

2 Materials

2.1 Chemicals

Acetic acid	Merck (Darmstadt)
Acrylease	Stratagene(La Jolla, USA)
Agar Agar	Serva(Heidelberg)
Agarose (LMP, ultrapure)	Gibco BRL (Gaithersburg, USA)
Agarose NA	Pharmacia (Uppsala, Schweden)
Agarose-Pulse Field	Amersham (Braunschweig)
Ammoniumacetate	Sigma (München)
Ammoniumpersulfate(APS)	Serva (Heidelberg)
Ampicilline	Bayer (Leverkusen)
Bacto Peptone	Difco (Detroit, USA)
Bacto Yeast Extract	Difco (Detroit,USA)
Boric acid	Merck (Darmstadt)
Bovine Serum Albumin(BSA)	Sigma (München)
5-Brom-4-chloro-3-indolyl- β -	Roth (Karlsruhe)
D-Galactopyranoside (X-gal)	
Bromphenolblue	Merck (Darmstadt)
Calciumchloride	Merck (Darmstadt)
Chloramphenicol	Boehringer (Mannheim)
Chloroform	Merck (Darmstadt)
Dextransulfate	Sigma (München)
D(+)-Glucose	Sigma (München)
Dimethylformamide	Merck (Darmstadt)
1,4-Dithiothret (DTT)	Merck (Darmstadt)
D(+)-Saccharose	Roth (Karlsruhe)
Ethanol	Merck (Darmstadt)
Ethidiumbromide (EtBr)	Serva (Heidelberg)
Ethylendiaminteracetate (EDTA-2Na)	Merck (Darmstadt)
Ficoll type 400	Amersham (Braunschweig)
Formaldehyde (37%)	Merck (Darmstadt)
Formamide	Merck (Darmstadt)
Glutamine	Merck (Darmstadt)
Glycerin (87%)	Merck (Darmstadt)
Glycogen	Boehringer (Mannheim)
Hydrogenchloride (HCl)	Merck (Darmstadt)
HEPES	Sigma (München)
Isopropanol	Merck (Darmstadt)
Isopropyl- β -D-Thio-Galactopyranoside (IPTG)	Roth (Karlsruhe)
Kanamycin	Merck (Darmstadt)
Polyvinylpyrrolidone	Sigma (München)
Potassiumchloride(KCl)	Merck (Darmstadt)
Potassiumacetate	Sigma (München)
L-Arginin-HCl	Roth (Karlsruhe)
L-Histidine	Roth (Karlsruhe)
L-Isoleucine	Roth (Karlsruhe)
L-Leucine	Roth (Karlsruhe)
L-Methionine	Roth (Karlsruhe)

L-Phenylalanine	Roth (Karlsruhe)
L-Threonine	Roth (Karlsruhe)
L-Tryptophan	Roth (Karlsruhe)
L-Tyrosine	Roth (Karlsruhe)
L-Valine	Roth (Karlsruhe)
Lysine	Merck (Darmstadt)
Long Ranger gel solution	Amersham (Braunschweig)
MDE gel solution	FMC (Maine, USA)
Magnesiumchloride	Merck (Darmstadt)
Manganchloride	Merck (Darmstadt)
3-[N-Morpholino] propansulfonate (MOPS)	Sigma (München)
Mineral oil	Sigma (München)
N,N,N',N'-Tetramethylethyldiamin (TEMED)	Serva (Heidelberg)
Sodiumchloride	Merck (Darmstadt)
Sodiumdodecylsulfate (SDS)	Serva (Heidelberg)
Sodiumhydroxide (NaOH)	Merck (Darmstadt)
Sodiumpyrophosphate	Sigma (München)
Phenol	Merck (Darmstadt)
Rotiphorese Gel 40	Roth (Karlsruhe)
Rubidiumchloride	Sigma (München)
Sigmacote	Sigma (München)
Spermidin	Sigma (München)
Tris,[Tris(hydroxymethyl)-aminoethan]	USB (Braunschweig)
Tri-sodiumcitrate	Merck (Darmstadt)
Triton X-100	Merck (Darmstadt)
Urea	Merck (Darmstadt)
Whatman 3 MM-Paper	Schleicher u. Schuell (Dassel)
Xylene cyanol	Merck (Darmstadt)

