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◆ 연구관심분야 :

Human Genetics ; Genetic Susceptibility of Disease

Energy metabolism as a predisposing factor in Human Disease and Aging

Diagnosis of Human Disease using Bioinformatics and DNChip technology

◆ 학 력

1982. 3. ~ 1988. 2. 부산대학교 의과대학 의학과, 의학사

1983. 3. ~ 1990. 2. 부산대학교 대학원 의학과, 의학석사

1990. 3. ~ 1993. 2. 부산대학교 대학원 의학과, 의학박사

◆ 주요경력

2002. 1. ~ 현 재 중앙약사심의위원회 소분과위원회 위원

2001. 2. ~ 현 재 부산유전체연구센터장

2001. 7. ~ 현 재 한국나노바이오연구센터 제3부장

2000. 1. ~ 2000. 3. 일본 국립신경정신센터 국립신경과학연구소
COE researcher (Center Of Excellence)

1998. 9. ~ 현 재 부산대학교 의과대학 생화학교실 조교수

1996. 7. ~ 1998. 9. 국립마산결핵병원 임상연구소 의무사무관

1995. 3. ~ 1996. 4. 국립마산결핵병원 임상연구소 공중보건과의사

1993. 4. ~ 1995. 2. 국립과학수사연구소 남부분소 법의학과 공중보건과의사

1990. 11 ~ 1990. 12. 일본 국립암센터연구소 약리학부 방문연구원

◆ 학회 활동 / 수상 / Honors

2000. 5. 20 대한임상병리학회 제39차 춘계학술대회의 Roche 학술상

2001. 3. 대한남성과학회 학술상 (Pharmacia award)

1999. 10. 대한임상병리학회 제38차 춘계학술대회의 Roche 학술상

2001. 9. ~ 현 재 국립보건원 중앙유전체 센터 운영위원



Identification and Genome Analysis of A Pathogenic Microbe in Fish Farming

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Department of Biochemistry
College of Medicine
Pusan National University



- ☒ Introduction
 - Why flounder? Why *Lymphocystivirus* ?
- ☒ Whole Genome Analysis of *lymphocystivirus*
 - Isolation of virus from lymphocystis
 - Whole genome shotgun strategy
 - Assembly
 - Annotation
- ☒ What characteristics ?



수산동식물-1

- **원양어류 (목) ; 가자미, 금눈돔, 꼬리치, 농어, 달고기, 당멸치, 대구, 동발상어, 동갈치, 멍장어, 메기, 바다빙어, 뱀장어, 북어, 색줄멸, 셋비늘치, 수영상어, 송어, 신락상어, 썸뱅이, 아귀, 악상어, 앵통이, 연어, 은상어, 이악어, 전자리상어, 참치, 청어, 큰가시고기, 톱상어, 해와, 흥메치, 흥어, 흥상어**
- **연근해어류 ; 가오리, 가자미, 갯이상어, 금눈돔, 농어, 달고기, 대구, 동발상어, 동갈치, 메기, 뱀장어, 북어, 셋비늘치, 시락상어, 살고기, 아귀, 악상어, 양볼락, 연어, 은상어, 청어, 칠성장어, 큰가시고기, 학치**
- **내수면어류 ; 뱀장어, 북어, 색줄멸, 셋비늘치, 수영상어, 송어, 신락상어, 살고기, 썸뱅이, 아귀, 악상어, 앵통이, 양볼락, 연어, 은상어, 이악어, 전자리상어, 참치, 청어, 칠성장어, 큰가시고기, 톱상어, 학치, 해와, 흥메치, 흥어, 흥상어**
- **패류 ; 고이치, 고다치, 고이치, 기안, 나새, 두순, 무순, 병안, 뽕조개, 신복족, 심완, 연두군부, 원시복족, 중복족, 진다치, 털군부, 팔완**
- **해조류 ; 국수나물, 다시마, 돌가사리, 디크티오시폰, 디크티오타, 로오디메니아, 마디털, 무자반, 보라털, 불복크스, 비단풀, 산말, 스파셀라리아, 스포로크누스, 시포노클라두스, 에크토키아르푸스, 우룻가사리, 홀로드락스, 지누아리, 채찍말, 청각, 코르다리아, 크로우코크스, 클라도포라**

Why Flounder ? 넙치 광어, flat fish, flounder

양어류 (목) 가자미(과) 넙치(종) • 연근해어류 (목) 가자미(과) 넙치(종)



분류	어류
목	Pleuronctiformes
과	Paralichthyidae
영어명	Flatfish
일어명	Hirame
학명	<i>Paralichthys olivaceus</i>
명명자	Temmick et Schlegel

분류	어류 (연근해어류)
목(영어)	Pleuronectiformes
과(영어)	Paralichthyidae
단축명	넙치
영어명	Bastard, Flatfish
일어명	Hirame
학명	<i>Paralichthys olivaceus</i>
명명자	Temminck et Schlegel

가주넙치

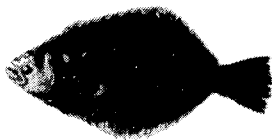
점넙치

별넙치

별넙치



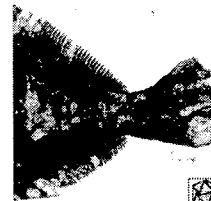
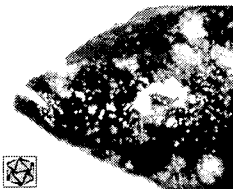
Why Flounder ? Why Lymphocystivirus ?



