			Name	Type	Description	Designer	Length
❤		W	BBa_K1077000	Intermediate	fim switch inverted repeat left IRL natural	Mike Ferguson	291
❤		W	BBa_K1077001	Intermediate	fim switch inverted repeat right IRR natural	Mike Ferguson	37
❤		W	BBa_K1077002	Composite	Inducible fimE and hbiF recombinase generator (tet and lux).	Mike Ferguson	3265
❤		W	BBa_K1077003	Composite	J23100 fim switch b0034 GFP	Mike Ferguson	1125
❤		W	BBa_K1077005	Composite	J23100 fim switch ON orientation	Mike Ferguson	379
❤		W	BBa_K1077007	Composite	J23100 fim switch b0034 amilCP ON orientation	Mike Ferguson	1074
			BBa_K1077004	Device	natural fim switch OFF orientation	Mike Ferguson	378
			BBa_K1077006	Composite	nat fim switch b0034 GFP OFF orientation	Mike Ferguson	1124

Click here to see parts that we significantly improved and reviewed

K1077000
K1077001
K1077002
K1077003
K1077004
K1077005
K1077006
K1077007

A complete, customizable, fim transcriptor system:

We submitted the parts necessary for teams to insert any sequence of their choosing within the fim switch. We redesigned the inverted repeats to include the IHF and LRP sites as well as the rest of the natural sequence. Using K1077000 and K1077001, teams can insert any sequence of their choosing within the switch. Given that we have successfully shown these new natural sequences to function in K1077003 and K1077007, enabling the fim switch to flip completely, we have improved the 2008 caltech igem team's inverted repeat parts K137008 and K137010, which showed very little flipping (fig. 1). Additionally, we used these parts to engineer a completely flipping, J23100 promoter containing, fim switch which is an improvement on both the 2008 caltech igem fim switch (K137057) and the 2012 michigan igem team's engineered fim switch (K880002), which showed no or little flipping. K1077003 is the engineered switch, producing GFP in the ON orientation. K1077007 is the engineered switch, producing amilCP in the ON orientation, which aided immensely in determining the orientation of the switch by eye. This was a big concern for us, since we did not readily have access to a fluorimeter or flow cytometer. K1077005 is the engineered switch without any parts upstream or downstream of it. Finally, we engineered the hbiF and fimE recombinases to be inducible by HSL and aTc respectively (K1077002). We successfully characterized this part and submitted it in both

pSB1C3 and pSB1K3. To use the system, one can co transform K1077002 with a switch of their design.

Design and Construction of the new fim switch:

Given the importance of the LRP and IHF binding sites in the switch (see background, fig. 1), and the lack of these sites in the engineered fim switch we created last year, we decided to copy the natural fim switch as close as possible. The sequence of the switch varies slightly from strain to strain in E. coli. We chose to use the natural switch sequence from E. coli CFT073 because that is the strain that has the most characterization data on hbiF. When swapping out the fimA promoter, we ran in to two problems. The first was that part of the fimA promoter overlapped with the IRR-internal half site specifically where the recombinases had been shown to bind via DNA footprinting (fig 2). We solved this by removing the fimA promoter only up to and including the "AT" before the "GATAT...", seen in fig. 2, and taking out the rest of the fimA promoter, including the -35. With the -35 and most of the fimA promoter gone, we hypothesized that the fimA promoter would be inactive. The second problem was that once the promoter was swapped out, the switch would be bigger than it was before and that the spacing between the important binding sites of the fim switch would be altered. Given how much is unknown about the mechanism of inversion, we decided to conserve the distance between the LRP and IHF binding sites by removing the minimal amount of non IHF and LRP site sequence from the switch.

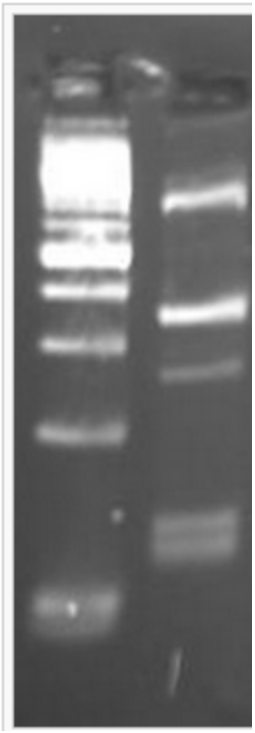

Fig 1: Incomplete flipping of the fim switch without IHF and LRP sites. The ~350bp band corresponds to unflipped switch whereas the ~250bp band corresponds to flipped switch. Source: http://2012.igem.org/Team:Michigan/Results

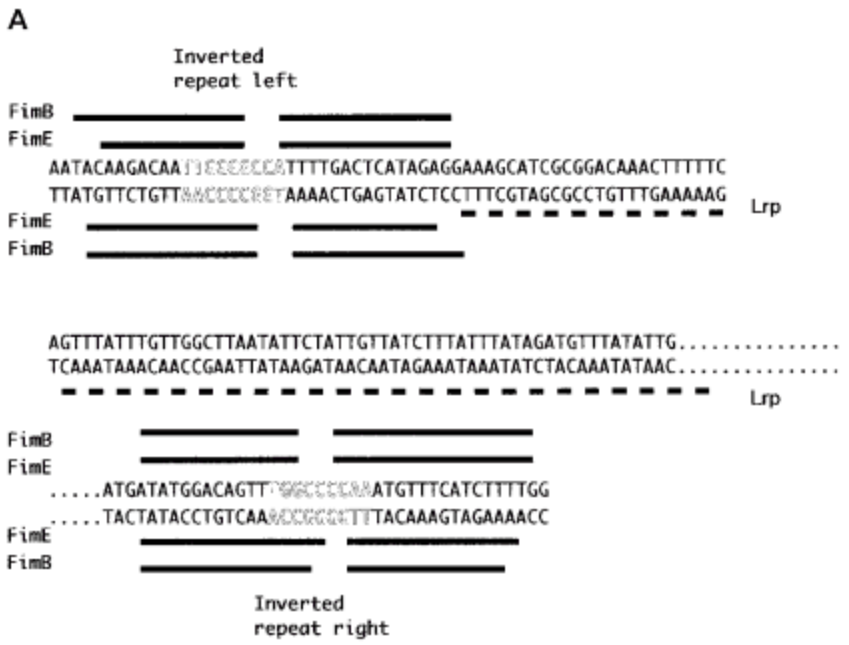

Fig. 4. Organization of the *fim* switch. A. The regions of the *fim* switch protected by FimB and FimE from phen(Cu)$^{2+}$-activated cleavage. The switch is in the on orientation and shows the juxtapositioning of the recombinases and the previously identified position of Lrp binding within the switch. B. The position and sequence alignment of the four half-sites.

Fig 2: FimB and fimE binding sites indicated by the solid black lines. Source: [7]

Parts that we significantly improved:

K137010
K137008
K137057
K880001
K880002
K880003

Parts Reviewed:

K137010
K137008
K137057
K880001
K880002
K880003

I13500
K592009
K137007
K880000
F2622
K173007

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