2.2 Media

I dYT medium : For 1 l medium	16 g Tryptone 10 g Yeast Extract 10 g NaCl
II LB medium : For 1 l medium	10 g Tryptone 5 g Yeast Extract 10 g NaCl
III LB plate : For 1 l preparation	1 l LB medium 15 g Agar-Agar
IV NZYC-Broth: For 1 l :	5 g NaCl 2 g MgCl ₂ .6H ₂ O 10 g NZ amino acids (casein hydrolysate N-Z Amine A) 5 g Bacto yeast extract 1 g Bacto casamino acids (adjust pH to 7.4)

PCR stop buffer: 10 mM NaOH
 1 mM EDTA
 80 % Formamide
 0.1 % Bromophenolblue
 0.1 % Xylencyanol

III Buffers for preparation of competent cells

TFB I-Buffer: 100 mM RbCl₂
 50 mM MnCl₂
 30 mM KOAc
 10 mM CaCl₂ pH 5.8

TFB II Buffer: 10 mM MOPS
 10 mM RbCl
 75 mM CaCl₂
 15 % Glycerol pH 7.0

IV Buffers for DNA preparations

(1) Buffers for genomic DNA isolation of human blood

Nuclei Extraction Buffer: For 100 ml 10.95 g Sucrose
 0.5 ml 1 M MgCl₂
 1 ml Triton X-100
 1 ml 1 M Tris, pH 8.0
 Filter sterile and store at 4°C

DNA Extraction Buffer: For 100 ml 4 ml 1 M Tris, pH 8.0
 4 ml 0.5 M EDTA, pH 8.0
 2 ml 5 M NaCl

The nuclei extraction buffer and DNA extraction buffer are sterilized with filters and stored at 4°C.

(2) Buffers for plasmid DNA miniprep

Lysis Buffer I: 50 mM Glucose
 10 mM EDTA
 25 mM Tris-HCl
 2 mg/ml Lysozyme pH 8.0

Lysis Buffer II: 0.2 N NaOH
 1% (w/v) SDS

Lysis Buffer III: 3 M Na-Acetate, pH 4.8

RNase A : 10 mg/ml

Ribonuclease A is dissolved in 10 mM Tris/HCl, pH7.5 / 15 mM NaCl, and heated for 15 min at 100°C. After slowly cooling down to room temperature, the solution is aliquoted and stored at -20°C.

(3) Buffers for PAC and BAC DNA isolations

P1(filter sterilized, store at 4°C): 15 mM Tris,pH8.0
 10 mM EDTA ,pH 8.0
 100 µg/ml RNase A

P2 (filter sterilized, store room temperature) 0.2 N NaOH
 1 % SDS

P3(autoclaved,store at 4°C) 3 M KOAc, pH5.5

V Solutions for hybridization

100 x Denhardt's solution: For 1 l: 20 g Ficoll
 20 g Polyvinylpyrrolidone
 20 g BSA

Salmon sperm DNA: 5 mg/ml dH₂O
 The solution is autoclaved for 10 min to shear DNA and stored at -20°C.

