Pattern Recognition In Clinical Data

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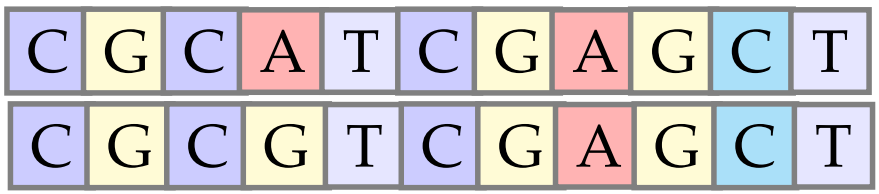
Dual Degree Project

Guide: Prof. Santosh Noronha



October 30, 2013

Introduction

OBJECTIVE

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Next Generation Sequencing & Cancer Research

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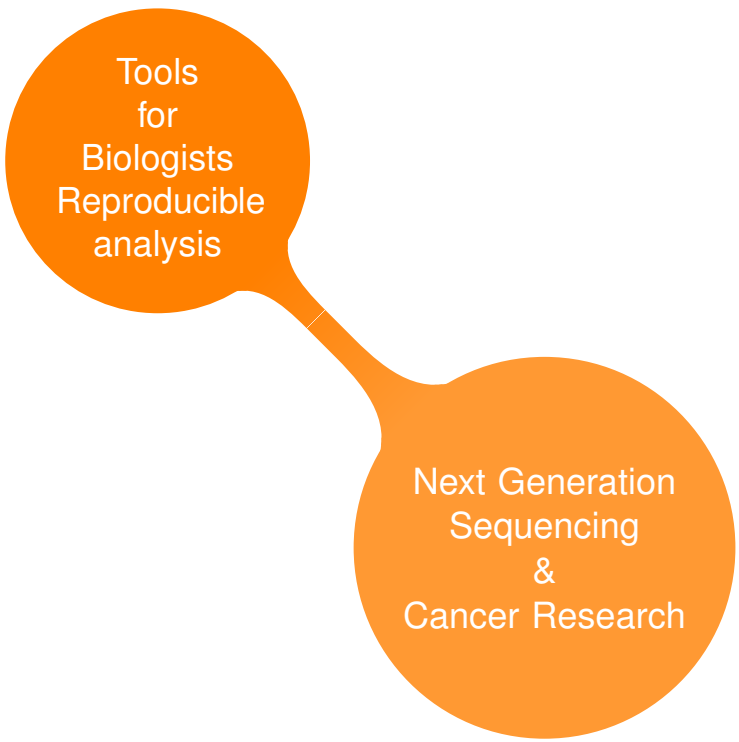
Tools
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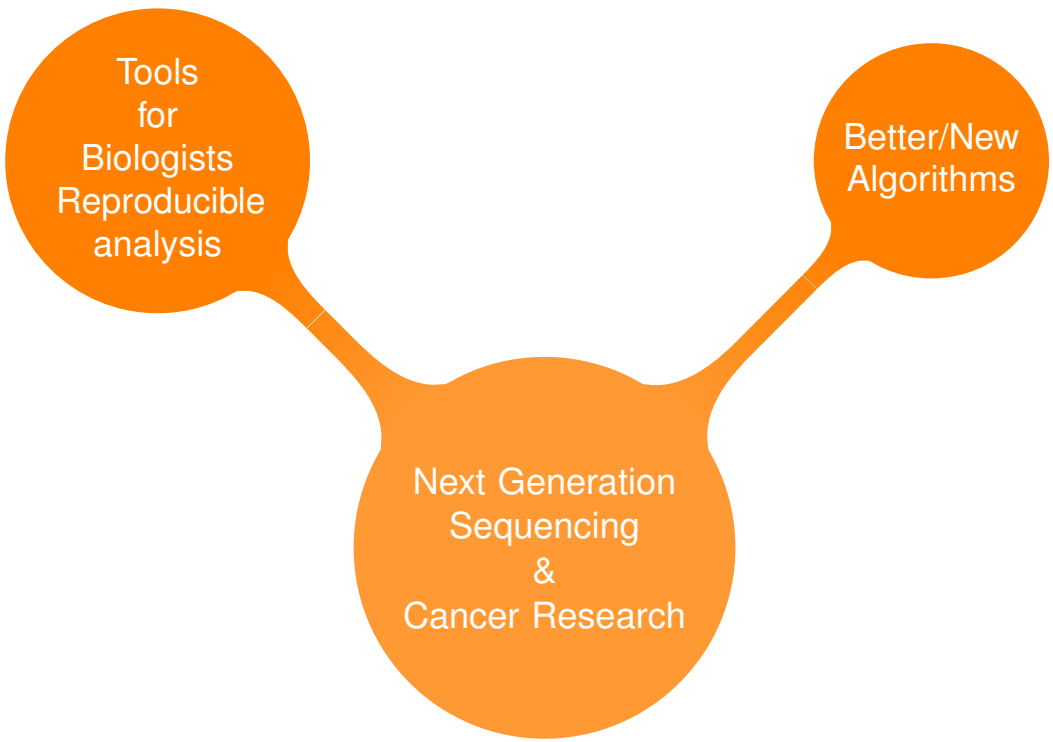
