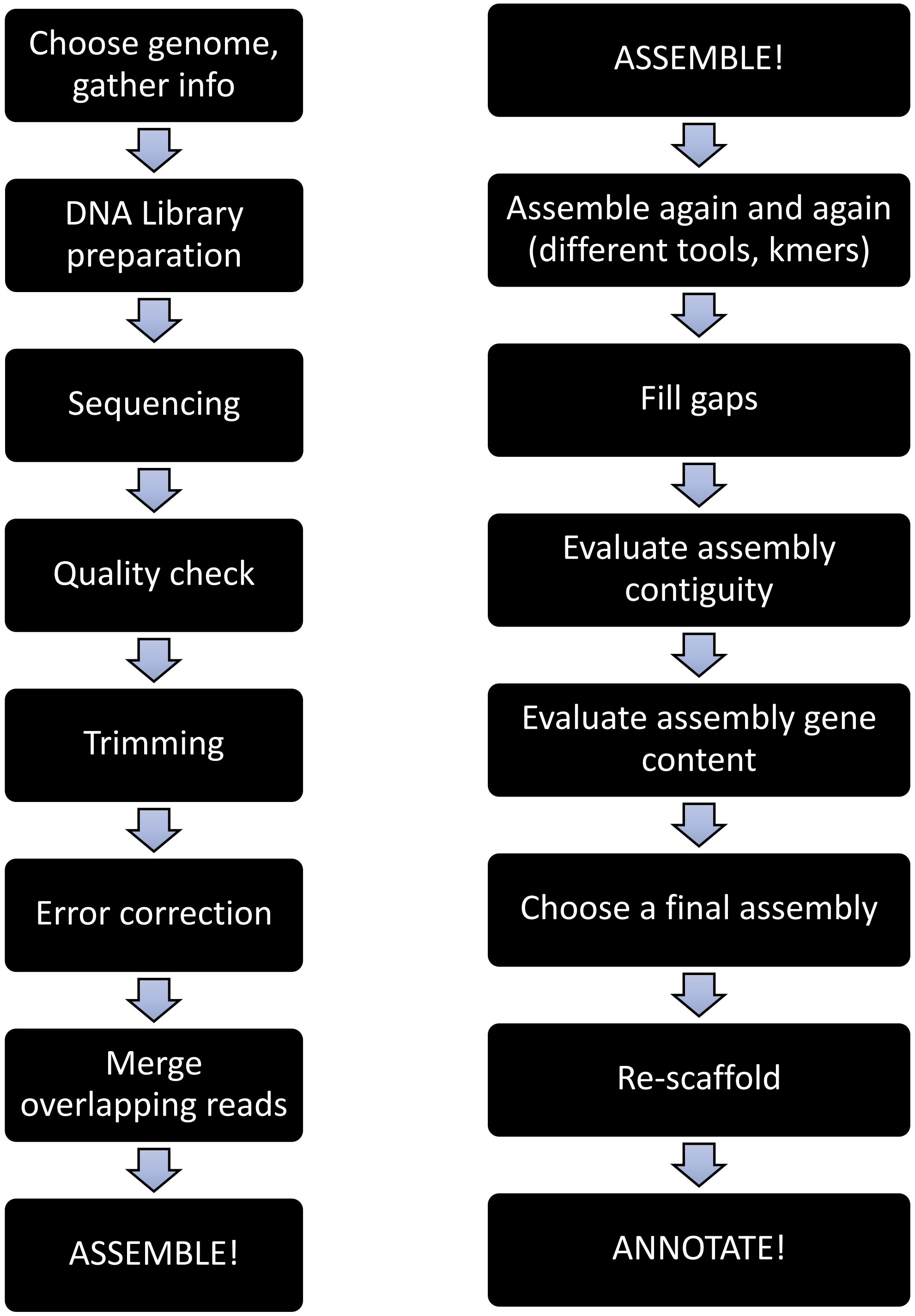
Genome Annotation



Choose genome, gather info

DNA Library preparation

Sequencing

Quality check

Trimming

Error correction

Merge overlapping reads

ASSEMBLE!

ASSEMBLE!

Assemble again and again (different tools, kmers)

