Important note on the medium type and plating procedure

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ABSTRACT

Important Information for the two tetA dual selection protocols "Gene knockout strategy" and "Tuning the expression of native genes".

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ABSTRACT

Important Information for the two tetA dual selection protocols "Gene knockout strategy" and "Tuning the expression of native genes".

- While working with *tetA*, we made some important observations to ensure its functionality. In M9 medium, the concentration of $NiCl_2$ that is necessary to ensure counterselection is 50 µM. When we tried to assay the nickel sensitivity in LB medium, required concentrations to inhibit growth were as high as 2 mM. We speculate that differences in osmolarity between the different media might be responsible for the varying sensitivity of *tetA* harboring cells towards $NiCl_2$. Since LB medium is undefined and its composition can vary between batches, we recommend using M9 medium for more reproducible results.

- Further, for counterselection it is important to wash the cells in sterile water before they can be plated on $NiCl_2$ to remove traces of LB medium, otherwise efficiencies decrease. Before plating, cells need to be recovered overnight before they can be plated on $NiCl_2$ to allow the cell population without the membrane protein TetA to grow. Recovery of 4 h is also possible but with decreased efficiencies. Lastly, plates should not be incubated for longer than three days, since background colonies that still contain *tetA* appear on the plates. This background could be explained by the presence of nickel efflux pumps in *E. coli* that are possibly upregulated after a prolonged incubation. Nonetheless, even if background colonies appear, correct colonies are easily distinguishable based on their larger size.

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