Sequence Analysis
Lab Protocol

You will need this handout of instructions

The sequence of your plasmid from the ABI

The Accession number for Lambda DNA J02459

The Accession number for pUC 18 is L09136

The Accession number for Bacillus licheniformis is NC_006270

Prepared by E.Lyons
Revised May 09, Oct 10 RGM (ABI change)
Go to Black Board for your Sequencing Results

– NOTE: EcoR I, Hind III and Xba I sites are in color – identified with the FIND tool of MSWord.

E01: pBD1 100 fm

TTGCCAGCCTGCATGGCCTGCCAGGTCGAACTCTAAGAGGATCCCCG
GGTACCAGCTCCAAATTCCGGCCTTTTCGGGGCAGGTTGCCCCCAATCC
CGTGGCCCATACAGATATACGTTTTCGCTCTGGCTACGTCCTGAATGCGAGG
GGCAGGCGTTTGAGCAAAATCAGCCTACCCAAAAACTTGGCTGTGCGTATGCT
CATCGGGGTGCTTCTCTGATATGCGAGGCTGGAACAATCAAGGGGA
CCCAGCAGCGCTGGTGCTGTATTTGTCTCAGGAACAGGATGGAATTAAG
TCGCACACCCACAGTGCCAGTGCATCCCAGTACGGGATTTGGGGACGAA
AACCACATCGCTGTGGATTACGGGACGAACCAACAGGCGATTTCCGA
TTACGGCACCATAACAGAGGAATACACACGCCGCTCATGCTCACAGTCT
GAGCGGTTCACACAGGGGGGCCGCCGCTGGTCATGCCCACACAAGTTGTT
TAAGGGATGAACAGTTCTGGGCTGAGTCAGTATGGAACAGCAACATT
CAGGAAGTTATCCACACAGTTAAAGGAACCAGCACACACAGGGTTATGGCTT
ATTTATCGAAAAGGGACAAGTCGAGGGGAGCAGGCCCCAGCTACATTGTTCCGG
TACAGGCGTGAGTGCCGCTGCACATGCTACGTGGTATGGGTGGCACC
ACATCGGTGTACGCTGCTCATCTCATCAGTGACGTCGACGA
ACCGCAAAACGTAATTGTCAATCCGCCTATTTCCAA

Select the sequence and copy.
Observe the information on Lambda while on Blackboard

- **LOCUS** LAMCG 48502 bp DNA linear PHG 18-OCT-2007
- **DEFINITION** Enterobacteria phage lambda, complete genome.
- **ACCESSION** J02459 M17233 M24325 V00636 X00906
- **VERSION** J02459.1 GI:215104
- **KEYWORDS** DNA-binding protein; circular; coat protein; complete genome; origin of replication; repressor; unidentified reading frame.
- **SOURCE** Enterobacteria phage lambda
- **ORGANISM** Enterobacteria phage lambda
  - Viruses; dsDNA viruses, no RNA stage; Caudovirales; Siphoviridae;
  - Lambda-like viruses.
- **REFERENCE** 2 (bases 1 to 12)
- **AUTHORS** Wu,R. and Taylor,E.
- **TITLE** Nucleotide sequence analysis of DNA. II. Complete nucleotide sequence of the cohesive ends of bacteriophage lambda DNA
- **JOURNAL** J. Mol. Biol. 57 (3), 491-511 (1971)
- **PUBMED** 4931680

ETC. – all publications that contributed sequence of Lambda are given.

This information is from a search for Lambda DNA on ncbi.
BLAST® (Basic Local Alignment Search Tool) is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query is protein or DNA. The BLAST programs have been designed for speed, with a minimal sacrifice of sensitivity to distant sequence relationships. The scores assigned in a BLAST search have a well-defined statistical interpretation, making real matches easier to distinguish from random background hits. BLAST uses a heuristic algorithm that seeks local as opposed to global alignments and is therefore able to detect relationships among sequences that share only isolated regions of similarity (Altschul et al. 1990). For a better understanding of BLAST, you can refer to the BLAST Course, which explains the basics of the BLAST algorithm. There is also a description of BLAST services located here. Also for details on BLAST and theory of similarity search, see the References section.

