DOCUMENT 1

Culture medium

Cells were grown in a defined mineral salts medium ($CaSO_4$, $MgSO_4$, NaCl, KNO_3, $NaNO_3$, $ZnSO_4$, H_3BO_3, $CuSO_4$, $NaMoO_4$, $CoCl_2$, $FeCl_3$, $MnSO_4$, Na_2HPO_4) containing 0.3 % glutamic acid, biotin and thiamin (0.1 mg/L each) and nicotinic acid (0.05 mg/L).

Growth conditions

Cells were grown initially in 500 mL Erlenmeyer flasks at 75°C in a water bath shaker. When the cultures reached a density of approximately 170 Klett units, 1 liter of these cells was transferred to 16-liter bioreactor, which were placed in hot-air incubators. In place of shaking, sterile air was bubbled through the cultures, and the temperature was maintained at 75°C. The cells were allowed to grow for 20 h before they were collected with continuous-flow centrifuge.

DOCUMENT 2

2A

Summary of the purification procedure

Fraction	volume (mL)	Total polymerase activity (U)	Total protein (mg)	Yield of activity (%)	Specific activity (U/mg)	Purification fold
« Crude »	176	2,080	975	100		1
DEAE-Sephadex	210	1,982	197			
Phosphocellulose	132	1,900	16			
DNA-cellulose	63	0,685	*ND*			

ND : non déterminable par suite de l'ajout de BSA indispensable pour enrayer la perte d'activité.

2B

DNA polymerase assays

The reaction mixture (125 µL) contained : tris buffer (pH 8, 25 mM) ; $MgCl_2$, 10 mM ; KCl, 25 mM ; 2-mercaptoethanol, 1 mM ; dCTP, dGTP and dTTP (150 µM each) ; [^{3}H]dATP (150 µM, specific activity : 38 mCi/mmol) and activated calf thymus DNA template, 12.5 µg.

Activated calf thymus DNA template is a double-stranded molecule which has gaps in portions of either strand, which gaps are filled by the appropriate dNTPs using the DNA polymerase.

After 30 minutes of incubation with enzymatic extracts at 80°C in sealed tubes, the assay was stopped by chilling the tubes in an ice bath. Samples of 100 µL were then pipetted onto filter paper disks and immediatly dropped into ice-cold 10% trichloracetic acid containing 0.1 M sodium pyrophosphate for at least 1 h. Finally, the disks were washed in ether-ethanol, air dried and the amount of [^{3}H]dATP incorporated into an acid-insoluble product was measured in a scintillation spectrometer.

One unit of enzyme is defined as the amount of enzyme that will incorporate 10 nmol of [^{3}H]dATP into acid-insoluble material at 80°C in 30 min.

DOCUMENT 3

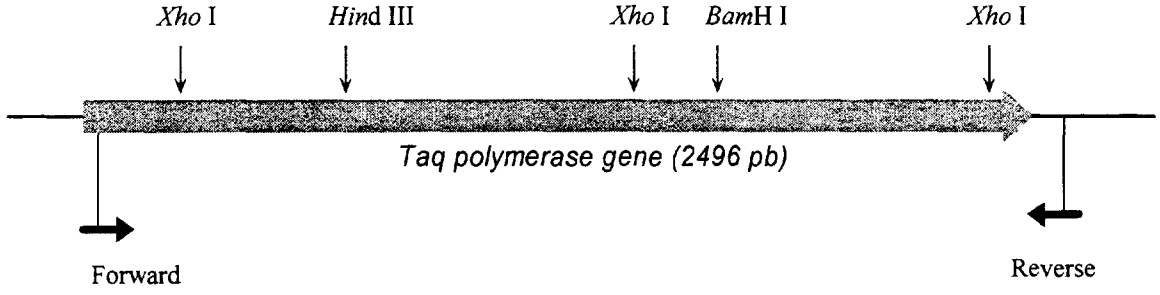
Construction of the Expression Vector

Briefly, genomic DNA was isolated from *T. aquaticus* and used as a template to amplify the Taq DNA polymerase gene by Polymerase Chain Reaction (PCR). The Forward primer creates a unique *Eco*R I restriction cut site and the Reverse primer create a unique *Bgl* II restriction cut site.
The resulting amplification product was digested with *Eco*R I and *Bgl* II and cloned into pUC18 digested with *Eco*R I and *Bam*H I.
This vector was designated pTAQ and transformed into *DH5 α E. coli* by Hanahan's method for bacterial transformation.

