

1                    **A simple method for constructing magnetic *Escherichia coli***

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## 31 **Abstract**

32 Magnetic force can serve as an ideal way to control the spatial behavior of microorganisms,  
33 because of its flexibility and penetrability. By incubation with the biocompatible compound,  
34 ammonium ferric citrate, as an iron source, we magnetized *Escherichia coli*, the most  
35 programmable chassis in synthetic biology. To enhance the magnetization efficiency, the ferritin  
36 protein, FtnA, from *E. coli* was cloned and overexpressed in strain BL21(DE3). The  
37 magnetization effect was observed within 30 min after harvest of bacteria, and the concentration  
38 of ammonium ferric acid used could be as low as 0.5 mM. Using different shapes of magnetic  
39 fields, different patterns could be generated easily. Our method may set up the foundation for a  
40 rational design of spatial structure of cell communities, which is important for their actual  
41 application.

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43 **Keywords:** Ammonium ferric citrate; *E. coli*; FtnA; Magnetization

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## 62 **Introduction**

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64 Controlling the movement and location of cells is one of the most important topics in synthetic  
65 biology. Pattern formation, synthetic consortia and the novel cell array sensor heavily rely on the  
66 precise spatial arrangement of cells (Melamed et al. 2012; Brenner et al. 2008). The traditional  
67 method relied on modifying the chemotaxis mechanism of cells, which was both slow and  
68 irreversible (Goldberg et al. 2009). Recently, rapid control of cells has been achieved by  
69 introducing light controllable proteins into organisms, hence the name optogenetics (Herrou and  
70 Crosson 2011). However, the heat effect of light prevented its use in prolonged control.  
71 Furthermore, the low penetrability of light hindered its application. Compared with chemical and  
72 light control, magnetic force is safe, fast, reversible, highly penetrative and easy to design  
73 (Pankhurst et al. 2003), and thus would be an ideal method for controlling cells if the cells could  
74 be magnetized. Therefore, several methods to magnetize cells have been developed, including  
75 methods that rely on artificial synthesis of magnetic nanoparticles (Cho et al. 2012), and  
76 transferring the cassette controlling magnetosome synthesis in *Magnetospirillum*  
77 *gryphiswaldense* into the related species, *Rhodospirillum rubrum* (Kolinko et al. 2014).  
78 However, these methods are both laborious and hard to generalize. In 2011, Silver's group  
79 (Nishida and Silver 2012) reported a simple method for magnetizing budding yeast, by  
80 incubation with ferric citrate and introduction of a ferritin protein.

81 So far, no simple method for magnetizing the most programmable chassis organism in synthetic  
82 biology has been reported. Here, we extended Silver's method to *Escherichia coli*. After  
83 incubation with ammonium ferric citrate, we showed the magnetization by the formation of  
84 defined patterns under strong magnets. By cloning and overexpressing FtnA, a ferritin protein  
85 that helps store iron in *E. coli* (Bitoun et al. 2008), we showed that the magnetization effect

86 could be amplified even under a low concentration of  $\text{Fe}^{3+}$ . Using different shapes of magnets,  
87 we also showed the flexibility of patterns formed by bacteria. Our method provides a versatile  
88 tool for spatial control of *E. coli*, which may aid the development of synthetic biology.

89

## 90 **Methods**

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### 92 **Strains, culture medium and conditions**

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94 The *E. coli* strains used for cloning FtnA, plasmid construction and the attraction test were K12,  
95 DH5 $\alpha$  and BL21(DE3), respectively. The medium used was LB. Ampicillin was added when  
96 necessary. Except for induction, the bacteria were cultured at 37°C with agitation at 220 rpm.

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### 98 **Plasmid construction**

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100 The *ftnA* gene was amplified from strain K12 and cloned into plasmid pUC18. A mutation was  
101 introduced to eliminate the NdeI restriction site in the original *ftnA* sequence using the  
102 QuickChange kit (Stratagene). Then, the coding sequence was ligated into the pET-22b+ vector  
103 using the NdeI and BamHI restriction sites (Fig. 1).

### 104 **Insert Figure 1**

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### 106 **Growth test**

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108 Experiments were performed at 37°C with agitation at 220 rpm. OD<sub>600</sub> values were measured at  
109 several time points, and converted into dry weight. The experiments were performed in triplicate.

