Genetic backgrounds of each *Escherichia coli* strain used in The ST²OOL Project

DH5α (Derived from *E. coli* K-12 strain)

Ref: http://wiki.chem.virginia.edu/ColumbusLab/images/0/03/E_coli_host_strains_genotypes.pdf

F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dl*acZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(rK- mK+), λ–

F-: This strain does not carry the F plasmid (DNA plasmid called Fertility Factor or Sex Factor).

endA1: This strain lacks Endonuclease I (non-specific digestion) for cleaner preparations of DNA and better results in downstream applications.

glnV44: In this strain a suppression of amber (UAG) stop codons (required for some phage growth) by insertion of glutamine was carried out.

thi-1: This strain requires thiamine (thiamine auxotroph, cannot produce its own thiamine).

recA1: RecA is a protein used by *E. coli* to repair and maintain DNA. RecA1 is an inactivated form of RecA. RecA1 is deficient in all known function of the RecA gene specifically in ATPase activity, binding with DNA in the presence of ATP, and changing conformation in the presence of ATP and repressor cleavage. Cells of this genotype are UV sensitive due deficiencies in DNA repair mechanisms. **Why select this mutation in *E. coli* lab strains?** For reduced occurrence of unwanted recombination in cloned DNA.

Ref: http://2011.igem.org/RecA_Project

relA1: *Escherichia coli* (relA1) develop a lipid structure that radically differs from the wild type and is characterized by accumulation of neutral phospholipids and saturated fatty acids. The membrane is more fragile with respect to sonication and osmotic chock. Protein leakage and cell lysis is, however, lower in the mutant most likely due to the increased amounts of saturated fatty acids, which might be a possible strategy to overcome the reduced amounts of membrane-strengthening cardiolipinrelaxed phenotype; it also permits RNA synthesis in absence of protein synthesis.

Ref: http://www.ncbi.nlm.nih.gov/pubmed/16718493

gyrA96: The strain has a mutation in DNA gyrase which conveys nalidixic acid resistance (the gyrase mutation gyrA96 gives *E. coli* a ccdB resistance).

Ref: http://parts.igem.org/Part:BBa_P1010:Experience

deoR: The transcriptional repressor DeoR, for "Deoxyribose Regulator", is involved in the negative expression of genes related to transport and catabolism of deoxyribonucleoside nucleotides. This is a regulatory gene that allows constitutive expression of deoxyribose synthesis genes which allows uptake of large plasmids.

http://biocyc.org/ECOLI/NEW-IMAGE?type=GENE&object=EG10223

nupG: NupG is one of two high-affinity nucleoside transporters in *E. coli*. As the one described above, this regulatory gene allows constitutive expression of deoxyribose synthesis genes and permits uptake of large plasmids.

http://ecocyc.org/ECOLI/NEW-IMAGE?type=ENZYME&object=NUPG-MONOMER

Φ80dlacZΔM15: Allows for blue white screening.

Δ(lacZYA-argF)U169: The strains that have this deletion in their genome exhibit a high level of resistance to hydrogen peroxide compared with its undeleted parent.

Ref: http://www.ncbi.nlm.nih.gov/pubmed/8113168

hsdR17 (rK- mK+): The hsdR17 mutation eliminates the restriction endonuclease of the restriction-modification system, EcoKI, so DNA lacking the EcoKI methylation will not be degraded (prevents cleavage of heterologous DNA by an endogenous endonuclease), but the corresponding methylase gene (hsdM) is present. To sum up, this strain is restriction deficient but still methylate's DNA.

λ–: Lambda lysogen deletion.

DH5a is an Hoffman-Berling 1100 strain derivative (Meselson68).