There are many different types of BLAST available from the main BLAST web page. Choosing the right one depends on the type of sequence you are searching with (long, short; nucleotide protein), and the desired database. In order to help guide you to the right service for your search please consult the "BLAST Program Selection Guide".

This guide provides information on what database and BLAST service (megaBLAST, translation BLAST etc) is ideal for your search. Plus there is also additional information describing how the tools work and why they are best for certain types of searches.

WWW BLAST:
The easiest way to use BLAST is through the Web. Users may simply point their browsers at the NCBI home page (http://www.ncbi.nlm.nih.gov) and link to the /BLAST pages for any number of different types of searches. A complete description of BLAST services is available at this location.
CEQ: Seq Lab: NCBI, Go to BLAST app

Click on BLAST
CEQ: Nucleotide Blast Search

Click nucleotide blast
If you want to see what Matches your sequence.
1. Paste your copied sequence into this box.

2. Make sure this box is NOT checked.

3. Click on BLAST.
CEQ: Sequence Lab; BLAST query of Nucleotide

NCBI Blast:Nucleotide Sequence (533 letters) - Windows Internet Explorer

BLAST
Home Recent Results Saved Strategies Help

Nucleotide Sequence (533 letters)

Query ID: gi|35463
Description: None
Molecule type: nucleic acid
Query Length: 533

Database Name: nr
Description: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences)
Program: BLASTN 2.2.26+

Distribution of 106 Blast Hits on the Query Sequence

Mouse over to see the detail, click to show alignments

Color key for alignment scores:
- <40
- 40-80
- 80-120
- 120-160
- >=160
For an alignment with sequence in the database, scroll down...

Scroll down to bottom of page.
Click on Align
CEQ: Sequence Lab: BLAST, Paste Plasmid Sequence

Paste your plasmid sequence into the Sequence 1 box.
This is E01: pBD1 100 fm

To align this with Sequence 2, *Lambda* DNA, type the Accession number for what you are trying to match into the box.
CEQ: Sequence Lab; BLAST, There is Alignment

There is alignment!
The position on the known DNA is given.

Percent identity is given.

The nucleotide numbers of alignment are given. In this case, alignment starts at nt 21254 and ends at nt 21816.
CEQ: Sequence Lab: Where is this on the Lambda map?

Ta-Da!

bp sizes:
- 4878
- 5643
- 7421
- 5804
- 3530
- 21226
- 26104
- 31747
- 39168
- 44972
- 37253

Lambda DNA Eco RI Digest
(total size = 48,502 bp)

This is the Lambda fragment that is inserted in this plasmid.
But there was no Hind III or Xba I sites in this sequence – why not?
There are 752 nucleotides in this sequence (easily counted in the WORD document using ‘Tools’ on the menu bar and selecting ‘Word Count’).

E01: pBD1 100 fm

- TTGCCAGCCTGCAATGGGCCCTGCGCCAGGTCTGAATCTCTAAGGAGGATCCGG
  GGTACCAGCTCAATTCCGGGCCTTTCGGGCTCCAGGTTCAGTTGGCTACGCTCTGATATGACGG
  GGCAACGCGTTTGACAAATCAGCCTACCCAAAAACCTTGCTGTCGCTATC
  CATCGGGGTGTGCTTTATGATATGCGAGGCTGGACACATCAAGGGGAA
  CCGCCAGCGGTCGTCGATATTGCTCGCACAGGAACAGGATGGAATTAAG
  TCGCACACCACAGTGCCAGTGCATCCCGGATACGGAATTTGGGGACGAA
  ACCCACATCGTCGTTTGATTACGGGACGAAAACACACAGGCGAGTTTCGA
  TTACGGCAACCAATCGACGAAATAAACACAGGGGCTCATGCTCAGACTCT
  GAGCGGTTCACACAGGGGCGCCGGGTGCTGTATCAGGCCCACAAACAGTTGTT
  TAAGGATAGAACAGTTTGCTGGGAGTCAAGTATGGAACAGCACAACATTA
  CAGGAAGTTTATCCACACAGTTAAGGAACCCAGCACACACAGGGGTATTGCTT
  ATTTATCGAAACCGGACAGTCAGGGCGGACACACAGTCTCATGCTGTCGG
  TACAGCCCGTGAGTGCCCGTGACATGCCATACAGTGGTATTGGTGCCAC
  ACATCGGTGTTCATCGGCCTCATTATCCTCAGGTAAGTACGTACTCGACGA
  ACCGCAAACAGTAAATGGTCAGTCAATCCGCTATTTCGAAA
Because the Hind III sites are more than 752 nt from each end of this fragment, they are not part of these sequence results.
Plasmid: pUC18 (p=plasmid, U of CA, ID#)