Hybridization solution: For 1 l: 50 g Dextran Sulfate
 500 ml Formamide
 300 ml 20 x SSC
 50 ml 20% SDS
 50 ml 100 x Denhardt's solution
 4 ml 5mg/ml Salmon sperm DNA

Prehybridization and hybridization solution for oligo-hybridization:
 For 500 ml: 150 ml 20 x SSC
 5ml 100 x Denhardt's solution
 2.5 ml 10% Sodiumpyrophosphate
 12.5 ml 20% SDS
 2.5 ml Salmon sperm DNA(5mg/ml)

VI Solutions for Southern blot

Denaturing solution: 0.5 M NaOH ,
 0.5 M NaCl

Neutralization solution: 0.5 M Tris-HCl, pH7.0
 1 M NaCl

VII 50 x TAE buffer: 2 M Tris-base, pH8.0 (adjust pH with acetic acid)
 50 mM EDTA-diNa

VIII 10 x TBE buffer: 0.5 M Tris-HCl , pH8.0

	0.5 M Boric acid
	0.5 M EDTA
IX 20 x SSC buffer:	0.3 M Tri-sodium citrate, pH7.0 3.0 M NaCl
X TE buffer :	10 mM Tris/HCl, pH7.6 1 mM EDTA-diNa
XI X-gal stock solution:	20 mg/ml in Dimethylformamide
XII Proteinase K solution :	1 mg/ml Proteinase K 0.5 M EDTA 1% Sarcosyl
XIII 10 x TAE DNA loading buffer:	50 % (v/v) Glycerol 0.25 % (w/v) Bromophenolblue 0.25 % (w/v) Xylencyanolblue 10 x TAE
XIV Ethidiumbromide stock solution:	10 mg/ml dH ₂ O

2.4 Dideoxyribonucleic acids (DNA)

Fish sperm DNA	Boehringer (Mannheim)
Lambda DNA	New England BioLabs (Boston)

2.5 Nucleotides

Adenosine-5'-triphosphate-disodium (ATP)	Boehringer (Mannheim)
dNTPs	Roth (Karlsruhe)

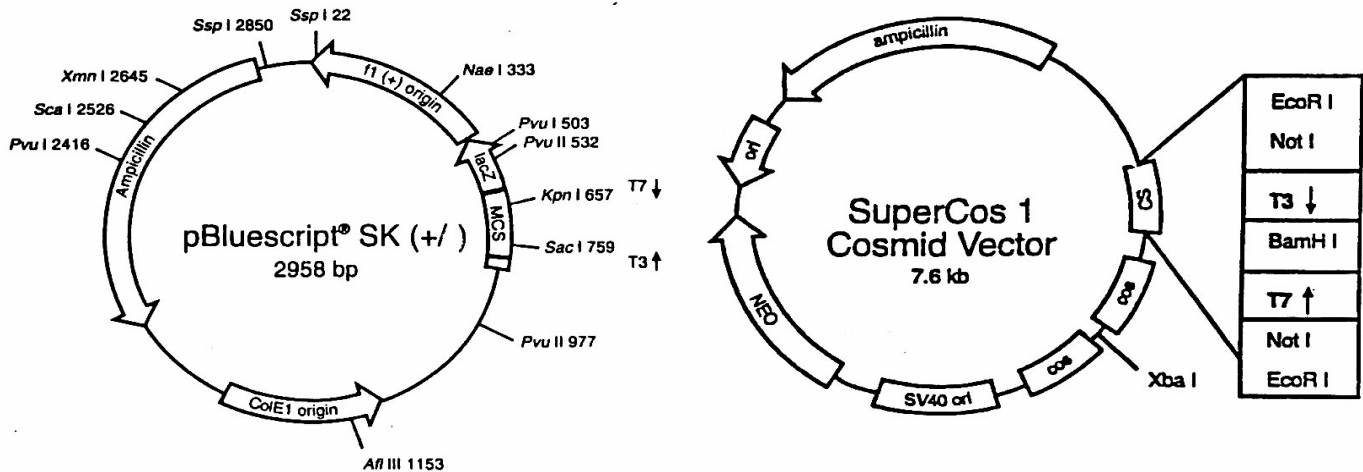
2.6 Radioactive Labeled Substances

α - ³² P-dCTP (~3000 Ci/mmol)	Amersham (Braunschweig)
γ - ³² P-ATP (~3000 Ci/mmol)	Amersham (Braunschweig)
α - ³³ P-ddNTP (~1000 Ci/mmol)	Amersham (Braunschweig)
α - ³⁵ S-dATP (~1000 Ci/mmol)	Amersham (Braunschweig)

2.7 Cloning Vectors

pBluescript II KS (+)	Stratagene (La Jolla, USA.)
SuperCos 1	Stratagene (La Jolla, USA.)

