Lecture7: Chromosome and Genome

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1. Lessons, questions and answers

Lesson 1:

Under endosymbiosis, mobile elements in endosymbionts became transposons in chromosomes which later lead to the advent of RNA splicing. However, primary mRNA wasn't supposed to be translated due to some non-coding information, such as transposons, in it. Hence, it was necessary for the separation between ribosomes and primary mRNAs. Nowadays, we can see that the ancestor created nuclear envelope to achieve the goal. Besides, some retrotransposons without LTR added at the terminals of chromosomes, subsequently, made telomeres.

Lesson 2:

In chromosomes, DNA sequences which cannot be expressed contain introns, repeated sequences, non-coding RNAs(tRNA, rRNA, snRNA, miRNA, long non-coding RNA), transposons, and psuedogenes. Among these DNA sequences not expressed, transposons were, in effect, not only ubiquitous for its appearance from bacteria to human beings, but found to jump from one locus to another. In terms of mechanisms, they can be categorized into two classes, Class I elements and Class II elements. In Class I elements, long terminal repeats(LTR) existed and acted as well functional promoters which strongly prompted RNA pol II to transcribe the contents of Class I elements. The mRNAs of Class I elements were then retrotranscribed by reverse transcriptase, so the new DNA sequences of Class I elements were produced. Finally, these new DNA sequences of Class I elements would be integrated into the new positions on chromosomes by integrase. The illustration of Class I elements was shown in Fig. 1. As for Class II elements, instead of LTR, terminal inverted repeats(TIR) existed. Besides, compared to Class I elements, Class II elements lacked the process of reverse transcription. In contrast, they underwent excision and were, subsequently, integrated into the new positions on chromosomes by transposase. The illustration of Class II elements was also shown in Fig. 1; the illustration of excision was shown in Fig. 2.

