

REFERENCES

1. Miller RW, Young JL Jr, Novakovic B. (1994). Childhood cancer. Cancer, 75: 395-405.
2. Pui C-H, Evans WE. (1998). Acute Lymphoblastic Leukemia. N Engl J Med, 339: 605-15.
3. F D Groves, M S Linet and S S Devesa. (1995). Patterns of occurrence of the leukemias. Eur J of Cancer, 31A: 941-949.
4. Partow Kebriaei, John Anastasi, Richard A. Larson. (2003). Acute lymphoblastic leukemia: diagnosis and classification. Best Pract Res clin Hematol, 15(4): 597-621.
5. Martin J. Cline. (1994). The molecular basis of leukemia. N Engl J Med, 330(5): 328-336.
6. Greaves M. Differentiation-linked leukemogenesis in lymphocytes. (1986). Science, 234: 697-704.
7. Compana D, Pui C-H. Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. (1995). Blood 85:1416-1434.
8. Wright J, Poplack D, Bakhski A, et al. (1987). Gene rearrangements as markers of clonal variation and minimal residual disease in acute lymphoblastic leukemia. J Clin Oncol, 5:735-41.
9. M Greaves. (2002). Childhood Leukemia. BMJ, 324: 283-287.
10. D L Preston, S Kusumi and M Tomonaga. (1994). Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma. Radiat Res, 137: S68-S97.
11. Magaret A. Tucker, Anna T. Meadows, John D. Boice, et al. (1987). Leukemia after therapy with Alkylating agents for childhood cancer. J. Natl cancer Inst, 78(3): 459-464.
12. Y Ben-David and A Bernstein. (1991). Friend virus-induced erythroleukemia and the multistage nature of cancer. Cell, 66: 831-834.

13. T.H. Rabitts. (1994). Chromosomal translocations in human cancer. Nature, 372: 143-149.
14. A. Thomas Look. (1997). Oncogenic Transcription Factors in the human acute leukemias. Science, 278: 1059-1064.
15. Stefan Faderl, Hagop M. Kantarjian, Moshe Talpaz. (1998). Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia. Blood, 91: 3995-4019.
16. Susana Catalina Raimondi. (1993). Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood, 81(9): 2237-2251.
17. Ramesh A. Shivdasani and stuart H. Orkin. (1996). The transcriptional control of Hematopoiesis. Blood, 87(10): 4025-4039.
18. Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts and Peter Walter. (2002). Molecular Biology of the Cell. 4th(ed). New York: Garland Science publishing.
19. S.Fear, Gavin, D.-E. Zhang, C. Hetherington, et al. (1997). Functional characterization of ETV6 and ETV6/CBFA2 in the regulation of the MCSFR proximal promoter. Proc. Natl. Acad. Sci. USA, 94: 1949-1954.
20. Thomas W. McLean, Sarah Ringold, Donna Neuberg, Kimberly Stegmaier. (1996). TEL-AML1 dimerizes and is associated with a favorable outcome in childhood acute lymphoblastic leukemia. Blood, 88(11): 4252-4258.
21. S Avigad. (1999). TEL-AML1 fusion transcript designates a favorable outcome with and intensified protocol in childhood acute lymphoblastic leukemia. Leukemia, 13: 481-498.
22. Scott W. Hiebert, Wuhua Sun, J. Nathan David, et al. (1996). The t(12;21) translocation converts AML1B from an activator to a repressor of transcription. Mol Cell Biol, 16(4): 1349-1355.
23. Tarin Niimi, Jukka Kanerva, Kim Vettenranta, et al. (2000). AML1 gene amplification: a novel finding in childhood acute lymphoblastic leukemia. Hematologica, 85: 362-366.
24. <http://www.ncbi.nlm.nih.gov/>
25. Bishop JM. (1987). The molecular genetics of cancer. Science, 235: 305-11.

26. Solomon E, Borrow J, Goddard AD. (1991). Chromosome aberrations and cancer. *Science*, 254: 1153-60.
27. Rowley JD. (1990). Molecular cytogenetics. *Cancer Res*, 50: 3816-25.
28. Pui C-H. (1995). Childhood leukemias. *N Engl J Med*, 332: 1618-30.
29. Louise Kelly, Jennifer Clasrk and D, Gary Gilliland. (2002). Comprehensive genotypic analysis of leukemia: clinical and therapeutic Implications. *Curr Opin Oncol*, 14: 10-18.
30. A K Stewart, A C Schuh. (2002). White cells 2: impact of understanding the molecular basis of haematological malignant disorders on clinical practice. *Lancet*, 355: 1447-53.
31. Hisamaru Hirai. (1999). Signal transduction and leukemias, Education session 7: mechanism of cell control. *Congress of ISH*, Asian-pacific division: 24-28.
32. http://www.ivillagehealth.com/experts/fertility/qas/0,,256850_153004,00.html
33. JE Rubnitz, C-H Pui and JR Downing. (1999). The role of TEL fusion genes in pediatric leukemias. *Leukemia*, 13: 6-13.
34. Helene Poirel, Cecile Oury, Clemence Carron, et al. (1997). The TEL gene products: nuclear phosphoproteins with DNA binding properties. *Oncogene*, 14: 349-357.
35. Christine Jousset, Clemence Carron, Anthiny Boureux, et al. (1997) A domain of TEL conserved in a subset of ETS proteins defines a specific oligomerization interface essential to the mitogenic properties of the TEL-PDGFR β oncoprotein. *EMBO J*, 16(1): 9-82.
36. Todd R. Golub, George F. Barker, Michael Lovett and D. Gary Gilliland. (1994). Fusion of PDGF receptor β to a novel ets-like Gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell*, 77: 307-316.
37. Lwona Wlodarska, Roberta La Starza, Mathijs Baens, et al. (1998). Fluorescence In situ Hybridization characterization of new translocations involving TEL(ETV6) in a wide spectrum of hematologic malignancies. *Blood*, 91(4): 1399-1406.

