

112年度食品微生物檢驗業務推廣訓練班

食品中A型肝炎病毒之檢驗

研究檢驗組 食品生物科
袁巧璇 112年10月27日



衛生福利部
食品藥物管理署
Taiwan Food and Drug Administration

<http://www.fda.gov.tw/>

課程大綱

A型肝炎病毒檢驗流程

檢驗結果分析方法 (NCBI Blast)

病毒檢驗注意事項及常見問題

食因性病毒檢驗流程

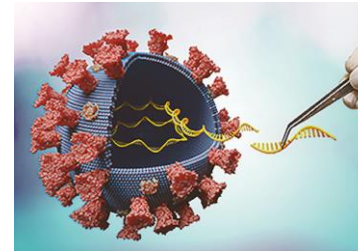
病毒自基質分離



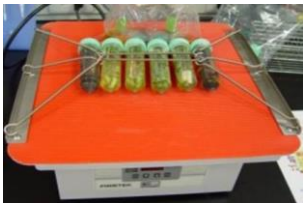
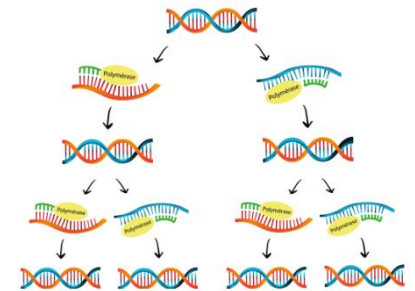
病毒濃縮



