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PROJECT | IN PROGRESS

Improving knowledge of Asian pteridophytes through DNA sampling of specimens in regional collections

1 September 2021 - 28 February 2023 € 20,000

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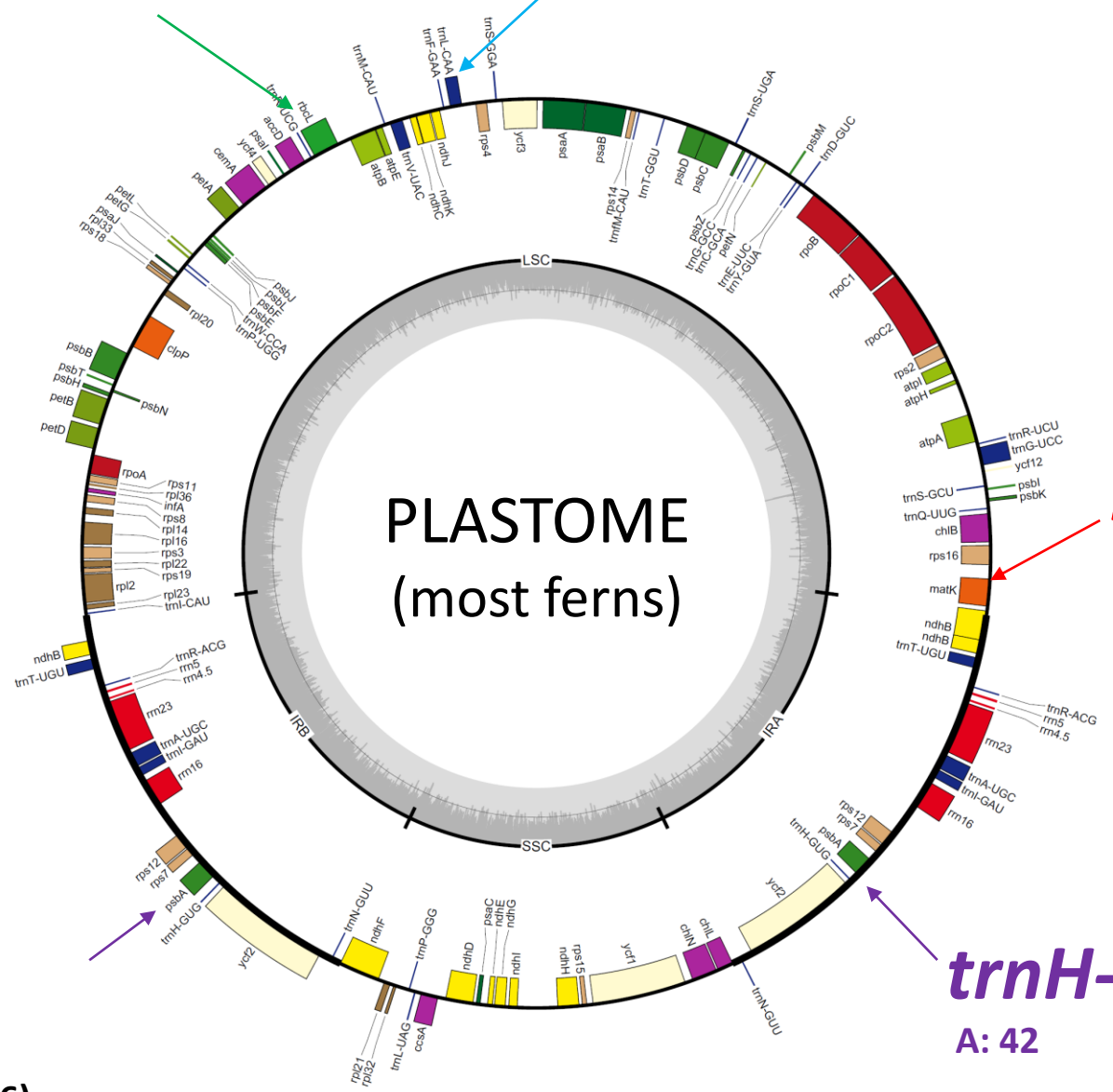
Brainea insignis. Photo © 2021 Li-Yaung Kuo. All rights reserved.

