김 철 민 박사

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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 DNAchip 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 공중보건의사

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

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 Pathogenic Microbe in Fish Farming

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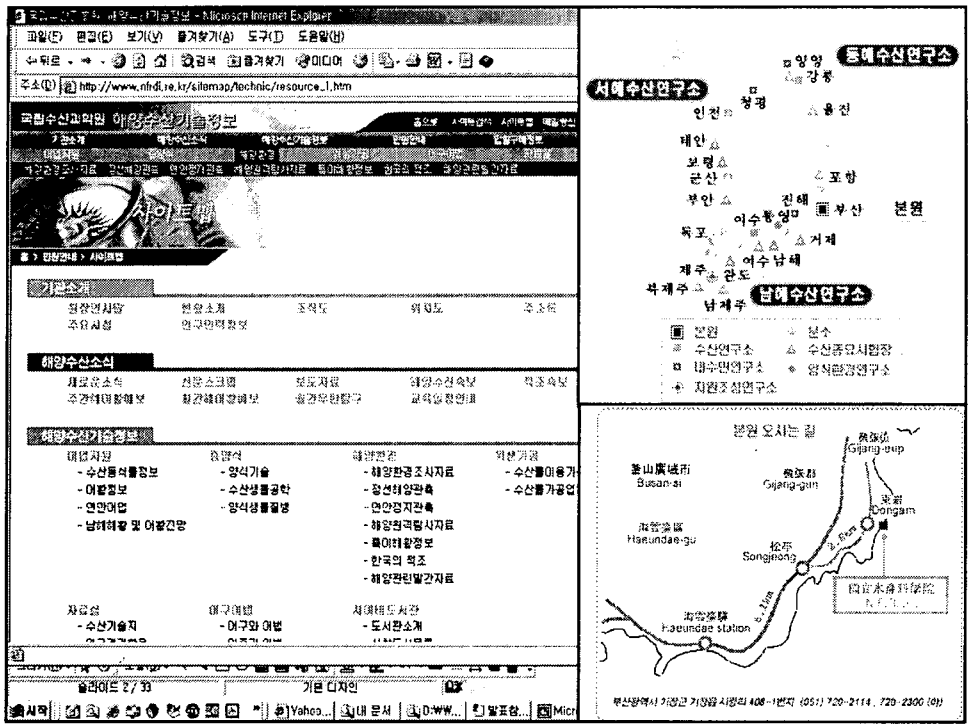
Department of Biochemistry
College of Medicine
Pusan National University

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

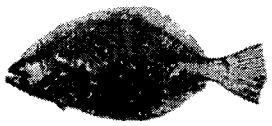


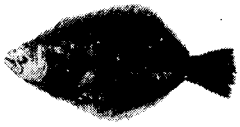
수산동식물-1

- 원양어류 (목) ; 가자미,금눈돔, 꼬리치, 농어, 달고기, 당멸치,대구, 돔발상어, 동갈치, 먹장어, 메기, 바다빙어, 뱀장어, 복어, 색줄멸, 샛비늘치, 수염상어, 숭어, 신락상어, 쏨뱅이, 아귀, 악상어, 앨퉁이, 연어, 은상어, 이악어, 전자리상어, 첨치, 청어, 큰가시고기, 톱상어, 해와, 홍메치, 홍어, 흉상어
- 연근해어류 ; 가오리, 가자미, 괭이상어, 금눈돔, 농어, 달고기, 대구, 돔발상어, 동갈치, 메기, 뱀장어, 복어, 샛비늘치, 시락상어, 실고기, 아귀, 악상어, 양볼락, 연어, 은상어, 청어, 칠성장어, 큰가시고기, 학치
- 내수면어류 ; 뱀장어, 복어, 색줄멸, 샛비늘치, 수염상어, 숭어, 신락상어, 실고기, 쏨뱅이, 아귀, 악상어, 앨퉁이, 양볼락, 연어, 은상어, 이악어, 전자리상어, 첨치, 청어, 칠성장어, 큰가시고기, 톱상어, 학치, 해와, 홍메치, 홍어, 흉상어
- 패류 ; 고이치, 고다치, 고이치, 기안, 나새, 두순, 무순, 병안, 뿔조개, 신복족, 십완, 연두군부, 원시복족, 중복족, 진다치, 털군부, 팔완
- 해조류 ; 국수나물, 다시마, 돌가사리, 디크티오시폰, 디크티오타, 로오디메니아, 마디털, 모자반, 보라털, 붉복크스, 비단풀, 산말, 스파셀라리아, 스포로크누스, 시포노클라두스, 에크토카르푸스, 우뭇가사리, 울로드릭스, 지누아리, 채찍말, 청각, 코르다리아, 크로오콕크스, 클라도포라

Why Flounder ? 넙치 광어, flat fish, flounder

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



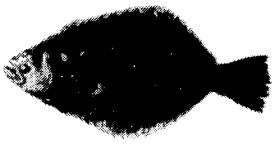


분류	어류
목	Pleuronetiformes
과	Paralichthyidae
영어명	Flatfish
일어명	Hirame
학명	*Paralichthys olivaceus*
명명자	Temmick et Schlegel

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

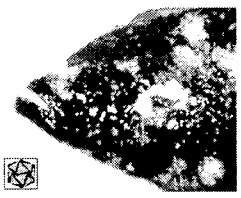
가주넙치 점넙치 별넙치 별넙치

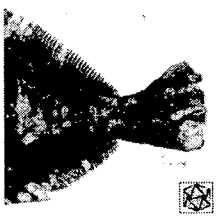
Why Flounder ? Why Lymphocystivirus ?



Lymphocystivirus

Lymphocystis (림포시스티스 바이러스병)





Lymphocystis?