病毒核酸萃取



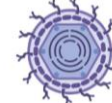
病毒基因體偵測



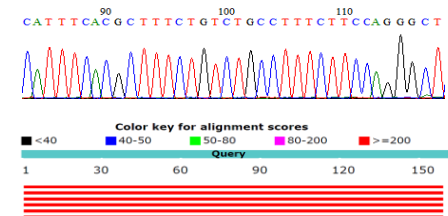
Norovirus



Hepatitis A virus



Rotavirus



公告檢驗方法

当前位置：首頁 > 業務專區 > 研究檢驗 > 公告檢驗方法

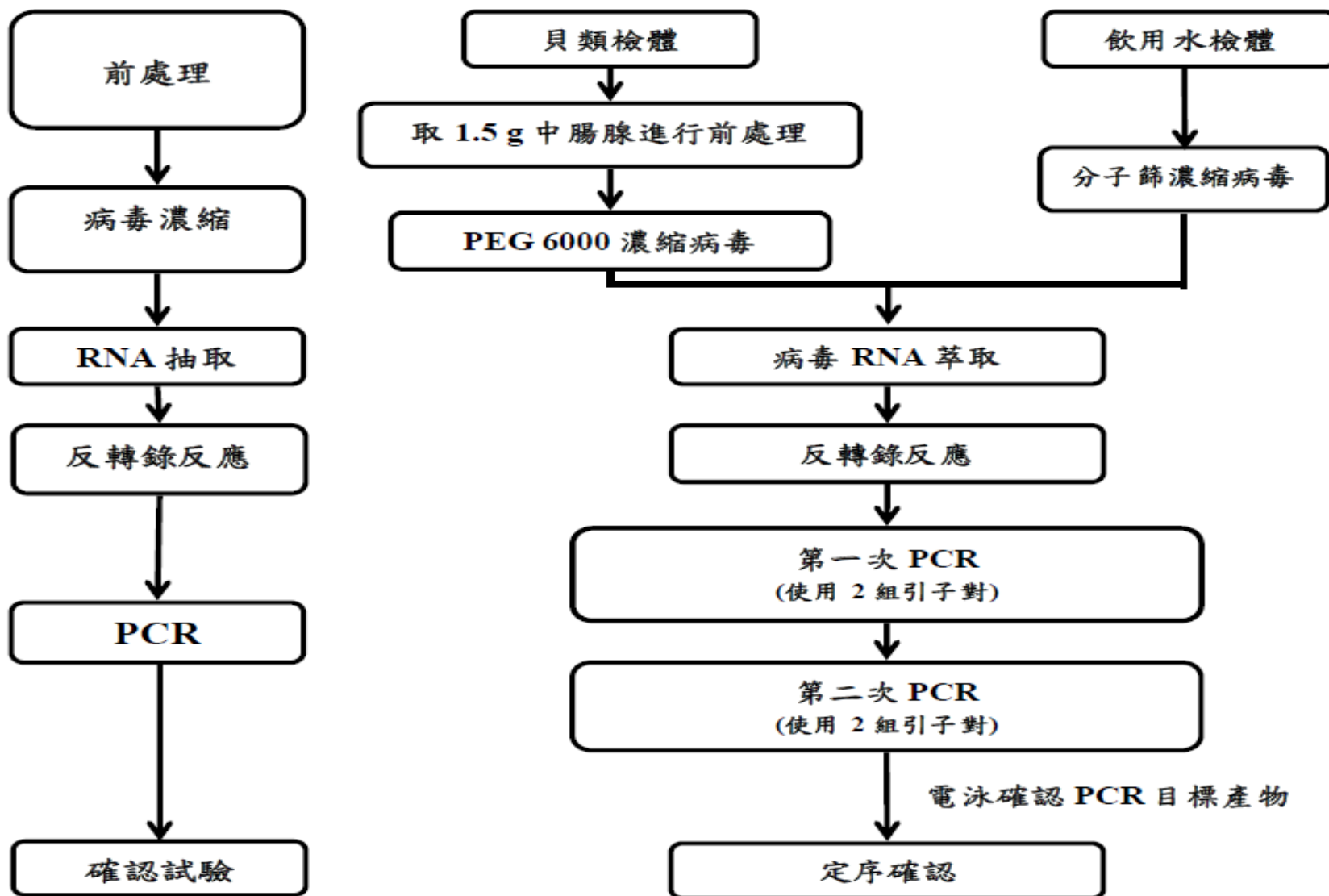
分類：

區域檢索：

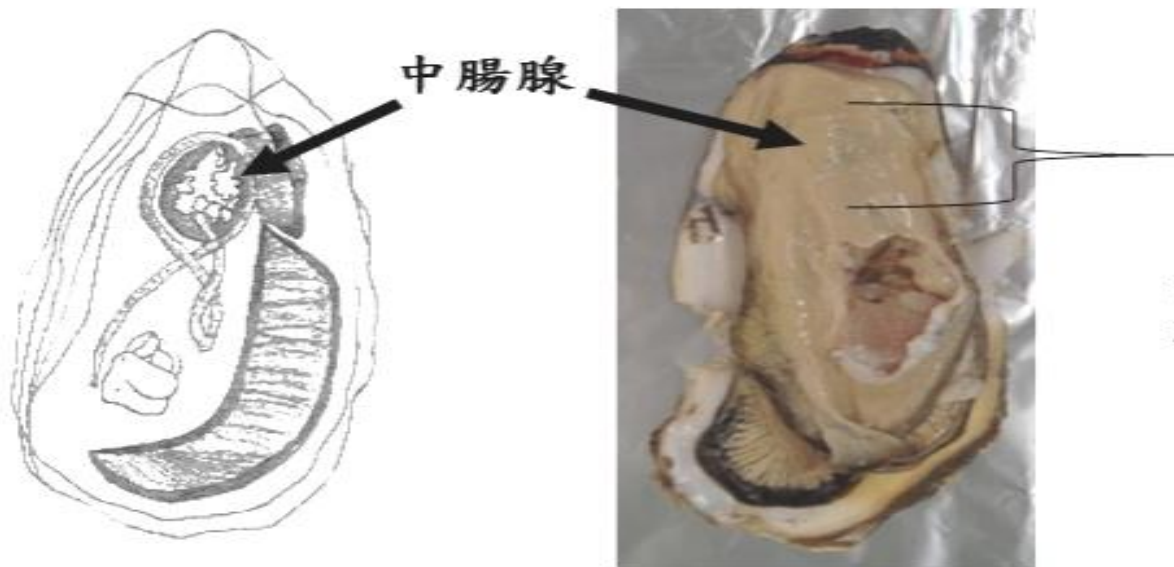
搜尋

序號	標題	發布日期
11	食品微生物之檢驗方法 - 曲狀桿菌之檢驗(MOHWM0007.02)	2015-10-02
12	食品微生物之檢驗方法 - 志賀氏桿菌之檢驗(MOHWM0003.02)	2015-04-29
13	食品微生物之檢驗方法 - 阪崎腸桿菌之檢驗(MOHWM0004.02)	2015-01-07
14	食品中微生物之檢驗方法 - 星狀病毒之檢驗(MOHWM0001.01)(自103年10月1日生效)	2014-06-27
15	食品中微生物之檢驗方法 - 諾羅病毒之檢驗(MOHWM0018.03)(自103年10月1日生效)	2014-06-27
16	食品中微生物之檢驗方法 - A型肝炎病毒之檢驗(MOHWM0027.00)(自103年9月1日生效) 	2014-06-16
17	食品微生物之檢驗方法 - 沙門氏桿菌之檢驗(MOHWM0025.01)	2013-12-23