Fill gaps

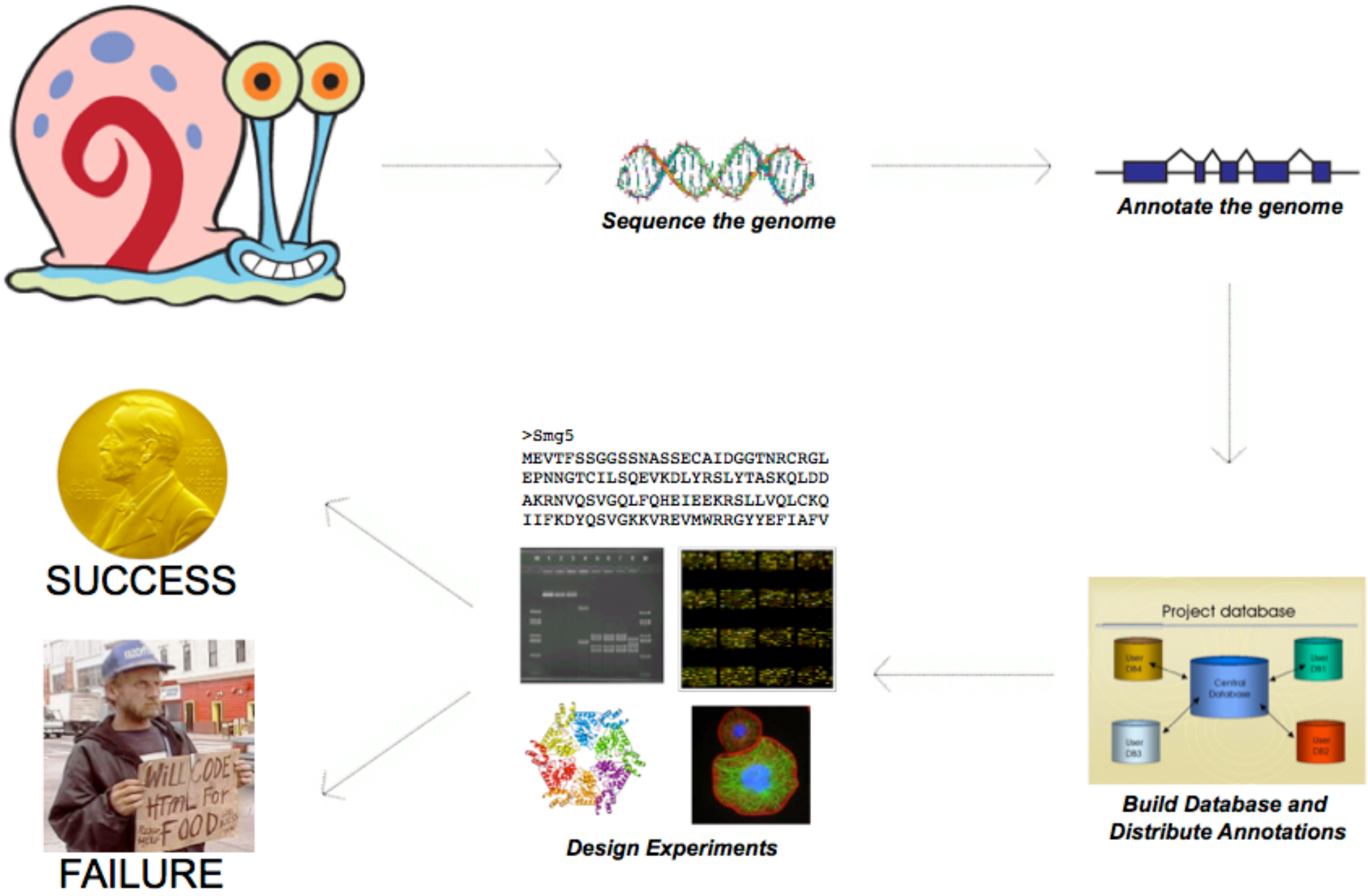
Evaluate assembly contiguity

Evaluate assembly gene content

Choose a final assembly

Re-scaffold

ANNOTATE!



Sequence the genome

Annotate the genome

Build Database and Distribute Annotations

```
>Smg5
MEVTFSSGGSSNASSECAIDGGTNRCRGL
EPNNGTCILSQEVKDLYRSLYTASKQLDD
AKRNVQSVGQLFQHEIEEKRSLLVQLCKQ
IIFKDYQSVGKKVREVMWRRGYYEFIAFV
```

Design Experiments

SUCCESS

FAILURE

http://weatherby.genetics.utah.edu/MAKER/wiki/index.php/MAKER_Tutorial

```
ACGGGTTCGCTACAGATGAACTGAATTTATACACGGACAACTCATCGCCCATTTGGGCGTGGCACCGCAGATCA
AAAGTGGCAGATTAGGAGTGCTTGATCAGGTTAGCAGGTGGACTGTATCCAACAGCGCATCAAACTTCAATAAAT
CCAAAGCGTTGTAGTGGTCTAAGCACCCCTGAACAGTGGCGCCCATCGTTAGCGTAGTACAACCCTTCCCCCTTG
AGGTGCGACATGGGGCCAGTTAGCCTGCCCTATATCCCTTGCACACGTTCAATAAGAGGGGCTCTACAGCGCCGC
TTTTTAAATTAGGATGCCGACCCCATCATTGGTAACTGTATGTTCATAGATATTTCTTCAGGAGTAATAGCGACA
AGCTGACACGCAAGGGTCAACAATAATTTCTACTATCACCCCGCTGAACGACTGTCTTTGCAAGAACCAACTGGG
CTTAGATTCGCGTCCTAACGTAGTGAGGGCCGAGTCATATCATAGATCAGGCATGAGAAACCGACGTCGAGTCTA
CACACGAGTTGTAAACAACTTGATTGCTATACTGTAGCTACCGCAAGGATCTCCTACATCAAAGACTACTGGGCG
```

A GENOME ASSEMBLY IS JUST A TEXT FILE IN FASTA FORMAT.

```
CTAGGTATTCACGCAACCGTCGTAACATGCACTAAGGATAACTAGCGCCAGGGGGGCATACTAGGTCCCGGAGCT
AAAGACTACCCTATGGATTCCTTGGAGCGGGGACAATGCAGACCGGTTACGACACAATTATCGGGATCGTCTAGA
GGTATTATTAGCAAGACAATAAAGGACATTGCACAGAGACTTATTAGAATTCAACAAACAGGATCATATCATGCG
GTGTTGGGTCGGGCAAGTCCCCGAAGCTCGGCCAAAAGATTCGCCATGGAACCGTCTGGTCCTGTTAGCGTGTAC
GCCTGCTCCTGTTCCGGGTACCATAGATAGACTGAGATTGCGTCAAAAAATTGCGGCGAAAATAGAGGGCTCCT
TGTAGAAATACCAGACTGGGGAATTTAAGCGCTTTCCACTATCTGAGCGACTAAACATCAACAAATGCGTCTACT
CGAATCCGCAGTAGGCAATTACAACCTGGTTCAGATCACTGGTTAATCAGGGATGTCTTCATAAGATTATACTTG
CCCCGACGCGACAGCTCTTCAAGGGGCCGATTTTTGGACTTCAGATACGCTAGAATTTAAAGGGTCTCTTACACC
TGCTGCGGCCTGCAGGGACCCCTAGAACTTGCCGCCTACTTGTCTCAGTCTAATAACGCGCGAAGCCGTGGGGCA
CGTGACCTTAAGTCGCAGAGCGAGTGATGAATTTGGGACGCTAATATGGGTGAATAGAGACTTATATCATCAGGG
```

```
ACGGGTTCGCTACAGATGAACTGAATTTATACACGGACAACTCATCGCCCATTTGGGCGTGGCACCGCAGATCA
AAAGTGGCAGATTAGGAGTGCTTGATCAGGTTAGCAGGTGGACTGTATCCAACAGCGCATCAAACTTCAATAAAT
CCAAAGCGTTGTAGTGGTCTAAGCACCCCTGAACAGTGGCGCCCATCGTTAGCGTAGTACAACCCTTCCCCCTTG
AGGTGCGACATGGGGCCAGTTAGCCTGCCCTATATCCCTTGCACACGTTCAATAAGAGGGGCTCTACAGCGCCGC
TTTTTAAATTAGGATGCCGACCCCATCATTGGTAACTGTATGTTCATAGATATTTCTTCAGGAGTAATAGCGACA
AGCTGACACGCAAGGGTCAACAATAATTTCTACTATCACCCCGCTGAACGACTGTCTTTGCAAGAACCAACTGGG
CTTAGATTCGCGTCCTAACGTAGTGAGGGCCGAGTCATATCATAGATCAGGCATGAGAAACCGACGTCGAGTCTA
CACACGAGTTGTAAACAACTTGATTGCTATACTGTAGCTACCGCAAGGATCTCCTACATCAAAGACTACTGGGCG
```

