



gene



**an international journal focusing on gene
cloning and gene structure and function**



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Ex. 1066

Page 3 of 10

AUTHOR INDEX VOLUME 167 (1995)

Airenne, K.J. and Kulomaa, M.S., Rapid purification of recombinant proteins fused to chicken avidin	167 (1995) 63
Aitken, R., see Catherine Sinclair, M.	167 (1995) 285
Albani, D. and Robert, L.S., Cloning and characterization of a <i>Brassica napus</i> gene encoding a homologue of the B subunit of a heteromeric CCAAT-binding factor	167 (1995) 209
Altamura, S., see Cabibbo, A.	167 (1995) 41
Amemura, A., see Fujita, M.	167 (1995) 93
Anton, D.L., see Payne, M.S.	167 (1995) 215
Arai, H., see Kawasaki, S.	167 (1995) 87
Arrecubieta, C., García, E. and López, R., Sequence and transcriptional analysis of a DNA region involved in the production of capsular polysaccharide in <i>Streptococcus pneumoniae</i> type 3	167 (1995) 1
Bakhshi, M.R., see Janel, N.	167 (1995) 291
Baralle, F.E., see Tisminetzky, S.	167 (1995) 313
Barbé, J., see Jordan, A.	167 (1995) 75
Barbier, G., see Bouyoub, A.	167 (1995) 147
Barek, L., see Janel, N.	167 (1995) 291
Barlatti, S., see Tisminetzky, S.	167 (1995) 313
Baud, E., see Schwaab, V.	167 (1995) 25
Bayles, K.W., see Domanski, T.L.	167 (1995) 111
Benitez, E., see Bosch, T.C.G.	167 (1995) 191
Bennett, J., Sun, D. and Karikó, K., Sequence analysis of the 5.34-kb 5' flanking region of the human rhodopsin-encoding gene	167 (1995) 317
Bianchi, G., see Tisminetzky, S.	167 (1995) 313
Bosch, T.C.G., Benitez, E., Gellner, K., Praetzel, G. and Salgado, L.M., Cloning of a <i>ras</i> -related gene from <i>Hydra</i> which responds to head-specific signals	167 (1995) 191
Bouyoub, A., Barbier, G., Querellou, J. and Forterre, P., A putative SOS repair gene (<i>dinF</i> -like) in a hyperthermophilic archaeon	167 (1995) 147
Brickner, A.G., Gossage, D.L., Dusing, M.R. and Wiginton, D.A., Identification of a murine homolog of the human adenosine deaminase thymic enhancer	167 (1995) 261
Cabibbo, A., Sporeno, E., Toniatti, C., Altamura, S., Savino, R., Paonessa, G. and Ciliberto, G., Monovalent phage display of human interleukin (hIL)-6: selection of superbinder variants from a complex molecular repertoire in the hIL-6 D-helix	167 (1995) 41
Caricasole, A., see Ward, A.	167 (1995) 239
Carvajal, E., see McGuire, T.M.	167 (1995) 151
Chang, Y.C., Wickes, B.L. and Kwon-Chung, K.J., Further analysis of the <i>CAP59</i> locus of <i>Cryptococcus neoformans</i> : structure defined by forced expression and description of a new ribosomal protein-encoding gene	167 (1995) 179
Chepelinsky, A.B., see Wang, X.Y.	167 (1995) 321
Chernoff, J., see Creasy, C.L.	167 (1995) 303
Chevallet, M., see Dupuis, A.	167 (1995) 99
Ciliberto, G., see Cabibbo, A.	167 (1995) 41
Cole, G.T., see Pishko, E.J.	167 (1995) 173
Colombi, M., see Tisminetzky, S.	167 (1995) 313
Courtney, M.-A., see Simpson-Haidaris, P.J.	167 (1995) 273
Coyne Jr., M.J. and Goldberg, J.B., Cloning and characterization of the gene (<i>rfe</i>) encoding O-antigen polymerase of <i>Pseudomonas aeruginosa</i> PAO1	167 (1995) 81
Creasy, C.L. and Chernoff, J., Cloning and characterization of a member of the MST subfamily of Ste20-like kinases	167 (1995) 303
Cundliffe, E., see Thiara, A.S.	167 (1995) 121
Darrouzet, E., see Dupuis, A.	167 (1995) 99
Dashper, S., see Jackson, C.A.	167 (1995) 127
Delrot, S., see Montamat, F.	167 (1995) 197
Devescovi, G., see Tisminetzky, S.	167 (1995) 313
Dew-Jager, K., Yu, W.-Q. and Huang, W.M., The <i>recA</i> gene of <i>Borrelia burgdorferi</i>	167 (1995) 137
DiCosimo, R., see Payne, M.S.	167 (1995) 215
Dietel, M., see Holm, P.S.	167 (1995) 221
Domanski, T.L. and Bayles, K.W., Analysis of <i>Staphylococcus aureus</i> genes encoding penicillin-binding protein 4 and an ABC-type transporter	167 (1995) 111
Drevet, J.R., see Schwaab, V.	167 (1995) 25
Driscoll, J., see Rowland, B.	167 (1995) 105