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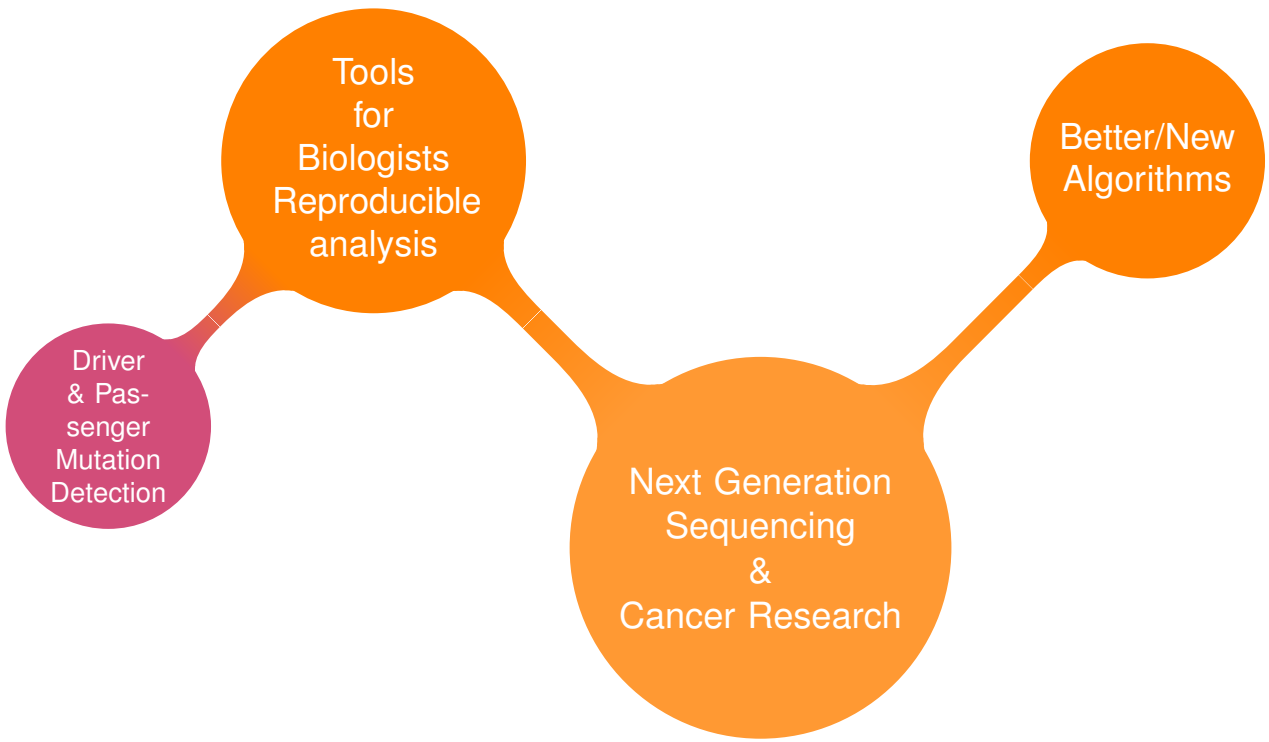
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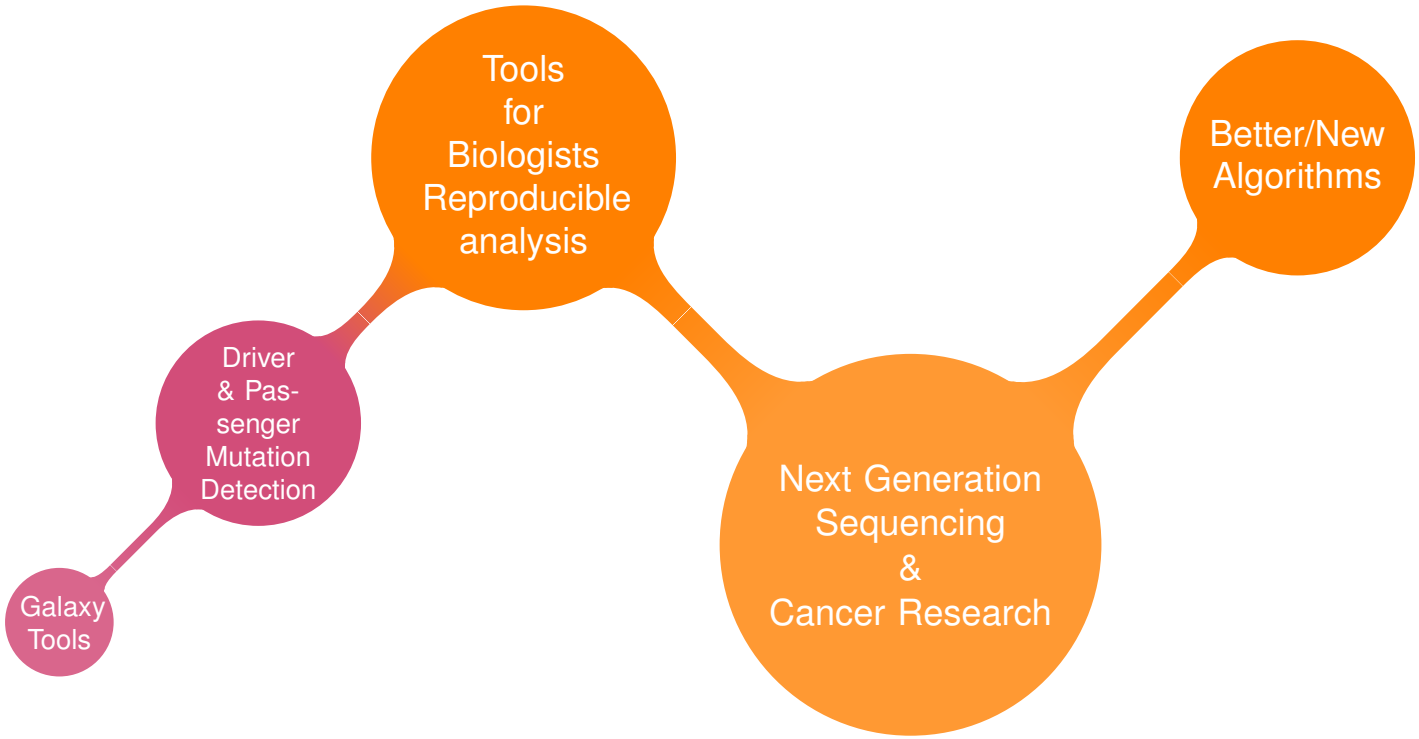
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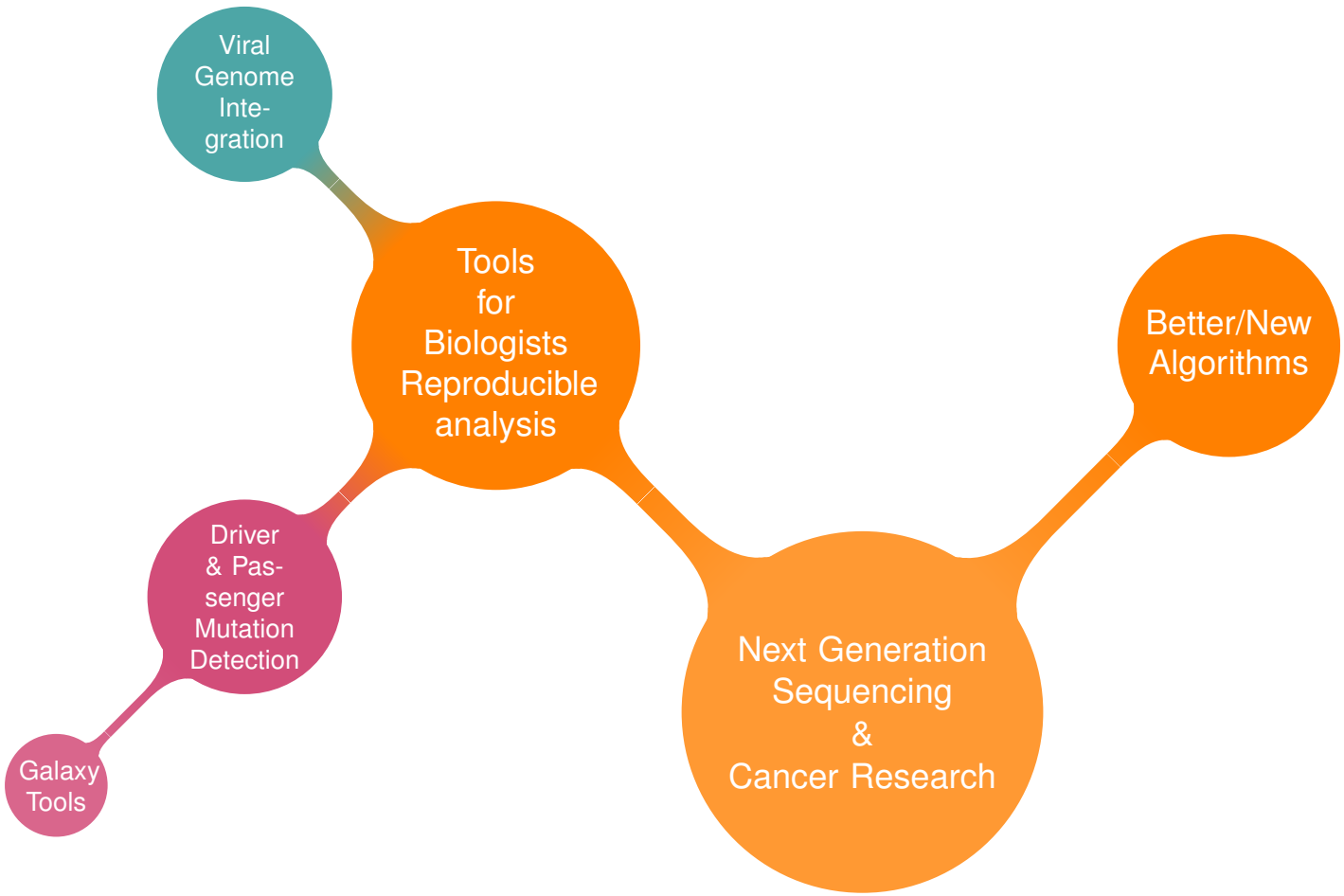
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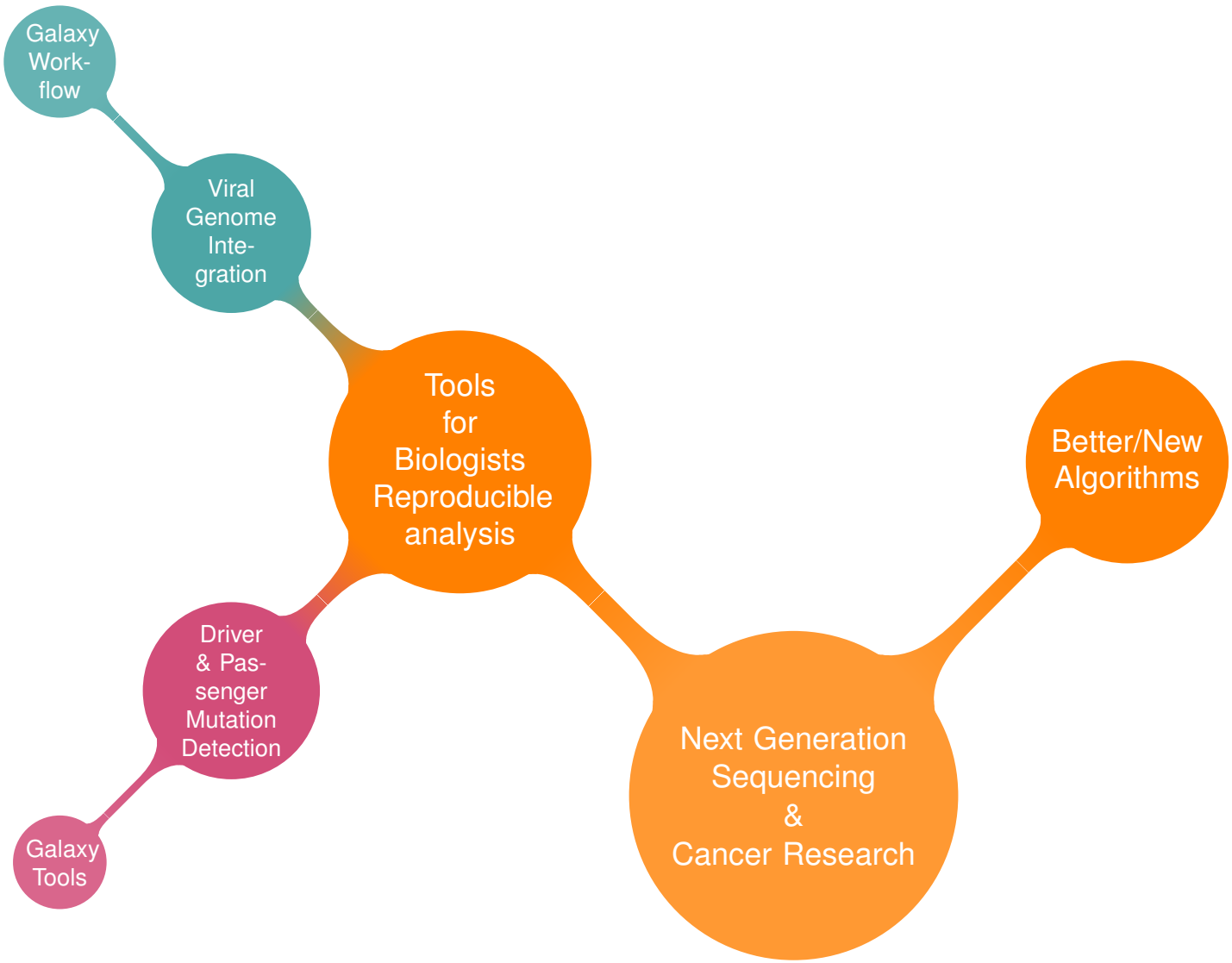
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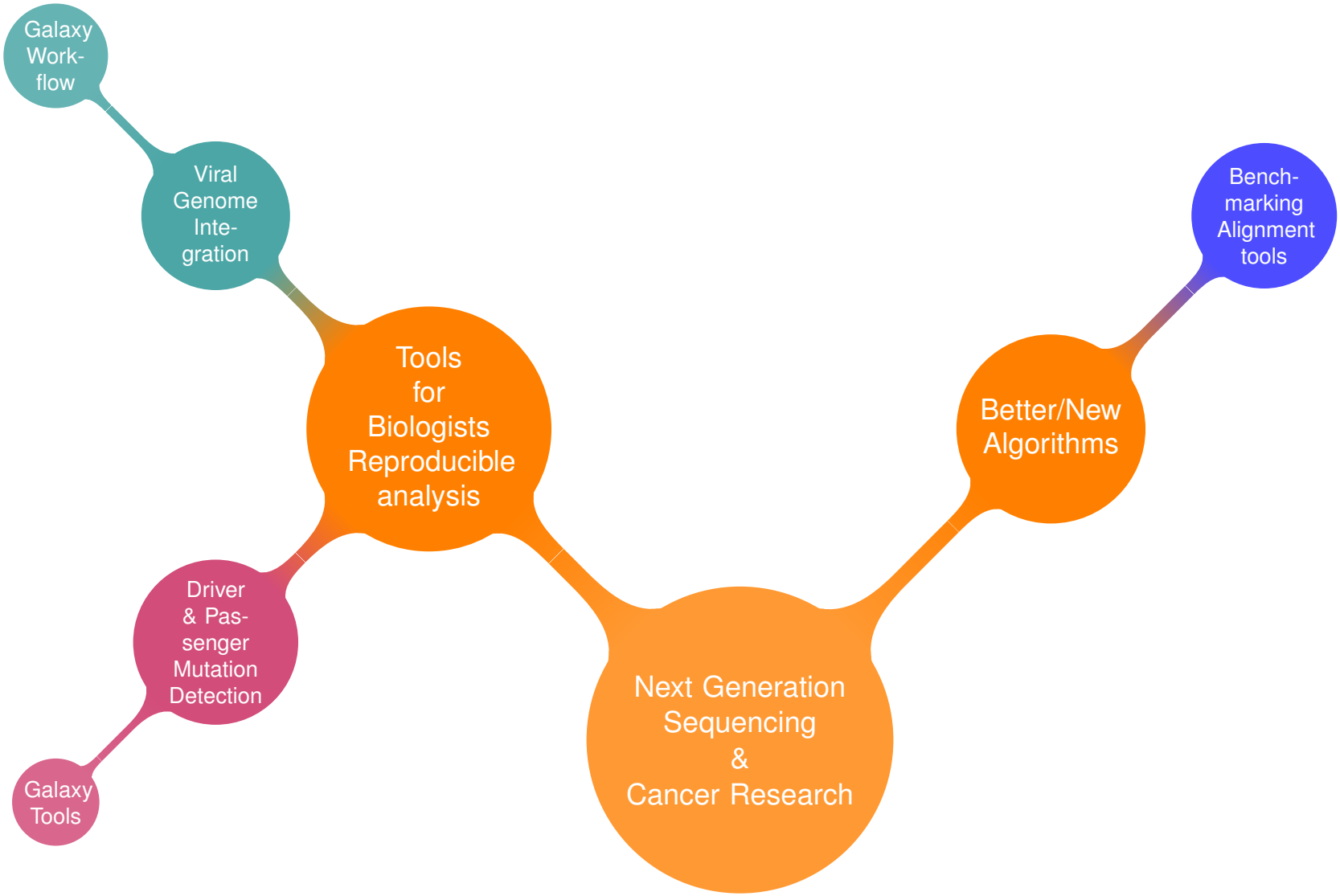