IT IS MUCH MORE INTERESTING TO HAVE AN ANNOTATION.

```
CTAGGTATTCACGCAACCGTCGTAACATGCACTAAGGATAACTAGCGCCAGGGGGCATACTAGGTCCCGGAGCT
AAAGACTACCCTATGGATTCCTTGGAGCGGGACAATGCAGACCGGTTACGACACAATTATCGGGATCGTCTAGA
GGTATTATTAGCAAGACAATAAAGGACATTGCACAGAGACTTATTAGAATTCAACAAACAGGATCATATCATGCG
GTGTTGGGTCGGGCAAGTCCCCGAAGCTCGGCCAAAAGATTCGCCATGGAACCGTCTGGTCCTGTTAGCGTGTAC
GCCTGCTCCTGTTCCGGGTACCATAGATAGACTGAGATTGCGTCAAAAAATTGCGGCGAAAATAGAGGGGCTCCT
TGTAGAAATACCAGACTGGGGAATTTAAGCGCTTTCCACTATCTGAGCGACTAAACATCAACAAATGCGTCTACT
CGAATCCGCAGTAGGCAATTACAACCTGGTTCAGATCACTGGTTAATCAGGGATGTCTTCATAAGATTATACTTG
CCCCGACGCGACAGCTCTTCAAGGGGCCGATTTTTGGACTTCAGATACGCTAGAATTTAAAGGGTCTCTTACACC
TGCTGCGGCCTGCAGGGACCCCTAGAACTTGCCGCCTACTTGTCTCAGTCTAATAACGCGCGAAGCCGTGGGGCA
CGTGACCTTAAGTCGCAGAGCGAGTGATGAATTTGGGACGCTAATATGGGTGAATAGAGACTTATATCATCAGGG
```

Annotation Goals

Identifying repeats

- Biologically interesting
- Technically very important. Repeats are often "masked" in downstream analyses to avoid false positives and other issues

Identifying protein coding genes

- Build inventory of genes
- Identify boundaries of introns, exons, promoters
- Predict mRNA structure (remember Central Dogma)