Ref: http://www.pnas.org/content/87/12/4645.full.pdf

http://www.nature.com/nature/journal/v217/n5134/abs/2171110a0.html

http://openwetware.org/wiki/E._coli_genotypes

XL1Blue (Derived from *E. coli* K-12 strain)

Ref: http://wiki.chem.virginia.edu/ColumbusLab/images/0/03/E_coli_host_strains_genotypes.pdf

endA1 gyrA96(nalR) thi-1 recA1 relA1 lac glnV44 F'[::Tn10 proA^{+}B^{+} lacI^{q} Δ(lacZ)M15] hsdR17(rK- mK+)

endA1: This strain lacks Endonuclease I (non-specific digestion) for cleaner preparations of DNA and better results in downstream applications.

gyrA96: The strain has a mutation in DNA gyrase which conveys nalidixic acid resistance (the gyrase mutation gyrA96 gives *E. coli* a ccdB resistance).

Ref: http://parts.igem.org/Part:BBa_P1010:Experience

thi-1: This strain requires thiamine (thiamine auxotroph, cannot produce its own thiamine).

recA1: RecA is a protein used by *E. coli* to repair and maintain DNA. RecA1 is an inactivated form of RecA. RecA1 is deficient in all known function of the RecA gene specifically in ATPase activity, binding with DNA in the presence of ATP, and changing conformation in the presence of ATP and repressor cleavage. Cells of this genotype are UV sensitive due deficiencies in DNA repair mechanisms. **Why select this mutation in *E. coli* lab strains?** For reduced occurrence of unwanted recombination in cloned DNA.

Ref: http://2011.igem.org/RecA_Project

relA1: *Escherichia coli* (relA1) develop a lipid structure that radically differs from the wild type and is characterized by accumulation of neutral phospholipids and saturated fatty acids. The membrane is more fragile with respect to sonication and osmotic chock. Protein leakage and cell lysis is, however, lower in the mutant most likely due to the increased amounts of saturated fatty acids, which might be a possible strategy to overcome the reduced amounts of membrane-strengthening cardiolipinrelaxed phenotype; it also permits RNA synthesis in absence of protein synthesis.

Ref: http://www.ncbi.nlm.nih.gov/pubmed/16718493

lac: Deletion of the entire lac operon.

glnV44: In this strain a suppression of amber (UAG) stop codons (required for some phage growth) by insertion of glutamine was carried out.

F'[…]: Host contains an F' episomal plasmid with the stated features.

- **Tn10**: Transposon normally carrying tetracycline resistance.
  Ref: http://www.ncbi.nlm.nih.gov/pubmed/6260375
- **proA^{+}B^{+}**: The genes proA and proB encoding the first two enzymes of the proline biosynthetic sequence in *Escherichia coli*.

- **lacI^q**: Overproduction of the lac repressor protein (-35 site in promoter upstream of lacI is mutated from GCGCAA to GTGCAA).
- **Δ(lacZ)M15**: This E. *coli* strain carries the lacZ deletion mutant which contains the ω-peptide: a mutant β-galactosidase derived from the M15 strain of *E. coli* that has its N-terminal residues 11—41 deleted and is unable to form a tetramer so it is inactive. The plasmids used in transformation process carry the lacZa sequence which encodes the first 59 residues of β-galactosidase (the α-peptide). Neither is functional by itself, however, when the two peptides are expressed together they form a functional β-galactosidase enzyme. Required for blue/white selection on XGal plates.

hsdR17(rK- mK+): The hsdR17 mutation eliminates the restriction endonuclease of the restriction-modification system, EcoKI, so DNA lacking the EcoKI methylation will not be degraded (prevents cleavage of heterologous DNA by an endogenous endonuclease), but the corresponding methylase gene (hsdM) is present. To sum up, this strain is restriction deficient but still methylate's DNA.

Other interesting features:

Permits bacteriophage M13 superinfection.