Duarte, A., see Ward, A.	167 (1995)	239
Dubin, R.A., Kazmi, M.A. and Ostrer, H., Inverted repeats are necessary for circularization of the mouse testis <i>Sry</i> transcript	167 (1995)	245
Dufaure, J.-P., see Schwaab, V.	167 (1995)	25
Dupuis, A., Peinnequin, A., Chevallet, M., Lunardi, J., Darrouzet, E., Pierrard, B., Procaccio, V. and Issartel, J.-P., Identification of five <i>Rhodobacter capsulatus</i> genes encoding the equivalent of ND subunits of the mitochondrial NADH-ubiquinone oxidoreductase	167 (1995)	99
Dusing, M.R., see Brickner, A.G.	167 (1995)	261
Dyer, D.W., see Giardina, P.C.	167 (1995)	133
Earnest, B.J., see Simpson-Haidaris, P.J.	167 (1995)	273
Faerman, M., Filon, D., Kahila, G., Greenblatt, C.L., Smith, P. and Oppenheim, A., Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles	167 (1995)	327
Feltens, R., see Pfeiffer, T.	167 (1995)	141
Filon, D., see Faerman, M.	167 (1995)	327
Forterre, P., see Bouyoub, A.	167 (1995)	147
Foster, L.-A., see Giardina, P.C.	167 (1995)	133
Frandsen, N., see Guérout-Fleury, A.-M.	167 (1995)	335
Frye, J.G., see Stamm, L.V.	167 (1995)	339
Fujita, M., Hanaura, Y. and Amemura, A., Analysis of the <i>rpoD</i> gene encoding the principal sigma factor of <i>Pseudomonas putida</i>	167 (1995)	93
García, E., see Arrecubieta, C.	167 (1995)	1
Gautvik, K.M., see Mathavan, S.	167 (1995)	33
Gautvik, V.T., see Mathavan, S.	167 (1995)	33
Gavagan, J.E., see Payne, M.S.	167 (1995)	215
Gellner, K., see Bosch, T.C.G.	167 (1995)	191
Ghyselinck, N., see Schwaab, V.	167 (1995)	25
Giancotti, V., see Manfioletti, G.	167 (1995)	249
Giardina, P.C., Foster, L.-A., Toth, S.I., Roe, B.A. and Dyer, D.W., Identification of <i>alcA</i> , a <i>Bordetella bronchiseptica</i> gene necessary for alcaligin production	167 (1995)	133
Gibert, I., see Jordan, A.	167 (1995)	75
Gigliotti, F., see Wright, T.W.	167 (1995)	185
Goffeau, A., see McGuire, T.M.	167 (1995)	151
Goldberg, J.B., see Coyne Jr., M.J.	167 (1995)	81
Golightly, E.J., see Thompson, S.A.	167 (1995)	337
Golin, J., see McGuire, T.M.	167 (1995)	151
Goodwin, G.H., see Manfioletti, G.	167 (1995)	249
Gossage, D.L., see Brickner, A.G.	167 (1995)	261
Greenblatt, C.L., see Faerman, M.	167 (1995)	327
Grenet, J., see Li, H.	167 (1995)	341
Guérout-Fleury, A.-M., Shazand, K., Frandsen, N. and Stragier, P., Antibiotic-resistance cassettes for <i>Bacillus subtilis</i>	167 (1995)	335
Guilfoyle, T.J., see Ulmasov, T.	167 (1995)	203
Guilloton, M., see Montamat, F.	167 (1995)	197
Haas, H., see Marx, F.	167 (1995)	167
Haas, R., see Kahrs, A.F.	167 (1995)	53
Habib, G.M., see Shi, Z.-Z.	167 (1995)	233
Haidaris, C.G., see Wright, T.W.	167 (1995)	185
Hanaura, Y., see Fujita, M.	167 (1995)	93
Hansen, K.R., Resta, R., Webb, C.F. and Thompson, L.F., Isolation and characterization of the promoter of the human 5'-nucleotidase (CD73)-encoding gene	167 (1995)	307
Hardham, J.M., see Stamm, L.V.	167 (1995)	339
Hartmann, R.K., see Pfeiffer, T.	167 (1995)	141
Hashimoto-Gotoh, T., Yasojima, K. and Tsujimura, A., Plasmids with a kanamycin-resistance gene for site-directed mutagenesis using the oligodeoxyribonucleotide-directed dual amber method	167 (1995)	333
Hastie, N.D., see Ward, A.	167 (1995)	239
Heuermann, D., see Kahrs, A.F.	167 (1995)	53
Hill, K., see Rowland, B.	167 (1995)	105
Holm, P.S., Dietel, M. and Krupp, G., Similar cleavage efficiencies of an oligoribonucleotide substrate and an <i>mdr1</i> mRNA segment by a hammerhead ribozyme	167 (1995)	221
Horikoshi, M., see Umehara, T.	167 (1995)	297
Huang, W.M., see Dew-Jager, K.	167 (1995)	137
Hui, Z., see Simpson-Haidaris, P.J.	167 (1995)	273

