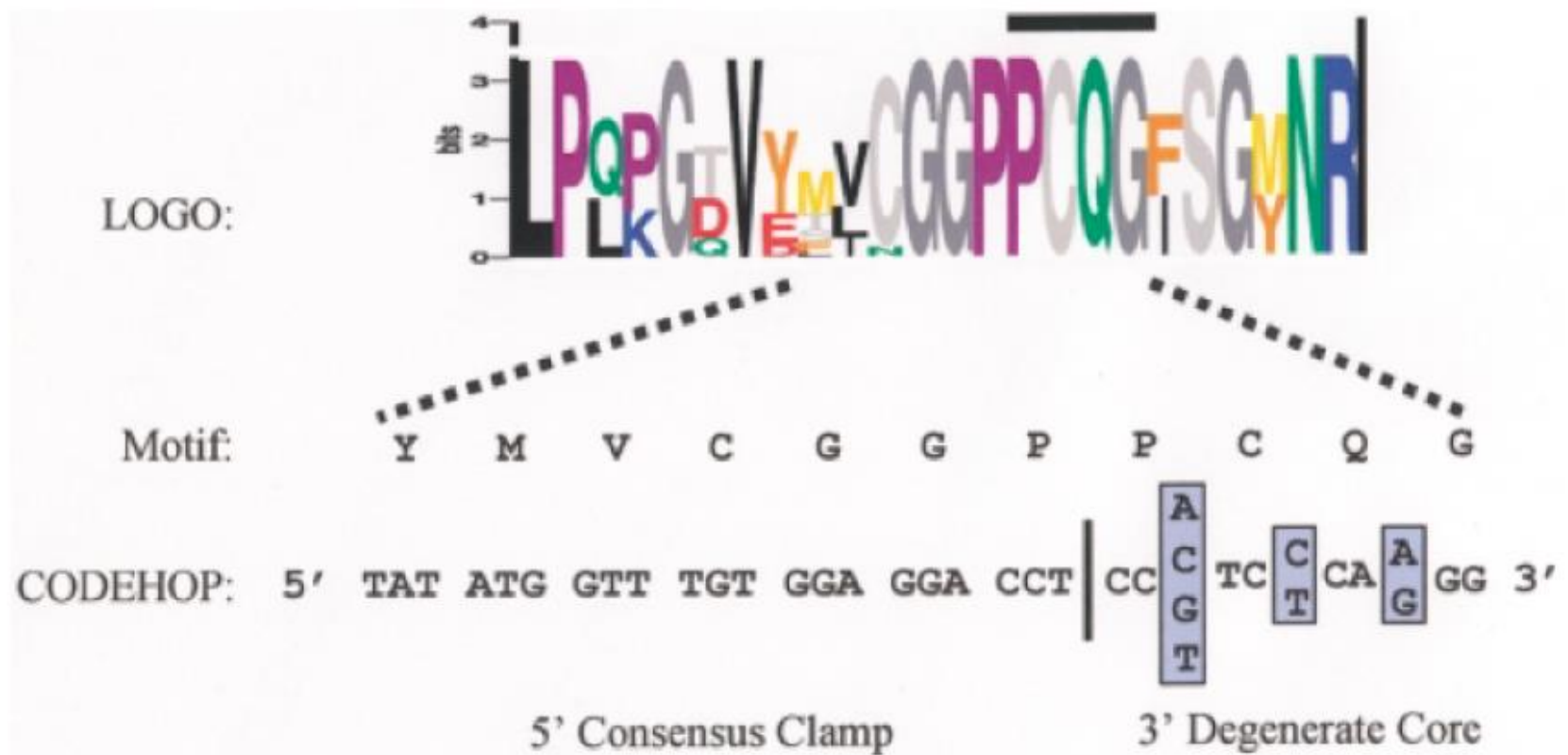
R	A or G
Y	C or T
S	G or C
W	A or T
K	G or T
M	A or C
B	C or G or T
D	A or G or T
H	A or C or T
V	A or C or G
N	any base

Bases

Manual primer design



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



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

Michelle M. Moore^{1*}, Sara M. Handy², Christopher J. Haney^{3†}, Gabrielle S. Pires¹, Lynda L. Perry¹, Jonathan R. Deeds², and Haile F. Yancy⁴

3.2 Reagents for both PCR master mixes

A. M13-tailed CO1 primers:

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

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

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

Michelle M. Moore^{1*}, Sara M. Handy², Christopher J. Haney^{3†}, Gabrielle S. Pires¹, Lynda L. Perry¹, Jonathan R. Deeds², and Haile F. Yancy⁴