Lymphocystivirus



Lymphocystis (림포시스티스 바이러스병)



Lymphocystis?

림포시스티스 바이러스병

- 우리나라 양식 해산 어류에 증양을 유발하여 폐사 초래
치어 중요 입식 후 45일째부터 증양 형성이 관찰됨
섬유아세포와 근아세포 비대로 피부와 지느러미에 증양(양성) 유발
- cells can increase in volume by a factor of 10⁶
- 연중 지속적으로 발병
특히 저수온기(12월에서 4월까지)에 가장 많이 발생
- 육안으로 증양을 확인하기 전까지는 바이러스의 감염여부를 알 수
없어 조기진단이 힘들
- 조기진단법 개발 및 방역법 개발 연구가 활발하지만 LCDV 자체에
대한 생물학적 지식의 부족으로 근본적인 대책을 마련하지 못하고
있어 유전체 연구 등의 보다 근본적이고 집중적인 기초 연구가
절실히 요구됨

Lymphocystivirus ?

'Virus Taxonomy' - the 6th Report of the ICTV, 1995

Group I: dsDNA Viruses

Group II: The ssDNA Viruses

Group III: dsRNA Viruses

Group IV: (+)sense RNA Viruses

Group V: (-) sense RNA Viruses

Group VI: RNA Reverse Transcribing Viruses

Group VII: DNA Reverse Transcribing Viruses

Subviral Agents: Satellites, Viroids, and Agents of Spongiform Encephalopathies

Adenoviridae
Baculoviridae
Corticoviridae
Fuselloviridae
Herpesviridae
Iridoviridae
Lipothrixviridae
Myoviridae
Papovaviridae
Phycodnaviridae
Plasmaviridae
Podoviridae
Polydnaviridae
Poxviridae
Siphoviridae
Tectiviridae

Iridoviridae

Introduction

- a diverse array of large icosahedral viruses
- "rainbow like"
- isolated from both invertebrate and non-mammalian vertebrate hosts
- hosts is the aquatic or moist environment in which they are found



Morphology

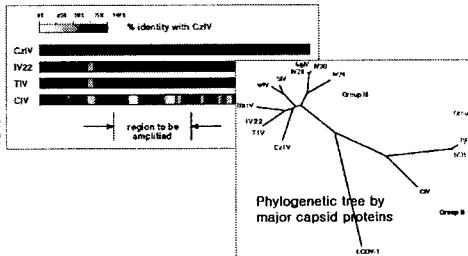


- large (120 to 300 nm in diameter) non-occluded viruses with icosahedral symmetry
- three concentric domains
- Fibrillar structures have also been observed protruding from capsid subunits of LCDV-1, MIV, and CIV, but not from FV3
- a single copy linear dsDNA genome that ranges in size from 150 to 280 kbp depending on viral species

taxonomy

Genus	Vernacular name	Host species	Type species
<i>Iridovirus</i>	Small iridescent insect virus	Invertebrates (mainly insects)	<i>Chilo</i> iridescent virus (IV6)
<i>Chloriridovirus</i>	Large iridescent insect viruses	Mosquitos	(IV3) Mosquito iridescent virus
<i>Lymphocystivirus</i>	Lymphocystis disease virus	Fish	Lymphocystivirus type 1 (LCDV-1)
<i>Ranavirus</i>	Frog virus	Amphibia	Frog Virus 3 (FV3)

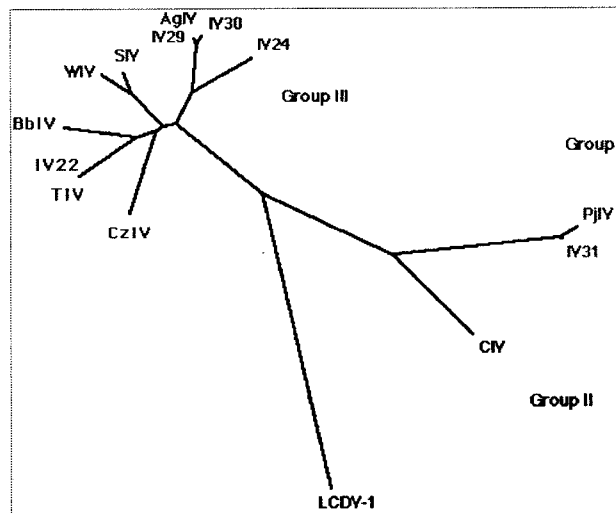
- ICTV list of recognised Iridoviridae members contains much redundant data
- The wide host range displayed because of their isolation from different hosts
- host of isolation also confuses taxonomy
- pitfalls in iridovirus taxonomy are only just beginning to be addressed using molecular techniques





Iridoviridae



Phylogenetic tree by major capsid proteins



Iridoviridae Taxonomy

Genus	Vernacular name	Host species	Type species
<i>Chilo virus</i>	 Small iridescent insect virus	Invertebrates (mainly insects)	<i>Chilo</i> iridescent virus (IV6)
<i>Iridovirus</i>	Large iridescent insect viruses	Mosquitos	Mosquito iridescent virus (IV3)
<i>Lymphocystivirus</i>	 Lymphocystis disease virus	Fish	Lymphocystivirus type 1 (LCDV-1)
<i>Ranavirus</i>	Frog virus	Amphibia	Frog Virus 3 (FV3)
<i>Goldfish virus 1-like viruses</i>		Vertebrates	goldfish virus 1

Current status of genome analysis

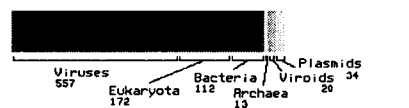
The whole genomes of over 900 organisms in Entrez Genomes.

