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Overnight liquid culture BL21 DE3

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Making BL21 DE3 competent

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Making KAN plates; TOP10+DVK_AF transformation

1/6

Liquid culture to amplify TOP10+DVK_AF

2/6

Maxiprep to extract DVK_AF from TOP10

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Digestion and gel electrophoresis to check DVK_AF

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Making KAN + IPTG + X-Gal plates

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Digestion and gel electrophoresis to re-check DVK_AF

12/6

Collecting DVK_AF/DVK_FG DH5alpha from MoClo kit; streaking onto plates

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Liquid cultures to amplify DVK_AF and DVK_FG DH5alpha colonies

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Miniprep DVK_AF, DVK_FG; digestion and gel electrophoresis to check DVK_AF and DVK_FG

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Resuspension of IDT protospacer constructs; MoClo of DVK_AF+KPC+GFP; transformation of TOP10 with KPC MoClo; PCR to amplify protospacer constructs

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KPC MoClo results; check protospacer PCR products on gel; nanodrop to check protospacer construct resuspension concentration

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Repeating PCR amplification of protospacer constructs; checking protospacer PCR products on gel; MoClo of DVK_AF+GFP+vanA/ampC; transformation of TOP10 with vanA and ampC MoClo; liquid culture of KPC MoClo TOP10

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ampC MoClo and vanA MoClo results; PCR of vanA protospacer using gradient of annealing temperatures; miniprep and digestion of KPC MoClo; gel to check KPC MoClo digest, and vanA gradient PCR products

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PCR purification of vanA PCR; check vanA purification on gel; running gel for extraction of vanA and ampC PCR products (gel slices stored in fridge o/n); PCR purification of KPC PCR

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Gel extraction of vanA and ampC PCR products; nanodrop of gel extracted vanA and ampC PCR products; MoClo of DVK_AF+GFP+vanA; transformation of TOP10 with vanA MoClo; liquid culture of KPC MoClo TOP10; liquid culture of ampC MoClo TOP10

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vanA MoClo results; miniprep, digestion and gel electrophoresis to check ampC MoClo; glycerol stock of KPC MoClo TOP10

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Miniprep, digestion and gel electrophoresis of vanA MoClo

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Resuspension of T7 flank DNA; MoClo of DVK_FG+T7 flanks+KPC; transformation of DH5alpha with T7 KPC MoClo, liquid cultures of vanA MoClo TOP10 and KPC MoClo TOP10

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Checking T7 KPC MoClo results; DVK_FG DH5alpha liquid culture, Glycerol stock of vanA MoClo; UV light examination of vanA and KPC MoClo liquid cultures

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Miniprep of DVK_FG; digestion of DVK_FG and ampC MoClo; gel for DVK_FG and ampC MoClo digests

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MoClo of DVK_FG+T7 flanks+KPC; transformation of TOP10 with T7 KPC MoClo

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MoClo of DVK_FG+T7 flanks+vanA; transformation of TOP10 with T7 vanA MoClo; T7 KPC MoClo results; making KAN+IPTG+X-Gal plates; liquid culture of T7 KPC MoClo

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Miniprep, digestion and gel electrophoresis of T7 KPC MoClo; T7 vanA MoClo results; liquid cultures of T7 vanA MoClo

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Miniprep, digestion and gel electrophoresis of T7 vanA MoClo; making KAN stock

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pCas9 digestion and gel electrophoresis; liquid culture of T7 KPC MoClo TOP10

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pCas9 gel extraction; resuspension of T7 spacer oligo DNA; phosphorylation and annealing of T7 spacer oligos; making up 1M NaCl; ligation T7 spacer oligos into pCas9

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T7 KPC MoClo TOP10 glycerol stock; transformation of BL21 DE3 with pCas9+T7 spacer ligation; making chloramphenicol plates

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pCas9+T7 spacers BL21 DE3 transformation results

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Gel to check T7 oligo annealing, repeating T7 oligo phosphorylation

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Resuspension of primers for T7 flanks and new Anderson, PCR to amplify T7 homology flanks and replace Anderson promoter with strong version, 2nd attempt at T7 oligo annealing, gel to check PCR reaction success, 2nd attempt at T7 oligo ligation into pCas9

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Nanodrop of T7 flanks and new Anderson KPC/vanA, repeat of T7 vanA MoClo and transformation into TOP10, gel extraction of new Anderson KPC/vanA, nanodrop of gel extraction product, transformation of pCas9_spacer ligation into BL21 DE3

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T7 vanA TOP10 transformation results, pCas9_spacer BL21 DE3 transformation results, KPC_new_Anderson+GFP+DVK_AF MoClo and transformation into TOP10

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KPC_new_Anderson transformation results, liquid cultures of KPC_new_Anderson and T7 vanA

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Digestion and gel to check T7 vanA and KPC_new_Anderson success

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Annealing unphosphorylated oligos, ligating unphosphorylated oligos into pCas9, liquid cultures of KPC_new_Anderson and T7 vanA

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Digestion and gel to re-check T7 vanA and KPC_new_Anderson success, transformation of BL21 DE3 with unphosphorylated oligo ligation

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Unphosphorylated ligation transformation results

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GFP fluorescence microscopy of *E. coli* mock pathogens

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Prepared samples to be sent for Sanger sequencing at Edinburgh Genomics