E. coli genotypes

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Nomenclature & Abbreviations

A listed gene name means that gene carries a loss of function mutation, a Δ preceding a gene name means the gene is deleted. If a gene is not listed, it is not known to be mutated. Prophages present in wt K-12 strains (F, λ , e14, rac) are listed only if absent. E. coli B strains are naturally lon- and dcm-.

- **F** = Does not carry the F plasmid
- F+ = Carries the F plasmid. The cell is able to mate with F- through conjugation.
- **F'[]** = Carries an F plasmid that has host chromosomal genes on it from a previous recombination event. This cell can also mate with F- through conjugation. Chromosomal genes carried in the F plasmid are listed in brackets.
- $\mathbf{r}_{B/K}^{+/-}$ = The (B/K) defines the strain lineage. The +/- indicates whether the strain has or hasn't got the restriction system.
- $m_{B/K}^{+/-}$ = The (B/K) defines the strain lineage. The +/- indicates whether the strain has or hasn't got the modification (methylation) system.
- <u>hsdS</u> = Both restriction and methylation of certain sequences is deleted from the strain. If you transform DNA from such a strain into a wild type strain, it will be degraded.
- <u>hsdR</u> = For efficient transformation of cloned unmethylated DNA from PCR amplifications
- **INV()** = chromosomal inversion between locations indicated
- <u>ahpC</u> = mutation to alkyl hydroperoxide reductase conferring disulfide reductase activity
- **ara-14** = cannot metabolize arabinose
- <u>araD</u> = mutation in L-ribulose-phosphate 4-epimerase blocks arabinose metabolism
- <u>cycA</u> = mutation in alanine transporter; cannot use alanine as a carbon source
- <u>dapD</u> = mutation in succinyl diaminopimelate aminotransferase leads to
 - succinate or (lysine + methionine) requirement
- Δ (≠ chromosomal deletion of genes between the listed genes (may include unlisted genes!)
- <u>dam</u> = adenine methylation at GATC sequences exist; high recombination efficiency; DNA repair turned on

- **dcm** = cytosine methylation at second C of CCWGG sites exist. dam & dcm • are the default properties and always elided, while dam-or dcm-should be declare explicitly
- **DE3** = Lysogen that encodes T7 RNA polymerase. Used to induce expression in T7-driven expression systems
- **deoR** = regulatory gene that allows constitutive expression of deoxyribose synthesis genes; permits uptake of large plasmids. See Hanahan D, US Patent 4,851,348. ***This has been called into question, as the DH10B genome sequence revealed that it is deoR+. See Durfee08, PMID 18245285.
- dnaJ = one of the chaparonins inactivated; stabilizes some mutant • proteins
- **dut1** = dUTPase activity abolished, leading to increased dUTP concentrations, allowing uracil instead of thymine incorporation in DNA. Stable U incorporation requires ung gene mutation as well.
- **endA1** = For cleaner preparations of DNA and better results in • downstream applications due to the elimination of non-specific digestion by Endonuclease I
- (e14) = excisable prophage like element containing mcrAgene; present in K-12 but missing in many other strains
- 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/TOP10 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 -> Phe conversion near the enzyme's active site. See van Die, et al. PMID 6373734, Hanahan, et al. PMID 1943786, and EcoSal ISBN 1555811647. --Dcekiert 16:56, 23 January 2008 (CST)
- <u>**galk**</u> = mutants cannot metabolize galactose and are resistant to • 2-deoxygalactose. galK16 is an IS2 insertion ~170bp downstream of the galK start codon. See EcoSal ISBN 1555811647. --Dcekiert 16:56, 23 January 2008 (CST)
- **<u>galU</u>** = mutants cannot metabolize galactose
- gor = mutation in glutathione reductase; enhances disulphide bond formation
- **gInV** = suppression of amber (UAG) stop codons by insertion of
- glutamine; required for some phage growth
- gyrA96 = mutation in DNA gyrase; conveys nalidixic acid resistance
- gyrA462 = mutation in DNA gyrase; conveys resistance to ccdB colicin gene product
- **hfIA150** = protease mutation stabilizing phage cll protein; high
 - frequency of lysogenization by λ

