Troubleshooting Guide for Acella™ Chemically Competent Cells, Acella™ pLysS Chemically Competent Cells

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Transformation efficiencies lower than expected	There may be DNA impurities present.	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 number of clones 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 a very large plasmid.	Transformation efficiencies are calculated using pUC19. Larger plasmids will result in lower transformation efficiencies.
	The ligation may have been inefficient.	Double check the cloning strategy, enzymes and concentrations of vector and insert. Set up a control for each step of the cloning procedure.
	You may have too little DNA in your ligation mix.	Increase the amount of vector and/or insert in your ligation.
	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 the wrong antibiotic concentration.	Some plasmids with very low copy number require lower antibiotic concentrations than standard high copy number plasmids. Verify the antibiotic concentration is optimal for your vector.
	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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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 one 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.
Little or no protein expression detected following induction	The vector may have the wrong promoter or there may be a cloning design problem.	• Ensure that the promoter is compatible with the T7 RNA polymerase-based expression systems. • Ensure that the gene of interest has an initiation codon or it is in the correct frame with the fusion tag and stop elements. Sequence the construct to ensure that no undesired changes have been introduced during the cloning steps.
	You may not have added any or enough IPTG to induce protein expression.	Check the induction requirements for your vector.
	The protein does not express well in *E. coli*.	Use a different expression system.

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Insoluble protein	Temperature, IPTG concentration, fusion tags and denaturing conditions can affect solubility.	• Lower the temperature during induction. • Lower the IPTG concentration. • Use a different fusion tag. • Use denaturing conditions to solubilize the protein.

For additional troubleshooting assistance, please contact Edge BioSystems at:

Telephone: (301) 990-2685 or (800) 326-2685	Fax: (301) 990-0881
Email: info@edgebio.com	Web: http://www.edgebio.com