The genomes represent both completely sequenced organisms and those for which sequencing is in progress

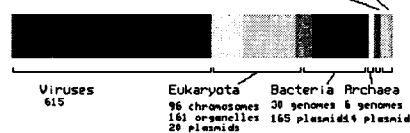
- All three main domains of life – bacteria, archaea, and eukaryota
- many viruses and organelles

Entrez Genomes statistics

Total species (908)



Total records (1161)



2001.10

Public Viral Genome Database

• Entrez Genome Database

819 genome list

A screenshot of the NCBI Entrez Genome Database search results for the keyword 'Virus'. The results list various viral genomes with columns for accession numbers, lengths, and dates. A callout box highlights '819 genome list'.

• EMBL Completed Viral Genomes

628 genome list

A screenshot of the EMBL Completed Viral Genomes database. It displays a table titled 'Completed genomes: VIRUSES' with columns for 'No.', 'Description', and 'EMBL/GenBank/CCDS Accession'. A callout box highlights '628 genome list'.

No.	Description	EMBL/GenBank/CCDS Accession
1	Adenovirus type 1	U00001.1
2	Adenovirus type 2	U00002.1
3	Adenovirus type 3	U00003.1
4	Adenovirus type 4	U00004.1
5	Adenovirus type 5	U00005.1
6	Adenovirus type 6	U00006.1
7	Adenovirus type 7	U00007.1
8	Adenovirus type 8	U00008.1
9	Adenovirus type 9	U00009.1
10	Adenovirus type 10	U00010.1
11	Adenovirus type 11	U00011.1
12	Adenovirus type 12	U00012.1
13	Adenovirus type 13	U00013.1
14	Adenovirus type 14	U00014.1
15	Adenovirus type 15	U00015.1
16	Adenovirus type 16	U00016.1
17	Adenovirus type 17	U00017.1
18	Adenovirus type 18	U00018.1
19	Adenovirus type 19	U00019.1
20	Adenovirus type 20	U00020.1
21	Adenovirus type 21	U00021.1
22	Adenovirus type 22	U00022.1
23	Adenovirus type 23	U00023.1
24	Adenovirus type 24	U00024.1
25	Adenovirus type 25	U00025.1
26	Adenovirus type 26	U00026.1
27	Adenovirus type 27	U00027.1
28	Adenovirus type 28	U00028.1
29	Adenovirus type 29	U00029.1
30	Adenovirus type 30	U00030.1

LCDV1

Virology 1993 Apr 30;126(2):456-79

Analysis of the genome of fish lymphocystis disease virus isolated directly from epidermal tumours of pleuronectes.

Daral G, Anders K, Koch HG, Dellus H, Gelderblom H, Samalencos C, Flugel RM.

vol 126, Iss 2, pp 456-479 (1993)
of refs 10, # of refs 10

The Complete DNA Sequence of Lymphocystis Disease Virus

CHRISTIAN A. TIDONA and GHOLANREZA DARAL

Institut für Medizinische Virologie, Universitätsklinikum, Im Neuenheimer Feld 324, D-69120 Heidelberg, F.R.G.

• Overview

• Protein View

A screenshot of the NCBI Overview view for Lymphocystis disease virus 1. It shows the complete DNA sequence in a text format with various annotations and features.

A screenshot of the NCBI Protein View for Lymphocystis disease virus 1. It displays the protein structure with a legend for coding regions, open reading frames, and overlapping regions.

LCDV 1

Lymphocystis disease virus 1, complete genome

Accession: **NC_001824**
 Total Bases Sequenced: 102653 bp
 Completed: Mar 16, 2001.

Legend:
 - CDS
 - 53988-54089...

ORF features:
 orf20 orf35 orf36 orf orf
 orf58 orf129 orf orf
 orf32 orf65 orf orf
 orf125 orf138 orf144

Legend:
 - Coding region on plus strand
 - Coding region on minus strand
 - Other regions

ORF Finder (Open Reading Frame Finder)
 Lymphocystis disease virus 1, complete genome - 30796..41061

Legend:
 - Coding region on plus strand
 - Coding region on minus strand
 - Other regions

Lymphocystis disease virus 1, complete genome - 30796..41061

Legend:
 - Coding region on plus strand
 - Coding region on minus strand
 - Other regions

ORF Finder (Open Reading Frame Finder)
 Lymphocystis disease virus 1, complete genome - 30796..40796

Legend:
 - Coding region on plus strand
 - Coding region on minus strand
 - Other regions

Why LCDV genome of Korean flounder should be reanalyzed?