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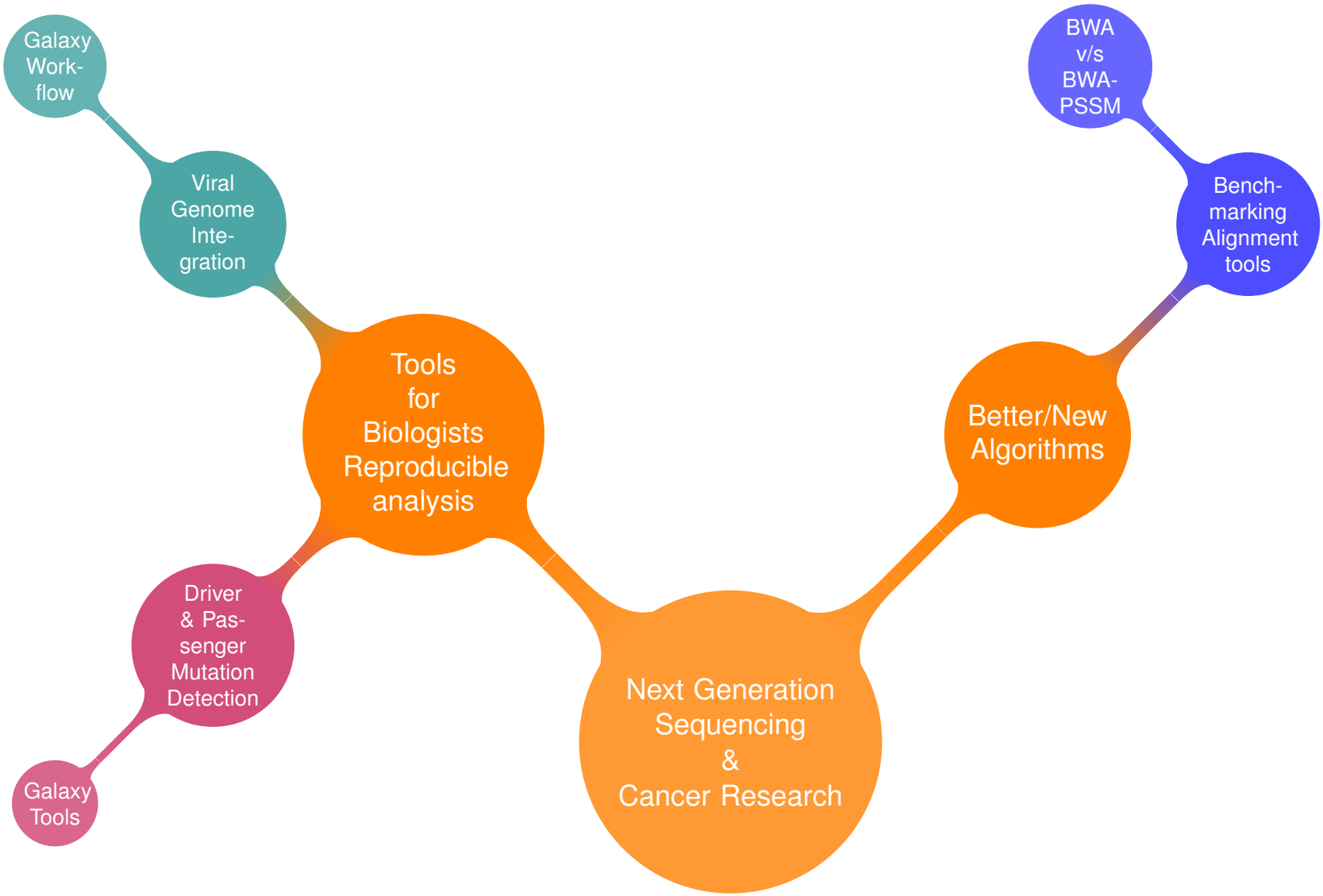
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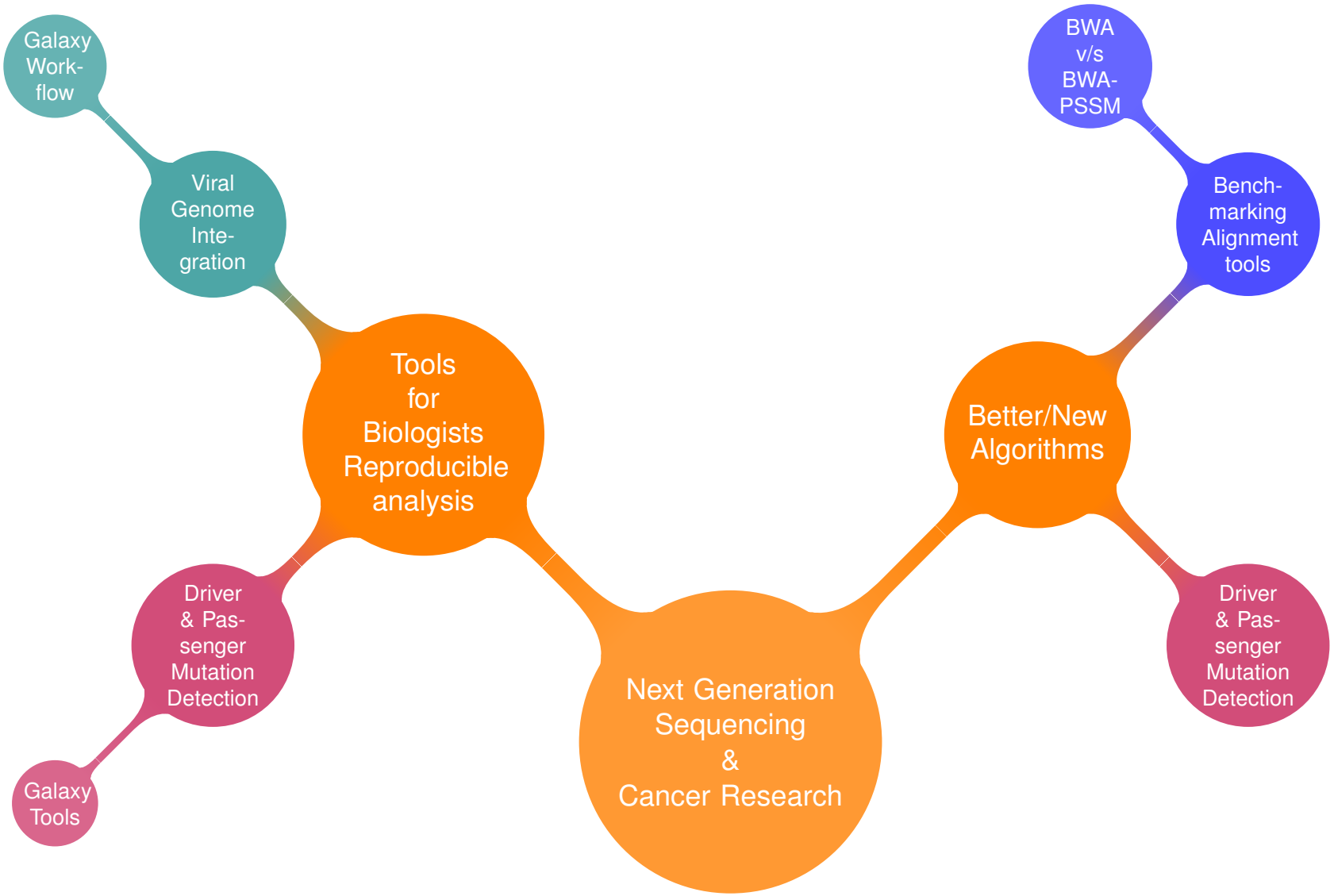
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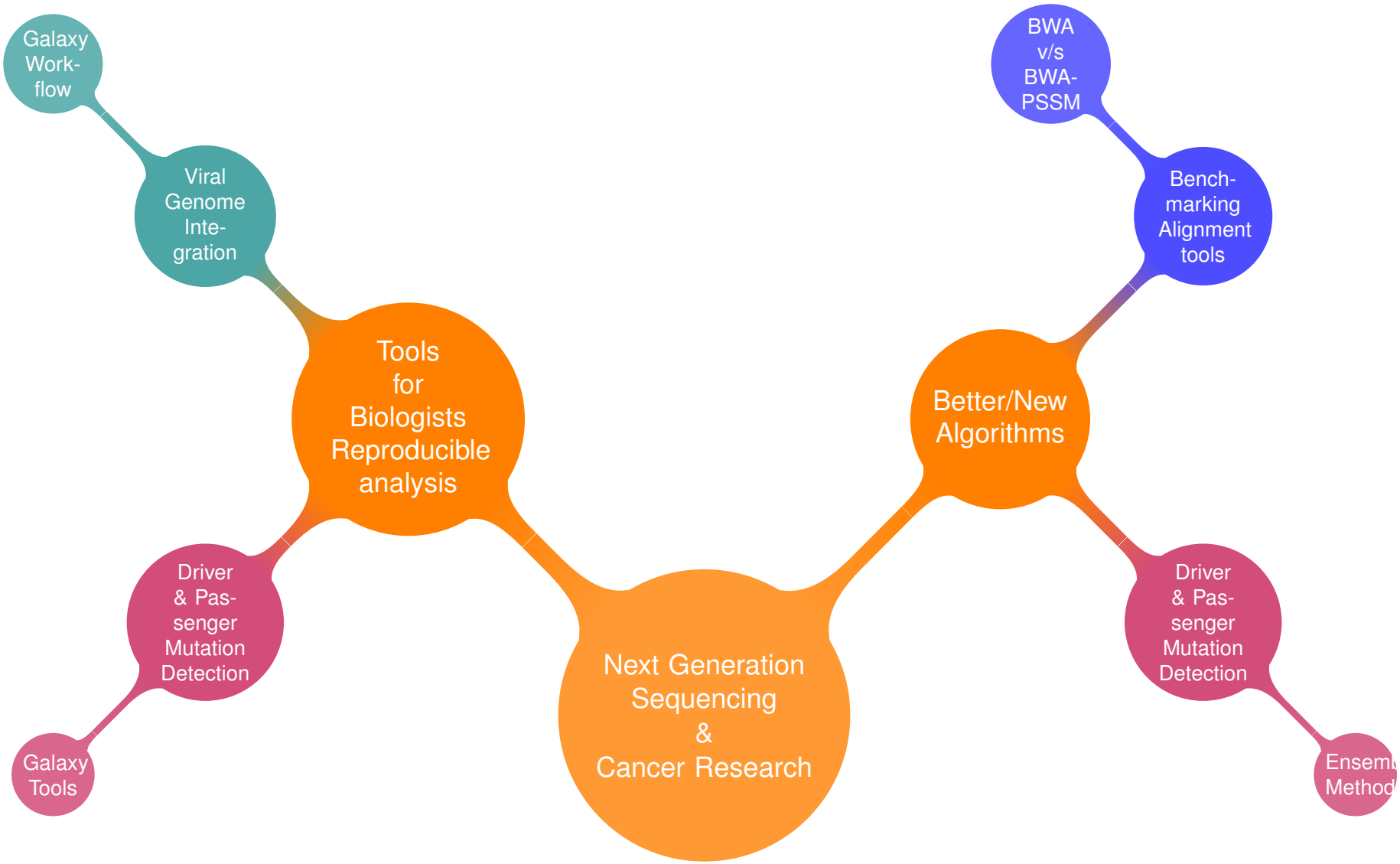
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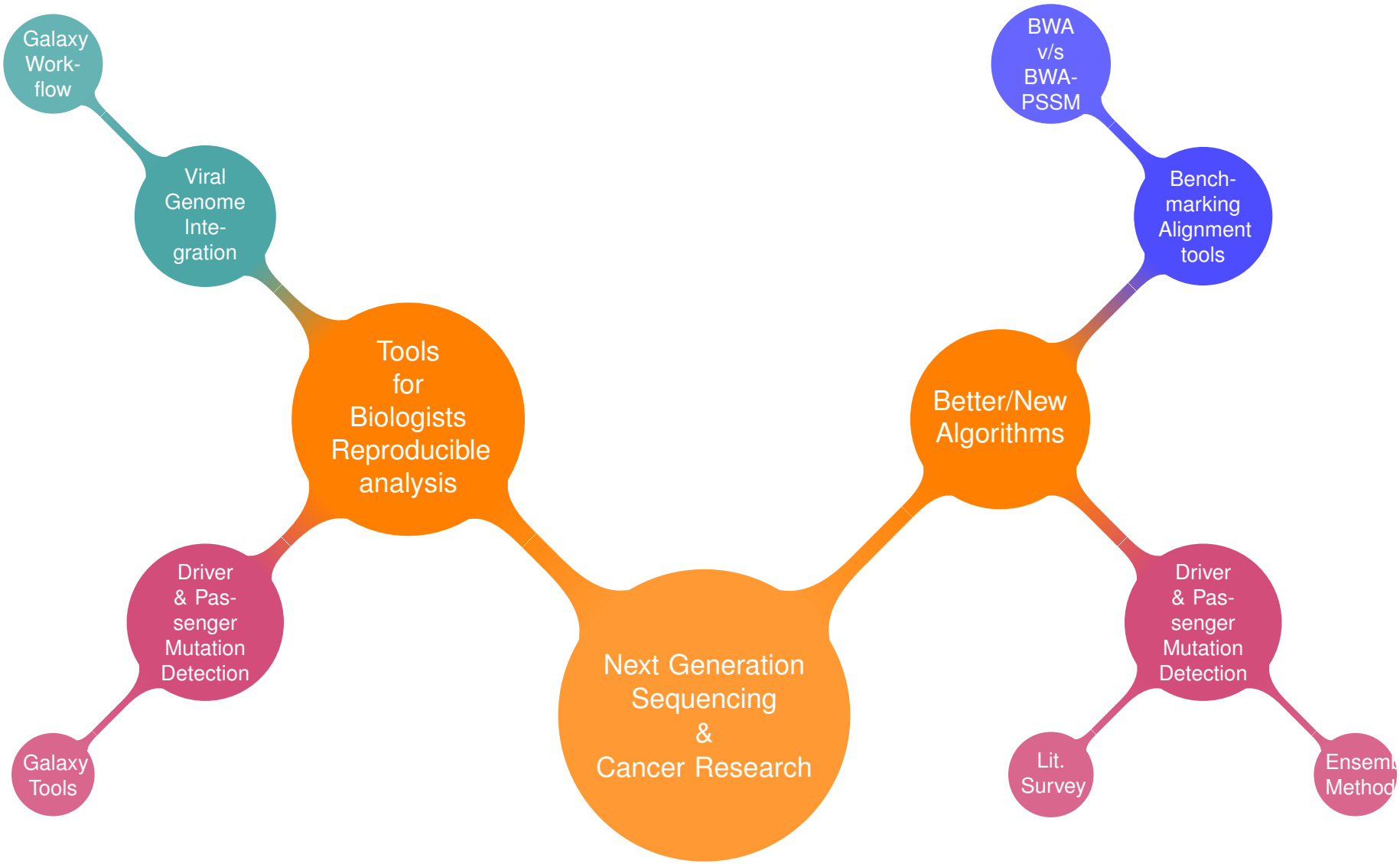
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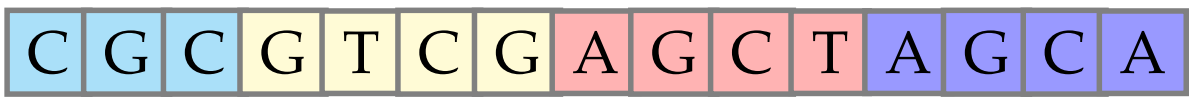
Lit. Survey

