Automatic Restriction Strategy Finder for Synthetic Biology Constructs

Jorge Gomes

Project Supervisor: Björn Johansson

Project in Bioinformatics and Systems Biology

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Synthetic Biology

✄ The assembly of DNA constructs from small fragments into large constructs.

✄ Convergence of molecular biology with engineering principles and advances in biological technologies.

✄ Synthesis/ Manipulation of:

    ✄ Full length genes   ✄ Proteins   ✄ Pathways   ✄ Complete genomes

✄ Applications:

    ✄ Vaccine and Antibody production   ✄ BioFuels   ✄ Crop Improvement

    ✄ Industrial Enzymes: Food Quality   ✄ BioBased Chemicals: Plastics and Textiles
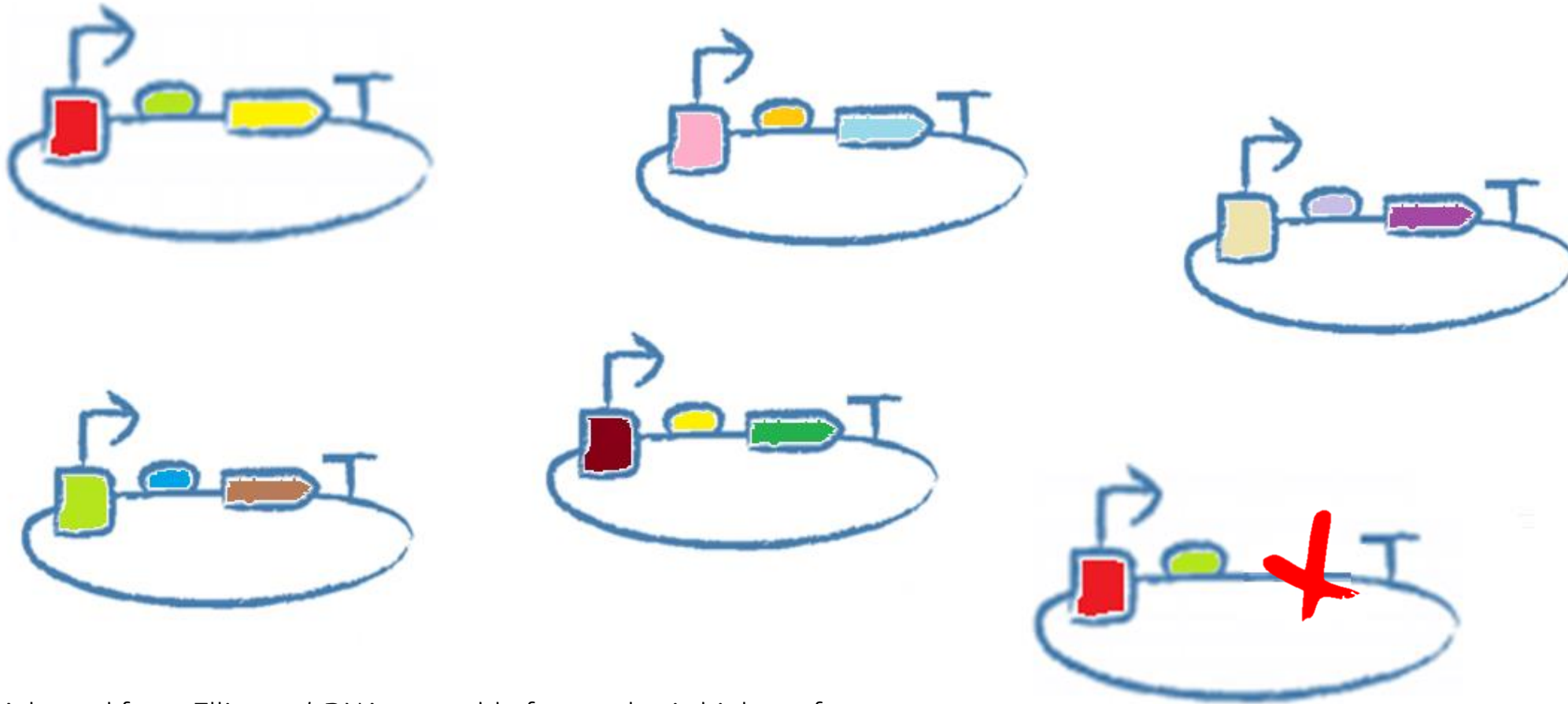
Synthetic Biology
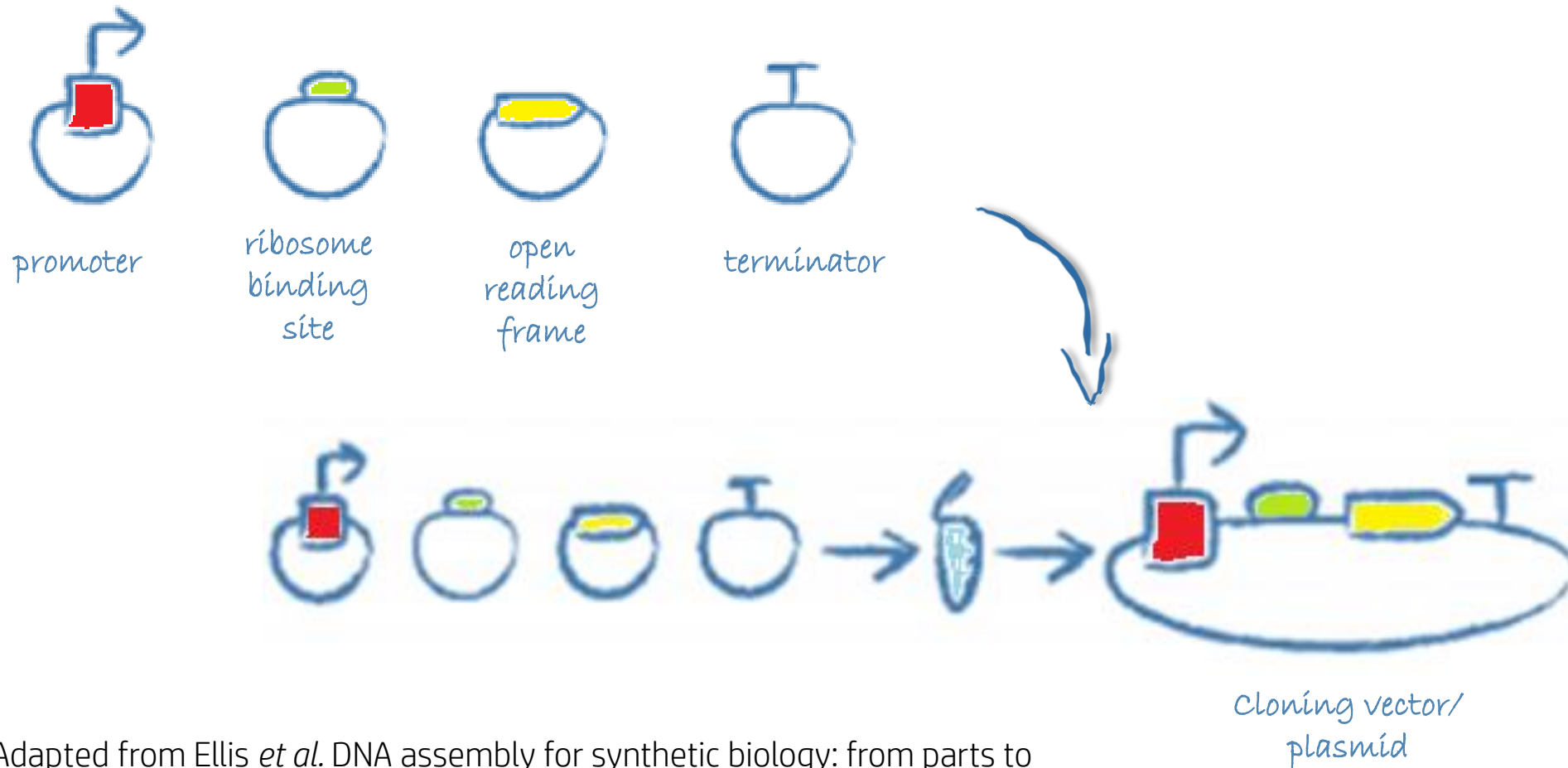
promoter