림포시스티스 바이러스병

- 우리나라 양식 해산 어류에 종양을 유발하여 폐사 초래
  치어 종묘 입식 후 45일째부터 종양 형성이 관찰됨
  섬유아세포와 근아세포 비대로 피부와 지느러미에 종양(양성) 유발
- cells can increase in volume by a factor of 10^6

- 연중 지속적으로 발병
  특히 저수온기(12월에서 4월까지)에 가장 많이 발생

- 육안으로 종양을 확인하기 전까지는 바이러스의 감염여부를 알 수 없어 조기진단이 힘듦

- 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

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

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

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

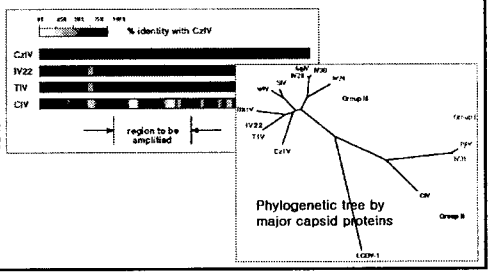
• Morphology

- 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

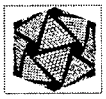
Taxonomy

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

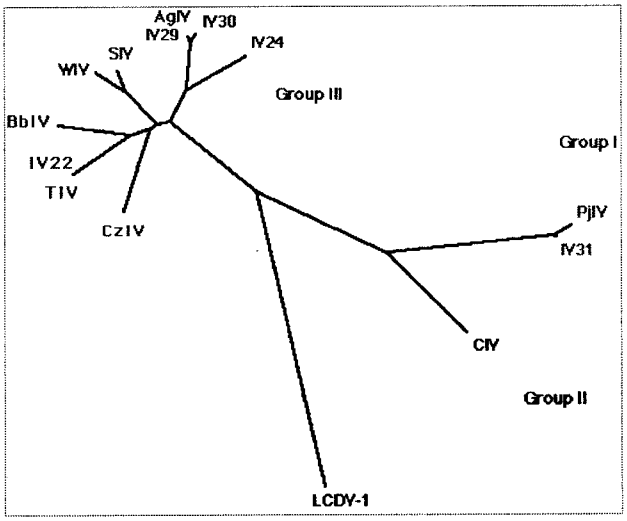
- ICTV list of recognised Iridoviridae members contains much redundant data
- The wide host range displayed because of their isolation from different hosts
- host of isolation also confuses taxonomy
- pitfalls in iridovirus taxonomy are only just beginning to be addressed using molecular techniques



Iridoviridae



Phylogenetic tree by major capsid proteins



Iridoviridae Taxonomy

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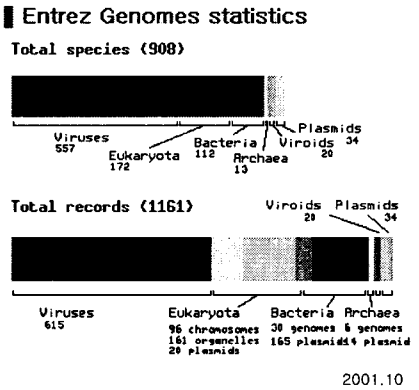
Ranavirus	Frog virus	Amphibia	Frog Virus 3 (FV3)
Goldfish virus 1-like viruses		Vertebrates	goldfish virus 1

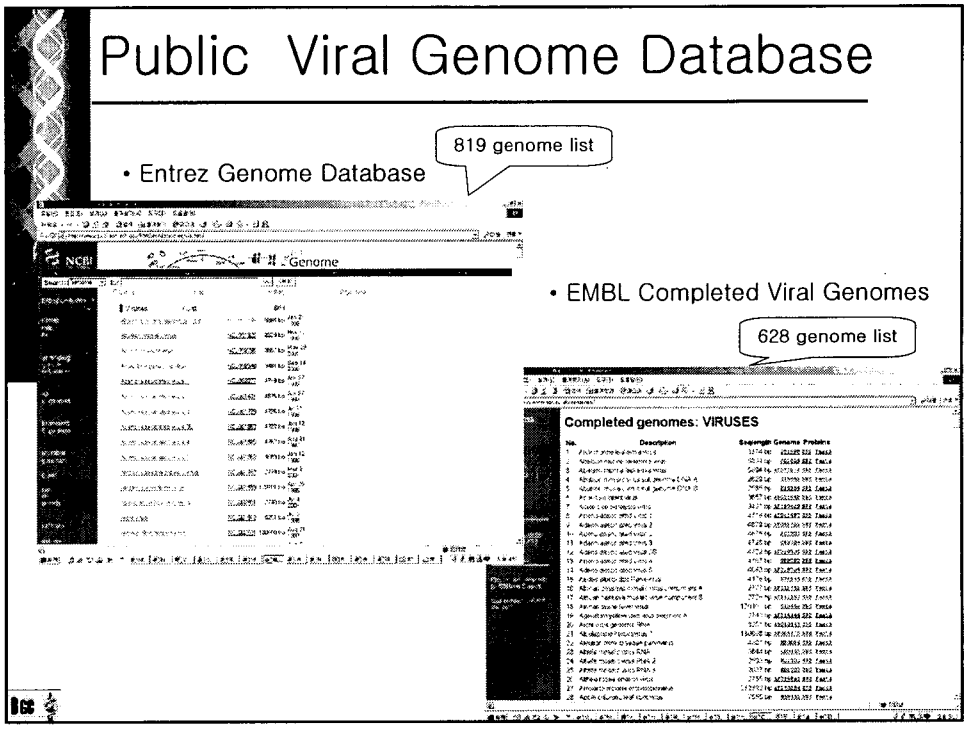
Current status of genome analysis

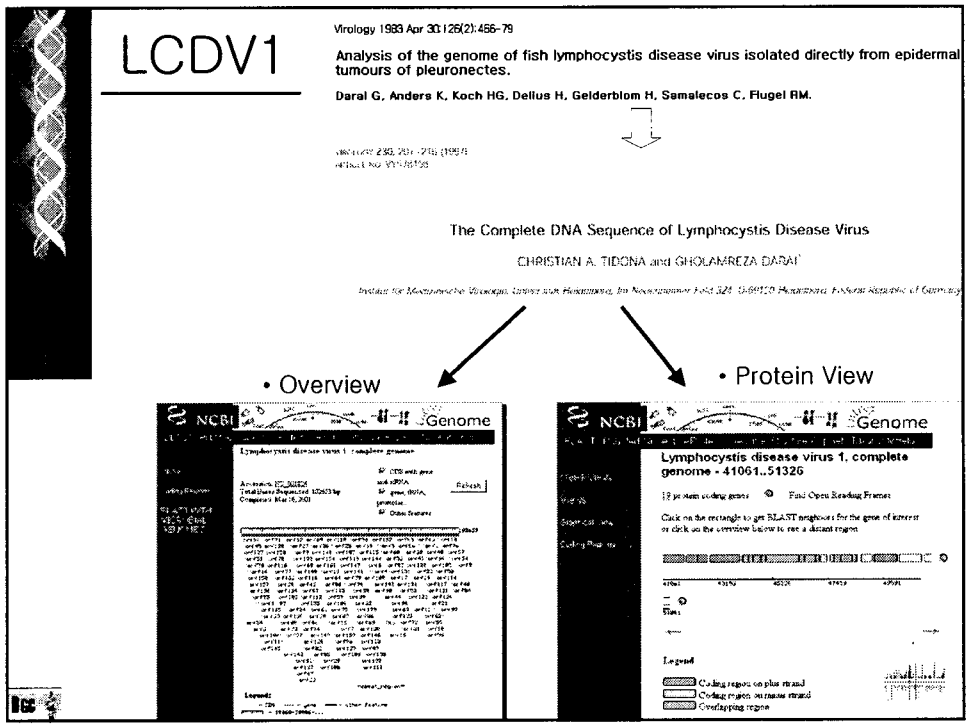
The whole genomes of over 900 organisms in Entrez Genomes.

