A

A (1) Adenine (base, nucleoside or nucleotide). (2) L-Alanine (alternative to Ala).

Å Ångström unit, 10^{-10} m; a unit of length used e.g. to indicate intermolecular distances.

A_{260} See ULTRAVIOLET ABSORBANCE.

A-DNA One of the major conformations adopted by dsDNA: a right-handed helix with ~11 base-pairs per turn.

A site (of a ribosome) The aminoacyl or 'acceptor' site at which tRNA molecules carrying the second and subsequent amino acids bind during translation. (cf. P SITE.)

A-tract In genomic DNA: a nucleotide motif associated with regions of the most pronounced curvature of the molecule. In the *Escherichia coli* genome, A-tracts were found to be distributed 'quasi-regularly' throughout, in both coding and non-coding sequences; the A-tracts occur in clusters of ~100 bp in length, with consecutive A-tracts exhibiting a periodicity of 10–12 bp.

It was suggested that the clusters of A-tracts may constitute a form of 'structural code' for the compaction of DNA in the NUCLEOID [Nucleic Acids Res (2005) 33(12):3907–3918].

AAA ATPases 'ATPases associated with diverse cellular activities'. These ATPases occur in various locations – including, for example, in proteasomes and peroxisomes.

AAAVs Avian adeno-associated viruses (see AAVS).

AAS Aminoalkylsilane (3-aminopropyltriethoxysilane; APES): a reagent used e.g. to bind tissue sections to glass (for *in situ* hybridization etc.).

***aat* gene** In *Escherichia coli*: a gene encoding the enzyme that catalyzes the addition of a leucine or phenylalanine residue to the N-terminal of proteins which are synthesized with an N-terminal arginine or lysine residue; such addition facilitates degradation of the protein.

(See also N-END RULE.)

AatII A RESTRICTION ENDONUCLEASE from *Acetobacter aceti*. Recognition sequence/cutting site: GACGT↓C.

AAUAAA In mRNAs: a polyadenylation signal upstream of the site at which the molecule is cut and polyadenylated; the sequence is similar in mRNAs from many organisms.

Other *cis*-acting elements may be involved in regulating the polyadenylation of human mRNAs, including upstream U-rich sequences similar to those identified in yeast and plants [RNA (2005) 11:1485–1493].

AAV Helper-Free System A commercial gene-delivery system (Stratagene, La Jolla CA) in which the genes in two plasmids provide functions necessary for production of infective AAV virions (see AAVS); these virions are used to transfect target cells within which viral DNA, containing the gene of interest, integrates in the host cell's DNA.

Essentially, the gene/fragment of interest is first cloned in a plasmid cloning vector in which the insert is bracketed by a pair of inverted terminal repeats (ITRs) which are necessary for subsequent viral packaging. This plasmid is then used to transfect appropriate cells – which are *co-transfected* with two other plasmids: (i) a plasmid containing the genes that encode viral capsid and replication functions, (ii) a plasmid containing genes that encode the lytic phase of AAV. The resulting infective (*but still replication-deficient*) virions are used to infect the required target cells (in which the gene of interest can be expressed).

[Use of method for genetic modification of cultured human cells: Nucleic Acids Res (2005) 33(18):e158.]

AAVs Adeno-associated viruses (also known as: adeno-satellite viruses): defective viruses that are able to replicate only when certain functions are provided by a co-infecting *helper virus* (adenovirus or herpesvirus) – or, in certain *in vitro* systems, when these functions are provided by plasmid-borne genes (see e.g. AAV HELPER-FREE SYSTEM). (Functions provided by adenovirus type 5 for AAV type 5 include both positive and negative effects; for example, the E4Orf6 function – involved in replication of AAV5 genomic DNA – also (with E1b) acts to degrade AAV5 capsid proteins and Rep52 [J Virol (2007) 81(5):2205–2212].)

The AAVs are parvoviruses in which the genome is linear ssDNA. Positive and negative strands of the viral DNA are encapsidated in separate virions.

It was reported earlier that, in human cells, AAV DNA (in the absence of helper virus) integrates in the genome with an apparent preference for CPG ISLANDS. More recently, AAVs have been reported to integrate, site-specifically, into a locus on chromosome 19, and the occurrence of such integration is apparently influenced by the TRP-185 protein [J Virol (2007) 81(4):1990–2001].

The AAVs infect a wide range of vertebrates. Initial stages of infection, including internalization of DNA, occur without a helper virus.

AAVs are used, for example, in GENE THERAPY. A caprine AAV, resistant to (human) neutralizing antibodies and with a marked tropism for (murine) lung tissue, has been suggested as a vector in CYSTIC FIBROSIS [J Virol (2005) 79:15238–15245].

AAV vectors, encoding genes of the α and the β subunits of hexosaminidase, were used in a mouse model (with intracranial inoculation) to evaluate the potential of gene therapy for the treatment of human GM2 gangliosidoses such as Tay–Sachs disease and Sandhoff disease [Proc Natl Acad Sci USA (2006) 103(27):10373–10378].

[Cloning of an avian AAV – an AAAV – and generation of recombinant AAAV particles: J Virol (2003) 77:6799–6810.]

AB1380 A strain of the yeast *Saccharomyces cerevisiae* (see entry SACCHAROMYCES).

(See also YEAST ARTIFICIAL CHROMOSOME.)

abacavir A NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR used e.g. in antiretroviral therapy; CSF–plasma ratios indicate that it may reach therapeutic levels in the CSF [Antimicrob Agents Chemother (2005) 49(6):2504–2506].

abasic site *Syn.* AP SITE.

ABC excinuclease See UVRABC-MEDIATED REPAIR.

Abelson murine leukemia virus See ABL.

abl (*ABL*) An ONCOGENE first identified in the Abelson murine leukemia virus. The v-*abl* product has TYROSINE KINASE activity. The human homolog of v-*abl*, c-*abl*, is usually present on chromosome 9; however, in the majority of patients with CHRONIC MYELOGENOUS LEUKEMIA it has been translocated to chromosome 22, forming a chimeric gene, known as *bcr-abl*, that encodes a tumor-specific tyrosine kinase (designated P210). Chromosome 22 containing the chimeric *bcr-abl* gene is called the Philadelphia chromosome (also referred to as Ph^1).

Subcellular localization of c-Abl protein at an early stage in myogenic differentiation was reported to be influenced by its acetylation [EMBO Rep (2006) 7(7):727–733].

abortive transduction TRANSDUCTION in which the transduced DNA persists in a recipient cell as a stable, extrachromosomal but non-replicating molecule; when the recipient divides only one daughter cell receives the DNA fragment.

absorbance (ultraviolet) See ULTRAVIOLET ABSORBANCE.

abzyme *Syn.* CATALYTIC ANTIBODY.

Abzyme® A reagent kit (Abbott Laboratories) used for detecting antibodies in the context of hepatitis B.

acceptor splice site (acceptor splice junction) In pre-mRNA: the splice site (consensus AG) at the 3′ end of an intron.

(cf. DONOR SPLICE SITE.)

accession number (of a gene) A number that refers to the database entry for a specific gene. Some examples: (i) GenBank accession number X17012 refers to data on the gene for rat insulin-like growth factor II (IGF II); (ii) GenBank accession number AY024353 refers to data on the *ftsZ* gene of the bacterium *Sodalis glossinidius*; (iii) GenBank accession number AM160602 refers to data on the mRNA of the gene for cinnamyl alcohol dehydrogenase in the oak species *Quercus ilex*.

AccuPrime™ GC-rich DNA polymerase A DNA polymerase (Invitrogen, Carlsbad CA) optimized for DNA synthesis on difficult-to-amplify templates, including those with a GC content >65%.

AccuProbe® A family of PROBES (Gen-Probe, San Diego CA) used for identifying certain medically important bacteria by detecting specific sequences of nucleotides from lysed cells. The method involves a *hybridization protection assay*. In this assay, an added reagent cleaves the acridinium ester label on all *unbound* probes. Labels on the *bound* probes (which are protected from cleavage by virtue of their position in the probe–target duplex) react with a second reagent, producing a chemiluminescent (light) signal. The light produced by this reaction is measured in RLUs (i.e. relative light units). The threshold value (in RLUs) for a positive result must be carefully examined [see for example: J Clin Microbiol (2005) 43: 3474–3478].

(See also PACE 2C and TMA.)

acetosyringone An agent used e.g. for induction of the *virB* promoter in the plant pathogen *Agrobacterium tumefaciens*.

[Example of use: Infect Immun (2006) 74(1):108–117.]

(See also CROWN GALL.)

***N*-acetyl-L-cysteine** See MUCOLYTIC AGENT.

acetylation (of histones) HISTONE acetylation is regulated e.g. by the opposing effects of: (i) histone deacetylases (HDACs) and (ii) histone acetyltransferases (HATs); (de)acetylation of histones can affect CHROMATIN structure, and may alter the accessibility of DNA for processes such as transcription and repair.

In a genomewide study of HDACs in *Schizosaccharomyces pombe* (a fission yeast), the patterns of histone acetylation, HDAC binding and nucleosome density were compared with gene expression profiles; it was found that different HDACs can have different roles in repression and activation of genes [EMBO J (2005) 24(16):2906–2918].

In (human) nucleosomes, the acetylation of certain lysine residues depends primarily on HATs, but the effect of these enzymes appears to be promoted by binding protein HMGN1 [EMBO J (2005) 24(17):3038–3048].

Acetylation of histone *chaperone* protein NUCLEOPHOSMIN (in conjunction with histone acetylation) has been reported to play a role in the enhancement of transcription [Mol Cell Biol (2005) 25(17):7534–7545].

The c-Abl protein (see ABL) has been identified as a substrate for the p300 and other histone acetyltransferases.

(See also TRICHOSTATIN A and 'Elongator complex' in the entry WOBBLE HYPOTHESIS.)

***N*-acetylmuramidase** See LYSOZYME.

ACF APOBEC-1 complementation factor: see RNA EDITING.

Achilles' heel technique Any technique in which a RESTRICTION ENDONUCLEASE is targeted to one *particular* recognition site when multiple copies of that site are freely available. One method uses a triplex-forming oligonucleotide to mask the required cleavage site. While masked, the remaining sites are methylated in order to inhibit subsequent cleavage; when the triplex is removed specific cleavage can be carried out.

(See also PROGRAMMABLE ENDONUCLEASE.)

aciclovir Alternative spelling for ACYCLOVIR.

acid-fast bacilli Those bacilli (i.e. rod-shaped bacteria) which, when stained with the Ziehl–Neelsen (or similar) stain, resist decolorization with mineral acid or an acid–alcohol mixture. This kind of staining method is used for screening respiratory specimens (such as samples of sputum) for *Mycobacterium tuberculosis* (a so-called acid-fast species).

Ac*M*NPV *Autographa californica* NPV: see NUCLEAR POLYHEDROSIS VIRUSES.