- Igarashi, Y., see Kawasaki, S. 167 (1995) 87
- Issartel, J.-P., see Dupuis, A. 167 (1995) 99
- Ito, M., see Izuhara, M. 167 (1995) 267
- Izuhara, M., Ito, M. and Takagi, Y., Transcription of the rat liver uricase-encoding gene is regulated via a *cis*-acting element responsive to cAMP 167 (1995) 267
- Jackson, C.A., Kirszbaum, L., Dashper, S. and Reynolds, E.C., Cloning, expression and sequence analysis of the genes encoding the heterodimeric methylmalonyl-CoA mutase of *Porphyromonas gingivalis* W50 167 (1995) 127
- Janel, N., Schwachtgen, J.-L., Bakhshi, M.R., Barek, L., Meyer, D. and Kerbiriou-Nabias, D., Comparison of the 5'-flanking sequences of the human and bovine von Willebrand factor-encoding genes reveals alternation of highly homologous domains with species-specific *Alu*-type repeats 167 (1995) 291
- Jannatipour, M., see Labbé, J.-C. 167 (1995) 227
- Jaworski, C.J., see Wang, X.Y. 167 (1995) 321
- Jorcke, D., see Pfeiffer, T. 167 (1995) 141
- Jordan, A., Gibert, I. and Barbé, J., Two different operons for the same function: comparison of the *Salmonella typhimurium* *nrdAB* and *nrdEF* genes 167 (1995) 75
- Kahila, G., see Faerman, M. 167 (1995) 327
- Kahrs, A.F., Odenbreit, S., Schmitt, W., Heuermann, D., Meyer, T.F. and Haas, R., An improved *TnMax* mini-transposon system suitable for sequencing, shuttle mutagenesis and gene fusions 167 (1995) 53
- Kareem, B.N., see Mathavan, S. 167 (1995) 33
- Karikó, K., see Bennett, J. 167 (1995) 317
- Karst, F., see Montamat, F. 167 (1995) 197
- Katzmann, D., see McGuire, T.M. 167 (1995) 151
- Kawasaki, S., Arai, H., Igarashi, Y. and Kodama, T., Sequencing and characterization of the downstream region of the genes encoding nitrite reductase and cytochrome *c-551* (*nirSM*) from *Pseudomonas aeruginosa*: identification of the gene necessary for biosynthesis of heme *d1* 167 (1995) 87
- Kazmi, M.A., see Dubin, R.A. 167 (1995) 245
- Kerbiriou-Nabias, D., see Janel, N. 167 (1995) 291
- Kida, S., see Umehara, T. 167 (1995) 297
- Kidd, V.J., see Li, H. 167 (1995) 341
- Kimura, H., see Takizawa, N. 167 (1995) 343
- Kirkland, T.N., see Pishko, E.J. 167 (1995) 173
- Kirszbaum, L., see Jackson, C.A. 167 (1995) 127
- Kodama, T., see Kawasaki, S. 167 (1995) 87
- Köhler, C., see Liang, X. 167 (1995) 17
- Kortt, A.A., see Riffkin, M.C. 167 (1995) 279
- Krueger, K.E., see Oberto, A. 167 (1995) 255
- Krupp, G., see Holm, P.S. 167 (1995) 221
- Kulomaa, M.S., see Airene, K.J. 167 (1995) 63
- Kwon-Chung, K.J., see Chang, Y.C. 167 (1995) 179
- Labbé, J.-C., Jannatipour, M. and Rokeach, L.A., The *Caenorhabditis elegans rop-1* gene encodes the homologue of the human 60-kDa Ro autoantigen 167 (1995) 227
- Larkin, R.M., see Ulmasov, T. 167 (1995) 203
- Lebovitz, R.M., see Shi, Z.-Z. 167 (1995) 233
- Lee, C.Y., Association of staphylococcal type-1 capsule-encoding genes with a discrete genetic element 167 (1995) 115
- Lee, N.S., see Valinluck, B. 167 (1995) 59
- Li, H., Grenet, J. and Kidd, V.J., Structure and gene expression of avian cyclin D2 167 (1995) 341
- Li, K., see Liang, X. 167 (1995) 17
- Liang, X., Oono, Y., Shen, N.F., Köhler, C., Li, K., Scolnik, P.A. and Theologis, A., Characterization of two members (*ACS1* and *ACS3*) of the 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana* 167 (1995) 17
- Lieberman, M.W., see Shi, Z.-Z. 167 (1995) 233
- Liras, P., see Rodríguez-García, A. 167 (1995) 9
- Longone, P., see Oberto, A. 167 (1995) 255
- López, R., see Arrecubieta, C. 167 (1995) 1
- Lottspeich, F., see Marx, F. 167 (1995) 167
- Lunardi, J., see Dupuis, A. 167 (1995) 99
- Maeda, S., see Mathavan, S. 167 (1995) 33
- Manfioletti, G., Rustighi, A., Mantovani, F., Goodwin, G.H. and Giancotti, V., Isolation and characterization of the gene coding for murine high-mobility-group protein HMGI-C 167 (1995) 249
- Mantovani, F., see Manfioletti, G. 167 (1995) 249
- Martín, J.F., see Rodríguez-García, A. 167 (1995) 9