ribosome binding site
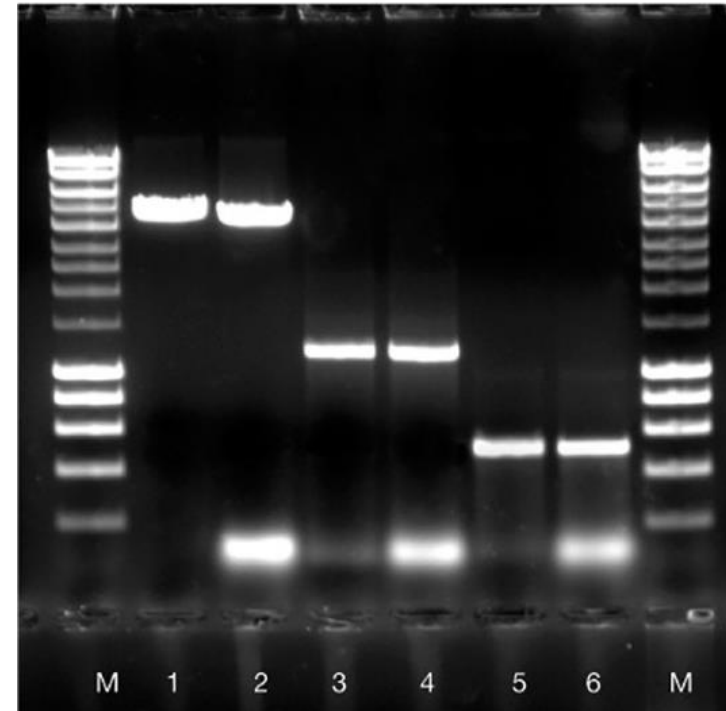
open reading frame

terminator

Cloning vector/ plasmid

Adapted from Ellis *et al.* DNA assembly for synthetic biology: from parts to pathways and beyond. *Integrative Biology*, *3*(2), 109-118.