Sites de restriction des enzymes utilisées

***Bgl* II**	A/GATCT
***Eco*R I**	G/AATTC
***Bam*H I**	G/GATCC

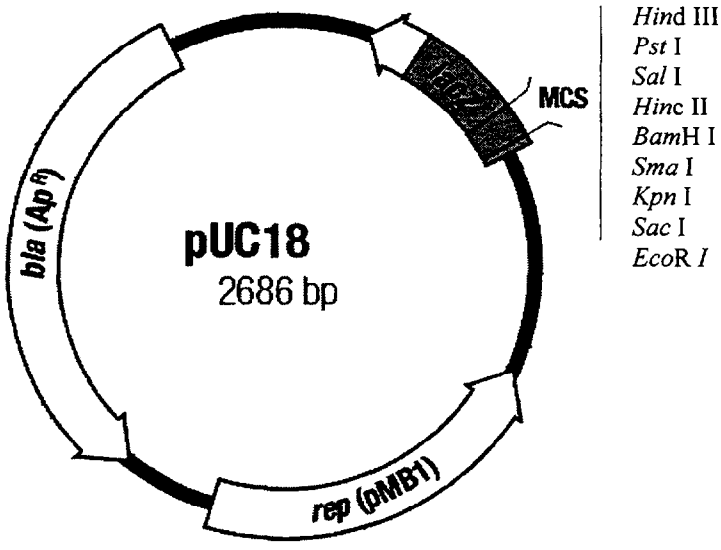
Carte de restriction partielle du gène Taq et positions des amorces utilisées



Séquences des amorces utilisées

Forward primer : 5'-CAC**GAATTC**GGGGATGCTGCC-CCTCTTTGAGCCCAAG
Reverse terminal primer : 5'-GTG**AGATCT**ATCACTCCTTGGCGGAGAGCCAGTC

Carte du plasmide pUC18



DOCUMENT 4

Buffer Compositions (1X):
Buffer 1 : 10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT (pH 7.0 at 25°C).
Buffer 2 : 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT (pH 7.9 at 25°C).
Buffer 3 : 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT (pH 7.9 at 25°C).

	% activity in Buffer 1	% activity in Buffer 2	% activity in Buffer 3
***Bam*HI**	75	100	50
***Bgl*II**	10	75	100
***Eco*RI**	100	100	100

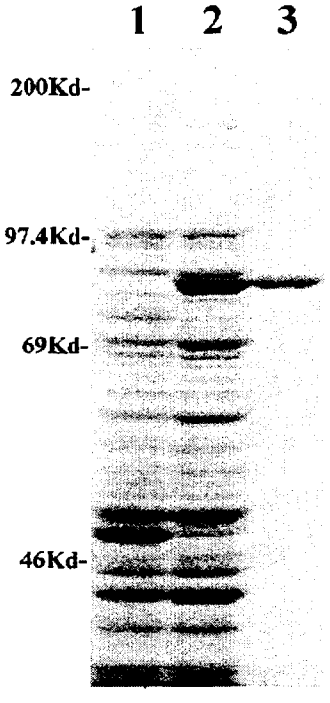
DOCUMENT 5

5A

Purification of DNA Polymerase

Taq DNA polymerase was purified from 100 mL cultures. Super Broth media plus ampicillin was inoculated with 100 µL of a log phase culture of DH5α containing the pTAQ construct described above. This culture was grown to an OD600 of 0.3 at 37 °C, then induced with 0.5 mM IPTG and allowed to grow for 16 hours.
Cells were centrifuged, resuspended in three mL of buffer A (50 mM Tris-HCl, pH 7.9, 50 mM dextrose, 1 mM EDTA) containing 4 mg/mL lysozyme, and incubated 15 minutes at room temperature.
Three mL of buffer B was added (10 mM Tris-HCl, pH 7.9, 50 mM KCl, 1 mM EDTA, 0.5% Tween 20, 0.5% Nonidet P40), and the mixture was incubated 60 minutes at 75 °C in a shaking water bath.
Cell debris and denatured protein were removed by centrifugation at 12,000 x g for 10 minutes at 4 °C.
This **lysate** was then mixed with an equal volume of storage buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.1 mM EDTA, 0.5 mM DTT, 1% Triton X-100) containing 50% glycerol, followed by addition of an equal volume of storage buffer containing 75% glycerol. This mixture was then stored at -20 °C.