NEXT GENERATION SEQUENCING



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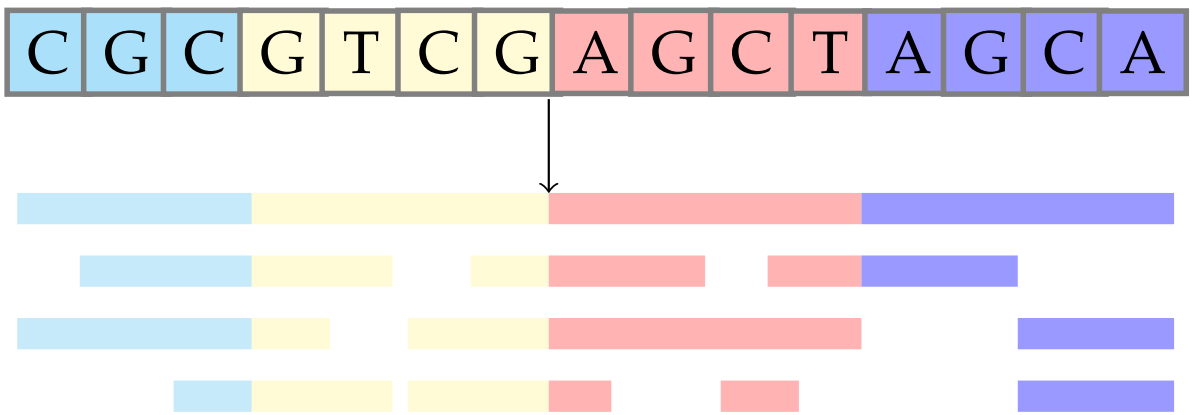

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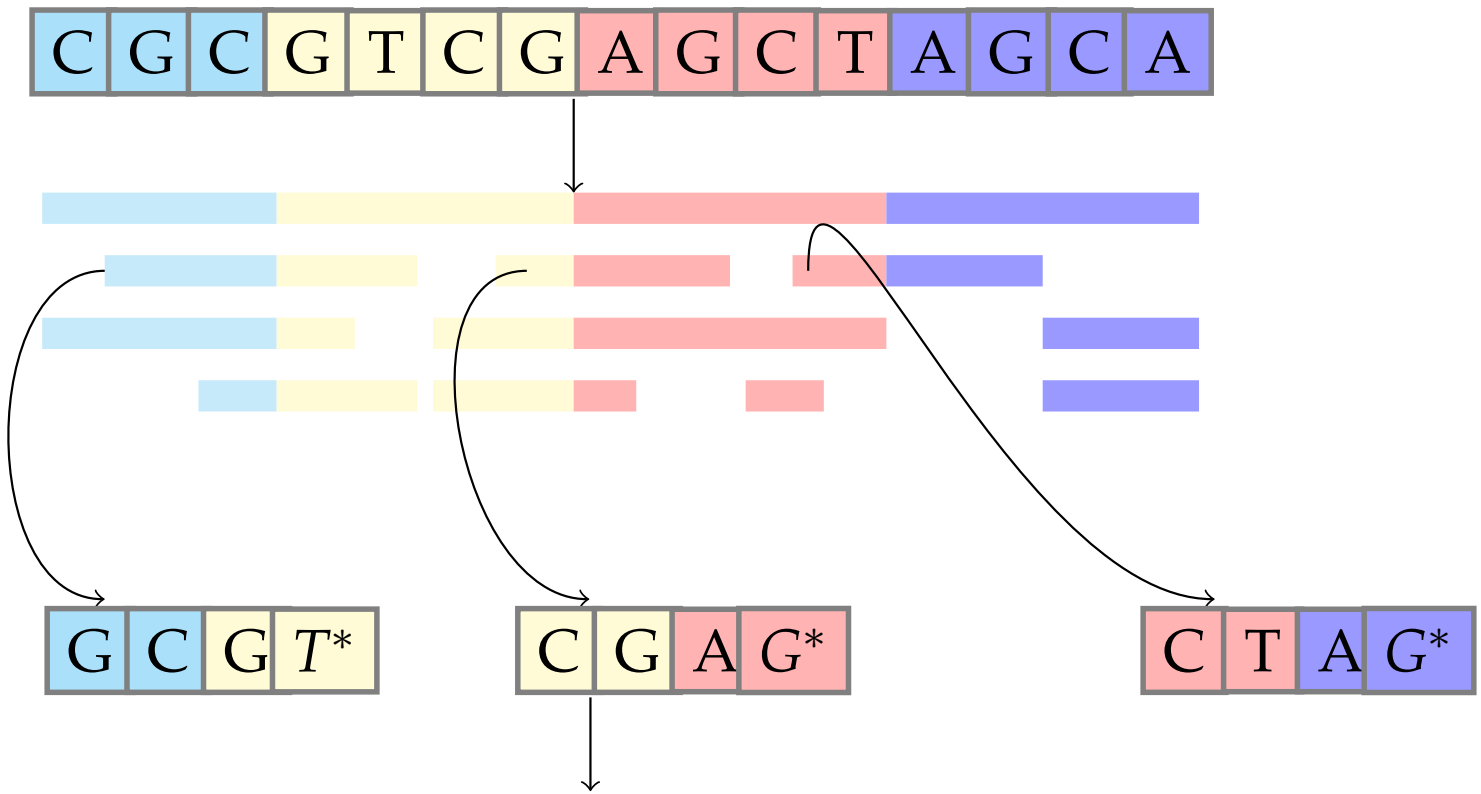

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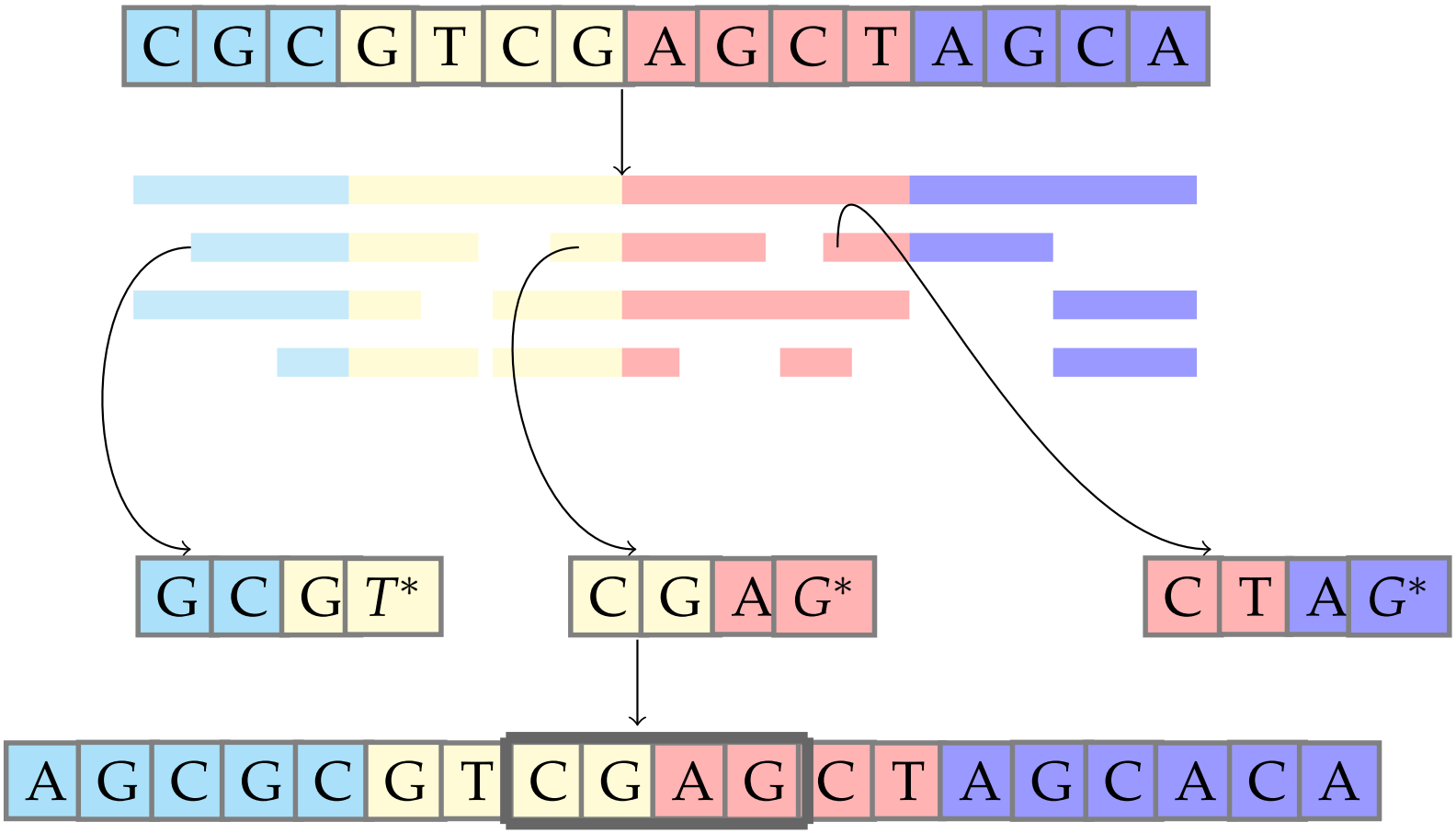

NGS: WHY?

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NGS: MUTATIONS

- $3x10^9$ base pairs
- We are all 99.9% similar, at DNA level
- More than 2 million SNPs
- No particular pattern of SNPs
- If a certain mutation causes a change in an amino acid, it is referred to as non synonymous(nsSNV)

Drivers and Passengers I

Cancer is known to arise due to **mutations**
Not all mutations are equally important!