The structures of vectors are given below.



2.8 Bacterial strains

All bacteria used in this study were derived from *Escherichia coli* K12 strains

XL1-Blue MR	<i>recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, lac[F'proAB, lac I^q ZΔM15, Tn10, (Tet^r)]^c</i>
JM 83	<i>F⁻, ara, Δ(lac-proAB), rpsL (Str^R), (Φ80dΔ(lacZ)M15)</i>
DH5α-MCR	<i>F⁻ mcrAΔ(mrr-hsdRMS-mcrBC)φ80dlacZΔM15Δ(lacZYA-argF) U169endA1 recA1deoR thi-1 phoA supE44λ⁻ gyrA96 relA1</i>
DH10B	<i>F⁻ mcrAΔ(mrr-hsdRMS-mcrBC)φ80dlacZΔM15ΔlacX74end A1 recA1 deoRA(ara, leu)7697 araD139galU galK nupG rpsLλ⁻</i>

2.9 DNA length standards

100 bp ladder(100-1000bp)	Invitrogen
500 bp ladder (500- 8000bp)	Appligene-Oncor
1 Kb ladder(506-12216bp)	GibcoBRL (Eggenstein)
50 Kb ladder (48.5-1000Kb)	Bio-Rad (München) and New England BioLabs (Boston)
λ/Hind III (0.5-23Kb)	New England BioLabs (Boston)

2.10 Enzymes

Restrictionendonucleases	New England Biolab (Boston)
	Boehringer (Mannheim)
	Gibco/BRL (Eggenstein)
	AGS (Heidelberg)
Taq DNA polymerase	Institute of Human Genetics, Giessen
T4 ligase	Boehringer (Mannheim)
T4 polynucleotide kinase	Amersham (Braunschweig)
Calf alkaline phosphatase (CIP)	Boehringer (Mannheim)
Superscript II RNase H - reverse transcriptase	Gibco/BRL (Eggenstein)

Proteinase K
Lysozyme

Merck (Darmstadt)
Sigma (München)

2.11 Kits

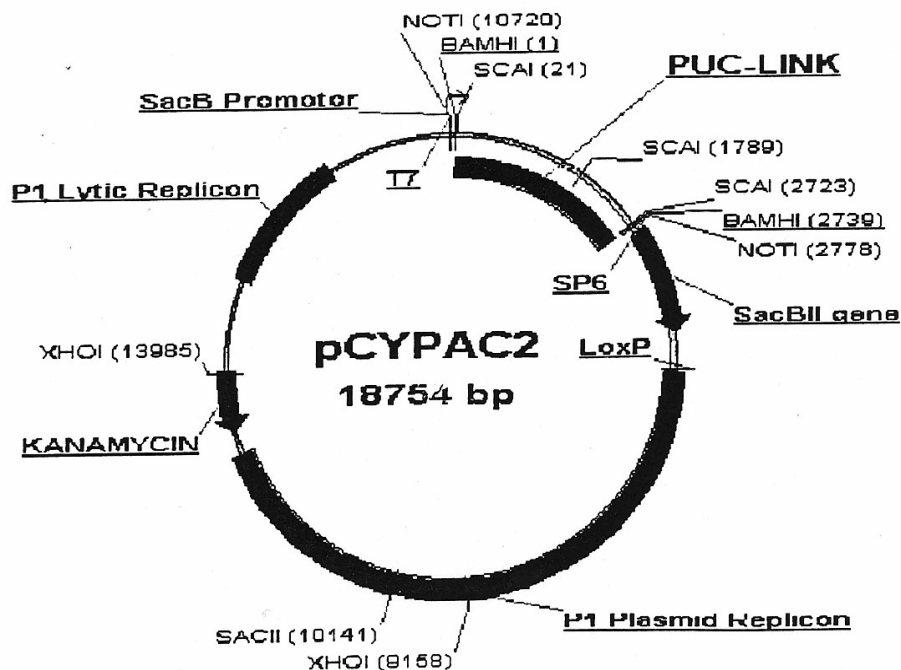
Nonaprimer labelling kit
T7 sequenase
Thermal sequenase for fluorescent labeled primers
Thermal sequenase for radioterminator cycle sequencing
Nucleospin extraction kit
Gigapack III Gold Packaging kit
QIAamp RNA Blood Mini kit