5B



12% SDS-PAGE gel was used to analyze each step of the purification process.
Lanes contain :
1) sonicated *E. coli* host cells not expressing *Taq* DNA pol I,
2) sonicated *E. coli* expressing *Taq* DNA pol I,
3) cell lysate cleared by thermal denaturation.

(Protocoles pour 1) et 2) non fournis)

DOCUMENT 6

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pol   ----MVQIPQNPLILVDGSSYLYRAYHAFPPLTNSAGEPTGAMYGVLNMLRSLIMQYKPT
taq   MAMLPLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEPVQAVYGFAKSLLKALKEDG-D      60

pol   HAAVVFDAKGKTFRDELFEHYKSHRPPMPDDLRAQIEPLHAMVKAMGLPLLAVSGVEADD
taq   VVVVVFDAKAPSFRHEAYEAYKAGRAPTPEDFPRQLALIKELVDLLGLVRLEVPGFEADD     120

pol   VIGTLAREAEKAGRPVLISTGDKDMAQLVTPNITLINTMTNTILGPEEVVNKYGVPPELI
taq   VLATLAKRAEKEGYEVRILTADRDLYQLLSERIAILHPEG-YLITPAWLYEKYGLRPEQW     180

pol   IDFLALMGDSSDNIPGVPGVGEKTAQALLQGLGGLDTLYAEPEKIAGLSFRGAKTMAAKL
taq   VDYRALAGDPSDNIPGVKGIGEKTAQRLIREWGSLENLFQHLDQVK-------PSLREKL     240

pol   EQNKEVAYLSYQLATIKTDVELELTCEQLEVQQPAAEELLGLFKKYEFKRWTADVEAGKW
taq   QAGMEALALSRKLSQVHTDLPLEVDFG-RRRTPNLEGLRAFLERLEFGSLLHEFGLLEG300

pol   LQAKGAKPAAKPQETSVADEAPEVTATVISYDNYVTILDEETLKAWIAKLEKAPVFAFDT
taq   PKAAEEAPWPPPE----------------------------------------------     360

pol   ETDSLDNISANLVGLSFAIEPGVAAYIPVAHDYLDAPDQISRERALELLKPLLEDEKALK
taq   ---------GAFLGFSFSRPEPMWAELLALAGAWEG-------RLHRAQDPLRGLRDLKG     420

pol   VGQNLKYDRGILANYGIELRGIAFDTMLESYILNSVAGRHDMDSLAERWLKHKTITFEEI
taq   VRGILAKDLAVLALREGLDLFPEDDPMLLAYLLDPSN-----------------TTPEGV     480

pol   AGKGKNQLTFNQIALEEAGRYAAEDADVTLQLHLKMWPDLQKHKGPLNVFENIEMPLVPV
taq   ARRYGG----------EWTEDAGERALLAERLFQTLKERLKGEERLLWLYEEVEKPLSRV     540

pol   LSRIERNGVKIDPKVLHNHSEELTLRLAELEKKAHEIAGEEFNLSSTKQLQTILFEKQGI
taq   LARMEATGVRLDVAYLQALSLEVEAEVRQLEEEVFRLAGHPFNLNSRDQLERVLFDELGL     600

pol   KPLKKTPG-GAPSTSEEVLEELALDYPLPKVILEYRGLAKLKSTYTDKLPLMINPKTGRV
taq   PAIGKTEKTGKRSTSAAVLEALREAHPIVDRILQYRELTKLKNTYIDPLPALVHPKTGRL     660

pol   HTSYHQAVTATGRLSSTDPNLQNIPVRNEEGRRIRQAFIAPEDYVIVSADYSQIELRIMA
taq   HTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFVAEEGWVLVVLDYSQIELRVLA     720

