Stock culture heterogeneity rather than new mutational variation complicates short-term cell physiology studies of *Escherichia coli* K-12 MG1655 in continuous culture

By: Ranno Nahku, Karl Peebo, Kaspar Valgepea, Jeffrey E. Barrick, Kaarel Adamberg and Raivo Vilu

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. S1. Reproducibility of the *glyA* (serine hydroxymethyltransferase) subpopulation at the end (D=0.48 h^{-1}) of three A-stat experiments. Mixed population samples were Sanger-sequenced to detect a predicted subpopulation with a single-nucleotide polymorphism (SNP) in *glyA* (G→A). The genome position of the SNP is indicated with a red rectangle, where the green peak corresponds to the *glyA* subpopulation. The negative control is a single colony of stock culture not containing the mutation in *glyA*. A-stat 1 is the experiment that was analysed with high-throughput (HT) DNA sequencing.

Supplementary Fig. S2. Reproducibility of the *cspH/cspG* (stress protein, member of the CspA family/cold-shock protein) subpopulation at the end (D=0.48 h^{-1}) of three A-stat experiments. Mixed population samples were Sanger-sequenced to detect a predicted subpopulation with an SNP in the intergenic region between *cspH* and *cspG* (T→A). The genome position of the SNP is indicated with a red rectangle, where the green peak corresponds to the *cspH/cspG* subpopulation. The negative control is a single colony of stock culture not containing the mutation in *cspH/cspG*. A-stat 1 is the experiment that was analysed with HT DNA sequencing.

Supplementary Fig. S3. Reproducibility of the *betA* (choline dehydrogenase) subpopulation at the end (D=0.48 h^{-1}) of three A-stat experiments. Mixed population samples were Sanger-sequenced to detect a predicted subpopulation with an SNP in *betA* (C→T). The genome position of the SNP is indicated with a red rectangle, where the red peak corresponds to the *betA* subpopulation. The negative control is a single colony of stock culture not containing the mutation in *betA*. A-stat 1 is the experiment that was analysed with HT DNA sequencing.

Supplementary Fig. S4. Presence of the *dppD* (ATP-binding component of the dipeptide ABC transporter) subpopulation in the stock culture that was used in all A-stat experiments. The mixed population sample was Sanger-sequenced to detect a predicted subpopulation with an SNP in *dppD* (G→A). The genome position of the SNP is indicated with a red rectangle, where the green peak corresponds to the *dppD* subpopulation. The negative control is a single colony of stock culture not containing the mutation in *dppD*.

Supplementary Fig. S5. Presence of the *yahE* (predicted protein) subpopulation in the stock culture that was used in all A-stat experiments. A mixture of four single colonies was Sanger-sequenced to detect a predicted subpopulation with an SNP in *yahE* (T→C). The genome position of the SNP is indicated with a red rectangle, where the blue peak corresponds to the *yahE* subpopulation. The negative control is a single colony of stock culture not containing the mutation in *yahE*.

Supplementary Fig. S6. Rejection of the *allD* (ureidoglycolate dehydrogenase) and *recB* (exonuclease V, beta subunit) subpopulations in the stock culture that was used in all A-stat experiments. The stock culture was Sanger-sequenced to detect predicted subpopulations with either an SNP in *allD* (A→G) or an SNP in *recB* (T→G). The genome position of each SNP is indicated with a red rectangle. A black peak is expected for the SNP in both cases.