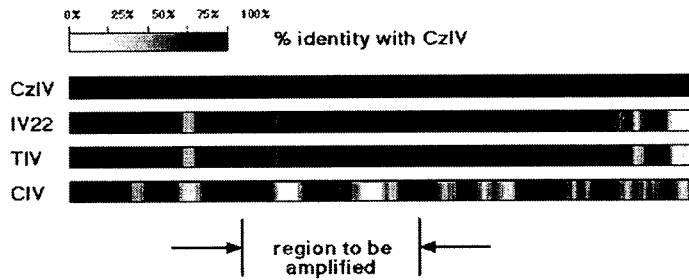
1. Regional variety and host variety

Countries of virus isolation

Virus	Genus	Host	Country of isolation
TIV (IV1)	<i>Iridovirus</i>	crane fly	England
SIV (IV2)	<i>Iridovirus</i>	scareb grub	Australia
CIV (IV6)	<i>Iridovirus</i>	rice stem borer	Japan
WIV (IV9)	<i>Iridovirus</i>	porina caterpillar	New Zealand
CzIV (IV16)	<i>Iridovirus</i>	grass grub	New Zealand
IV22	<i>Iridovirus</i>	black fly	Wales
BbIV (IV23)	<i>Iridovirus</i>	black beetle	South Africa
IV24 (AIV)	<i>Iridovirus</i>	honey bee	India
IV29	<i>Iridovirus</i>	meal worm	USA
IV30	<i>Iridovirus</i>	corn carworm	USA
IV31	<i>Iridovirus</i>	woodlice	USA
AgIV	<i>Iridovirus</i>	Velvetbean caterpillar	Argentina
PjIV	<i>Iridovirus</i>	Japanese beetle	Azores
LCDV-1	<i>Lymphocystivirus</i>	flounder	World wide

Why LCDV genome of Korean flounder should be reanalyzed?

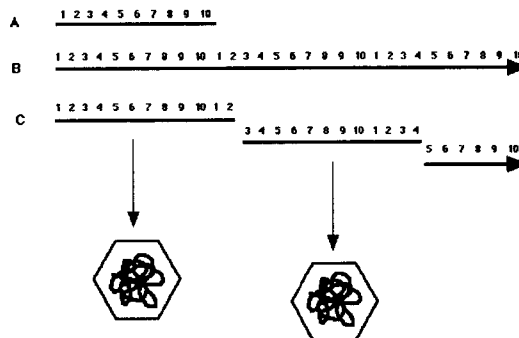
Genotyping is essential for early detection of outbreak




Homologies between invertebrate iridovirus major capsid genes, highlighting the region used for phylogenetic analysis. Bars represent the major capsid proteins from each virus with greyscales ranging from black for 100% homology to the CzIV protein to white for 0% homology.

Why LCDV genome of Korean flounder should be reanalyzed?

Unique genome structure within the eucaryotic viruses '**terminal redundancy**' and '**cyclic permutation**'



terminal redundancy ; a complete genome as well as duplicated copies of some genes
cyclic permutation ; The ends of each of these packaged DNAs differs from one virus particle to the next




Why LCDV genome of Korean flounder should be reanalyzed?

4. Little is known about the pathogenesis of iridoviruses

1. temperature dependent (poikilothermic hosts)
Why? How?
2. Some iridoviruses are lethal, some are not lethal.
FV3 and LCDV-1 appear to be non-lethal
What differences?
Really nonlethal?



- 
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Whole genome shotgun strategy
Assembly
Annotation
 3. What characteristics ?

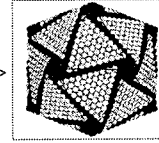
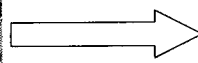