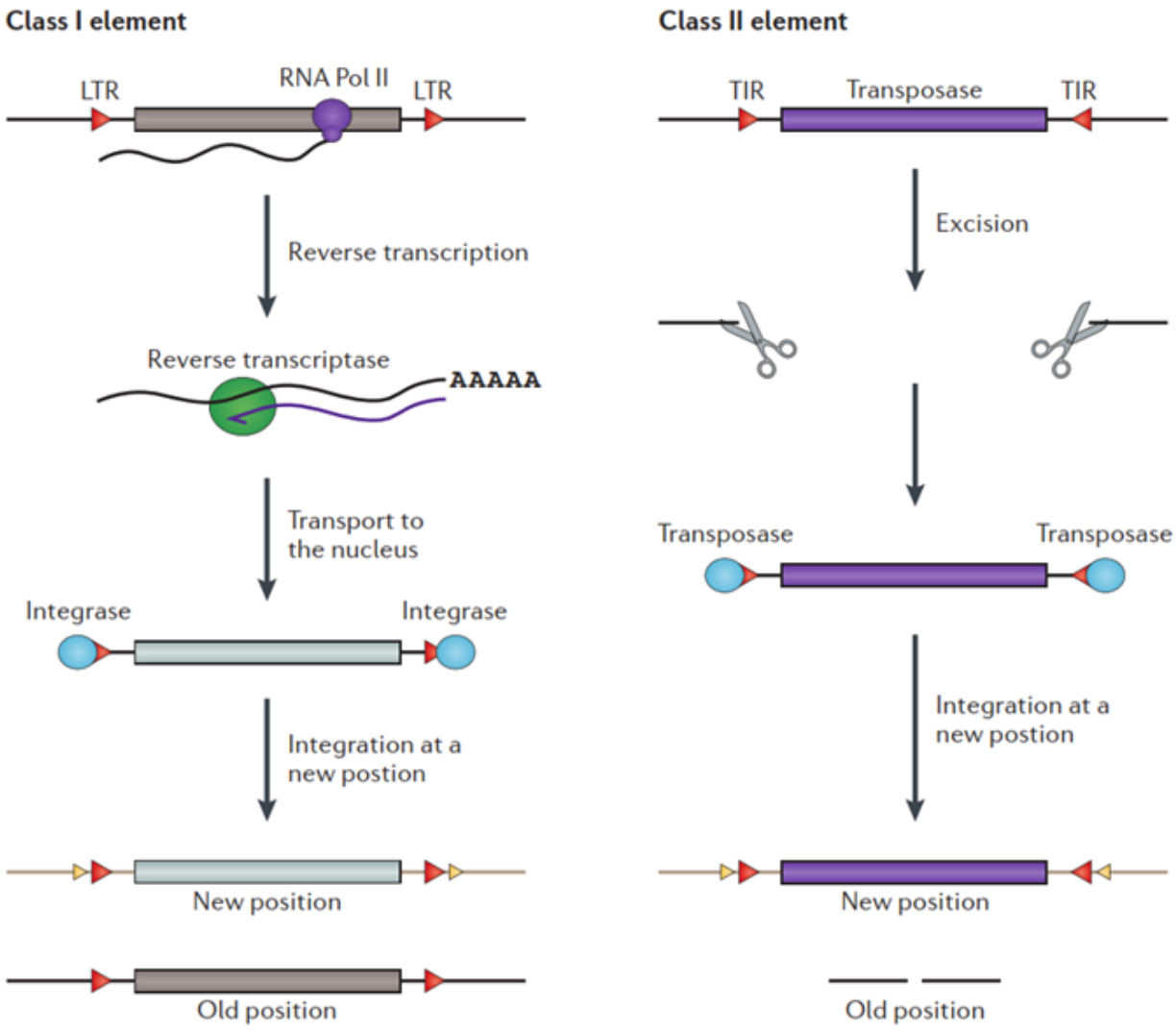


Figure 1. Mechanism of Class I elements and Class II elements(excerpted from teacher's slide)

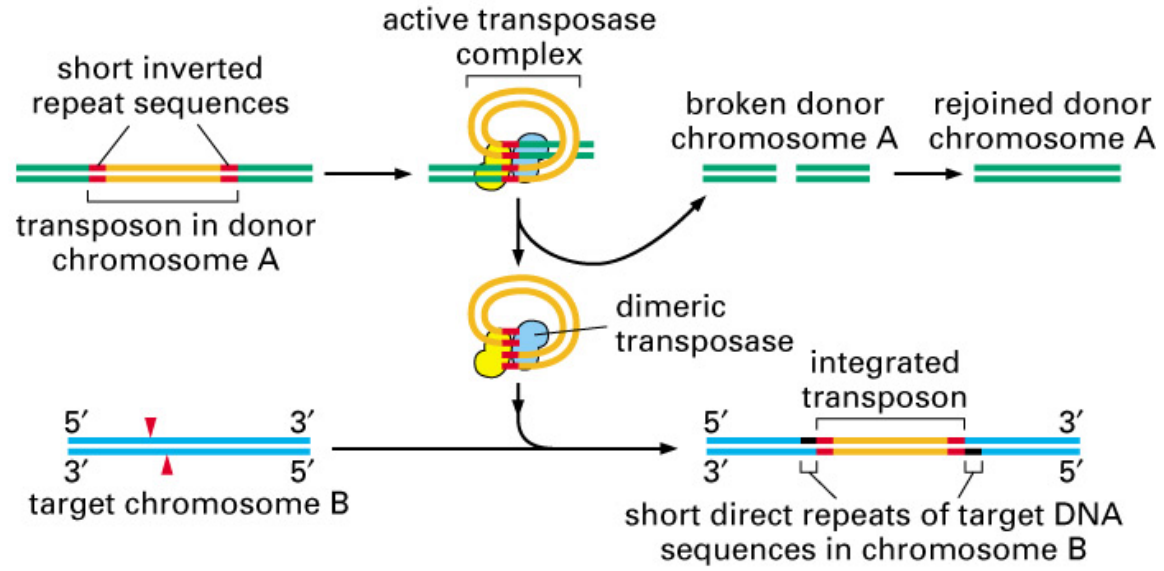


Figure 5–70. Molecular Biology of the Cell, 4th Edition.