Somatic Mutations

Set of mutations *acquired* after zygote formation, over and above the **germline** mutations

Driver Mutations

Mutations that confer growth advantages to the cell, being selected positively in the tumor tissue

DRIVERS AND PASSENGERS

Drivers are **NOT** simply *loss of function* mutations, but more than that:

- **Loss of function**: Inactivate tumor suppressor proteins
- **Gain of function**: Activates normal genes transforming them to oncogenes
- **Drug Resistance Mutations**: Mutations that have evolved to overcome the inhibitory effect of drugs

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DRIVER MUTATIONS: WHY?

Identify **driver mutations** $\longrightarrow$ better therapeutic targets
But how does one zero down upon the exact set? $\longrightarrow$ experiments are too costly, probably infeasible for 2 million+ SNPs $\longrightarrow$ Leverage computational analysis

- Low cost of NGS comes with a heavier roadblock of data analysis
- Searching among 2 million+ SNPs is a non-trivial, and a computationally intensive problem
- Softwares have a low consensus ratio amongst them selves $\longleftrightarrow$ Defining a driver, computationally is non-trivial
- However there is no tool that allows one to visualise the results on an input across the cohort of tools

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MACHINE LEARNING I

Two datasets:

- **Training**: *Labeled* dataset, containing a table of features with mutations labelled as "drivers/passengers"
- **Test**: 'Learning' from training dataset, test the prediction model

Table: Training Dataset

Chromosome	Position	Ref	Alt	Type
1	27822	A	G	Driver
1	27832	T	G	Driver
2	47842	G	C	Passenger
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.

Machine Learning II

Table: Test Dataset

Chromosome	Position	Ref	Alt	Type
1	27824	A	G	?
1	47832	T	G	?

MACHINE LEARNING: FEATURE SELECTION I

Machine Learning relies on a set of **features** for training
Redundant features should be avoided
CHASM [1] makes use of
$p(X_i)$ represents the probability of occurrence of an event X_i
Considering a series of events X_1, X_2, X_3...,X_n analogous 'series of packets' in communication theory , the information received at each step can be quantified on a log scale by:

$$\frac{1}{log_2(X_i)} = -log_2(p(X_i)) \tag{1}$$

The expected value of information from a series of events is called shannon entropy: $H(X)$:

$$H(X) = -\sum_i p(X_i)\log_2 p(X_i) \tag{2}$$

MACHINE LEARNING: FEATURE SELECTION II

Mutual Information between two random variables X, Y is defined as the amount of information gained about random variable X due to additional information gained from the second, Y:

$$I(X, Y) = H(X) - H(X|Y) \tag{3}$$

Here:
X: Class Label[Driver/Passenger]
Y: Predictive Feature
and hence $I(X, Y)$ represents how much information was gained about the class label Y from knowledge of a feature X
Simplifying :

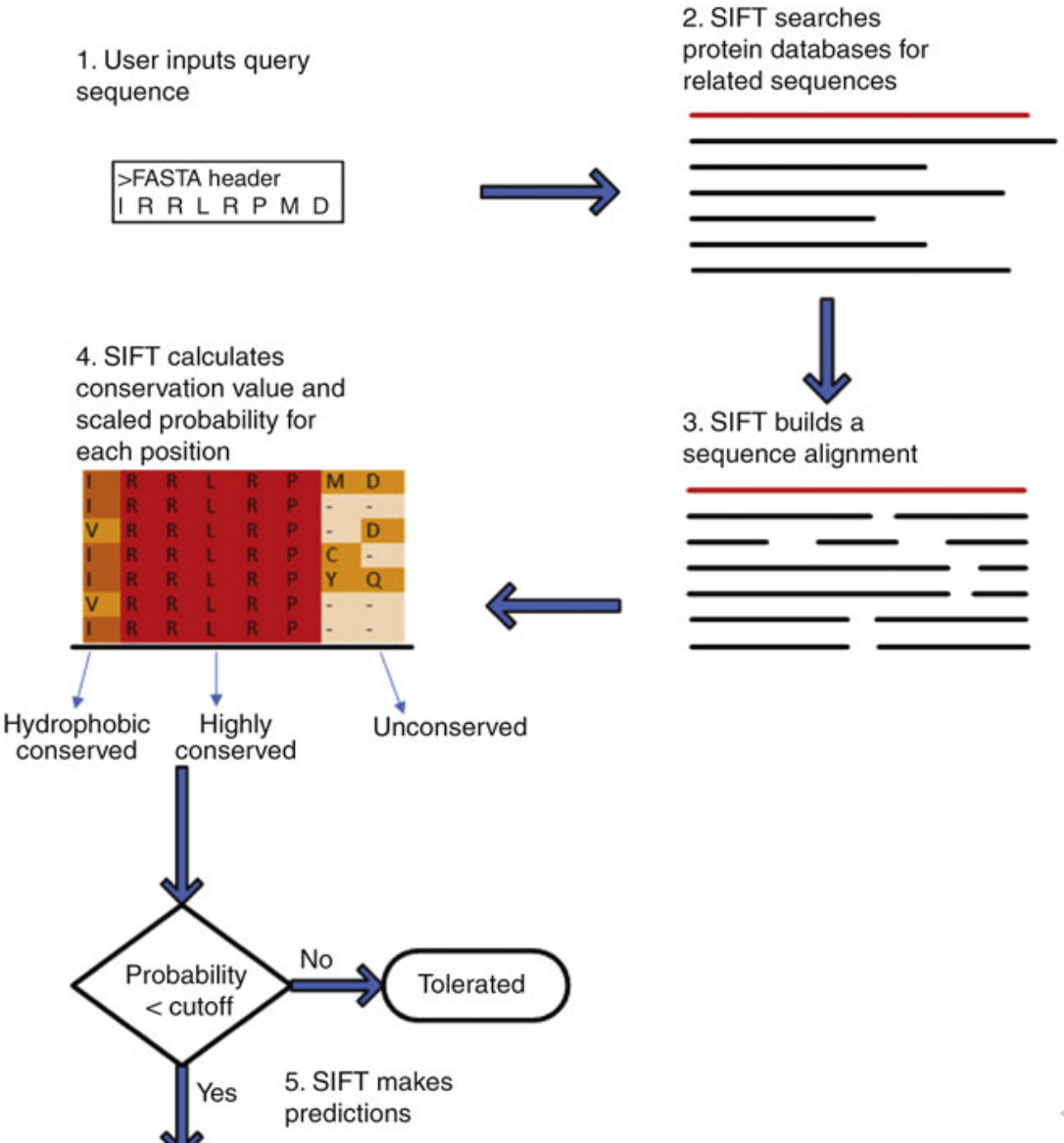
$$I(X, Y) = \sum p(x, y) log_2 \frac{p(x, y)}{p(x)p(y)} \tag{4}$$

FUNCTIONAL IMPACT I

- If a certain mutation confers an advantage to the cell in terms of replication rate, it is probably going to be selected while all those mutations that reduce its fitness have a higher chance of being eliminated from the population.
- Certain residues in a MSA of homologous sequences are more conserved than others. A highly conserved if mutated is possibly going to **cost a lot** since what had 'evolved' is disturbed!
- Scores can be assigned based on this "conservation" parameter.

FUNCTIONAL IMPACT II

Figure: SIFT [?] algorithm



Some of the common tools/algorithms used for driver mutation prediction:

- SIFT
- Polyphen
- Mutation Assesor
- TransFIC
- Condel

FRAMEWORK FOR COMPARING VARIOUS TOOLS I

- Different tools use different formats, give different outputs for similar input
- Running analysis on multiple tools $\longrightarrow$ keep shifting data formats
- Concordance?

Polyphen2 Input

chr1:888659 T/C
chr1:1120431 G/A
chr1:1387764 G/A
chr1:1421991 G/A
chr1:1599812 C/T
chr1:1888193 C/A
chr1:1900186 T/C

FRAMEWORK FOR COMPARING VARIOUS TOOLS II

SIFT Input
1,888659,T,C
1,1120431,G,A
1,1387764,G,A
1,1421991,G,A
1,1599812,C,T
1,1888193,C,A
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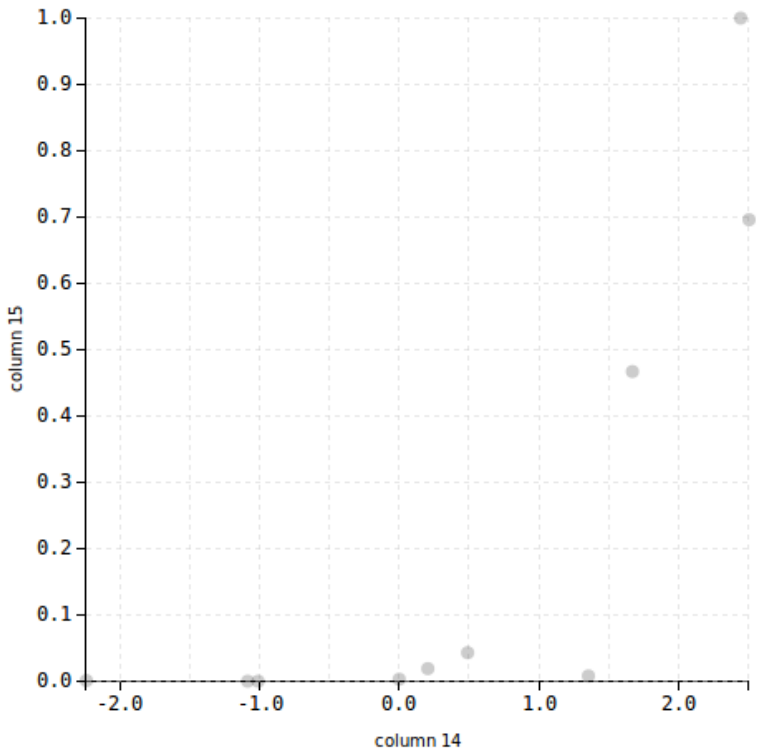
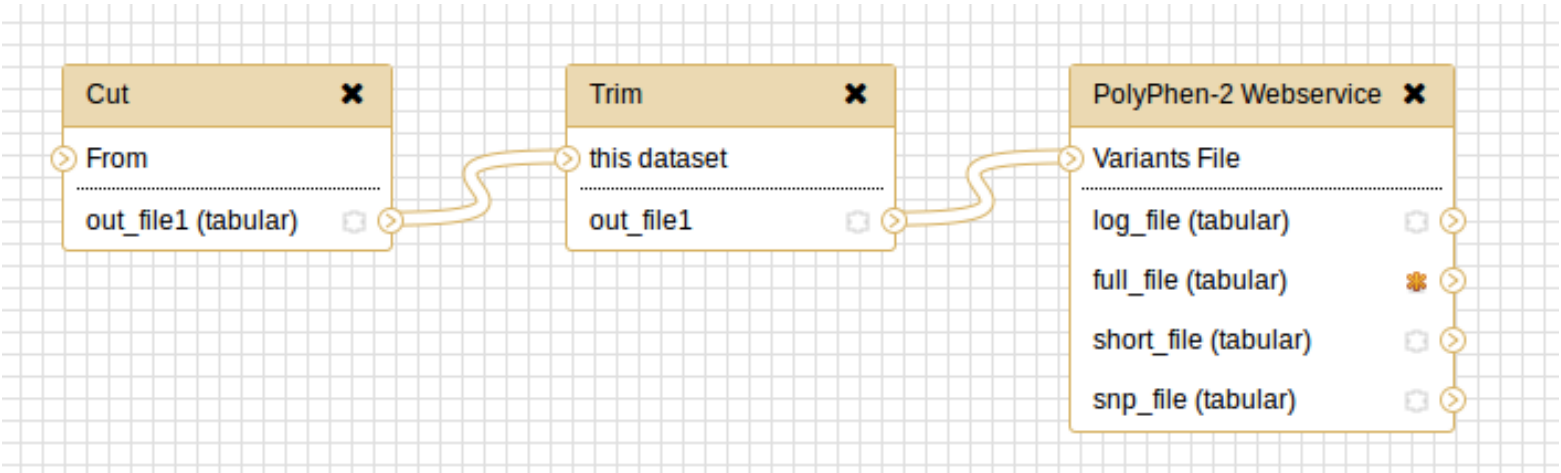
DRIVER MUTATIONS: TOOLS DON'T AGREE