3.2 Reagents for both PCR master mixes

A. M13-tailed CO1 primers:

1. FISHCO1LBC_ts:

CACGACGTTGTAAAACGACTCAACYAATCAYAAAGATATYGGCAC

2. FISHCO1HBC_ts:

GGATAACAATTTACACACAGGACTTCYGGGTGRCCRAARAATCA

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:** GGATAACAATTTACACACAGG

Primer sets comprising major analytical pipelines at CCDB

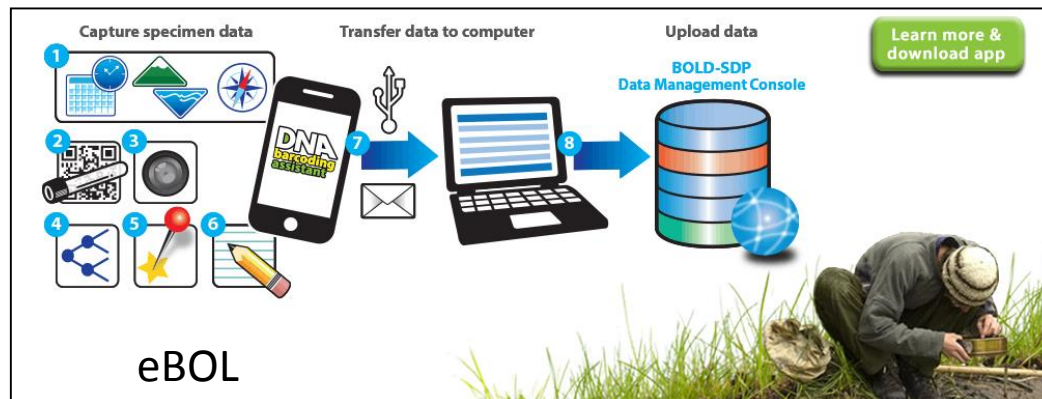
Name	Ratio	Cocktail name / Primer sequence 5'-3'	Taxonomic Groups	Reference
Regular primer pairs				
		Folmer primers	various phyla	Folmer et al. 1994
LCO1490		GGTCAACAAATCATAAAGATATTGG		
HCO2198		TAACTTCAGGGTGACCAAAAAATCA		
		Lepidoptera primers	insects, amphibians	
LepF1		ATTCAACCAATCATAAAGATATTGG		Hebert et. al. 2004a
LepR1		TAACTTCTGGATGTCCAAAAATCA		Hebert et. al. 2004a
MLepF1		GCTTTCCACGAATAATAATA (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

BARCODE OF LIFE DATA SYSTEM v4

Advancing biodiversity science through DNA-based species identification.

EXPLORE THE DATA

DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA



Primer Database

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

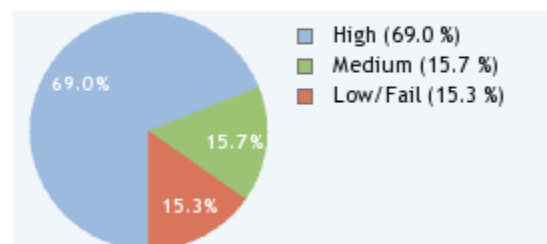


Primer Database

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

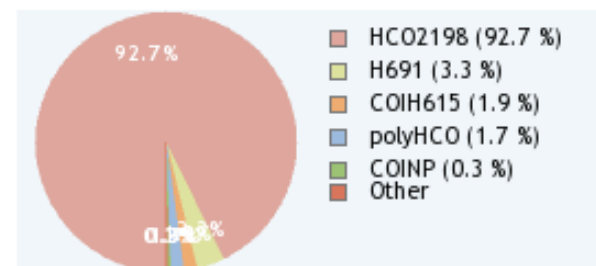
Paired Primers Summary

Primer	High	Medium	Low/Failed
HCO2198	161594275	4019	
H691	580	24	15
COIH615	326	75	39
polyHCO	297	204	421
COINP	51	4	7
CRYP COIR18	2	0	
C1-N-21918	0	0	
MLepR1	1	4	28
LepR1	0	0	5

Paired Primer Stats

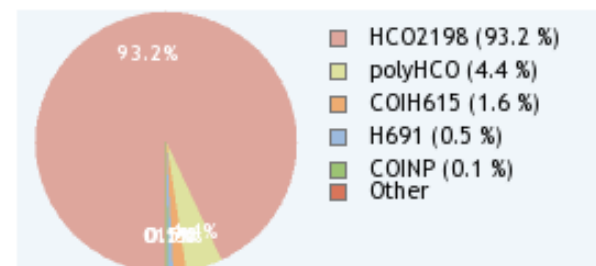
High Quality Runs:

17440 samples



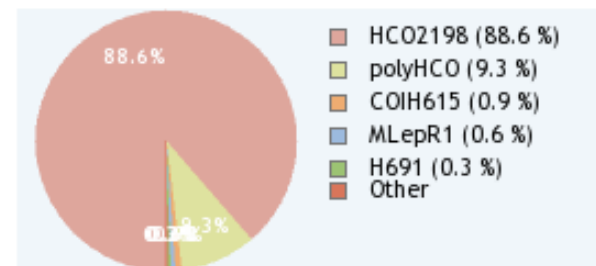
Medium Quality Runs:

4588 samples



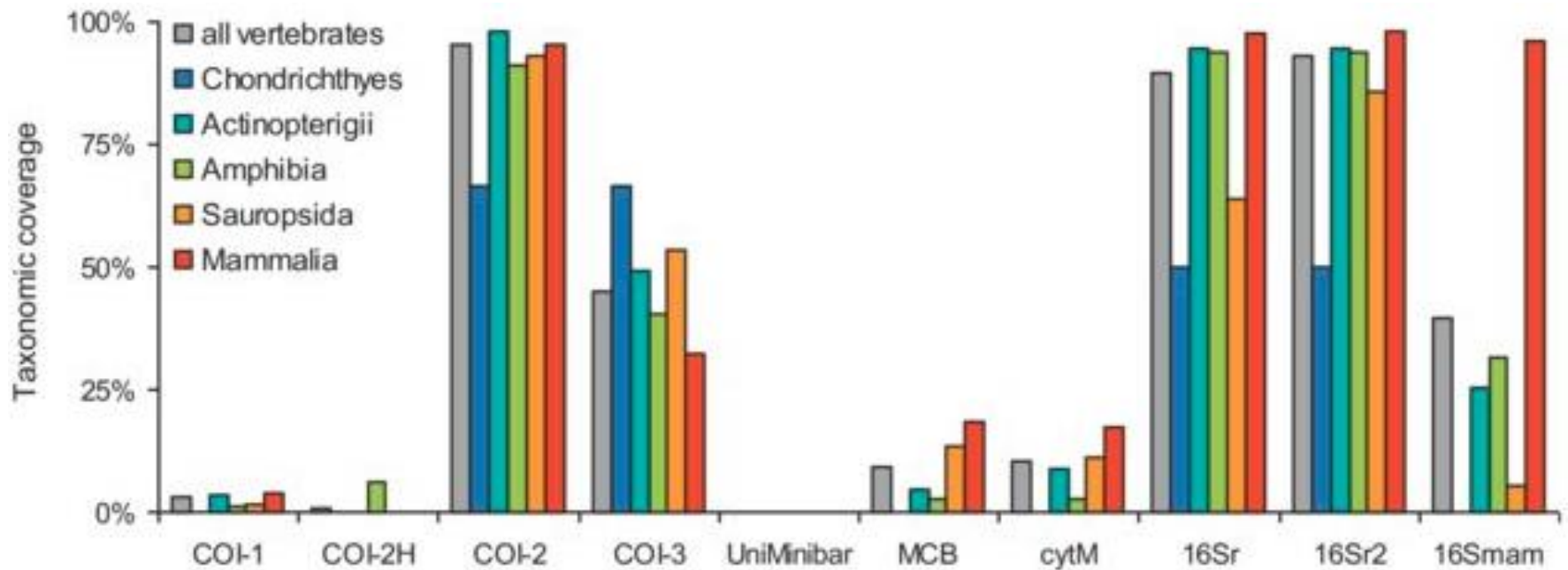
Low Quality/Failed Runs:

4534 samples

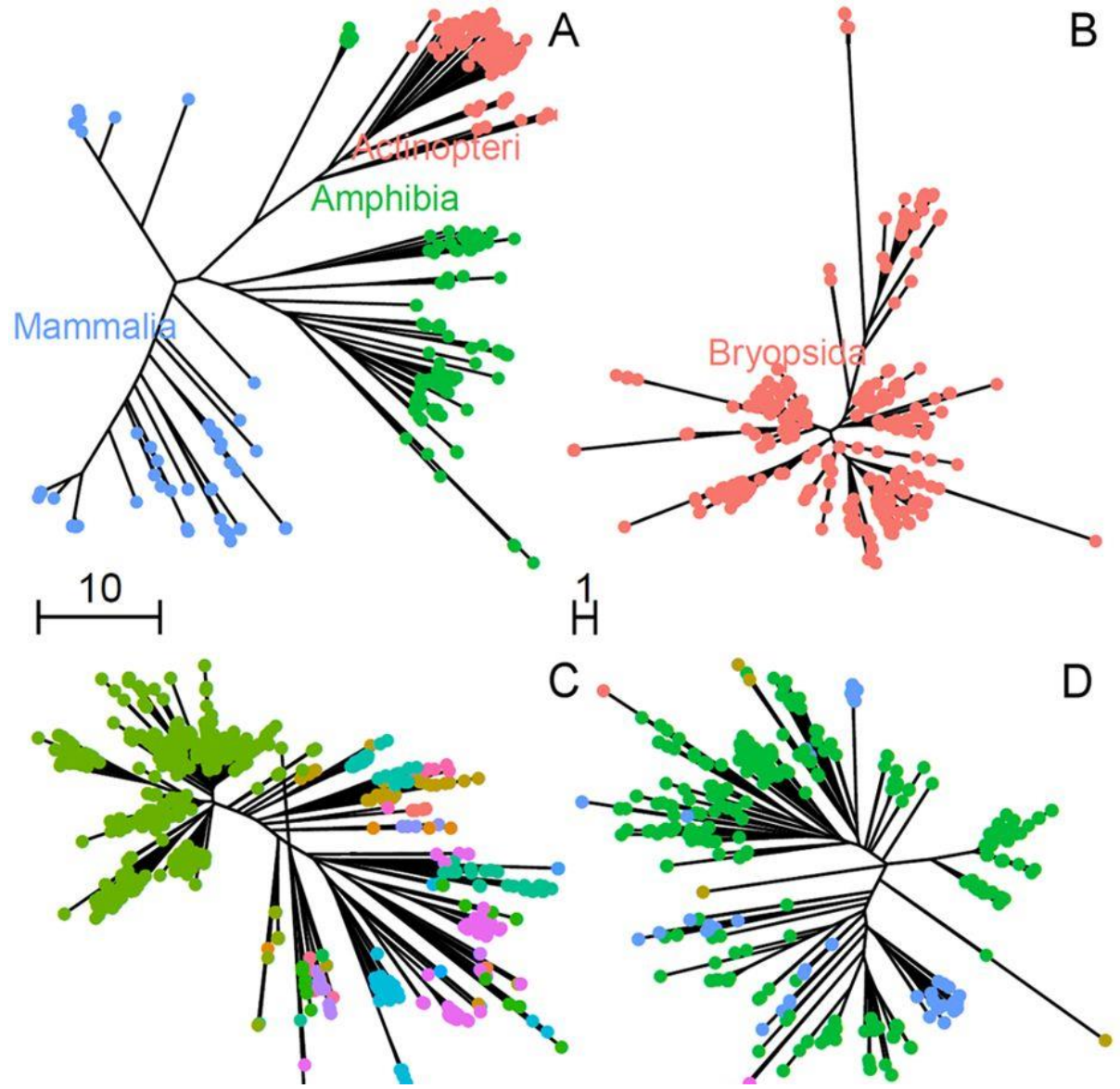


In silico comparison of primers for DNA barcoding

(Ficetola et al. 2010)