AcNPV *Syn.* Ac*M*NPV – see entry NUCLEAR POLYHEDROSIS VIRUSES.

acridines Heterocyclic, fluorescent compounds which bind to dsDNA (primarily as an INTERCALATING AGENT) and also to single-stranded nucleic acids (and to the backbone chains of double-stranded nucleic acids). Acridines have antimicrobial activity and are mutagenic; they are also used as stains for nucleic acids and can be used for CURING plasmids.

acridinium ester label (on probes) See ACCUPROBE.

acrocentric Refers to a CHROMOSOME in which the CENTROMERE is located close to one end.

acrydite hybridization assay An assay in which molecules of labeled ssDNA or ssRNA, passing through a polyacrylamide gel by electrophoresis, are captured (bound) by complementary oligonucleotides immobilized in a (central) 'capture zone' within the gel; all the molecules of nucleic acid that are *not* complementary to the capture oligos pass through the central capture zone and continue their migration to the end of the gel strip. The complementary oligos are synthesized with a 5′ terminal acrydite group which binds them to the polyacrylamide matrix so that they are immobilized in the gel. (Note that the central region of the gel strip is prepared separately.)

acrylamide A toxic, water-soluble agent (CH_2=CH–$CONH_2$) which can be polymerized to POLYACRYLAMIDE by catalysts such as *N*,*N*′-methylene-*bis*-acrylamide ('Bis') which promote cross-linking.

actinomycin C_1 *Syn.* ACTINOMYCIN D.

actinomycin D An antibiotic (a substituted phenoxazone linked to two pentapeptide lactone rings) produced by some species of *Streptomyces*; it acts as an INTERCALATING AGENT, binding to DNA and inhibiting DNA-dependent RNA polymerase. The drug has low affinity for AT-rich promoter regions; *initiation* of transcription from such promoters may be little affected by the antibiotic.

activation-induced cytidine deaminase (AID) An enzyme that occurs in germinal center B lymphocytes (B cells) and which is an absolute requirement for affinity maturation and class switching. HYPER-IGM SYNDROME has been associated with a deficiency of AID.

(See also CYTIDINE DEAMINASE and RNA EDITING.)

acyclovir (alternative spelling: aciclovir) 9-(2-hydroxyethoxymethyl) guanine: an antiviral agent which is active against a number of herpesviruses, including herpes simplex. In cells, acyclovir is phosphorylated to the monophosphate by (viral) thymidine kinase; subsequently it is converted to the (active) triphosphate form via host-encoded enzymes. The active drug inhibits *viral* DNA polymerase; the host cell's polymerase is much less sensitive.

In cells that are not virally infected, acyclovir appears not to be significantly phosphorylated.

Acyclovir has been used topically and systemically.

***N*-acyl-homocysteine thiolactone** See QUORUM SENSING.

***N*-acyl-L-homoserine lactone** (AHL) See QUORUM SENSING.

Ada protein (in *Escherichia coli*) See DNA REPAIR.

adaptamer See ORFMER SETS.

adaptive response (to alkylating agents) See DNA REPAIR.

AdEasy™ XL adenoviral vector system A system (Stratagene, La Jolla CA) which can be used for creating adenoviral vectors containing a specific gene/insert of interest.

Initially, the gene/insert is cloned in a small shuttle vector (~7 kb) which includes: (i) the left and right ITRs (inverted terminal repeats) of the adenovirus genome; (ii) two regions homologous to two sequences in another plasmid, pAdEasy-1 (see later), (iii) a gene encoding resistance to kanamycin; and (iv) a recognition site for the restriction endonuclease PmeI. After cloning, the shuttle vector is linearized (by cleavage with PmeI); linearization leaves the two homologous regions (see above) in terminal positions.

The linearized shuttle vector is inserted, by transformation, into a strain of *Escherichia coli*, BJ5183-AD-1, that already contains the (circular) plasmid vector pAdEasy-1. pAdEasy-1 (~33 kb) includes modified genomic DNA of human adenovirus serotype 5 (containing deletions in both the E1 and E3 regions). Homologous recombination occurs (intracellularly) between the linearized shuttle vector and homologous regions in pAdEasy-1; cells containing the recombinant plasmids are selected on kanamycin-containing media.

Recombinant plasmids are cleaved by restriction enzyme PacI, at selected sites, yielding a linear construct with adenoviral terminal sequences. This construct is used to transfect specialized, competent AD-293 cells – within which infective adenovirus virions are produced and released for subsequent use in gene-transfer and gene-expression studies in mammalian cells.

The AdEasy™ vector system has been used e.g. in studies on aptamer-regulated control of intracellular protein activity [Nucleic Acids Res (2006) 34(12):3577–3584].

The underlying principle of the AdEasy™ system has been exploited in the production of oncolytic adenovirus vectors [BMC Biotechnol (2006) 6:36].

adefovir A NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR.

adenine phosphoribosyltransferase An enzyme (EC 2.4.2.7) which forms adenosine monophosphate (AMP) from adenine and 5-phosphoribosyl-1-diphosphate.

In humans, a deficiency of adenine phosphoribosyltransferase (an autosomal recessive disorder) can cause excretion of adenine (in the urine) and the formation of a highly insoluble product, 2,8-dihydroxyadenine, which can give rise to kidney stones and renal failure.

adeno-associated viruses See AAVS.

adeno-satellite viruses See AAVS.

adenosine A RIBONUCLEOSIDE.

adenosine deaminase An enzyme (EC 3.5.4.4) which catalyzes the conversion of adenosine to inosine.

adenosine deaminase deficiency The congenital deficiency of a functional adenosine deaminase (EC 3.5.4.4) characterized by lack of normal development of T cells and a (consequent) marked immunodeficiency in which the patient is susceptible to infection by opportunist pathogens. This disorder has been treated successfully by GENE THERAPY.

(See also GENETIC DISEASE (table).)

adenovirus Any member of a family of non-enveloped, icosahedral viruses (genome: linear dsDNA) that infect mammals and birds; each type of adenovirus is commonly specific for one or a limited range of closely related host species.

Adenoviruses are widely used as vectors in various types of investigation, including GENE THERAPY. (See also ADEASY XL ADENOVIRAL VECTOR SYSTEM.) Adenoviruses have also been studied for their oncolytic potential [BMC Biotechnol

(2006) 6:36].

The adenovirus virion is ~70–90 nm in diameter; the capsid encloses a core containing genomic DNA (which is closely associated with an arginine-rich polypeptide). The 5′ end of each strand of the DNA is covalently linked to a hydrophobic 'terminal protein' (TP). The ends of the DNA are characterized by an inverted terminal repeat (ITR) – which varies in length in different types of adenovirus; the 5′ terminal residue is commonly dCMP.

During infection, the core enters the nucleus, releasing viral DNA. Replication of viral DNA involves TP and also a virus-encoded DNA polymerase as well as other virus- and host-encoded proteins. TP, synthesized in precursor form, binds covalently to DNA during replication and is later cleaved to the mature (DNA-bound) TP. A TP-mediated form of DNA replication also occurs in PHAGE φ29 (q.v.).

Expression of late viral genes, encoding structural proteins, is accompanied by the cessation of cellular protein synthesis.

Some 10^5 virions may be formed within a single cell.

adenylate cyclase An enzyme (EC 4.6.1.1) which catalyzes the conversion of ATP to CYCLIC AMP.

In *Escherichia coli*, the activity of adenylate cyclase (*cya* gene product) is regulated e.g. via CATABOLITE REPRESSION.

In mammals, the enzyme forms part of a plasma membrane complex and is regulated e.g. via certain G proteins; it is activated by some bacterial exotoxins (e.g. PERTUSSIS TOXIN).

Anthrax toxin (EF component) and *cyclolysin* (a virulence factor synthesized by the Gram-negative bacterial pathogen *Bordetella pertussis*) both have adenylate cyclase activity which is stimulated by CALMODULIN.

adenylate kinase An enzyme (EC 2.7.4.3) which catalyzes the (reversible) conversion of two molecules of ADP to ATP and AMP.

ADP-ribosylation The transfer, to a protein, of an ADP-ribosyl group from NAD^+, mediated by ADP-ribosyltransferase (EC 2.4.2.30). In eukaryotes this can e.g. regulate the properties of HISTONES. In *Escherichia coli*, the RNA polymerase is ADP-ribosylated (with change in activity) following infection with bacteriophage T4.

ADP-ribosylation is an intracellular effect of some bacterial exotoxins (e.g. cholera toxin and PERTUSSIS TOXIN).

Polymerized ADP-ribosyl subunits (up to 50) may be found on certain eukaryotic proteins.

affinity capture electrophoresis Electrophoresis in a medium containing immobilized capture probes; it is used e.g. for the isolation of a given fragment of ssDNA, or a fragment of triplex-forming dsDNA.

(See also ACRYDITE HYBRIDIZATION ASSAY.)

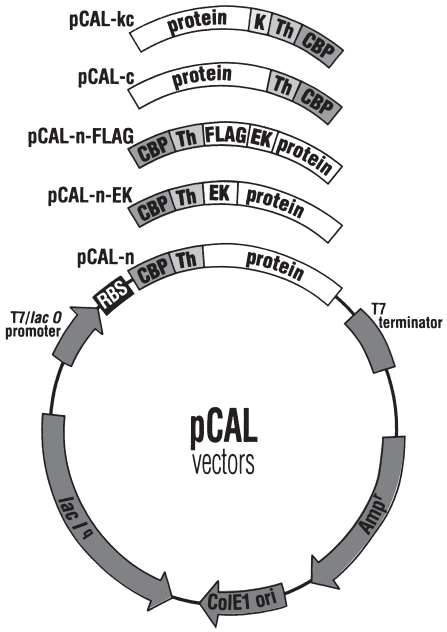
affinity chromatography Chromatography in which specific molecules are isolated (adsorbed) owing to their affinity for an immobilized ligand – any non-specific unbound molecules being removed from the immobilized matrix. This procedure may be used e.g. for isolating/purifing a given type of molecule (see e.g. GENE FUSION (uses)).

Affinity® protein expression and purification A product of Stratagene (La Jolla CA) designed to facilitate the expression and purification of proteins expressed in prokaryotic systems; the product includes various pCAL plasmid vectors, shown in the diagram, each encoding a unique CALMODULIN-binding affinity tag.

Protein expression is maximized by a vector that includes a T7/LacO promoter system. In suitable strains of *Escherichia coli* (e.g. BL21(DE3)), T7 RNA polymerase is expressed in the presence of the inducer IPTG and drives expression from the T7/LacO promoter system on the plasmid. Tight control of expression is achieved with a plasmid-borne copy of *lacI*^{q}. Efficient translation of the protein of interest is promoted by using the strong ribosome-binding site (RBS) of T7 gene 10.

pCAL vectors contain a ColE1 origin of replication and an ampicillin-resistance gene.

All pCAL vectors encode a CALMODULIN-binding peptide (CBP) tag which forms a fusion product with the expressed protein and permits high-level purification following a single passage through CALMODULIN-AFFINITY RESIN. The (small)



Affinity® PROTEIN EXPRESSION AND PURIFICATION The range of pCAL vectors (see entry for details of the method). CBP (in each vector) refers to calmodulin-binding peptide. EK = enterokinase; K = Kemptide sequence; Th = thrombin proteinase. (See also entry for FLAG.)

Courtesy of Stratagene, La Jolla CA, USA.

size of the CBP tag (about 4 kDa) may be expected to have a smaller effect on the protein of interest compared with larger tags such as 26-kDa glutathione *S*-transferase (GST) affinity tag.

One of the pCAL vectors includes a KEMPTIDE SEQUENCE which can be used e.g. for *in vitro* labeling of the expressed protein with protein kinase A (PKA) and ^{32}P.