Isolation of virus from lymphocystis

- Sucrose density gradient centrifugation



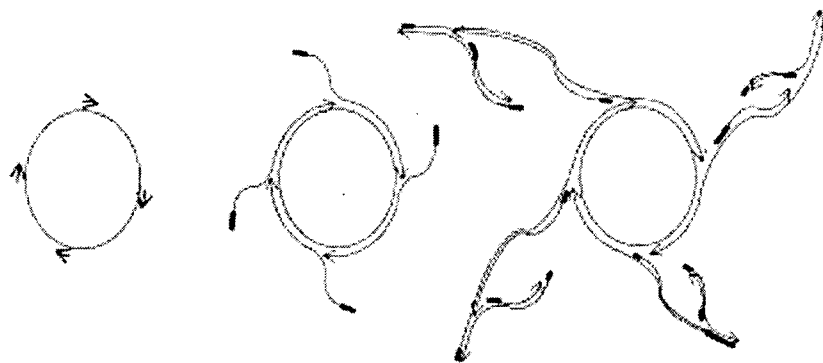
3g tissue



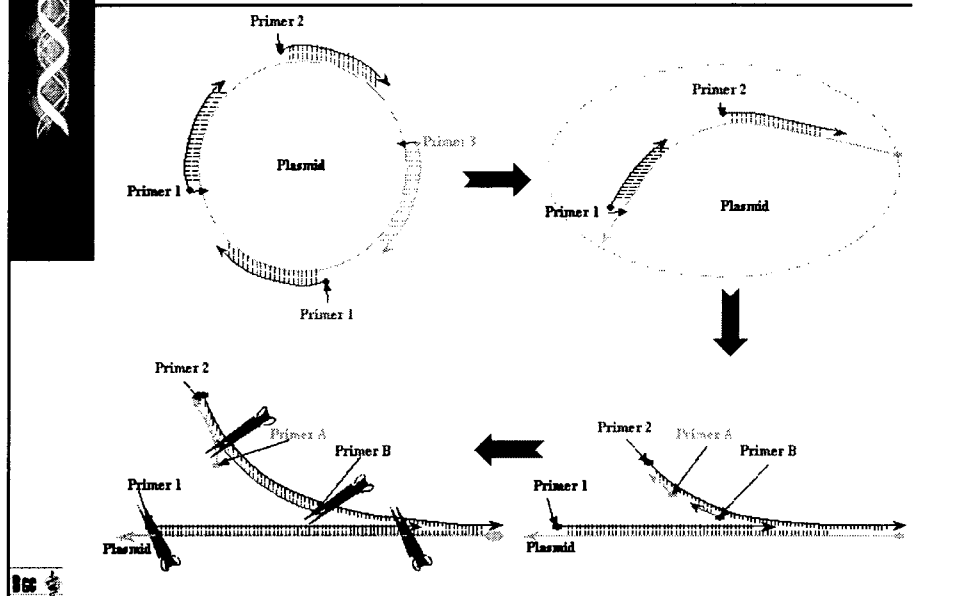
50 ug DNA

- Limited amount of starting material
The first obstacle for whole genome analysis
⇒ Overcome by amplification of whole genome
using DNA polymerase with strand displacement function

Scheme for multiply-primed rolling circle amplification using phi29 DNA polymerase

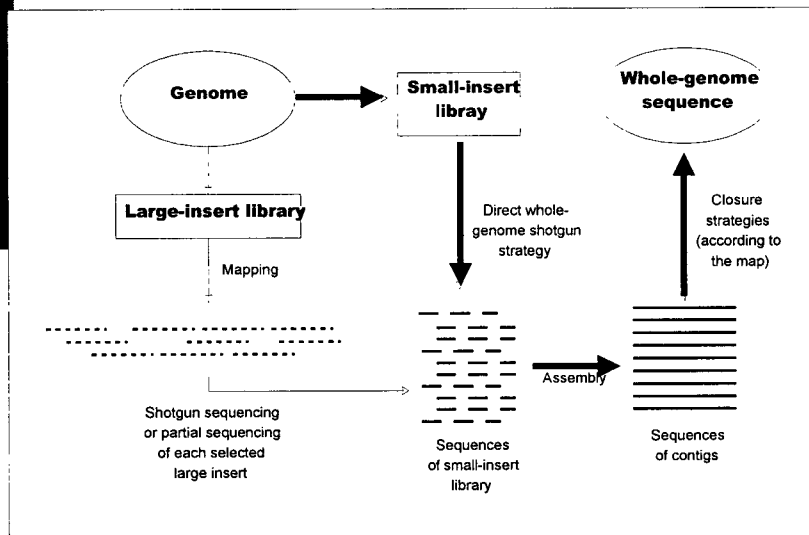


Amplification of whole viral genomic DNA Using phi29 DNA polymerase



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Strategy for genome analysis



Introduction

Why flounder? Why *Lymphocystivirus* ?

Whole Genome Analysis of *lymphocystivirus*

Isolation of virus from lymphocystis

Whole genome shotgun strategy

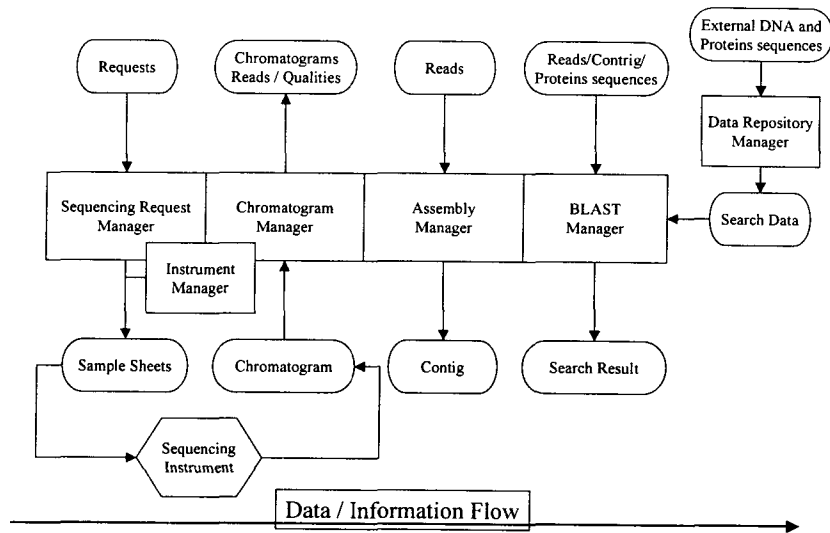
Assembly

Annotation

What characteristics ?