Variability

trnL-L-F ≥
matK >>
trnH-psbA ≥
rbcL

Primer universality

trnL-L-F ~=
trnH-psbA ≥
rbcL >>
matK



rbcL A: 3558 B: 3146

trnL-L-F A: 7 B: 2559

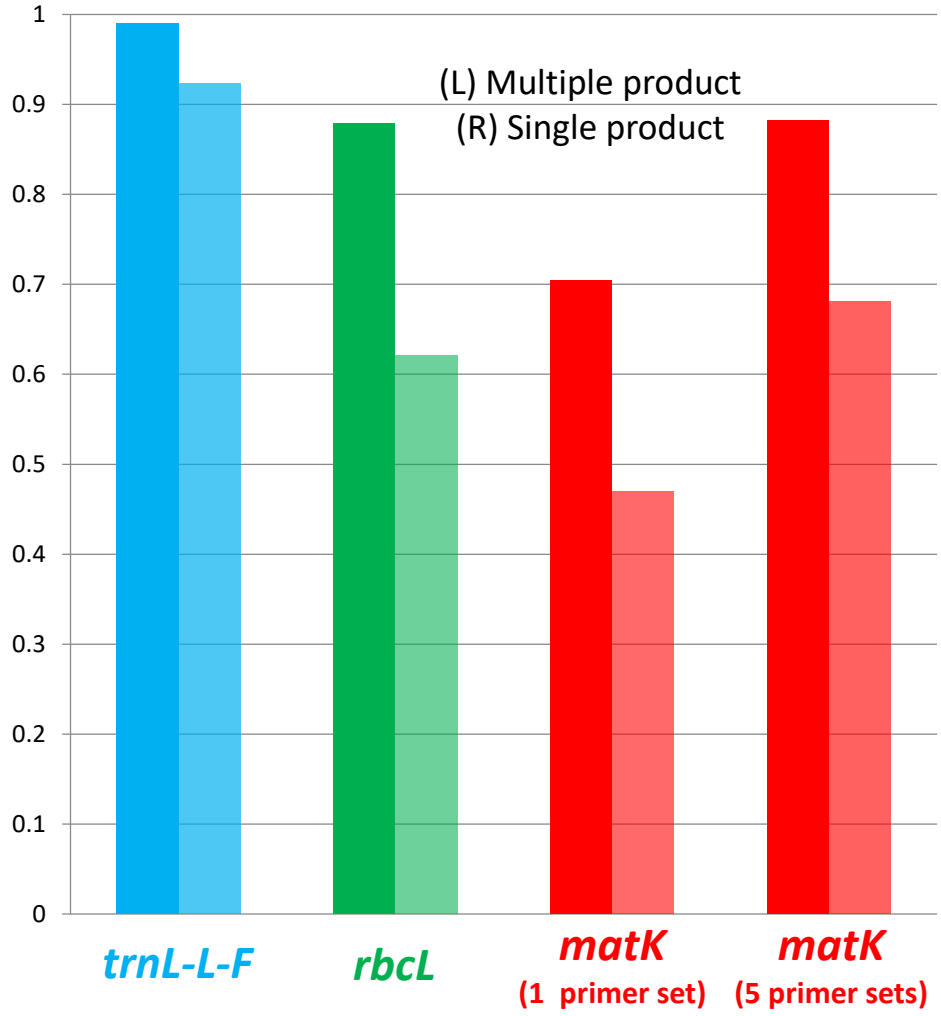
matK A: 240

trnH-psbA A: 42

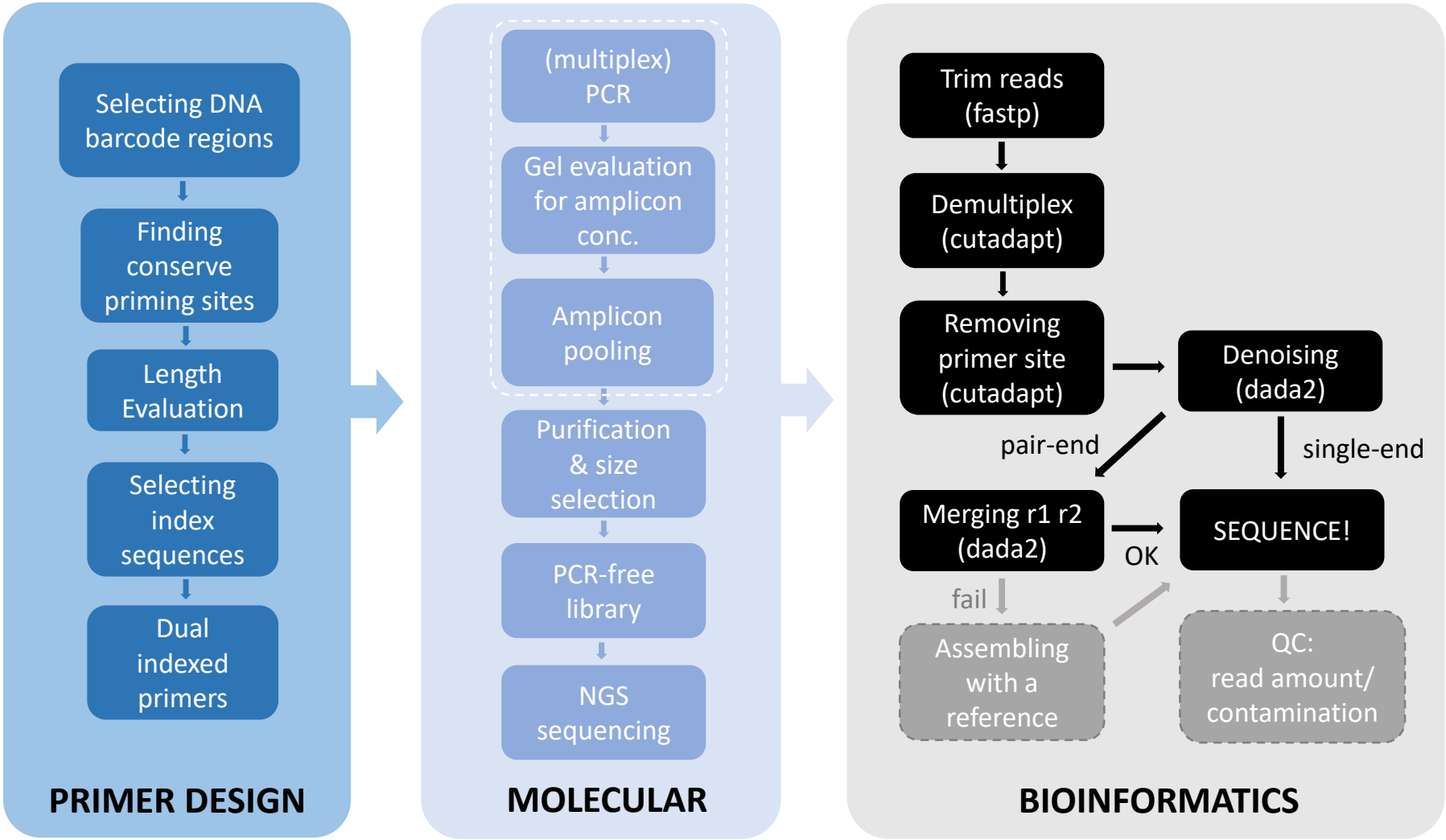
A: BOLD (2021)
B: Testo & Sundue (2016)

High Universality of *trnL-L-F* Primer Set

Family	*trnL-L-F*	*rbcL*	*matK*
Hymenophyllaceae	100.00%	100.00%	54.55%
Gleicheniaceae	100.00%	100.00%	100.00%
Plagiogyriaceae	100.00%	100.00%	50.00%
Lindsaeaceae	100.00%	50.00%	0.00%
Dennstaedtiaceae	100.00%	100.00%	80.00%
Pteridaceae	80.00%	60.00%	80.00%
Dryopteridaceae	100.00%	91.67%	16.67%
Polypodieaceae	100.00%	76.92%	7.69%
Aspleniaceae	100.00%	85.71%	14.29%
Thelypteridaceae	100.00%	22.22%	44.44%
Arthyriaceae	100.00%	90.91%	100.00%