Marx, F., Haas, H., Reindl, M., Stöffler, G., Lottspeich, F. and Redl, B., Cloning, structural organization and regulation of expression of the <i>Penicillium chrysogenum</i> <i>paf</i> gene encoding an abundantly secreted protein with antifungal activity	167 (1995)	167
Masuda, Y., Park, S.M., Ohta, A. and Takagi, M., Cloning and characterization of the <i>POX2</i> gene in <i>Candida maltosa</i>	167 (1995)	157
Mathavan, S., Gautvik, V.T., Rokkones, E., Olstad, O.K., Kareem, B.N., Maeda, S. and Gautvik, K.M., High-level production of human parathyroid hormone in <i>Bombyx mori</i> larvae and BmN cells using recombinant baculovirus	167 (1995)	33
Mattei, M.-G., see Schwaab, V.	167 (1995)	25
McGuire, T.M., Carvajal, E., Katzmman, D., Wagner, M., Moye-Rowley, W.S., Goffeau, A. and Golin, J., Analysis of second-site mutations that suppress the multiple drug resistance phenotype of the yeast <i>PDR1-7</i> allele	167 (1995)	151
Menzel, R., see Taylor, S.T.	167 (1995)	69
Meyer, D., see Janel, N.	167 (1995)	291
Meyer, T.F., see Kahrs, A.F.	167 (1995)	53
Miller, P., see Rowland, B.	167 (1995)	105
Miyagawa, K., see Ward, A.	167 (1995)	239
Montamat, F., Guilloton, M., Karst, F. and Delrot, S., Isolation and characterization of a cDNA encoding <i>Arabidopsis thaliana</i> 3-hydroxy-3-methylglutaryl-coenzyme A synthase	167 (1995)	197
Moro, L., see Tisminetzky, S.	167 (1995)	313
Moye-Rowley, W.S., see McGuire, T.M.	167 (1995)	151
Muro, A., see Tisminetzky, S.	167 (1995)	313
Neroni, L.A., see Simpson-Haidaris, P.J.	167 (1995)	273
Oberto, A., Longone, P. and Krueger, K.E., Identification of three transcriptional regulatory elements in the rat mitochondrial benzodiazepine receptor-encoding gene	167 (1995)	255
Odenbreit, S., see Kahrs, A.F.	167 (1995)	53
Ohta, A., see Masuda, Y.	167 (1995)	157
Ohtaka-Maruyama, C., see Wang, X.Y.	167 (1995)	321
Olstad, O.K., see Mathavan, S.	167 (1995)	33
Oono, Y., see Liang, X.	167 (1995)	17
Oppenheim, A., see Faerman, M.	167 (1995)	327
Ostrer, H., see Dubin, R.A.	167 (1995)	245
Pannekoek, H., see van Zonneveld, A.-J.	167 (1995)	49
Paonessa, G., see Cabibbo, A.	167 (1995)	41
Park, S.M., see Masuda, Y.	167 (1995)	157
Payne, M.S., Petrillo, K.L., Gavagan, J.E., Wagner, L.W., DiCosimo, R. and Anton, D.L., High-level production of spinach glycolate oxidase in the methylotrophic yeast <i>Pichia pastoris</i> : engineering a biocatalyst	167 (1995)	215
Peinnequin, A., see Dupuis, A.	167 (1995)	99
Petrillo, K.L., see Payne, M.S.	167 (1995)	215
Pfeiffer, T., Jorcke, D., Feltens, R. and Hartmann, R.K., Direct linkage of <i>str-</i> , <i>S10-</i> and <i>spc</i> -related gene clusters in <i>Thermus thermophilus</i> HB8, and sequences of ribosomal proteins L4 and S10	167 (1995)	141
Pierrard, B., see Dupuis, A.	167 (1995)	99
Pisano, M.M., see Wang, X.Y.	167 (1995)	321
Pishko, E.J., Kirkland, T.N. and Cole, G.T., Isolation and characterization of two chitinase-encoding genes (<i>cts1</i> , <i>cts2</i>) from the fungus <i>Coccidioides immitis</i>	167 (1995)	173
Pooler, J.-A., see Ward, A.	167 (1995)	239
Practzel, G., see Bosch, T.C.G.	167 (1995)	191
Procaccio, V., see Dupuis, A.	167 (1995)	99
Querellou, J., see Bouyoub, A.	167 (1995)	147
Redl, B., see Marx, F.	167 (1995)	167
Reindl, M., see Marx, F.	167 (1995)	167
Resta, R., see Hansen, K.R.	167 (1995)	307
Reynolds, E.C., see Jackson, C.A.	167 (1995)	127
Rifkin, M.C., Wang, L.-F., Kortt, A.A. and Stewart, D.J., A single amino-acid change between the antigenically different extracellular serine proteases V2 and B2 from <i>Dichelobacter nodosus</i>	167 (1995)	279
Robert, L.S., see Albani, D.	167 (1995)	209
Rodríguez-García, A., Martín, J.F. and Liras, P., The <i>argG</i> gene of <i>Streptomyces clavuligerus</i> has low homology to unstable <i>argG</i> from other actinomycetes: effect of amplification on clavulanic acid biosynthesis	167 (1995)	9
Roe, B.A., see Giardina, P.C.	167 (1995)	133
Rokeach, L.A., see Labbé, J.-C.	167 (1995)	227
Rokkones, E., see Mathavan, S.	167 (1995)	33
Rowland, B., Hill, K., Miller, P., Driscoll, J. and Taber, H., Structural organization of a <i>Bacillus subtilis</i> operon encoding menaquinone biosynthetic enzymes	167 (1995)	105
Rustighi, A., see Manfioletti, G.	167 (1995)	249
Ryu, J., see Valinluck, B.	167 (1995)	59