Automatic Restriction Finder Strategy for Synthetic Biology Constructs
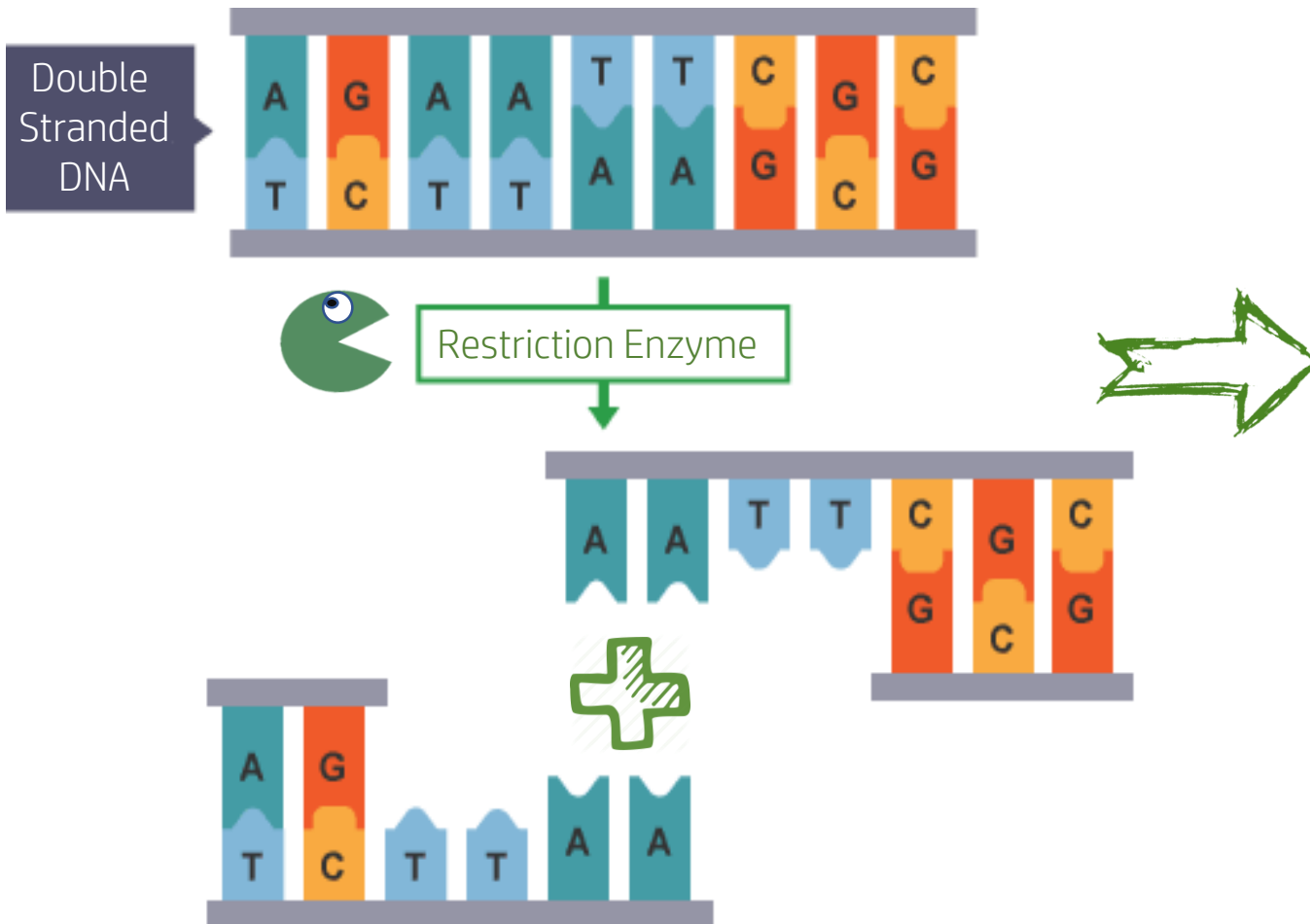
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Problem

Adapted from Ellis *et al.* DNA assembly for synthetic biology: from parts to pathways and beyond. *Integrative Biology*, *3*(2), 109-118.

Solution

Double Stranded DNA

Restriction Enzyme

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Goals

✂ Design an algorithm capable of automatically selecting the most effective restriction enzyme(s)