38. Jeffrey E. Rubnitz, James R. Downing, Ching-Hon Pui, Sheila A. Shurtleff, et al. (1997). TEL gene rearrangement in acute lymphoblastic leukemia: A new genetic marker with prognostic significance. *J Clin Oncol*, 15(3): 1150-1157.
39. S.P. Romana, M. Mauchauffe, M. Le Coniat, et al. (1995). The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. *Blood*, 85(12): 3662-3670.
40. Hiroyuki Miyoshi, Kimiko Shimizu, Tomoko Kozu, et al. (1991). t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. *Proc. Natl. Acad. Sci. USA*, 88(23): 10431-10434.
41. Francesco Lo Coco, Simona Pisegna, Daniela Diverio. (1997). The AML1 gene: a transcription factor involved in the pathogenesis of myeloid and lymphoid leukemias. *Hematologica*, 82: 364-370.
42. Bart Lutterbach, Jennifer Westendorf, Bryan Linggi, et al. (2000). A mechanism of repression by Acute Myeloid Leukemia-1, the target of multiple chromosomal transcriptions in acute leukemia. *J Biol Chem.*, 275(1): 651-656.
43. S.P. Romana, H. Poirel, M. Le Coniat, et al. (1995). High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. *Blood*, 86(11): 4263-4269.
44. Karlheinz Seeger, Hans-peter Adams, Dirk Buchwald, Birgit Beyermann, et al. (1998). TEL-AML1 fusion transcript in relapsed childhood acute lymphoblastic leukemia. *Blood*, 91(5): 1716-1722.
45. Mignon L. Loh, Lewis B. Silverman, Mary L. Young, Donna Neuberg, Todd R. Golub, et al. (1998). Incidence of TEL-AML1 fusion in children with relapsed acute lymphoblastic leukemia. *Blood*, 92(12): 4792-4797.
46. David J. Amor, Elizabeth M. Algar, Howard R. Slater, Peter. Smith. (1998). High frequency of t(12;21) in childhood acute lymphoblastic leukemia detected by RT-PCR. *Pathology*, 30: 381-385.
47. Shurtleff, A Buijs, FG Behm, JE Reznitz, SC Raimondi, et al. (1995). TEL-AML1 fusion resulting from a cryptic t(12;21) is the most common genetic region

- in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia, 9: 1985-1989.
48. Carlo Lanza, Gisella Volpe, Giuseppe Basso, et al. (1997). Outcome and lineage involvement in t(12;21) childhood acute lymphoblastic leukemia. Br J Haematol., 97: 460-462.
49. S Raynaud, g Brunie, M Bakkus, P Cochaux, et al. (1997). ETV6 is the target of chromosome 12p deletions in t(12;12) childhood acute lymphoblastic leukemia. Leukemia, 11: 1459-1464.
50. S Avigad. (1999). TEL-AML1 fusion transcript designates a favorable outcome with and intensified protocol in childhood acute lymphoblastic leukemia. Leukemia, 13: 481-498.
51. Heba M. Shaker, Iman A. Sidhom and Inasa A. El-Attar. (2001). Frequency and clinical relevance of TEL-AML1 fusion gene in childhood acute lymphoblastic leukemia in Egypt. Journal of the Egyptian Nat. Cancer Inst, 13(1): 9-18.
52. C Liang, T-B Chou, J-S Chen, SA Shurtliff, JE Rubnitz, JR Downing, C-H Pui and L-Y Shih. (1996). High incidence of TEL-AML1 fusion resulting from a cryptic t(12;21) in childhood B-lineage acute lymphoblastic leukemia in Taiwan. Leukemia, 10: 991-993.
53. Noriko Satake, Hirofumi Kobayashi, Yukiko Tsunematsu, et al. (1997). Minimal residual disease with TEL-AML1 fusion transcript in childhood lymphoblastic leukemia with t(12;21). Br J Hematol., 97: 607-611.
54. Todd R. Golub, George F. Barker, Stefan K. Bohlander, Scott W. Hiebert, et al. (1995). Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc. Natl. Acad. Sci. USA, 92: 4917-4921.
55. S.P. Romana, M. Mauchauffe, M. Le Coniat, I. Chumakov, et al. (1995). The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood, 85(12): 3662-3670.
56. Rodolphe G. Lopez, Clemence Carron, Cecile Oury, Paola Gardellin, et al. (1999). TEL is a sequence-specific transcription repressor. J Biol Chem., 274(42): 30132-30138.