- Δ(lac)X74=Deletion of the entire *lac* operon as well as some flanking DNA (complete deletion is Δcod-mhpF; see Mol.Micro., 6:1335, and J.Bact., 179:2573)
- **lacl**q or **lacl**q = overproduction of the lac repressor protein; -35 site in promoter upstream of *lacl* is mutated from GCGCAA to GTGCAA
- **laclo1** = overproduction of the lac repressor protein; contains a 15 bp deletion to create optimal -35 site in promoter upstream of *lacl*
- **lacY** = deficient in lactose transport; deletion of lactose permease (M protein)
- **lacZ\DeltaM15** = partial deletion of the lacZ gene that allows α complementation of the β -galactosidase gene; required for blue/white selection on XGal plates. Deletes the amino portion of lacZ (aa 11-41).
- LAM-or λ-=lambda lysogen deletion; approximate map location: 17.40; information from CGSC *---Karmella 13:02, 21 October 2012 (EDT):
- LamR = mutation in malT1 conferring lambda resistance; synonym malT1(LamR) [1] *---Karmella 13:35, 21 October 2012 (EDT):
- <u>leuB</u> = requires leucine
- Δlon = deletion of the lon protease
- <u>malA</u> = cannot metabolize maltose
- <u>mcrA</u> = Mutation eliminating restriction of DNA methylated at the sequence CmCGG (possiblymCG). Carried on the e14 prophage (q.v.)
- <u>mcrB</u> = Mutation eliminating restriction of DNA methylated at the sequence RmC
- <u>metB</u> = requires methionine
- <u>metC</u> = requires methionine
- <u>mrr</u> = Mutation eliminating restriction of DNA methylated at the sequence CmAG or GmAC
- **mtIA** = cannot metabilize mannitol
- (Mu) = Mu prophage present. Mu δ means the phage is defective.
- mutS mutation inhibits DNA repair of mismatches in unmethylated newly synthesized strands
- <u>nupG</u> = same as <u>deoR</u>
- <u>ompT</u> = mutation in outer membrane protein protease VII, reducing proteolysis of expressed proteins
- (P1) = Cell carries a P1 prophage. Cells express the P1 restriction system.
- (P2) = Cell carries a P2 prophage. Allows selection against Red+ Gam+
- Λ
- (φ80) = Cell carries the lambdoid prophage φ80. A defective version of this phage carrying lacZM15 deletion (as well as wild-type lacl, lacYA, and flanking sequences) is present in some strains. The φ80 attachment site is just adjacent totonB.
- <u>pLysS</u> = contains pLysS plasmid carrying chloramphenicol resistance and phage T7 lysozyme, effective at attenuating activity of T7 RNA

polymerase, for better inhibition of expression under non-induced conditions. The sequence can be found here.

- proA/B = requires proline
- **recA1** = For reduced occurrence of unwanted recombination in cloned DNA; cells UV sensitive, deficient in DNA repair
- **recA13** = as for recA1, but inserts less stable.
- <u>recBCD</u> = Exonuclease V; mutation in RecB or RecC reduces general recombination by a factor of 100; impaired DNA repair; UV sensitive, easier propagation of inverted repeats
- **<u>recJ</u>** Exonuclease involved in alternate recombination
- relA = relaxed phenotype; permits RNA synthesis in absence of protein synthesis
- <u>rha</u> = blocked rhamose metabolism
- <u>rnc</u> = encodes RnaseIII (rnc-14 is a common null mutant)
- <u>rne</u> = encodes RnaseE (rne-3071 is a common temperature sensitive mutant)
- <u>rpsL</u> = mutation in ribosomal protein S12 conveying streptomycin resistance; also called strA, rpsL135(strR), strA135[2]*---Karmella 13:27, 21 October 2012 (EDT):
- **sbcBC** = Exol activity abolished; usually present in recBC strains; recombination proficient, stable inverted repeats
- <u>sr1</u> = cannot metabolize sorbitol
- supE = glnV
- supF = tyrT
- <u>thi</u> = requires thiamine
- <u>thyA</u> = requires thymidine
- **Tn10** = transposon normally carrying Tetracyclineresistance
- **Tn5** = transposon normally carrying Kanamycin resistance
- <u>tonA</u> = Mutation in outer membrane protein conveying resistance to phage T1 and phage T5
- <u>traD</u> = Mutation eliminating transfer factor; prevents transfer of F plasmid
- <u>trxB</u> = mutation in thioredoxin reductase; enhances disulphide bond formation in the cytoplasm
- tsx = outer membrane protein mutation conveying resistance to phage T6 and colicin K
- <u>tyrT</u> = suppression of amber (UAG) stop codons by insertion of tyrosine; needed for some phage infection such as λ gt11.
- **ung1** = allows uracil to exist in plasmid DNA
- **xyI-5** = blocked xylose metabolism
- **SmR** = Streptomycin resistance

