

The Language of Recombinant DNA Technology

Complementary DNA (cDNA): a synthetic DNA made by a special DNA polymerase enzyme known as reverse transcriptase, which uses messenger RNA (mRNA) as a template. Used to refer either to a single-stranded copy or to its double-stranded derivative. Usage: “a cDNA clone,” “a cDNA library,” or “to isolate a cDNA.”

Clone: a recombinant DNA molecule containing a gene or other DNA sequence of interest. Also, the act of generating such a molecule. Usage: “to isolate a clone” or “to clone a gene.”

Host: the organism used to isolate and propagate a recombinant DNA molecule. Usually a strain of the bacterium *Escherichia coli* or the yeast *Saccharomyces cerevisiae*. Usage: “What host did they clone in?”

Hybridization: the act of two complementary single-stranded nucleic acid molecules forming bonds according to base-pairing rules (A with T or U, G with C) and becoming a double-stranded molecule. Usage: “The probe hybridized to a gene.”

Insert: a fragment of foreign DNA cloned into a particular vector. Usage: “They purified the insert.”

Library: a collection of recombinant clones from a source known to contain the gene, cDNA, or other DNA sequences of interest. In principle, a library may contain all the DNA or cDNA sequences represented in the original cell, tissue, or chromosome. Usage: “a muscle cDNA library” or “a human genomic library.”

Ligation: the act of forming phosphodiester bonds to join two double-stranded DNA molecules with the enzyme DNA ligase. Usage: “The fragments were ligated together.”

Northern blot: a filter to which RNA has been transferred after gel electrophoresis to separate the RNA molecules by size, named for the compass point, as a pun on Southern blot (see later); also, the act of generating such a filter and hybridizing it to a specific probe. Usage: “to probe a Northern blot” or “they did a Northern.”

Oligonucleotide: a short strand of nucleic acid, ranging in length from a few base pairs to a few dozen base pairs, often synthesized chemically. Often referred to as an “oligo.”

Polymerase chain reaction (PCR): enzymatic amplification of a fragment of DNA located between a pair of primers. Usage: “I PCR’d the fragment” or “I isolated the fragment using PCR.”

Primers (for PCR): two oligonucleotides, one on each side of a target sequence, designed so that one of the primers is complementary to a segment of DNA on one strand and the other is complementary to a segment of DNA on the other strand of a double-stranded DNA molecule. A specific pair of primers serves to prime synthesis of DNA in a PCR reaction. Usage: “I designed primers for PCR.”

Probe: a cloned DNA or RNA molecule, labeled with radioactivity or another detectable tracer, used to identify its complementary sequences by molecular hybridization; also, the act of using such a molecule. Usage: “the β -globin probe” or “to probe a patient’s DNA.”

Restriction endonucleases (restriction enzymes): enzymes that recognize specific double-stranded DNA sequences and cleave the DNA at or near the recognition site. Usage: “a restriction enzyme digest” (or just “a restriction digest”) or “the restriction enzyme *EcoRI*.”

Southern blot: a filter to which DNA has been transferred, usually after restriction enzyme digestion and gel electrophoresis to separate DNA molecules by size (named after the developer of the technique, Ed Southern); also, the act of generating such a filter and hybridizing it to a specific probe. Usage: “to probe a Southern blot” or “they did a Southern.”

Vector: the DNA molecule into which the gene or other DNA fragment of interest is cloned; the resulting recombinant molecule is capable of replicating in a particular host. Examples include plasmids, bacteriophage lambda, cosmids, and yeast or bacterial artificial chromosomes. Usage: “a cloning vector” or “the cosmid vector.”

Western blot: a filter to which protein molecules have been transferred after gel electrophoresis to separate the protein molecules by size (named, tongue in cheek, for a direction on a compass other than Southern or Northern); also, the act of generating such a filter and exposing it to a specific antibody. Usage: “to probe a Western blot” or “they did a Western.”

specific six base-pair sequence 5'-GAATTC-3' wherever it occurs in a double-stranded DNA molecule. The enzyme cleaves the DNA at that site by introducing a nick on each strand between the G and the

adjacent A. Cleavage generates two fragments, each with a four-base, single-stranded overhang at the end (Fig. 4-2). Such “sticky” ends are useful for subsequent joining reactions in the construction of recom-