Salgado, L.M., see Bosch, T.C.G.	167 (1995) 191
Savino, R., see Cabibbo, A.	167 (1995) 41
Schmitt, W., see Kahrs, A.F.	167 (1995) 53
Schwaab, V., Baud, E., Ghyselinck, N., Mattei, M.-G., Dufaure, J.-P. and Drevet, J.R., Cloning of the mouse gene encoding plasma glutathione peroxidase: organization, sequence and chromosomal localization	167 (1995) 25
Schwachtgen, J.-L., see Janel, N.	167 (1995) 291
Scolnik, P.A., see Liang, X.	167 (1995) 17
Shazand, K., see Guérout-Fleury, A.-M.	167 (1995) 335
Shen, N.F., see Liang, X.	167 (1995) 17
Shi, Z.-Z., Habib, G.M., Lebovitz, R.M. and Lieberman, M.W., Cloning of cDNA and genomic structure of the mouse γ -glutamyl transpeptidase-encoding gene	167 (1995) 233
Simpson-Haidaris, P.J., see Wright, T.W.	167 (1995) 185
Simpson-Haidaris, P.J., Wright, T.W., Earnest, B.J., Hui, Z., Neroni, L.A. and Courtney, M.-A., Cloning and characterization of a lung-specific cDNA corresponding to the γ chain of hepatic fibrinogen	167 (1995) 273
Sinclair, M.C. and Aitken, R., PCR strategies for isolation of the 5' end of an immunoglobulin-encoding bovine cDNA	167 (1995) 285
Smith, P., see Faerman, M.	167 (1995) 327
Sporeno, E., see Cabibbo, A.	167 (1995) 41
Stamm, L.V., Frye, J.G. and Hardham, J.M., Sequence of the <i>Leptospira biflexa</i> serovar patoc <i>recA</i> gene	167 (1995) 339
Stewart, D.J., see Riffkin, M.C.	167 (1995) 279
Stöffler, G., see Marx, F.	167 (1995) 167
Stragier, P., see Guérout-Fleury, A.-M.	167 (1995) 335
Sugimoto, K., see Takizawa, N.	167 (1995) 343
Sun, D., see Bennett, J.	167 (1995) 317
Taber, H., see Rowland, B.	167 (1995) 105
Takagi, M., see Masuda, Y.	167 (1995) 157
Takagi, Y., see Izuhara, M.	167 (1995) 267
Takizawa, N., Kimura, H. and Sugimoto, K., Sequence of mouse <i>CDC47</i> , a member of the minichromosome maintenance (Mcm) family involved in the DNA replication licensing system	167 (1995) 343
Taylor, S.T. and Menzel, R., The creation of a camptothecin-sensitive <i>Escherichia coli</i> based on the expression of the human topoisomerase I	167 (1995) 69
Theologis, A., see Liang, X.	167 (1995) 17
Thiara, A.S. and Cundliffe, E., Analysis of two capreomycin-resistance determinants from <i>Streptomyces capreolus</i> and characterization of the action of their products	167 (1995) 121
Thompson, L.F., see Hansen, K.R.	167 (1995) 307
