**CS123A Midterm #2 Study Guide**

By: Zayd Hammoudeh

**The Big Jaw**

|  |  |  |
| --- | --- | --- |
| **Big Jaw** – Constraint that inhibited brain growth | **Powerful masticatory muscles:** Found in most primates including chimpanzees and gorillas  **Human Masticatory Muscles** – Much smaller compared to other animals in the Homo genus. | **Myosin Heavy Chain (MYH):** Gene expressed in masticatory muscles. **Inactivated in humans** by a frameshift mutation after the lineage of humans and chimpanzees diverged.  Mutation **removed a barrier for the remodelling of the hominid cranium** which consequently **allowed for an increase in the size of the brain**. |

**Viruses**

|  |  |  |
| --- | --- | --- |
| **Virus** – Small living particles that can infect cells and change how the cells function. The effect on the cell’s function depends on the type of virus and the cells that are infected.  **Surrounded by protein case.** | **Pathogen:** A disease product. It can include both infectious organisms (bacteria, fungi, etc.) as well as viruses. | **Virulence:** Ability of an infectious agent (i.e. pathogen) to cause a disease.  Many viruses are virulent sometimes and asymptomatic at other times. |

|  |  |
| --- | --- |
| **Immunodeficiency** – The result when the immune system is unable to protect the host from disease causing agents or from malignant cells. | **Acquired Immunodeficiency:** Loss of immune function because the genetic or development deficiency was not acquired at birth. It results from exposure to various agents. |

|  |  |  |
| --- | --- | --- |
| **Virus** – A single stranded RNA virus that employs a **double stranded DNA** (**dsDNA**) intermediate for replication. | **Reverse Transcriptase:** Turns viral RNA into DNA. It turns the RNA strand into DNA. It then uses the DNA to make it complementary strand. | **cDNA** – Complementary DNA made from mRNA by reverse transcriptase. |

|  |  |
| --- | --- |
| **Capsid** – Surrounds mRNA in virus particle. | **Viral DNA** is integrated into the DNA of the host cell. |

**HIV**

|  |  |  |
| --- | --- | --- |
| **HIV** – Human Immunodeficiency Virus  **Type of retrovirus.**  **Inherited from:**   * **Chimpanzees** * **Mangabyes**   Transmitted through **bodily fluids** (e.g. blood, semen) when the virus of an infected individual contacts the **mucous membrane** or **enters the blood stream** of an uninfected individual. | **Cells Affected by HIV:**   * **Macrophages** - Large immune cells that devours invading pathogens and other intruders. Stimulates other immune cells by presenting them small pieces of the invaders. * **CD4+ T Cells (aka T-Helper Cells)** – White blood cells that orchestrate the immune response. They signal other cells to perform their special functions. | **Lentivirus** – “**Slow viruses**” where the period between initial infection and the onset of serious symptoms is long.  **Other Lentiviruses:**   * **FIV** – Feline Immunodeficiency Virus * **SIV** – Simian Immunodeficiency Virus (Infects monkeys and nonhuman primtates) |

|  |  |  |
| --- | --- | --- |
| **Body’s Immune Response to HIV**  Destroys the **virions** floating in the bloodstream before they can infect new cells.  **Destroys the infected CD4 helper T-cells** depleting the body’s ability to fight disease. This causes an immune system collapse. This leads to **AIDS** (**Acquired immunodeficiency Syndrome**). | **Three Types of Proteins Involved in Viral (Virion) Replication**   * **GAG –** Encodes for **core proteins** and structural virion components. * **POL** – Encodes for **reverse transcriptase, integrase**, and **protease**. * **Env** – Encodes for the **structural protein components** that surrounds the virus. Needed for the virus to leave the cell. | **Miscellaneous HIV Notes**  Many subgroups of HIV-1 exist.  Within a single subtype and in a single infected person, the virus changes constantly.  Transmission from chimp to humans happened multiple times in the past. |

**Sanger Sequencing**

|  |  |  |
| --- | --- | --- |
| Developed by Frederick Sanger in **1977**. Most widely used technology for ~25 years.  Replaced by “Next Generation Sequencing” techniques.  Still widely used for **smaller scale projects** and for **long contiguous DNA sequences** (>500 nucleotides). | **Dideoxynucleotides** – Nucleotides where the OH molecule on the 3’ carbon of the sugar is modified to simply an –H making a subsequent phosphodiester linkage impossible.  These are floating in the gel and sometimes DNA polymerase selects a normal nucleotide and other times the **dideoxy analog** which terminate the sequence. **This sugar can flouresce**. | DNA polymerase makes a complement of a partial sequence within a DNA molecule. **Synthesis is primed from a chemically synthesized fragment** (i.e. **primer**) that is complementary to a part of the DNA sequence known from other studies.  DNA polymerase **builds strand from 5’ to 3’**. |