pol   HLSRDKGLLTAFAEGKDIHRATAAEVFGLPLETVTSEQRRSAKAINFGLIYGMSAFGLAR
taq   HLSGDENLIRVFQEGRDIHTQTASWMFGVSPEGVDPLMRRAAKTINFGVLYGMSAHRLSG     780

pol   QLNIPRKEAQKYMDLYFERYPGVLEYMERTRAQAKEQGYVETLDGRRLYLPDIKSSNGAR
taq   ELSIPYEEAVAFIERYFQSYPKVRAWIEGTLEEGRRRGYVETLFGRRRYVPDLNARVKSV     840

pol   RAAAERAAINAPMQGTAADIIKRAMIAVDAWLQAEQPRVRMIMQVHDELVFEVHKDDVDA
taq   REAAERMAFNMPVQGTAADLMKLAMVRLFPRLQELG--ARMLLQVHDELVLEAPKDRAER     900

pol   VAKQIHQLMENCTRLDVPLLVEVGSGENWDQAH-
taq   VAALAKEVMEGVWPLQVPLEVEVGLGEDWLSAKE                               934
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DOCUMENT 7

Procedure for producing monoclonal antibodies specific for a given antigen developed by G. Kohler and C. Milstein.

Spleen cells from an antigen-primed mouse are fused with mouse myeloma cells (HGPRT - and Ig -).
The spleen cells provides the necessary enzymes for growth on HAT medium, while the myeloma provides immortal-growth properties. Unfused myeloma cells or myeloma-myeloma fusions fail to grow due of lack of HGPRT. Unfused spleen cells have limited growth in vitro and therefore do not need an enzyme deficiency for elimination with the HAT selection procedure.
After 7-10 days of culture in the HAT medium, most of the wells contain dead cells, but a few wells contain small clusters of viable cells, which could be visualized by using an inverted phase contrast microscope.

DOCUMENT 8

Microtiter well plates are coated with *Thermus aquaticus* DNA polymerase (50 µL/plate well of 2µg/mL) were incubated at room temperature for one hour, contacted with gelatin 1% and tween 20 0,05% in phosphate buffered salin solution (200µL/plate well) and stored frozen until needed.
Conventionnal ELISA is performed for initial screening for antibody by the addition of the hybridoma culture supernatant (50µL/plate well) followed by incubation at room temperature with constant agitation. All incubations were followed by washing five times with tween 20 0,05% in phosphate buffered saline solution using a microtiter plate washer.
The detection reagents (50µL/plate well) included goat anti mouse IgG horseradish peroxydase conjugate (diluted 1/3000 in gelatin/phosphate buffered salin solution) were incubated for one hour at room temperature. After washing five times, the peroxydase substrate used to produce dye (ABTS) was incubated for 15 minutes at room temperature. The dye signal was evaluated at 414nm using a plate reader.
Enzymatic reaction was stopped with 2 M NaOH.

DOCUMENT 9

Protocole 1

L'ADN polymérase est incubée avec chaque anticorps 10 minutes à température ambiante. L'activité enzymatique est ensuite mesurée après incubation à 37°C pendant 240 minutes en présence d'ADN matrice et des quatre dNTPs. L'activité ADN polymérase est exprimée en % d'activité par rapport au contrôle sans anticoprs.

Anticorps	Activité ADN polymérase % de contrôle A
Pas d'anticorps (contrôle A)	100 %
Pas d'anticorps. Pas d'enzyme (contrôle B)	0,3 %
Mab1	28 %
Mab2	87 %
Mab3	64,3 %
Mab4	1,4 %

Protocole 2

L'ADN polymérase est incubée avec chaque anticorps à température ambiante puis le mélange est chauffé à 85°C pendant 1 min avant d'être refroidi 5 minutes dans la glace. L'activité enzymatique est ensuite mesurée après incubation pendant 10 min à 75° C en présence d'ADN matrice et des quatre dNTPs.
L'activité ADN polymérase est exprimée en % d'activité par rapport au contrôle sans anticorps.

Anticorps	Activité : ADN polymérase % de contrôle A
Pas d'anticorps (contrôle A)	100 %
Pas d'anticorps. Pas d'enzyme (contrôle B)	0 %
Mab1	99,4 %
Mab2	104 %
Mab3	98,5 %
Mab4	101 %