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### 111 **Magnetic cells preparation**

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113 For attraction tests without plasmid induction, bacteria were cultured in LB medium inoculated  
114 with 20 mM Fe<sup>3+</sup> derived from ammonium ferric citrate. Cells were harvested after 24 h and  
115 washed several times, then pelleted by centrifugation and resuspended in double-distilled H<sub>2</sub>O.  
116 For induction experiments, cells were exposed to 20 mM Fe<sup>3+</sup> and induced with 1 mM isopropyl  
117 β-D-1-thiogalactopyranoside (IPTG) at 25°C for 24 h, and then harvested as described above.

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### 119 **Attraction test**

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121 Three milliliters of cell suspension were added to 3.5-cm dishes. For characterizing the  
122 magnetization effect, a circular Rubidium iron boron magnet (2-cm in diameter) was put  
123 underneath each dish, representing a magnetic field of 1.2 T near the magnet edge. For  
124 generating different patterns, different shapes of magnets were used, accordingly. Photographs  
125 were taken at appropriate time points.

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## 127 **Results and Discussion**

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### 129 **Effect of ammonium ferric citrate on *E. coli* growth**

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131 To obtain magnetic *E. coli* as much as possible, we used ammonium ferric citrate, since citrate  
132 has been reported as an effective microorganism siderophore for iron absorption (Guerinot et al.  
133 1990). We compared the growth of *E. coli* under high iron concentration (20 mM ammonium  
134 ferric citrate added from a fresh stock of 500 mM) and normal conditions by measuring the  
135 OD<sub>600</sub> at several time points after inoculation. As shown in Fig. 2, no significant difference was  
136 observed between the control group and the experimental group, demonstrating that ammonium  
137 ferric citrate is not toxic to *E. coli* cells. Hence, ammonium ferric citrate could serve as the iron  
138 source without decreasing the amount of *E. coli* harvested. This enabled us to easily maintain a

139 population that is much larger than any other chassis organism for a synthetic cell-cell  
140 community (Youk and Lim 2014).

## 141 **Insert Figure 2**

### 142 **Magnetization of *E. coli* by incubation with ammonium ferric citrate**

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144 Next, we tested whether *E. coli* could be magnetized by incubation with ammonium ferric  
145 citrate. Cells were harvested 24 h after inoculation. As shown in Fig. 3a, cells harvested from  
146 cultures containing 20 mM Fe<sup>3+</sup> formed a white circle where the magnetic force was strongest,  
147 near the edge of the magnet. No noticeable cluster of *E. coli* was formed in the control group.  
148 This result demonstrated that incubation with ammonium ferric citrate indeed magnetized *E.*  
149 *coli*. The short time and ease of preparation of the magnetic *E. coli* enabled a massive  
150 experiment to be performed in a short time.

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### 152 **The time course of the attraction phenomena**

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154 As shown in Fig. 3b, the attraction ring appeared 1 h after putting the magnet under the culture  
155 plate, and the pattern was stable after 2 h. Note that the pattern was formed in a liquid  
156 environment, which could not be accomplished by the traditional method with the chemotaxis  
157 mechanism, because the chemicals would quickly become uniform by diffusion. The *E. coli*  
158 could respond to the magnetic fields so quickly, because of the high efficiency of iron absorption  
159 of bacteria (Andrews et al. 2003). It is also well known that *E. coli* can express ferritin, a  
160 ubiquitous iron storage protein that is conserved from bacterium to human. This protein becomes  
161 paramagnetic when iron is incorporated (Bauminger and Nowik 1989). Note that in our  
162 experiment, the time required to form the attraction ring was slightly longer than that in Silver's  
163 experiment using yeast, probably because *E. coli* cells are much lighter than yeast cells (Nishida  
164 and Silver 2012).

## 165 **Insert Figure 3**

## 166 **Improving the magnetization effect by introducing FtnA**

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168 To achieve a higher magnetization efficiency of *E. coli*, we cloned and overexpressed FtnA, a  
169 ferritin protein, in *E. coli* to enhance the efficiency of ferrite incorporation. This protein also  
170 increases the iron stores, which could be used during iron deficiency (Abdul-Tehrani et al. 1999).  
171 As shown in Fig. 4, the attraction ring was formed within 30 min in the experimental group,  
172 while in the control group no observable ring was formed in such a short time. Note that the  
173 concentration of  $\text{Fe}^{3+}$  used could be as low as 0.5 mM. Thus, the introduction of FtnA  
174 significantly increased the magnetization efficiency. This result also revealed a remarkable  
175 advantage of our system: the concentration of the iron source used could be as low as 0.5 mM,  
176 which was much lower than that required for magnetizing yeast (Nishida and Silver 2012).