Figure 2. Mechanism of excision(excerpted from teacher's slide)

Question 1:

Is it possible to utilize the mechanism of transposable elements as a tool of gene editing?

Answer 1:

Lesson 3:

Alu is the most prevalent short interspersed element(SINE), equaling about 11% of the genome; *Alu* and other SINEs are all "defective" transposons, so they entirely depend on the enzymes of other transposons, like L1, for mobility. Besides, *Alu* elements also encode no protein, for having no open reading frames(ORFs). As far as their composition is concerned, *Alu* elements are characterized by a sequence with two G/C rich regions: the left(L-*Alu*) and the right(R-*Alu*) monomers, both of which are connected by an A-rich linker. Furthermore, every *Alu* element ends with a poly-A tail, and is, respectively and immediately, flanked by A+T rich sequences.

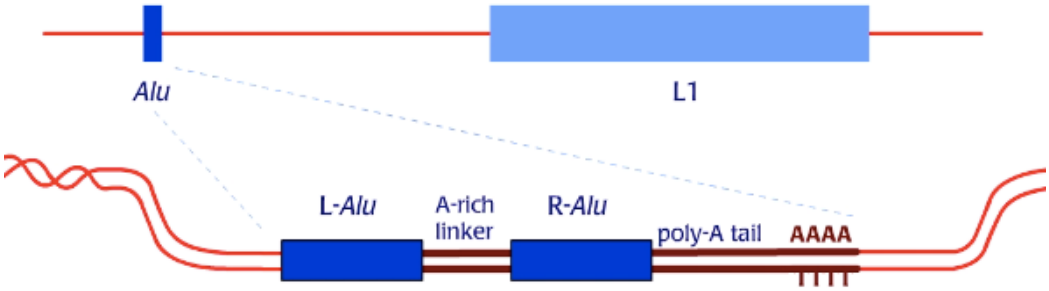
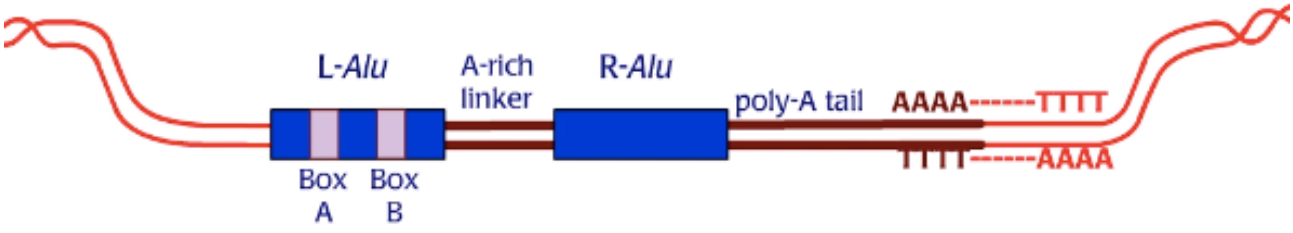


Figure 3. an *Alu* element in genome[1]

In L-*Alu* region, two specific features, Box A and Box B, exist and also act as the binding sites for transcription factors and RNA polymerase III. In the process of transcription, its halt occurs while RNA polymerase III meets the stretch of T's in the genome. Subsequently, a reverse transcriptase(rt), encoded by L1, makes the *Alu* RNA a template for the formation of a new *Alu* element that can insert itself in a new genome location.