A new workflow adopting NGS platforms



$$ > Sanger's

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Sequencing Costs (incl. library)

	Miseq PE 300	PacBio sequel, SMART
Min. per run	22,000 NTD— 1.66 M reads— 1 GB (1/10lane)	92,000 NTD— 0.75 M reads[b]— 15 GB (one flow cell)
Max. sample per run	16667 (50X)[a] 8333 (100X)[a]	15000 (50X)[b]
Coverage 50X	1.32 NTD per sample	6.13 NTD per sample
Coverage 100X	2.65 NTD per sample	12.26 NTD per sample

[a]Separate into two amplicons within ~1kb region
[b]CCS reading 20 times per DNA molecular

Selecting DNA barcode regions

↓

Finding conserve priming sites

↓

Length Evaluation

↓

Selecting index sequences

↓

Dual indexed primers

PRIMER DESIGN

Fern Plastome List

Paul G. Wolf Lab
Biology | Ferns | Genetics | Phylogeny

THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Here is a list of fern plastomes published in genbank. You can downoad the csv below. The list includes complete plastomes as well as fragments greater than 10 kb. This list is based on a search using a Biopython script written by Carol Rowe and Paul Wolf, then rewritten in R by Tanner Robison Sept 2018.

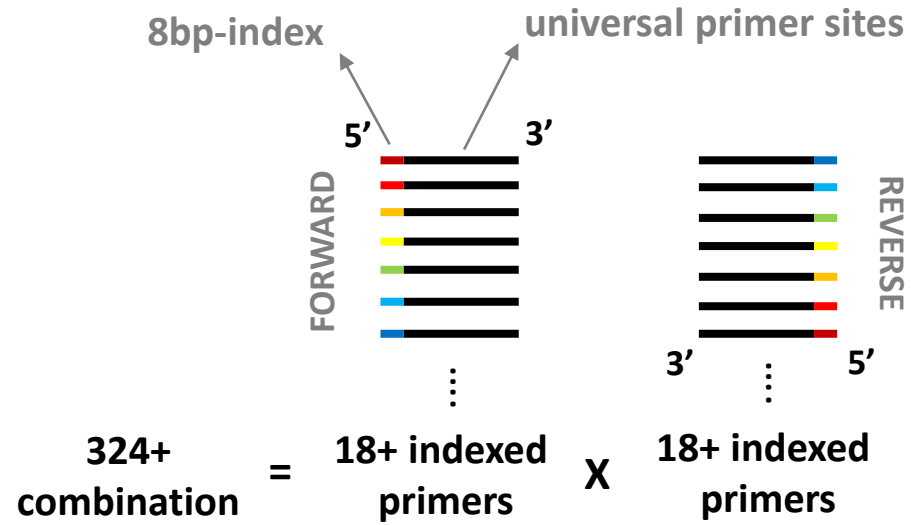
The list can be download here: all_fern_plastomes.csv

250 plastomes (2020/9/24)