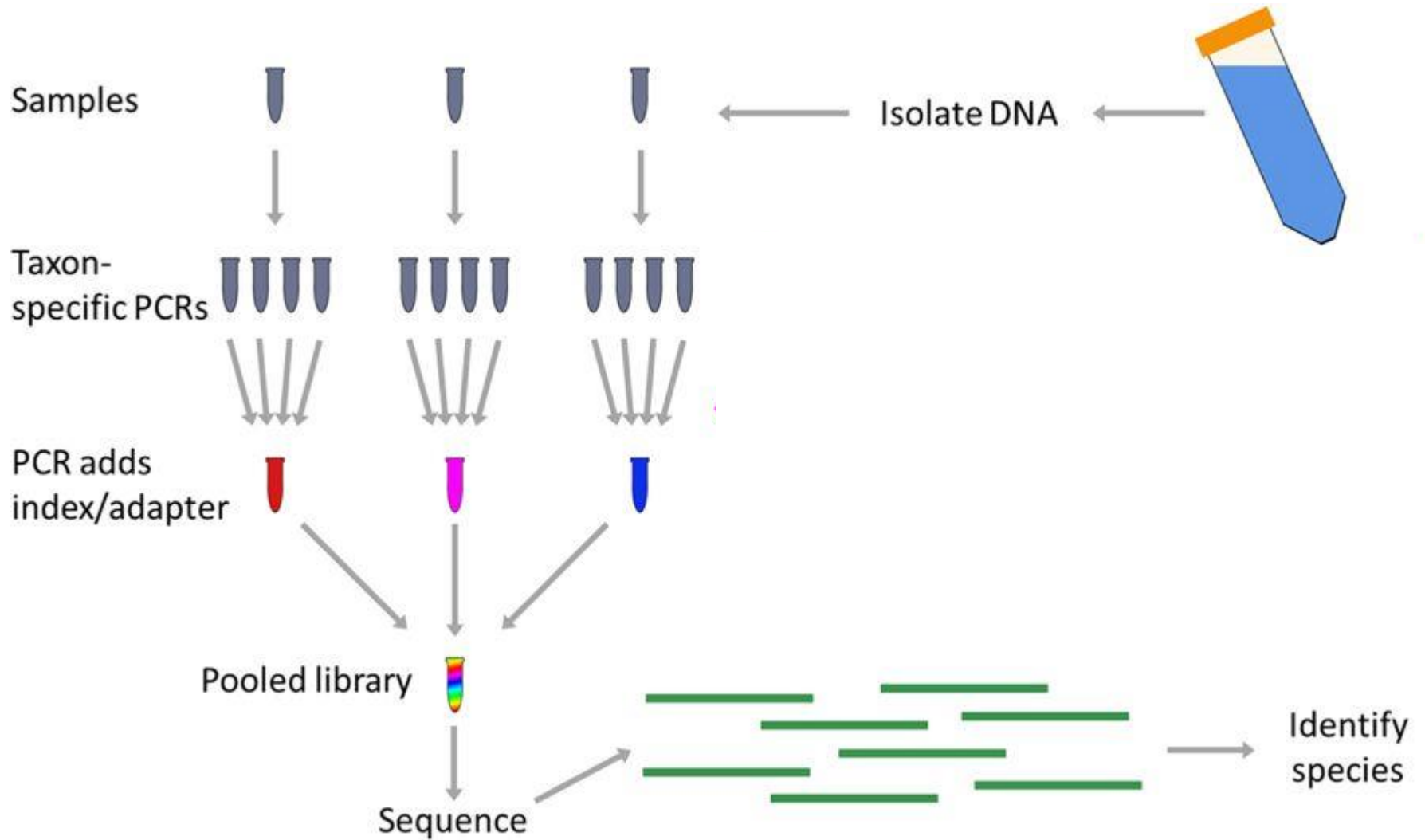
primerTree



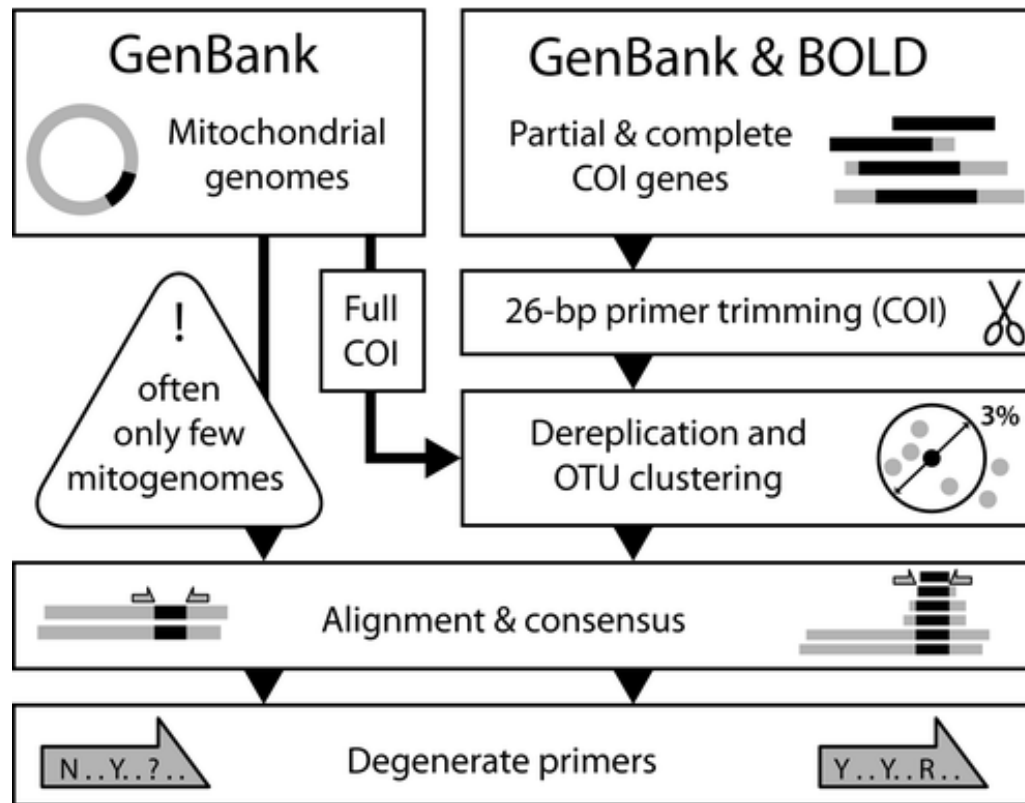
Description

`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`

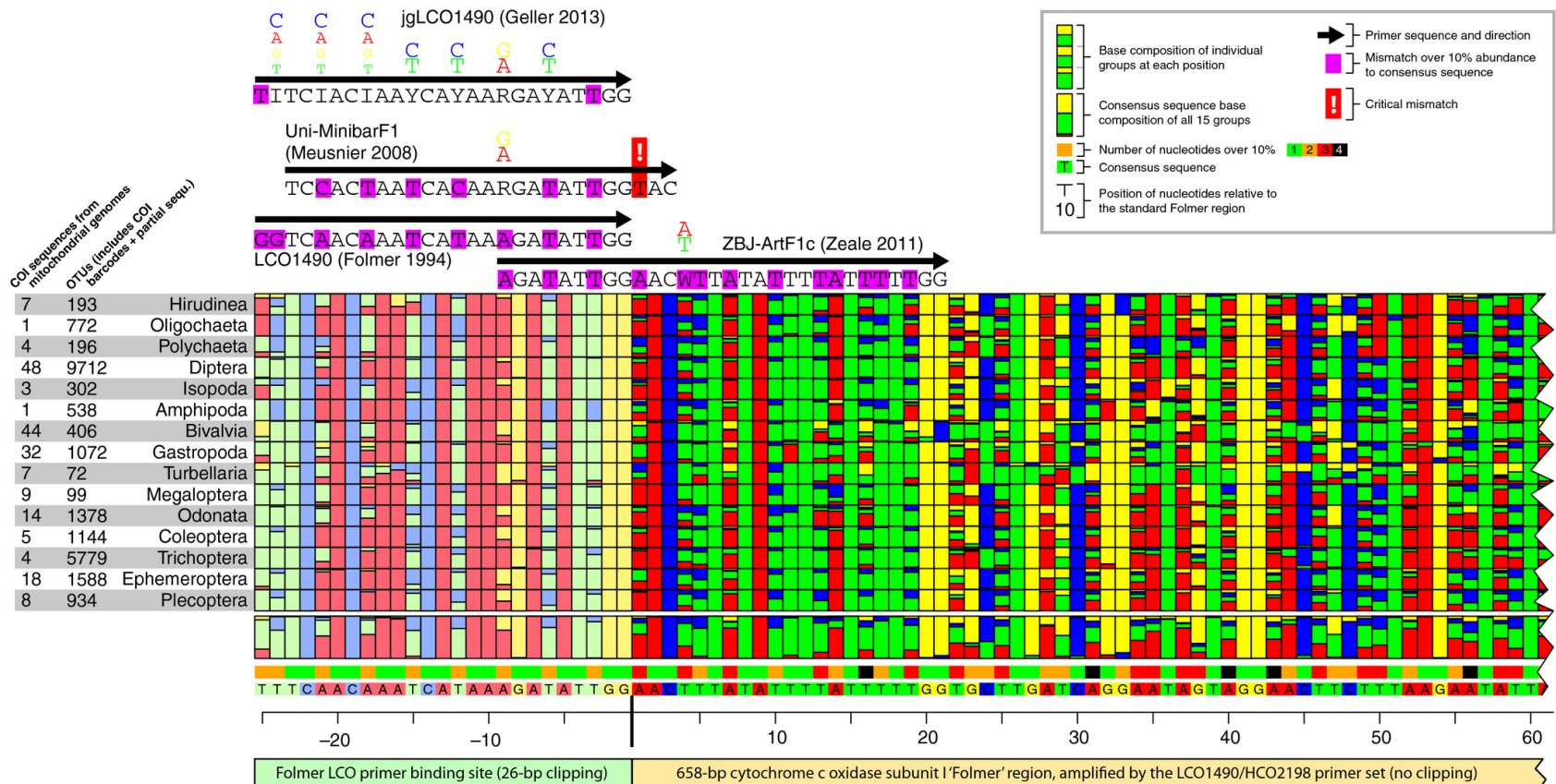
primerTree



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



File Edit View Tools Sequence Annotate & Predict Help

Back Forward BLAST Workflows Align/Assemble Tree Primers Cloning Back Up Support Help