All of the pCAL vectors include a cleavage site for enterokinase and/or thrombin proteinase.

One of the pCAL vectors includes a FLAG sequence.

affinity resin See e.g. NICKEL-CHARGED AFFINITY RESIN.

affinity tag *Syn.* AFFINITY TAIL.

affinity tail (affinity tag) That part of a FUSION PROTEIN (sense 2) which facilitates detection/isolation of the protein e.g. by an affinity resin or by AFFINITY CHROMATOGRAPHY.

Some affinity tails are small peptides. One advantage of using a small affinity tail is that it is less likely to interfere with the function of the fusion protein – so that its removal may not be necessary.

(See also FLAG, PESC VECTORS and SIX-HISTIDINE TAG.)

Large (protein) tails, for example glutathione *S*-transferase, may improve the solubility of the fusion protein but they may need subsequent removal in order to avoid interference with the function of the recombinant target protein.

(See also CHAMPION PET SUMO VECTOR.)

A highly temperature-stable affinity tail (a lectin, stable up to 80°C) may be useful for proteins originating from thermophilic organisms; the fusion proteins bind specifically to an agarose matrix containing D-mannose, and the affinity tail can by cleaved by an enterokinase [BioTechniques (2006) 41 (3):327–332].

(See also AFFINITY PROTEIN EXPRESSION AND PURIFICATION.)

aflatoxins Heat-stable toxins produced by certain fungi (strains of *Aspergillus flavus* and *A. parasiticus*); the molecule of an aflatoxin contains a bifuran moiety fused with a substituted coumarin. Aflatoxins have been associated with some cases of hepatocellular carcinoma. Different species may be affected in different ways by these toxins.

Aflatoxins may give rise to errors in DNA replication by reacting with guanine bases.

[Aflatoxin (biosynthesis genes): Appl Environ Microbiol (2005) 71:3192–3198.]

AFLP Either of *two distinct* PCR-based approaches for TYPING bacteria.

One approach ('amplified fragment length polymorphism') includes a number of variant forms of arbitrarily primed PCR (AP-PCR), including e.g. RAPD analysis.

The other approach, outlined here, involves initial digestion of genomic DNA by two types of RESTRICTION ENDONUCLEASE; it is sometimes called 'amplified restriction fragment length polymorphism' but this is incorrect [see original paper: Nucleic Acids Res (1995) 23:4407–4414].

In the digested genome each fragment is flanked by STICKY ENDS produced by one or other of the two types of restriction enzyme. Two types of adaptor molecule (A, B) are added; the A molecules have *one* sticky end which binds to sites cleaved by one of the restriction enzymes, and B molecules have *one* sticky end that binds to sites cleaved by the other enzyme. A site cleaved by EcoRI (left) and a matching adaptor (right) is shown below:

```
5′-----NNG          AATTGNNNNN-3′
  -----NNCTTAA            CNNNNN
```

Fragment–adaptor binding is followed by ligation – but the cleavage site of EcoRI is *not* regenerated, avoiding repeated restriction. Fragments (with their adaptors) are amplified by PCR; each primer is complementary to a sequence covering part of an adaptor and the (contiguous) restriction site of the fragment. Each primer's 3′ end extends beyond the restriction site for one (or several) nucleotides; thus, a given primer will be extended only if the primer's 3′ terminal 'selective' nucleotide(s) align with *complementary* base(s) in the fragment. In the example given above, one template strand is:

```
5′-----NNGAATTGNNNNN-3′
```

and a primer with a deoxycytidine (C) selective 3′ nucleotide will bind as follows:

```
5′-----NNGAATTGNNNNN-3′
       CC TTAACNNNNN-5′   ← primer
```

The same principle holds for each primer-binding site: only some of the primers will be extended owing to the selective 3′ end. The PCR products undergo gel electrophoresis; bands of products (made visible e.g. by the use of labeled primers) form the *fingerprint*.

AFLP was highly discriminatory e.g. for *Clostridium botulinum* types A, B, E and F [Appl Environ Microbiol (2005) 71:1148–1154] but only limited intraspecies differences were reported in isolates of *Brucella* [J Clin Microbiol (2005) 43: 761–769].

AFM ATOMIC FORCE MICROSCOPY.

agar A complex mixture of galactans obtained from certain red algae (e.g. *Gelidium*); these compounds form part of the cell wall and/or intercellular matrix.

Agar comprises two main components: AGAROSE and agaropectin. Agaropectin is a mixture of sulfated galactans, some of which contain glucuronic acid and/or other constituents.

An agar *gel* is used as a matrix in many types of solid and semi-solid microbiological medium. This translucent, jelly-like material is prepared by heating a mixture of agar (e.g. 1.5% w/v) and water to >100°C and then cooling it to room temperature; gelling begins at ~40–45°C.

Agar can be an inhibitory factor in PCR (cf. GELLAN GUM).

agarose The major constituent of AGAR: a non-sulfated linear polymer consisting of alternating residues of D-galactose and 3,6-anhydro-L-galactose. Agarose is used e.g. as a medium in

GEL ELECTROPHORESIS for the separation of large fragments of nucleic acid.

(cf. POLYACRYLAMIDE.)

agnoprotein A regulatory protein, encoded by the JC VIRUS, which plays an important role in the infective cycle; it may be involved in facilitating the transport of virions from the nucleus (where virus assembly occurs) to the cytoplasm. It has been found that mutant, phosphorylated forms of agnoprotein are unable to sustain the viral infective cycle [J Virol (2006) 80(8):3893–3903].

Agrobacterium A genus of motile, Gram-negative bacteria that are found primarily in the rhizosphere (root environment of plants). GC% of genomic DNA: 57–63. Optimal temperature for growth: 25–28°C. Various mono- and disaccharides can be metabolized; glucose is metabolized e.g. via the Entner–Doudoroff pathway and the hexose monophosphate pathway.

Colonies which develop on media containing carbohydrates are generally mucilaginous, copious slime being formed.

Most species are pathogenic to plants. *A. tumefaciens* and *A. vitis* are causal agents of CROWN GALL.

(See also BINARY VECTOR SYSTEM.)

agroinfection A method for introducing viral DNA (or cDNA) into plant cells.

In the original procedure, viral DNA is inserted into the T-DNA region of the Ti plasmid of bacterium *Agrobacterium tumefaciens* (see the entry CROWN GALL for details of the Ti plasmid); thus, when infecting a plant, *A. tumefaciens* injects viral DNA (within the T-DNA) into plant cells.

Currently, the commonly used procedure involves the use of a *binary vector* system. In this approach the gene/sequence of interest is inserted into a small (binary) vector in which it is flanked, on each side, by the left border and right border of T-DNA. The binary vector, containing the gene, is inserted into an engineered strain of *Agrobacterium tumefaciens* that contains the *vir* (virulence) region of the Ti plasmid which is concerned with the transfer of DNA into plant cells. This strain is then used to infect a plant. When the *vir* genes are activated, DNA from the *binary vector* (specifically, DNA from the section bracketed by the left and right borders of T-DNA) is transferred into the plant cells, such transfer being mediated by factors encoded by the *vir* region acting in *trans*.

[Example of agroinfection: J Virol (2003) 77:3247–3256.]

In the development of transgenic plants it is desirable to minimize the content of extraneous DNA which is transferred to plant cells through the vector system, particularly when such plants are to be made available in a general agricultural setting; the content of extraneous DNA is covered by certain laws that relate to transgenic plants. To this end, a number of minimal T-DNA vectors have been proposed [BioTechniques (2006) 41(6):708–710].

(See also FLORAL DIP METHOD.)

AGT O^6-alkylguanine-DNA alkyltransferase: see 'Uses of gene fusion' in the entry GENE FUSION.

AHL (*N*-acyl-L-homoserine lactone) See QUORUM SENSING.

***ahpC* gene** See ISONIAZID.

AHT (*N*-acyl-homocysteine thiolactone) See QUORUM SENSING.

AID ACTIVATION-INDUCED CYTIDINE DEAMINASE.

AIDS Acquired immune deficiency syndrome. An HIV^+ person with AIDS has counts of $CD4^+$ T cells below a certain level and, additionally, the presence of one or more types of AIDS-defining disease (such as: candidiasis of the *lower* respiratory tract, retinitis with CMV (cytomegalovirus), *extra*pulmonary infection with *Mycobacterium tuberculosis*, pneumonia due to *Pneumocystis carinii* etc.).

alanine scan mutagenesis A method for studying the binding properties (or other characteristics) of particular residues in a protein by replacing them with alanine residues; replacement can be achieved during synthesis of the protein (from a modified mRNA) or by exchanging residues in the protein itself. After insertion of alanine residues the protein is examined for properties/function.

alarmone Any of various low-molecular-weight molecules that are able to mediate some change in cellular metabolism as a response to a particular type of stress. One example is ppGpp which is formed in the stringent response in *Escherichia coli*.

albamycin *Syn*. NOVOBIOCIN.

(See also ANTIBIOTIC (table).)

AlgZ In *Pseudomonas aeruginosa*: a DNA-binding protein reported to regulate twitching motility (due to type IV fimbriae) as well as alginate biosynthesis [J Bacteriol (2006) 188(1): 132–140].

AlkA protein See DNA REPAIR.

alkaline phosphatase (AP) An enzyme (EC 3.1.3.1; maximum stability at pH ~7.5–9.5) used e.g. for removing 5′ terminal phosphate groups from nucleic acids. When it is bound e.g. to streptavidin, it is also used as a label (see BIOTIN) which can be detected by various types of chromogenic substrate.

(See also CHEMILUMINESCENCE.)

AttoPhos™ is used as a substrate for alkaline phosphatase in a CROSS-LINKING ASSAY.

(See also ELISA.)

alkaline stripping Stripping of hybridized RNA probes from a DNA MICROARRAY by degradation with buffers that contain NaOH (sodium hydroxide) under carefully regulated conditions (e.g. temperatures of 60–62°C). A stripped microarray can be used again, reducing costs, but a microarray cannot be stripped *twice* without loss of quality. Microarrays which had been stripped once gave results similar to those from virgin (non-stripped) arrays [BioTechniques (2005) 38:121–124].

***O^6*-alkylguanine-DNA alkyltransferase** (AGT) See 'Uses of gene fusion' in the entry GENE FUSION.

allele (allelomorph) Any one of two or more different versions of a particular GENE; the product or function of a given allele may exhibit qualitative and/or quantitative difference(s) from the product or function of other alleles of that gene.

In a diploid cell or organism, if an allelic pair (i.e. the two alleles of a given gene) consists of two identical alleles then the cell/organism is said to be *homozygous* for that particular gene; if different, the cell/organism is said to be *heterozygous*

for the gene.

allele-specific DNA methylation analysis A method, designed for determining allele-specific methylation, which involves PCR-amplification of BISULFITE-treated DNA followed by PYROSEQUENCING of each allele, individually – using allele-specific primers. The method was demonstrated by analyzing methylation of the *H19* gene in which only the paternal allele is usually imprinted [BioTechniques (2006) 41(6):734–739].

allele-specific PCR A variant form of PCR designed to amplify a particular allele of a given gene – but no other allele(s) of that gene. One of the two primers is designed with a 3′ terminal nucleotide that pairs with a *specific* base in the required allele – a base which is known to be different from that in the other allele(s) at this location; this primer can be extended on the required allele, which will be amplified. Other alleles will not be amplified because the mismatch at the primer's 3′ terminal will inhibit extension.