### 177 **Insert Figure 4**

## 178 **The flexibility of the pattern formed by magnets of different shapes**

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180 To show the flexibility of the cell pattern formation, we used different shapes of magnets to  
181 induce different pattern shapes. As shown in Fig. 5, a rectangular shape and small double circles  
182 were formed under the attraction of rectangular magnets and small circular magnets,  
183 respectively. Interestingly, our method generated accurate pattern edges comparable to the  
184 synthetic edge detection circuit in *E. coli* (Tabor et al. 2009), without complex genetic design or  
185 mathematical modeling.

### 186 **Insert Figure 5**

## 187 **Conclusion**

188

189 Here, we established a simple method for producing magnetized *E. coli* by incubation with  
190 ammonium ferric citrate. The attraction ring pattern could be formed within 1 h. Overexpression  
191 of FtnA in *E. coli* significantly improved the magnetization effect. Furthermore, different  
192 patterns could easily be generated by different shapes of magnets, demonstrating the flexibility

193 of our method. This method sheds light on using the magnetic force to control the most  
194 programmable microorganism, *E. coli*, in synthetic biology.

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200 The authors declare no conflict of interest.

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## 254 **Figure Legends**

255

256 **Fig. 1** The map of plasmid pET-22b+-FtnA, which was constructed by inserting the FtnA coding  
257 sequence immediately after the ribosomal binding site

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260 **Fig. 2** Growth test of *E. coli* inoculated with ammonium ferric acid. The OD<sub>600</sub> values were  
261 measured at 3, 5, 7 and 12 h. Experiments were performed in triplicate

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263

264 **Fig. 3** The test of the magnetization effect of inoculation with ammonium ferric acid by  
265 attraction ring formation. Bacteria resuspended in water were added to 3.5-cm blank dishes. A  
266 circular magnet was placed underneath each dish to attract magnetized bacteria. **(a)** The  
267 attraction ring was formed only in the group inoculated with ammonium ferric citrate. **(b)** A time  
268 course of the attraction ring formation