57. Shari Meyers, Noel Lenny and Scott W. Hiebert. (1995). The t(8;21) fusion protein interferes with AML-1B dependent transcriptional activation. Mol Cell Biol., 15(4): 1974-1982.
58. Ting-Lei Gu, Tamara L. Goetz, Barbara J. Graves and Nancy A. Speck. (2000). Auto-Inhibition and partner proteins, Core binding factor β (CBF β) and Ets-1, Modulate DNA binding by CBF α 2 (AML1). Mol Cell Biol, 2000: 91-103.
59. Fabien Guidez, Kevin Petrie, Anthony M. Ford, Huafeng Lu, Arthur Zelent, et al. (2000). Recruitment of the nuclear receptor corepressor N-CoR by the TEL moiety of the childhood leukemia-associated TEL-AML1 oncogene. Blood, 96(7): 2557-2561.
60. Randy Fenrick, Joseph M. Amann, Bart Lutterbach, Lilin Wang, Scott W. Hiebert. (1999). Mol Cell Biol, 19(10): 6566-6574.
61. Ayoubi TA, Van De Ven WJ. (1996). Regulation of gene expression by alternative promoters. FASEB J, 10: 453-460.
62. Strachan, T. and Read, A.P. (1999). Human Molecular Genetics. 2nd(ed). North Yorkshire: The Bath press.
63. Clark, S.C. and Kamen. R. (1987). The human hematopoietic colony-stimulating factors. Science, 236: 1229-1237.
64. Schrander, J.W. (1986). The panspecific hemopoietin of activated T cells(interleukin-3). Annu. Rev. Immunol., 4: 205-230.
65. Simon Musyoka Wangi, Linda Logan-Henry, Colin McInnes and Bea Mertens. (1995). Cloning of bovine interleukin-3-encoding cDNA. Gene, 162: 309-312.
66. Jacques J.M. van Dongen and Ingrid L.M. Wolvers-Tettero. (1991). Analysis of Immunoglobulin and T cell receptor genes. Clin Chim Acta, 198: 93-174.
67. T.R. Dykman, J.A. Hatch, M.S. Aqua, J.P. Atkinson. (1985). Polymorphism of the C3b/C4b receptor (CR1): characterization of a fourth allele. J Immunol, 134: 1787-1789.
68. M. Aegeerter-Shaw, J.L. Cole, L.B. Klickstein, W.W. Wong, D.T. Fearon, et al. (1987). Expansion of the complement receptor gene family: identification in

- the mouse of two genes related to the CR1 and CR2 family. J Immunol, 138: 3488-3494.
69. Nishikawa M, Shirakawa S. (1992). The expression and possible roles of protein kinase C in Hematopoietic cells. Leuk Lymphoma, 8(3): 201-11.
70. Lee SW, Kwak HB, Chung WJ, Cheong H, Kim HH, Lee ZH. (2003). Participation of protein kinase c beta in osteoclast differentiation and function. Bone, 32(3): 217-27.
71. Hidaka M, Nakakuma H, Kawaguchi T, Nagakura S, Horikawa K. (1992). Altered expression of protein kinase C in adult T-cell leukemia cells. Int J Hematol, 56(2): 135-41.
72. Fumihiko Komada, Masakatsu Nishikawa, Yasuhiro Uemura, et al. (1991). Expression of three major protein kinase C isoenzymes in various types of human leukemic cells. Cancer Res, 51: 4271-4278.
73. Yasutomi Nishizuka. (1984). The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature, 308: 693-698.
74. Schatz, D.G., M.A. Oettinger, and D. Baltimore. (1989). The V(D)J recombination activating gene, RAG-1. Cell, 59: 1035-1048.
75. David G. Schatz. (1999). Developing B-cell theories. Nature, 400: 614-617.
76. Kazuhiro Nishii, Kenkichi Kita, Hiroshi Miwa, Masato Shikami, et al. (2000). Expression of —cell-associated transcription factors in B-cell precursor acute lymphoblastic leukemia cells: Association with PU.1 expression, phenotype, and immunogenotype. Int J Hemato, 71: 372-378.
77. Volm M, Sauerbrey A, Zintl F, koomagi R, Efferth T. (2002). Protein expression profiles of newly diagnosed acute lymphoblastic leukemia in children developing relapses. Oncol Rep, 9(5): 965-9.
78. Uchida H, Zhang J and Nimer SD. (1997). AML1A and AML1B can transactivate the human IL-3 promoter. J Immunol, 158(5): 2251-2258.
79. Song H, Kim JH, Rho JK, Park SY, et al. (1999). Functional characterization of TEL-AML1 fusion protein in the regulation of human CR1 gene promoter. Mol Cells, 9(5): 560-3.

80. Jae Hyun Kim, Sooyeon Lee, Jae Kyun Rho, Soo Young Choe. (1999). AML1, the target of chromosomal rearrangements in human leukemia, regulates the expression of human complement receptor type 1(CR1) gene. Int J Biochem Cell Biol, 31: 933-940.
81. Hideo Uchida, James R Downing, Yasushi Miyazaki, Richard Frank, Jim Zhang and Stephen D Nimer. (1999). Three distinct domains in TEL-AML1 are required for transcriptional repression of the IL-3 promoter. Oncogene, 18: 1015-1022.
82. Neal S. Young, Photis Beris. (2001). Flow cytometry in the diagnosis of Acute Leukemia. Semin Hematol, 38(2): 124-137.
83. Sophie Raynaud, Helene Cave, Mathijs Baensm Christian Bastard, et al. (1996). The 12;21 translocation involving TEL and deletion of the other TEL allele: two frequently associated alterations found in childhood acute lymphoblastic leukemia. Blood, 87(7): 2891-2899.
84. Takahashi Y, Horibe K, Kiyoi H, et al. (1998). Prognostic significance of TEL-AML1 fusion transcript in childhood B-precursor lymphoblastic leukemia. J Pediatr Hematol Oncol, 20: 190-195
85. Fear S, Vignon C, Bohlander SK, Smith S, Rowley JD, et al. (1996). Correlation between the ETV6/CBFA2 (TEL-AML1) fusion gene and karyotypic abnormalities in children with B-cell precursor acute lymphoblastic leukemia. Genes Chromosomes Cancer, 17: 127-135
86. Germano G, del Giudice L, Palatron S, et al. (2003). Clonality profile in relapsed precursor-B-All children by GeneScan and sequencing analyses. Consequences on minimal residual disease monitoring. Leukemia, 17(8): 1573-82.
87. Philip J. Moos, Elizabeth A. Raetz, Marlee A. Carlson, Aniko Szabo, et al. (2002). Identification of Gene expression profiles that segregate patients with childhood leukemia. Clin Cancer Res, 8: 3118-3130.



APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

BUFFERS AND REAGENTS

1. 10% SDS solution

Sodium dodecyl sulfate	10	g
Distilled water to	100	ml

Mix the solution and store at room temperature

2. 0.5 M EDTA (pH 8.0)

Disodium ethylenediamine tetraacetate.2H₂O 186.6 g

Dissolve in distilled water and adjust pH to 8.0 with NaOH

Distilled water to 1,000 ml

Sterilize the solution by autoclaving and store at room temperature

3. 50X Tris acetate buffer (50X TAE buffer)

Tris base	242	g
Glacial acetic	57.1	ml
0.5 M EDTA pH 8.0	100	ml
Distilled water to	1,000	ml

4. 6X loading dye

Bromphenol blue	0.25	g
Xylene cyanol	0.25	g
Glycerol	50	ml
1 M tris (pH 8.0)	1	ml
Distilled water to	100	ml

Mix and stored at 4 °C

5. 2% agarose gel (w/v)

Agarose	1.6	g
1X TAE	80	ml

Dissolve by heating in microwave oven and mix occasionally until no granules of agarose are visible.

6. Ethyldium bromide

Ethyldium bromide	10	mg
Distilled water	1	ml

Mix the solution and store at 4 °C

7. Denaturing solution

NaOH 0.5 M	10	g
NaCl 1.5 M	43.8	g
Distilled water to	500	ml

Mix the solution and store at room temperature

8. Neutralizing solution

NaCl 1.5 M	43.8	g
Tris base 1 M pH 7.2	60.5	g
Distilled water to	500	ml

Mix the solution and store at room temperature

9. 20X SSC

NaCl 3 M	87.6	g
Sodium citrate 0.3 M	44.1	g
Distilled water to	500	ml

Mix the solution and store at room temperature

10. 10% Sodium-N-Lauroylsarcosine

Sodium-N-Lauroylsarcosine	10	g
Distilled water to	100	ml

Mix the solution and store at room temperature

APPENDIX B

CHEMICAL AGENTS AND INSTRUMENTS

Materials

1. Pipette tip: 10 μl , 200 μl 1000 μl (Axygen Scientific, USA and Euro Lab[®] Labortechnik KG, German)
2. Microcentrifuge tube: 0.2 ml, 0.5 ml, 4.5 ml (Axygen Scientific, USA)
3. Polypropylene conical tube: 15 ml, 50 ml (Corning, USA)
4. Polystyrene round-bottom tube: 5 ml (Becton Dickinson, USA)
5. Beaker: 50 ml, 100 ml, 200 ml, 500 ml, 1,000 ml (Pyrex)
6. Flask: 250 ml, 500 ml, 1,000 ml (Pyrex)
7. Reagent bottle: 100 ml, 250 ml, 500 ml, 1,000 ml (Duran, USA)
8. Cylinder: 25 ml, 50 ml, 100 ml, 250 ml, 500 ml, 1,000 ml (Witeg, Germany)
9. Glass pipette: 5 ml, 10 ml (Witeg, Germany)
10. Pipette rack (Eppendorf, Germany)
11. Thermometer (Precision, Germany)
12. PARAFILM (American National Can, USA)
13. Plastic wrap
14. Aluminum foil
15. Acridisc syringe filter 0.2 μm (PALL Gelman Laboratory)
16. Nylon membrane (PALL Gelman Laboratory)
17. 3MM whatman paper
18. Stirring-magnetic bar
19. Combs (Bio-RAD)
20. Electrophoresis chamber set (Bio-RAD)
21. Timer (Canon, China)

Equipment

1. Automatic adjustable micropipette: P2 (0.1-2.5 μ l), P10 (0.5-10 μ l), P20 (2-20 μ l), P100 (10-100 μ l), P200 (10-200 μ l), P1000 (0.1- 1 ml) (Eppendorf, Germany)
2. Pipette boy (Tecnomara, Switzerland)
3. Vortex (Scientic Industry, USA)
4. pH meter (Ecomet, UK)
5. Stirring hot plate (Corning, USA)
6. Microcentrifuge (Eppendorf, Germany)
7. Thermal centrifuge (Heraeus, Germany)
8. Dynal MPC (Dynal, Norway)
9. DNA Thermal cycler 480 (Applied Biosystems, USA)
10. Beta shield (C.B.S Scientific. Co.)
11. Heat block (Boekel Scientific, UK)
12. Shaker (Armed)
13. Incubator (Memmert, Germany)
14. Spectrophotometers (Bio-RAD, USA)
15. UV-absorbing face shield (Bio-RAD, USA)
16. Gel-doc (Bio-RAD, USA)
17. Refrigerator 4 °C (Hitachi, Sanya, Japan)
18. Deep freeze -20 °C, -80 °C (Sanyo, Japan)
19. Water bath
20. Microscope (Leica, USA)
21. Storm 840 and ImageQuaNT software (Molecular Dynamics)
22. FACScan and CELLquest software (Becton Dickinson, USA)

