Transposition Mutagenesis Flow Chart

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Part I: Conjugation

What	Why
Resuspend donor cells by vortexing	mix with nutrient broth
Vortex recipient briefly	
Put $500\mu L$ donor and $500\mu L$ recipient in mating tube and vortexing briefly	mate two strains
Incubate in 37°C for 60 min (with balance)	start mating
Label information on the plate (name, day, medium, solution, dilution)	
Add $900\mu L$ saline to 7 tube labeled with $10^{-1} \sim 10^{-7}$	prepare to dilute
Add $100\mu L$ recipient to 10^{-1}, vortex	dilute
Repeat until 10^{-7}	
Put $100\mu L$ last three tube onto nutrient agar plates and spread in circular fasion	count number of recipient
Put $100\mu L\, 10^0$ donor onto a Nal plate	verify all donor is sensitive to Nal
Put $100\mu L\, 10^0$ recipient onto a Cm plate	verify all recipient is sensitive to Cm
Vortex mating tube for 60s	break pilus between donor and recipient
Dilute to 10^{-3}	
Plate $10^0 \sim 10^{-3}$ to 4 Cm + Nal plates	count number of mated recipient

What	Why
Divide 9 plates to 4/5 two stack. Incubate in 37°C incubator	let colony grow

Part II: Transposition Mutagenesis

What	Why
add $1\mu L$ IPTG, $1\mu L$ chloramphenicol to $998\mu L$ nutrient broth	make inducing broth
Inoculate with small loopful colonies from 10^0 Cm+Nal plate	
Vortex briefly	
Incubate suspension in 37°C for 60 min	induce transposition
Label plates with information, make 7 dilution tube with $900\mu L$ saline	
Stop incubation and vortex briefly	
Dilute unitl 10^{-7}	
Spread $10^0 \sim 10^{-3}$ onto 4 X-gal + kan plates	count number of transposition
Spread $10^{-4} \sim 10^{-7}$ onto 4 nutrient agar plates	count cm^R, nal^R cells
Place in stacks of 4/4 and incubate in 37°C incubator	incubate