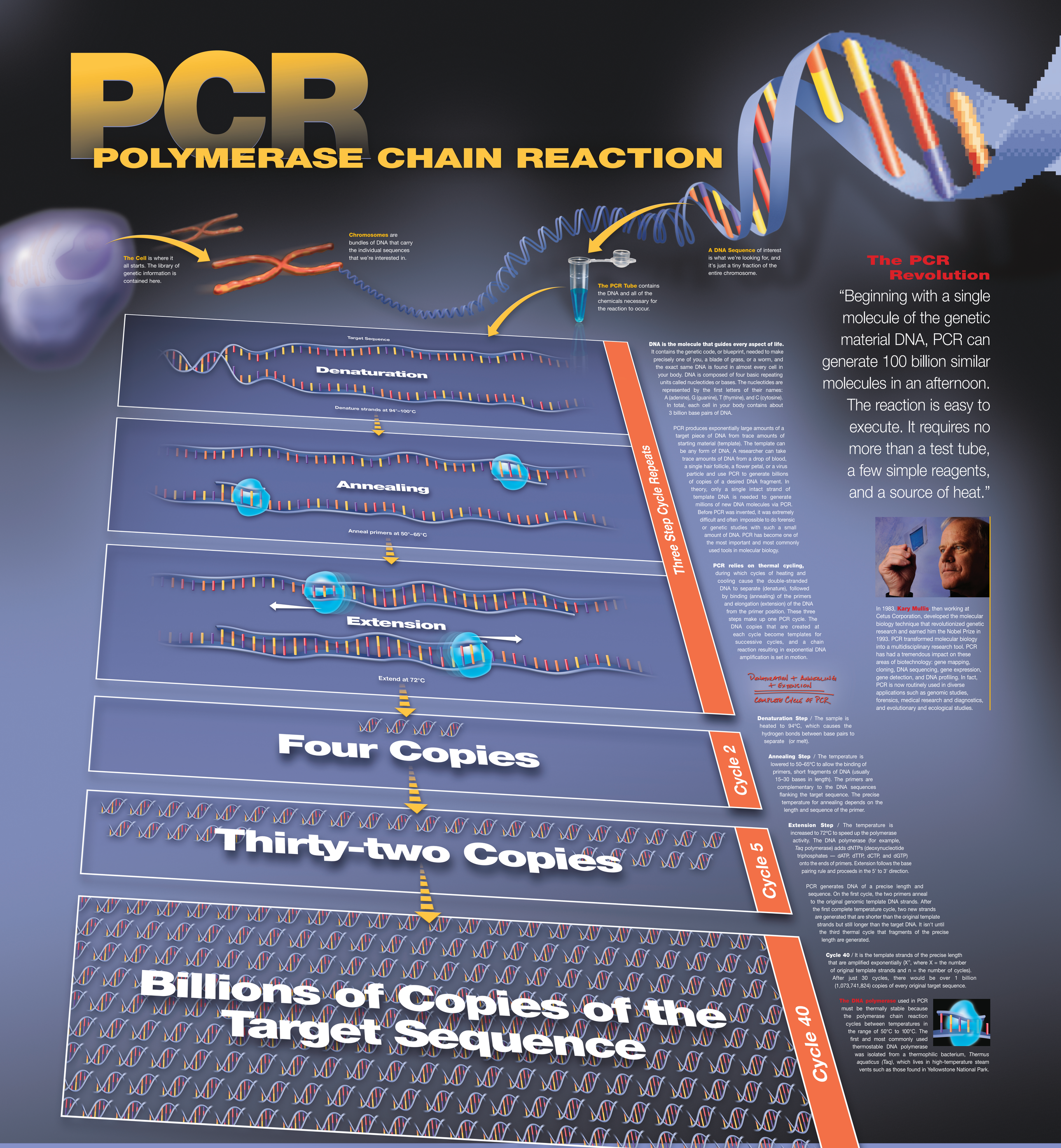
PCR
POLYMERASE CHAIN REACTION



DNA is the molecule that guides every aspect of life. It contains the genetic code, or blueprint, needed to make precisely one of you, a blade of grass, or a worm, and the exact same DNA is found in almost every cell in your body. DNA is composed of four basic repeating units called nucleotides or bases. The nucleotides are represented by the first letters of their names: A (adenine), G (guanine), T (thymine), and C (cytosine). In total, each cell in your body contains about 3 billion base pairs of DNA.