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

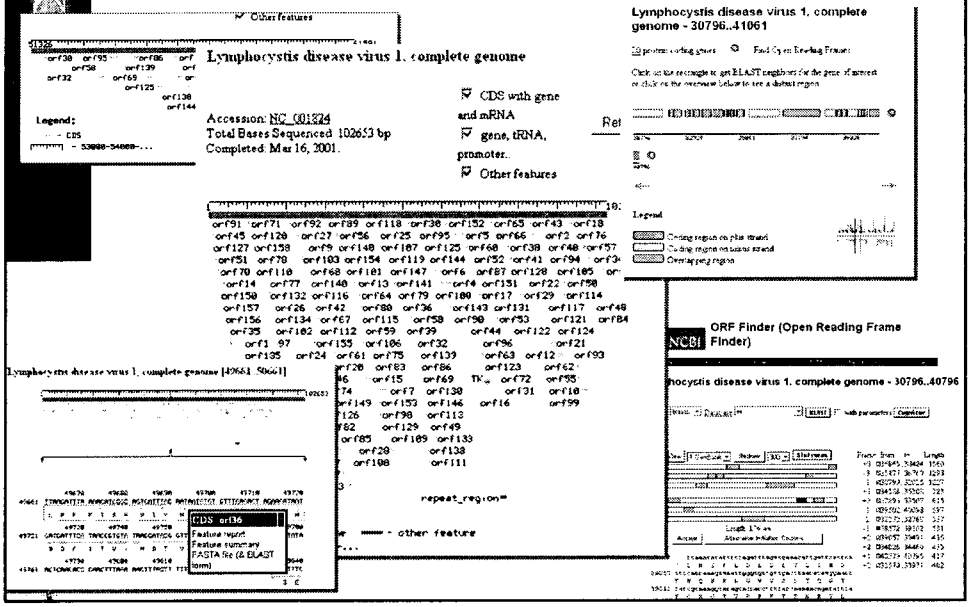
- All three main domains of life – bacteria, archaea, and eukaryota
- many viruses and organelles




Public Viral Genome Database
• Entrez Genome Database
819 genome list
• EMBL Completed Viral Genomes
628 genome list
Completed genomes: VIRUSES


LCDV1
Virology 1988 Apr 30;126(2):466-79
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, Samalecos C, Flugel RM.
The Complete DNA Sequence of Lymphocystis Disease Virus
CHRISTIAN A. TIDONA and GHOLAMREZA DARAI
• Overview
• Protein View
Lymphocystis disease virus 1, complete genome - 41061..51326

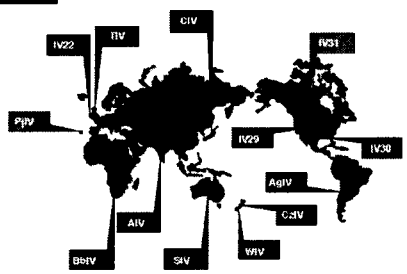
LCDV 1



Why LCDV genome of Korean flounder should be reanalyzed?

1. Regional variety and host variety

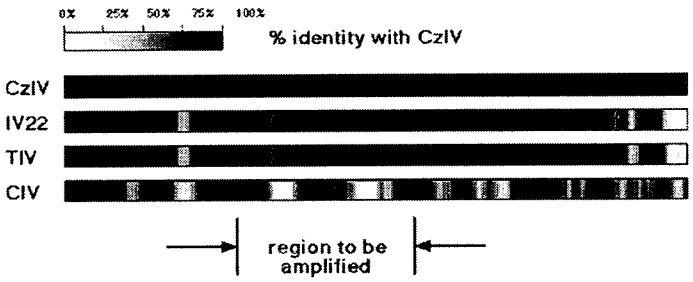
Countries of virus isolation



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

Why LCDV genome of Korean flounder should be reanalyzed?

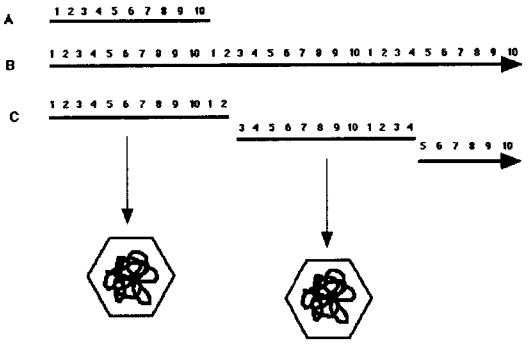
Genotyping is essential for early detection of outbreak



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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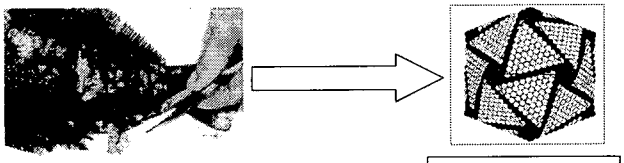
Isolation of virus from lymphocystis