Methylation Issues in E. coli

- Type I methylation systems:
 - $_{\odot}$ *E. coli* K-12 restricts DNA which is **not** protected by adenine methylation at sites AA*C[N₆]GTGC or GCA*C[N₆]GTT, encoded by the hsdRMS genes(EcoKI). Deletions in these genes removes either the restriction or methylation or both of these functions.
 - $_{\circ}$ *E. coli* B derivative strains contain an hsdRMS system (EcoBI) restricting and protecting the sequence TGA*[N₈]TGCT or AGCA*[N₈]TCA.
- The mcrA gene (carried on the e14 prophage) restricts DNA which is methylated in CmCWGG or mCG sequences (methylation by the dcm gene product).
- The mcrBC genes restrict RmC sequences.
- The **mrr** gene product restricts adenine methylated sequences at CAG or GAC sites.
- E. coli methylates the adenine in GATC (and the corresponding A on the opposite strand) with the **dam** gene product.
- M.EcoKII methylates the first A at the palindromic site ATGCAT (as well as the corresponding A on the opposite strand), see (Kossykh VG (2004) J. Bact 186: 2061-2067 PMID 15028690) Note that this article has been retracted; the retraction appears to center on textual plagarism, not experimental results. The homology to AvaIII is real. I think I believe it. tk 20:28, 9 December 2005 (EST). Rich Roberts reports: "We have tried ourselves to detect activity with this gene product and cannot detect any methyltransferase activity. In our case we used antibodies able to detect N6-methyladenine or N4 methylcytosine in DNA. The ones we have are very sensitive and should have been able to detect 5 methyl groups in the whole E. coli chromosome. Nothing was detected in an over expressing strain."
- For additional information see E. <u>coli restriction-modification system and</u> the NEB technical information on methylation.

Commonly used strains

AG1

endA1 recA1 gyrA96 thi-1 relA1 glnV44 hsdR17($r_{K^-}m_{K^+}$)

AB1157

thr-1, araC14, leuB6(Am), Δ(gpt-proA)62, lacY1, tsx-33, qsr'-0, glnV44(AS), galK2(Oc), LAM-, Rac-0, hisG4(Oc), rfbC1, mgl-51, rpoS396(Am), rpsL31(strR), kdgK51, xylA5, mtl-1, argE3(Oc), thi-1

• Bachmann BJ: Derivation and genotypes of some mutant derivatives of Escherichia coli K-12.

Escherichia coli and Salmonella typhimurium. Cellular and Molecular Biology (Edited by: F C Neidhardt J L Ingraham KB Low B Magasanik M Schaechter H E Umbarger). Washington, D.C., American Society for Microbiology 1987, 2:1190-1219.

See <u>CGSC#1157</u>

B2155

thrB1004 pro thi strA hsdsS lacZD M15 (F`lacZD M15 laclq traD36 proA+ proB+) D dapA::erm (Erm^r) pir::RP4 [::kan (Km^r) from SM10]

An *E. coli* strain carrying the *pir* sequence required for maintenance of plasmids containing R6K ori. Also, this strain is auxotrophic for DAP (diaminopimelic acid - a lysine precursor). The auxotrophy helps in removal of this strain from a bi-parental mating setup after conjugation.