for DNA constructs analysis and distinction;

✂ Minimize the number of enzymes necessary;

✂ Make it's use both simple and quick.

Pydna

✂ Python package providing human-readable description and simulation of cloning and assembly strategies;

✂ Includes simulations for:

    ✂ <u>Restriction Digestion</u>   ✂ Ligation   ✂ PCR

    ✂ Assembly   ✂ Homologous recombination   ✂ Primer Design

    ✂ <u>Gel electrophoresis of DNA with generation of gel images</u>

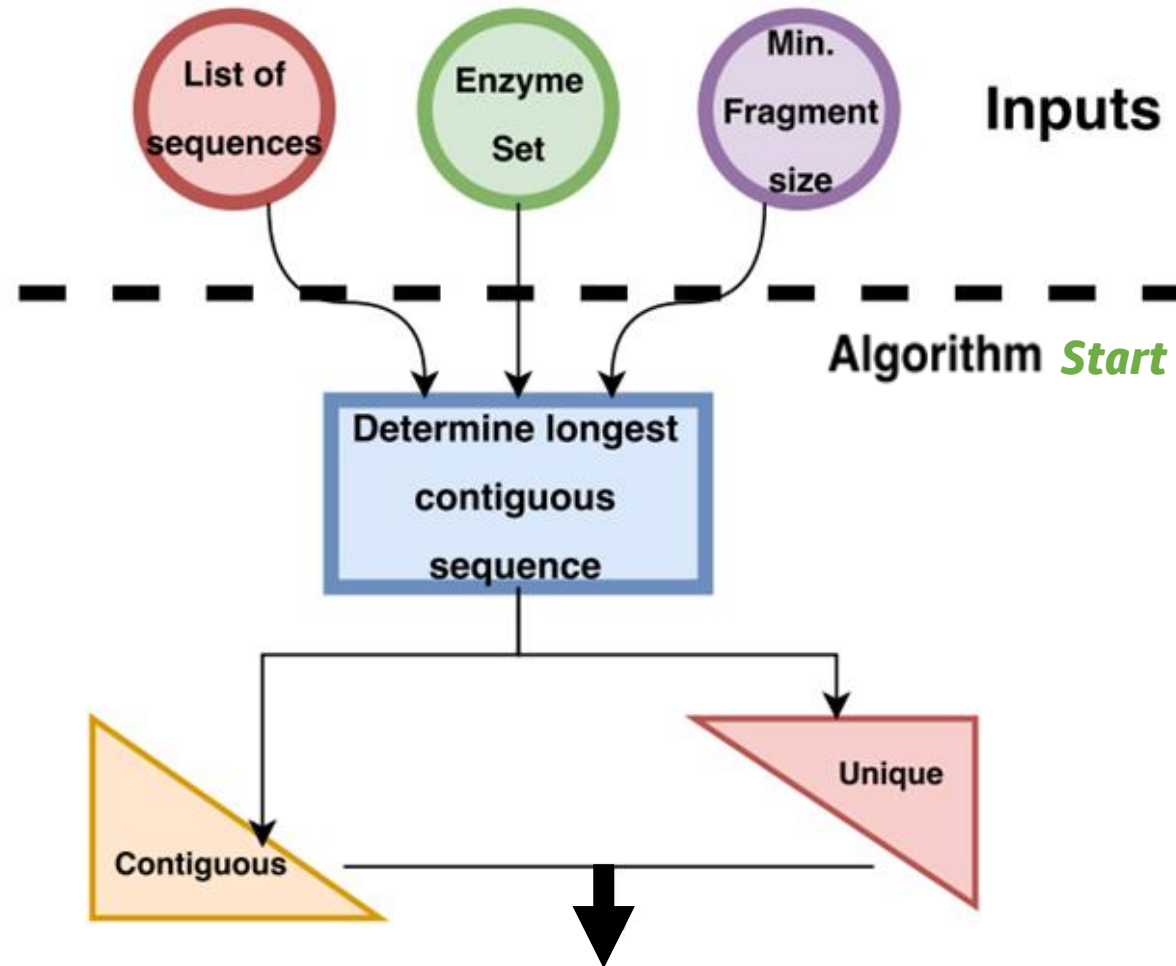✂ Command Line interface;