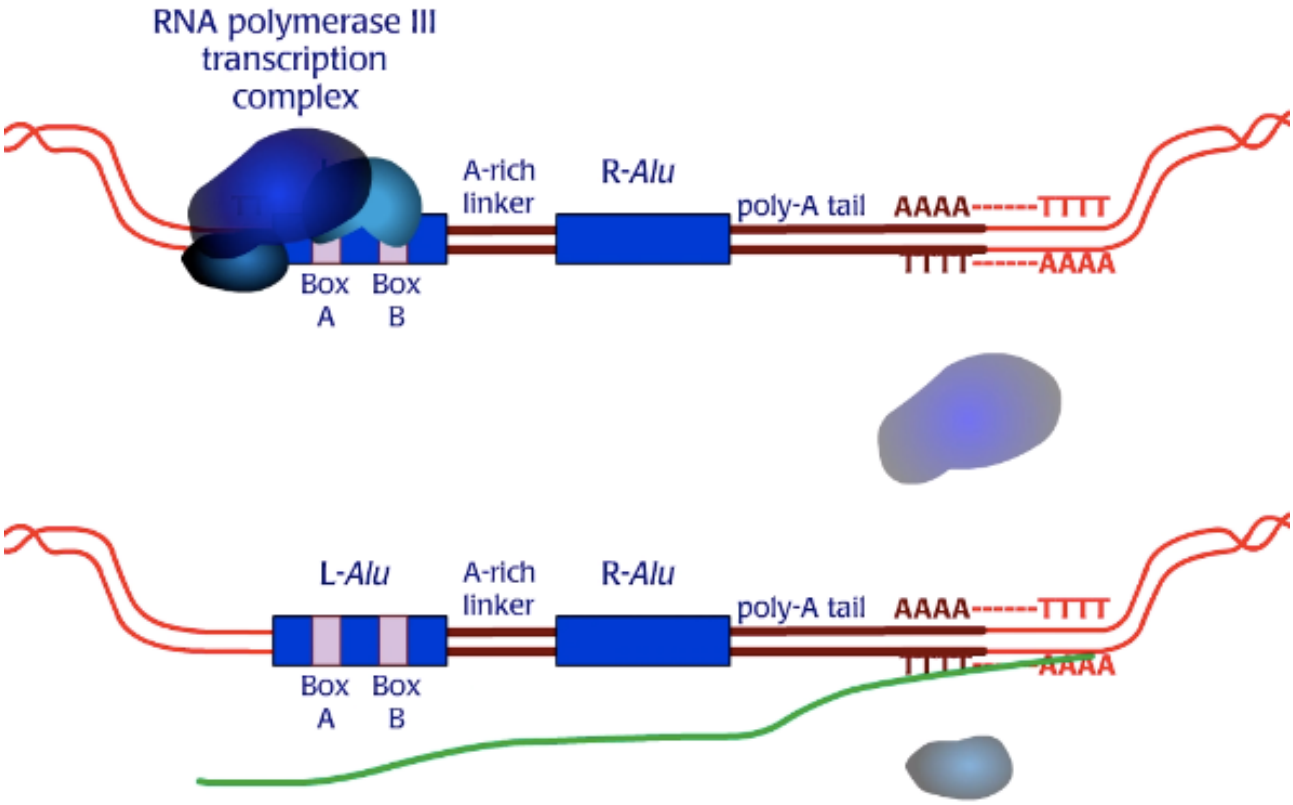


Figure 4. Transcription of an *Alu* element[1]

At the outset of reverse transcriptoin, L1 rt has the unique ability to nick DNA in a site-preferential manner. Most often the nick is made at the consensus sequence TTAAAA. This can create a single-stranded sequence of Ts that hydrogen bonds with the poly-A tail of the *Alu* RNA to form a short RNA/DNA heteroduplex. The hereroduplex later can serve as a primer for L1 rt to synthesize a new complementary DNA strand.