PCR produces exponentially large amounts of a target piece of DNA from trace amounts of starting material (template). The template can be any form of DNA. A researcher can take trace amounts of DNA from a drop of blood, a single hair follicle, a flower petal, or a virus particle and use PCR to generate billions of copies of a desired DNA fragment. In theory, only a single intact strand of template DNA is needed to generate millions of new DNA molecules via PCR. Before PCR was invented, it was extremely difficult and often impossible to do forensic or genetic studies with such a small amount of DNA. PCR has become one of the most important and most commonly used tools in molecular biology.

PCR relies on thermal cycling, during which cycles of heating and cooling cause the double-stranded DNA to separate (denature), followed by binding (annealing) of the primers and elongation (extension) of the DNA from the primer position. These three steps make up one PCR cycle. The DNA copies that are created at each cycle become templates for successive cycles, and a chain reaction resulting in exponential DNA amplification is set in motion.

Denaturation + Annealing + Extension
Complete Cycle of PCR

Denaturation Step / The sample is heated to 94°C, which causes the hydrogen bonds between base pairs to separate (or melt).

Annealing Step / The temperature is lowered to 50–65°C to allow the binding of primers, short fragments of DNA (usually 15–30 bases in length). The primers are complementary to the DNA sequences flanking the target sequence. The precise temperature for annealing depends on the length and sequence of the primer.

Extension Step / The temperature is increased to 72°C to speed up the polymerase activity. The DNA polymerase (for example, *Taq* polymerase) adds dNTPs (deoxynucleotide triphosphates — dATP, dTTP, dCTP, and dGTP) onto the ends of primers. Extension follows the base pairing rule and proceeds in the 5' to 3' direction.

PCR generates DNA of a precise length and sequence. On the first cycle, the two primers anneal to the original genomic template DNA strands. After the first complete temperature cycle, two new strands are generated that are shorter than the original template strands but still longer than the target DNA. It isn't until the third thermal cycle that fragments of the precise length are generated.

Cycle 40 / It is the template strands of the precise length that are amplified exponentially (X^n, where X = the number of original template strands and n = the number of cycles). After just 30 cycles, there would be over 1 billion (1,073,741,824) copies of every original target sequence.

The DNA polymerase used in PCR must be thermally stable because the polymerase chain reaction cycles between temperatures in the range of 50°C to 100°C. The first and most commonly used thermostable DNA polymerase was isolated from a thermophilic bacterium, *Thermus aquaticus (Taq)*, which lives in high-temperature steam vents such as those found in Yellowstone National Park.

The PCR Revolution

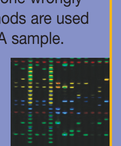
"Beginning with a single molecule of the genetic material DNA, PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat."

In 1983, **Kary Mullis**, then working at Cetus Corporation, developed the molecular biology technique that revolutionized genetic research and earned him the Nobel Prize in 1993. PCR transformed molecular biology into a multidisciplinary research tool. PCR has had a tremendous impact on these areas of biotechnology: gene mapping, cloning, DNA sequencing, gene expression, gene detection, and DNA profiling. In fact, PCR is now routinely used in diverse applications such as genomic studies, forensics, medical research and diagnostics, and evolutionary and ecological studies.

Applications

Genomics / The Human Genome Project (HGP) was one of the greatest scientific endeavors in history. Completed in 2003, it took a coordinated international research effort to sequence and map the complete human genome. One surprising outcome of the HGP was just how few genes are encoded in the human genome, and how much of the genome is made up of noncoding sequences. Knowing the sequence is just the beginning.

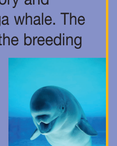
Forensics / Forensic sciences describe the boundary between science and the law. Science can as easily convict someone of a crime as free someone wrongly convicted. Today, molecular genetic methods are used to determine the exact genotype of a DNA sample. Using PCR, it is possible to analyze DNA from the tiniest of biological samples and to distinguish any two people on the planet (with the exception of identical twins), living or dead.



Medicine / With increased understanding of molecular genetics, it has become clear that many diseases have a genetic component. Certain genes are linked to diseases such as sickle cell anemia or breast cancer (BRCA1 gene). In other cases, environmental damage (UV light, cigarette smoke) can cause mutations in DNA that lead to cancer. In the future, our unique genetic makeup will determine which treatments will be most effective in treating our diseases.

Evolution / Why is there a duck-billed platypus (*Ornithorhynchus anatinus*)? This unusual animal — which both lays eggs and has mammary glands — appears to be an evolutionary bridge between mammals and reptiles. Genomic studies provide molecular evidence of the genetic divergence between mammals and their reptilian relatives. The duck-billed platypus data have been used to deepen our understanding of developmental biology, comparative physiology, and neurobiology.