Reagents

1. General reagents

- 1.1 Fetal Bovine Serum (GIBCO, USA)

- 1.2 Phosphate Buffer Saline (PBS)
- 1.3 Hydrochloric acid (Merck)
- 1.4 Sodium chloride (Merck)
- 1.5 Tris base (USB)

2. Reagents for Immunophenotyping

- 2.1 Ficoll-PaqueTM PLUS (Amershampharmacia Biotech)
- 2.2 FACSTM lysing solution (Becton Dickinson, USA)
- 2.3 Paraformaldehyde (Sigma)
- 2.4 FACSFlow (Becton Dickinson)
- 2.5 Immunophenotype panel (Becton Dickinson, USA)
 - 2.5.1 γ1 FITC / γ1 PE / CD45
 - 2.5.2 CD10 FITC / CD19 PE / CD45
 - 2.5.3 CD5 FITC / CD20 PE / CD45
 - 2.5.4 CD3 FITC / CD22 PE / CD45
 - 2.5.5 CD7 FITC / CD34 PE / CD45
 - 2.5.6 HLA-DR FITC / CD13 PE / CD45
 - 2.5.7 GPA FITC / CD14 PE / CD45
 - 2.5.8 CD71 FITC / CD33 PE / CD45

3. Reagents for Immunomagnetic Selection

- 3.1 Monoclonal antibody CD10 (Immunotech, France)
- 3.2 Dynabead IgG (Dynal, Norway)
- 3.3 Dynabead M-450 CD19 (PanB) (Dynal, Norway)

4. Reagents for RNA extraction

- 4.1 TRIzol LS reagent (GIBCO, USA)
- 4.2 Chloroform (Merck)
- 4.3 Isopropyl alcohol (BDH Laboratory Supplies, Germany)
- 4.4 Absolute ethanol (BDH Laboratory Supplies, Germany)

5. Reagents for cDNA synthesis (Promega)

- 5.1 Oligonucleotide(dT)₁₅ primers
- 5.2 Nuclease-free water
- 5.3 ImProm-II™ 5X reaction buffer
- 5.4 Magnesium chloride
- 5.5 Deoxynucleotide triphosphates (dNTPs)
- 5.6 Recombinant RNasin® Ribonuclease Inhibitor
- 5.7 ImProm-II™ Reverse Transcriptase
- 5.8 RNA template

6. Reagents for PCR analysis

- 6.1 10X PCR buffer (500 mM KCL, 200 m tris-HCl pH 8.4)
- 6.2 Magnesium Chloride
- 6.3 Deoxynucleotide triphosphates (dNTPs)
- 6.4 Oligonucleotide primers (BSU)
- 6.5 Taq DNA polymerase (Fermentus)
- 6.6 cDNA sample

7. Reagents for Southern Blot Hybridization

- 7.1 T₄ kinase (New England Biolabs)
- 7.2 10X Kinase buffer (New England Biolabs)
- 7.3 [γ^{32} P] ATP (Amershampharmacia Biotech)
- 7.4 Denaturing solution (0.5 M NaOH, 1.5 M NaCl)
- 7.5 Naturizing solution (1.5 M NaCl, 1 M Tris)
- 7.6 20X SSC (tri-sodium citrate)(3 M NaCl, 0.3 M Sodium Citrate)
- 7.7 10%SDS (Sodium dodecyl sulfate 10 g)
- 7.8 10%Sodium-N-Lauroylsarcosine (USB) filtrate
- 7.9 Blocking reagent (Salmon sperm DNA)(GIBCO)

8. Reagents for electrophoresis

- 8.1 Agarose, molecular grade (Promega)

- 8.2 Ethyldium bromide (Sigma, USA)
- 8.3 6X loading dye
- 8.4 50X TAE (242 g Tris base, 57.1 ml glacial acetic, 100 ml 0.5M EDTA pH 8.0) add H₂O until 1000 ml)
- 8.5 100 base pair DNA ladder (Biolabs)



APPENDIX C

OLIGONUCLEOTIDE PRIMERS AND TARGET GENES

1. Partial human *TEL* mRNA, (GENBANK Accession number U11732)

```

481 gaagaagata actgtgtcca gaggaccccc aggccatccg tggataatgt gcaccataac
541 cctcccacca ttgaactgtt gcaccgcgtcc aggtcaccta tcacgacaaa tcaccggcct
601 ttccttgacc ccgagcagcg gccctccgg tccccctgg acaacatgtat ccgcggcctc
661 tccccggctg agagagctca gggaccagg ccgcaccagg agaacaacca ccaggagtcc
721 tacccctctgt cagtgtctcc catggagaat aatcaactgcc cagcgtcctc cgagtccac
781 ccgaagccat ccagcccccg gcaggagagc acacgcgtga tccagctgat gcccagcccc
841 atcatgcacc ctctgatcct gaaccccccgg cactccgtgg atttcaaca gtccaggctc 1
901 tccgaggacg ggctgcatacg ggaagggaaag cccatcaacc tctctcatcg ggaagacactg
961 gcttacatga accacatcat ggtctctgtc tcccccgtg aagagcacgc catgcccatt 2
1021gggagaatag cag      |-----breakpoint-----|