X Axis: Condel Score Y Axis: MA Score

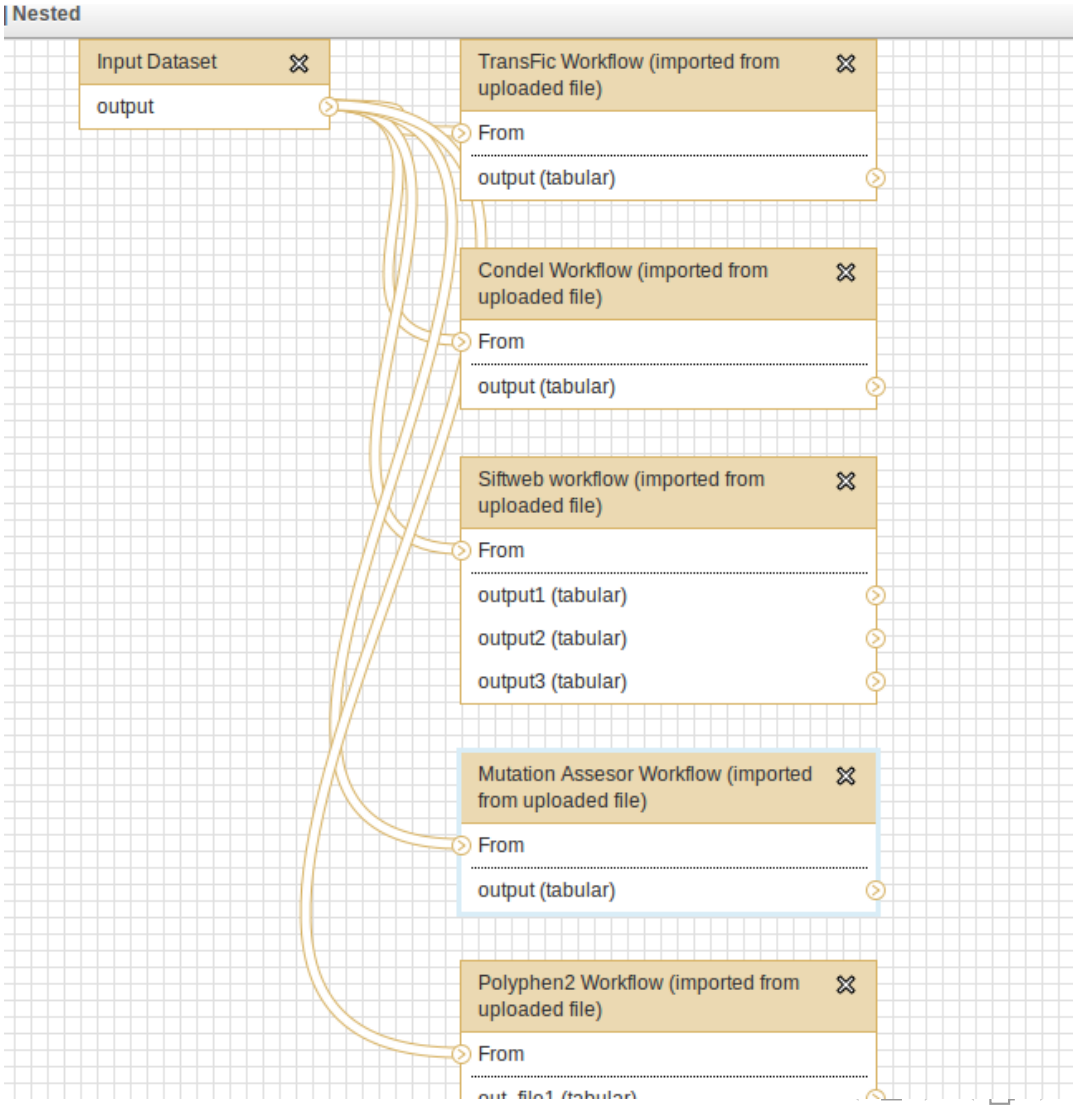
Solution?:
Galaxy[**?**], an open source web-based platform for bioinformatics, makes it possible to represent the entire data analysis pipeline in an intuitive graphical interface

Figure: Galaxy Workflow polyphen2 algorithm



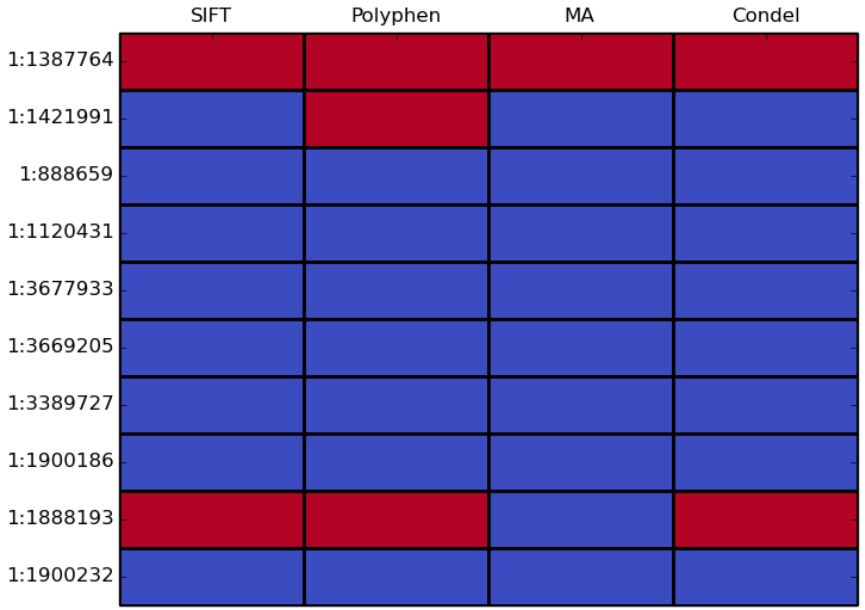
Run all tools in one go:

Figure: Run all tools



Compare all tools:

Figure: Compare all tools



VIRAL GENOME DETECION

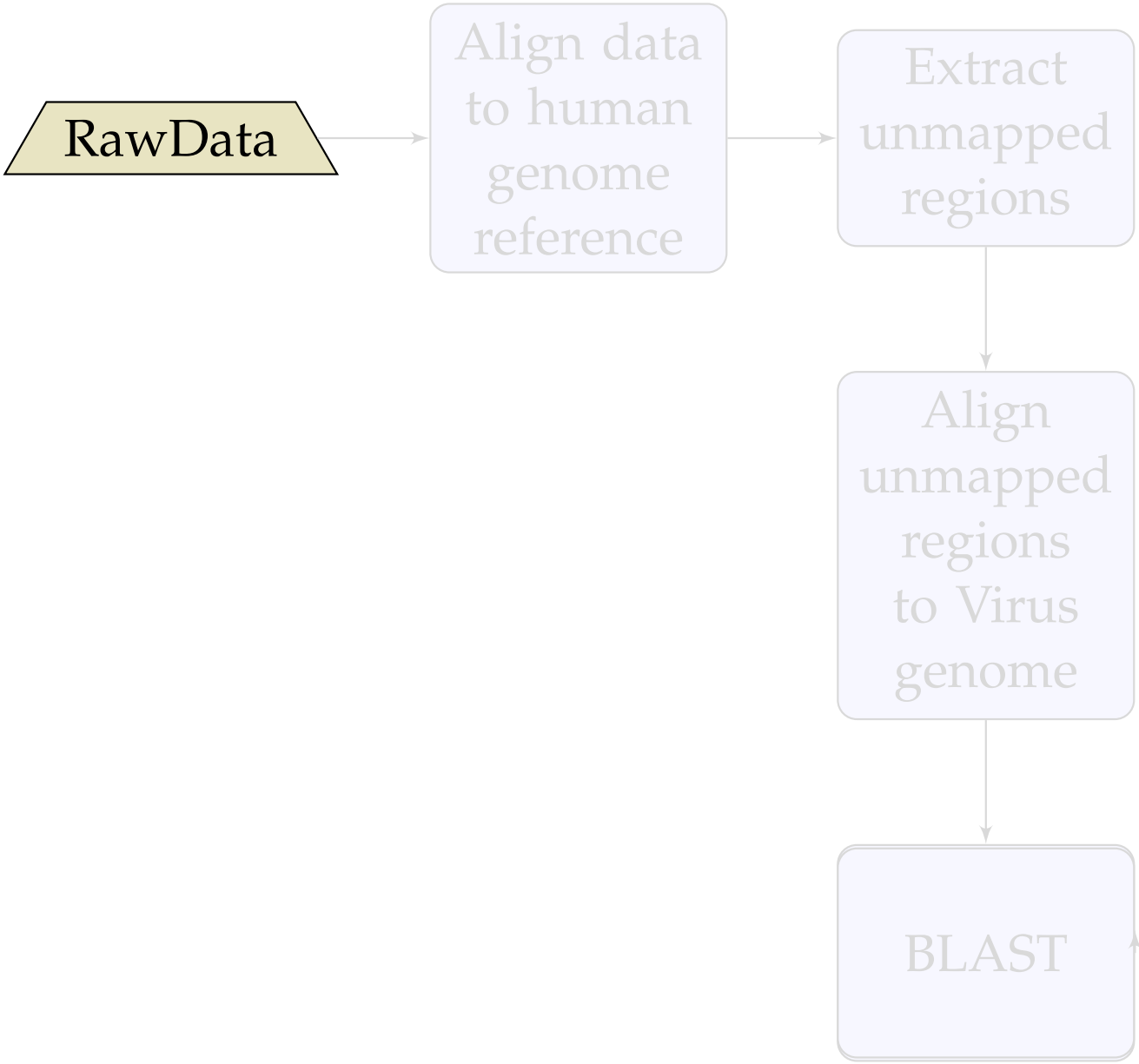
Cervical cancers have been proven to be associated with Human Papillomavirus(HPV)
Cervical cancer datasets from Indian women was put through an analysis to detect :

1. Any possible HPV integration
2. Sites of HPV integration

Who Cares?

- Replacing whole genome sequencing, by targeted sequencing at the sites where these virus have been detected in a cohort of samples, thus speeding up the whole process.

