技术与方法

全染料染色法测定末端脱氧核苷酰转移酶活力

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摘要 介绍一种末端脱氧核苷酰转移酶活力测定的新方法. 利用全染料 (stains-all) 对单链寡聚核苷酸片段灵敏度较高的特点，建立了电泳-全染料染色法测定 TdT 酶活力. TdT 酶促加长的寡聚核苷酸片段样本. 经聚丙烯酰胺凝胶电泳分离、全染料染色后即可根据寡聚核苷酸片段的泳动距离推算 TdT 酶活力. 此法所用试剂便宜，操作简便，无需大型仪器及灵敏度较高，适用于临床上白血病患者白细胞中 TdT 酶活力的半定量检测及基因工程中 TdT 工具酶活力的测定.

关键词 末端脱氧核苷酰转移酶 (TdT)，全染料 (stains-all)，白血病分型

末端脱氧核苷酰转移酶 (terminal deoxynucleotidyl transferase，TdT) 的活力在健康者及不同类型白血病患者外周血白细胞中有极大的差异，此特点可用于白血病的诊断、分型及缓解后病人的追踪复发，有助于确定临床治疗方案[1,2]，最常用的酶活力测定方法为放射性同位素掺入法[3]，此法虽灵敏但存在局限性，不易临床推广应用.

全染料(stains-all)是一种羰花青阳离子染料，它之所以被称为全染料是因为能与多种生物大分子结合，产生不同颜色复合物[5—7]. 全染料对单链 DNA 尤以寡聚核苷酸片段更为灵敏，远高于用溴化乙锭染色的结果，0.1μg DNA 即可分辨. 因此，若配合特定的凝胶电泳分离条件，在一定灵敏度范围内，全染料染色法可代替放射自显影法，大大地简化了实验步骤. 本实验正是利用这一性质，建立了一种新方法即电泳-全染料染色法，可半定量地检测 TdT 酶活力. 这一方法的建立将为白血病的诊断与分型提供一种简便、易行的辅助方法.

1 材料与方法

1.1 材料

全染料 (Sigma)；丙烯酰胺 (Serva)；N，N，N′，N′-甲叉双丙烯酰胺 (Fluka AG)；oligo $(dT)_{20}$ (南开大学分子生物研究所刘福森老师赠)；甲酰胺 (Fluka AG)；TdT 酶液 (BRL)；其它化学试剂均为国产分析纯.

1.2 TdT 酶活力测定标准曲线

酶活力测定体系中含 0.025μg/μl oligo $(dT)_{20}$，2mmol/L dTTP (deoxythymidine triphosphate)，0.2mol/L 二甲胂酸缓冲液 (pH7.5)，8mmol/L $MgCl_2$ 巯基乙醇及一定量标准 TdT 酶液，总反应体积为 12μl. 反应混合物 37℃保温 1h 后，冰浴终止反应，加甘油至终浓度为 20%备用. 15%聚丙烯酰胺凝胶，200V 电压预电泳 45min. 将上述 TdT 酶反应混合物点样，电泳 (250V，2.5h) 用 0.05%全染料溶液 (50%甲酰胺溶液) 染色过夜，于 25%甲酰胺溶液中脱色. 绘制寡聚核苷酸片段泳动距离与加入 TdT 量的关系曲线.

1.3 电泳-全染料染色法测定白血病患者外周血白细胞中 TdT 酶活力

分别取健康人及白血病患者静脉血 3—7ml (视患者外周血白细胞浓度而定). 加入抗凝剂后将血样加至 3ml 白细胞分离液中 (2.4

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：1，9% Ficall 400：34% Hypaque 混合液），1000r/min，离心 30min，吸取界面层的细胞，加入等体积生理盐水，1000r/min，离心 30min，收集白细胞（如混有少量红细胞，可用 0.87% NH_4Cl 溶液除去）计数．加入白细胞匀浆液（0.25mol/L磷酸钾缓冲液，pH7.5及1mmol/L巯基乙醇），调整白细胞的浓度为10^7—10^8个/ml，冻融 3—5 次破碎白细胞，18 000r/min，离心 60min，收取上清液备用．

酶活力测定体系中含：3μl 0.25μg/μl oligo $(dT)_{20}$，3μl 20mmol/L dTTP 溶液，20μl 白细胞冻融上清液、2.9μl 0.5mol/L Tris-HCl，pH7.5．反应体系 37℃保温 45min，加甘油至终浓度 15%．经 15%聚丙烯酰胺凝胶电泳分离，0.05%全染料染色，25%甲酰胺溶液脱色，观察结果．