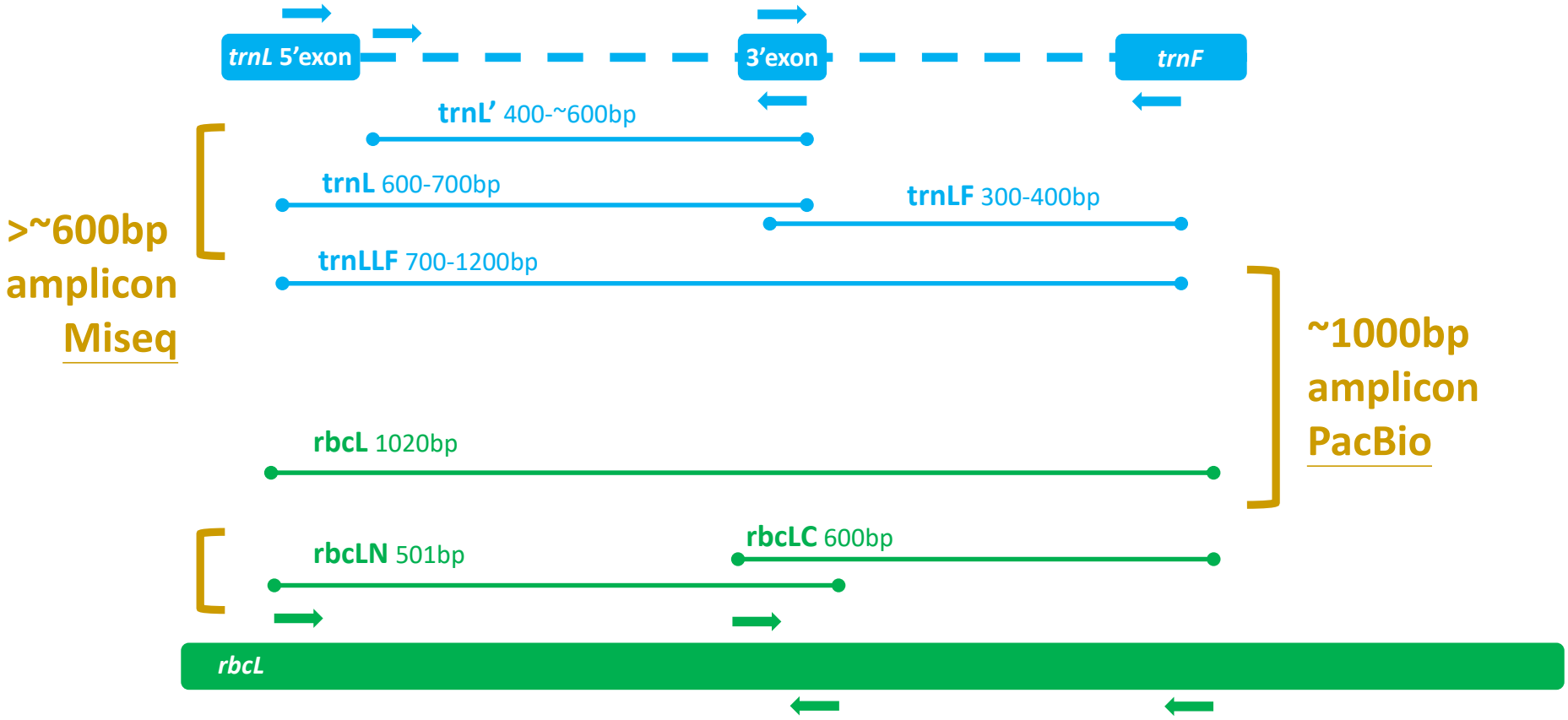
A custom Python script finding 20-25bp conserved primering sites (Wu et al. 2022)

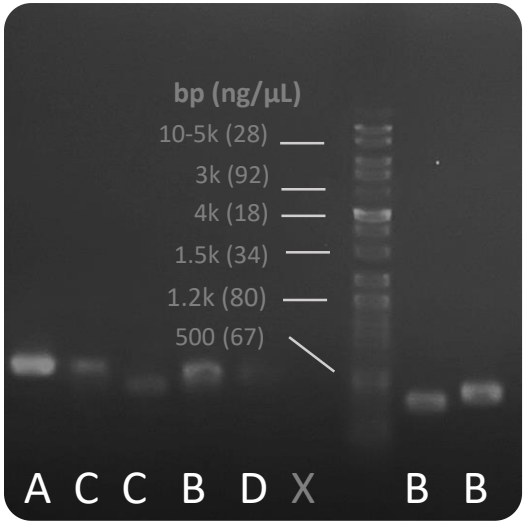
Target region ~1,000bp or < 540bp

A custom Python script evaluate mismatches of 18+ 8bp-indexes at 5' of primering sites



Primer sets & Amplicon Design





(multiplex) PCR

Gel evaluation for amplicon conc.