Thompson, S.A., Golightly, E.J. and Yaver, D.S., Nucleotide sequence of the <i>Aspergillus niger</i> <i>srpA</i> gene	167 (1995) 337
Tisminetzky, S., Devescovi, G., Tripodi, G., Muro, A., Bianchi, G., Colombi, M., Moro, L., Barlati, S., Tuteja, R. and Baralle, F.E., Genomic organisation and chromosomal localisation of the gene encoding human beta adducin	167 (1995) 313
Toniatti, C., see Cabibbo, A.	167 (1995) 41
Toth, S.I., see Giardina, P.C.	167 (1995) 133
Tripodi, G., see Tisminetzky, S.	167 (1995) 313
Tsujimura, A., see Hashimoto-Gotoh, T.	167 (1995) 333
Tuteja, R., see Tisminetzky, S.	167 (1995) 313
Ulmasov, T., Larkin, R.M. and Guilfoyle, T.J., <i>Arabidopsis</i> expresses two genes that encode polypeptides similar to the yeast RNA polymerase I and III AC40 subunit	167 (1995) 203
Umehara, T., Kida, S., Yamamoto, T. and Horikoshi, M., Isolation and characterization of a cDNA encoding a new type of human transcription elongation factor S-II	167 (1995) 297
Valinluck, B., Lee, N.S. and Ryu, J., A new restriction-modification system, <i>KpnBI</i> , recognized in <i>Klebsiella pneumoniae</i>	167 (1995) 59
Van den Berg, B.M.M., see Van Zonneveld, A.-J.	167 (1995) 49
Van Meijer, M., see Van Zonneveld, A.-J.	167 (1995) 49
Van Zonneveld, A.-J., Van den Berg, B.M.M., Van Meijer, M. and Pannekoek, H., Identification of functional interaction sites on proteins using bacteriophage-displayed random epitope libraries	167 (1995) 49
Wagner, L.W., see Payne, M.S.	167 (1995) 215
Wagner, M., see McGuire, T.M.	167 (1995) 151
Wang, L.-F., see Riffkin, M.C.	167 (1995) 279
Wang, X.Y., Ohtaka-Maruyama, C., Pisano, M.M., Jaworski, C.J. and Chepelinsky, A.B., Isolation and characterization of the 5'-flanking sequence of the human ocular lens <i>MIP</i> gene	167 (1995) 321
Ward, A., Pooler, J.-A., Miyagawa, K., Duarte, A., Hastie, N.D. and Caricasole, A., Repression of promoters for the mouse insulin-like growth factor II-encoding gene (<i>Igf-2</i>) by products of the Wilms' tumour suppressor gene <i>wt1</i>	167 (1995) 239
Webb, C.F., see Hansen, K.R.	167 (1995) 307
Wickes, B.L., see Chang, Y.C.	167 (1995) 179
Wiginton, D.A., see Brickner, A.G.	167 (1995) 261
Wright, T.W., Gigliotti, F., Haidaris, C.G. and Simpson-Haidaris, P.J., Cloning and characterization of a conserved region of human and Rhesus macaque <i>Pneumocystis carinii</i> <i>gpA</i>	167 (1995) 185