An essential requirement is the use of a polymerase which *lacks* proofreading ability (i.e. one which lacks 3′-to-5′ exonuclease activity); such an enzyme (e.g. the *Taq* polymerase) is not able to remove the primer's terminal nucleotide (i.e. it cannot correct the mismatch) and does not amplify unwanted alleles.

It is also important to carry out the reaction with an appropriate level of stringency.

A modified form of this method is found e.g. in SNP GENOTYPING.

allelic pair See ALLELE.

allelomorph *Syn.* ALLELE.

allolactose β-D-Galactopyranosyl-(1→6)-D-glucopyranose: the natural inducer of the LAC OPERON in *Escherichia coli*; it is formed as a minor product during the cleavage of lactose by β-glucosidase.

(See also IPTG.)

allosteric effect The effect produced when the binding of a ligand to a particular target molecule affects the properties of other site(s) on the same molecule; allosteric effects are due to conformational changes that result from the binding of the ligand.

allosteric nucleic acid enzymes Nucleic acid enzymes (see e.g. APTAZYME) whose function can be subject to regulation e.g. by the activity of a low-molecular-weight molecule – or the activity of an oligonucleotide.

(See BINARY DEOXYRIBOZYME LIGASE and MAXIZYME.)

alpha (α, *Lk*) A symbol for LINKING NUMBER.

alpha peptide (α-peptide) See entry α-PEPTIDE.

alternation of generations See PLOIDY.

alternative splicing SPLICING of a given pre-mRNA which can proceed in at least two different ways; the different modes of splicing produce mRNAs with different sequences.

Alternative splicing is a common (i.e. natural) phenomenon [Nucleic Acids Res (2004) 32(13):3977–3983] which allows a given gene to encode extra information. Thus, e.g. a given gene may encode two mutually antagonistic messages (whose expression must be under appropriate control). Exceptionally, it is reported that a single gene may potentially give rise to >100 different transcripts.

Events that may occur during alternative splicing include the splicing out (loss) of an exon (*exon skipping*), inclusion of an intron (*intron retention*) and/or splicing at a site *within* an exon.

The regulation of (normal) alternative splicing involves a balance between certain factors which promote or inhibit the use of specific splicing sites in pre-mRNA.

Splicing of pre-mRNA of the *bcl-x* gene can produce a long (anti-apoptotic) mRNA and a short (pro-apoptotic) mRNA; the content of these mRNAs in the cell is a key determinant of cancer progression. Splicing has been modulated, *in vitro* and *in vivo*, by means of a short antisense PNA conjugated to an oligopeptide containing eight serine–arginine repeats (cf. SR PROTEINS); this procedure produced apoptosis in HeLa (cancer) cells [Nucleic Acids Res (2005) 33(20):6547–6554].

In public databases, sequences of many cancer-associated genes are reported to reflect tumor-associated, *atypical* splice forms (rather than normal splice forms) [Nucleic Acids Res (2005) 33(16):5026–5033].

In a comparative study on eight organisms, the extent of alternative splicing was reported to be higher in vertebrates than in invertebrates [Nucleic Acids Res (2007) 35(1):125–131].

(See also EXON TRAPPING.)

Alu sequences (*Alu* sequences) In (at least) some mammalian genomes: a family of related sequences, each typically about 300 nt long and commonly having a recognition site for the RESTRICTION ENDONUCLEASE AluI (AG↓CT); the human genome may contain about one million copies. Alu sequences are RETROTRANSPOSONS; owing to their ability to generate insertional mutations they are regarded as potential factors in genetic disorders.

Alu sequences in the human genome may be undergoing active transposition [amplification of Alu sequences: PLoS Comput Biol (2005) 1(4):e44 and Genome Res (2005) 15(5): 655–664.]

(See also SINE and LINE.)

AluI A RESTRICTION ENDONUCLEASE from *Arthrobacter luteus*; recognition site: AG↓CT.

α-amanitin A cyclic peptide which, at low concentrations, can inhibit (eukaryotic) DNA-dependent RNA polymerase II.

amber codon See NONSENSE CODON.

amber mutation A mutation that creates an amber codon (see NONSENSE CODON).

amber suppressor See SUPPRESSOR MUTATION.

ambisense RNA Viral ssRNA in which some gene(s) occur in positive-sense form and other(s) in negative-sense form.

amelogenin A protein associated with dental development. A gene encoding human amelogenin (*AMELX*) occurs on the X chromosome (at location Xp22.3–Xp22.1) and another gene encoding amelogenin (*AMELY*) occurs on the Y chromosome (location Yp11.2).

The amelogenin genes are exploited e.g. in a gender ident-

ification assay: see DNA SEX DETERMINATION ASSAY.

***AMELX* gene** See AMELOGENIN.

***AMELY* gene** See AMELOGENIN.

Ames strain (of *Bacillus anthracis*) See BACTERIA (table).

Ames test (Mutatest; *Salmonella*/microsome assay) A test used to determine whether a given agent is mutagenic (and therefore possibly carcinogenic) by investigating its ability to *reverse* an auxotrophic mutation in *Salmonella typhimurium*; the mutation in *S. typhimurium* makes the organism dependent on an exogenous source of histidine, and reversal of the mutation would allow the organism to synthesize its own histidine (i.e. it would revert to prototrophy). Different strains of *S. typhimurium* may be used, each with a different type of mutation in the histidine operon. Some of these test strains may also contain mutations which make them more permeable to certain chemicals and/or which prevent them from carrying out DNA repair. Moreover, the test strains may contain the plasmid pKM101 which includes genes for so-called error-prone repair and which therefore promotes the mutagenic effects of any DNA-damaging agents present in the reaction.

Because some chemical agents exhibit mutagenic activity only after their metabolic activation, the test system generally includes microsomal enzymes from a liver homogenate (the 9000 ***g*** supernatant, fraction 'S9') from rats pre-treated with a carcinogen to induce production of the appropriate enzymes.

When performed as a 'plate incorporation test', a culture of *S. typhimurium*, an S9 preparation and the substance under test are mixed with soft agar (which includes a low level of histidine) and this is poured onto a plate of *minimal agar* – which is then incubated at 37°C in the dark. (Minimal agar permits the growth of prototrophs but does not permit growth of the (auxotrophic) test strain of *S. typimurium*.) The low level of histidine in the soft agar allows (only) limited growth of the (auxotrophic) *S. typhimurium* and this results in a light, confluent growth of this organism in the upper layer of agar (the 'top agar').

If the test substance had caused reversion to prototrophy in any cells of the test strain, those cells (whose growth would not be limited) can grow and form visible colonies.

When interpreting the results, several factors must be borne in mind. An absence of growth in the 'top agar' would suggest that the substance under test has general antibacterial activity, and that any colonies which develop on the plate are unlikely to be true revertants. Again, before drawing conclusions from a number of *apparent* revertant colonies, it is necessary to take into account the known *spontaneous* reversion rate of the particular mutation in the given test strain.

Various modifications of the basic Ames test are used for specific purposes.

[Ames test with a derivative of carbamic acid: Antimicrob Agents Chemother (2005) 49:1160–1168.]

(See also SOS CHROMOTEST.)

amikacin See AMINOGLYCOSIDE ANTIBIOTICS.

amino acid A term which, in the present context, usually refers to one of the 20 compounds whose residues are components of oligopeptides, polypeptides and proteins (see table).

Ornithine is just one example of an amino acid which is not represented by a CODON but which is nevertheless found e.g. in certain oligopeptide antibiotics; this kind of oligopeptide is synthesized by a ribosome-free enzyme system (rather than by translation) (see NON-RIBOSOMAL PEPTIDE SYNTHETASE).

aminoglycoside antibiotic Any of a group of broad-spectrum antibiotics in which the molecular structure typically includes an aminosugar and either 2-deoxystreptamine or streptidine; these antibiotics bind to the bacterial 30S ribosomal subunit and inhibit protein synthesis.

The aminoglycosides include amikacin, framycetin, gentamicin, hygromycin B, kanamycin, neomycin, streptomycin and tobramycin; they are typically bactericidal (at appropriate concentrations) and are active against a wide range of Gram-positive and Gram-negative species.

Resistance to aminoglycosides can arise e.g. by (i) mutation in proteins of the ribosomal 30S subunit (affecting the binding of antibiotics); (ii) inactivation of antibiotics by bacterial enzymes which e.g. carry out *N*-acetylation or *O*-phosphorylation; (iii) decreased uptake by the cell.

(See also G418 SULFATE.)

AMP CT Amplified *Chlamydia trachomatis* test: a TMA-based assay used for detecting the pathogen *Chlamydia trachomatis* in clinical specimens (Gen-Probe, San Diego CA). One early study [J Clin Microbiol (1997) 35:676–678] examined urine specimens from female patients as a non-invasive method of diagnosing chlamydial infection; both the AMP CT assay and a PCR-based method were found to be sensitive and specific methods for detecting *C. trachomatis* and it was concluded that both methods were suitable screening procedures.

ampholyte Any electrolyte with both acidic and basic groups.

ampicillin 6(α-aminobenzylamido)-penicillanic acid: a semi-synthetic PENICILLIN used in media e.g. as a selective agent for bacteria containing a vector with an ampicillin-resistance marker gene.

amplicon (1) A specific (precise) sequence of nucleotides, part of a larger nucleic acid molecule, which is copied (amplified) by an *in vitro* nucleic acid amplification process – such as NASBA or PCR.

(2) One of the copies of a sequence of nucleotides which has been copied (amplified) by methods such as NASBA or PCR.

(3) One of a number of elements of linear DNA (~100 kb) formed in attempts to obtain a null mutation in the *JBP1* gene of *Leishmania tarentolae* [term used in: Nucleic Acids Res (2005) 33 (5):1699–1709].

(4) *Formerly*: a term for a defective virus vector [example of use: Proc Natl Acad Sci USA (1985) 82:694–698].

amplicon containment One approach to the minimization of contamination by amplicons from previous assays in methods such as PCR. Essentially, this involves division of the working environment into several dedicated areas, each of which is used for only certain specific stage(s) of the procedure. For example, when working with PCR, it is usual to carry out the thermal cycling and the analysis of products (e.g. electro-

AMINO ACIDS: symbols, molecular weights and codons

Amino acid	1-letter symbol	3-letter symbol	Molecular weight	Codons
Alanine	A	Ala	89	GCA, GCC, GCG, GCU
Arginine	R	Arg	174	AGA, AGG, CGA, CGC, CGG, CGU
Asparagine	N	Asn	150	AAC, AAU
Aspartic acid	D	Asp	133	GAC, GAU
Cysteine	C	Cys	121	UGC, UGU
Glutamic acid	E	Glu	147	GAA, GAG
Glutamine	Q	Gln	146	CAA, CAG
Glycine	G	Gly	75	GGA, GGC, GGG, GGU
Histidine	H	His	155	CAC, CAU
Isoleucine	I	Ile	131	AUA, AUC, AUU
Leucine	L	Leu	131	CUA, CUC, CUG, CUU, UUA, UUG
Lysine	K	Lys	146	AAA, AAG
Methionine	M	Met	149	AUG
Phenylalanine	F	Phe	165	UUC, UUU
Proline	P	Pro	115	CCA, CCC, CCG, CCU
Serine	S	Ser	105	AGC, AGU, UCA, UCC, UCG, UCU
Threonine	T	Thr	119	ACA, ACC, ACG, ACU
Tryptophan	W	Trp	204	UGG
Tyrosine	Y	Tyr	181	UAC, UAU
Valine	V	Val	117	GUA, GUC, GUG, GUU

phoresis) in separate areas, and separate areas may also be specified for extracting target nucleic acid and for preparing reagents.

