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The Technique of Temperature Gradient Gel Electrophoresis and Its Applications. CHEN Hanr-Kui, FENG Xin (*Guangzhou Cancer Hospital, Guangzhou* 510095, China).

Abstract Temperature gradient gel electrophoresis (TGGE) is a new and powerful electrophoresis method for separation of nucleic acids (DNA and RNA) and analysis of sequence variations. The TGGE method uses the melting temperature (T_m) as an important parameter to identify DNA, which differs in sequence among a mixture of molecules of the same size but different conformation. With the advantages of high-resolution capability, high reproducibility and timesaving process, the TGGE technique has been applied broadly in the field of molecular biology.

Key words temperature gradient gel electrophoresis, melting temperature, sequence variation, point mutation

一种改良的从银染的聚丙烯酰胺凝胶中回收 DNA 的方法

Clin Chem, 1998, **44** (9) 报道了 Steven 等介绍的从聚丙烯酰胺凝胶上将 DNA 转移到 DEAE 膜上的技术。此技术能同时进行多个样品的处理和纯化，被纯化的差异显示法 (DDA) 产物电泳条带单一，分离的范围为 100~ 700 bp 的 DNA，回收的 DNA 足够作数次重扩增反应，且重新扩增的条件不变，重复性高。此技术不需要特殊仪器和试剂，操作简便在 DDA 中应用十分广泛。

细胞遗传学正常的羊水细胞在二倍的常氏 (CISM) 细胞培养液中生长。信使 RNA 用寡聚 dT/链霉抗生物素蛋白磁珠吸附系统分离，以 MMLV 逆转录酶、寡聚 dT 为引物 (5′-TTTTTTTTTTTTGC)，将其转变成 cDNA。DDA 分析，用 20 μl 反应液。1 × PCR 缓冲液，含 1.5 mmol/L $MgCl_2$，20 μmol/L dNTP，0.5 μmol/L 寡聚 dT 引物，0.25 μmol/L 上游引物 (5′-CTGCTTGATG，5′-GATCCAGTC，5′-GATCGCATTG，和 5′-AAACTCCGTC)，30 ng cDNA 和 0.625 U Taq 酶，40 个热循环，95 ℃ 30 s，40 ℃ 1 min，72 ℃ 1 min。DNA 产物用乙醇沉淀，再水溶解，用 5% 非变性聚丙烯酰胺凝胶电泳分离，银染显色。

含 DDA 扩增产物的聚丙烯酰胺干胶用小刀切下，放入 2% 琼脂糖凝胶中近阴极端的孔内，压平，小心地用溶化的较凉的 2% 琼脂糖胶填满。将 DEAE 膜裁成 0.5 cm × 0.75 cm 的条，插入离孔 0.5 cm 处，每孔后插一条，可用小刀帮助插入干 DEAE 条。DNA 以 10 V/cm 电泳 90 min 后，将 DEAE 膜取出，放入小离心管内，用 500 μl 低盐 NET (0.15 mmol/L NaCl, 0.1 mmol/L EDTA, 20 mmol/L Tris, pH 8.0) 缓冲液浸泡，然后用 50 μl 高盐 NET 缓冲液 (1 mol/L NaCl, 0.1 mmol/L EDTA, 20 mmol/L Tris, pH 8.0)，在 65 ℃温育 30 min 以洗脱 DNA。DNA 用 2.5 倍的乙醇沉淀，然后用 15 μl 水溶解。用 5 μl 洗脱 DNA，以上述条件进行扩增，产物连接上 T-载体 (Promega) 并测序。

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