Installed assembly system

Finch-Suite™



BaseCalling -1

Using Phred

Select Good Candidate for Assembling

- Trimed length
- Quality Check
- Is Vector
- Short Insert
- Screen Pos



Reads Assembly -1

- Using Phrap
- Contig Quality Check
 - Consecsus Quality
 - Discrepancy Quality
 - Coverage Depth



Pilot Mapping

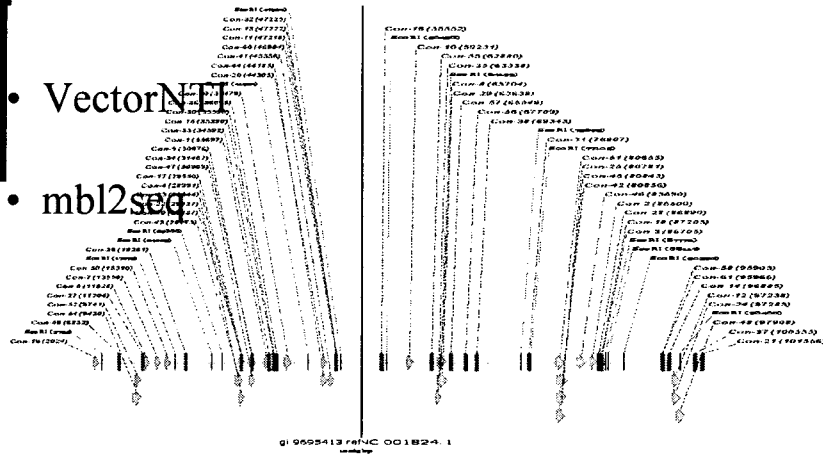
- For Mutiplex PCR
- Mapping to Reference Sequence
 - Pairwise Alingment
 - ClustalW
 - BL2SEQ



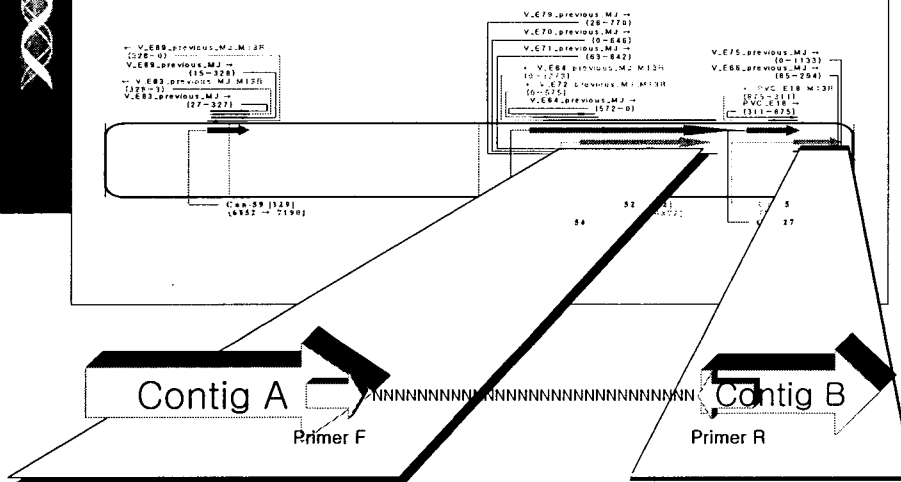
From U.S. Department of Energy Human Genome Program

Pilot Mapping Results

- VectorNEI
- mb12seq



Lymphocystis disease virus (LCDV) Contigs Position



Introduction
Why flounder? Why *Lymphocystivirus* ?

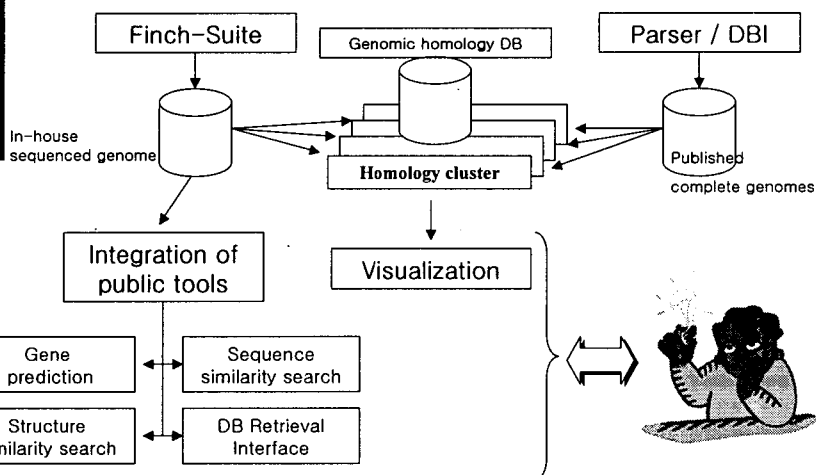
Whole Genome Analysis of *lymphocystivirus*
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Annotation

What characteristics ?