Ref: Maintenance of broad-host-range incompatibility group P and group Q plasmids and transposition of Tn5 in Bartonella henselae following conjugal plasmid transfer from Escherichia coli Dehio, C. & Meyer, M. (1997) J. Bacteriol. 179, 538–540

BL21

E. coli B F- dcm ompT hsdS($r_{B}^{-} m_{B}^{-}$) gal [malB+]_{K-12}(λ s)

- The "malB region" was transduced in from the K-12 strain W3110 to make the strain Mal+λs. See Studier et al. (2009) J. Mol. Biol. 394(4), 653 for a discussion of the extent of the transfer.
- Stratagene E. coli Genotype Strains



F-ompT gal dcm lon $hsdS_B(r_{B^-}m_{B^-})$ araB::T7RNAP-tetA

- an *E. coli* B strain carrying the T7 RNA polymerase gene in the *araB* locus of the *araBAD* operong.
 - Transformed plasmids containing T7 promoter driven expression are repressed until L-arabinose induction of T7 RNA polymerase.

- Maximal expression is lower than that of BL21(DE3)(customer support 10/2012)
- Derived from BL21.
- See the <u>product page</u> for more information.
- Brian Caliendo (Voigt lab) reported trouble getting the Datsenko and Wanner (2000) plasmid pCP20 to transform into this strain, when other strains transformed fine. Cause is unknown.

BL21(DE3)

F- ompT gal dcm lon hsdS_B($r_{B^-} m_{B^-}$) λ (DE3 [lacl lacUV5-T7 gene 1 ind1 sam7 nin5])

- an E. col B strain with DE3, a λ prophage carrying the T7 RNA polymerase gene and laclq
- Transformed plasmids containing T7 promoter driven expression are repressed until IPTG induction of T7 RNA polymerase from a lac promoter.
- Derived from B834 (Wood, 1966) by transducing to Met+.
- See the original Studier paper or the summary in Methods in Enzymology for more details.
- Whole genome sequence available [3]

BL21 (DE3) pLysS

F-ompT gal dcm lon hsdS_B($r_{B} - m_{B} - \lambda$ (DE3) pLysS(cmR)

- pLysS plasmid chloramphenicol resistant; grow with chloramphenicol to retain plasmid
- Chloramphenicol resistant
- The pLysS plasmid encodes T7 phage lysozyme, an inhibitor for T7 polymerase which reduces and almost eliminates expression from transformed T7 promoter containing plasmids when not induced.
- see Moffatt87 for details of pLysS and pLysE plasmids

BNN93

F-tonA21thi-1thr-1leuB6lacY1glnV44rfbC1fhuA1mcrBe14-(mcrA-)

hsdR(r_κ-m_K+) λ-

• Some C600 strains are really BNN93

BNN97

• BNN93 (λgt11)

A λ gt11 lysogen producing phage at 42C 0

BW26434, CGSC Strain #7658

 Δ (araD-araB)567, Δ (lacA-lacZ)514(::kan), lacIp-4000(lacIq), λ -, rpoS396(Am)?, rph-1, Δ (rhaD-rhaB)568, hsdR514

- This information is from a printout sent by the *E. coli* Genetic Stock Center with the strain.
- B.L. Wanner strain
- rph-1 is a 1bp deletion that results in a frameshift over last 15 codons and has a polar effect on pyrE leading to suboptimal pyrimidine levels on minimal medium. (Jensen 1993 J Bact. 175:3401)
- Δ (araD-araB)567 was formerly called Δ araBAD_{AH33} by Datsenko and Wanner
- Am = amber(UAG) mutation
- Reference: Datsenko and Wanner, 2000, PNAS, 97:6640

NOTE:

- This promoter driving the expression of lacl was sequenced in this strain using a primer in mhpR (upstream of lacl) and a primer in the opposite orientation in lacl. The lac promoter was found to be identical to wildtype. Thus, the -35 sequence was GCGCAA not GTGCAA as expected with laclq. Therefore this strain (or at least the version obtained from the *E. coli* Genetic Stock Center) does NOT appear to be laclq. According to Barry Wanner, this is an unexpected result. -Reshma 13:19, 5 May 2005 (EDT)
- "We have now confirmed that BW25113, BW25141, and BW26434 are all lacl+, and not laclq. We thank you for alerting us to the error with respect to BW26434. Apparently, the lacl region was restored to wild-type in a predecessor of BW25113." (from Barry Wanner November 18, 2005)
- The genotype has been corrected at the CGSC

C600

F-tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1λ-

- There are strains circulating with both e14+(mcrA+) and e14-(mcrA-)
- General purpose host
- See CGSC#3004
- References: Appleyard, R.K. (1954) Genetics 39, 440; Hanahan, D. (1983) J. Mol. Biol. 166, 577.