2　结果与讨论

2.1　TdT 酶活力与寡聚核苷酸片段泳动距离的关系

由图 1，图 2 可见到 TdT 酶活力与寡聚核苷酸引物泳动的距离有一定的线性关系，即酶

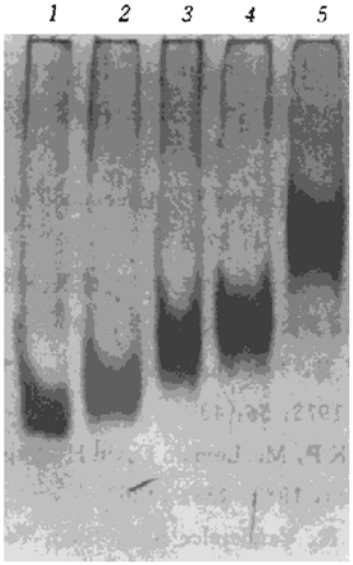

图 1　寡聚核苷酸片段泳动距离与 TdT 酶活力的关系

1—5：TdT 酶活力为 0，3，6，9 和 15 单位（U）．

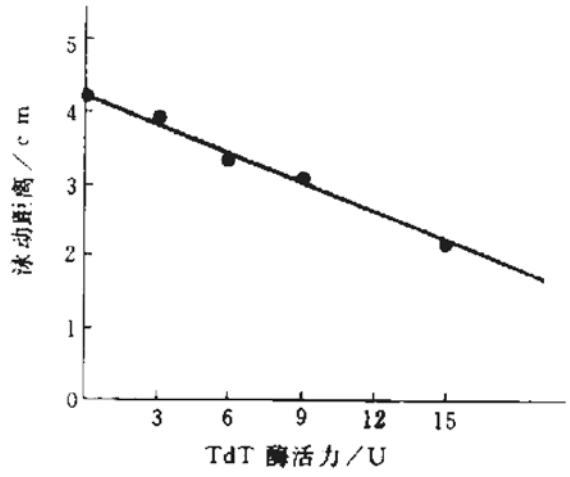

图 2　寡聚核苷酸引物的泳动距离与 TdT 酶活力的关系曲线

量越大，dTTP 掺入量也越多；寡聚核苷酸引物加长越大，而其泳动距离则越短．因此根据寡聚核苷酸片段泳动距离及标准曲线可半定量甚至定量地测定 TdT 酶活力．

2.2　电泳-全染料染色法测定白血病患者外周血中 TdT 酶活力

图 3 结果表明点样槽 5 的样品为不加酶只有寡聚核苷酸引物及底物，其电泳带仅一条位于前沿，与点样槽 4 的最前沿带泳动距离相同，表明健康人白细胞中未检测到 TdT 酶活力，另外几条电泳带泳动较慢可能是人血细胞中的组成蛋白；而样品 1—4 中寡核苷酸片段区带之前的涂布状物亦是血样中蛋白及核酸降解物所致，这些电泳带并不干扰 TdT 酶活力的测定．样品 1，2，3 中寡聚核苷酸引物电泳区带位置的改变是 TdT 酶催化底物加接至引物的作用结果，并非是血样中杂蛋白非特异性作用．

假设血样中寡聚核苷酸引物泳动距离为 d'，空白对照中引物泳动距离为 d_0'；标准曲线中空白对照引物泳动距离为 d_0，待测血样中引物的实际泳动距离为 $d=\frac{d_0 d'}{d_0'}$；根据图 2 所示标准曲线及 d 值可推算出待测血样中 TdT 酶活力．

上述 3 例患者血样用本法和用放射性同位素掺入法测定结果基本相符，如表 1 所示．

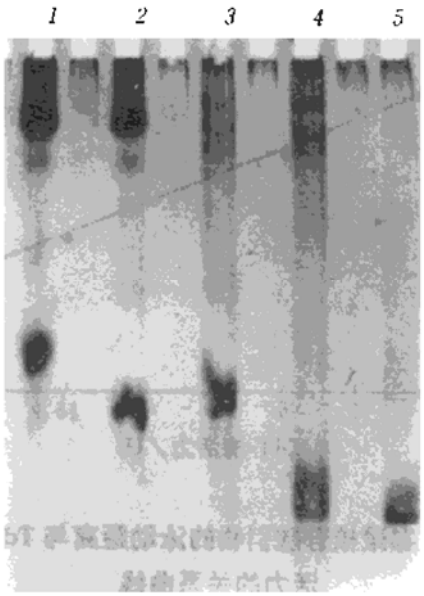

图 3 健康人及白血病患者外周血白细胞 TdT 酶活力的测定

1—3: 分别为三例白血病患者外周血白细胞冻融液; *4*: 健康人外周血白细胞冻融液; *5*: 对照 (只加入 oligo $(dT)_{20}$, dTTP, 不加酶).