✂ Integration with IPython Notebooks;

Methodology

✂ **0. Inputs**

✂ **1. Sequence Alignment Step**

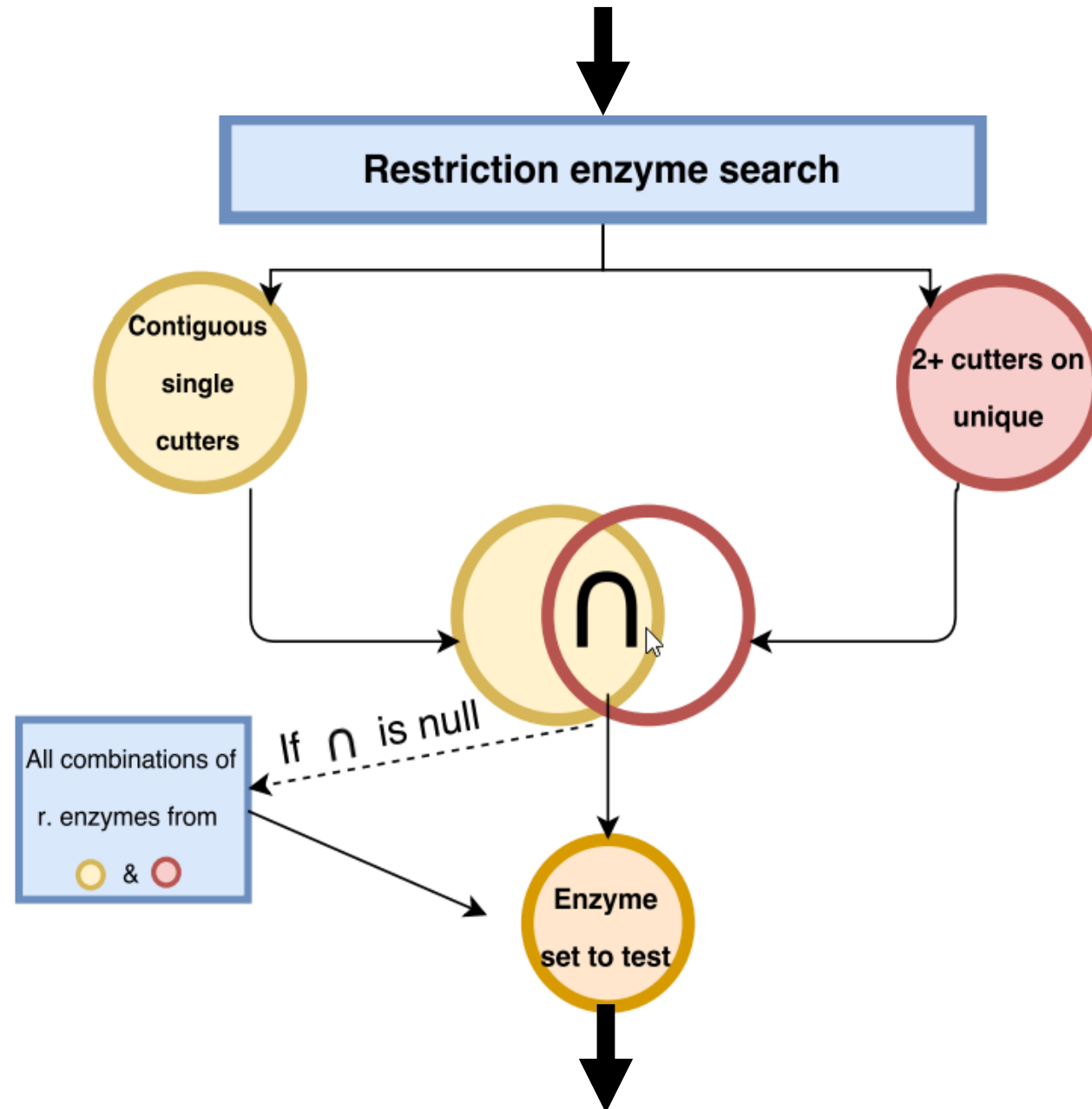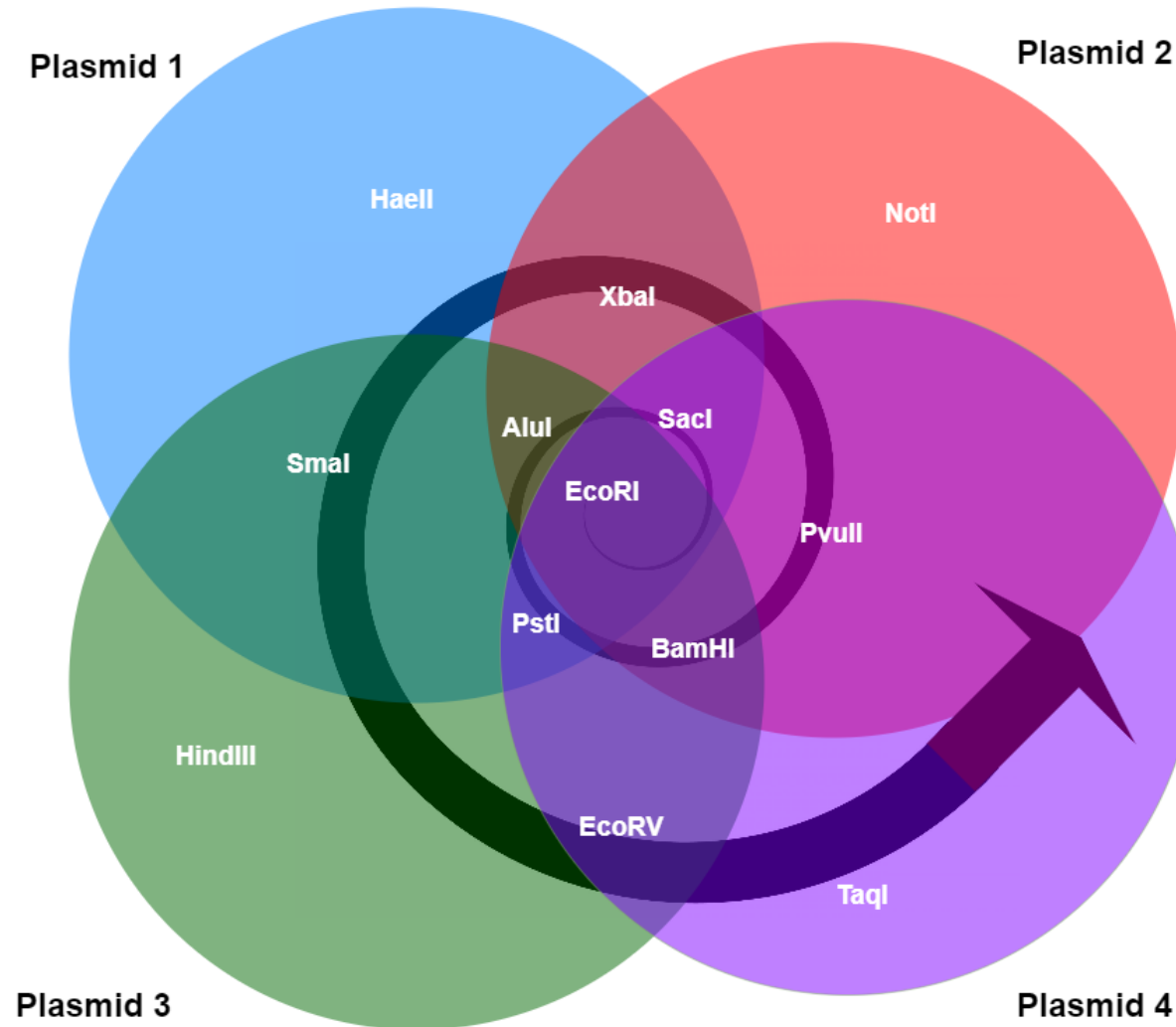 ✂ Contiguous sequence;

  ✂ "Insert" sequence.

Methodology

✂ **2. Restriction Enzymes Search Step**

✂ Single cutters for Contiguous

sequence;

✂ 2 ≤ cuts for "Insert" sequence;

✂ Intersection or combination of

the above.

Methodology

✂ 2 ≤ cuts for "Insert" sequence?