Sources

- Local (909,900 unread)
 - Anna (12)
 - 4 documents Assembly - Nu
 - Xzmt1076.ab1 - Nucleotide
 - Jakiul (1)
 - Sample Documents (50, 1 unread)
 - 14 documents Assembly Co
 - Alignments (8)
 - Batch search of 14 - Nucleo
 - 01.Xzmt1031.ab1 - bases
 - 02.Xzmt1070.ab1 - bases
 - 03.Xzmt1036.ab1 - bases
 - 04.Xzmt1032.ab1 - bases
 - 05.Xzmt1069.ab1 - bases
 - 06.Xzmt1035.ab1 - bases
 - 07.Xzmt1038.ab1 - bases
 - 08.Xzmt1033.ab1 - bases
 - 09.Xzmt1034.ab1 - bases
 - 10.Xzmt1037.ab1 - bases
 - 11.Xzmt1071.ab1 - bases
 - 12.Xzmt1030.ab1 - bases
 - 13.Xzmt1047.ab1 - bases
 - 14.Xzmt1048.ab1 - bases
 - Cloning (12)
 - Contig Assembly (7)
 - Genomes (233)
 - PlasMapper Features (314)
 - Plasmids from NEB (27)
 - Primers (12)
 - Protein Documents (6)
 - Tree Documents (4)
 - Deleted Items (2)
 - Shared Databases
 - Operations
 - NCBI
 - Gene
 - Genome
 - Nucleotide
 - PopSet
 - Protein
 - PubMed
 - SNP
 - Structure
 - Taxonomy
 - UniProt

Name	Description	Modified	Sequenc...	# Sequen...	Min Sequ...	Max Sequ...	% Pairwi...	% Identic...	Alignme...
COXII CDS	Multiple alignment of 50 Cytochrome C Oxidase Subunit II genes	27 Aug 2013 10:28 pm	705	50	684	705	72.6%	35.6%	-
HIV env	20 HIV envelope genes	06 Dec 2012 9:23 am	-	20	2,520	2,628	-	-	-
Ig variable region	5 immunoglobulin heavy chain variable regions	06 Dec 2012 9:23 am	-	5	264	314	-	-	-
LysR family	7 LysR protein se				306	353	-	-	-
Pairwise protein	Pairwise alignme				212	220	46.6%	47.1%	Geneious ...
People	Multiple alignme				364	386	85.3%	72.1%	MUSCLE
Three Kingdoms	Multiple alignme				730	990	37.0%	7.7%	MUSCLE A...
Transcript variants	Multiple alignme				2,155	2,698	88.7%	74.3%	-

Select Task: ☒ Design New ☐ Design with Existing

Primer design uses a modified version of Primer3 2.3.7. Please cite [Primer3](#) if you publish results

☒ Forward Primer ☐ DNA Probe ☒ Reverse Primer

Task: Generic

☒ Included Region: 5,331 To 6,878

☐ Target Region: 5,331 To 6,878

☒ Product Size Between: 200 And 300

☒ Optimal Product Size: 250

Number of pairs to generate: 1

Tm Calculation

Characteristics

Primer DNA Probe

Size Min: 18 Optimal: 20 Max: 27

Tm Min: 57 Optimal: 60 Max: 63

%GC Min: 20 Optimal: 50 Max: 80

Product Tm Min: 0 Optimal: 0 Max: 0

Max Tm Difference: 2 GC Clamp: 0

Max Dimer Tm: 47 Max Poly-X: 5

Max 3' Stability: 9

☐ Allow primers inside target with penalty: 0

Primer Picking Weights

☐ Allow Degeneracy: 1

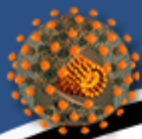
Advanced

Annotations and Tracks

☒ Show Annotations (0 of 3)

Filter

Source (3)



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

No file selected.

Or use premade HIV or SIV
sequence alignment [?](#)

☐

Gap stripping [?](#)

Remove columns having greater than % gaps

Sequence, Adaptor, and Barcode Options

Region of Interest [?](#)

Start

Stop

Multiple fragments [?](#)