Appigene-Oncor (Heidelberg)
Amersham (Braunschweig)
Amersham (Braunschweig)
Amersham (Braunschweig)
Macherey-Nagel (Düren)
Stratagene (La Jolla, USA)
Qiagen (Düsseldorf)

2.12 Human genomic DNA library

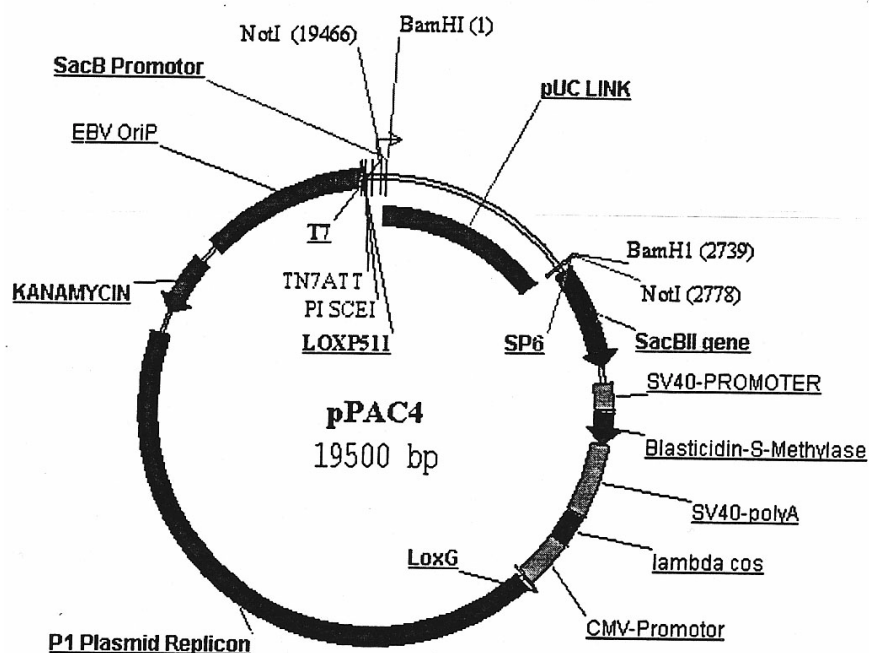
I. Human PAC library : RPCI1 high density gridded filters

The human PAC library RPCI1 provided by the UK HGMP Resource Center was constructed by Pieter de Jong and his group at the Roswell Park Cancer Institute, Buffalo. The library is constructed in vector pCYPAC2. The source of insert DNA is a normal male blood donor, and the average insert size is about 110kb. There are 25% clones in this library without inserts. The library consists of approximately 120,000 clones in 315 384-well microtitre plates and has been gridded in a 4 x 4 array on 22.2 x 22.2 cm Hybond N nylon membranes (Amersham). Each clone is spotted twice to give 36,864 spots on each membrane. The library consists of 7 membranes. The structure of pCYPAC2 is shown below.