- Sucrose density gradient centrifugation



3g tissue

50 ug DNA

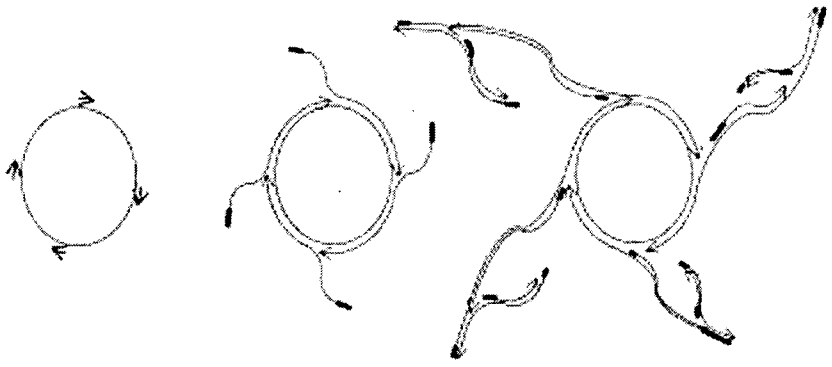
- Limited amount of starting material

  The first obstacle for whole genome analysis

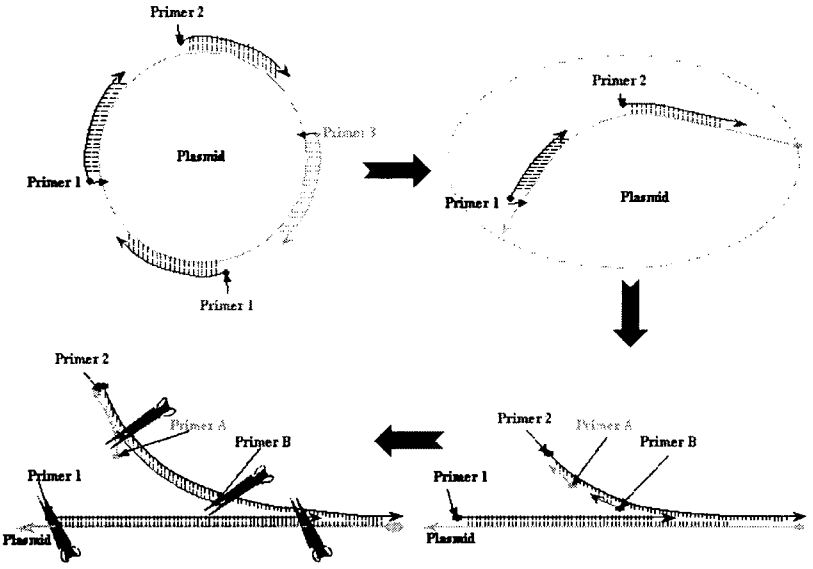
⇒ Overcome by amplification of whole genome

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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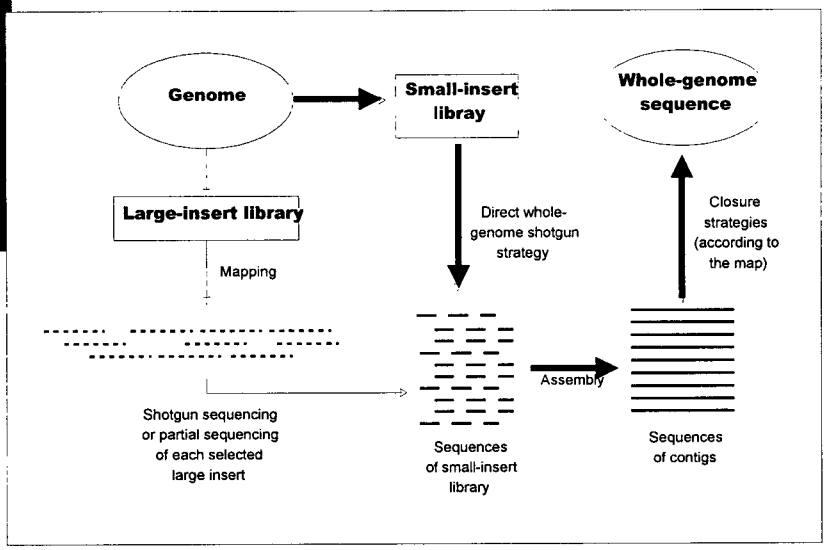
Strategy for genome analysis



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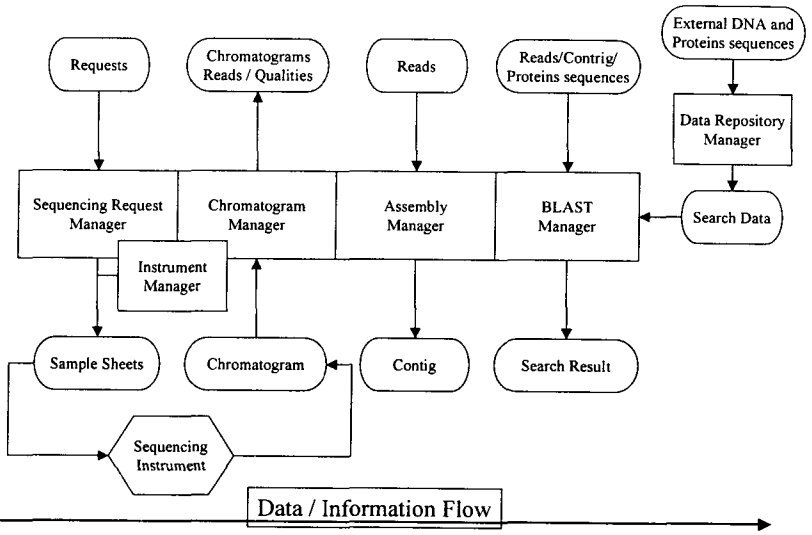
Installed assembly system