Identifying other regions

- Noncoding RNAs
- Promoters
- Cis-regulatory regions

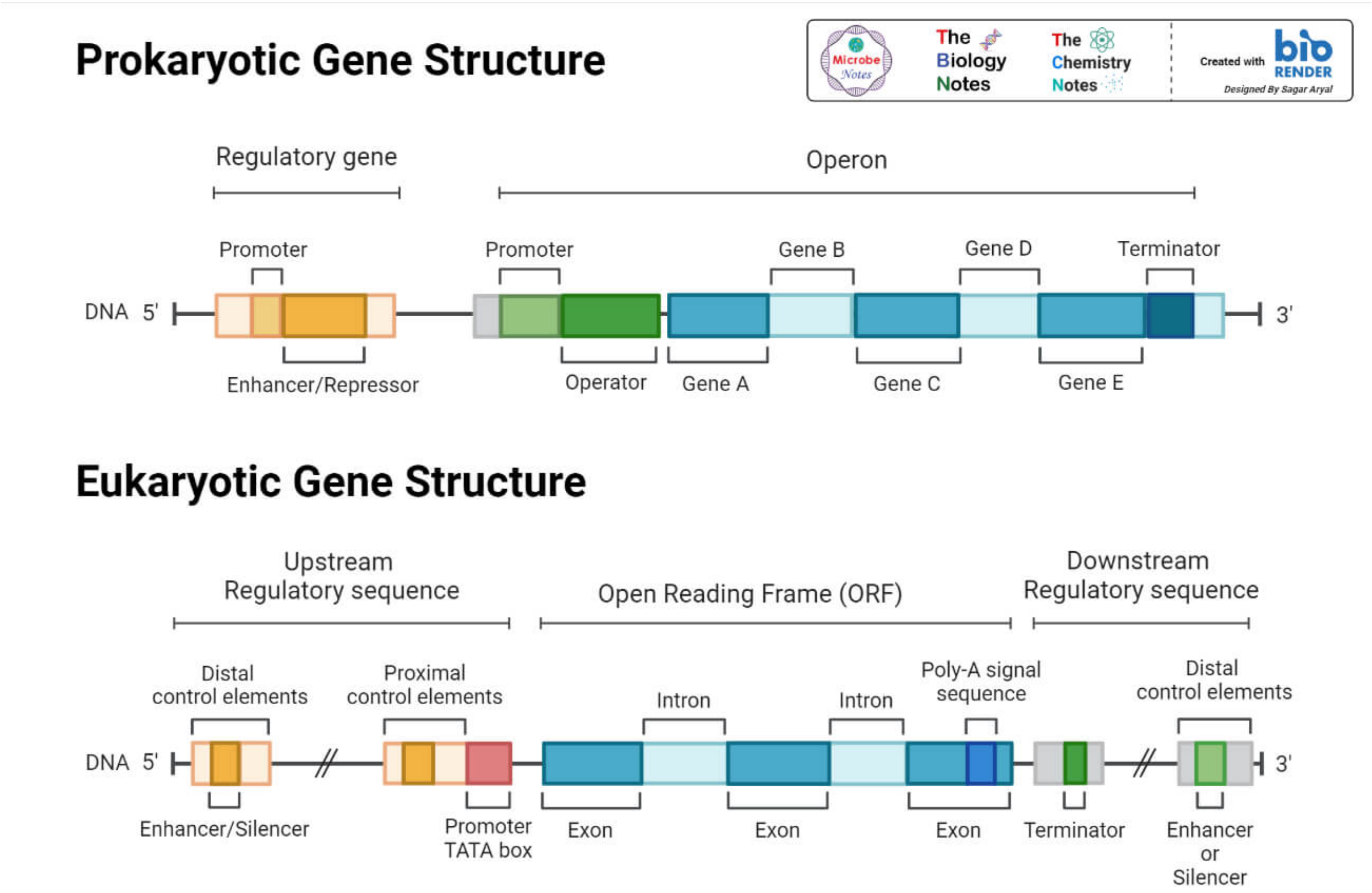
Understanding genome structure

- Centromeres
- Telomeres
- Mapping scaffolds to chromosomes

Community

- Creating a useful resource
- Output needs to be in a standard format

What is the stuff? *Where* is it?



Nidhi Abhay Kulkarni, *The Biology Notes*

BED Format

The first three fields in each feature line are required:

1. **chrom** - name of the chromosome or scaffold. Any valid seq_region_name can be used, and chromosome names can be given with or without the 'chr' prefix.
2. **chromStart** - Start position of the feature in standard chromosomal coordinates (i.e. first base is 0).
3. **chromEnd** - End position of the feature in standard chromosomal coordinates
4. **name** - Label to be displayed under the feature, if turned on in "Configure this page".
5. **score** - A score between 0 and 1000. See track lines, below, for ways to configure the display style of scored data.
6. **strand** - defined as + (forward) or - (reverse).
7. **thickStart** - coordinate at which to start drawing the feature as a solid rectangle
8. **thickEnd** - coordinate at which to stop drawing the feature as a solid rectangle
9. **itemRgb** - an RGB colour value (e.g. 0,0,255). Only used if there is a track line with the value of itemRgb set to "on" (case-insensitive).
10. **blockCount** - the number of sub-elements (e.g. exons) within the feature
11. **blockSizes** - the size of these sub-elements
12. **blockStarts** - the start coordinate of each sub-element

https://m.ensembl.org/info/website/upload/bed.html

BED Format

BED (9-column):

```
chr7  127471196  127472363  Pos1  0  +  127471196  127472363  255,0,0
chr7  127472363  127473530  Pos2  0  +  127472363  127473530  255,0,0
chr7  127473530  127474697  Pos3  0  +  127473530  127474697  255,0,0
chr7  127474697  127475864  Pos4  0  +  127474697  127475864  255,0,0
```

https://m.ensembl.org/info/website/upload/bed.html

GFF-3 Format

Fields **must** be tab-separated. Also, all but the final field in each feature line must contain a value; "empty" columns should be denoted with a '.'

1. **seqid** - name of the chromosome or scaffold; chromosome names can be given with or without the 'chr' prefix.
2. **source** - name of the program that generated this feature, or the data source (database or project name)
3. **type** - type of feature. Must be a term or accession from the SOFA sequence ontology
4. **start** - Start position of the feature, with sequence numbering starting at 1.
5. **end** - End position of the feature, with sequence numbering starting at 1.
6. **score** - A floating point value.
7. **strand** - defined as + (forward) or - (reverse).
8. **phase** - One of '0', '1' or '2'. '0' indicates that the first base of the feature is the first base of a codon, '1' that the second base is the first base of a codon, and so on..
9. **attributes** - A semicolon-separated list of tag-value pairs, providing additional information about each feature. Some of these tags are predefined, e.g. ID, Name, Alias, Parent

https://m.ensembl.org/info/website/upload/gff3.html

GFF-3 Format

GFF3:

```
##gff–version 3
ctg123 . mRNA            1300  9000  .  +  .  ID=mrna0001;Name=sonichedgehog
ctg123 . exon            1300  1500  .  +  .  ID=exon00001;Parent=mrna0001
ctg123 . exon            1050  1500  .  +  .  ID=exon00002;Parent=mrna0001
ctg123 . exon            3000  3902  .  +  .  ID=exon00003;Parent=mrna0001
ctg123 . exon            5000  5500  .  +  .  ID=exon00004;Parent=mrna0001
ctg123 . exon            7000  9000  .  +  .  ID=exon00005;Parent=mrna0001
```

https://m.ensembl.org/info/website/upload/gff3.html

Annotations can be made

With ***ab initio*** methods based on the understanding of particular properties of different genome features

Based on direct evidence, such as RNA-seq

Based on comparison to a reference of similar sequences

- Looking for repetitive DNA elements
- **Blasting** known protein coding genes

BLAST – Basic Local Alignment Search Tool

Requirements

- Query (sequence you want to identify)
- Database (reference)