```

2. Partial human *AML1* mRNA, (GENBANK Accession number U19601)

	-----breakpoint-----	aa
61 tgcatacttg gaatgaatcc ttcttagagac gtccacgatg ccagcacgag ccgcgcgttc		
121 acgccgcctt ccaccgcgtc gagcccaggc aag <u>atgagcg aggcgttgcc gctggcgcc</u> 3		
181 ccggacgccc gcgctgcctt ggccggcaag ctgaggagcg gcgaccgcag catggtgag		
241 gtgctggcccg accacccggg cgagctggc cgcaccgaca gccccaactt cctctgctcc		
301 gtgctgccta cgcactggcg ctgcaacaag accctgccca tcgctttcaa ggtggtgcc		
361 cttagggatg ttccagatgg cactctggc actgtgatgg ctggcaatga tgaaaactac		
421 tcggctgagc tgagaaaatgc taccgcagcc atgaagaacc aggttgcag atttaatgac		

Figure 20 Partial TEL-AML1 fusion mRNA nucleotide sequence. 1 is TELS primer, 2 is TEL primer⁸³, 3 is AML1 primer.⁸³

3. Homo sapiens *Interleukin 3* (colony-stimulating factor, multiple) (IL3), mRNA.
(GENBANK Accession number NM_000588)

```

1   cagagccccca cgaaggacca gaacaagaca gagtgccctc tgccgatcca aacatgagcc
61  gcctgcccgt cctgctcctg ctccaactcc tggtccgccc cggactccaa gctcccatga 1
121 cccagacaac gccctgaag acaagctggg ttaactgctc taacatgatc gatgaaattt
181 taacacactt aaagcagcca ccttgcctt tgctggactt caacacctc aatggggaag 2
241 accaagacat tctgatggaa aataaccttc gaaggccaaa cctggaggca ttcaacaggg
301 ctgtcaagag tttacagaac gcatcagcaa ttgagagcat tctaaaaat ctcctccat
361 gtctgcccct ggccacggcc gcacccacgc gacatccaat ccatatcaag gacggtgact
421 ggaatgaatt cggaggaaa ctgacgttct atctgaaaac ccttgagaat ggccaggctc 3
481 aacagacgac tttgagcctc gcatctttt gagtccaaacg tccagctcgt tctctggcc
541 ttctcaccac agagcctcgg gacatcaaaa acagcagaac ttctgaaacc tctgggtcat
601 ctctcacaca ttccaggacc agaagcattt cacctttcc tgccgcatca gatgaatttgt
661 taatttatcta atttctgaaa tgtcagctc ccatttggcc ttgtcggtt gtgttctcat
721 ttttatccca ttgagactat ttatttatgt atgtatgtat ttatttattt attgcctgga
781 gtgtgaactg tatttattt agcagaggag ccatgtcctg ctgcttctgc aaaaaactca
841 gagtggggtg gggagcatgt tcatttgct ctcgagttt aaactggttc ctagggatgt
901 gtgagaataa actagactct gaac

```

Figure 21 IL-3 mRNA nucleotide sequence. 1 is IL-3 EX1 primer, 2 is IL-3 EX2 primer, 3 is IL-3 EX5 primer.

4. Human T-cell receptor gamma-chain mRNA. (GENBANK Accession number Y00790)

```

1 tggtccttt cttccaagg ccccgagag gaaggcatgc ggtggccct agtggtgctt
61 ctagcttcc tgtctcctgc cagtcagaaa tcttccaact tggaaggag aacgaagtca
121 gtcaccaggc agactgggtc atctgctgaa atcacttgcg atcttactgt aacaaatacc
181 ttctacatcc actggtacct acaccaggag gggaggccc cacagcgtct tctgtactat
241 gacgtctcca ccgcaaggga tgtgttgaa tcaggactca gtccaggaaa gtattatact
301 catacaccca ggaggtggag ctggatattg agactgcaaa atctaattga aaatgattct
361 ggggtctatt actgtgccac ctggacagg ccccgcccta agaaactctt tggcagtgga
421 acaacacttg ttgtcacaga taaacaactt gatgcagatg tttccccaa gcccactatt
481 tttcttcctt cgattgctga aacaaaactc cagaaggctg gaacataacct ttgtcttctt
541 gagaatttt tccagatattattaagata cattggcaag aaaagaagag caacacgatt
601 ctggatccc aggaggggaa caccatgaag actaacgaca catacatgaa atttagctgg 1
661 ttaacgggtgc cagaagatc actggacaaa gaacacagat gtatcgtag acatgagaat
721 aataaaaacg gaattgatca agaaatttac tttcctccaa taaagacaga tgtcaccaca
781 gtggatccca aatacaatta ttcaaaggat gcaaattgatg tcatcacaat ggatcccaa
841 gacaatttgtt caaaagatgc aaatgataca ctactgctgc agtcacaaa cacctctgca
901 tattacacgt acctcctcct gctcctcaag agtgtggtct atttgccat catcacctgc
961 tgtctgctta gaagaacggc tttctgctgc aatggagaga aatcataaca gacggtgca
1021caaggaggcc atctttcctt catcggttat tgtccctaga agcgtcccg aattcaaggt 2

```

Figure 22 TCR γ mRNA nucleotide sequence. 1 is TCR γ forward primer, 2 is TCR γ reverse primer.