HaeII
NotI
XbaI
SacI
AluI
SmaI
EcoRI
PvuII
PstI
BamHI
HindIII
EcoRV
TaqI

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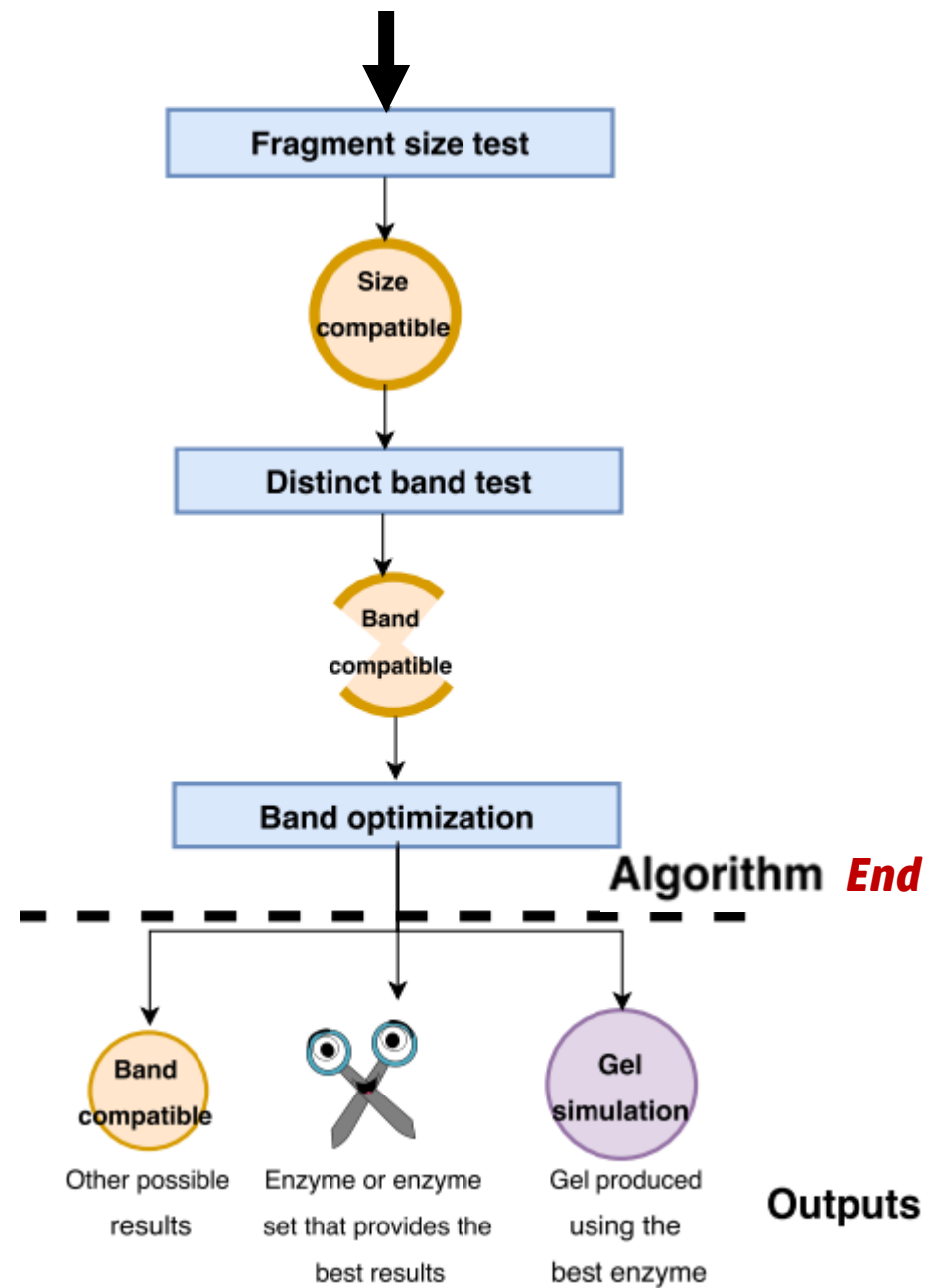
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Methodology

3. Restriction Enzymes Testing Step

- Fragment size;

- Distinc band/fragment;

- Band/pattern optimization.

4. Outputs

Methodology

✂ **How to use it?**

> Restriction_Finder(**seqs**, min_size, r_enzymes, optim, gel, iso)

seqs: List of sequences as Dseqrecord objects;

min_size: Minimum fragment size allowed after digestion;

r_enzymes: Set of restriction enzymes to be used;

optim: Should the band optimization step be performed?;

gel: Should the output be presented on an agarose gel?;
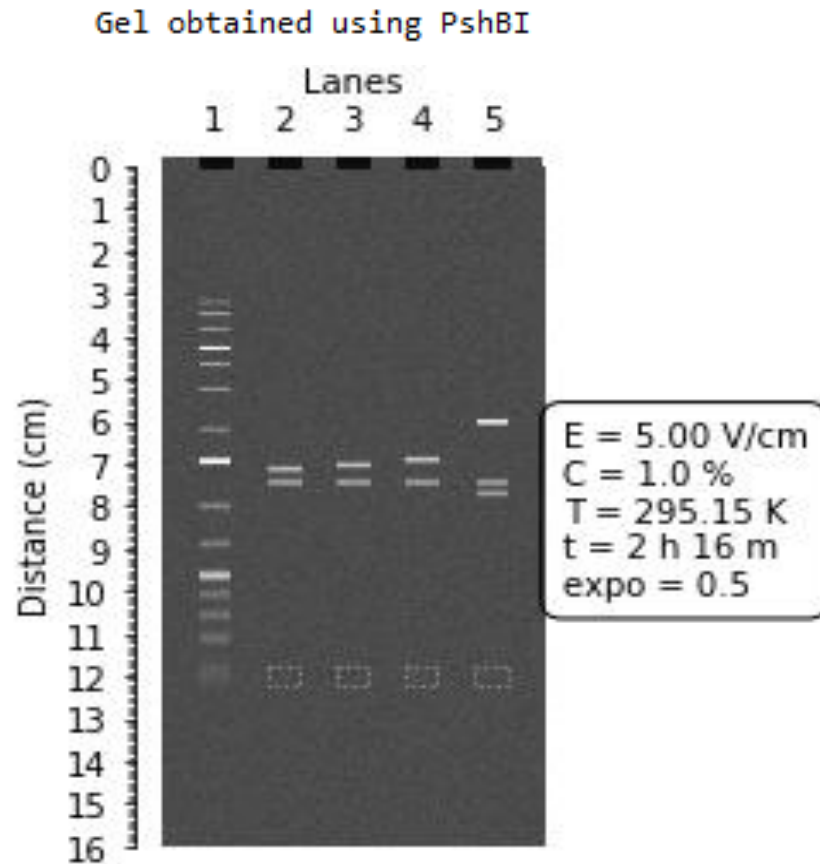
iso: Should the optimal enzyme be an isoschizomer?

```
>rf = Restriction_Finder(seqs)
>rf.lanes()
>rf.solutions()
>rf.is_isoschizomer()
>rf.all_best()
```

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Results

✂ **Using 4 pUC18 cloning vectors with different inserted genes, minimum fragment size of 50bp.**
✂ Elapsed time: 10.753 seconds.