Ecology / Research groups like NOAA's National Marine Fisheries Service use variation in molecular genetic markers to understand the migratory and dispersal patterns of species like the beluga whale. The genetic data provide tools to understand the breeding behavior and social organization of the species. By combining genetic data with traditional ecological information, NOAA researchers are better able to design species management plans.



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PCR — Polymerase Chain Reaction

Bio-Rad Explorer Classroom Kits, Curricula, and Supplies.

Bio-Rad offers a range of reliable, high-quality classroom kits, reagents, and curriculum supplies to introduce students to the polymerase chain reaction (PCR). Students use these kits to amplify and analyze DNA from agricultural, human, forensic, and fish samples.

Crime Scene Investigator PCR Basics Kit[1] (1662600EDU or **1662650EDU)** — an introductory PCR laboratory that is performed without the need for complex genomic DNA extraction steps but still allows students to simulate DNA profiling as it is commonly used in forensics, diagnostics, and archaeological procedures. (Versions of the Crime Scene Investigator and GMO Investigator Kits are also available for real-time PCR. Visit explorer.bio-rad.com for more information.)

PV92 PCR Informatics Kit (1662100EDU) — students extract DNA from their hair follicles or cheek cells and then use PCR amplification and electrophoresis to fingerprint their own DNA at a specific genetic locus. Using their results, students test the Hardy-Weinberg equilibrium theory within their classroom population, then go online to compare their classroom results to genetic data of populations worldwide

GMO Investigator Kit[1] (1662500EDU or 1662550EDU) — students gather sample food items from the grocery store, extract DNA from the samples, amplify the DNA using PCR, and use agarose gel electrophoresis to identify the presence or absence of amplified GMO sequences

GAPDH PCR module (1665010EDU) — a component of the Cloning and Sequencing Explorer Series, this module is used to amplify a portion of the plant GAPC gene. Students amplify genomic and control plasmid DNA from Arabidopsis and may also amplify DNA from other plants. Students can explore how using degenerate primer sequences and multiple rounds of PCR can overcome the problems of the degradation of genetic code

Fish DNA Barcoding Kit (1665100EDU) and **DNA Barcoding Sequencing Module (1665115EDU)** — students collect fish samples, extract DNA, amplify the cytochrome c oxidase I gene, analyze the product using gel electrophoresis, and then use sequencing and bioinformatics to determine the fish species and contribute to global initiatives to barcode life

[1] *Versions of the Crime Scene Investigator and GMO Investigator Kits are also available for real-time PCR. Visit* ***explorer.bio-rad.com*** *for more information*

Science Framework **Kit Selection Guide**

	Bio-Rad Explorer Kit (Catalog #)
ThiNQ!	**Photosynthesis and Cellular Respiration Kit for General Biology** (12005534EDU)
	Photosynthesis and Cellular Respiration Kit for AP Biology (17001238EDU)
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	Biofuel Enzyme Reactions Kit for AP Biology (17001235EDU)
	Giant Panda Problem Kit for AP Biology (17002878EDU)
Introductory	**Engineering Solutions for Global Health Kit** (17005278EDU)
	Genes in a Bottle Kit (1662300EDU)
	Got Protein? Kit (1662900EDU)
	IDEA Kit — Inquiry Dye Electrophoresis Activity (1665075EDU)
	Microbes and Health Kit (1665030EDU)
	pGLO Bacterial Transformation Kit (1660003EDU)
	Size Exclusion Chromatography Kit (1660008EDU)
	ELISA Immuno Explorer Kit (1662400EDU)
	STEM Electrophoresis Classroom Kit (1665090EDU)
Intermediate	**Analysis of Precut Lambda DNA Kit** (1660001EDU)
	Restriction Digestion and Analysis of Lambda DNA Kit (1660002EDU)
	Forensic DNA Fingerprinting Kit (1660007EDU)
	***C. elegans* Behavior Kit** (1665120EDU)
	Science of Opioid Dependence Kit (17005316EDU, 17005297EDU, or 17005313EDU)
	Biofuel Enzyme Kit (1665035EDU)
	pGLO Kit SDS-PAGE Extension (1660013EDU)
	Green Fluorescent Protein Chromatography Kit (1660005EDU)
	Secrets of the Rainforest Kit (1660006EDU)
	Comparative Proteomics Kit I: Protein Profiler Module (1662700EDU)
Advanced	**Cloning and Sequencing Explorer Series** (1665000EDU)
	Crime Scene Investigator PCR Basics Kit (1662600EDU)
	Crime Scene Investigator PCR Basics Real-Time PCR Starter Kit (1662660EDU)
	Fish DNA Barcoding Kit and DNA Barcoding Sequencing Module (1665100EDU and 1665115EDU)
	GMO Investigator Kit (1662500EDU)
	GMO Investigator Real-Time PCR Starter Kit (1662560EDU)
	PV92 PCR Informatics Kit (1662100EDU)
	Comparative Proteomics Kit II: Western Blot Module (1662800EDU)
	Protein Expression and Purification Series (1665040EDU, 1665045EDU, or 1665050EDU)
	Rapid Blotting — V3 Western Workflow Starter Kit (1662800EDU)

