

Asymmetrical Digest *fim* switch Assay¹

1. Co-transform *E. coli* 10-Beta(NEB), or any other $\Delta fimEB\Delta hbiF$ strain, with an asymmetrical digest reporter (K880001 or K880003), and strong, constitutive FimE (K880005-K137007)
2. Pick several colonies for 20uL colony PCR amplification with primers VF and VR with an elongation time of 45 seconds
3. Digest the PCR amplification products with AgeI-HF and EcoRI-HF (5 units of each) in NEBuffer 4 in a 30uL reaction
4. Run the digest products on a wide-laned 2% electrophoresis gel
5. Image the gel

Expected Results:

Reporter orientation	Recombinase Specificity	EcoRI and AgeI Digest Fragments*
Unflipped	FimE	357bp, 136bp, and 123bp
Flipped	HbiF	255bp, 238bp, and 123bp

*Fragment size only indicative of PCR amplification of this part in the plasmid pSB3C5 with the primers VF2 and VR, must be recalculated otherwise.

References:

1. Teng et al. Infect. Immun. 2005, 73(5):2923.