Gel obtained using PshBI


```
E = 5.00 V/cm
C = 1.0 %
T = 295.15 K
t = 2 h 16 m
expo = 0.5
```

```
In [5]: rf.solutions()
The best enzyme is an isoschizomer so if you run
Restriction_Finder() multiple times you will get different
best enzymes but same digestion result

Best enzyme:
 PshBI
Other possible solutions:
[AseI, VspI]

In [6]: print(rf.all_best())
[PshBI, AseI, VspI]

In [7]: rf.lanes()
Lane order:
['L08752.1', 'U03991', 'LC129268.1', 'U07164.1']
```

Results

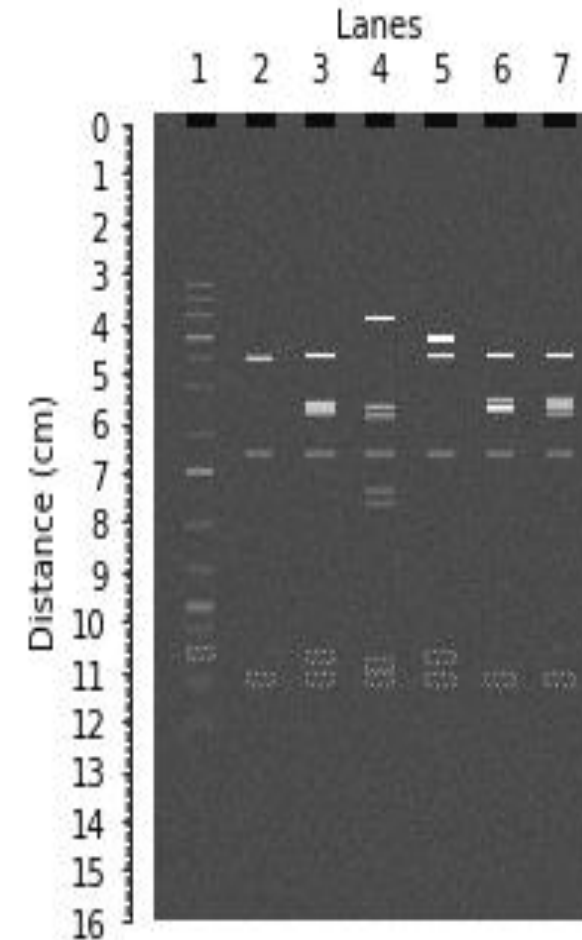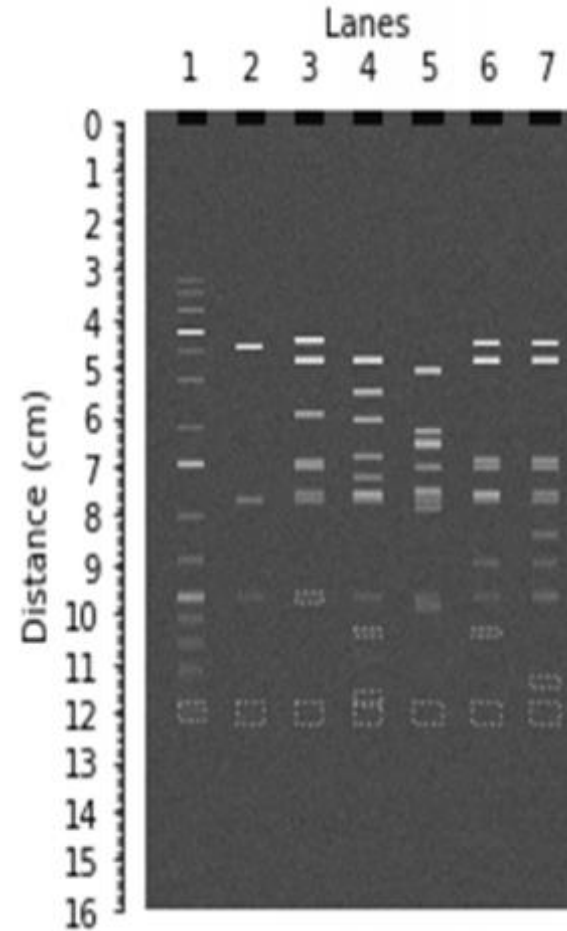✂ Using 6 yeast based cloning vectors with different inserted genes, and different parameter values for *optim* and *iso.*
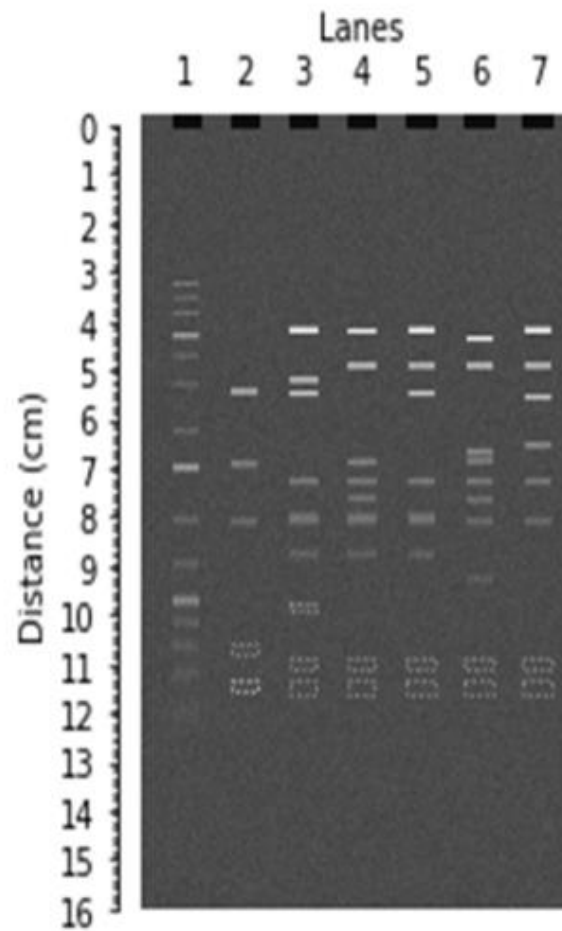