(See also AMPLICON INACTIVATION.)

amplicon inactivation In PCR: any method which avoids contamination by destroying carry-over amplicons from previous assays. Such contamination can be a major problem e.g. in those clinical laboratories in which specimens are examined routinely for only a small number of target sequences; under these conditions new specimens may risk contamination if amplicons are allowed to build up in the laboratory environment (e.g. in/on equipment or in reagents).

Methods for amplicon inactivation

The uracil-N-glycosylase method. In this method, *all* assays are conducted in the normal way except that deoxythymidine triphosphate (dTTP) is replaced by deoxyuridine triphosphate (dUTP) in the reaction mixture. All amplicons produced in each assay therefore contain dUMP instead of dTMP. These amplicons can be analysed in the normal way by gel electrophoresis etc.

In addition to the use of dUTP, the reaction mixture also includes the enzyme URACIL-N-GLYCOSYLASE (UNG). Thus, if an assay is contaminated with amplicons from a previous assay, these amplicons will act as substrates for the enzyme, uracil being cleaved from each dUMP; this, in itself, does not bring about strand breakage, but the amplicons are degraded to non-amplifiable pieces by the high temperature used for the initial denaturation of target DNA. The high temperature also inactivates UNG; this is necessary in order to avoid degradation of amplicons from the current assay. (The target DNA in the reaction is not affected by UNG as it contains dTMP.)

Normally this method cannot be used in PCR-based studies of DNA methylation in which the sample DNA is treated with BISULFITE; this is because bisulfite treatment converts non-methylated cytosines to uracil, so that the template DNA

itself would be subjected to degradation. However, unlike the usual form of bisulfite treatment (in which DNA is sulfonated and is subsequently desulfonated), it has been found that non-desulfonated DNA can be amplified by PCR with the UNG method of decontamination because this (sulfonated) form of DNA is resistant to UNG. Desulfonation of DNA is achieved by a prolonged (30-minute) initial stage of denaturation (at 95°C) [Nucleic Acids Res (2007) 35(1)e4].

The isopsoralen method. In this method isopsoralen is added to the reaction mixture. Isopsoralen is a heterocyclic compound which, when bound to DNA, can form covalent interstrand crosslinks when photoactivated by ultraviolet radiation (e.g. 365 nm/15 min/4°C); activation of isopsoralen at low temperatures has been reported to be more efficient than at room temperature. Because the (double-stranded) amplicons are covalently crosslinked they cannot be denatured to single strands; this means that they cannot serve as templates, and if they were to contaminate a subsequent assay the outcome would not be affected.

Amplicons produced by this method are suitable for examination by processes such as gel electrophoresis and staining (e.g. for confirming the presence of a given target sequence in the sample DNA). However, they cannot be used for any process (such as SSCP analysis) that requires single-stranded samples.

In a different approach to amplicon inactivation, all the primers have a 5′ tag which incorporates a binding site for a type IIS restriction endonuclease; this enzyme, present in the reaction mixture, cleaves any contaminating amplicons and is itself inactivated at the initial high-temperature stage of PCR (see APSR).

amplicon primer site restriction See APSR.

amplification (of DNA *in vitro*) See DNA AMPLIFICATION.

amplification-refractory mutation system See ARMS.

amplified fragment length polymorphism (AFLP) See AFLP.

amplified restriction fragment length polymorphism (AFLP) See AFLP.

Ampligase® See DNA LIGASE.

amplimer Any primer used in PCR.

AmpliWax™ See HOT-START PCR.

AMPPD® A 1,2-dioxetane substrate that emits light when dephosphorylated by ALKALINE PHOSPHATASE (AP). It is used e.g. for detecting AP-labeled probes.

(See also CHEMILUMINESCENCE.)

amprenavir See PROTEASE INHIBITORS.

AMTDT Amplified *Mycobacterium tuberculosis* direct test: a TMA-based assay for detecting *Mycobacterium tuberculosis* in clinical specimens (Gen-Probe, San Diego CA).

The AMTDT was approved in 1995 by the American FDA (Food and Drug Administration) for use with *smear-positive* respiratory specimens; a smear-positive specimen is one from which a smear showing ACID-FAST BACILLI can be prepared. In the original form of the test, the sample was initially treated with a MUCOLYTIC AGENT, then 'decontaminated' with sodium hydroxide, and finally subjected to sonication to lyse organisms and release nucleic acids. The specimen was then heated (95°C/15 minutes) to remove intra-strand base-pairing in the rRNA.

The reaction mixture contained 45 μL or 50 μL of sample, and amplification (at 42°C) was conducted for 2 hours. The amplification product was detected by the addition of target-specific probes (for details see entry ACCUPROBE).

An attempt was made to adapt AMTDT for the detection of *Mycobacterium tuberculosis* in *non*-respiratory specimens [J Clin Microbiol (1997) 35:307–310], and studies were made to compare the original and subsequent (improved) versions of AMTDT for the detection of *M. tuberculosis* in respiratory and non-respiratory specimens [J Clin Microbiol (1998) 36: 684–689].

The new-format 'enhanced' AMTDT was approved by the FDA in 1998. Among other changes, this version involved the use of a 450 μL aliquot of the sample.

One study reported false-positive results in tests on sputa from patients infected with *Mycobacterium kansasii* and *M. avium*; these species of *Mycobacterium* are not infrequently isolated from infections in immunocompromised patients. The authors of this study suggested a change in the threshold value of luminometer readings considered to be an indication of a positive result [J Clin Microbiol (1999) 37:175–178].

anaerobic respiration RESPIRATION (q.v.) in the absence of oxygen.

analyte In a test system: the component whose properties are studied/measured.

anchor primer A primer that binds to an ANCHOR SEQUENCE.

anchor sequence Commonly, a sequence of nucleotides, with a known composition, which is present in a given molecule or which is ligated to another sequence (or added by tailing) in order to serve a particular function – e.g. as a primer-binding site. For example, in ANCHORED PCR an anchor sequence is used to provide an otherwise unavailable site for priming the amplification of an unknown sequence.

Certain natural sequences – e.g. the poly(A) tail on many mRNA molecules – are referred to as anchor sequences.

anchored PCR A form of PCR used for amplifying an unknown sequence of nucleotides adjacent to a known sequence on a fragment of DNA; this approach addresses the problem of the lack of a primer-binding site in the unknown sequence.

To each end of the fragment is ligated a short segment of DNA of known sequence (e.g. a linker). (If the fragment has 3′ or 5′ overhangs then these can be eliminated enzymically in order to prepare the fragment for blunt-ended ligation to the linkers). After ligation, the linker contiguous with the unknown region provides an ANCHOR SEQUENCE which can serve as a primer-binding site for one of the PCR primers. The second primer is designed to bind at a site within the known sequence. If PCR is primed in this way, the resulting amplicons will include the unknown sequence and at least part of the known sequence. If the known sequence occurs in the *center* of the fragment, then amplification, as described above, can be carried out for both of the unknown flanking

regions.

(See also VECTORETTE PCR.)

anchoring enzyme In SAGE (q.v.): a name sometimes given to the enzyme used for initial cleavage of the cDNAs.

aneuploid Refers to a genome that has one or more chromosomes in excess of, or less than, the number characteristic of the species (see e.g. DOWN'S SYNDROME).

Angelman syndrome A genetically based disorder which may be caused by any of various mechanisms – see table in entry GENETIC DISEASE for further details.

annealing The hybridization of two complementary (or near-complementary) sequences of nucleotides to form a double-stranded molecule or a double-stranded region within a larger molecule (e.g. the binding of primers to primer-binding sites in PCR).

annotation The assignment of a (predicted) function to an uncharacterized gene (and/or attempted characterization of the gene product) based on sequence homology with a gene of known function present in another organism.

antagomir Any of a range of synthetic, chemically engineered oligonucleotides that bind to, and antagonize, specific types of MICRORNA molecule. Intravenous administration (in mice) of antagomirs directed against particular miRNAs resulted in efficient and specific silencing of the given miRNAs [Nature (2005) 438:685–689].

anthrax toxin A toxin, produced by the Gram-positive pathogen *Bacillus anthracis*, which gives rise to the symptoms of anthrax. It comprises three protein components – each, alone, being unable to function as a toxin; these three proteins are encoded by the plasmid pXO1. Another plasmid, pXO2, is needed for the pathogenicity of *B. anthracis*; this encodes an essential anti-phagocytic capsule which protects the organism from the host's immune system.

One component of the toxin localizes in the cell membrane and permits internalization of the other two components – a zinc protease (which disrupts intracellular signaling) and an ADENYLATE CYCLASE (which e.g. promotes edema).

anti Abbreviation for ANTICLINAL.

antibiotic Any of an extensive range of natural, semi-synthetic and fully synthetic compounds which, in low concentrations, are able selectively to inhibit or kill specific types of microorganism and, in some cases, other types of cell – e.g. tumor cells; an antibiotic acts at specific site(s) in a susceptible cell. (Compounds that are active against *viruses* are usually called 'antiviral agents' rather than antibiotics.) Natural antibiotics (see e.g. BACTERIOCIN) have ecological roles. Some types of antibiotic have medical/veterinary uses in the prevention and/or treatment of infectious diseases, while some are used e.g. as food preservatives.

In nucleic-acid-based technology, antibiotics are used in a variety of selective procedures. In one common scenario, the presence of a given antibiotic in a growth medium permits positive selection of cells which are expressing a gene that confers resistance to that particular antibiotic. The gene may be included e.g. in a vector used for the transformation of a population of cells – those cells which internalize the vector (and express the antibiotic-resistance gene) being selected by growth on an antibiotic-containing medium.

Some antibiotics are MICROBICIDAL, others are MICROBISTATIC; a microbicidal antibiotic may behave as a microbistatic antibiotic at lower concentrations.

A mixture of antibiotics may behave synergistically or antagonistically (or may not display either effect).

Synergism is shown when different antibiotics, that are acting simultaneously on a given organism, produce an effect which is greater than the sum of their individual effects. For example, the antibiotics sulfamethoxazole and trimethoprim block different reactions in the same major metabolic pathway: sulfamethoxazole inhibits the formation of dihydrofolic acid (DHF), and trimethoprim inhibits the conversion of DHF to the important coenzyme tetrahydrofolate (THF); these two antibiotics act synergistically and they are used, together, in the therapeutic agent *cotrimoxazole*.

Antagonism, the converse of synergism, can occur in different ways. In one form, an antibiotic that inhibits growth (e.g. CHLORAMPHENICOL) antagonizes those antibiotics (such as the penicillins and other β-lactam antibiotics) whose activity depends on growth in the target cell. In a different form of antagonism, certain antibiotics stimulate cells to produce enzymes that inactivate *other* antibiotics; for example, the β-lactam imipenem (or cefoxitin) induces the synthesis of β-lactamases – enzymes which can inactivate certain other β-lactam antibiotics.