ATCC 37253

MCS

lacZ'

ampR

pUC18
2686 bp

lacZ' promoter

pMB1 ori
HindIII is a type II site-specific deoxyribonuclease restriction enzyme isolated from Haemophilus influenzae that cleaves the palindromic DNA sequence AAGCTT in the presence of the cofactor Mg2+ via hydrolysis.[1]

The cleavage of this sequence between the AA's results in 5' overhangs on the DNA called sticky ends:

5'-A | A G C T T-3'

3'-T T C G A | A-5'

Restriction endonucleases are used as defense mechanisms in prokaryotic organisms in the restriction modification system. Their primary function is to protect the host genome against invasion by foreign DNA, primarily bacteriophage DNA. There is also evidence that suggests the restriction enzymes may act alongside modification enzymes as selfish elements, or may be involved in genetic recombination and transposition.[2]

HindIII as well as other type II restriction endonucleases are very useful in modern science, particularly in DNA sequencing and mapping. Unlike type I restriction enzymes, type II restriction endonucleases perform very specific cleaving of DNA. Type I restriction enzymes recognize specific sequences, but cleave DNA randomly at sites other than their recognition site whereas type II restriction enzymes cleave only at their specific recognition site.[7] Since their discovery in the early 1970s, type II restriction enzymes have revolutionized the way scientists work with DNA, particularly in genetic engineering and molecular biology.

Major uses of type II restriction enzymes include gene analysis and cloning. They have proven to be ideal modeling systems for the study of protein-nucleic acid interactions, structure-function relationships, and the mechanism of evolution.[2] They make good assays for the study of genetic mutations by their ability to specifically cleave DNA to allow the removal or insertion of DNA. Through the use of restriction enzymes, scientists are able to modify, insert, or remove specific genes, a very powerful tool especially when it comes to modifying an organism's genome.
A plasmid is a DNA molecule that is separate from, and can replicate independently of, the chromosomal DNA.[1] They are double stranded and in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms (e.g., the 2-micrometre-ring in Saccharomyces cerevisiae).

Plasmid size varies from 1 to over 1,000 kilobase pairs (kbp).[2][3][4] The number of identical plasmids within a single cell can range anywhere from one to even thousands under some circumstances. Plasmids can be considered to be part of the mobilome, since they are often associated with conjugation, a mechanism of horizontal gene transfer. The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952.[5]

Plasmids are considered transferable genetic elements, or "replicons", capable of autonomous replication within a suitable host. Plasmids can be found in all three major domains, Archea, Bacteria and Eukarya.[1] Similar to viruses, plasmids are not considered a form of "life" as it is currently defined.[6] Unlike viruses, plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host. Plasmid host-to-host transfer requires direct, mechanical transfer by "conjugation" or changes in host gene expression allowing the intentional uptake of the genetic element by "transformation".[1] Microbial transformation with plasmid DNA is neither parasitic nor symbiotic in nature, since each implies the presence of an independent species living in a commensal or detrimental state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances. Plasmids also can provide bacteria with an ability to fix elemental nitrogen or to degrade calcitrant organic compounds which provide an advantage under conditions of nutrient deprivation.[1]