RawData
Align data to human genome reference
Extract unmapped regions
Align unmapped regions to Virus genome
BLAST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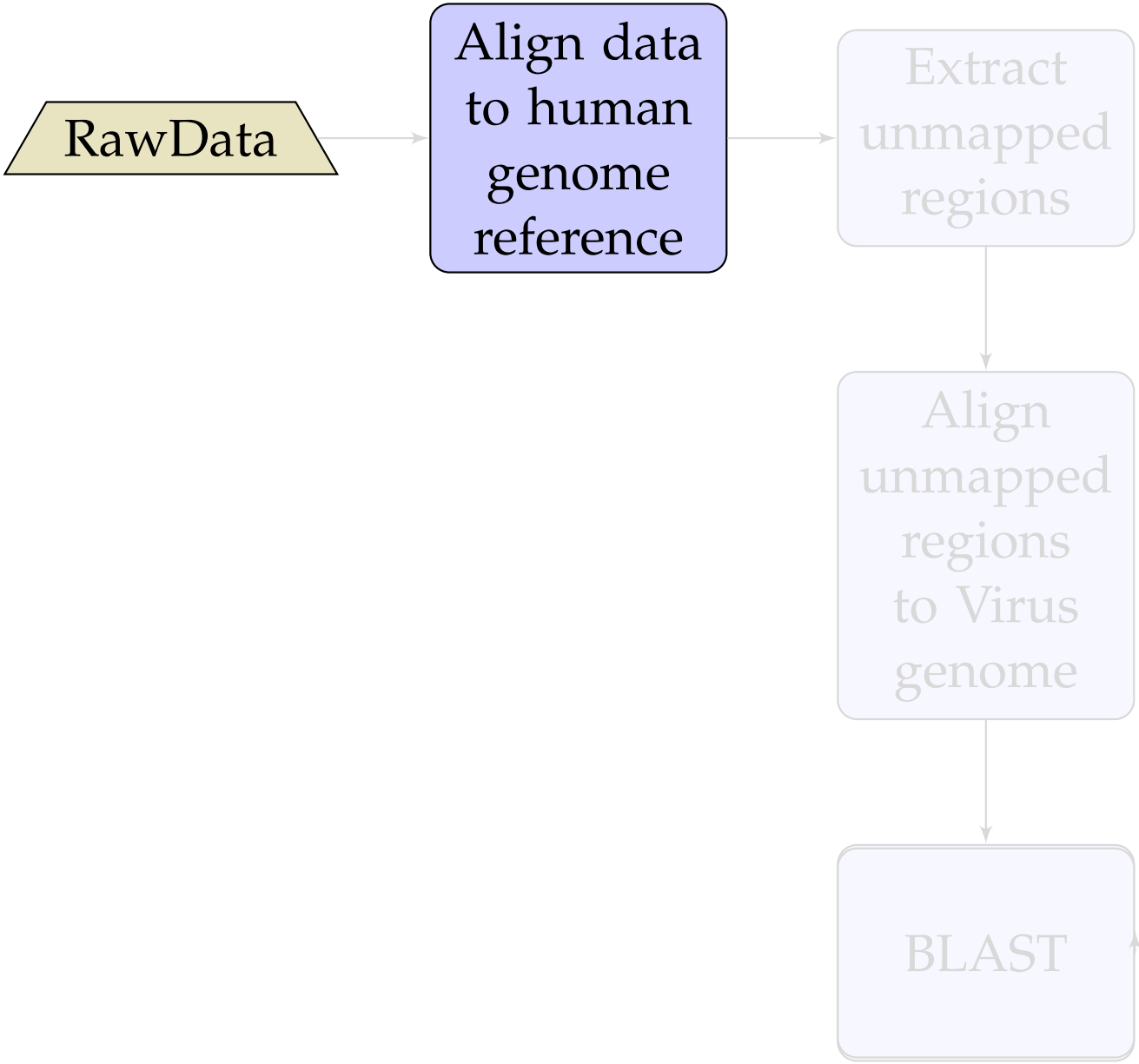
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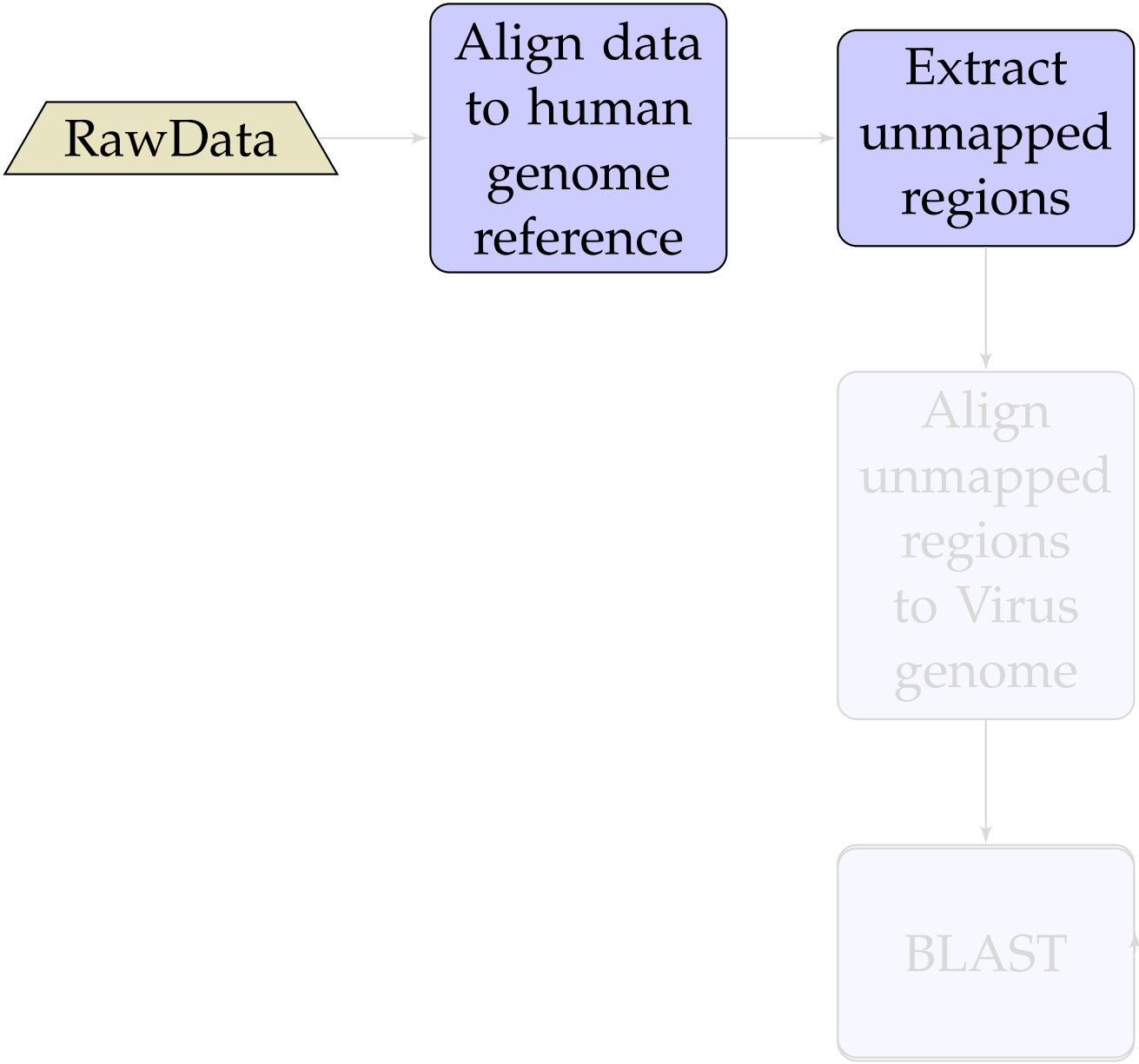
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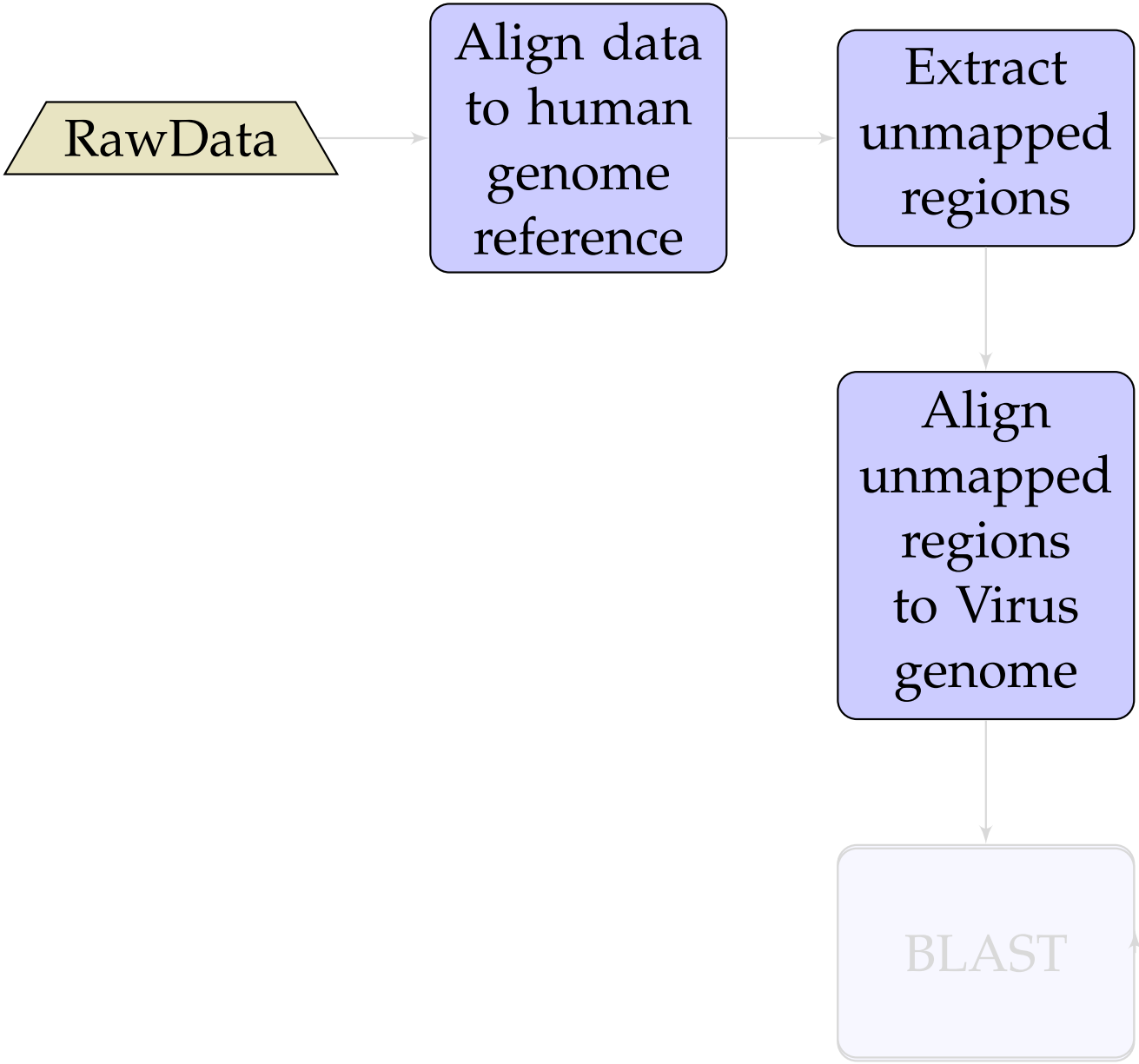
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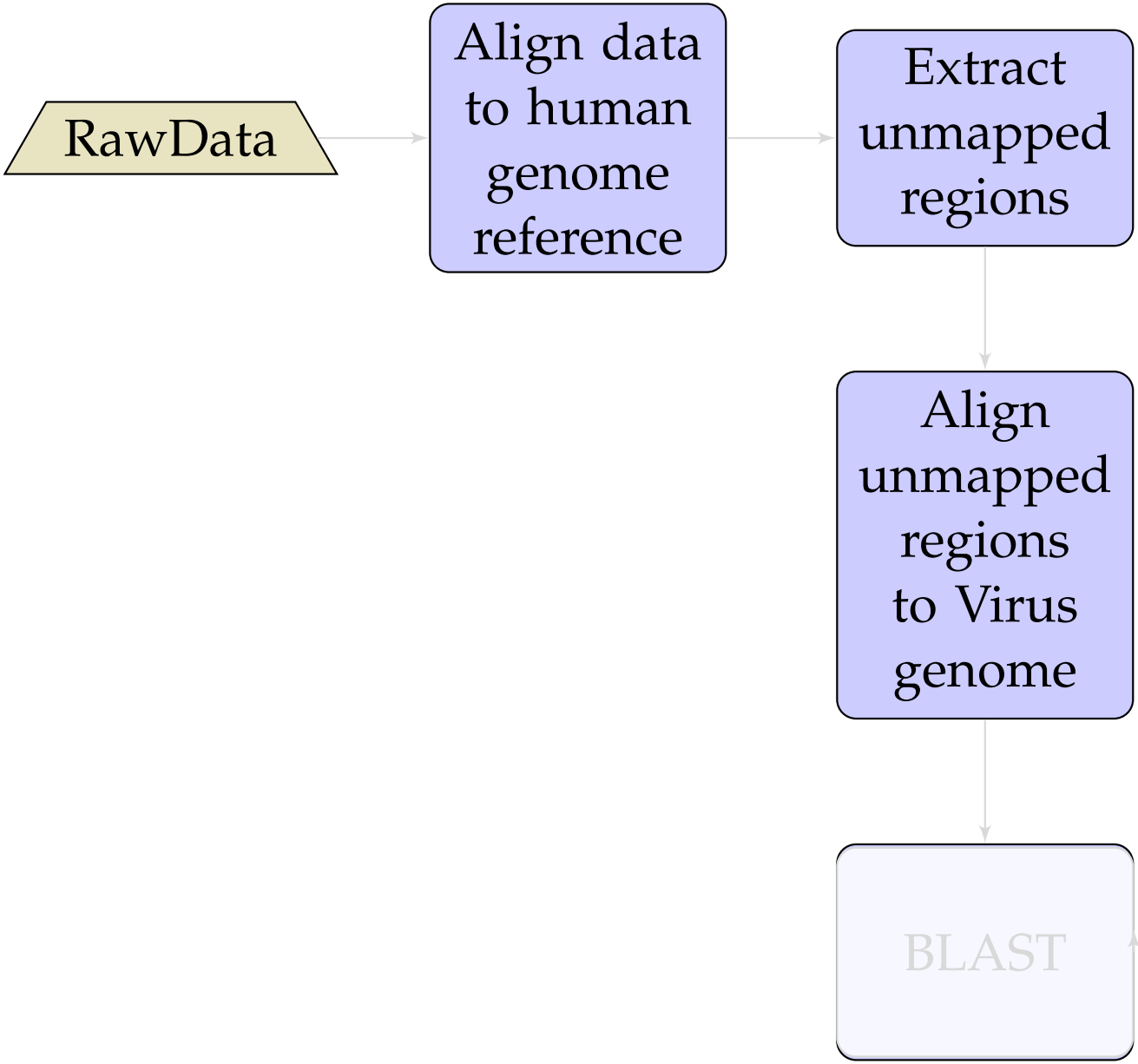
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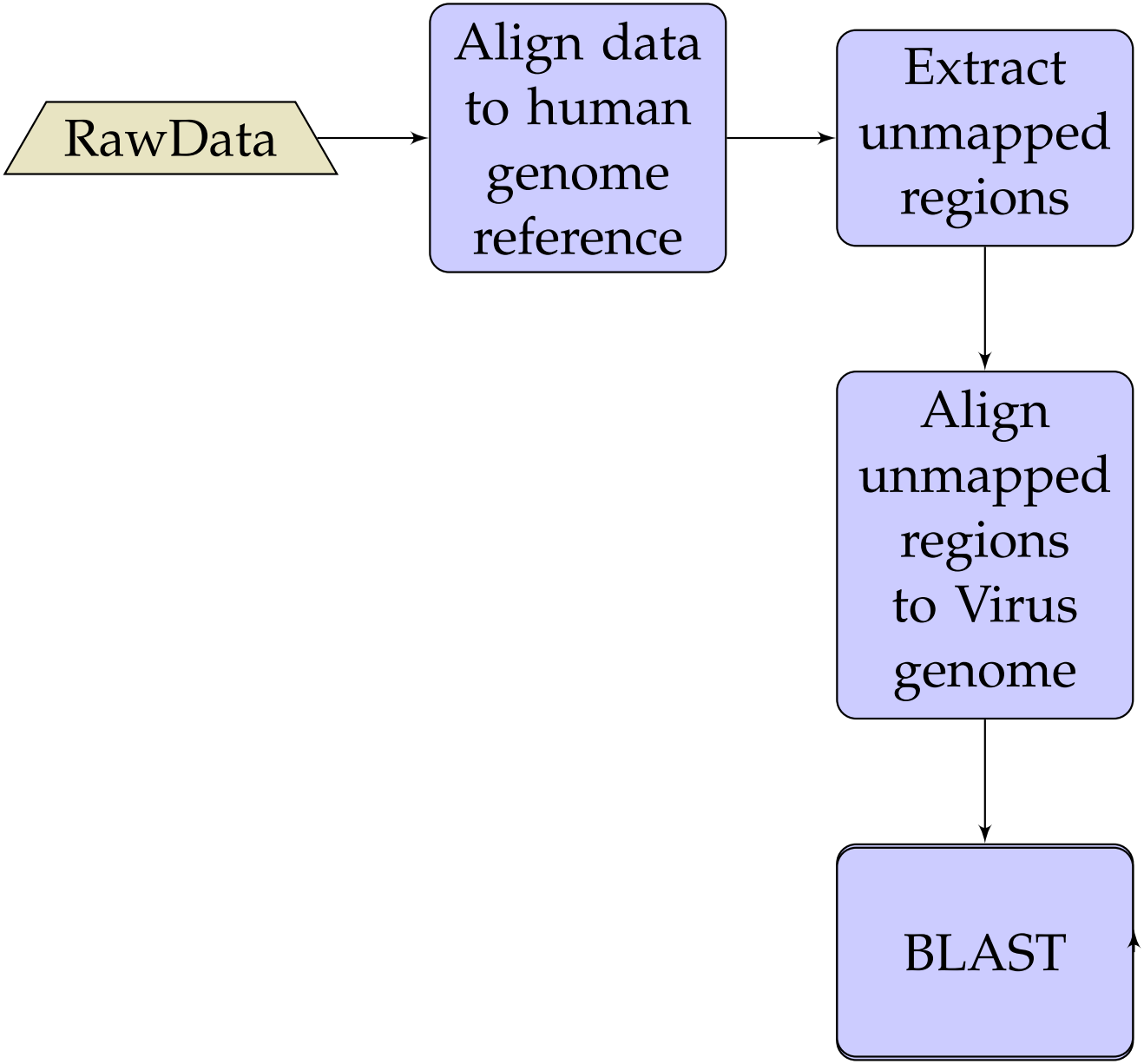
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GALAXY WORKFLOW