Wright, T.W., see Simpson-Haidaris, P.J.	167 (1995) 273
Yamamoto, T., see Umehara, T.	167 (1995) 291
Yanagi, H., see Yoshida, H.	167 (1995) 157
Yasojima, K., see Hashimoto-Gotoh, T.	167 (1995) 333
Yaver, D.S., see Thompson, S.A.	167 (1995) 337
Yoshida, H., Yanagi, H. and Yura, T., Cloning and characterization of the mitochondrial HSP60-encoding gene of <i>Schizosaccharomyces pombe</i>	167 (1995) 167
Yu, W.-Q., see Dew-Jager, K.	167 (1995) 137
Yura, T., see Yoshida, H.	167 (1995) 163

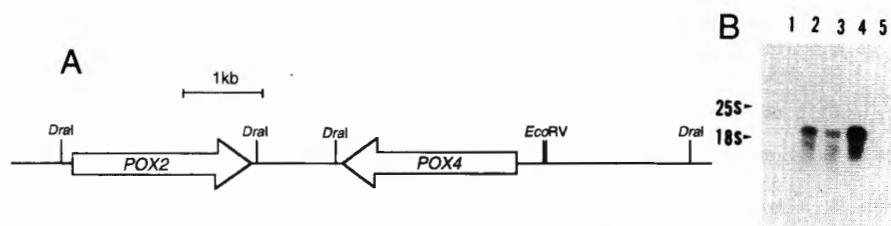


Fig. 2. Arrangement of *POX* genes and Northern blot analysis. (A) Arrangement of the *POX2* and *POX4* genes. ORF of the respective genes are indicated by open arrows. (B) Northern blot analysis of the *POX2* gene. *Cm* wt strain IAM12247 was grown on glucose (lane 1), *n*-tetradecane (lane 2), *n*-tetradecanol (lane 3), oleic acid (lane 4) and glycerol (lane 5). **Methods:** The strains and media used in this study were *C. maltosa* IAM12247 (a wt strain) and SD (0.67% Yeast Nitrogen Base w/o amino acids (Difco)/2% glucose). Glucose in SD was replaced by 1% *n*-tetradecane, *n*-tetradecanol or oleic acid when necessary. Northern blot analysis was performed by the previously described procedure (Masuda et al., 1994).

