Creating Point Mutations in Histones

Edidiong Essienton, Rashmi Panigrahi, Mark Glover
Department of Biochemistry, University of Alberta

Introduction

- In the cell, 2 meter long DNA is packaged into chromatin assisted by nucleosomal condensation. This packaging helps in protecting the genetic information.
- The nucleosome is composed of a histone protein core wrapped around by 145 base pairs double stranded DNA (Figure 1A, B).
- The DNA wraps around the histones 1.6 times.
- The histone octameric core is composed of two copies of each dimer: H2A-H2B and H3-H4 (Figure 1C).

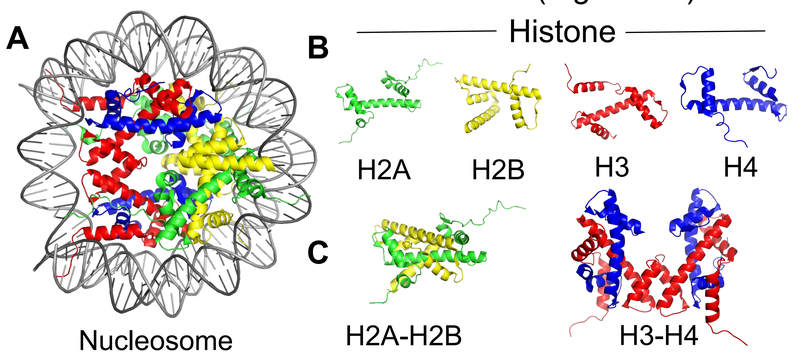


Figure 1. Components of Nucleosomes

Objective

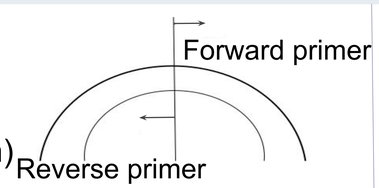
Figure 2. Genetic code

First base position	Second base position: U		C		A		G		Third base position
U	UUU	[1]P	UCU	S	UAU	Y	UGU	C	U
	UUC		UCC		UAC		UGC		C
	UUA	L	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG		UGG	W	G
C	CUU	L	CCU	P	CAU	H	CGU	R	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Q	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	I	ACU	T	AAU	N	AGU	S	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	K	AGA	R	A
	AUG	M	ACG		AAG		AGG		G
G	GUU	V	GCU	A	GAU	D	GGU	G	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	E	GGA		A
	GUG		GCG		GAG		GGG		G

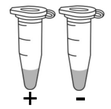
In this project, various point mutations were designed, cloned and sequenced to verify mutations in histones H2A and H2B. The overarching aim is to study the effect of these mutations on function when compared with the wild type.

Methods

1. Substitution (point mutation)



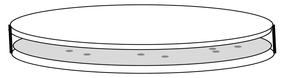
2. Exponential amplification (PCR)
   - Q5 Hot Start High-Fidelity 2X Master Mix
   - Primer Mix
   - Template



Primers

3. Kinase, ligase & DpnI treatment
   - 10 minutes at room temperature
   - 10X KLD Enzyme Mix
   - 2X KLD Reaction Buffer

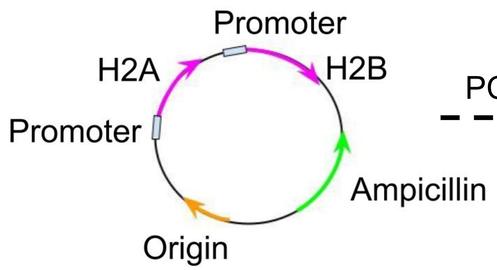
4. High-efficiency transformation



5. Inoculation and miniprep
6. Agarose gel electrophoresis

7. Sanger sequencing

Results



PCR

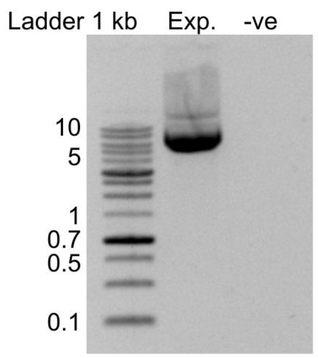


Figure 3. pETDuet-1 map with histones H2A and H2B as insert.

Figure 4. An agarose gel showing PCR amplification.

Transformation

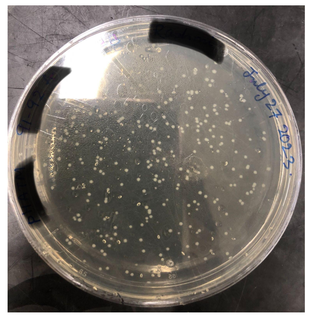


Figure 5. Agar plate showing bacterial colonies containing PCR amplified clone.

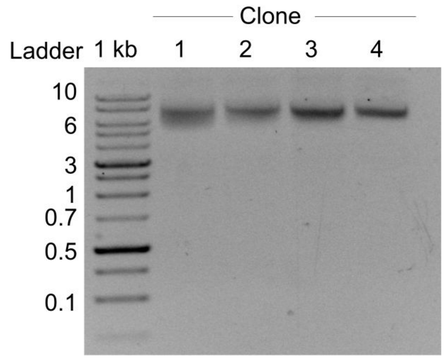


Figure 6. Verification of clones using agarose gel electrophoresis.

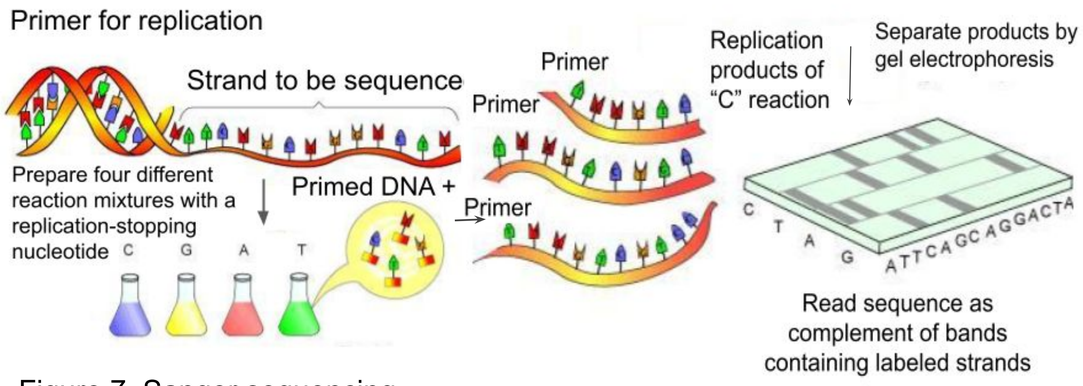


Figure 7. Sanger sequencing.

Conclusion

- 5 H2A point mutations and 1 point mutation on H2B were successfully obtained.

Acknowledgements

- I would like to thank Dr. Rashmi Panigrahi, Professor Mark Glover, the WISEST team, and my sponsors for their support.

References

- Antal, P., *et al.*, Bioinformatics 2014
- Kornberg, D., Science 1974