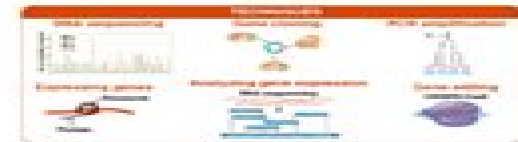


DNA Tools Part I

- There are many tools for manipulating DNA
 - DNA sequencing* - to directly sequence a site of variation
 - Vectors* - to carry specific DNAs and make identical copies
 - Restriction endonucleases* - to cut and join different DNAs
 - Gel electrophoresis* - to visualize the size of PCR products
 - PCR* - to amplify specific regions of a person's genome
 - RT PCR* - to characterize mRNAs expressed in a cell
 - Allele specific oligonucleotides* - to genotype SNPs

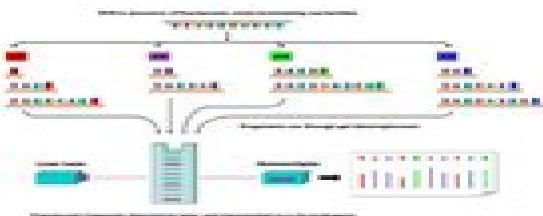


DNA sequencing

- A useful tool
 - A gene's complete nucleotide sequence can be determined using this process
 - The first automated procedure was based on a technique called dideoxy or chain termination sequencing
 - It was developed by Frederick Sanger
 - In the last 15 years, "next generation sequencing" techniques have been developed that are much faster



- Sanger sequencing is by chain termination
 - This is still used for sequencing single genes and shorter fragments
 - Put all components in a tube and send it out
 - If we have the sequence on the top, a ten base sequence, we're going to do the PCR in presence of fluorescent chain terminating nucleotides
 - Most of our nucleotides are dNTPs, but some are ddNTPs (dideoxynucleic acids) and they have two effects: fluorescent and stop PCR chain
 - Added at a low concentration, so sporadically added in and not extremely frequently
 - When this PCR occurs, when the first base is added it complements our A and in many of them it will get the normal dNTP, but in small percentage some get ddNTP and stop the chain and be one nucleotide long and it will fluoresce
 - We'll add more base pairs once again and most will be the normal base pair but some get the ddNTP A, and what happens is that every position gets a small amount of PCR product that is terminated → run the fragments through gel electrophoresis which separates them out from smallest to biggest and that can be run across a laser beam which will excite it and it can be read
 - In every position the different fluorescent tag will be read → in this case we got a red T, purple A, blue C, green G, etc
- Entire genomes can be sequenced
 - If we did this with Sanger sequencing it would take forever → there are other techniques that can be used



Manipulating Dna Study Guide

JS Bruner



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Table of Contents Manipulating Dna Study Guide

1. Understanding the eBook Manipulating Dna Study Guide
 - The Rise of Digital Reading Manipulating Dna Study Guide
 - Advantages of eBooks Over Traditional Books
2. Identifying Manipulating Dna Study Guide
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Manipulating Dna Study Guide
 - User-Friendly Interface
4. Exploring eBook Recommendations from Manipulating Dna Study Guide
 - Personalized Recommendations
 - Manipulating Dna Study Guide User Reviews and Ratings
 - Manipulating Dna Study Guide and Bestseller Lists
5. Accessing Manipulating Dna Study Guide Free and Paid eBooks
 - Manipulating Dna Study Guide Public Domain eBooks
 - Manipulating Dna Study Guide eBook Subscription Services
 - Manipulating Dna Study Guide Budget-Friendly Options
6. Navigating Manipulating Dna Study Guide eBook Formats

- ePub, PDF, MOBI, and More
- Manipulating Dna Study Guide Compatibility with Devices
- Manipulating Dna Study Guide Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Manipulating Dna Study Guide
 - Highlighting and Note-Taking Manipulating Dna Study Guide
 - Interactive Elements Manipulating Dna Study Guide
- 8. Staying Engaged with Manipulating Dna Study Guide
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Manipulating Dna Study Guide
- 9. Balancing eBooks and Physical Books Manipulating Dna Study Guide
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Manipulating Dna Study Guide
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Manipulating Dna Study Guide
 - Setting Reading Goals Manipulating Dna Study Guide
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Manipulating Dna Study Guide
 - Fact-Checking eBook Content of Manipulating Dna Study Guide
 - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
- 14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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