double auxotroph, CHA1, was transformed with the 4.7-kb *Pst*I-*Sac*I fragment of pPD2H (Fig. 3A) and histidine prototrophs were selected on adenine-supplemented SD plates. Southern analysis of total DNA from a His⁺ transformant revealed a 1.6-kb disrupted *Dra*I band and the 2.4-kb band of the undisrupted allele (Fig. 4A, lanes 1 and 2). This *POX2* single gene disruptant was designated P2D (*POX2/pox2::C-HIS5*). Then, the P2D was transformed with the 4.0-kb *Pst*I-*Sac*I fragment of pPD2A. Southern analysis of total DNA from a His⁺Ade⁺ transformant revealed only a 1.6-kb *Dra*I band (Fig. 4A, lane 3), indicating that both alleles of the *POX2* gene were disrupted. This *POX2* double disruptant was termed P2DD (*pox2::C-HIS5/pox2::C-ADE1*).

The *POX4* disruptants P4D (*POX4/pox4::C-HIS5*) and P4DD (*pox4::C-HIS5/pox4::C-ADE1*) were constructed using the same procedure by using two fragments, the 5.7-kb *Xba*I fragment derived from pPD4H plasmid for construction of the P4D strain and the 5.1-kb *Xba*I fragment derived from pPD4A plasmid for P4DD (Fig. 4D). Southern analysis of P4DD strain showed the appearance of 4.4 and 3.3-kb *Dra*I-*Eco*RI disruptant bands and the disappearance of the 2.9-kb band of the undisrupted allele (Fig. 4B, lane 3).

(d) Effect of *POX2* disruption on assimilation of various carbon sources

Picataggio et al. (1991) reported the sequential disruption of the *POX4* and *POX5* genes in *C. tropicalis* and functional block of the β -oxidation pathway. In contrast to these results, the importance of *POX2* gene in assimilation of hydrophobic carbon sources is not clear. As shown in Table I, the P2DD strain grew on *n*-tetradecane, *n*-tetradecanol and oleic acid. In contrast, the P4DD strain was not able to grow on *n*-tetradecane, *n*-tetradecanol and oleic acid but able to grow in media containing glucose or glycerol. Considering the growth phenotype of the P2DD strain, the function of the *POX2* gene on assimilation of *n*-tetradecane, *n*-tetradecanol or oleic acid appears not to be important. Okazaki et al. (1987) described that the cell extract preparation containing both products of *C. tropicalis* *POX4* and *POX2* showed only half of the acyl-CoA oxidase activity exhibited by the preparation that was composed of the product of *POX4* gene alone. The possible explanation is that the *POX2* gene product may regulate the activity of the *POX4* gene.

(e) Conclusions

(1) *Cm POX2* encoded an ORF encompassing 2172 nt and a 84-kDa polypeptide (724 aa) was predicted.

TABLE I

Growth of the wt and CHA1 strains, and of *POX* gene disruptants on various carbon sources

Strain	Genotype ^a	Growth ^b				
		Glucose	<i>n</i> -tetradecane	<i>n</i> -tetradecanol	Oleic acid	Glycerol
IAM12247	wt	+	+	+	+	+
CHA1	<i>his5, ade1</i>	+	+	+	+	+
P2D	<i>pox2::HIS5/POX2</i>	+	+	+	+	+
P2DD	<i>pox2::HIS5/pox2::ADE1</i>	+	+	+	+	+
P4D	<i>pox4::HIS5/POX4</i>	+	+	+	+	+
P4DD	<i>pox4::HIS5/pox4::ADE1</i>	+	—	—	—	+

^a Slash (/) specifies 'diploid' genotype. The DD strains are double disruptants.

^b Growth activity was determined by colony formation on SD plate and liquid culture in SD media. Glucose in SD was replaced by other carbon sources as specified.