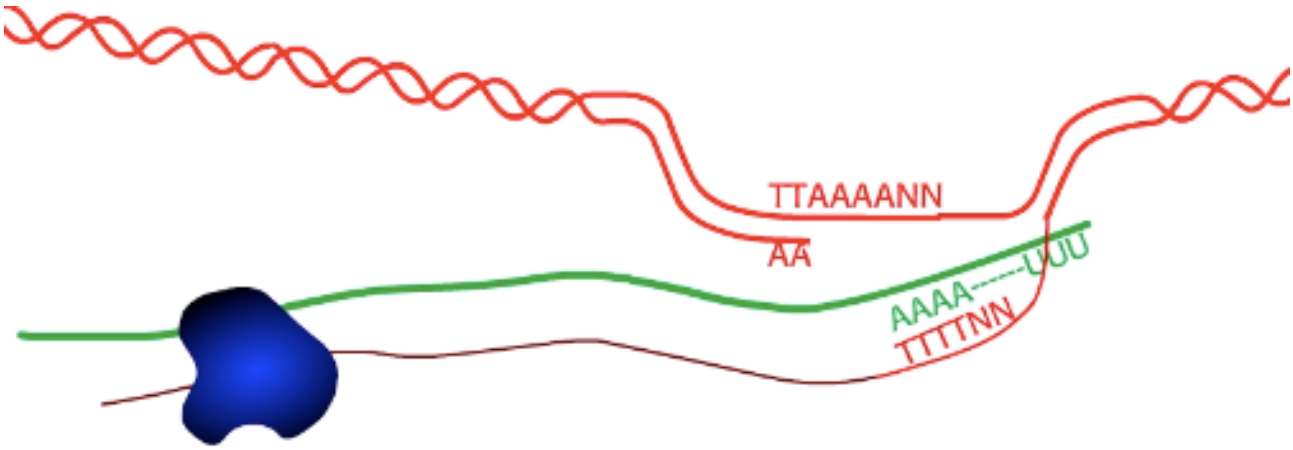


Figure 5. Reverse transcription of *Alu* RNA[1]

A second nick is then made on the opposite strand, a variable distance from the initial cleavage site. Either the L1 rt or a cellular DNA polymerase, likewise, synthesizes the second DNA strand. This results in an *Alu* element inserted into a novel position of the genome. The insertion also creates a direct repeat sequence on either side if the element.