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

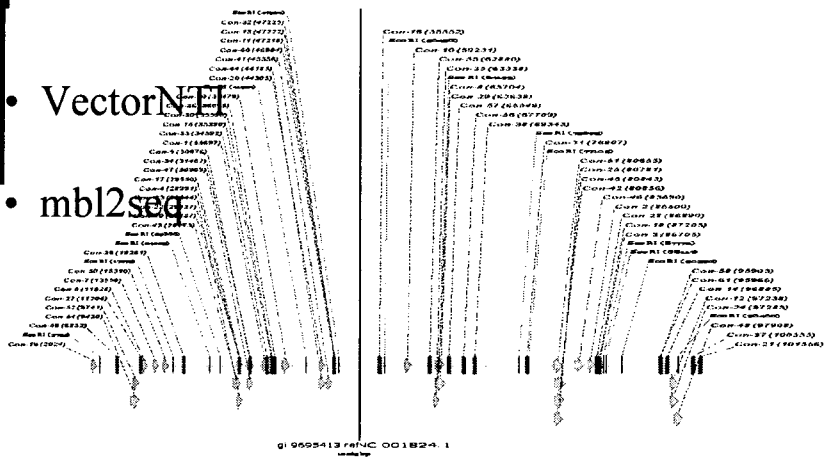
Pilot Mapping

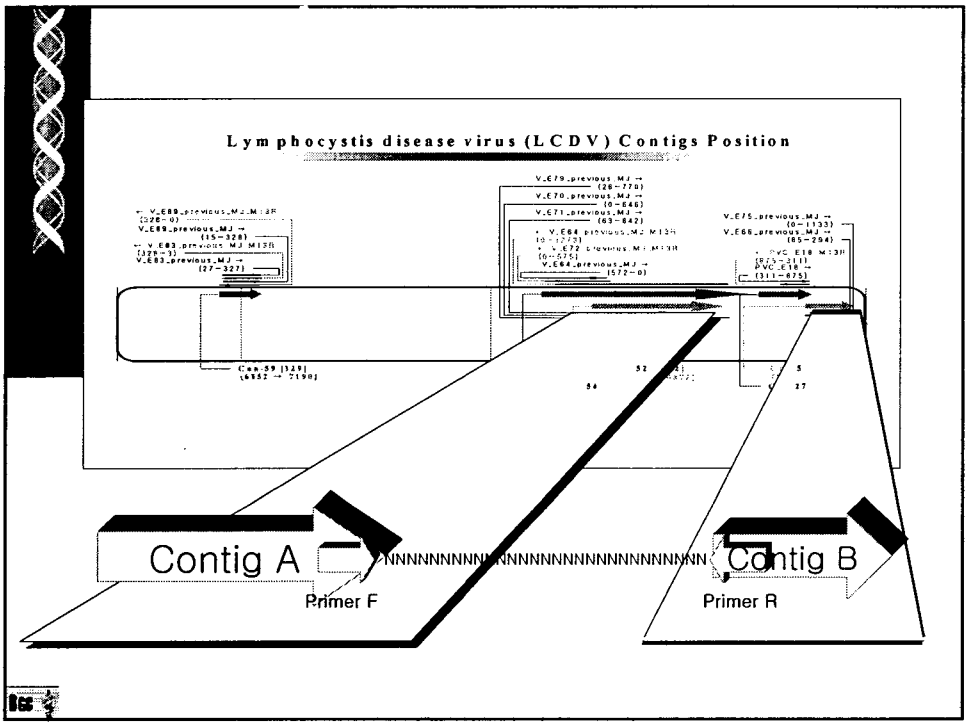
- For Mutiplex PCR
- Mapping to Reference Sequence
  - Pairwise Alingment
    - ClustalW
    - BL2SEQ

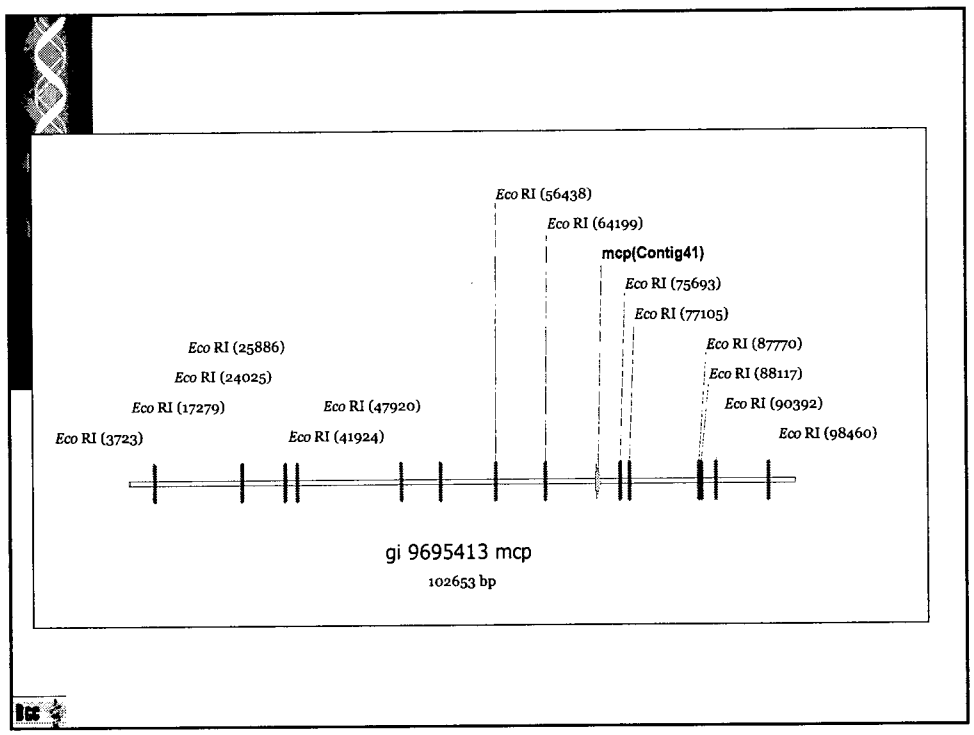
From U.S. Department of Energy Human Genome Program