Local alignment

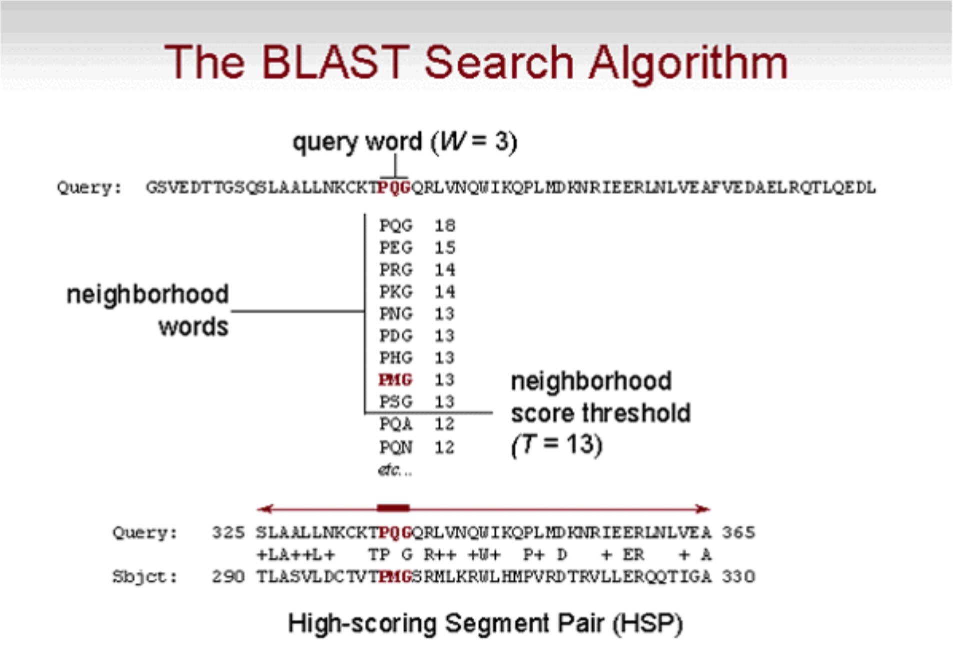
- proteins are modular, genes contain exons and introns
- versus global alignment

Neighborhood

- BLAST considers exact words, but also similar ones according to BLOSUM26 matrix
- these are aligned and then *extended*
- cumulative score is tallied

Goal

- when score drops significantly, extension is trimmed
- this results in the high scoring segment pair



```
Query:  GSVEDTTGSQSLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVEDAELRQTLQEDL
```

```
Query:  325 SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA 365
            +LA++L+    TP G R++ +W+   P+ D    + ER    + A
Sbjct:  290 TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA 330
```

BLAST is used for many things.

BLAST searching is fundamental to understanding the relatedness of any favorite query sequence to other known proteins of DNA sequences.

Applications include:

- identifying orthologs and paralogs
- discovering new genes and proteins
- discovering variants of genes or proteins
- investigating expressed sequence tags (ESTs)
- exploring protein structure and function

Types of BLAST searches

blastn (nucleotide BLAST) -> DNA query to DNA database

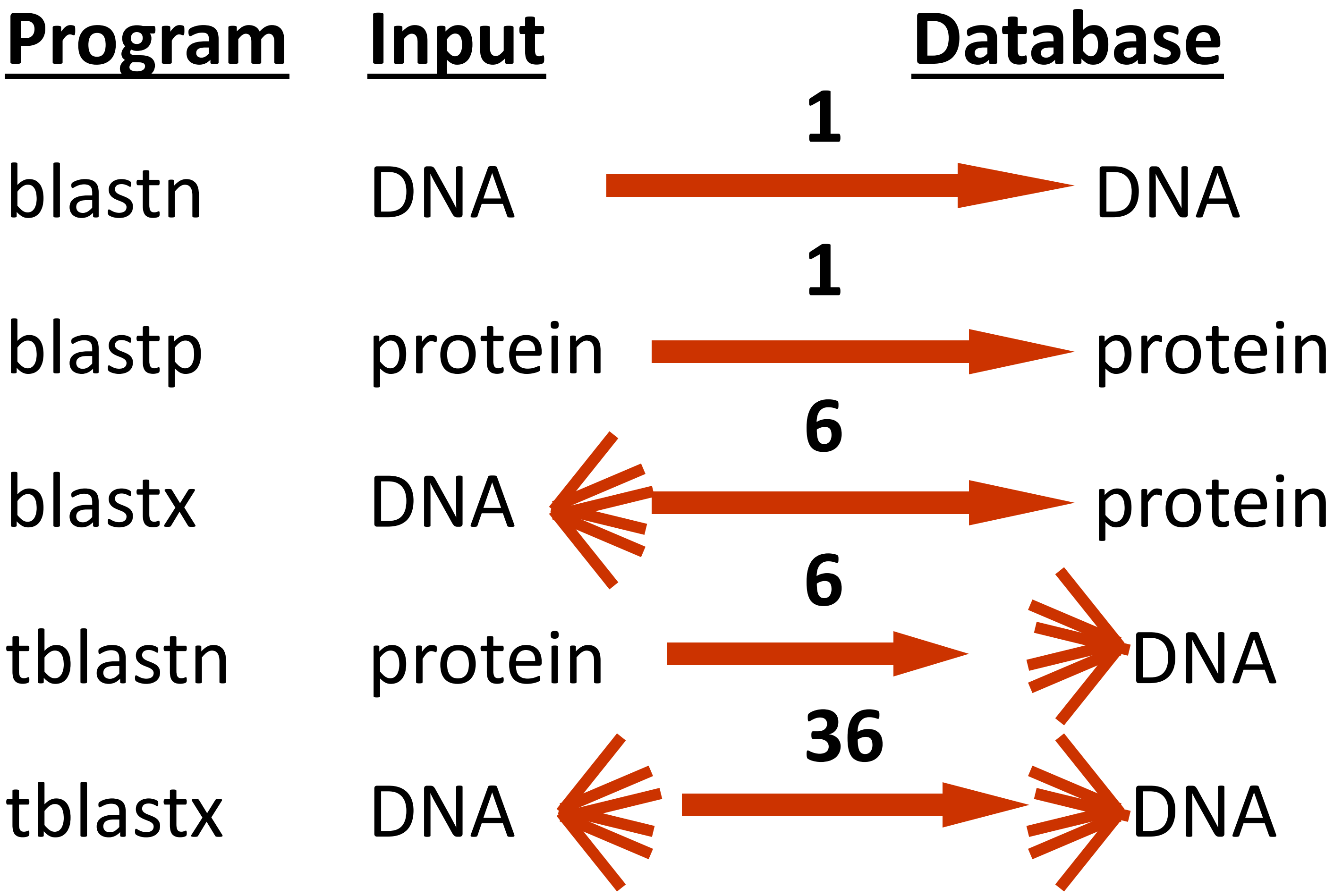
blastp (protein BLAST) -> protein query to protein database

blastx (translated BLAST) -> DNA query to protein database

tblastn (translated BLAST -> protein query to DNA database

tblastx (translated BLAST) -> translated DNA query to translated DNA database

Choose a BLAST program based on your needs



Program	**Input**		**Database**
blastn	DNA	**1**	DNA
blastp	protein	**1**	protein
blastx	DNA	**6**	protein
tblastn	protein	**6**	DNA
tblastx	DNA	**36**	DNA