☒

Single fragment

☐

Multiple fragments

Alignment Position

70

68

66

64

62

60

$Tm(.C)$

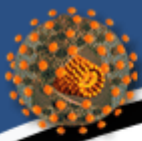
58

56

54

52

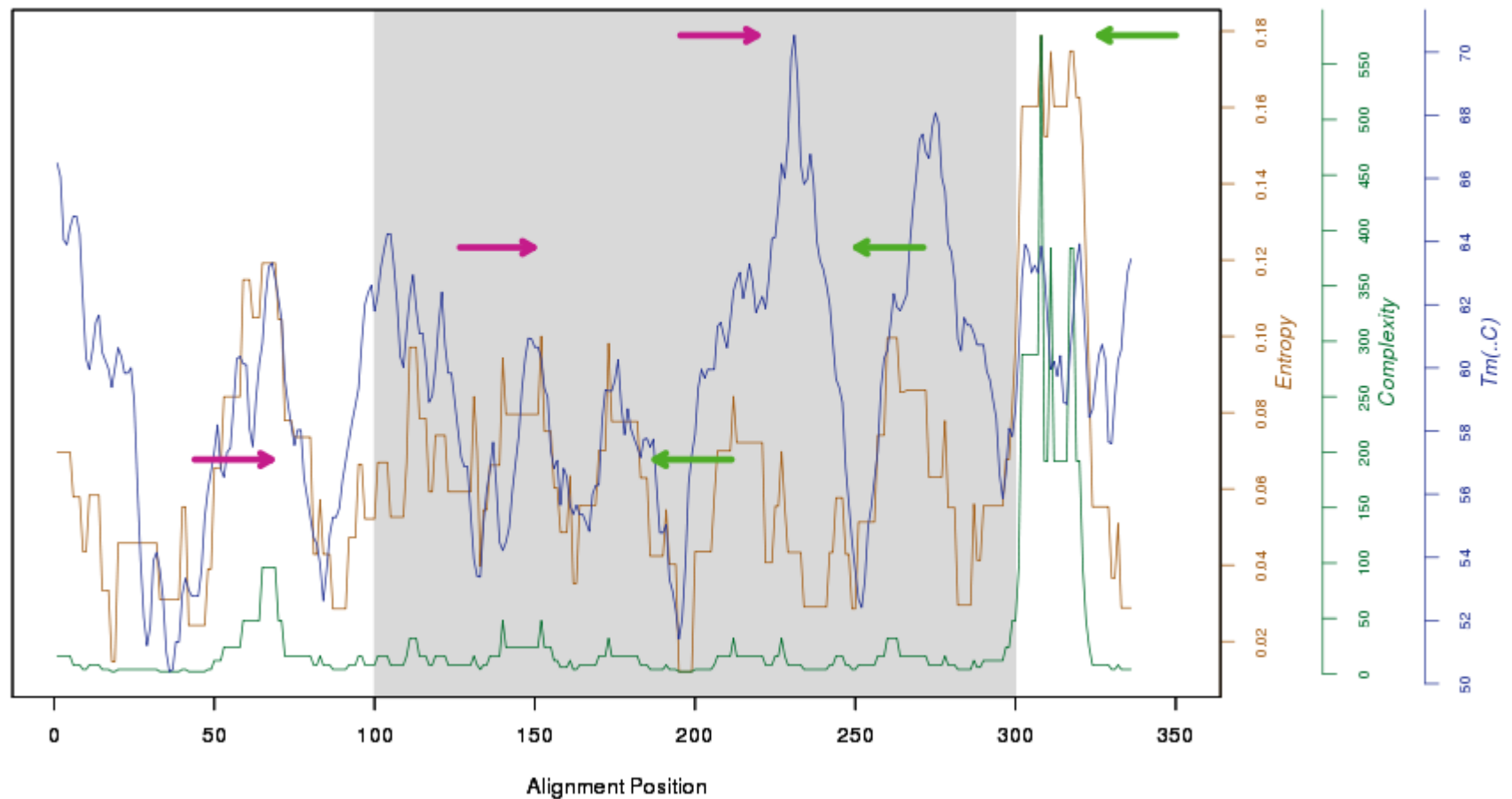
50



HIV sequence database

[DATABASES](#)[SEARCH](#)[ALIGNMENTS](#)[TOOLS](#)[PUBLICATIONS](#)[GUIDES](#)

FragmentSummary



Primer-BLAST

A tool for finding specific primers

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

PCR Template

[Reset page](#)[Save search parameters](#)[Retrieve recent results](#)[Publication](#)[Tips for finding specific primers](#)

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

[Clear](#)

Or, upload FASTA file

No file selected.

Range

Forward primer

From

To

Reverse primer

[Clear](#)

Primer Parameters

Use my own forward primer
(5'→3' on plus strand)

[Clear](#)

Use my own reverse primer
(5'→3' on minus strand)

[Clear](#)

PCR product size

Min

Max

of primers to return

Primer melting temperatures
(T_m)

Min

Opt

Max

Max T_m difference

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

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

Primer Pair Specificity Checking Parameters

Specificity check

☒

Enable search for primer pairs specific to the intended PCR template ⓘ

Search mode

Automatic



Primer design part II: Obitools

Veronique Helfer