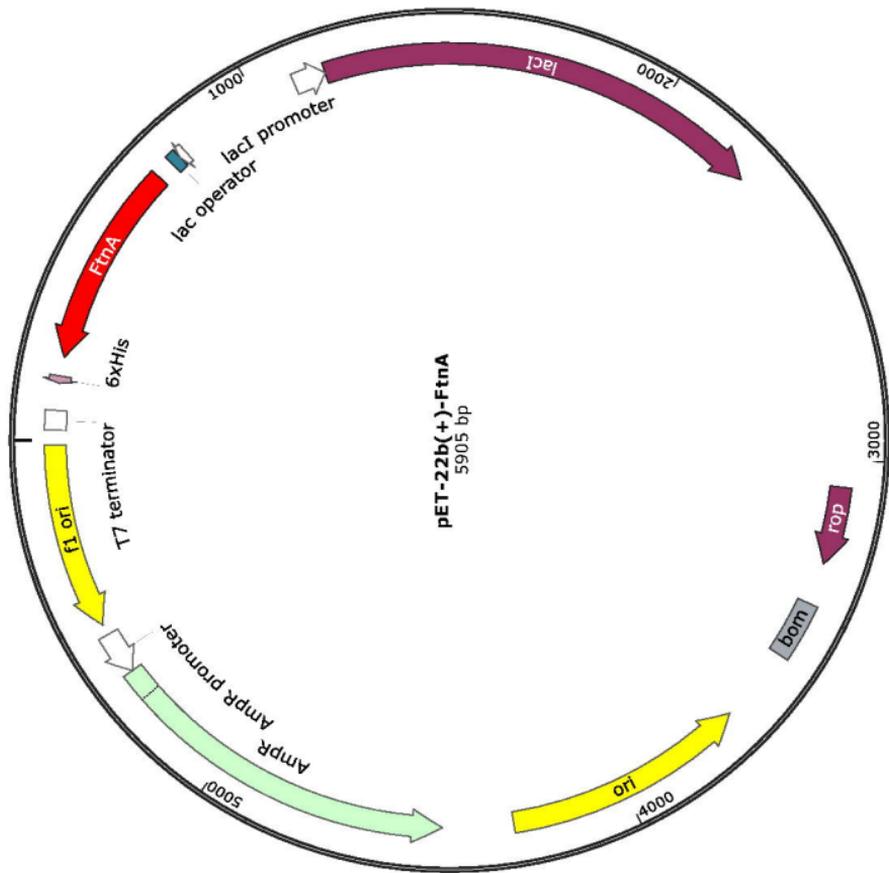
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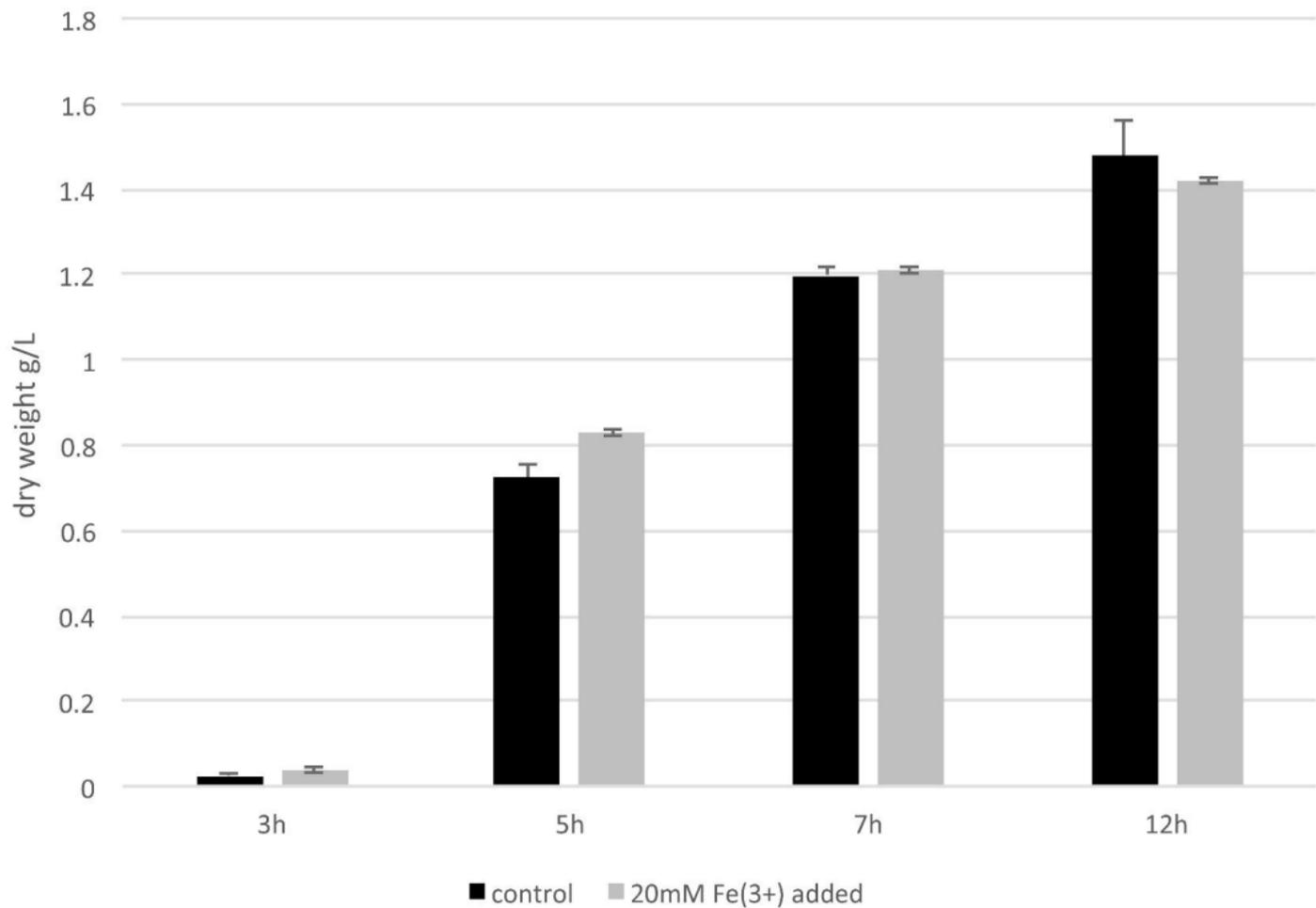
270 **Fig. 4** Overexpressing FtnA improves the magnetization effect

