A genetically encoded system for programmable cell assembly using surface-displayed DNA oligonucleotides

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1. Introduction

Project Goal: to develop a method that enables cell to display DNA and facilitates their assembly.

- Precise patterning of cells in three dimensions is highly desirable in both basic and applied research for tissue engineering.
- Current bottom-up methods require complicated chemical functionalization protocols to attach oligonucleotides.
- A genetically encoded system is devised to covalently attach customizable single-stranded DNA (ssDNA) onto Escherichia coli cell surface.

2.1 Strains construction

- Phage protein ø174 A* gene was fused with E. coli outer membrane lipoprotein gene Blw.
- Three reverse transcriptase (RT) genes were cloned separately.
- Customized oligonucleotide sequences were introduced in the plasmids.
- Protein expression was induced by IPTG under the control of the lac operon.
- The plasmids were confirmed by diagnostic digestion and DNA sequencing.

2.2 ssDNA detection

- Flow cytometry was used to examine transformed cells.
- ssDNA was stained using a membrane impermeable dye (propidium iodide, PI), so that only cell surface-displayed DNA can be stained.
- Treatment with exonuclease I (ExoI) will digest ssDNA at cell surfaces and reduce fluorescence.
- Sequence-complementary ssDNA was expressed in two cells and stained with different fluorophores (DAPI and FITC). Mixing two kinds of cells promotes cell binding that can be detected with flow cytometry.

References


3. Results

- DNA sequencing results of plasmids revealed expected nucleotide sequences of inserted fragments.
- Digestion with ExoI reduced fluorescence intensities for all systems, confirming the existence of ssDNAs on cell surfaces.
- Complementary ssDNA molecules were expressed on different cells to allow hybridization and cell assembly, revealed by dual fluorescence staining.

Fig. 3.2 Flow cytometry profile of differently stained cell surface ssDNA

Conclusion

- Designed plasmids were successfully constructed and expressed in E. coli cells.
- Cells were enabled to display custom ssDNA sequences onto cell surfaces without complex modification or chemical functionalization.
- The existence of surface-bound ssDNAs and cell self assembly were confirmed using flow cytometry, confirming a functional genetic system was established for in vivo displaying of ssDNA molecules on cell surfaces.

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