Annotation strategy-1

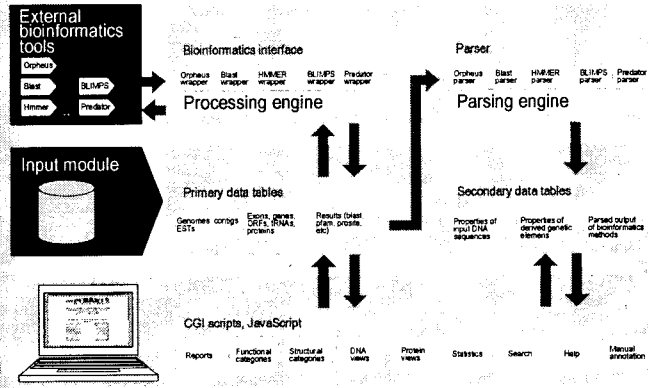
High-throughput Genome Analysis

Construction Local DB
of Complete Genomes



Annotation strategy-2

Pedant-Pro Sequence Analysis Suite



Biomax Informatics AG *Bioinformatics designed with you in mind.*

PEDANT Web interface

Dataset overview Catalogues/tables DNA/protein viewer Report page Navigation strip Online help

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- Full annotation
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Search

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- Pattern search
- Word search

Protein function

- Clustal membership
- Accession numbers
- PDB structures
- Pfam domains
- Gene Ontology
- Protein families
- Protein motifs
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- Clusters
- Clusters

Specialized searches

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Protein structure

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DNA VIEWER | **PROTEIN VIEWER**

• Report = PreSeq + DnaSeq + Ab + BLOC IS + PROSITE + PFAM + 3D + BCDP + AUTOFUNCAT + COG + BLASTP + cell + ASIMULATE

Title: g_L9945820.DNA polyme ase III, beta chain [Pseudomonas aeruginosa]

General properties

Length (aa)	817
Molecular weight (kDa)	99394.9
Accession number	124844271.pep
Contig name	Pseudomonas
Position	2826-3129
GC content, %	52.0

Protein function

Closest homologues: TREM5_NEW_TREMBL_A509440.2_gene: 'dnaN'; product: 'DNA polymerase III, beta chain' [Pseudomonas aeruginosa DSM 50218] section 1 of 329 of the complete genome. 82

COGs: COG0602 DNA polymerase III beta subunit 14-192

PFAM domain: PF00713 DNA polymerase III beta subunit 14-192

BLOCKS: BLO18223 Adenylate cyclases class I proteins

Automatically derived PIR superfamily: PIR_DJF53P DNA-directed DNA polymerase III beta chain 14-165

Automatically derived EC number: PIR_DJF53P 2.2.7.2 DNA-directed DNA polymerase III beta chain 14-165

Homology of LCDV-K1 with other iridoviridae

Accession	Start	End	Gene	Notes
146L*	71256-71056	67	none	none
147L	72773-71398	450	none	major capsid protein of chilo iridovirus (Q95815) [Stehweger et al., 1993]
148R*	73414-73396	61	none	none
149L	73486-72965	374	zinc finger (C1 type) signature	none

bp index

Comparison of NCBI annotation and PEDANT using the genome of type strain

Gene	NCBI Start	NCBI Stop	Gene	PEDANT Start	PEDANT Stop
ORF1	1253	622	ORF1	213	619
ORF2	1483	819	ORF2	483	827
ORF3	2141	1469	ORF3	2141	1469
ORF4	4271	2719	ORF4	2731	2720
ORF5	4325	3429	ORF5	4325	3425
ORF6	4973	3476	ORF6	4973	3485
ORF7	5192	10202	ORF7	5192	10202
ORF8	10634	10222	ORF8	10234	10719
ORF9	10740	1484	ORF9	10740	1481
ORF10	11483	11384	ORF10	11778	12215
ORF11	11778	12219	ORF11	12104	12654
ORF12	12219	12597	ORF12	12594	12694
ORF13	12597	17004	ORF13	12594	17001
ORF14	17004	17511	ORF14	17001	17511
ORF15	17511	18104	ORF15	17511	18104
ORF16	18104	20725	ORF16	18104	20725
ORF17	18104	20725	ORF17	18104	20725
ORF18	20725	22461	ORF18	20725	22461
ORF19	22461	23371	ORF19	22461	23371
ORF20	23371	23371	ORF20	23371	23371
ORF21	23371	23371	ORF21	23371	23371
ORF22	23371	23371	ORF22	23371	23371
ORF23	23371	23371	ORF23	23371	23371
ORF24	23371	23371	ORF24	23371	23371
ORF25	23371	23371	ORF25	23371	23371
ORF26	23371	23371	ORF26	23371	23371
ORF27	23371	23371	ORF27	23371	23371
ORF28	23371	23371	ORF28	23371	23371
ORF29	23371	23371	ORF29	23371	23371
ORF30	23371	23371	ORF30	23371	23371
ORF31	23371	23371	ORF31	23371	23371
ORF32	23371	23371	ORF32	23371	23371
ORF33	23371	23371	ORF33	23371	23371
ORF34	23371	23371	ORF34	23371	23371
ORF35	23371	23371	ORF35	23371	23371
ORF36	23371	23371	ORF36	23371	23371
ORF37	23371	23371	ORF37	23371	23371
ORF38	23371	23371	ORF38	23371	23371
ORF39	23371	23371	ORF39	23371	23371
ORF40	23371	23371	ORF40	23371	23371
ORF41	23371	23371	ORF41	23371	23371
ORF42	23371	23371	ORF42	23371	23371
ORF43	23371	23371	ORF43	23371	23371
ORF44	23371	23371	ORF44	23371	23371
ORF45	23371	23371	ORF45	23371	23371
ORF46	23371	23371	ORF46	23371	23371
ORF47	23371	23371	ORF47	23371	23371
ORF48	23371	23371	ORF48	23371	23371
ORF49	23371	23371	ORF49	23371	23371
ORF50	23371	23371	ORF50	23371	23371
ORF51	23371	23371	ORF51	23371	23371
ORF52	23371	23371	ORF52	23371	23371
ORF53	23371	23371	ORF53	23371	23371
ORF54	23371	23371	ORF54	23371	23371
ORF55	23371	23371	ORF55	23371	23371
ORF56	23371	23371	ORF56	23371	23371
ORF57	23371	23371	ORF57	23371	23371
ORF58	23371	23371	ORF58	23371	23371
ORF59	23371	23371	ORF59	23371	23371
ORF60	23371	23371	ORF60	23371	23371
ORF61	23371	23371	ORF61	23371	23371
ORF62	23371	23371	ORF62	23371	23371