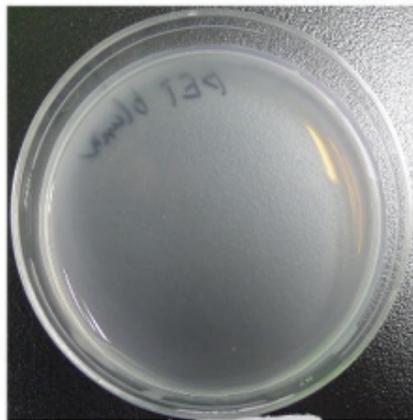
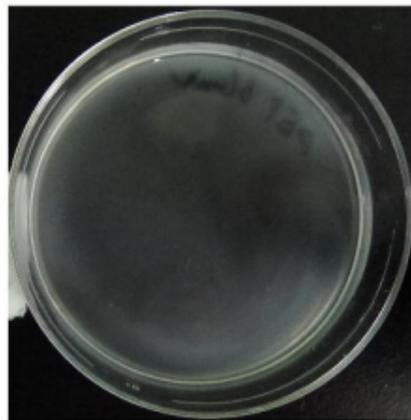
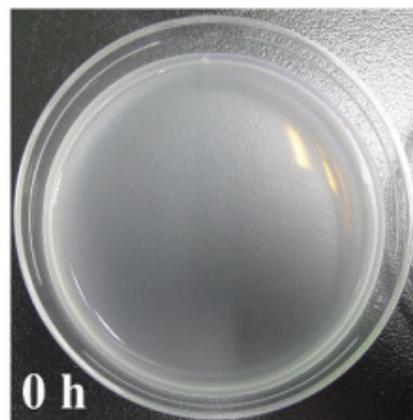
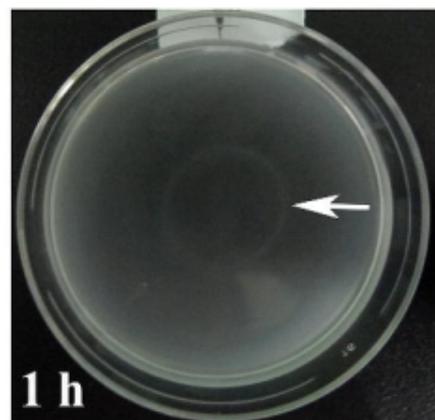
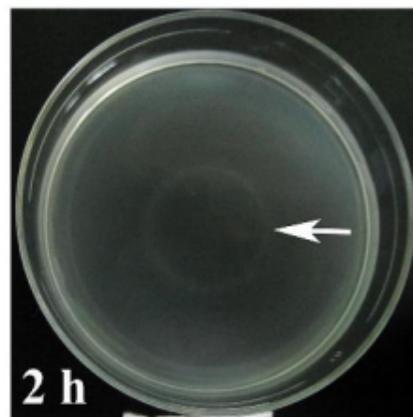
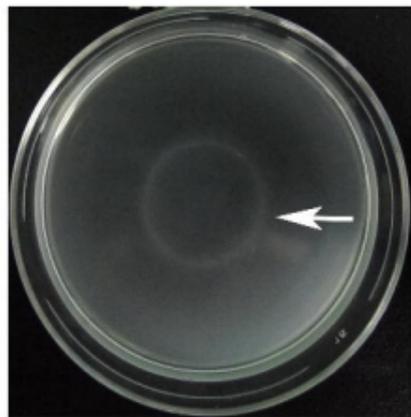
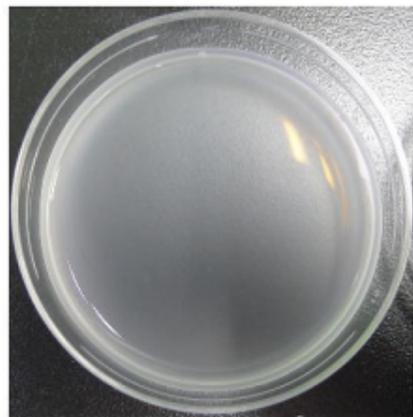
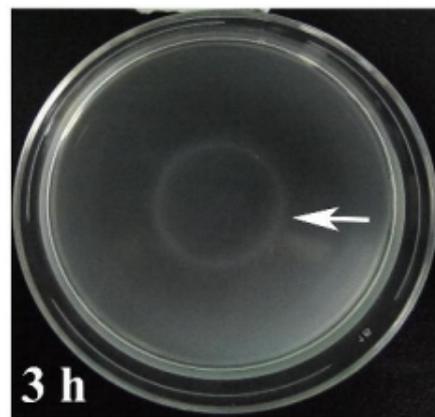
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273 **Fig. 5** Different attraction rings with a sharp edge under the action of different magnet shapes