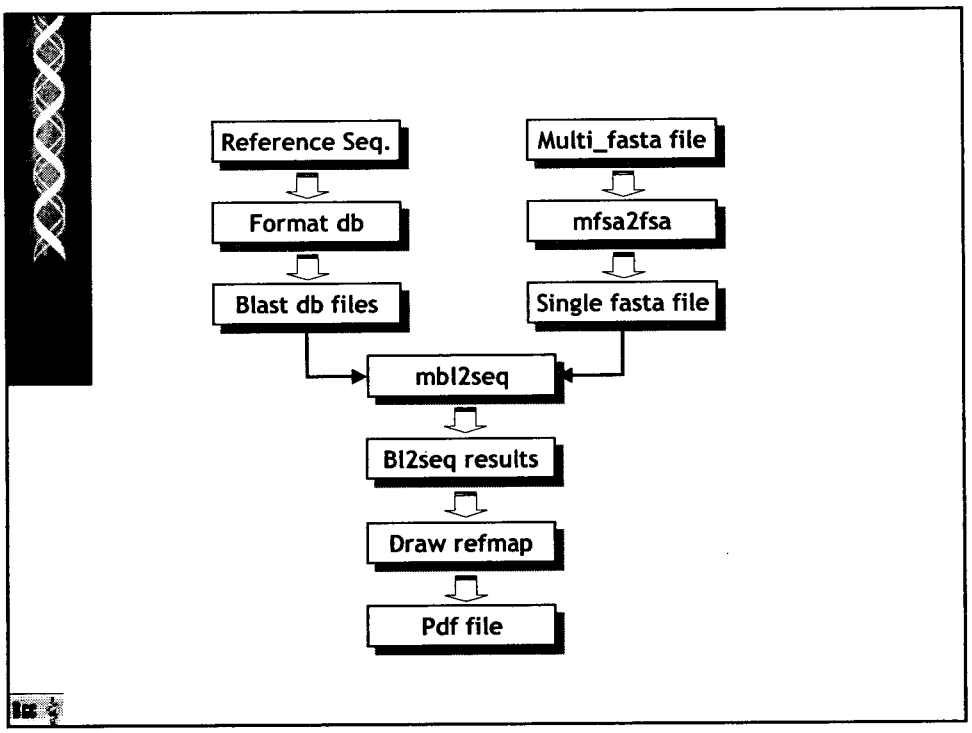
Pilot Mapping Results

- VectorNTI
- mbl2seq



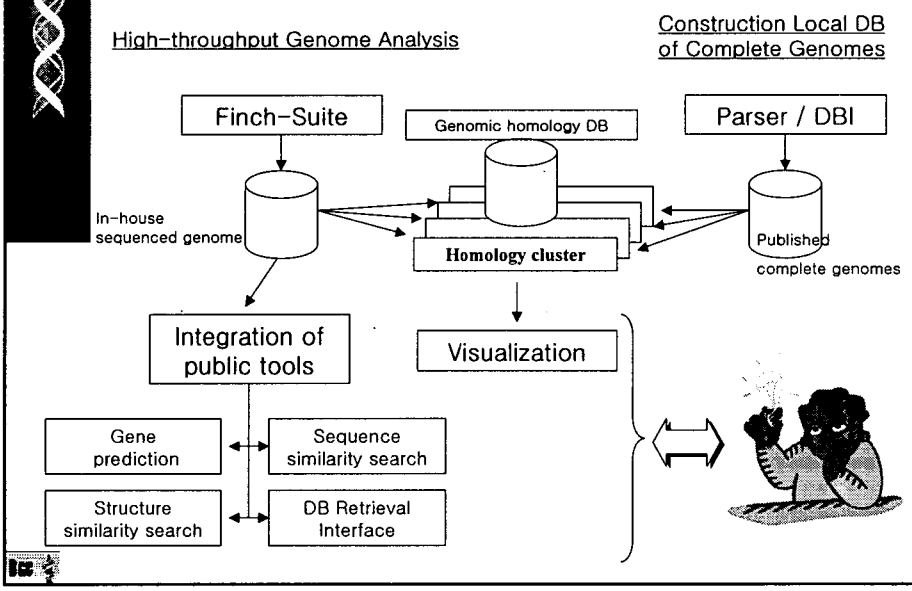



Eco RI (56438)
Eco RI (64199)
mcp(Contig41)
Eco RI (75693)
Eco RI (77105)
Eco RI (87770)
Eco RI (88117)
Eco RI (90392)
Eco RI (98460)
Eco RI (25886)
Eco RI (24025)
Eco RI (17279)
Eco RI (3723)
Eco RI (47920)
Eco RI (41924)
gi 9695413 mcp
102653 bp

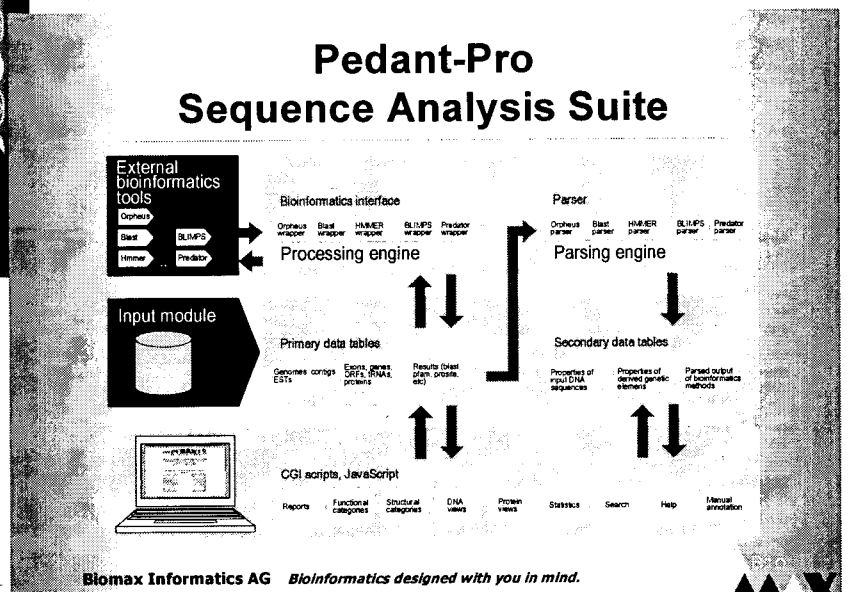

Reference Seq.
Format db
Blast db files
Multi_fasta file
mfsa2fsa
Single fasta file
mbl2seq
Bl2seq results
Draw refmap
Pdf file