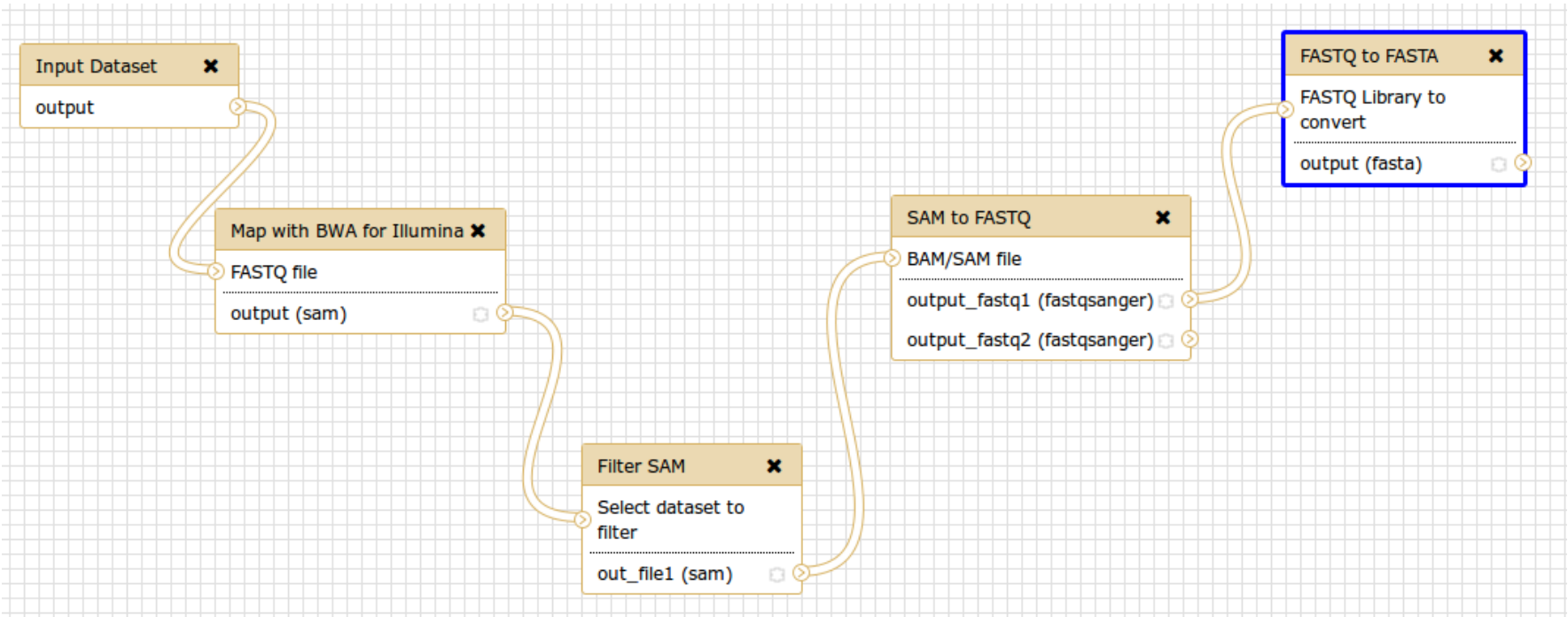
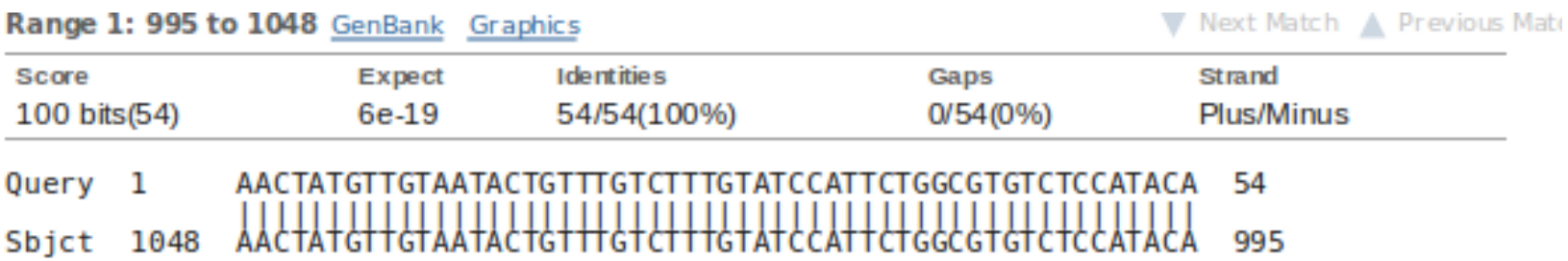


Figure: Aligned HPV genomes



Range 1: 995 to 1048 GenBank Graphics ▼ Next Match ▲ Previous Mat

Score	Expect	Identities	Gaps	Strand
100 bits(54)	6e-19	54/54(100%)	0/54(0%)	Plus/Minus

```
Query  1     AACTATGTTGTAATACTGTTTGTCTTTGTATCCATTCTGGCGTGTCTCCATACA  54
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1048  AACTATGTTGTAATACTGTTTGTCTTTGTATCCATTCTGGCGTGTCTCCATACA  995
```

REPRODUCIBILITY

- In pursuit of novel 'discovery', standardizing the data analysis pipeline is often ignored, leading to dubious conclusions
- Analysis should be reproducible and above all, correct
- Parameter's values can change the results by a big factor, they need to be documented/logged
- *Garbage in, Garbage out*

CONCLUSIONS

With the Galaxy tool box for identification of significant mutations and the study of the science behind the methods, the next steps would be to:

- **Open source the toolbox to the community**: A tool makes little sense if it is not in a usable form, community feedback will be used to add more tools and improve the existing ones
- **A new method for driver mutation prediction**: all the methods have low level of concordance. A new method that takes into account the available data at all levels : mutations, transcriptome and micro array data is possible. With the Galaxy toolbox in place, it would be possible to integrate information at various levels

FUTURE WORK

- Develop an algorithm that integrates machine learning approach with functional approach by zeroing down upon only those attributes that are *known* to have an impact
- The algorithm would also account for information at other levels: RNA expressions, Clinical data.
- Integrating information at all levels would provide a deeper insight
- The developed Galaxy toolbox will be used a the basic framework for integrating information

REFERENCES I

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