Directed evolution of human transcription factor SOX17 for pluripotency reprogramming

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Abstract

Sox17, a reprogramming incompetent transcription factor, shares high sequence similarity to its reprogramming competent paralog, Sox2. Engineered Sox17, carrying mutations at residue 46,53 and 57 in the HMG box, becomes a reprogramming factor that outperforms wild-type Sox2. This project adopts directed evolution to generate and screen for reprogramming competent engineered Sox17(eSox17). The high mobility group(HMG) box domain is subjected to random mutagenesis using an error-prone polymerase chain reaction(epPCR). Gibson assembly is used to clone HMG back to the expression vector, pHAGE2-TetO-AgeI-hSOX17-SalI. Higher annealing temperature for PCR,61°C for HMG box and 68° C for vector backbone, which is comparable to the melting temperature of primers, enhances amplification specificity. To give suitable mutation rate, the optimum cycle of epPCR is set to be 15 preliminarily. However, no colony can be observed after bacterial transformation implies the possible failure of the Gibson assembly. Further optimization or other cloning methods should be explored for the effective generation of the eSOX17 library.

Introduction

SRY-related high mobility group protein(Sox) transcription factor(TF) are involved in maintain stemness, cell fate determination and differentiation during embryonic development[1].

This family of protein process a highly conserved high mobility group(HMG) box domain which binds to the minor groove of DNA and interacting with other transcription factors.

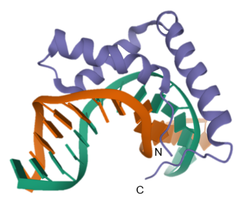


Fig1. DNA binding of Sox17(PDB ID: 3F27)

Sox2, one of the ingredients of Yamanaka factors, can reprogram adult somatic cells into induced pluripotent stem cells, whereas wild type Sox17 is involved in definitive endoderm (DE) specification and primordial germ cell (PGC) specification in humans[2,3]. Sox/Oct partnering account for the difference in cell fate by determining the preference of either forming Sox17-Oct4/ Sox2-Oct4 complex when binding to the two slightly different DNA motif, canonical vs. compressed[4].

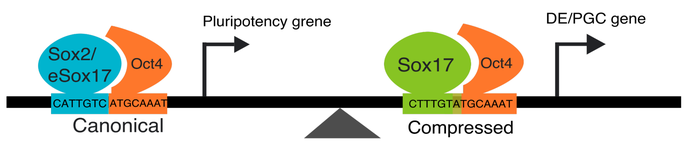


Fig2. schematic diagram of Sox2/eSox17-Oct4 or Sox17WT-Oct4 partnership

Rationally engineered Sox17 carrying mutations at residue 46,53 and 57 in the HMG box can turn the reprogram incompetent endodermal factor SOX17 into a reprogramming factor that outperform wild-type Sox2[5]. By mimicking the natural process of evolution, this project attempt to adopt directed evolution to screen for reprogramming competent engineered Sox17(eSox17) variants.