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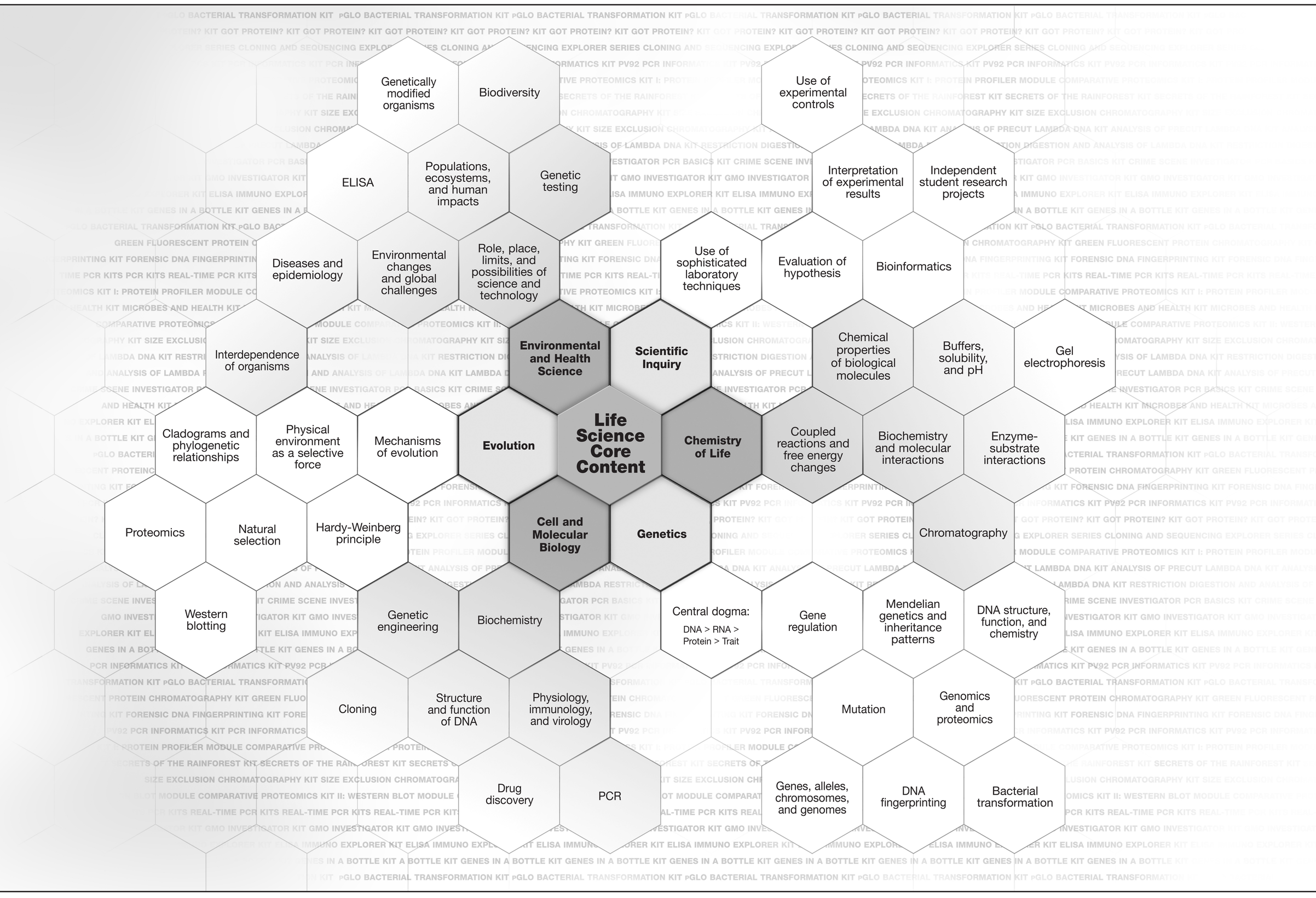

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