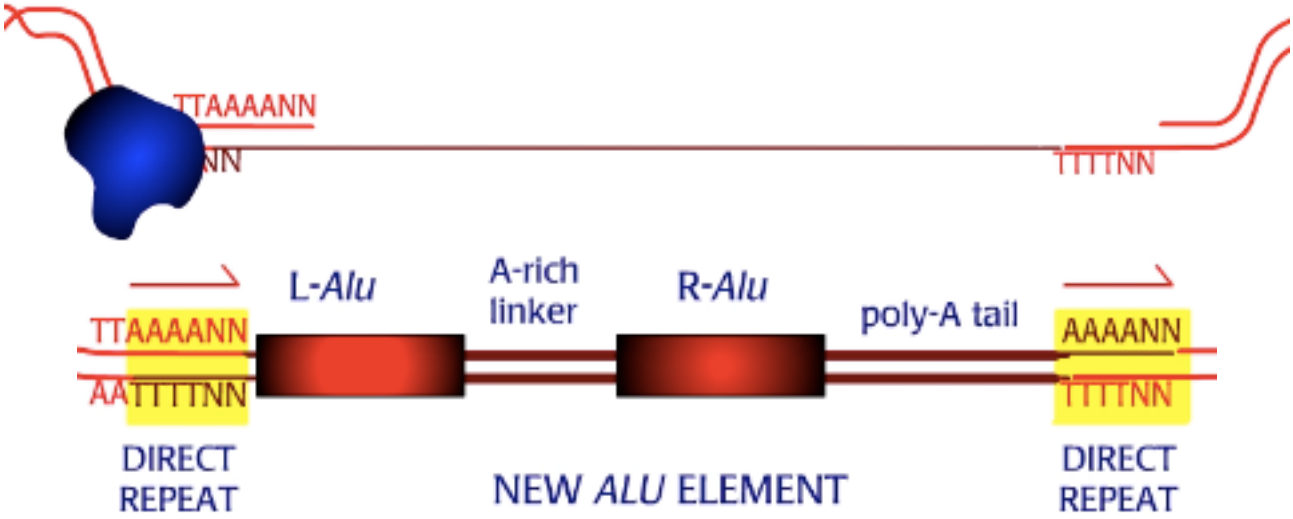


Figure 6. Formation of new *Alu* element[1]

Lesson 4:

Regulation in gene activity and expression is not only dependent upon transcription factor, promoter and something highly related to only DNA sequences, but also about modification on base pairs, histones…and so on. It was a novel subject, called epigenetics. Due to its complexity, I genuinely have no idea about how to introduce it any more.

2. Answer to quiz

(1) 作 Test cross 的目地是什麼? 怎麼作?

Test cross的目的在於確認目標個體之特定基因之基因型，透過將目標個體與該特定基因為隱性之個體交配，並觀察子代之表現型與每個表現型之個數，便能確定目標個體之該基因之基因型。

(2) 單細胞的酵母菌很適合作為 model organism to study how cell division is regulated. 設計一個實驗去找出會影響 cell division 的 gene mutation.

實驗步驟：

a. 形成變異：將突變因子加入test和control酵母菌。

b. 標記所有基因體：只將test培養在含有^{33}P的培養液持續一個世代。

c. 稀釋非目標基因體：將test和control培養在含有^{32}P的培養液數個世代。

d. 純化基因體：將test和control以離心分離出基因體。

e. 分離基因體：將前個步驟所得的samples進行agarose electrophoresis。

f. 獨立目標基因體：將test中和control不同的bands取出。

g. 確定突變基因：先透過PCR擴大樣本的量，再用DNA sequencing確定序列，最後將上個步驟中的樣本和wildtype的全部序列比較。

將以上步驟重複數遍，最常在g. 步驟被發現出問題的基因最可能是與cell division有關的基因。(以上步驟見Fig. 7)

解釋：

由於有cell division related gene mutation的個體與沒有這個mutation的個體最大的差別在於前者會停留在cell cycle的G2而不會分裂，後者則會不斷地進行著cell cycle。因此，有cell division related gene mutation的個體中會一直保持著原先保有的基因體，而沒有cell division related gene mutation的個體則會不斷地產生新的基因體。透過這個特性，我以步驟a., b., c. 標記出含有目標基因突變個體的所有基因體。

至於如何確定是哪一段基因突變造成cell division受到影響，我首先利用含^{33}P基因體比含^{32}P基因體還重，進而導致在agarose electrophoresis時會被分離的特性(步驟d., e.)，順利地將test中「不含有目標基因突變個體的所有基因體」與「含有目標基因突變個體的所有基因體」分離(步驟f.)。最後，再透過比對「含有目標基因突變個體的所有基因體」與wildtype的所有基