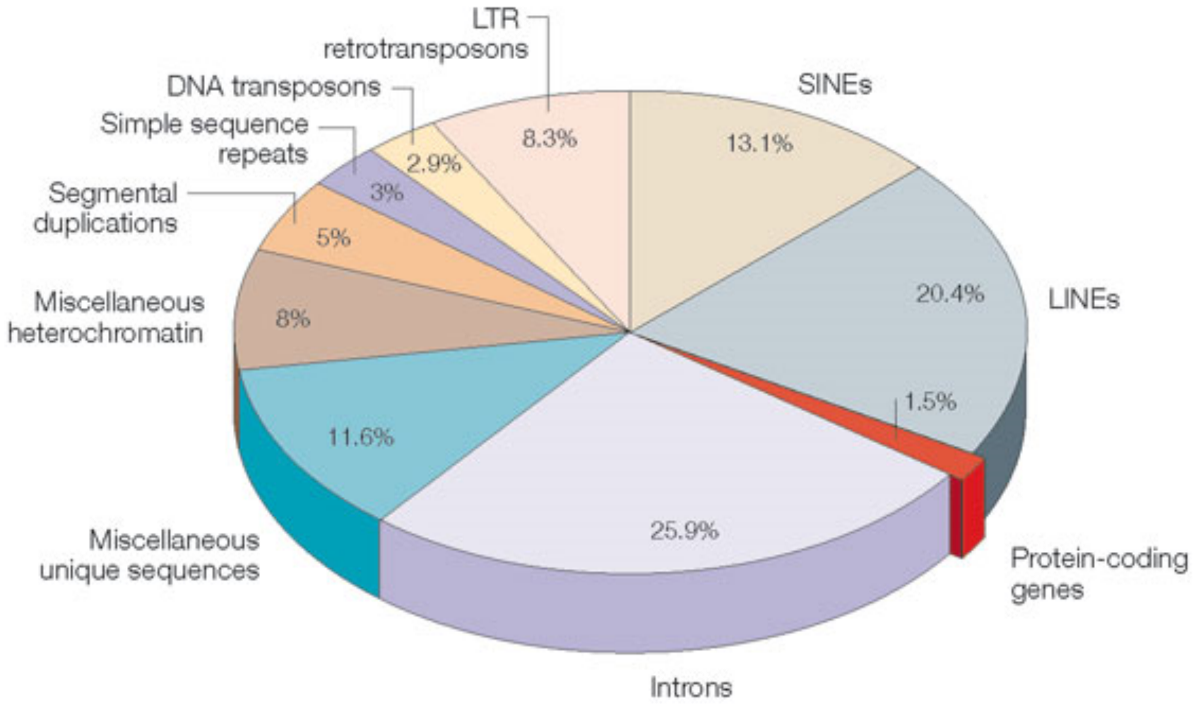
Repetitive DNA

Tandem repeats

- Adjacent along the chromosome
- Satellite DNA
  - minisatellites – 10-60bp
  - microsatellites - <10bp

Interspersed repeats

- Transposable elements



Nature Reviews | Genetics

Repeat Masking

Essential step before gene annotation

Many repeats contain ORFs, can be mistaken as genes.

Repeats should be “masked” before you try to annotate genes

- “NNNNN” = hardmasked
- “atcg” = softmasked
    - Better for downstream BLAST or genome alignment