C600 hflA150 (Y1073, BNN102)

F-thi-1 thr-1 leuB6 lacY1 tonA21 glnV44 λ-hflA150(chr::Tn10)

- host for repressing plaques of λ gt10 when establishing cDNA libraries
- Reference Young R.A. and Davis, R. (1983) Proc. Natl. Acad. Sci. USA 80, 1194.
- Tetracycline resistance from the Tn10 insertion

CSH50

F-λ-ara Δ (lac-pro) rpsL thifimE::IS1

- See CGSC#8085
- References: Miller, J.H. 1972. Expts.in Molec. Genetics, CSH 0:14-0; Blomfeld et al., J.Bact. 173: 5298-5307, 1991.

D1210

HB101 lacl_q lacY+

DB3.1

F- gyrA462 endA1 glnV44 Δ (sr1-recA) mcrB mrr hsdS20(r_B-, m_B-) ara14 galK2 lacY1 proA2 rpsL20(Smr) xyl5 Δleu mtl1

- useful for propagating plasmids containing the ccdB operon.
- gyrA462 enables ccdB containing plasmid propagation
- streptomycin resistant •
- appears to NOT contain lacl (based on a colony PCR) -- Austin Che • 16:16, 18 June 2007 (EDT)
- 1. Bernard P and Couturier M. . pmid:1324324. PubMed HubMed [Bernard-JMolBiol-1992]
- 2. Miki T, Park JA, Nagao K, Murayama N, and Horiuchi T. pmid:1316444. PubMed HubMed [Miki-JMolBiol-1992]

All Medline abstracts: PubMed HubMed

DH1

endA1 recA1 gyrA96 thi-1 glnV44 relA1 hsdR17($r_{\kappa} m_{\kappa}^+$) λ -

- parent of DH5α
- An Hoffman-Berling 1100 strain derivative (Meselson68)

- more efficient at transforming large (40-60Kb)plasmids
- nalidixic acid resistant
- Reference: Meselson M. and Yuan R. (1968) Nature 217:1110 PMID 4868368.

DH5α

F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d/acZΔM15 Δ (*lacZYA-argF*)U169, hsdR17(r_{K⁻} m_{K⁺}), λ –

- An Hoffman-Berling 1100 strain derivative (Meselson68)
- Promega also lists phoA
- nalidixic acid resistant
- References:
 - FOCUS (1986) 8:2, 9.
 - Hanahan, D. (1985) in DNA Cloning: A Practical Approach (Glover, D.M., ed.), Vol. 1, p. 109, IRL Press, McLean, Virginia.
 - Grant, S.G.N. et al. (1990) Proc. Natl. Acad. Sci. USA 87: 4645-4649 PMID 2162051.
 - Meselson M. and Yuan R. (1968) Nature 217:1110 PMID 4868368.

DH5α Turbo (NEB)

F' proA+B+ laclq Δ lacZ M15/ fhuA2 Δ (lac-proAB) glnV gal R(zgb-210::Tn10)Tets endA1 thi-1 Δ (hsdS-mcrB)5

- Also known as NEB Turbo
- T1 phage resistant
- Rapid growth: visible colonies on agar, ~6.5 hours; shaking liquid culture OD 600 = 2.0, ~4 hours
- Expresses the Lac repressor
- References:
 - New England Biolabs, product catalogue number C2984H

DH10B (Invitrogen)

F-endA1 recA1 galE15 galK16 nupG rpsL Δ lacX74 Φ80lacZ Δ M15 araD139 Δ (ara,leu)7697 mcrA Δ (mrr-hsdRMS-mcrBC) λ -

- suitable for cloning methylated cytosine or adenine containing DNA
- an MC1061 derivative (Casadaban80). Prepare cells for chemical transformation with CCMB80 buffer
- blue/white selection