 ศูนย์วิทยาศาสตร์พยาบาล
 จุฬาลงกรณ์มหาวิทยาลัย

5. *Homo sapiens recombination activating gene 1 (RAG1), mRNA. GENBANK accession number NM_000448)*

1	agagggcaag gagagagcag agaacacact ttgccttctc tttggattt agtaatatca	1
61	accaaattgc agac <u>atctca</u> <u>acactttggc</u> <u>cagg</u> cgcct gctgagcaag gtacccgc	2
121	cagcatggca gcctcttcc caccaccc gggactcagt tctgcc <u>ccag</u> <u>atgaaattca</u>	3
181	<u>gcaccc</u> acat attaaatttt cagaatggaa att <u>taagctg</u> <u>ttccgggtga</u> <u>gatc</u> cttga	
241	aaagacaccc gaagaagctc aaaaggaaaa gaaggattcc tttgagggga aaccctctc	
301	ggagcaatct ccagcagtcc tggacaaggc tgatggtag aagccagtcc caactcagcc	
361	attgttaaaa gcccaccta agtttcaaa gaaatttcac gacaacgaga aagcaagagg	
421	caaagcgatc catcaagcca accttcgaca tctctgccgc atctgtggga attcttttag	
481	agctgatgag cacaacagga gatatccagt ccatggctt gtggatggta aaaccctagg	
541	cctttacga aagaaggaaa agagagctac ttccctggccg gacctcattt ccaaggttt	
601	ccggatcgat gtgaaggcag atgttgactc gatccacccc actgagttct gccataactg	
661	ctggagcatc atgcacagga agtttagcag tgccccatgt gaggttact tcccgaggaa	

Figure 23 Partial RAG1 mRNA nucleotide sequence. 1 is RAG1 forward primer, 3 is RAG1 reverse primer, 2 is oligonucleotide sequence using as a probe in detection RAG1 mRNA by Southern blot hybridization.

6. Homo sapiens complement receptor 1 mRNA. GENBANK accession number XM_114735)

```

1 aaactgtgag tttggggatt gttgtgtcca ctaaccggac tcagaaggga cttccctgct
61 cggtggctt tcggtttctc tgctcacctc cgatataatc acggggtctc ccgcgccgct
121 catggcgctt cccgtccgtc tcgagcgtcc ctttccttcc cgccgttcc ctgggttgct
181 tctggggcc ctgggtttgc tgctgtcctc ctttccttcc caatgcaatg tccccgaatg
241 gcttccattt gccaggccta ccaacctaac tgatgacttt gagttccca ttgggacata
301 tctgaactat gaatgccc ctgggttattc cggaagaccg ttttctatca tctgcctaaa 1
361 aaactcagtc tggacaagtg ctaaggacaa gtgcaaacgt aaatcatgtc gtaatcctcc
421 agatcctgtg aatggcatgg cacatgtgat caaagacatc cagttcagat cccaaattaa
481 atattcttgt cctaaaggat accgactcat tggttcctcg tctgccacat gcatcatctc 2
541 aggcaacact gtcatttggg ataataaaac acctgtttgt gacagaatta tttgtgggct
601 acccccccacc atgcctaattg gagatttcac tagcatcagc agagagtatt ttcactatgg
661 atcagtggtg acctaccact gcaatcttgg aagcagaggg aaaaagggtgt ttgagcttgc
721 gggtagccc tccatataact gcaccagcaa agatgatcaa gtggcatct ggagtggccc
781 agccctcag tgcattatac ctaacaaatg cacgcctcca aatgtggaaa atggaatatt
841 ggtatctgac aacagaagct tatttcctt aaatgaagtt gtggagtttta ggtgtcagcc
901 tggcttggc atgaaaggc cctccatgt gaatgtccag gccctgaaca aatgggagcc
961 agagttacca agctgctcca ggatgttca gccacctcca gatgtcctgc atgctgagcc
1021tacccaaagg gacaaggaca actttcacc cgggcaggaa gtgttctaca gctgtgagcc
1081cggtacgac ctcagaggat ctacgtattt gcactgcaca ccccaaggag actggagccc

```

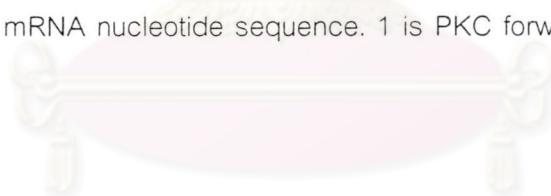
Figure 24 Partial CR1 mRNA nucleotide sequence. 1 is CR1 forward primer, 2 is CR1 reverse primer.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

7. Homo sapiens protein kinase C, mRNA. (GENBANK accession number NM_002738)

1 agcgggcgca cgcggccgc cagagccggc gcaggggaag cgcccggc cccgggtgca
 61 gcagcgcccg ccgcctcccc cgctccccg gcccgcagcc cgccgtcccc cgccccccgg
 121 gccggcacct ctccggctcc ggctccccgc gcgcaagatg gctgaccggg ctgcggggcc
 181 gcccggcggc gagggcgagg agagcaccgt gcgcttcgcc cgcaaaggcg ccctccggca
 241 gaagaacgtg catgaggtca agaaccacaa attcaccgcc cgcttcttca agcagccac
 301 cttctgcagc cactgcaccc acttcatctg gggcttcggg aagcagggat tccagtgc
 361 agtttgctgc ttgtggtgc acaagcggtg ccatgaattt gtcacattt cctgccttgg
 421 cgctgacaag ggtccagcct ccgatgaccc ccgcagcaaa cacaagttt agatccacac 1
 481 gtactccagc cccacgtttt gtgaccactg tgggtcaactg ctgtatggac tcattccacca
 541 gggatgaaa tgtgacacct gcatgatgaa tgtgcacaag cgctgcgtga tgaatgttcc
 601 cagcctgtgt ggcacggacc acacggagcg ccgcggccgc atctacatcc aggcccacat
 661 cgacagggac gtcctcattt tcctcgtaag agatgctaaa aaccttgtac ctatggaccc
 721 caatggcctg ttagatccct acgtaaaact gaaactgatt cccgatccca aaagtggagag 2
 781 caaacagaag accaaaaacca tcaaattgctc cctcaaccct gagtgaaatg agacatttag
 841 atttcagctg aaagaatcg acaaagacag aagactgtca gtagagattt gggattggga
 901 ttgaccaggc aggaatgact tcatggatc ttgtcctt gggatttctg aacttcagaa
 961 agccagtgtt gatggctgg ttaagttact gagccaggag gaaggcgagt acttcaatgt
 1021gcctgtgcca ccagaaggaa gtgaggccaa tgaagaactg cggcagaaat ttgagagggc
 1081caagatcgtt cagggaaacca aggtcccgaa agaaaaagacg accaacactg tctccaaatt

