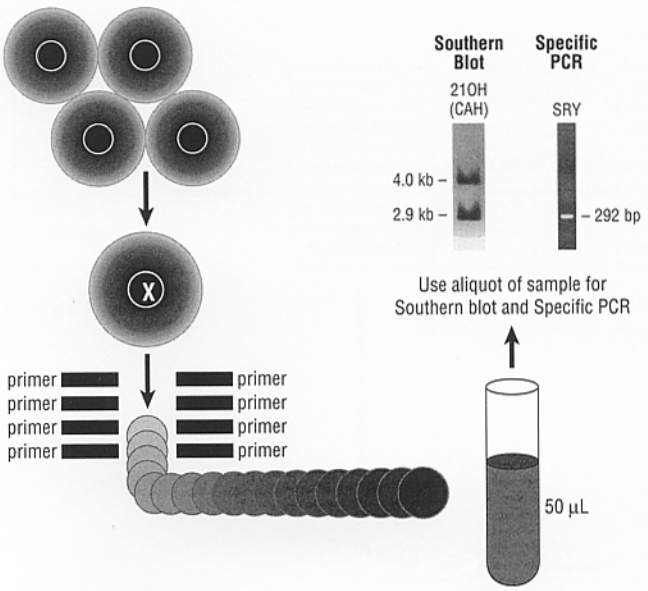


**Fig. 2** This cartoon illustrates the use of primer extension preamplification (PEP) to amplify the whole genome of a single cell many times. A series of degenerate oligonucleotide primers, approximately 15 base pairs in size are developed to amplify the entire genome of the cell, using a standard PCR reaction. Multiple "xerox" copies of the single cell's genome provides a generous amount of material (50 µL) for multiple diagnostic tests. The genes for congenital adrenal hyperplasia (21 hydroxylase) and Sex Related Y (SRY) are analyzed in a southern blot and a specific PCR reaction using an aliquot of the original 50 µL PEP product.

enzymes used for this purpose is unique in that it recognizes a specific base sequence, *i.e.* GAG, or ATAG, *etc.*, where it cuts the DNA. Some restriction enzymes consistently cut every six base pairs, others every eight, *etc.*, to generate different size fragments of DNA. Few diagnostic problems are solved by simply testing for the presence or absence of hybridization. The main application of probes is to detect changes in the pattern of fragments produced by restriction enzyme digestion (restriction analysis). If the DNA being analyzed has been changed or "mutated" so that there is a gain or loss of a restriction enzyme cut site, then the size of the fragments generated by enzymatic digestion will be altered. These changes in fragment size are diagnostic of a change in the nucleotide sequence of the gene or its flanking regions. Restriction enzymes are named after the bacterium from which they were isolated. The restriction enzyme *Hae*III is named after the bacterium *Hemophilus aedes egyptei*.

After the DNA has been cut or restricted into millions of fragments, the pieces (1–30 kilobase) are separated on the basis of molecular weight. This is achieved by running the pieces of DNA on an agarose gel through an electrical field. The smaller, low molecular weight fragments migrate quickly down the gel, whereas the larger, less mobile fragments remain at the top of the gel. Fragments of 500–30,000 base pairs (500 bp–30 kbp) are readily resolved, and the technique of pulse field or field inversion gel electrophoresis can extend the upper limits of resolution to several megabase pairs or fragments greater than 50 kbp. Electrophoresis results in a smear on the agarose gel of many fragments, whose position on the gel is a measure of their size. In order to test for hybridization, the smear of linearized fragments are next transferred to a nitrocellulose membrane or nylon filter without disturbing their pattern. This is the purpose of the Southern blot, named after its inventor, E.M. Southern.[7] After denaturing with alkali, the fragments are drawn from the gel and deposited on the filter by capillary action. A similar technique can be used to transfer RNA (Northern blotting), or proteins (Western blotting), from an electrophoretic gel onto a nitrocellulose filter.[8] At this juncture it is usually not possible to visualize any of the fragments as discrete bands. In order to detect or capture the fragment or gene of interest, one must have a piece of DNA corresponding to or complementary to the gene of interest. This piece of DNA or probe is a small fragment of single stranded (ss)DNA which usually represents all or part of a single gene sequence. The nucleic acid sequence is unique for that gene and that organism. Since ssDNA will recombine only with a nucleic acid strand whose sequence is homologous, a probe identifies a specific gene because it binds to a segment of DNA in the organisms or on the gel that is complementary to the gene of interest. Normally double-stranded in form, specimen DNA can be denatured to form single strands. Specially designed single-stranded probes can then seek out and bind to complementary sequences on the specimen DNA (Fig. 3). Hybridization formats are not limited to DNA-DNA. It can also occur between RNA and DNA strands that contain complementary sequences. In general, the nucleotide homology, temperature, and salt concentrations form the conditions of stringency for hybridization. More stringent conditions permit hybridization between only highly homologous sequences. A single base mismatch in a stretch of 20 base pairs may be sufficient to prevent hybridization under the most stringent conditions. Another factor influencing duplex stability is the sugar moiety in the backbone of the nucleic acid. Thus RNA-RNA duplexes are more stable than RNA-DNA ones, which are more stable than DNA-DNA. This property may be exploited in hybridization experiments in which very strong binding is desired and in which high stringency can be used.

A nucleic acid probe is a reagent that is typically labeled to permit detection of whether it has hybridized to target nucleic acids in a sample undergoing analysis.