Troubleshooting Guide forElectroEB10B Competent Cells

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Transformation efficiencies lower than expected	There may be impurities present in the DNA.	Ensure that the DNA does not contain any protein, detergents or ethanol.
	There may be too much DNA.	Transformation efficiencies are calculated using 10pg pUC19. Transformation efficiencies will decrease with increasing amounts of DNA; however, total numbers of colonies will increase with the amount of DNA.
	The cells may have been improperly handled or stored.	• Cells should be thawed in ice and used immediately. Do not refreeze cells and do not vigorously mix cells by vortexing. Cells must be stored at -80°C to guarantee stability. • Transformation should be done according to the recommended protocol to ensure the guaranteed transformation efficiency.
	You may be using an extremely large plasmid.	Transformation efficiencies are calculated using pUC19. Extremely large plasmids result in lower transformation efficiencies.
Few or no colonies seen after ligation	You may have too little DNA in your ligation mix.	Increase the amount of vector and/or insert in your ligation.
	The ligation may have been inefficient.	Double check the cloning strategy, enzymes and concentrations of both vector and insert. Set up a control for each step of the cloning procedure.
	You may have the wrong antibiotic concentration.	Some plasmids with very low copy number require lower antibiotic concentrations than standard high copy number plasmids. Check the antibiotic concentration that is optimal for your vector.
	You may have restriction enzymes or phosphatase remaining in your ligation mix.	Make sure that you inactivate or eliminate any trace of restriction enzymes or phosphatase before ligating your DNA.
	You may have diluted your transformation too much.	After ligation, calculated transformation efficiencies are usually much lower than transformation efficiencies calculated with supercoiled DNA. You may plate most of your transformations to ensure that you get colonies and dilute the rest to ensure that you obtain single colonies.

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Problems with blue and white screening	You may have incorrect amounts of X-gal and/or IPTG added to your agar plates.	Agar plates should include the appropriate antibiotic, 40ug/ml X-gal and 1mM IPTG.
Problems with arcing	You may be providing a conductive environment.	Avoid conductive ions by lowering the ratio of DNA-to-cells in the sample. Always avoid air bubbles and condensation that can accumulate on the electroporation cuvette.
	You may be using unclean DNA or too much DNA	Precipitate or dilute DNA before electroporation.
Low field strength	You may be using improper electroporation cuvettes.	Because field strength is critical, the choice of cuvette is important. In order to achieve the proper pulse, we suggest a cuvette of 1mm.
	You may be using improper conditions	Recheck that the equipment is providing the correct electroporation conditions.
Satellite colonies	The antibiotic concentration may be incorrect.	Check the antibiotic concentration added to the plates.
	The ampicillin may be degraded.	Make sure that ampicillin was not added to the medium when it was still too hot and that it was properly stored. Use carbanicillin, an antibiotic similar to ampicillin with improved stability. Check that plates are not more than 1 month old or have been stored above 4°C.
	You may have too many colonies in the plate.	Plate the transformation at a higher dilution.
	You may have incubated the plates too long.	Incubate the plates overnight (16-18 hours) at 37°C or for 2-3 days at room temperature in the case of temperature-sensitive plasmids.

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For additional troubleshooting assistance, please contact Edge BioSystems at:

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