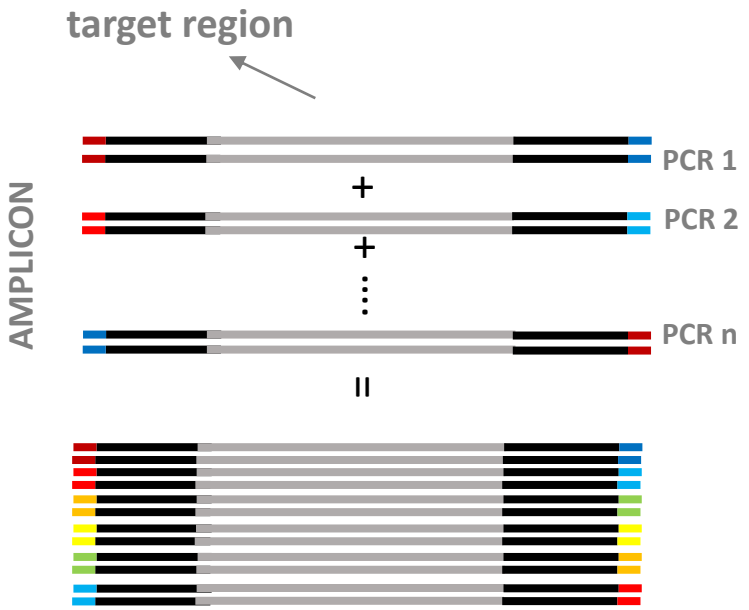
Amplicon pooling

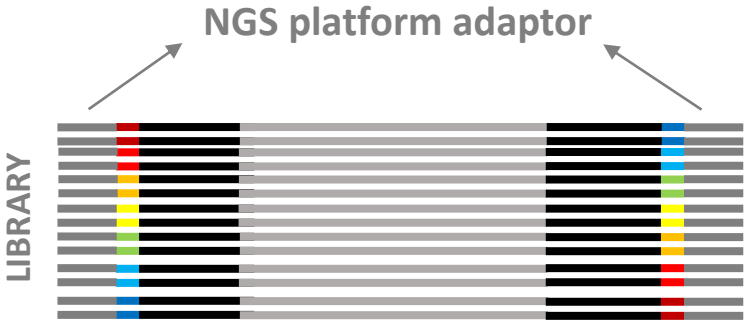
Purification & size selection

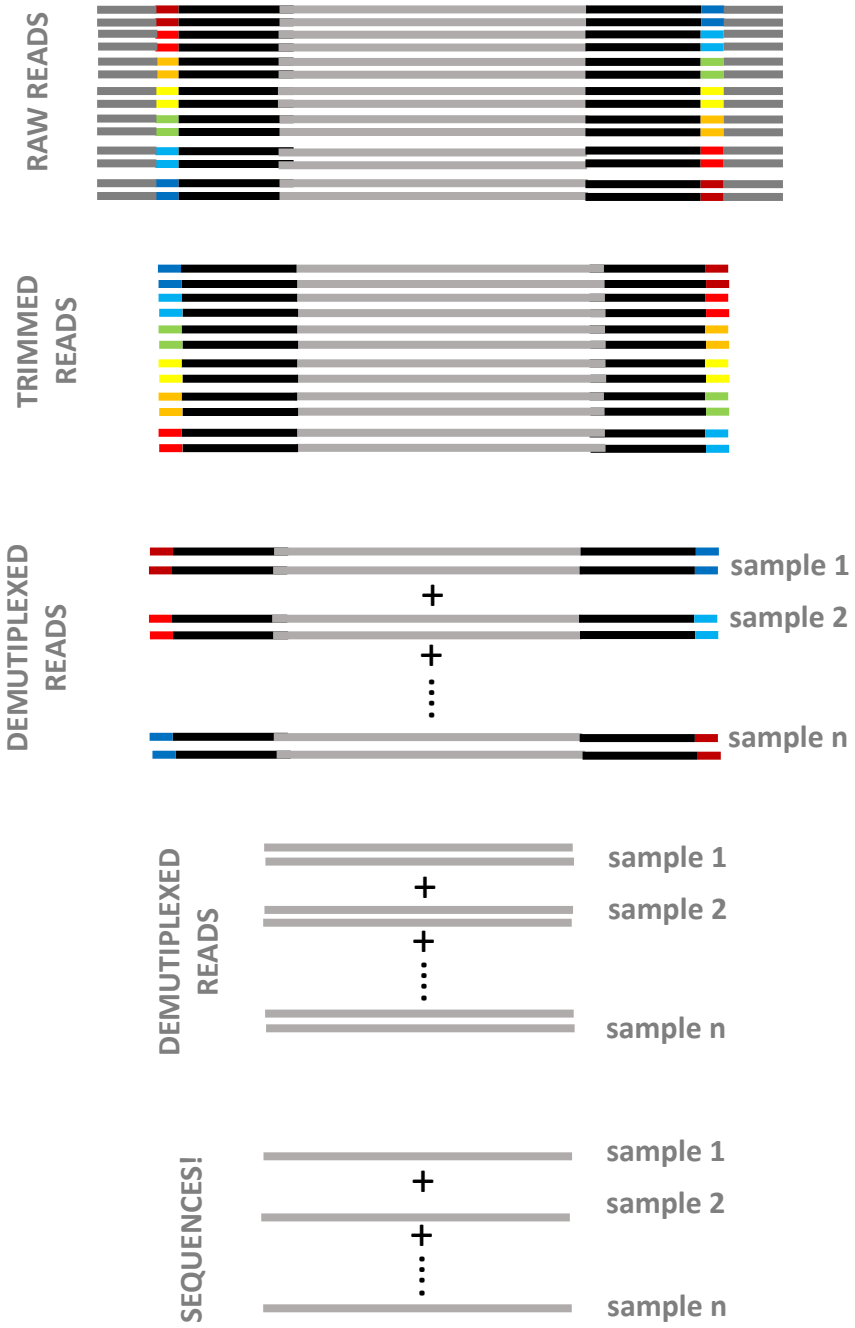