JM109 (Derived from *E. coli* K-12 strain)

Ref: http://wiki.chem.virginia.edu/ColumbusLab/images/0/03/E_coli_host_strains_genotypes.pdf

http://cgsc.biology.yale.edu/Strain.php?ID=74916

endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB^{+} Δ(lac-proAB) e14- [F' traD36 proAB^{+} lacI^{q} lacZΔM15] hsdR17($r_K^-m_K^+$)

endA1: This strain lacks Endonuclease I (non-specific digestion) for cleaner preparations of DNA and better results in downstream applications.

glnV44: In this strain a suppression of amber (UAG) stop codons (required for some phage growth) by insertion of glutamine was carried out.

thi-1: This strain requires thiamine (thiamine auxotroph, cannot produce its own thiamine).

relA1: *Escherichia coli* (relA1) develop a lipid structure that radically differs from the wild type and is characterized by accumulation of neutral phospholipids and saturated fatty acids. The membrane is more fragile with respect to sonication and osmotic chock. Protein leakage and cell lysis is, however, lower in the mutant most likely due to the increased amounts of saturated fatty acids, which might be a possible strategy to overcome the reduced amounts of membrane-strengthening cardiolipinrelaxed phenotype; it also permits RNA synthesis in absence of protein synthesis.

Ref: http://www.ncbi.nlm.nih.gov/pubmed/16718493

gyrA96: The strain has a mutation in DNA gyrase which conveys nalidixic acid resistance (the gyrase mutation gyrA96 gives *E. coli* a ccdB resistance).

Ref: http://parts.igem.org/Part:BBa_P1010:Experience

recA1: RecA is a protein used by *E. coli* to repair and maintain DNA. RecA1 is an inactivated form of RecA. RecA1 is deficient in all known function of the RecA gene specifically in ATPase activity, binding with DNA in the presence of ATP, and changing conformation in the presence of ATP and repressor cleavage. Cells of this genotype are UV sensitive due deficiencies in DNA repair mechanisms. **Why select this mutation in *E. coli* lab strains?** For reduced occurrence of unwanted recombination in cloned DNA.

Partly restriction-deficient; good strain for cloning repetitive DNA (RecA–).

Ref: http://2011.igem.org/RecA_Project

mcrB^{+}: McrB (modified cytosine restriction) system of *E. coli* interferes with incoming DNA containing methylcytosine. DNA from many organisms, including all mammalian and plant DNA, is expected to be sensitive, and this could interfere with cloning experiments.

Ref: http://www.ncbi.nlm.nih.gov/pubmed/2831502

Δ(lac-proAB): Deletion of the entire lac operon and also of the genes proA and proB that encode the first two enzymes of the proline biosynthetic sequence in *Escherichia coli* (so the cell requires proline for its growth).

e14-: Absence of the prophage like element containing mcrA gene (the McrA system, as the McrB described above, is involved in the restriction of DNA sequences containing methylated cytosine at particular sequences).

F'[…]: Host contains an F' episomal plasmid with the stated features.

- **traD36**: Mutation eliminating transfer factor; prevents transfer of F plasmid.
  Ref: *E. coli* Genetic Resources at Yale. CGSC, The Coli Genetic Stock Center
- **proA^{+}B^{+}**: The genes proA and proB encoding the first two enzymes of the proline biosynthetic sequence in *Escherichia coli*.
- **lacI^{q}**: Overproduction of the lac repressor protein (-35 site in promoter upstream of lacI is mutated from GCGCAA to GTGCAA).
- **lacZΔM15**: This *E. coli* strain carries the lacZ deletion mutant which contains the ω-peptide: a mutant β-galactosidase derived from the M15 strain of *E. coli* that has its N-terminal residues 11—41 deleted and is unable to form a tetramer so it is inactive. The plasmids used in transformation process carry the lacZa sequence which encodes the first 59 residues of β-galactosidase (the α-peptide). Neither is functional by itself, however, when the two peptides are expressed together they form a functional β-galactosidase enzyme. Required for blue/white selection on XGal plates.

hsdR17($r_K^-m_K^+$): The hsdR17 mutation eliminates the restriction endonuclease of the restriction-modification system, EcoKI, so DNA lacking the EcoKI methylation will not be degraded (prevents cleavage of heterologous DNA by an endogenous endonuclease), but the corresponding methylase gene (hsdM) is present. To sum up, this strain is restriction deficient but still methylate's DNA.