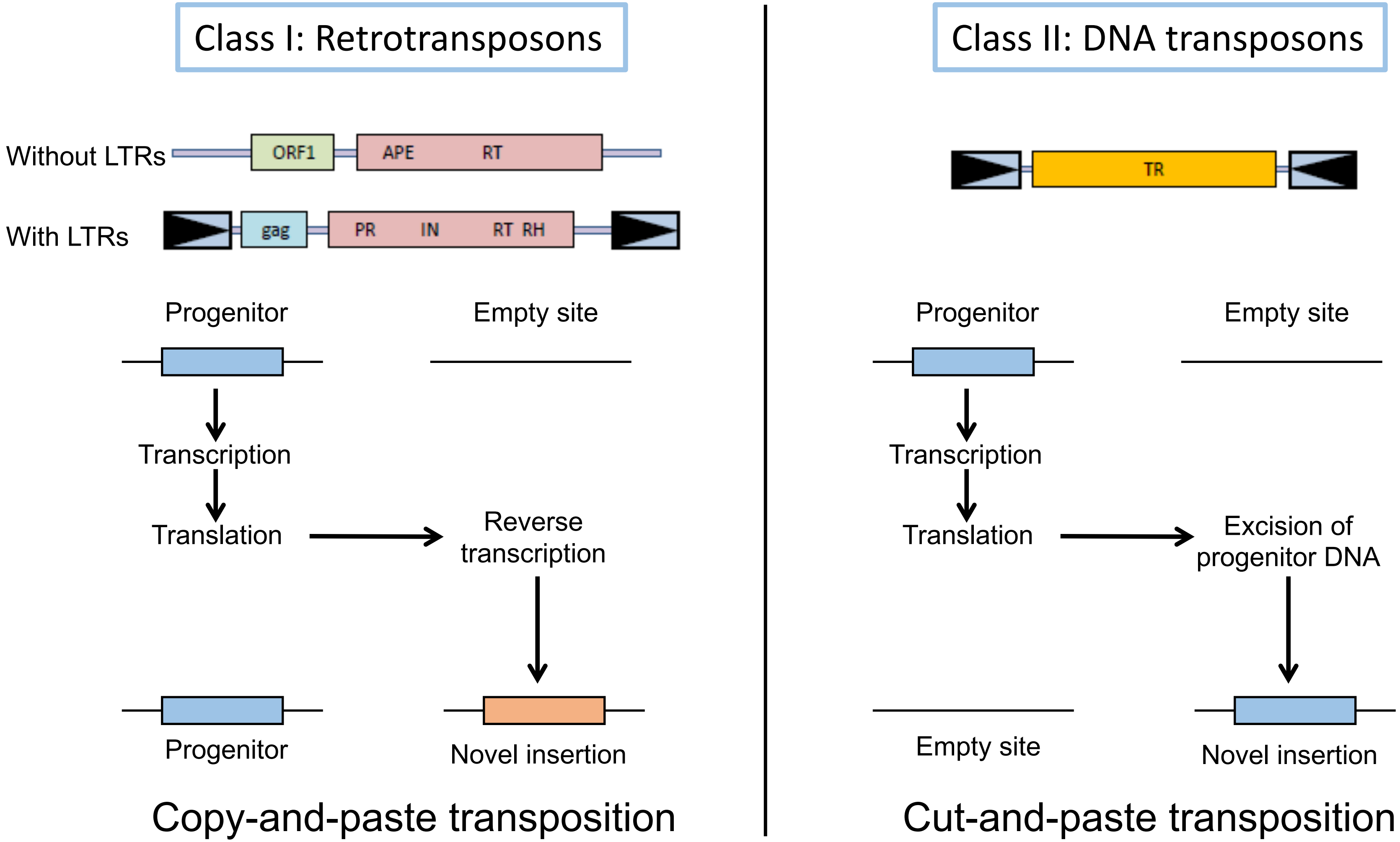
Two Methods of Repeat Finding

- ***Database method***
  - RepeatMasker (repeatmasker.org)
    - Blast RepBase elements to your genome
    - Good for mammals or model organisms
    - Ascertainment bias – species have unique repeats
      - Human: 50% masked with “homo sapiens” repeats.
      - Humpback whale: 38% masked with “mammalia” repeats.
      - Glass lizard: 13% masked with “vertebrate” repeats
- ***De novo method***
  - RepeatModeler (repeatmasker.org)
    - Blast your genome to itself
    - Models repeats without *a priori* knowledge
    - Good for finding species-specific repeats
    - May miss low-copy number repeats

What are Transposable Elements?

- DNA sequences that move about the genome
- Transposition
- “jumping genes”
- “junk DNA”, “selfish genes”