Figure 25 Partial PKC mRNA nucleotide sequence. 1 is PKC forward primer, 2 is PKC reverse primer.



 ศูนย์วิทยาศาสตร์
 จุฬาลงกรณ์มหาวิทยาลัย

8. Human *beta-actin* mRNA. (GENBANK accession number X00351)

1 ttgccgatcc ggcggccgtc cacacccgccc gccagctcac catggatgtatgc
 61 cgctcgctgt cgacaacggc tccggcatgt gcaaggccgg cttcgccggc gacgtatgc
 121 cccggccgt cttccccctcc atcgtggggc gccccaggca ccagggcgtg atggtggca
 181 tgggtcagaa ggattcctat gtggcgcacg aggcccagag caagagaggc atcctcaccc
 241 tgaagtaccc catcgagcac ggcacatgtca ccaactggga cgacatggag aaaatctggc 1
 301 accacacacctt ctacaattag ctgcgtgtgg ctcccggagga gcaccccggtg ctgctgaccg
 361 aggccccctt gaaccccaag gccaaccggc agaagatgac ccagatcatg tttgagacct
 421 tcaacaccccc agccatgtac gttgctatcc aggctgtgtct atccctgtac gcctctggcc
 481 gtaccactgg catcgatgacttccggtg acggggtcac ccacactgtg cccatctacg
 541 aggggtatgc cctccccat gccatcctgc gtctggaccc ggctggccgg gacctgactg
 601 actacccat gaagatcctc accgagcgcc gctacagctt caccaccacg gcccggcgg
 661 aaatcgatgc tgacatataag gagaagctgt gctacgtgc cctggacttc gagcaagaga
 721 tggccacggc tgcttccagg tcctccctgg agaagagacta cgagctgcct gacggccagg 2
 781 tcatcaccat tggcaatgag cgggtcccgct gcccctgaggc actcttccagg ccttccttcc
 841 tggcatgga gtcctgtggc atccacgaaa ctaccttcaa ctccatcatg aagtgtgacg
 901 tggacatccg caaagacctg tacgccaaca cagtgtgtc tggggcacc accatgtacc
 961 ctggcattgc cgacaggatg cagaaggaga tcactgcctt ggccacccagc acaatgaaga
 1021tcaagatcat tgctcctcct gagcgcaagt actccgtgtg gatcgccggc tccatcctgg

Figure 26 Partial β -actin mRNA nucleotide sequence. 1 is β -actin forward primer, 2 is β -actin reverse primer.

ศูนย์วิทยาศาสตร์
 จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX D

Table 6 Target gene expression in TEL-AML1 positive ALL.

No	IL-3	TCR γ	RAG1	CR1	PKC
1	+	-	+	+	+
2	+	-	+	+	+
3	+	-	+	+	+
4	+	-	+	+	+
5	+	+	+	-	+
6	+	-	+	-	-
7	+	-	+	+	+
8	+	+	+	-	+
9	+	-	+	+	+

+ , target genes are expressed

- , target genes are not expressed

**ศูนย์วิทยาศาสตร์พยาบาล
จุฬาลงกรณ์มหาวิทยาลัย**

Table 7 Target gene expression in TEL-AML1 negative ALL.

No	IL-3	TCRγ	RAG1	CR1	PKC
1	-	-	+	-	-
2	+	-	+	+	+
3	+	-	+	+	+
4	+	-	+	-	+
5	+	-	+	+	+
6	+	-	+	-	-
7	-	-	+	-	+
8	+	-	+	+	-
9	+	+	+	-	+
10	+	-	+	+	+
11	+	-	+	-	+
12	+	+	+	-	+
13	+	-	+	-	+
14	+	-	+	-	+
15	+	-	+	+	+
16	-	-	+	-	+
17	+	+	+	-	+
18	+	+	+	+	+
19	+	-	+	-	+
20	+	+	+	+	+
21	+	-	+	+	+
22	+	-	+	+	+
23	-	-	+	+	+
24	+	+	+	+	+
25	-	-	+	+	-
26	-	+	+	+	-
27	+	-	+	+	+

28	+	+	+	-	+
29	+	-	+	+	-
30	+	-	+	+	+

+, target genes are expressed

-, target genes are not expressed



BIOGRAPHY

Miss Tasawan Singhsilarak was born in Bangkok in 1978. She received her bachelor degree in Medical Technology from Chulalongkorn University in Bangkok, Thailand. Consequently, with her interest in human and molecular genetics, she made one of her vigorous decisions to study in the curriculum of the Medical Sciences in the Faculty of Medicine, Chulalongkorn University for her Master's degree. Next plan is to study in a Ph.D. program.