因體，我們可以得到可能的基因突變(步驟g.)，將重複實驗數次後，我們便幾乎能說每此都有出現的突變的基因非常可能正是影響cell division的基因。

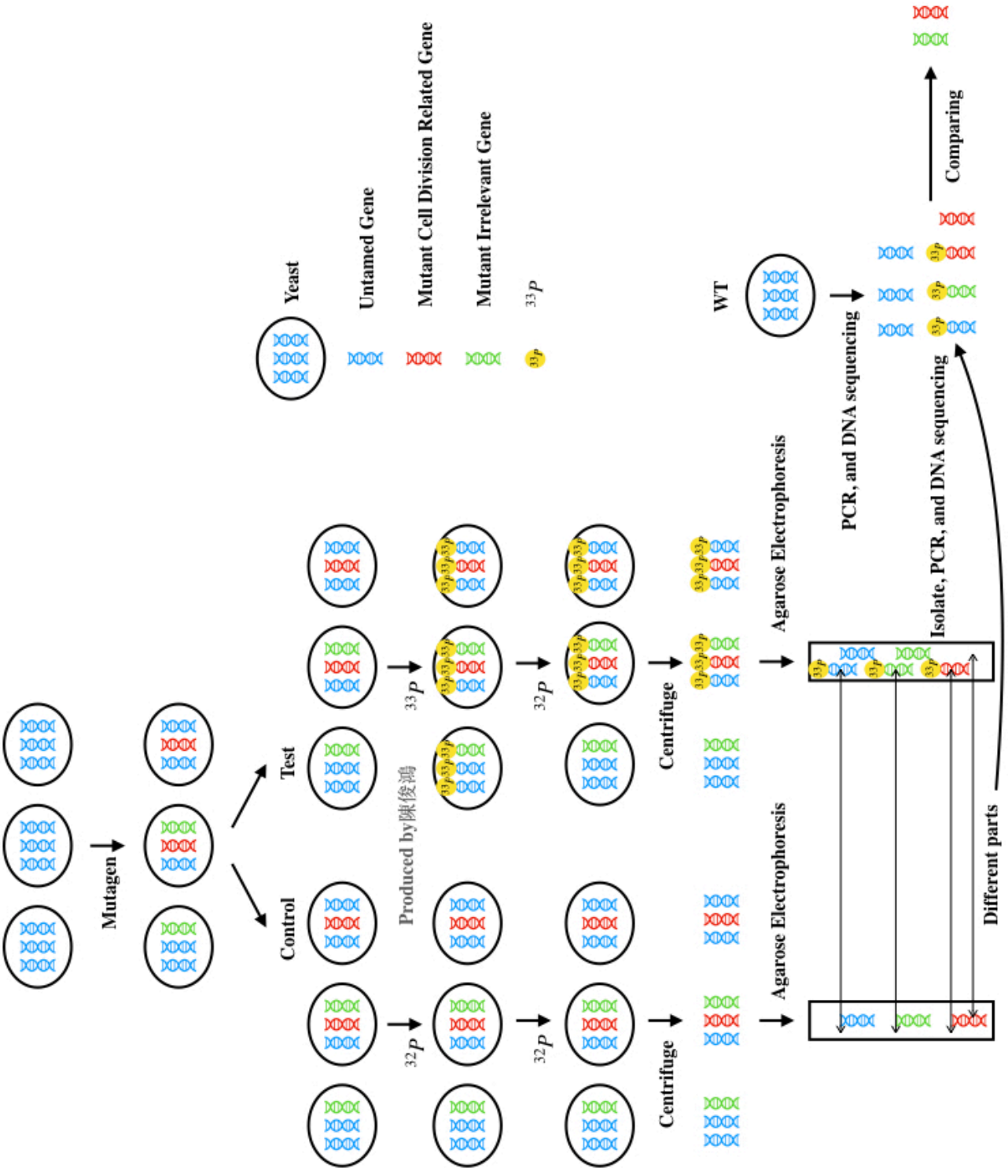


Figure 7. My experiment design(produced by myself)

(3) 假設第一步用經過突變劑處理過的 male fly (當中有些 sperm 會帶有的突變的基因） 與 wild type female mating, 產出的 F1 子代中你能否找到控制果蠅發育的基因突變種? 解釋你的答案。

能，以下是我找出控制果蠅發育的基因突變種的方法。由於果蠅的行動能力取決於翅膀的發育與步足的發育，此外果蠅具有趨光性。因此，我將會以強光與食物作為吸引果蠅的拉力，而其運動能力的極限作為推力，設計出如Fig. 8的迷宮。停留在原始chamber的果蠅只可能是控制發育基因突變種或是同時飛行與爬行能力發育基因突變種和視覺與嗅覺能力突變種，但後者機率極低。