Types of transposable elements and their mode of transposition



Class I: Retrotransposons

Without LTRs

With LTRs

Progenitor

Empty site

Transcription

Translation

Reverse transcription

Progenitor

Novel insertion

Copy-and-paste transposition

Class II: DNA transposons

Progenitor

Empty site

Transcription

Translation

Excision of progenitor DNA

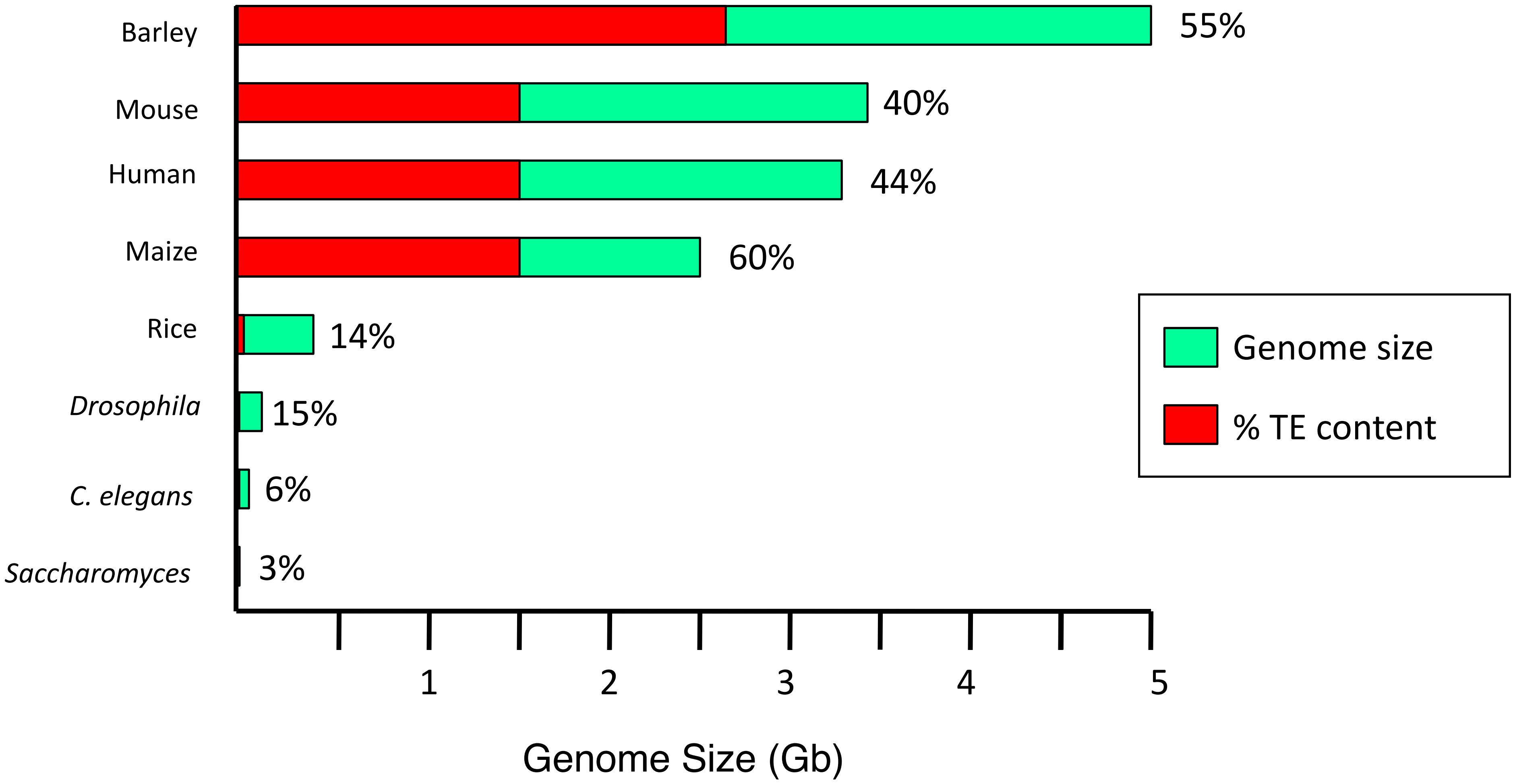
Empty site

Novel insertion

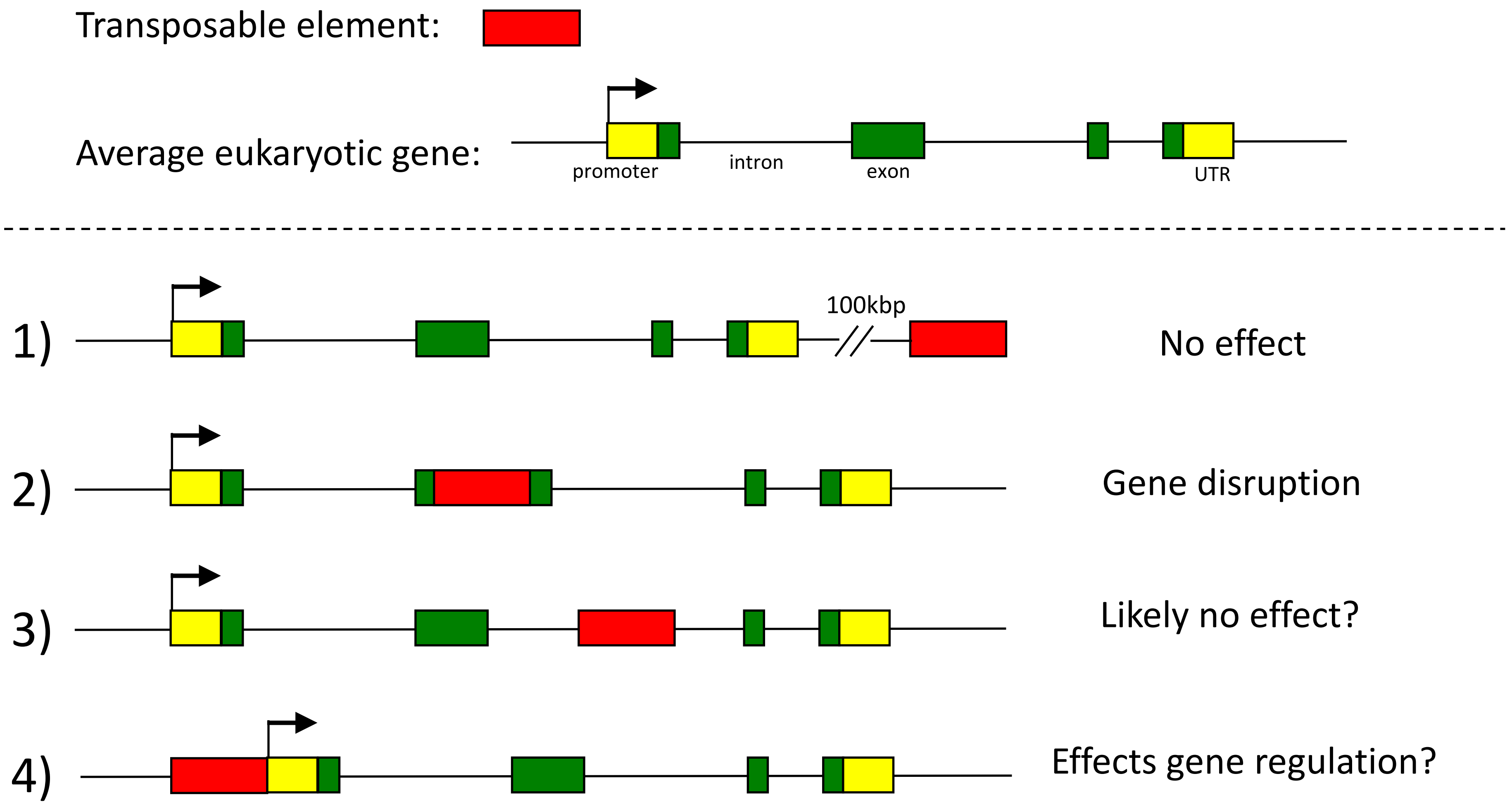
Cut-and-paste transposition

Tollis and Boissinot. ***The evolutionary dynamics of transposable elements in eukaryote genomes.*** 2012. *Genome Dynamics.*

Large genomes have a lot of transposable elements



TEs Effect Can Affect Gene Function and Regulation



Transposable element: 🟥

Average eukaryotic gene:

1) No effect

2) Gene disruption

3) Likely no effect?

4) Effects gene regulation?

Examples of Human Disease Caused by TE Mutation

	Gene	Disorder	Element	Mechanism
Alu	NF1	Neurofibromatosis	Alu Ya5	Intron/skipping
	BCHE	Acholinesterasemia	Alu Yb8	Exon insertion
	F9	**Hemophilia B**	Alu Ya5	Exon insertion
	CASR	Familial hypocalciuric hypercalemia	Alu Ya4	Exon insertion
	ADD1	**Huntington's disease**	Alu	Exon insertion
LINE-1	Factor VIII	**Hemophilia A**	L1	Exon insertion
	APC	FAP	L1	Exon insertion
	Dystrophin	**Muscular Dystrophy**	L1	Exon insertion
	Globin	Beta thalassemia	L1	Intron
	RP2	Retinitis Pigmentosis	L1	Intron
	Fukutin	**Muscular Dystrophy**	L1	Intron/skipping

Mills et al. (2007) *Trends Genet.* 23:183-91

Next

- We will discuss a beginner’s guide to genome annotation (Yandell and Ence 2012).

- We will have a computational lab on repeatmasking.

- Genome Annotation 2 will extend into gene-finding techniques.