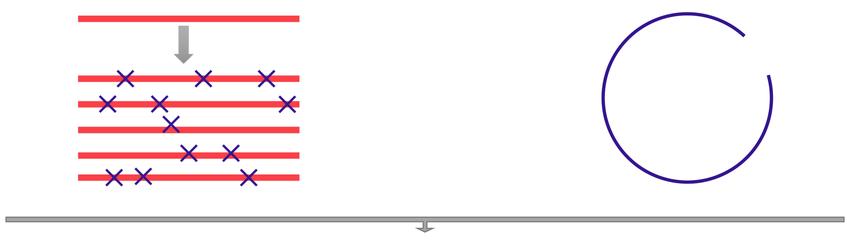
Objective

1. To set up a protocol for generation of eSox17 library
2. To screen for reprogramming competent eSox17

Materials and Methods

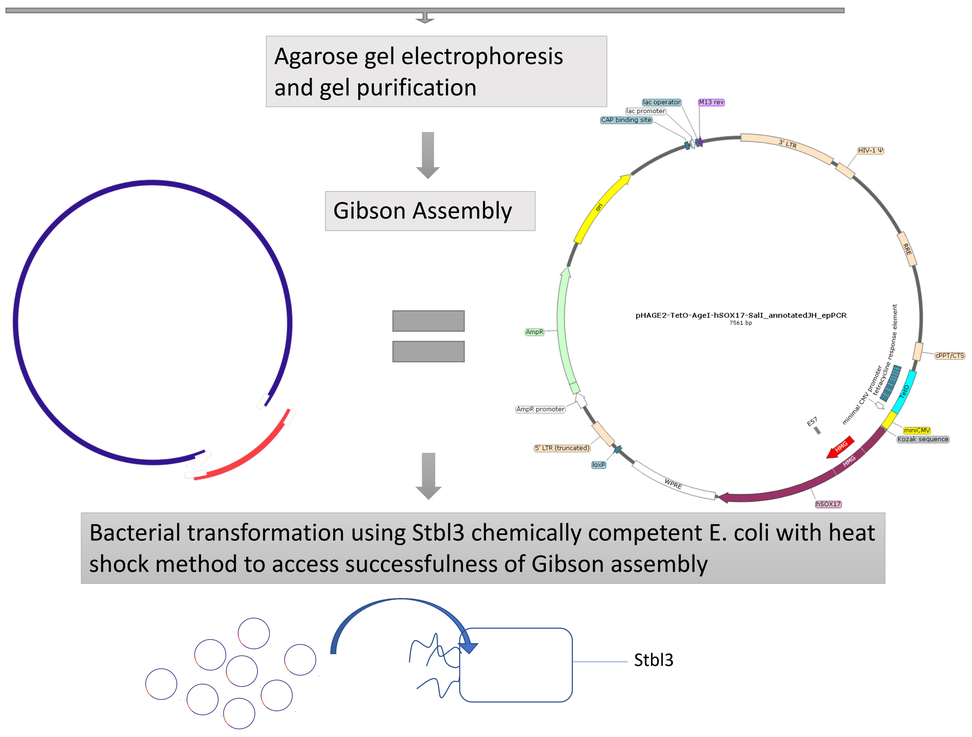
Error-prone PCR amplify HMG box of hSox17 using nucleotide analogue dPTP and 8-oxo-dGTP

Normal PCR to amplify the pHAGE2-TetO-AgeI-hSOX17-SalI vector backbone



Agarose gel electrophoresis and gel purification

Gibson Assembly

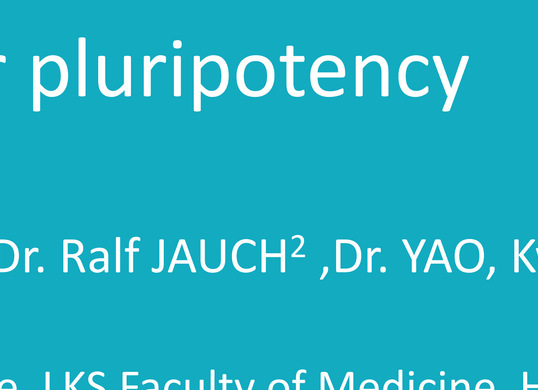


Bacterial transformation using Stbl3 chemically competent E. coli with heat shock method to access successfulness of Gibson assembly

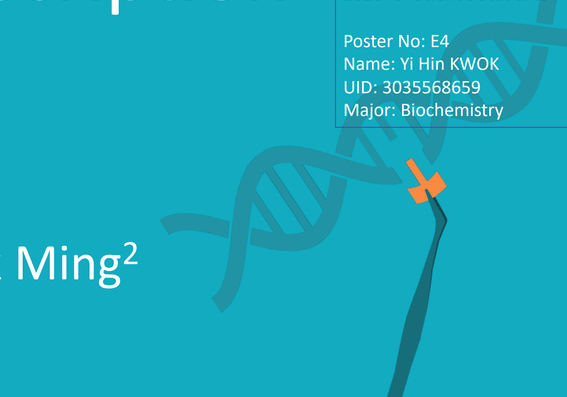
Fig3. Workflow of eSox17 library generation

Results and Discussion

4a



4b



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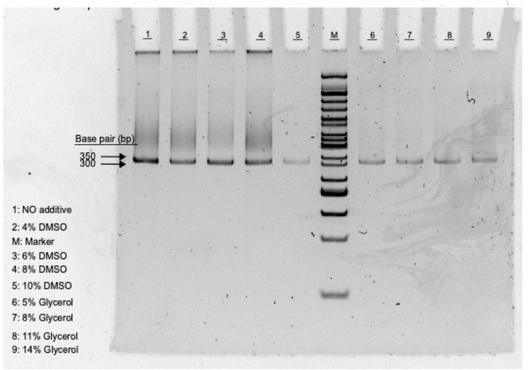

