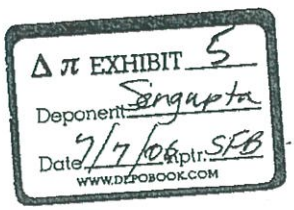

Subject

Basic Methods Molecular Biology Davis, Dibner Battey.
1986 Elsevier

Nature June 9, 1988

Master Mix (1ST 5 components)
Template
Taq Polymerase > Stored in freezer

Dilutions of Template
NL, 1/10, 1/100 dilution

In Tube Large
27 λ of Tris buffer (Blue pipet Man)
3 λ of Control Template (Brown pipet Man)

89.5 λ Master Mix
to 3 tubes (N, .1, .01)
3 λ of Taq Polymerase (Brown pipette)

10 λ Control Template into each tube (Brown pipette)
(From N and large tubes .1 → .1 .01 → .01 etc)

Covers with.
100 λ mineral oil to cover each tube

PCR Machine

Thermal cycle
1 min 30 sec @ 94°C

24 cycles
2 min 37°
3 min 72°
1 min 94°

25th
2 min 37°
10 min 72°

Signed Date

Subject 2

Agua Mixture into large Nalgene Flask

12 ml TBE (Tris Borate - EDTA) 5X

48 ml Distilled H_2O

1.8 g Nusieve

.6 g Seakem

Microwave until melted bubbling

12 c) EtBr (Ethidium Bromide) Stains DNA

To Make Gel
place blue tape over edges
pour liquid gel into stand
place slot bar in.

After PCR Reaction
Remove tubes from machine

Remove 20 λ of sample from tube place in large tube

Add 2.2 λ of loading Buffer to each tube

Marker
14 λ T.E.
6 λ Marker DNA - Hae III Digest pBR 322
2.2 λ Loading Buffer

Buffer for Gel
200 cc TBE
800 cc Distilled H_2O
Pour over gel (~ 5 mm over top of gel)

Signed Date

Place 20 λ of solution in each well.

Attach Electrodes to plate
Turn machine on 200 V 68 amps.
(Reaction ~ 2 hours).

↑ Marker N .1 .01
→

Angelena
^{32}P - labeling of Oligonucleotide

Oligonucleotide 70 Ng
T_4 Polynucleotide Kenase 5U
^{32}P ATP 7500 Ci/mmol 75 μCi

After labeling oligonucleotides were purified by chromatography on DE-50 cellulose (Whatman) then used as hybridization probes.

Hybridization
Membranes prehybridized for 1 hr @ 50°C in 5x NaCl/Citrate ; Na Phosphate 20 mM pH 7 ; 10x Denhardts 10% (wt/vol) dextran sulfate ; 7% (wt/vol) NaDodSO$_4$ and denatured herring sperm DNA @ 100 μg/ml.
Hybridization carried out overnight @ 50°C in the same solution containing ^{32}P - labeled oligonucleotide 10^6 cpm/ml.
Membranes washed successively for 1 hr @ 50°C in 3x NaCl/Citrate ; 10x Denhardts ; 5% w/v NaDodSO$_4$ Na Phosphate pH 7.5 25 mM then
1x NaCl/Citrate ; 1% w/v NaDodSO$_4$

Signed Date

Oligomer Restriction (OR)
end labeled oligonucleotide probe hybridizes in solution to a region of the amplified sequence.
Reconstitutes a specific endonuclease cleavage site.
Cleavage with the specific endonuclease generates an oligonucleotide of defined size.

J Virology Kwok 1987

1 µg cellular DNA amplified in total volume of 100 µl
for 20 cycles with either primer set SK01/SK02 or SK17/SK18

OR Analysis
10 µl of amplified DNA reaction volume
Annealed to ^{32}P ATP end labeled probe SK03 or SK19
Digested with BstNI
1/4 of this material analyzed by
Polyacrylamide gel electrophoresis and autoradiography.

Lancet Loche, March 1988

PBL separated by Ficoll gradient
Preparation of DNA
Proteinase K 500 µg/ml
Sodium Dodecyl sulphate 0.5%
Tris - HCl pH 7.5 10 mM/L
NaCl 30 mM/L
EDTA 20 mM/L
@ 50°C for 2 hours.

followed by 3 extractions with
Phenol/chloroform (50/50)
EtOH precipitation

Resuspension in
Tris - HCl pH 7.5 10 mM/L
EDTA 1 mM/L

Signed..... Date.....

Angelini (labeling of probe)
Proc Natl Acad Sci 1986: 83: 4489-93

PCR

DNA Sample 1 µg.	
dATP, dTTP, dCTP, dGTP	1.5 mM/L
Tris - HCl pH 8.8	7 mM/L
NH_4SO_4	16 mM/L
$MgCl_2$	10 mM/L
β-mercaptoethanol	10 mM/L
Gelatin	0.02 %
EDTA	7 µM/L
Primer I	1 µM/L
Primer II	1 µM/L

in a volume of 100 µl

Incubation @ 98°C for 7' (denaturation)
40°C for 1' (annealing of primers)

Taq polymerase 2U added
70°C for 3'

Cycle was Repeated 25 x
92°C for 1'
40°C for 1'
70°C for 3'

Hybridization
Membranes treated @ 80°C for 1 hour
Prehybridized in
4x SSC (NaCl 150 mM/L, Na citrate pH 7 15 mM/L)
5x Denhardt (Ficoll 0.02%, Polyvinylpyrolidone 0.02%,
0.02% BSA: bovine serum albumin)
Sodium Dodecyl sulfate 5%
Sodium Phosphate 20 mM/L
Sheared/Denatured Herring Sperm 20 mM/L
1 µCi (15×10^6 cpm) ^{32}PdCTP oligo probe

Signed Date

Membranes were washed twice in
3x SSC
8x Denhardt
Sodium dodecylsulphate 5%
@ 50°C for 15 min.

Autoradiographed for 2-6 hours with Cawo intensifier Screens.

Science Kwok, Sninsky 1988

Isolation of DNA → Maniatis Molecular Cloning A Lab Manual. CSH 1982

PCR

1 µg of PBMC DNA	
Primers	100 pmol
dATP, dTTP, dCTP, dGTP	10 mM/L
Tris · HCl pH 7.5	10 mM/L
NaCl	50 mM/L
$MgCl_2$	10 mM/L
Taq polymerase	0.6 U

Amplification as per Saiki et al Science

After PCR
1/10 of the reaction mixture removed
NaCl concentration adjusted to 0.15 M

Hybridization
32P ATP end labeled probe @ 56°C for 1 hour

Selective Restriction enzyme digestion — Bio Technology 1985 : 3 : 1008.

Primer Pair	Restriction Enzyme
SK 29/30	Hinf I
SK 38/39	Bst N I
SK 68/69	Hae III
CO 1/2	Hha I

Analysis

30% Polyacrylamide gel

Autoradiogram
KODAK XAR film @ -70°C. for 3-4 hours. ± intensifying screen.

Signed..... Date.....