Other interesting features:

Deficient in expression of the lon protease (ATP-dependent serine protease that mediates the selective degradation of mutant and abnormal proteins as well as certain short-lived regulatory proteins, including some antitoxins) due to IS186 transposon insertion. J Mairhofer 18:59, 24 March 2010 (CET)

Ref: http://www.uniprot.org/uniprot/P0A9M0

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0070516

DH10B (Derived from *E. coli* K-12 strain)

Ref: http://jb.asm.org/content/190/7/2597.full

F- endA1 recA1 galE15 galK16 nupG rpsL ΔlacX74 Φ80lacZΔM15 araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC) λ-

F-: This strain does not carry the F plasmid (DNA plasmid called Fertility Factor or Sex Factor).

endA1: This strain lacks Endonuclease I (non-specific digestion) for cleaner preparations of DNA and better results in downstream applications.

recA1: RecA is a protein used by *E. coli* to repair and maintain DNA. RecA1 is an inactivated form of RecA. RecA1 is deficient in all known function of the RecA gene specifically in ATPase activity, binding with DNA in the presence of ATP, and changing conformation in the presence of ATP and repressor cleavage. Cells of this genotype are UV sensitive due deficiencies in DNA repair mechanisms. **Why select this mutation in *E. coli* lab strains?** For reduced occurrence of unwanted recombination in cloned DNA.

Partly restriction-deficient; good strain for cloning repetitive DNA (RecA–).

Ref: http://2011.igem.org/RecA_Project

galE15: galE mutations are associated with high competence, increased resistance to phage P1 infection, and 2-deoxygalactose resistance. galE mutations block the production of UDP-galactose, resulting in truncation of LPS glycans to the minimal, "inner core". The exceptional competence of DH10B is thought to be a result of a reduced interference from LPS in the binding and/or uptake of transforming DNA. galE15 is a point mutation resulting in a Ser123 to Phe conversion near the enzyme's active site.

Ref: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC215500/pdf/jbacter00234-0372.pdf

galK16: Strains mutants in this gene cannot metabolize galactose and are resistant to 2-deoxygalactose.

nupG: NupG is one of two high-affinity nucleoside transporters in *E. coli*. As the one described above, this regulatory gene allows constitutive expression of deoxyribose synthesis genes and permits uptake of large plasmids.

http://ecocyc.org/ECOLI/NEW-IMAGE?type=ENZYME&object=NUPG-MONOMER

rpsL: Mutation in ribosomal protein S12. It confers streptomycin resistance.

ΔlacX74: Deletion of the entire lac operon as well as some flanking DNA.

Φ80dlacZΔM15: Allows for blue white screening.

araD139: Mutation in L-ribulose-phosphate 4-epimerase (arabinose metabolism blocked).

Δ(ara,leu)7697: Chromosomal deletion of the (b0059) b0060-b0079 genes. The strain lacks the leuLABCD operon (this operon encodes the enzymes responsible for the biosynthesis of leucine from valine), so DH10B is unable to grow on synthetic minimal medium.

Ref: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2293198/

mcrA: The McrA system is involved in the restriction of DNA sequences containing methylated cytosine at particular sequences

Δ(mrr-hsdRMS-mcrBC): DH10B *E. coli* strain lacks of the genes mrr (mutation in methyladenosine-specific restriction system which allows more efficient cloning of DNA containing methyladenines), hsdRMS (gene encoding the EcoKI restriction enzyme that attacks DNA not protected by adenine methylation) and mcrBC (gene encoding an endonuclease which cleaves DNA containing methylcytosine on one or both strands. McrBC does not act ypon unmethylated DNA).

λ-: Lambda lysogen deletion.

HB101 (Hybrid of *E. coli* K12 and *E. coli* B)

Ref: http://wiki.chem.virginia.edu/ColumbusLab/images/0/03/E_coli_host_strains_genotypes.pdf

https://cgsc2.biology.yale.edu/Strain.php?ID=130465

F- mcrB$^+$ mrr hsdS20(rB- mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20(SmR) glnV44 λ-

F-: This strain does not carry the F plasmid (DNA plasmid called Fertility Factor or Sex Factor).

mcrB$^+$: McrB (modified cytosine restriction) system of *E. coli* interferes with incoming DNA containing methylcytosine. DNA from many organisms, including all mammalian and plant DNA, is expected to be sensitive, and this could interfere with cloning experiments.