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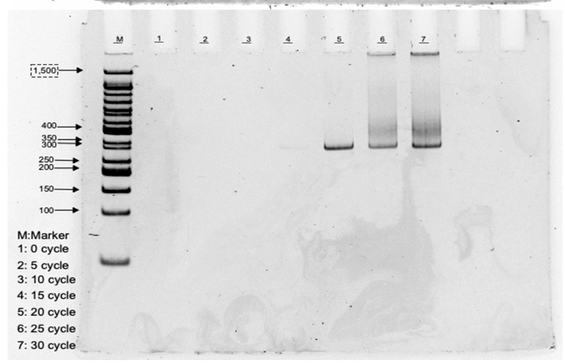
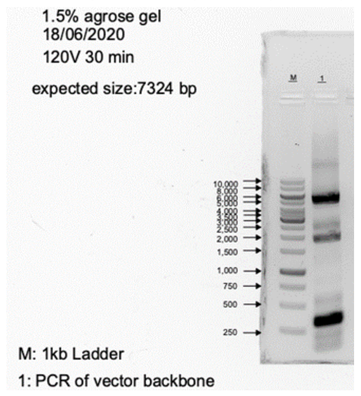


Fig4. Optimization of the ep-PCR on HMG box. Native polyacrylamide gel electrophoresis with 8% gel. Expected size=335bp

		Fig a	Fig b	Fig c			Fig d
Lane		Lane 7	Lane 7	Lane 1	Lane 2-5	Lane 6-9	Lane 4
Annealing temperature (°C)		**57**	**57**	**61**	**61**		**61**
Additives	DMSO	–	3%	–	4-10% increase at 2% interval	–	–
	Glycerol	–	–	–	–	5-14% increase at 3% interval	–
Band intensity		**no**	**weak**	**strong**	**Moderate to strong**	**moderate**	**weak**
Cycle number		30					15

Table 1. a summary of the set up of the epPCR amplifying HMG box,–: absence

5a



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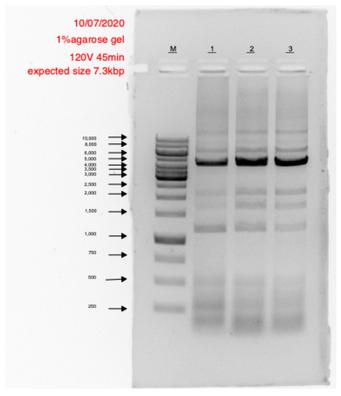

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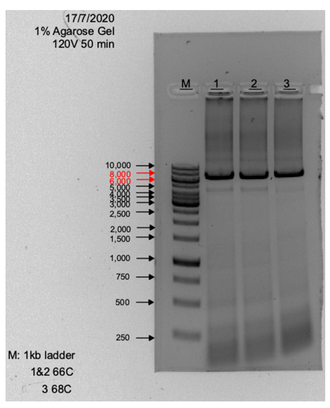
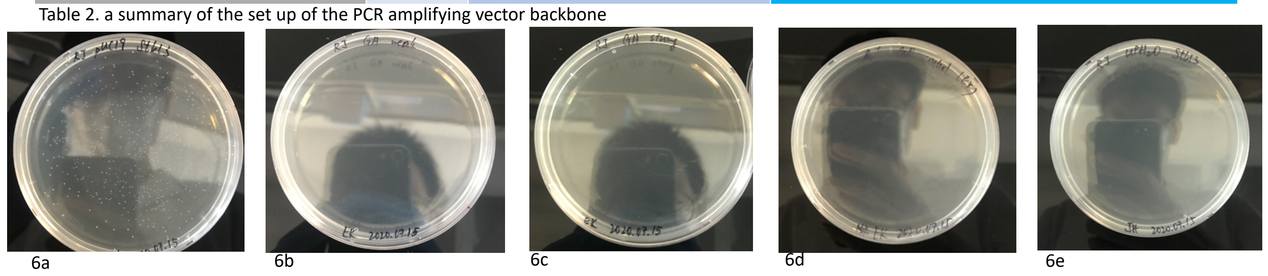


Fig6. Optimization of the PCR on vector backbone. Agarose gel electrophoresis with 1% gel. Expected size=7324p

	Fig a	Fig b			Fig c		
Lane	1	1	2	3	1	2	3
Annealing Temperature (°C)	61	60	61	62	66	66	68
Intense Band Size	5kbp	5kbp			7kbp		

Table 2. a summary of the set up of the PCR amplifying vector backbone



6a 6b 6c 6d 6e

Figure 6. bacterial transformation. a.) +ve control :pUC19. b.) sample1: GA master mix+ HMG fragment+7kbp vector backbone(correct size). c.) sample2 GA master mix+ HMG fragment+5kbp vector backbone (incorrect size) d.) Vector control: Water+ HMG fragment+ 7 kbp vector backbone f.) –ve control: UPH20

Conclusion

- Higher annealing temperature,61°C for HMG box and 68° C for vector backbone, which is comparable to melting temperature of primers, give high amplification specificity.
- The optimum cycle of epPCR is found to be 15
- no colony can be observed after bacterial transformation implies the possible failure of Gibson assembly

Reference

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