Modes of action

To be effective at all, an antibiotic must be able to enter, or pass through, the cell envelope in order to reach the relevant target site(s). Moreover, an antibiotic can be effective against a given population of cells only if its concentration is above the appropriate minimum level for that agent under the given conditions.

Modes of action include:

- Interference with DNA gyrase (a topoisomerase), with consequent inhibition of DNA synthesis (e.g. novobiocin, quinolone antibiotics).
- Depletion of guanine nucleotides (by inhibiting synthesis of GMP), affecting synthesis of nucleic acids (e.g. mycophenolic acid).
- Binding to ribosomes and inhibiting protein synthesis (e.g. aminoglycoside antibiotics, chloramphenicol, macrolide antibiotics (such as erythromycin), tetracyclines, viomycin).
- Binding to RNA polymerase, inhibiting transcription (e.g. rifamycins).
- Disruption of the bacterial cytoplasmic membrane, altered permeability affecting the cell's integrity (e.g. gramicidins, polymyxins).
- Inhibition of synthesis of the bacterial cell wall polymer peptidoglycan, leading to cell lysis (e.g. β-lactam antibiotics, vancomycin).
- Inhibition of the enzyme dihydrofolate reductase, thereby inhibiting tetrahydrofolate-dependent reactions (that include

ANTIBIOTIC: some antibiotics used in DNA technology (e.g. for marker selection)

Antibiotic	Group	Target organisms	Antibiotic action
Actinomycin D	–	Prokaryotic and eukaryotic	Intercalating agent; inhibits DNA-dependent RNA polymerase
Ampicillin	β-Lactams	Bacteria	Blocks synthesis of cell-wall polymer peptidoglycan
Blasticidin S	Nucleoside	Prokaryotic and eukaryotic	Inhibits protein synthesis by inhibiting the peptidyltransferase-mediated reaction at the ribosome
Carbenicillin	β-Lactams	Bacteria	Blocks synthesis of cell-wall polymer peptidoglycan
Cefotaxime	β-Lactams	Bacteria (mainly Gram-negative)	Blocks synthesis of cell-wall polymer peptidoglycan
Chloramphenicol (= chloromycetin)	–	Bacteria (broad spectrum), some fungi	Binds to prokaryotic and mitochondrial ribosomes; inhibits peptidyltransferase and (hence) protein synthesis
G418 sulfate	Related to gentamicin	Prokaryotes, yeasts, plants, mammalian cells	Inhibits protein synthesis
Gentamicin	Amino-glycosides	Bacteria	Binds to 30S subunit of bacterial ribosomes and inhibits protein synthesis
Hygromycin B	Amino-glycosides	Mammalian and plant cells	Inhibits protein synthesis
Kanamycin	Amino-glycosides	Bacteria	Binds to 30S subunit of bacterial ribosomes and inhibits protein synthesis
Kasugamycin	Amino-glycosides	Bacteria, some fungi	Inhibits polypeptide chain initiation and, hence, protein synthesis
Mycophenolic acid	–	Bacteria (also antitumor agent)	Inhibits synthesis of guanosine monophosphate, inhibiting synthesis of nucleic acids
Nalidixic acid	Quinolones	Bacteria (mainly Gram-negative)	Inhibits function of the A subunit of gyrase; inhibits DNA synthesis
Novobiocin	–	Bacteria	Inhibits binding of ATP to the B subunit of gyrase, inhibiting DNA synthesis
Penicillin G (= benzylpenicillin)	β-Lactams	Bacteria (mainly Gram-positive)	Blocks synthesis of cell-wall polymer peptidoglycan
Polymyxin B	Polymyxins	Bacteria (mainly Gram-negative)	Increases permeability of the cytoplasmic membrane and affects the integrity of the outer membrane
Puromycin	Nucleoside	Prokaryotic and eukaryotic	Inhibits protein synthesis by acting as an analog of part of an aminoacyl-tRNA
Rifampicin	Rifamycins	Bacteria (mainly Gram-positive)	Inhibits DNA-dependent RNA polymerase by binding to the β subunit of the enzyme
Streptomycin	Amino-glycosides	Bacteria, some fungi	Interacts with ribosomes and inhibits protein synthesis
Tetracycline	Tetracyclines	Bacteria	Binds to ribosomes; inhibits protein synthesis by blocking the binding of aminoacyl-tRNAs to the A site
Zeocin™	Bleomycin/ phleomycin	Bacteria, yeast, and mammalian cells	Binds to, and cleaves, DNA

synthesis of deoxythymidine – and, hence, DNA) (e.g. pyrimethamine, trimethoprim).

- Interference with DNA function by intercalating agents (e.g. actinomycin D, quinoxaline antibiotics).
- Interaction with sterols in the cytoplasmic membrane (in e.g. yeasts and other fungi), causing leakage (e.g. polyene antibiotics).
- Inhibition of the enzyme chitin synthase (in certain fungi), affecting cell wall synthesis (e.g. polyoxins).

Mechanisms of bacterial resistance to antibiotics

Resistance to a particular antibiotic is constitutive in those cells which (i) lack the antibiotic's specific target, (ii) have a variant form of the target which is not susceptible to the antibiotic, and (iii) are impermeable to the antibiotic. Examples: (i) *Mycoplasma* is resistant to β-lactam antibiotics because it lacks a cell wall; (ii) strains of *Staphylococcus aureus* known as MRSA (methicillin-resistant *S. aureus*) generally contain a modified target which is not susceptible to methicillin; (iii) typically, Gram-negative bacteria are insensitive to penicillin G (a β-lactam antibiotic) because this antibiotic is not able to penetrate the outer membrane of Gram-negative bacteria.

As well as constitutive resistance (see above) resistance can also be acquired e.g. by mutation or by the acquisition of plasmid or transposon gene(s) specifying resistance to one or more antibiotics. Examples include:

- Due to mutation, the target of a given antibiotic may be altered so that it fails to bind the antibiotic; consequently, the target (e.g. an enzyme) is not affected by otherwise inhibitory concentrations of that antibiotic. Thus, a mutant form of the ribosomal protein L22 in *Staphylococcus aureus* confers resistance to quinupristin/dalfopristin (Synercid®), a streptogramin, and in *Mycobacterium tuberculosis* point mutations in the *rpoB* gene (encoding the β subunit of RNA polymerase) can confer resistance to rifamycins (such as rifampin) for which RNA polymerase is the target.
- Transposon Tn*10* encodes an inducible efflux system that enables certain Gram-negative bacteria to externalize tetracycline via an 'efflux pump' located in the cell envelope.
- Mutant forms of some envelope proteins are associated with decreased permeability. For example, alteration in outer membrane porins in *Enterobacter aerogenes* increases resistance to certain antibiotics, and in *Pseudomonas aeruginosa* resistance to the aminoglycosides and other antibiotics can be determined through membrane permeability controlled by a TWO-COMPONENT REGULATORY SYSTEM.
- Degradation of antibiotics by plasmid-encoded or chromosome-encoded enzymes. Such enzymes include the inducible and constitutive β-lactamases that cleave the β-lactam ring in, and hence inactivate, β-lactam antibiotics such as penicillins and cephalosporins. The enzyme chloramphenicol acetyltransferase (which degrades CHLORAMPHENICOL) is another example – as are the acetyltransferases, adenylyltransferases and phosphotransferases that inactivate aminoglycoside antibiotics.
- Increased production of an affected metabolite. Thus, for example, synthesis of higher levels of *p*-aminobenzoic acid (PABA) may overcome the effect of competitive inhibition by sulfonamides.

antibody-labeling reagents See e.g. ZENON ANTIBODY LABELING REAGENTS.

anticlinal (*anti*) Of a nucleotide: the conformation in which the oxygen atom within the sugar ring (–O–) is the maximum distance from the 6-position of a purine (or the 2-position of a pyrimidine). (cf. SYNCLINAL.)

anticoagulant (*DNA technol.*) A term that usually refers to an agent which inhibits the coagulation (i.e. clotting) of blood.

The anticoagulants include sodium citrate, sodium oxalate, heparin and sodium polyanetholesulfonate (SPS); the latter two agents can inhibit PCR.

anticodon Three consecutive bases in a tRNA molecule complementary to a CODON which specifies the particular amino acid carried by that tRNA.

An anticodon is written in the 5′-to-3′ direction – as is a codon.

anti-downstream box (*or* antidownstream box) See DOWNSTREAM BOX.

antigenic variation Successive changes in surface antigens exhibited by certain types of microorganism (e.g. *Trypanosoma*). There are at least two distinct mechanisms: (i) the alternative antigens are transcribed from specific, *pre-existing* genes (see PHASE VARIATION), and (ii) the alternative antigens arise by ongoing recombinational events – i.e. they are encoded by newly formed, rather than pre-existing, genes. An example of the second mechanism is the formation of new versions of a subunit in the fimbriae of *Neisseria gonorrhoeae*; variant forms of this subunit arise through repeated recombination between the chromosomal subunit gene, *pilE*, and another chromosomal gene, *pilS*, as well as between *pilE* and any homologous DNA received e.g. by transformation.

Antigenic variation apparently helps a pathogen to evade a host's immunologic defense mechanisms.

antimutator gene Any gene whose activity reduces the rate of spontaneous mutation in a cell. For example, some strains of *Escherichia coli*, with a mutant form of DNA polymerase, have mutation rates below those of wild-type strains; in this case the antimutator activity presumably involves improved fidelity/proof-reading.

antiparallel (of strands in dsDNA) The (usual) arrangement in which a 5′-to-3′ strand is hybridized to a 3′-to-5′ strand, i.e. each end of a double-stranded DNA molecule has a 5′ terminal and a 3′ terminal.

(See also DNA.)

antiretroviral agents Agents with activity against retroviruses; some are useful e.g. in chemotherapy against AIDS. See e.g. NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS, NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS and PROTEASE INHIBITORS.

anti-reverse cap analog (ARCA) A chemically modified form of CAP ANALOG (Ambion, Austin TX) designed to maximize the efficiency of *in vitro* translation by ensuring that the cap

analog is incorporated in the transcript in the correct orientation (a cap analog incorporated in the reverse orientation does not support translation).

[Example of use of anti-reverse cap analog: Nucleic Acids Res (2005) 33(9):e86.]

antisense gene In a genetically engineered cell: any gene, inserted into the cell, whose presence is intended to inhibit or block the expression of an endogenous gene.

antisense RNA Any natural or synthetic RNA whose sequence permits interaction with a given sense sequence, in RNA or DNA, and which can affect the activity/expression of the target molecule (see FINOP SYSTEM, MICRORNA, MULTICOPY INHIBITION, POST-SEGREGATIONAL KILLING, R1 PLASMID, RNA INTERFERENCE).

While usually forming double-stranded structures, ssRNA can also form triple-stranded structures with dsDNA (see also TRIPLEX DNA), and may bind to ssDNA during transcription; in either case, transcription may be affected.

