TROUBLESHOOTING GUIDE
Transformation

EDVOTEK®

TRANSFORMATION TROUBLESHOOTING GUIDE

PROBLEM:	CAUSE:	ANSWER:
Poor cell growth on source plate	Incubation time too short	Continue to incubate source plate at 37ºC for a total of 16-20 hours.
	Antibiotic added to source plate	When pouring plates, be sure to add antibiotics & additives at the correct step.
	Incorrect incubation temperature	Use a thermometer to check incubator temperature. Adjust temp. to 37°C if necessary.
Satellite colonies seen on transformation plate	Incorrect concentration of antibiotics in plates	Ensure the correct concentration of antibiotic was added to plates - Make sure ReadyPour is cooled to 60° C before adding antibiotic.
	Antibiotic is degraded	Make sure ReadyPour is cooled to 60° C before adding antibiotic.
	Plates were incubated too long	Incubate the plates overnight at 37ºC (16-18 hours).
Colonies appeared smeary on transformation plate	Plates containing transformants were inverted too soon	Allow cell suspension to fully absorbed into the medium before inverting plates.
	Experimental plates too moist	After pouring plates, allow them dry overnight at room temp. Alternatively, warm plates at 37°C for 30 min. before plating cells
No colonies seen on transformation plates	Plasmid DNA not added to transformation mix	Ensure plasmid DNA was added to transformation tube.
		Make sure that pipets are used properly. If using micropipets, make sure students practice using pipets
	Incorrect host cells used for transformation	Confirm that correct bacterial strain was used for transformation
	Cells were not properly heat shocked	Ensure that temp. was 42ºC & heat shock step took place for no more than 90 seconds.
	Incorrect antibiotics	Be certain that the correct antibiotic was used.
Low transformation efficiency	Not enough cells used for transformation	Pick more colonies from source plate (15 colonies @ 1-2 mm width per 500µl $CaCl_2$)
	Source plates were incubated for more than 20 hours	Important that source cells grow no longer than 20 hrs. Refrigerate plates after 20 hrs if necessary. Do not use source plates that have been incubated longer than 24 hours, refrigerated or not).
	Experimental plates too old	Prepare transformation plate and use shortly after preparation
	Cells not well resuspended in $CaCl_2$	Completely resuspend the cells in the $CaCl_2$, leaving no cell clumps (vortex or mix vigorously to fully resuspend cells). Cell suspension should be cloudy.
	$CaCl_2$ solution not cold enough	Pre-chill $CaCl_2$ before adding cells to the $CaCl_2$
	Cell solution not cold enough	Extend incubation of cells in $CaCl_2$ + DNA on ice (extra 10-15 min. but not more than 30 min). This allows more DNA to adhere to outside of bacterial cell).
	Too much or too little plasmid DNA added to cell suspension	Ensure that correct volume of plasmid was added to the transformation tube. If using micropipets, make sure students practice using pipets.
	Cells were not properly heat shocked	Ensure that temperature was 42ºC and that heat shock step took place for no more than 90 seconds.
	Antibiotics were degraded prior to pouring plates	Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Incorrect concentration of antibiotics in plates	Ensure that the correct concentration of antibiotic was used