What characteristics ?

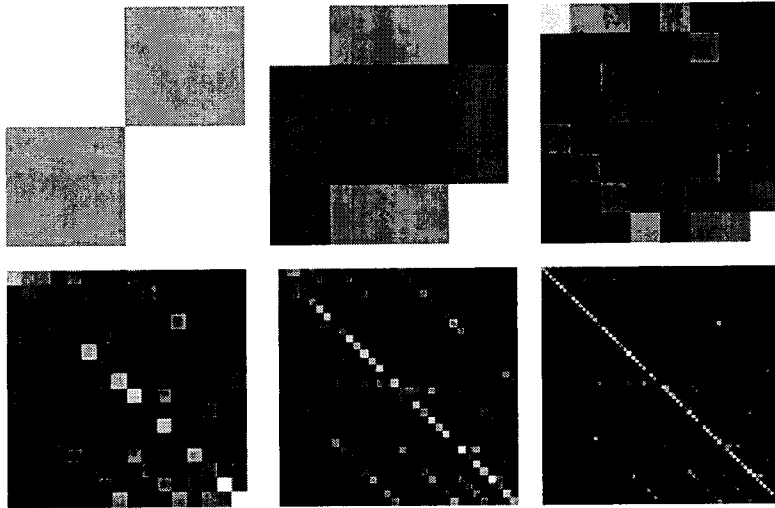
- Enough homology with LCDV1 type strain
⇒ to define the microbe we analyzed is '*Lymphocystivirus*'
- Enough discrepancy with LCDV1 type strain
⇒ to define the microbe we analyzed is a new strain different from type strain



LCDV-K1

The first Korean *Lymphocystivirus* analyzed in whole genome scale

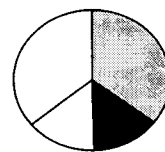
Analysis of base composition and oligonucleotide composition



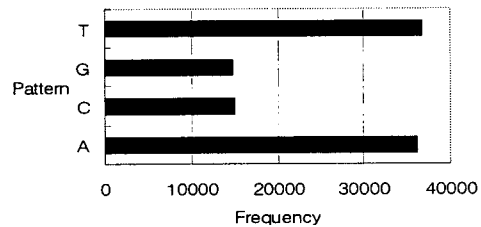
1-mer analysis

1-mer	Frequency
A	36102
C	15043
G	14799
T	36709

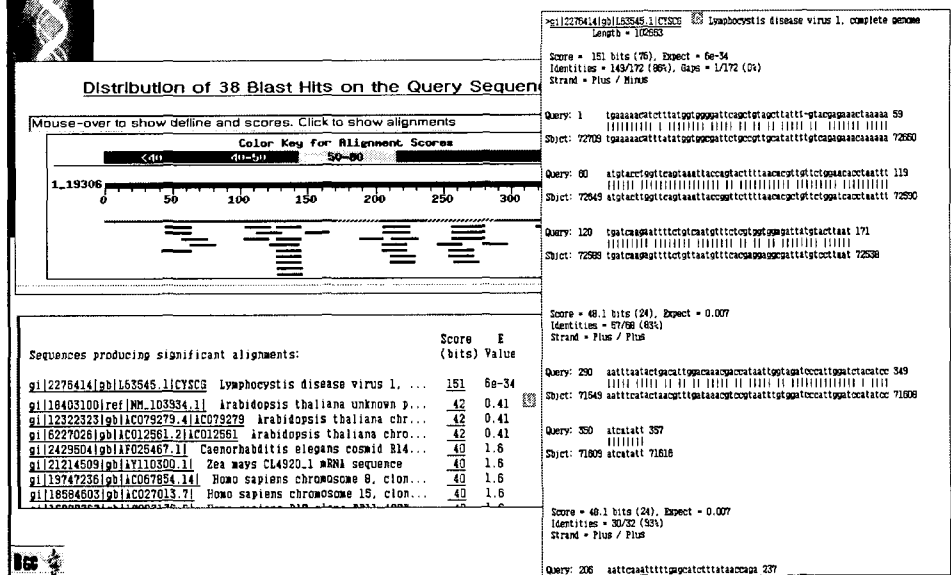
LCDV-Whole(1-mer)



LCDV-Whole(1-mer)



Annotation Results



What characteristics ?

Results from the annotation of LCVD-K1 will be presented at the workshop.

- This work is supported by the research grant of **NFRDI** (the National Fisheries Research and Development Institute under the Ministry of Marine Affairs and Fisheries)