II. Human PAC library: RPCI6 high density gridded filters and DNA pools

The human PAC library RPCI6 provided by the Resource Center/Primary Database of the German Human Genome Project was constructed by Pieter de Jong and his group at the Roswell Park Cancer Institute, Buffalo. It is constructed in the vector pPAC4 and *E.coli* DH10B is used as the host cell. The source of insert DNAs is a normal female blood donor, and the average insert size is about 140kb. The library consists of approximately 92160 clones in 240 384-well microtitre plates and has been gridded in a 5 x 5 array on 22 x 22 cm Hybond N+ nylon membranes. Each clone is spotted twice to give 27,648 spots on each membrane. The library consists of 4 membranes. The library is also available in DNA pools for PCR screens. The structure of pPAC4 is shown below.



Origin: <http://bacpac.med.buffalo.edu/>

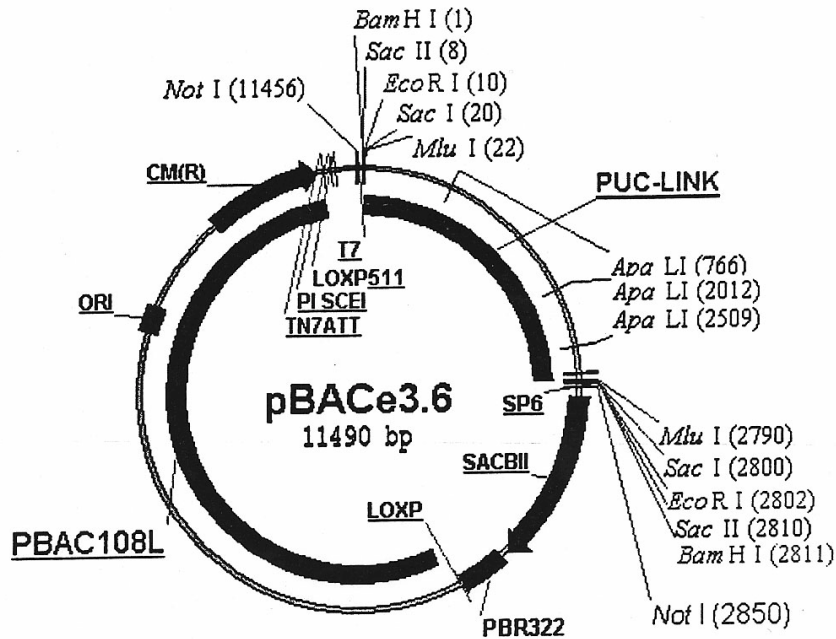
III. Human chromosome 2 PAC library

The library LL02NP04“AI“ provided by the Resource Center/Primary Database of the German Human Genome Project was constructed by Gingrich. Digests of DNA from flow-sorted human chromosome 2 of human-hamster hybrid somatic cell line GM10826 are cloned to vector pCYPAC2 and *E.coli* DH5 alpha MCR is used as the host cell. The library consists of approximately 5800 clones in 15 384-well microtitre plates, and has been gridded in a 3 x 3 array on 22 x 22 cm Hybond N+ nylon membranes. Each clone is spotted twice on one filter.

IV. Human BAC library : RPCI-11 DNA pools

The human BAC library RPCI-11 provided by Resource Center/Primary Database of the German Human Genome Project was constructed by Osoegawa and Tateno at the Roswell Park Cancer Institute, Buffalo. It is constructed in vector pBACe3.6 and *E.coli* DH10B is used

as the host cell. The source of insert DNA is a normal male blood donor, and the average insert size is about 174kb. The pools consist of 442368 clones in 1152 plates. The library comprises 144 primary pools and represents approximately 25.3 fold genomic coverage. The structure of pBACe3.6 is shown below.



Origin: [http:// bacpac.med.buffalo.edu/](http://bacpac.med.buffalo.edu/)

2.13 Patients

A large German pedigree with AD-HSP was investigated. This family consists of 66 living members (see Figure 1) and the blood samples were collected from 42 participating members of the family. In addition, *SPAST* was sequenced in nine affecteds of small families with pure HSP.