**Pyrosequencing**

|  |  |  |
| --- | --- | --- |
| Developed in **1996**.  Based off the detecting of released pyrophosphate during DNA synthesis. This detection is through the **detection of light**.  Sequences a single strand of DNA by synthesizing the strand’s complement. | **Benefits of Pyrosequencing:**   * “**Sequencing by synthesis**” * Accurate * Simple and robust * No labels or gels * Real time results.   **Nucleotides are dispensed sequentially** and then removed from the reaction (this is done by **apyrase**). Light is only produced when the solution complements the first unpaired base of the template strand.  A mini strand with a **magnetic bead** for DNA polymerase serves as a **primer**. | **Example Pryosequencing Instruments**  **PSQ96**   * 500 samples per hour * 4500 samples per day. * Includes CCD camera.   **PSQHS96A**   * 10,000 samples per day * 30,000 samples per day with triplex analysis. |

|  |  |  |
| --- | --- | --- |
| Reading a **Pyrogram**    Bases released sequentially. Depending on intensity of light you can determine the number of sequential bases. | **Single Nucleotide Polymorphism**   * Occurs every 500 to 1000 bases in DNA. * Most common cause of inter-individual variation.   **HPV – Human Papillomavirus**   * Sexually transmitted infection (STI) * Usually does not cause health problems but can cause cancer of the vulva, vagina, penis, and anus as well as in the back of throat. * Different primers used to detect the specific strain of HPV infection. | **Wild Type** – Original, non-mutated version of a gene. For bacteria, it would be the original non-drug resistant version.  Pyrosequencing begins with a primer that binds to the DNA sequence. Primer has a “**general primer site**.” |

**Primers**

|  |  |
| --- | --- |
| **Primer Design:** Required step before beginning pyrosequencing. This includes running PCR (polymerase chain reaction).  **General Primer** – Will anneal with all alleles.  Have a **magnetic bead** at **5’ end**. | **Polymerase Chain Reaction**  Used to amplify a specific DNA sequence. Exponentially increases number of copies of a DNA sequence.  **Step #1: Denaturing** – Heating the DNA sequence to render it single stranded. Example time: 1 minute at 94C.  **Step #2: Annealing** – Two primers bind the appropriate complementary strands.  **Step #3: Extension** – DNA polymerase extends the primers. |

**Primer Characteristics**

|  |  |  |  |
| --- | --- | --- | --- |
| * Lack of **secondary priming sites** (uniqueness) * **Absence of hairpin** formation (bends in single strand). Caused by intermolecular interaction within the primer. | * **Uniqueness:** There should be only one place the primer can bind in the template DNA. There should be no possible contaminant binding sites either (e.g. from other animals such as human, rat, mouse, etc. | **Length** – Related to uniqueness and melting/annealing temperatures. **The longer the primer, the more likely to be unique and the higher the melting/annealing temperatures.**  **Minimum Length:** 15 bases  **Ideal Length:** 17-28 bases | **Base Composition** – **Random base composition is best**. Best to avoid long A/T and G/C chains.  50-60% G+C content leads to the right annealing/melting temperatures. |

|  |  |  |  |
| --- | --- | --- | --- |
| **Melting Temperature**  Temperature at which half the DNA strands are single stranded and half are double stranded.  More G/C nucleotides in a strand means higher melting temperature since more hydrogen bonds.  Notation: | **Annealing Temperature**  Temperature at the primer anneals (bonds) to the DNA stand.  Calculated as: | **Internal Structure**  Primers can anneal to themselves or to other primers.  **Hairpin:** Primer bending back to **bind to itself**.  **Self Dimer:** Primer bonding to **another** **of the same primer**  **Dimer:** Primer binding to a **complementary strand primer**.  Stability at 5’ end of the primer is critical. | Primers **work in pairs**. Two types of primers:   * **Forward Primer** * **Reverse Primer**   **Annealing temperatures of the two strands must be compatible** (maximum 3 degrees) of each others. |

**Primer Design**

|  |  |  |
| --- | --- | --- |
| Generally done best by computers.  Example primer design tools:  **Primer3** (tool used in class from MIT), **BioTools**, **GCG**, **Oligo** | **Adjustable Features in Primer Design Tools**:   * **Primer Length** * **Melting Temperature** * **(G+C)%** | **Forward and Reverse Primer**  **Reverse** (**Left**) Primer  3'<-------GGAA---- 5'  Plus Strand ||||  5'-----ATCG--------=========------------CCTT---- 3'  |||| Target ||||  3'-----TAGC--------=========------------GGAA---- 5'  |||| Minus Strand  5'--ATCG----> 3'  **Forward** (**Right**) Primer |

|  |  |  |
| --- | --- | --- |
| **Multiplex PCR**  Multiple primer pairs are added together in PCR.  **This allows for the amplifying of multiple sites.**  **Challenges of this Approach:**   * Different melting temperatures. * Ensuring no **dimer** formation | Most primers are designed to **amplify a single product.**  **Universal Primer:** A single primer that can be used to amplify multiple products.  **Semi-universal Primer:** A single primer can be used to amplify a subset of template sequences from a large group of similar sequences. | **Guessmer**  In some cases, DNA sequences are unavailable or difficult to align. Example: **Back-translated a protein** which is degenerate so the nucleotide sequence cannot be known.  Procedural Differences in Primer Design:   * Length: Primer should be **longer than normal at about 30 bases** to offset decreased hybridization. * Set higher annealing temperature to **increase primer annealing stringency**. |