Ref: http://www.ncbi.nlm.nih.gov/pubmed/2831502

mrr: Mutation in methyladenosine-specific restriction system which allows more efficient cloning of DNA containing methyladenines.

hsdS20(rB- mB-): Restriction minus genotype which prevents cleavage of cloned DNA by endogenous restriction enzymes.

recA13: RecA is a protein used by *E. coli* to repair and maintain DNA. RecA1 is an inactivated form of RecA. RecA1 is deficient in all known function of the RecA gene specifically in ATPase activity, binding with DNA in the presence of ATP, and changing conformation in the presence of ATP and repressor cleavage. Cells of this genotype are UV sensitive due deficiencies in DNA repair mechanisms. RecA13 differs from RecA1 in the lower stability of inserts. **Why select this mutation in *E. coli* lab strains?** For reduced occurrence of unwanted recombination in cloned DNA.

Ref: http://2011.igem.org/RecA_Project

http://www.sciencedirect.com/science/article/pii/S0921877798000147

leuB6: This strains requires leucine.

ara-14: This strain is not able to metabolized arabinose.

proA2: proA gene encodes a gamma-glutamyl phosphate reductase (enzyme encoding one of the two enzymes of the proline biosynthesis pathway). So, the mutant in that gene cannot synthesize proline.

Ref: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC215281/pdf/jbacter00237-0209.pdf

http://www.ncbi.nlm.nih.gov/pubmed/16958849?dopt=Abstract

lacY1: This strain is deficient in lactose transport (a deletion of the lactose permease was carried out).

galK2: The mutants in this gene cannot metabolize galactose and are resistant to 2-deoxygalactose.

xyl-5: In this strain the xylose metabolism is blocked.

Ref: http://www.pnas.org/content/87/2/618.full.pdf

http://www.ncbi.nlm.nih.gov/pubmed/6330500

mtl-1: The HB101 strain has a mutation in a gene evolved in the mannitol metabolism pathway (the mannitol utilization is blocked).

rpsL20(SmR): Mutation in ribosomal protein S12 conveying streptomycin resistance.

glnV44: In this strain a suppression of amber (UAG) stop codons (required for some phage growth) by insertion of glutamine was carried out.

λ-: Lambda lysogen deletion.

BL21 [DE3] (Derived from *E. coli* B strain)

Ref: http://www.ncbi.nlm.nih.gov/pubmed/19765591

F– ompT gal dcm lon hsdSB(rB- mB-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])

F-: This strain does not carry the F plasmid (DNA plasmid called Fertility Factor or Sex Factor).

ompT: Mutation in outer-membrane protease that Improves yield of some recombinant proteins.

gal: Mutation in galactose metabolism. Blocks galactose utilization.

dcm: Cytosine methylation blocked (it makes DNA susceptible to cleavage at CC(A/T)GG sequences by some restriction enzymes).

lon: This strain is mutant for lon protease (ATP-dependent serine protease that mediates the selective degradation of mutant and abnormal proteins as well as certain short-lived regulatory proteins, including some antitoxins).

hsdSB(rB- mB-): Allows cloning of DNA without cleavage by endogenous restriction endonucleases.

λ(DE3[…]): λ prophage carrying the T7 RNA polymerase gene and $lacI^q$.

- **lacI**: Production of the lac repressor protein.
- **lacUV5-T7 gene**: The BL21(DE3) strain contains the T7 RNA polymerase gene controlled by the lacUV5 promoter (a strong promoter) in its chromosomal DNA.
- **ind1**: Mutant Ind1 resulted in a strong decrease in the prophage growth.
  Ref: http://dmm.biologists.org/content/6/5/1279.full
- **sam7**: The Sam7 lysis mutation makes the infectious phage particles remain intracellular.
  Ref: http://www.ncbi.nlm.nih.gov/pubmed/7763591
- **nin5**: Lysogenization frequency reduction due to the increase of Q gene activity.
  Ref: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC353291/pdf/jvirol00184-0011.pdf