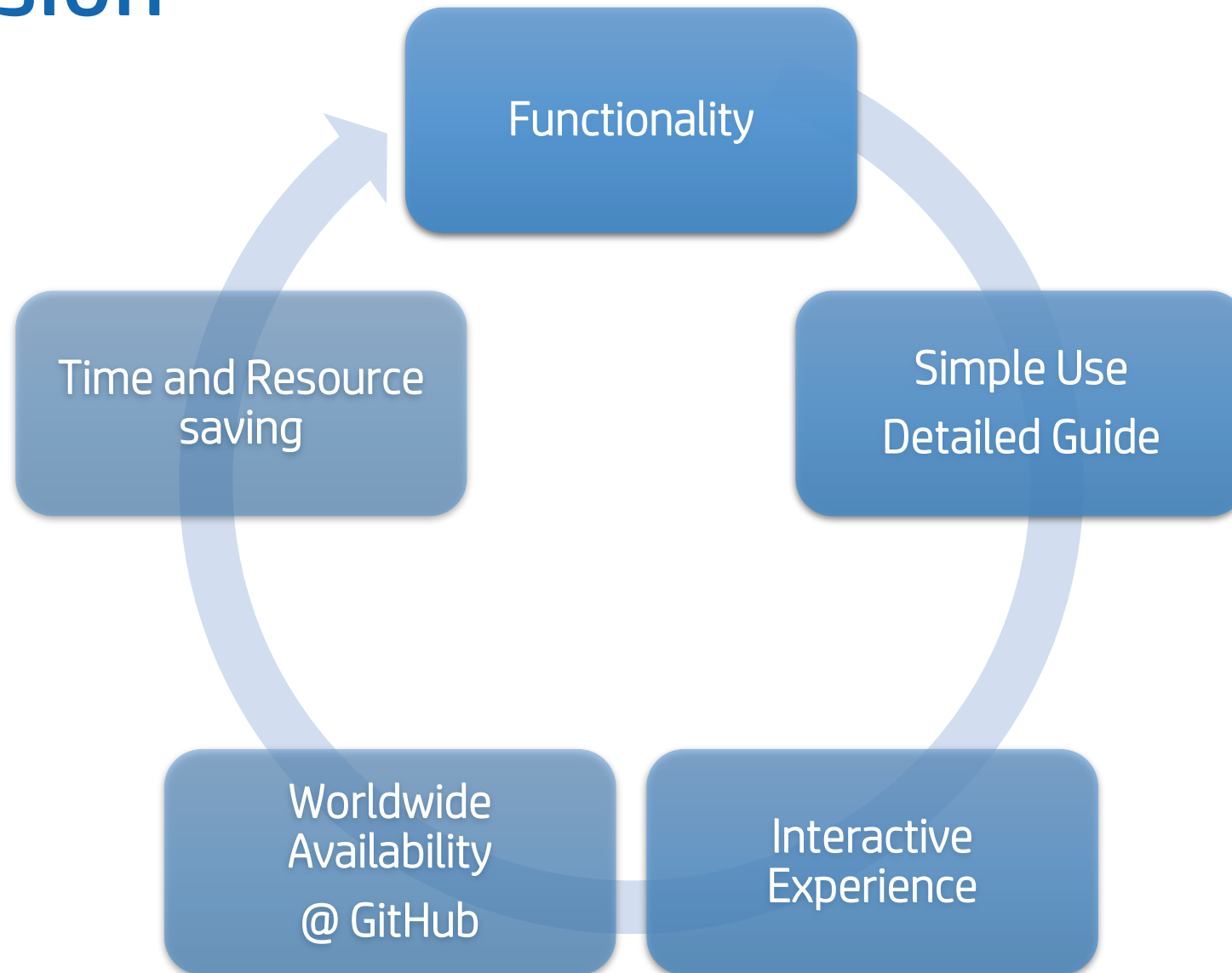| Test	Minimum fragment size (bp)	*iso*	*optim*	Best Restriction Enzyme	Other possible	Timing (s)
1	200	True	True	AanI	PsiI	26.96
2	150	True	True	BbsI	AanI, PsiI, BstV2I, BpiI	41.66
3	150	True	False	BpiI	AanI, BbsI, PsiI, BstV2I	33.28
4	50	True	True	BbsI	NspI, AanI, XceI, BsrFI, PsiI, BstNSI, TatI, and 9 others	61.59
5	50	False	True	TatI	NspI, AanI, XceI, BbsI, BsrFI, PsiI, BstNSI, Alw26I, Cfr10I, and 7 others	57.78

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Results

✂ **Restriction with BbsI, BpiI and TatI respectively.**
✂ Lane order: pJC170, pJC175, pJC178, pJC181, pJC184, pJC187

Conclusion

Functionality

Simple Use
Detailed Guide

Interactive
Experience

Worldwide
Availability

@ GitHub

Time and Resource
saving

Conclusion

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