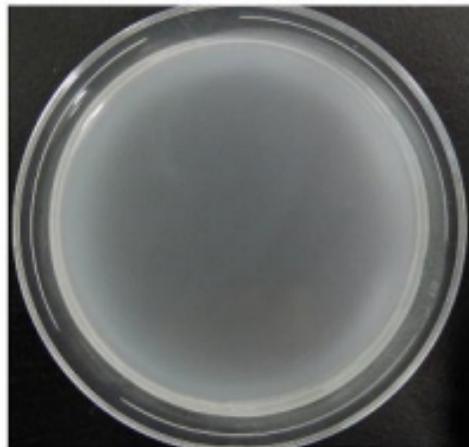
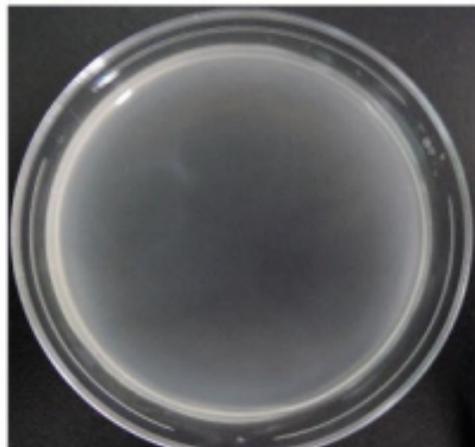


**a****0 h****0 mM Fe(3+)****3 h****b****0 h****1 h****20 mM Fe(3+)****2 h****3 h**

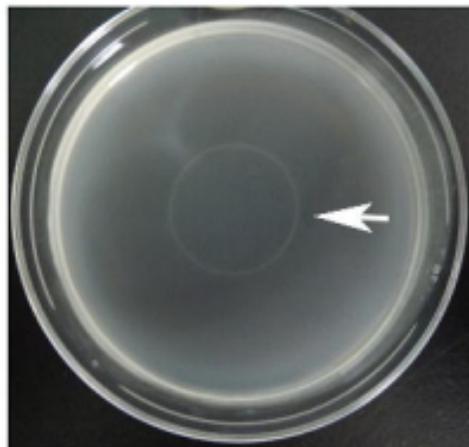
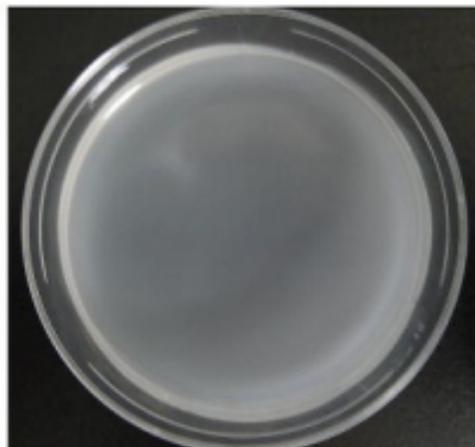
**0 h**

**0.5 h**

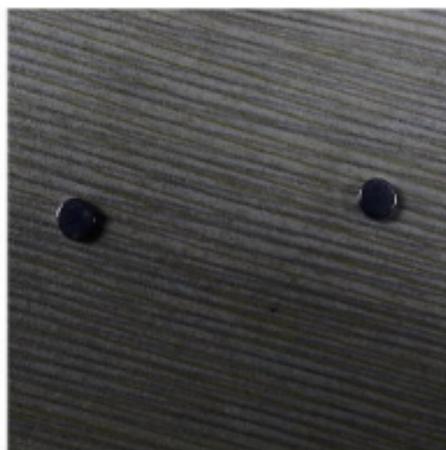
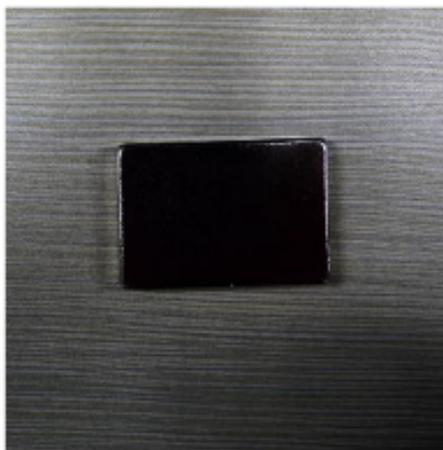
**pET-22b(+)**



**pET-22b(+)-FtnA**



# Magnets



# Patterns

