As single colony of WT Keio and ybeX deletion strains were grown at 37°C 210rpm shaking overnight. Peptide based media (PBM) supplemented with 100µM MgCl2 was used for the overnight cultures. Following morning the culture density according to the OD600 measurement was 7.7-8.3 (10x dilution measurement). I recommend to do 20x dilution of the overnight cultures for determining the saturated cultures density because when WT culture was diluted the media had the final OD600 0.121, meaning that the overnight culture was measured wrongly. The lag phase for WT was 40-60 min. ybeX deletion strain had longer lag phase, up to 4 hours. The actual experiment were done in PBM without addition of magnesium.

PBM – 10g/L Peptone, 3% Casein hydrolysate, 0.5x MOPS pH 7.2

The first sample collection performed when the culture density reached 0.5 50mL of the cells were cooled down on ice for max. 5min and the cells were pelleted at 9000rpm 4min at +4C (Eppendorf 5810F´R fixed angle rotor) and the cells were flash frozen in liqude nitrogen and stored at -80C.