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

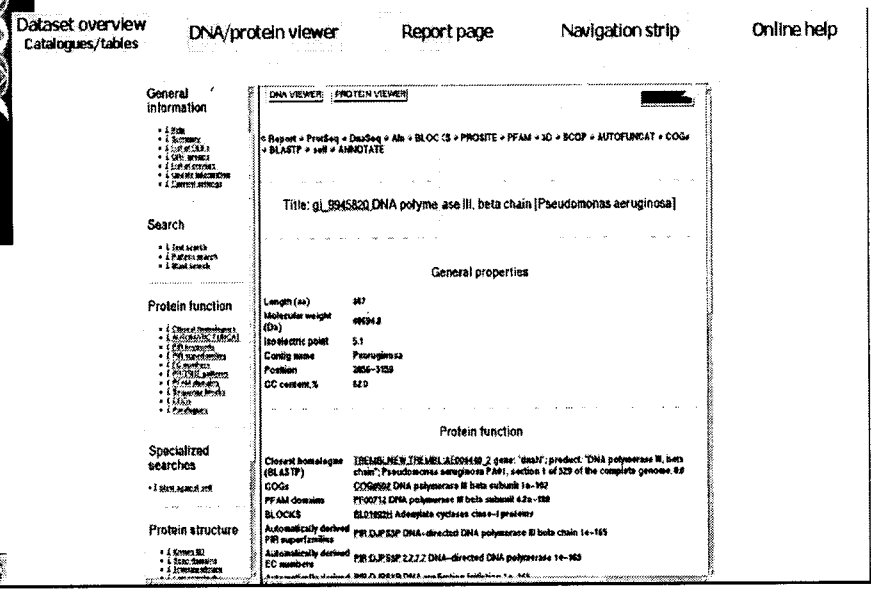
Annotation strategy-1



Annotation strategy-2



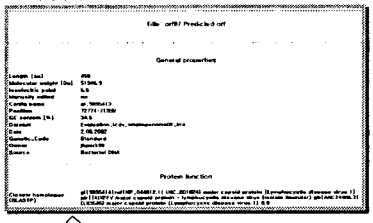
PEDANT Web interface

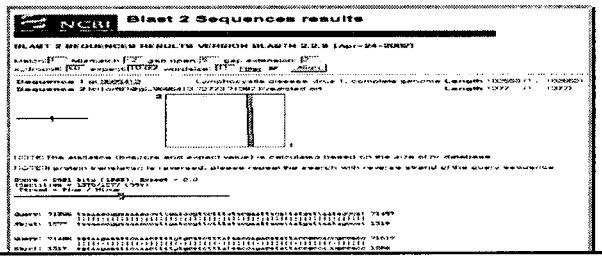


Comparison of MCP type strain and test strain

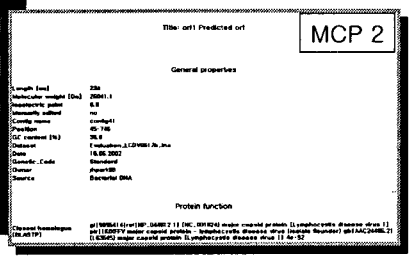
• Type strain

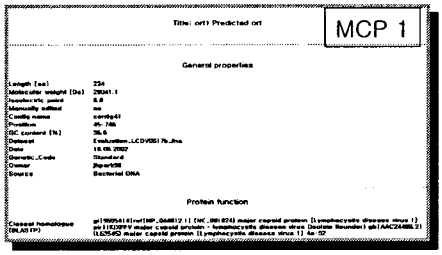
• Test strain

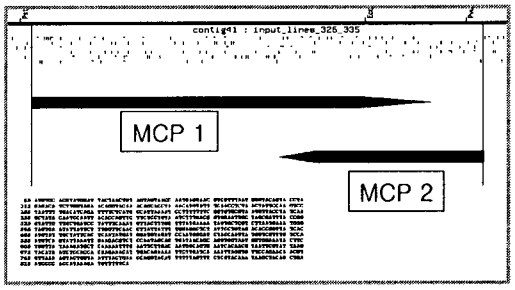


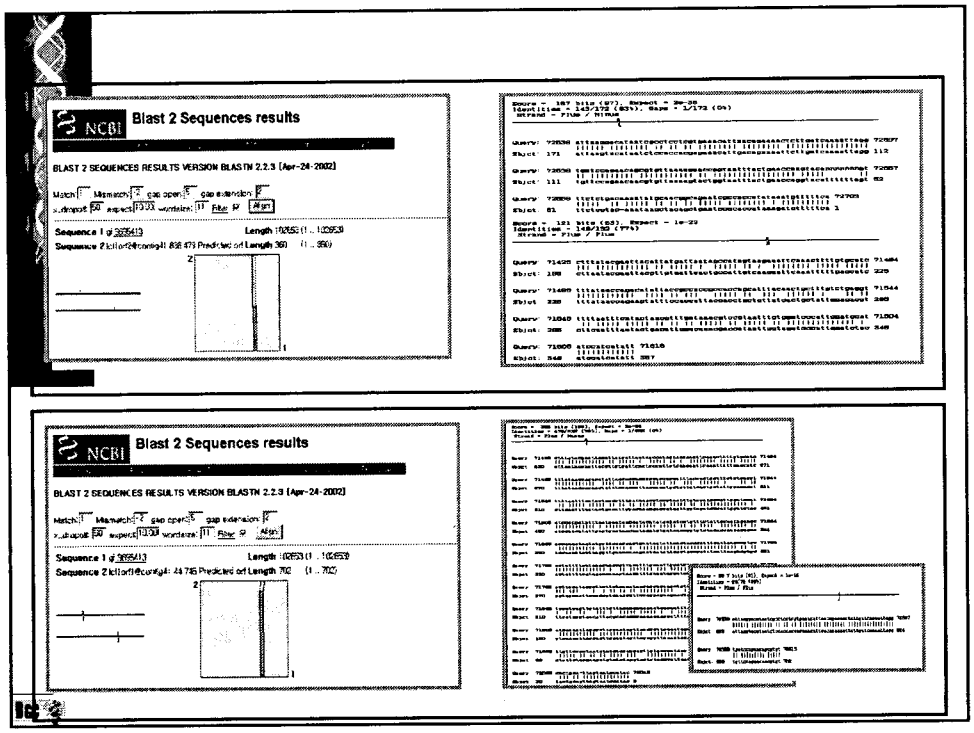


MCP ORF Predicted by PEDANT



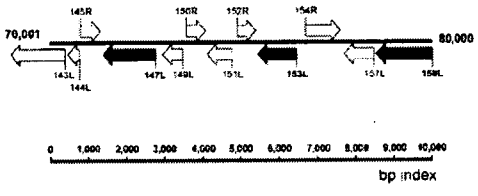


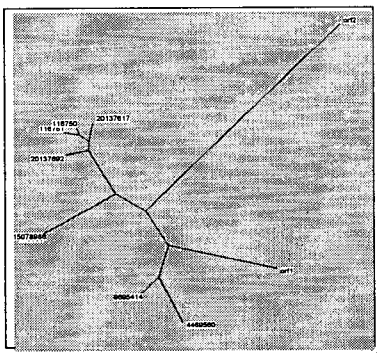




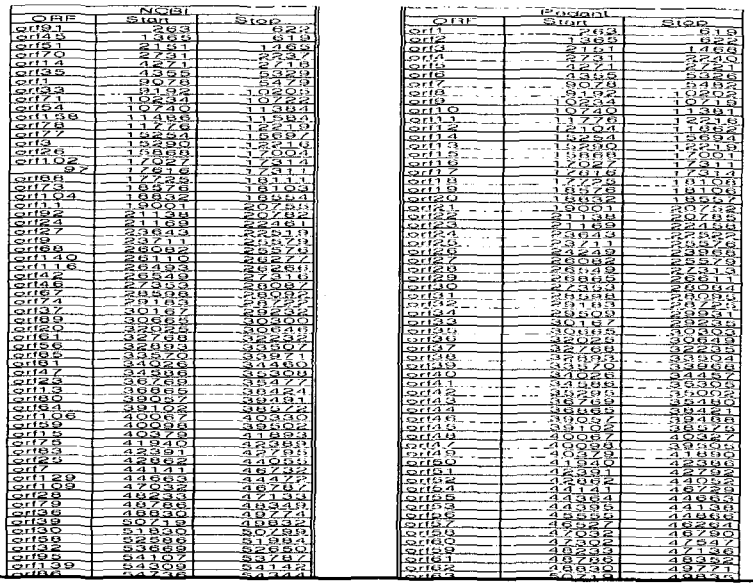
Homology of LCDV-K1 with other iridoviridae

146L*	71256-71056	67	none	none	
147L	72774-71398	459	none	major capsid protein of chilo iridescent virus (Q95815) [Stelnwasser *et al.*, 1993]	53.4/29.5
148R*	73414-73596	61	none	none	
149L	73486-72965	174	zinc finger (C4 type) signature	none	





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



What characteristics ?