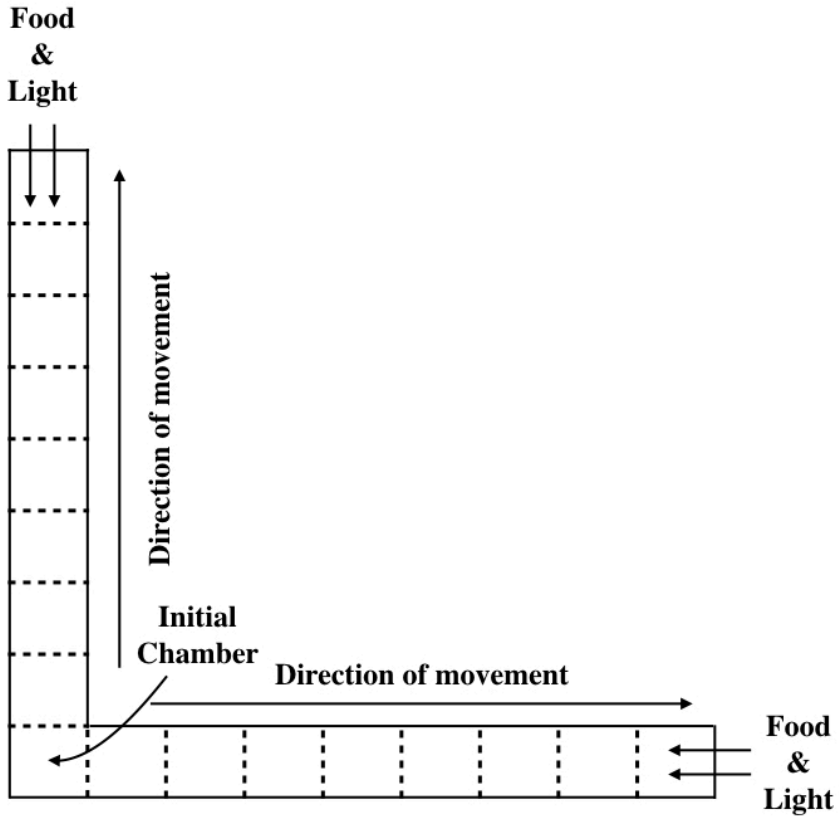


Figure 8. Design of puzzle for separation of fruit flies(produced by myself)

(4) 重返1000 年前的人類社會中會不會看到 Huntington's disease 的病人? 解釋你的答案。

我認為有可能。Huntington's disease基本上是一種遺傳性疾病，而且好發年齡在30~50歲，因此在古代非常可能是在患者已有子代後才發作。此外，雖然好發年齡在30~50歲，但其實可能在各年齡層發作，像是約有8%的患

者是在二十歲以前發作[2]。綜合以上資訊，在1000年前的人類社會粉可能存在Huntington's disease。

(5) Why IDH1 mutant has a better survival?

我猜測也許IDH1 mutant的某些代謝產物會對apoptosis相關的基因表現負面影響導致細胞雖然已經正在apoptosis卻因為進行得不順利導致較慢死亡(而我們便認為他有較好的survival)，類似於IDH1 mutant將α-ketoglutarate代謝為2-hydroxyglutarate(2HG) ，而2HG之後會進入細胞核抑制TET2 DNA hydroxylase和JmjC histone demethylase進而影響一些基因的表現。

3. 參考資料

[1] https://dnalc.cshl.edu/resources/animations/alu.html

[2] https://en.wikipedia.org/wiki/Huntington%27s_disease

(老師，我這次嘗試著部分段落用英文撰寫，這些內容已經有先給歸國的同學確認是否方便閱讀且不影響表達，希望您不會介意我這次大膽的嘗試)

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