- While DH10B has been classically reported to be galU galK, the • preliminary genome sequence for DH10B indicates that DH10B (and by their lineage also TOP10 and any other MC1061 derivatives) is actually galE galK galU+. Dcekiert 16:37, 23 January 2008 (CST)
- Genome sequence indicates that DH10B is actually deoR+. Presumably TOP10 and MC1061 are also deoR+.
- Streptomycin resistant •
- References:
 - o Casdaban, M. and Cohen, S. (1980) J Mol Biol 138:179 PMID 6997493.
 - o Grant, S.G.N. et al. (1990) Proc. Natl. Acad. Sci. USA 87: 4645-4649 PMID 2162051.
 - o E. coli Genetic Stock Center, MC1061 Record
 - DH10B Genome Sequencing Project, Baylor College of Medicine 0
 - Complete sequence is available, see Durfee08, PMID 18245285. 0

DH12S (Invitrogen)

mcrA Δ (mrr-hsdRMS-mcrBC) ϕ 80d lacZ Δ M15 Δ lacX74 recA1 deoR Δ (ara, leu)7697 araD139 galU galK rpsL F' [proAB+ laclqZΔM15]

- host for phagemid and M13 vectors •
- useful for generating genomic libraries containing methylated cytosine or adenine residues
- streptomycin resistant
- References: Lin, J.J., Smith, M., Jessee, J., and Bloom, F. (1991) FOCUS 13, 96.; Lin, J.J., Smith, M., Jessee, J., and Bloom, F. (1992) BioTechniques 12, 718.

DM1 (Invitrogen)

F-dam-13::Tn9(CmR)dcm-mcrBhsdR-M+gal1gal2ara-lac-thr-leu-tonR tsxR Su0

- Host for pBR322 and other non-pUC19 plasmids; useful for generating plasmids that can be cleaved with dam and dcm sensitive enzymes
- Chloramphenicol resistant
- Promega lists as F' not F-
- Reference: Lorow-Murray D and Bloom F (1991) Focus 13:20

E. cloni(r) 5alpha (Lucigen)

fhuA2Δ(argF-lacZ)U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17

• Common cloning strain.

E. cloni(r) 10G (Lucigen)

F- mcrA Δ (mrr-hsdRMS-mcrBC) endA1 recA1 Φ80dlacZ Δ M15 Δ lacX74 araD139 Δ (ara,leu)7697 galU galK rpsL nupG λ - tonA

- Common cloning strain.
- Resistant to phage T1.

E. cloni(r) 10GF' (Lucigen)

 $[F' pro A+B+lac lq Z\Delta M15::Tn10(Tet R)]/mcrA\Delta(mrr-hsd RMS-mcrBC)end A1 recA1 \Phi80dlac Z\Delta M15 \Delta lac X74 araD139 \Delta(ara, leu)7697 galU galK rpsL nupG\lambda tonA$

• Strain for cloning and single-strand DNA production.

E. coli K12 ER2738 (NEB)

F´proA+B+ laclq Δ (lacZ)M15 zzf::Tn10(TetR)/ fhuA2 glnV Δ (lac-proAB) thi-1 Δ (hsdS-mcrB)5

- Phage propagation strain
- Also available from Lucigen Corporation.

ER2566 (NEB)

F- λ - fhuA2 [lon] ompT lacZ::T7 gene 1 gal sulA11 Δ (mcrC-mrr)114::IS10 R(mcr-73::miniTn10-TetS)2 R(zgb-210::Tn10)(TetS) endA1 [dcm]

- Host strain for the expression of a target gene cloned in the pTYB vectors.
- Carry a chromosomal copy of the T7 RNA polymerase gene inserted into *lacZ* gene and thus under the control of the lac promoter. In the absence of IPTG induction expression of T7 RNA polymerase is suppressed by the binding of *lac* I repressor to the *lac* promoter.
- Deficient in both *lon* and *ompT* proteases.

ER2267 (NEB)

F' proA+B+ laclq Δ (lacZ)M15 zzf::mini-Tn10 (KanR)/ Δ (argF-lacZ)U169 glnV44 e14-(McrA-) rfbD1? recA1 relA1? endA1 spoT1? thi-1 Δ (mcrC-mrr)114::IS10

以上内容仅为本文档的试下载部分,为可阅读页数的一半内容。如要下载或阅读全文,请访问: https://d.book118.com/436214231050010110