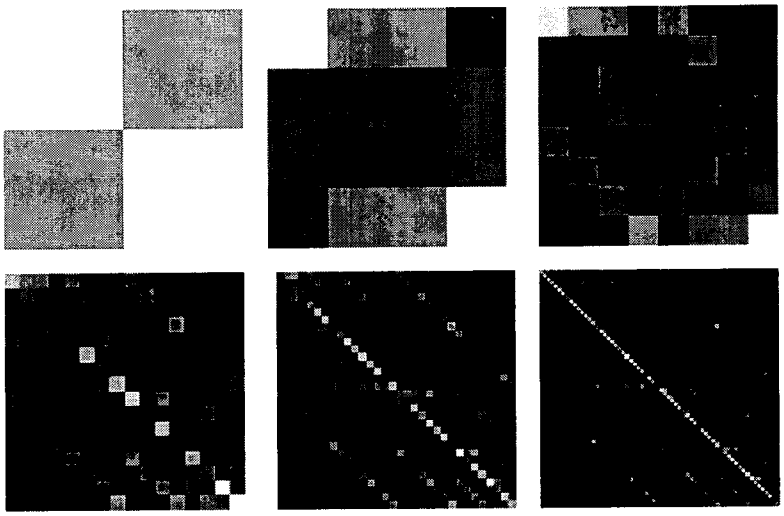
- Enough homology with LCDV1 type strain
  ⇒ to define the microbe we analyzed is '*Lymphocystivirus*'

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

⇨ LCDV-K1

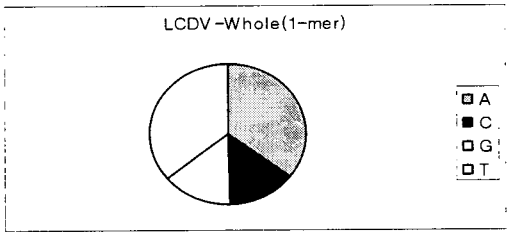
The first Korean *Lymphocystivirus* analyzed in whole genome scale

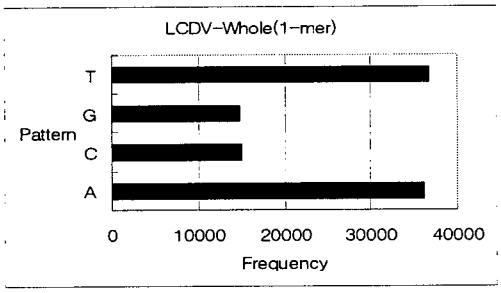
Analysis of base composition and oligonucleotide composition



1-mer analysis

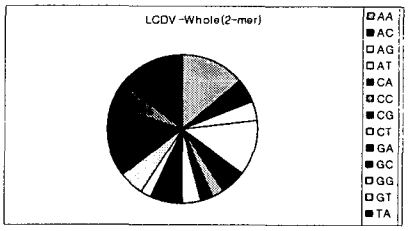
1-mer	Frequency
A	36102
C	15043
G	14799
T	36709

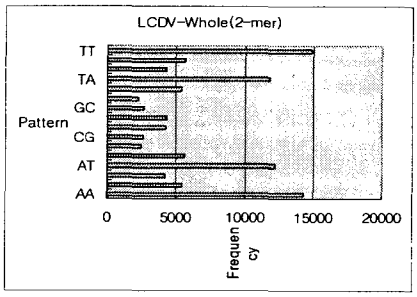




2-mer analysis

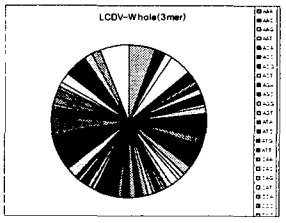
2-mer	Frequency
AA	14312
AC	5437
AG	4191
AT	12162
CA	5648
CC	2523
CG	2634
CT	4238
GA	4359
GC	2715
GG	2296
GT	5429
TA	11783
TC	4368
TG	5677
TT	14880

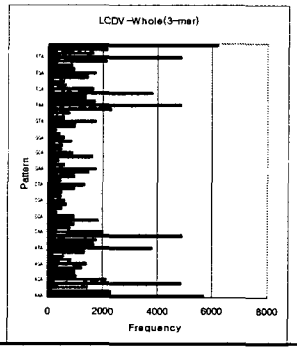




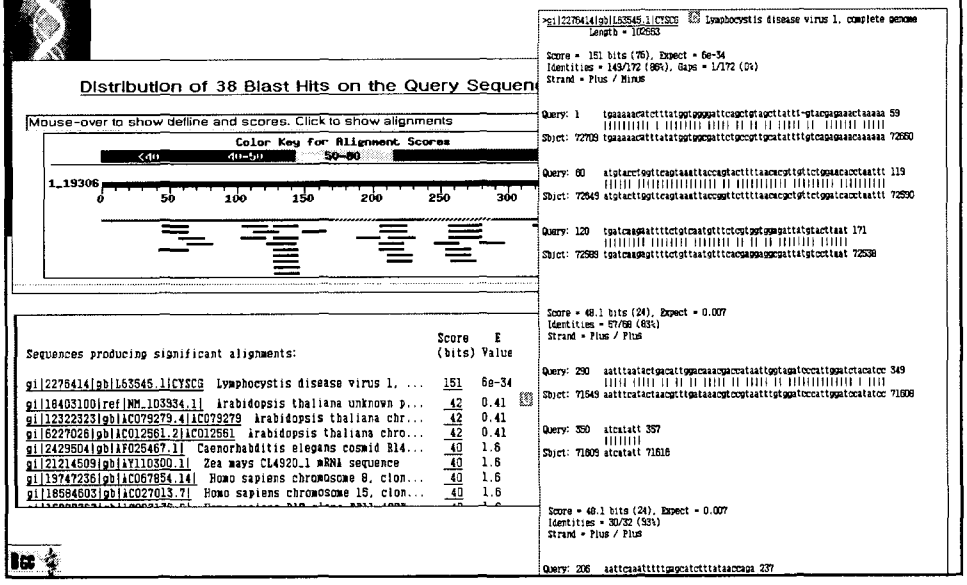
3-mer analysis

3-mer	Frequency
AAA	5685
AAC	2303
AAG	1453
AAT	4871
ACA	2151
ACC	1057
ACG	976
ACT	1253
AGA	1405
AGC	850
AGG	595
AGT	1341
ATA	3830
ATC	1666
ATG	1769
ATT	4897
CAA	2016
CAC	831
CAG	964
CAT	1837
CCA	964
CCC	326
CCG	536
CCT	697
CGA	640
CGC	492
CGG	518
CGT	984
CTA	1338
CTC	456
CTG	955
CTT	1489
GAA	1729
GAC	597
GAG	407
GAT	1626
GCA	877
GCC	479
GCG	514
GCT	845
GGA	584
GGC	434
GGG	300
GGT	978
GTA	1740
GTC	596
GTG	774
GTT	2319
TAA	4882
TAC	1706
TAG	1367
TAT	3828
TCA	1656
TCC	661
TCG	608
TCT	1443
TGA	1730
TGC	939
TGG	882
TGT	2126
TTA	4875
TTC	1650
TTG	2179
TTT	6175





Annotation Results



What characteristics ?

Results from the annotation of LCVD-K1 ill 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)