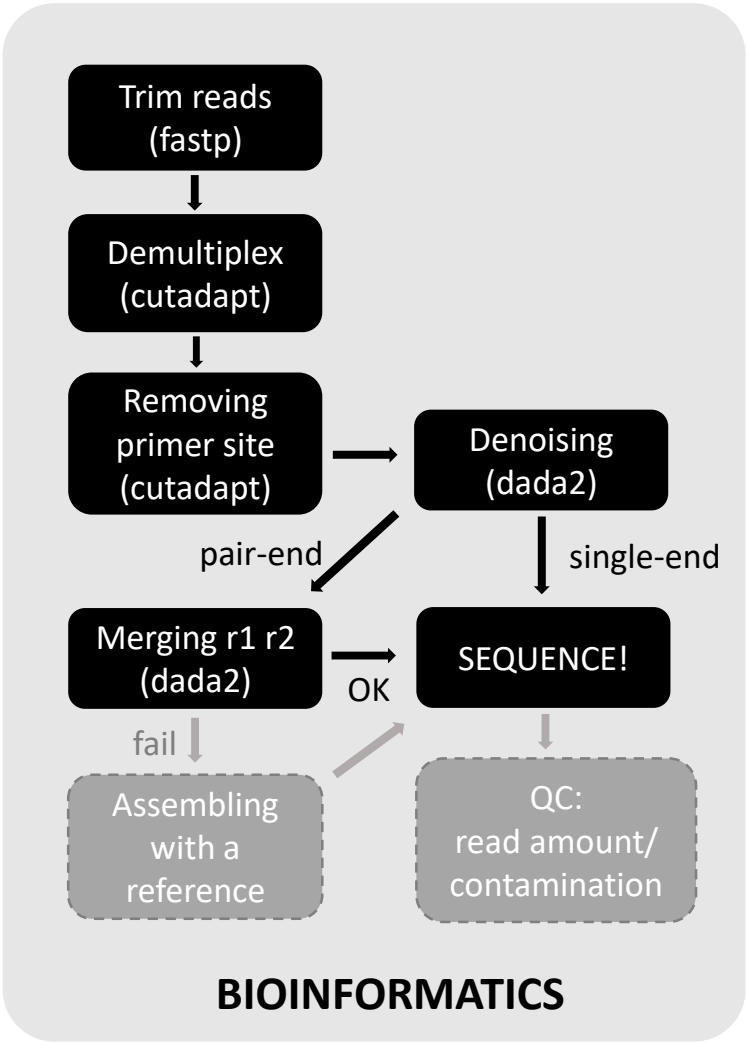
PCR-free library

NGS sequencing

MOLECULAR







A pipeline:
https://github.com/**PowerBarcoder**