The search for antisense RNAs may be hindered e.g. by their relatively small size and because their sequences may not exactly match those of their target molecules; moreover, in at least some cases a given antisense molecule may have more than one target. [Detection of 5′- and 3′-UTR-derived small RNAs and *cis*-encoded antisense RNAs in *Escherichia coli*: Nucleic Acids Res (2005) 33(3):1040–1050.]

antisense strand (of DNA) The non-CODING STRAND.

antizyme Any of a group of eukaryotic proteins associated with regulation of ornithine decarboxylase (ODC), an enzyme involved in the biosynthesis of polyamines. Functional antizyme is expressed in the presence of increased levels of polyamines; antizyme 1 promotes UBIQUITIN-dependent degradation of ODC in the 26S PROTEASOME.

The antizymes themselves are subject to regulation by antizyme inhibitor [Biochem J (2005) 385(1):21–28].

AOX1 In the (methylotrophic) yeast *Pichia pastoris*: a highly regulated, inducible gene which encodes alcohol oxidase – a peroxisomal enzyme involved in the metabolism of methanol.

(See also PPICZ VECTOR.)

AP ALKALINE PHOSPHATASE.

AP endonuclease Any enzyme with endonuclease activity that is involved in the excision of apurinic/apyrimidinic nucleotide residues (see e.g. BASE EXCISION REPAIR).

AP-PCR Arbitrarily primed PCR: any form of PCR which uses primers of arbitrary sequence and which amplifies random, but discrete, sequences of chromosomal DNA; AP-PCR has been used for TYPING bacteria.

PCR is initially carried out under low stringency, and the primers bind at various sites to each strand of heat-denatured chromosomal DNA; the binding of primers occurs at 'best-fit' sequences, and may include mismatches. In some cases two primers bind with relative efficiency, on opposite strands, at locations separated by a few hundred bases. If synthesis can occur normally from these two primers, a further round of cycling under low-stringency conditions, followed by many cycles under high-stringency conditions, may produce copies of an amplicon delimited by the two best-fit sequences. In the phase of high-stringency cycling not all the primers will bind to their best-fit sequences – so that only a proportion of the amplicons produced under low-stringency conditions will be amplified in the high-stringency phase.

The amplicons from a given sample are subjected to gel electrophoresis, and the stained bands of amplicons form the fingerprint. Strains are compared and classified on the basis of their fingerprints.

One advantage of this approach is that there is no need for prior knowledge of the genome sequence; there is no need to design specific primers, and any isolate is potentially typable.

Results are generally reproducible under standardized conditions in a given laboratory, but comparable results will not necessarily be obtained in other laboratories unless the procedures are *identical*; reproducibility of results depends not only on the primer sequence but also e.g. on the particular type of polymerase used and on the initial procedure used for preparing the sample DNA.

Some other named methods are based on the same principle – RAPD (random amplified polymorphic DNA) analysis and DAF (direct amplification fingerprinting). These methods may differ e.g. in the length of primers used, annealing temp-erature for the primers, and the type of gel used for electro-phoresis. The original AP-PCR employed primers of 20–50 nucleotides, with an annealing temperature of about 40°C, and used an agarose gel. In RAPD the primers are typically 10–20 nt, the annealing temperature is ~36°C, and products are separated in an agarose gel. DAF uses short primers (5–8 nt) at an annealing temperature of ~30°C; because there are many more, smaller products, electrophoresis is carried out in a polyacrylamide gel, and silver staining is used to detect bands in the fingerprint.

AP site (abasic site) In a nucleotide sequence: a site at which the base (purine or pyrimidine) is missing – the remainder of the nucleotide (sugar, phosphate) being present. (cf. GAP.)

APES See AAS.

aphidicolin A tetracyclic diterpenoid, isolated from a fungus, which strongly inhibits the eukaryotic α DNA polymerase. Bacterial DNA polymerases are unaffected, but aphidicolin was reported to inhibit DNA synthesis in at least some members of the domain ARCHAEA (e.g. some methanogens).

APOBEC-1 See CYTIDINE DEAMINASE and RNA EDITING.

apolipoprotein B See RNA EDITING.

APSR (amplicon primer site restriction) In PCR, a method for preventing the contamination of a reaction mixture with amplicons from previous assays. In APSR, *all* the assays are conducted with primers whose 5′ ends carry a recognition site for a (type IIS) RESTRICTION ENDONUCLEASE which cleaves both strands 3′ of its binding site. In a reaction mixture, the (added) restriction enzyme will cut any carry-over amplicons but will not cleave the template DNA (unless, by chance, it contains the given recognition site); the restriction enzyme is inactivated during PCR temperature cycling.

[Method: BioTechniques (2005) 39:69–73.]
(See also AMPLICON INACTIVATION.)

APT paper See SOUTHERN BLOTTING.

aptamer (1) Any of a large number of synthetic oligonucleotides which can adopt a three-dimensional structure and bind with high specificity to a given ligand. (See also INTRAMER.)

Selection of an RNA aptamer for a given target molecule can be achieved by SELEX (systematic evolution of ligands by exponential enrichment). Briefly, in SELEX, the immobilized target ligand (for example, a protein) is exposed to a large and diverse population of oligonucleotides synthesized with random sequences. After removal of unbound oligos, the relatively few bound oligos are eluted and then converted to cDNAs. The cDNAs are amplified by PCR using primers that incorporate a promoter; the resulting amplicons are transcribed, and the transcripts are used in another round of selection with the target molecule. The cycle is repeated, with target–RNA binding becoming more specific at each round; the range and affinity of selected aptamers can be determined e.g. by regulating the buffer conditions at the binding stage. (A principle similar to this is found in PHAGE DISPLAY.)

In general, SELEX is useful for analyzing protein–nucleic acid binding and e.g. interactions between RNA and various low-molecular-weight molecules.

An aptamer–shRNA fusion transcript has been used to regulate gene expression in mammalian cells; in this system, the activity of the shRNA moiety was controlled by interaction between the aptamer and its ligand – theophylline (1,3-dimethylxanthine) [RNA (2006) 12:710–716].

An aptamer – known to bind to prostate tumor cells – has been conjugated to an siRNA via a streptavidin bridge; on addition to cells, this conjugate was internalized, and the siRNA was able to inhibit gene expression as efficiently as when it was internalized by a lipid-based method [Nucleic Acids Res (2006) 34(10):e73.

(See also APTAZYME.)

[Aptamer database: Nucleic Acids Res (2004) 32(Database issue):D95–D100; online: http://aptamer.icmb.utexas.edu/]

(2) A *natural* sequence within a RIBOSWITCH.

aptazyme A composite APTAMER–RIBOZYME; the aptamer can bind certain molecules that may modify/regulate the activity of the ribozyme.

apurinic Lacking a purine residue: see AP SITE.

apyrase A nucleotide-degrading enzyme (EC 3.6.1.5) with various applications in technology (see e.g. PYROSEQUENCING).

apyrimidinic Lacking a pyrimidine residue: see AP SITE.

***araBAD* operon** See OPERON.

Arabidopsis thaliana A small cruciferous plant commonly used in plant genetics owing to its simple genome and its short generation time. [Genome of *A. thaliana*: Nature (2000) 408: 791–826.]

(See also FLORAL DIP METHOD.)

araC See OPERON.

Aranesp® See BIOPHARMACEUTICAL (table).

arbitrarily primed PCR See AP-PCR.

ARCA See ANTI-REVERSE CAP ANALOG.

Archaea One of the two domains of prokaryotic organisms, the other being Bacteria. Organisms in these two domains differ e.g. in 16S rRNA sequences, composition of cell wall macromolecules, composition of cytoplasmic membrane lipids and flagellar structure. The general features of gene expression in the two domains are also dissimilar.

(See also PROKARYOTE.)

archaean (*syn.* archaeon) An organism within the (prokaryotic) domain ARCHAEA.

Archaebacteria A now-obsolete kingdom of prokaryotes; the organisms formerly placed in this taxon are currently classified in the domain ARCHAEA.

ArchaeMaxx™ A polymerase-enhancing factor, marketed by Stratagene (La Jolla CA), which is designed to overcome the so-called DUTP POISONING effect.

This factor is used e.g. in association with the *PfuTurbo*® and Herculase® DNA polymerases (also marketed by Stratagene).

[Example of use: PLoS Biol (2006) 4(3):e73.]

archaeon See ARCHAEAN.

ARES™ See PROBE LABELING.

Argonaute 2 See RNA INTERFERENCE.

ARMS Amplification-refractory mutation system: a procedure used e.g. for demonstrating or detecting a point mutation *at a specific site* in DNA whose wild-type (non-mutant) sequence is known.

Essentially, use is made of a primer in which the 3′-terminal nucleotide is complementary to the *mutant* base at the given site. After hybridization, extension of the primer by a polymerase signals the presence of the mutation at that site, while the absence of extension indicates the presence of a wild-type (or other) nucleotide.

armyworm The insect *Spodoptera frugiperda*. Cell cultures of this organism are used e.g. for the synthesis of recombinant proteins encoded by baculovirus vectors.

(See also SF9 CELLS.)

aRNA Antisense RNA; for example of use see MESSAGEAMP ARNA AMPLIFICATION KIT.

array Often: a shortened version of MICROARRAY; also used to refer to other oligonucleotide- or tissue-based arrangements etc. with analogous or distinct uses.

ARS Autonomously replicating sequence: a genomic sequence which, if linked to a non-replicative fragment of DNA, promotes the ability of that fragment to replicate independently (extrachromosomally) in the cell. ARSs were first reported in the yeast *Saccharomyces cerevisiae*; this organism contains, on average, one ARS in every ~40 kb of genomic DNA, and these elements are also found in at least some yeast plasmids. Apparently some ARSs are active chromosomal origins while others are *silent origins*; some of the silent origins (and some active ones) may function as transcription silencers.

[Genome-wide hierarchy of replication origin usage in the yeast *Saccharomyces cerevisiae*: PLoS Genetics (2006) 2(9): e141.]

Factors reported to contribute to the efficient replication of ARS-containing plasmids in yeast cells include (i) the CEN (centromere) element and (ii) minichromosome maintenance protein 1 (Mcm1).

The circular dsDNA genome of human papillomavirus type 16 can replicate stably in *S. cerevisiae* independently of ARS or CEN; sequences in the viral DNA can substitute for both ARS and CEN [J Virol (2005) 79(10):5933–5942].

ARSs have also been described in species of the ARCHAEA [e.g. J Bacteriol (2003) 185(20):5959–5966].

(See also SIDD and YEAST ARTIFICIAL CHROMOSOME.)

ascospores See SACCHAROMYCES.

ascus See SACCHAROMYCES.

aseptic technique Measures taken to avoid contamination of cultures, sterile media etc. – and/or contamination of persons, animals or plants – by microorganisms that are present in the environment (e.g. in the air) and that may be associated with particular source(s).

In this approach, the vessels used for media etc. must be sterile before use (e.g. pre-sterilized Petri dishes), and sterile material should not be exposed to any non-sterile conditions before use.

The working surfaces of forceps and other types of metal instrument (such as bacteriological loops etc.) are sterilized by 'flaming' before use, and the rims of bottles etc. used for dispensing sterile (non-flammable) materials are also flamed.

Benches are regularly treated with disinfectants and/or with ULTRAVIOLET RADIATION (UVR). The so-called 'germicidal' lamps, which may emit UVR at ~254 nm, are used e.g. for the disinfection of air and exposed surfaces in enclosed areas. In general, UVR has rather poor powers of penetration, and its affects on microorganisms may be reversible by certain DNA repair processes (see e.g. UVRABC-MEDIATED REPAIR).