表 1 两种不同的方法测定白血病患者 TdT 酶活力的比较

(U)

序 号	电泳-全染料法	放射性同位素掺入法
样品 1	23.0	25.5
样品 2	10.0	11.0
样品 3	13.0	14.5

注: 1) 电泳-全染料法所测结果偏低, 原因是血样已放置了 6 个月, 酶活力降低; 2) 均为 10^8 细胞的酶活力.

我们认为若 TdT 纯酶不易获得时, 也可不制作标准曲线, 仅用患者与健康人血样对照, 即可用此法检测患者外周血白细胞中有无 TdT 酶活力, 做出初步诊断. 根据我们曾对几种不同类型的白血病、其它种类的癌症 (肝、肺、胃癌) 患者和健康人共 108 例的外周血白细胞中 TdT 酶活力的测定, 结果极其明显. 其中以初诊急淋患者最高 (20—100 酶单位每 10^8 细胞) 而健康人和其它类型白血病或其它癌症患者血样均未检测到 TdT 酶活力[4].

TdT 酶活力的测定方法有多种, 如生化定量法[3], 即通过测定掺入到引物的放射性同位素底物的量来计算所加入的 TdT 酶的活力; 间接免疫荧光法[7], 利用 TdT 有较宽的种属交叉免疫反应, 用兔抗人 TdT 血清做初级试剂, 加至待测细胞悬液的涂片上, 再用荧光素偶联的单一 F $(ab)_2$-抗兔 IgG 处理涂片反染后根据 TdT^+ 细胞百分率的高低, 以示其活力高低; 荧光底物掺入法[8], 即通过测定掺入到引物中的荧光底物的量来计算所加入的 TdT 酶活力.

上述几种测活方法都有一定的局限性. 如生化定量测定法必须使用放射性同位素标记物及液闪计数器; 间接免疫荧光法所用试剂要求很高并需荧光显微镜; 荧光底物掺入法所用主要试剂目前尚无商品并需荧光分光光度计, 所以限制了这些方法在临床的推广应用.

本文建立的电泳-全染料染色法, 在某些方面克服了上述几种方法的不足之处, 大大地简化了 TdT 测活操作, 直观性强, 重现性好, 为在临床以 TdT 酶为指标提高确诊率提供了现实的可能性.

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Tb (Ⅲ) as a fluorescent probe was used to study the surroundings of metal ions in FP (fibrinolytic principle). There is a high affinity site of Ca^{2+} in every FP molecule and Ca^{2+} ions are located near the Trp. When Tb (Ⅲ)-FP was excited by 222nm and 233nm, it is possible that energy transfer occurs are as follows: Trp → Tb^{3+}, Trp → Tb^{3+} → Phe, Tb^{3+} → Phe, Tb^{3+} → Trp. These results indicate that Phe may combine with Tb (Ⅲ). The results of calculations indicate that the distance between Trp and Tb (Ⅲ) is about 5.66 Å.

Key words agistrodon acutus, venoms, fibrinolytic principle (FP), fluorescent probe

A Study and Simulation on the Superposition Imaging in the Optical Modeling Compound Eye of Beijing Firefly. Wu Meiying, Mcintyre D. (*Institute of Biophysics, Academia Sinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 535—539

The dioptic system of compound eye of Beijing firefly was simulated on the basis of the real parameters measured from it's structure physiology and it's known GRIN lens properties. The size of the model and values of refractive index were based upon the measured values of actual compounc eye. It was proved that the compound eye of Beijing firefly was a refrac - ting superposition eye by the computer ray tracing model of the compound eye. Parallel light rays incident through the superposition aperture were focussed across the clear zone onto the retina and formed a blur-circle with a certain half-width value. The number of signal light rays and the ratio of signal and noise were increased as the distance to the image plane from exocone proxmal tip were increased. The maximun was at the retina level.