Some procedures, e.g. handling specimens likely to contain certain pathogens (such as *Mycobacterium tuberculosis*, or certain highly hazardous viruses such as Ebola virus or Lassa fever virus), are carried out in a SAFETY CABINET.

ASP APOBEC-1-stimulating protein: see RNA EDITING.

aspart See INSULIN ASPART.

asymmetric PCR A form of PCR in which the concentration of *one* of the primers is much lower than that of the other (e.g. a ratio of 1:50); during temperature cycling, this primer will be quickly used up – so that only one strand of the target sequence will be significantly amplified.

Uses of asymmetric PCR include the preparation of probes and the preparation of single-stranded DNA for sequencing.

ssDNA products from PCR can also be obtained in a different way. One of the two types of primer can be labeled with BIOTIN and the reaction carried out with both primers in their normal concentrations. At the end of the reaction, STREPTAVIDIN is added, and this binds only to the biotin-labeled strands; subsequent gel electrophoresis (in a denaturing gel) separates the two types of strand because the mobility of the streptavidin-bound strand is much lower. (The biotinylated primer forms the strand which is *not* required.)

AT type See BASE RATIO.

ATMS *p*-Aminophenyltrimethoxysilane: a reagent used for covalently binding DNA probes to a solid support when preparing a MICROARRAY. (In an earlier procedure, DNA was bound *non*-covalently to glass slides by the reagent poly-L-lysine.) [Method: Nucleic Acids Res (2001) 29:e107.]

(See also DENDRICHIP.)

atomic force microscopy (AFM; scanning force microscopy) A method for imaging *surfaces*, including those of molecules and of (living) cells, in e.g. air or liquid, at nanometer-scale resolution. Essentially, the object's surface is scanned in a raster pattern with a fine probe located underneath a traveling cantilever; a laser, reflected from the cantilever (and thus incorporating information on the cantilever's movements) is detected by a photodiode assembly, and the incoming signals are converted by computer into a surface profile.

***att* sites** Sites involved in the SITE-SPECIFIC RECOMBINATION that occurs e.g. when the PHAGE LAMBDA genome integrates into a bacterial chromosome.

Lambda *att* sites are used in commercial DNA technology systems: see e.g. GATEWAY SITE-SPECIFIC RECOMBINATION SYSTEM, MULTISITE GATEWAY TECHNOLOGY, BP CLONASE, LR CLONASE.

In addition to their commercial exploitation, *att* sites have been used e.g. for the production of excisable cassettes which (it has been suggested) may provide a new approach to the functional analysis of the genome of the Gram-positive bacterium *Streptomyces* [Appl Environ Microbiol (2006) 72(7): 4839–4844].

attaching and effacing lesion See PATHOGENICITY ISLAND.

***att*B, *att*P** See PHAGE LAMBDA.

(See also ATT SITES.)

attenuator control See OPERON.

***att*L, *att*R** See PHAGE LAMBDA.

(See also ATT SITES.)

atto- Prefix meaning 10^{-18}.

AttoPhos™ A commercial reagent (Promega) which can be cleaved by ALKALINE PHOSPHATASE to yield a fluorescent product. It has been used e.g. in a CROSS-LINKING ASSAY.

att*Tn*7 See TN7.

autoactivation (*syn*. self-activation) (two-hybrid systems) See e.g. BACTERIOMATCH TWO-HYBRID SYSTEM.

autocatalytic splicing See SPLICING.

autoclave An apparatus within which objects and/or materials are sterilized by saturated (air-free) steam under pressure; the conditions within a working autoclave are typically in the range 115°C (~69 kPa; 10 lb/inch^2) to 134°C (~207 kPa; 30 lb/inch^2).

STERILIZATION in an autoclave is carried out e.g. when preparing certain types of media. Heat-labile constituents of a medium (e.g. a solution of an antibiotic) may be membrane-filtered before being added to a sterile (autoclaved) medium.

Some steam-impermeable items that cannot be sterilized by autoclaving may be sterilized e.g. in a hot-air oven at ~160–170°C for ~1 hour.

autoclave tape A paper strip (usually self-adhesive) which is included with the objects being sterilized in an autoclave; it exhibits a visible change (e.g. in color) when it is subjected to appropriate sterilizing conditions, and can therefore act as a check on the correct operation of the autoclave.

***Autographa californica* NPV** See NUCLEAR POLYHEDROSIS VIRUSES.

autoinducer (in quorum sensing) See QUORUM SENSING.

automated sequencing (of DNA) A method used for rapidly sequencing DNA fragments of up to ~500 nt. Essentially, the process involves conventional chain-termination (i.e. Sanger) sequencing (see DIDEOXY METHOD) with fluorophore-labeled ddNTPs – each type of ddNTP (A, G, C, T) being labeled with a fluorophore that emits a distinctive color on excitation. The sequencing products are separated by polyacrylamide gel electrophoresis and the bands of products are scanned by laser; the positions of individual nucleotides, identified by the color of the fluorescence, are recorded automatically.

(See also DNA SEQUENCING and PYROSEQUENCING.)

autonomously replicating sequence See ARS.

autoplast A PROTOPLAST or SPHEROPLAST which develops as a result of activity of the organism's own autolytic enzymes.

autoradiography A procedure in which a radioactive source is detected or quantitated by its effect on a photographic film; a film is exposed to the radioactive source, in the dark, and for an appropriate period of time, and is subsequently processed.

Autoradiography is used e.g. for investigating intracellular processes (radioactive isotopes being incorporated into biomolecules) and also e.g. for detecting bands of products (such as isotopically labeled fragments of DNA), *in situ*, after gel electrophoresis.

In general, optimal resolution may require the use of those isotopes which have relatively low-energy emission (such as tritium, ^{3}H) rather than those (such as ^{32}P) which have high-energy emission.

autosomal dominant disorder Refers to a genetic disorder in which the phenotypic manifestation arising from expression of an abnormal autosomic allele occurs in the presence of the corresponding normal allele – that is, the influence of the abnormal allele overrides that of the normal allele. This type of disorder tends to exhibit a so-called *vertical* pattern of transmission from one generation to the next; in such cases, an abnormal trait is more likely (than in an autosomal recessive condition) to affect each successive generation.

Both males and females can be affected – and, unlike the situation in X-linked dominant disorders, father-to-son transmission can occur.

One example of this type of disorder: AXENFELD–RIEGER SYNDROME; another example is PEUTZ–JEGHERS SYNDROME. (See also CHARCOT–MARIE–TOOTH DISEASE.)

(cf. AUTOSOMAL RECESSIVE DISORDER and X-LINKED DISORDER.)

autosomal recessive disorder Refers to a genetic disorder in which manifestation of the abnormal phenotype is exhibited when the abnormal allele is not accompanied by the presence of the corresponding normal, wild-type allele; heterozygous individuals with one normal allele do not usually exhibit the abnormal phenotype.

Both males and females can be affected.

Only when mating occurs between two (homozygously) affected individuals will the trait necessarily appear in all the offspring; mating between two heterozygous individuals, or between one heterozygous and one normal individual, tends to spare some of the offspring, so that an autosomal recessive disorder may miss generation(s) and is said to exhibit a *horizontal* mode of transmission.

(cf. AUTOSOMAL DOMINANT DISORDER and X-LINKED DISORDER.)

autosome Any chromosome other than a HETEROSOME.

autotransporter See OMPT GENE.

auxins Phytohormones (plant hormones) which promote stem elongation and other aspects of plant development; the auxins are derivatives of tryptophan. Indole 3-acetic acid (IAA; also called 'auxin' or 'heteroauxin') is a major auxin; it is synthesized from the precursor indole 3-acetonitrile (IAN).

Abnormally high levels of auxins (*hyperauxiny*) are found in some plant diseases (e.g. CROWN GALL).

auxotrophic mutant Any microorganism which, as a result of a mutation, is unable to synthesize an essential nutrient and which therefore can grow only if provided with an exogenous source of that nutrient. (An organism which does *not* contain such a mutation, and which can synthesize all of its essential nutrients, is called a *prototroph*.)

One example of an auxotrophic mutant is mentioned in the entry AMES TEST.

Isolation of auxotrophic mutant bacteria

The usual (selective) procedures cannot be used to isolate auxotrophic bacteria from a mixture of prototrophs and auxotrophs because the requirements of auxotrophs are in excess of those of prototrophs.

The *limited enrichment* method uses an agar-based *minimal medium* enriched with *small* amounts of nutrients. A minimal medium is one which supports the growth of prototrophs but – because it lacks one or more essential nutrients – does not support the growth of auxotrophs. Any colony of auxotrophic cells will quickly exhaust the nutrients in its vicinity – so that the colony remains small; however, the unrestricted growth of prototrophs means that the colonies of prototrophs will be larger than those of auxotrophs. Hence, the small colonies indicate presumptive auxotrophs.

The *delayed enrichment* technique employs an agar-based minimal medium for initial growth, so that prototrophs (only) form colonies in the initial incubation. Complete medium is then poured onto the plate and allowed to set; the nutrients in this medium diffuse into the minimal medium below, allowing the growth of auxotrophs. Again, small colonies indicate presumptive auxotrophs.

For (penicillin-sensitive) bacteria, auxotrophic mutants can be isolated by virtue of their inability to grow in a (penicillin-containing) minimal medium; penicillin is an antibiotic that

acts only on *growing* cells. In this approach, a well-washed population of bacteria (that includes auxotrophs) is exposed to penicillin in a minimal medium; the prototrophs (which can grow) are killed by the penicillin. The remaining cells are washed and re-plated on a complete medium to recover any auxotrophs. It's important to note that, if the auxotrophs had developed through an *in vitro* process of mutagenization, it is essential that the cells be allowed to grow for several generations in complete medium prior to exposure to penicillin; this is because newly mutated cells contain a full complement of (prototrophic) enzymes, and the auxotrophic phenotype develops only after several rounds of cell division – during which the prototrophic enzymes are 'diluted out'. A further requirement in this method is that only a low concentration of cells be used; this is because auxotrophs should not be allowed to grow on nutrients released by lysed prototrophs – as this would render them susceptible to lysis by the penicillin. For this reason, STREPTOZOTOCIN may be used in place of penicillin.

Auxotrophs may also be isolated by REPLICA PLATING.

avian erythroblastosis virus See ERB.

avirulence gene See GENE-FOR-GENE CONCEPT.

Axenfeld–Rieger syndrome An autosomal dominant disorder involving eye defects and certain systemic abnormalities. The syndrome has been associated with mutations in the *PITX2* gene (which encodes a transcription factor). In some patients the disorder is reported to involve aberrant splicing of pre-RNA; it has been suggested that variability in the extent of the splicing fault may be reflected in the observed variability of phenotypic manifestations [BMC Med Genet (2006) 7:59].

5-aza-2′-deoxycytidine A cell-permeant agent used e.g. for studying DEMETHYLATION.

[Example of use: BioTechniques (2006) 41(4):461–466.]

azaserine (*O*-diazoacetyl-L-serine) An agent with antimicrobial and antitumor activity produced by *Streptomyces* sp (a Gram-positive bacterium). Azaserine inhibits the activity of certain enzymes, including phosphoribosylformylglycinamidine synthetase – thus inhibiting biosynthesis of purines and, hence, nucleotides.

(cf. DON; see also HADACIDIN.)

AZT See ZIDOVUDINE.