Key words compound eye of Beijing firefly, optical model, superposition image

Study of the Measurements with Acridine Orange in Protons Transportation of Lysosome H^+-ATPase. Zhang Guojiang, Wang Jia, Yao Junlan, Liu Xiaoqi. (*Institute of Biophysics, Academia Sinica, Beijing* 100101). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 539—543

The different range characteristics of acridine orange in absorption and fluorescence is dependent on its concentrations. The importance and mechanism as well as principles of choo - sing correct acridine orange concentration and rational amounts of lysosome in the assay of protons pumping are discussed. The obvious influences of the incubation time of acridine orange with lysosome and the K^+/H^+ exchange upon the measurements of proton transport are studied.

Key words acridine orange, lysosome, H^+-ATPase, proton

Stains-all staining Assay for Terminal Deoxynucleotidyl Transferase Activity. Zhang Jun, Lin Zhoukun. (*Department of Biology, Nankai University, Tianjin* 300071). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 544—546

A polyacrylamide gel electrophoresis-stains-all staining assay for terminal deoxynucleotidyl transferase (TdT) activity in purified TdT and leucocytic homogenate of normal and leukemic patients has been developed. The results show that stains-all [(1-ethyl-2-[3-(1-ethyl-naphtho

〔1, 2-d〕thiazolin-2 ylidene)-2-methylpropenyl〕naphtho〔1, 2-d〕-thiazolium bromide〕is highly specific for single strand oligo-deoxynucleotidyl fragment; no other cell component produces influence. The method permits the estimation of TdT activity as low as 3U. It is a simple assay suitable for clinical analysis of TdT in human leukemias and provding information useful in classifying haemaological neoplasms.

Key words terminal deoxynucleotidyl transferase (TdT), stains-all, classifying haematological neoplasms

Research on Phosphorus Content in Living Rabbits by *in vivo* Neutron Activation Analysis. Luo Xianqing, Wu Jingping, Wang Hai-ying, Huang Hanqiao, Liu Xiaohua, Lu Xihai, Yu Aiping, Luo Qiandi. (*Huazhong Agricultural University, Wuhan* 430070). *Prog. Biochem. Biophys.* (China), 1994: **21** (6): 547—549

Research on the determination of phosphorus content in living rabbits by *in vivo* neutron activation analysis (IVNAA) was carried out, and the average value of the phosphorus percentage content which were measured for ten rabbits was (1.26±0.01)%.

Key words *in vivo* neutron activation analysis, living rabbits, phosphorus content

A Non-radioactive Single-strand Conformation Polymorphisms of Asymmetric PCR ans its Application. Chen Jun, Wnag Huimin, Li Manzhi, Wu Yintang. (*Cancer institute, Sun Yat-Sen University of Medical Science, Guangzhou* 510060). *Prog. Biochem. Biophys.* (China). 1994: **21** (6): 550—553

PCR-single strand conformation polymorphisms is a powerful method for screening mutations and widely used in studying mutations of oncogene and tumor suppressor gene. The ordinary PCR-SSCP needs the use of radioactivity and sequencing appratus, thus compromises its application. Here, a non-radioaltive asymmetric PCR-SSCP was established. Single-stranded DNA was generated by asymmetric PCR, seperated by mini PAGE and silver stained. The exon 5, 6, 7, 8 of p53 gene in four cell lines of nasopharyngeal carcinoma-CNE1, CNE2, HK1 and SUNE1 were investigated. The method was proved to be succssful in screening mutations.

Key words asymmetric PCR, single-strand conformation, polymorphisms, mutation, p53, nasopharyngeal carcinoma

PCR Detection of Mycoplasma Contamination in Cell Culture. Wang Zhengsen, Wu Jianxin, Zhao Xiaoyuan, Sun Baoling, Guo Zhanggai, Li Min. (*Department of Biochemistry and Immunology of the Capital Institute of Pediatrics, Beijing* 100020). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 553—556

Mycoplasma contamination of cell culture is a serious problem in biomedical reseach. Three common PCR primers (F1, F2 and R1) were designed to amplify the spacer region between 16s and 23s DNA in rRNA operons of 6 species of mycoplasmas (*M. arginini*, *M. orale*, *M. hominis*, *M. hyorhinis*, *M. fementans* and *A. laidlawii*). When the DNA of 6 species was used as the template, primers F1 and R1 produced fragments of 340 to 468 bp, and primers F2 and R1 produced fragments of 145 to 211 bp. No discrete band was observed in electrophoretic gels when Hela cell or *E. coli* DNA was served as the template with the use of primers F1 and R1. As little as 8.5fg DNA of *M. arginini*, approximately 1 3 orga-