18	食品微生物之檢驗方法 - 包裝飲用水及盛裝飲用水中綠膿桿菌之檢驗(MOHWM0024.01)	2013-12-20
19	食品微生物之檢驗方法 - 包裝飲用水及盛裝飲用水中糞便性鏈球菌之檢驗(MOHWM0022.01)	2013-12-19
20	食品微生物之檢驗方法 - 包裝飲用水及盛裝飲用水中大腸桿菌群檢驗(MOHWM0020.00) 	2013-12-17

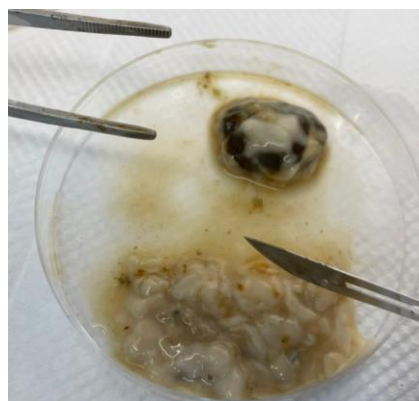
病毒檢驗流程圖(貝類及水)



貝類檢體-中腸腺取樣



圖二、自生壕中
取出之中腸腺



貝類檢體之檢驗流程

1 離心前

2 離心後

1.5 g oyster digestive tissues + 10 ml PBS

Homogenization

Chloroform-butanol (1/1)

1

1 hr, 4°C

12000 g

2

Virus concentration by PEG 6000-NaCl

Over-night, 4°C

3

離心後去除上清液，**pellet**以ddH₂O回溶作為
檢液，進行
Virus lysis and viral RNA extraction

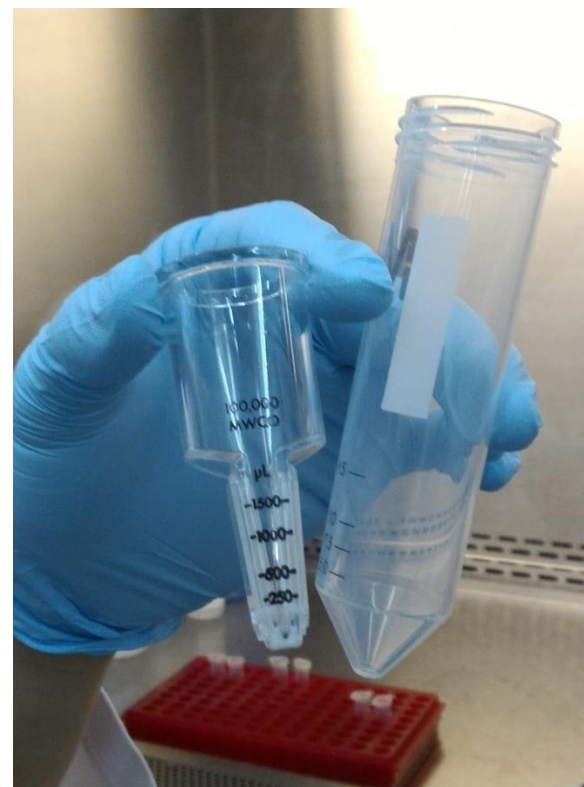
Reverse Transcription → 2X PCR → DNA analysis → DNA sequencing



水檢體-離心過濾管濃縮



50KDa-50,000MWCO (Molecular Weight Cutoff)



3,000 g 離心20-30分鐘

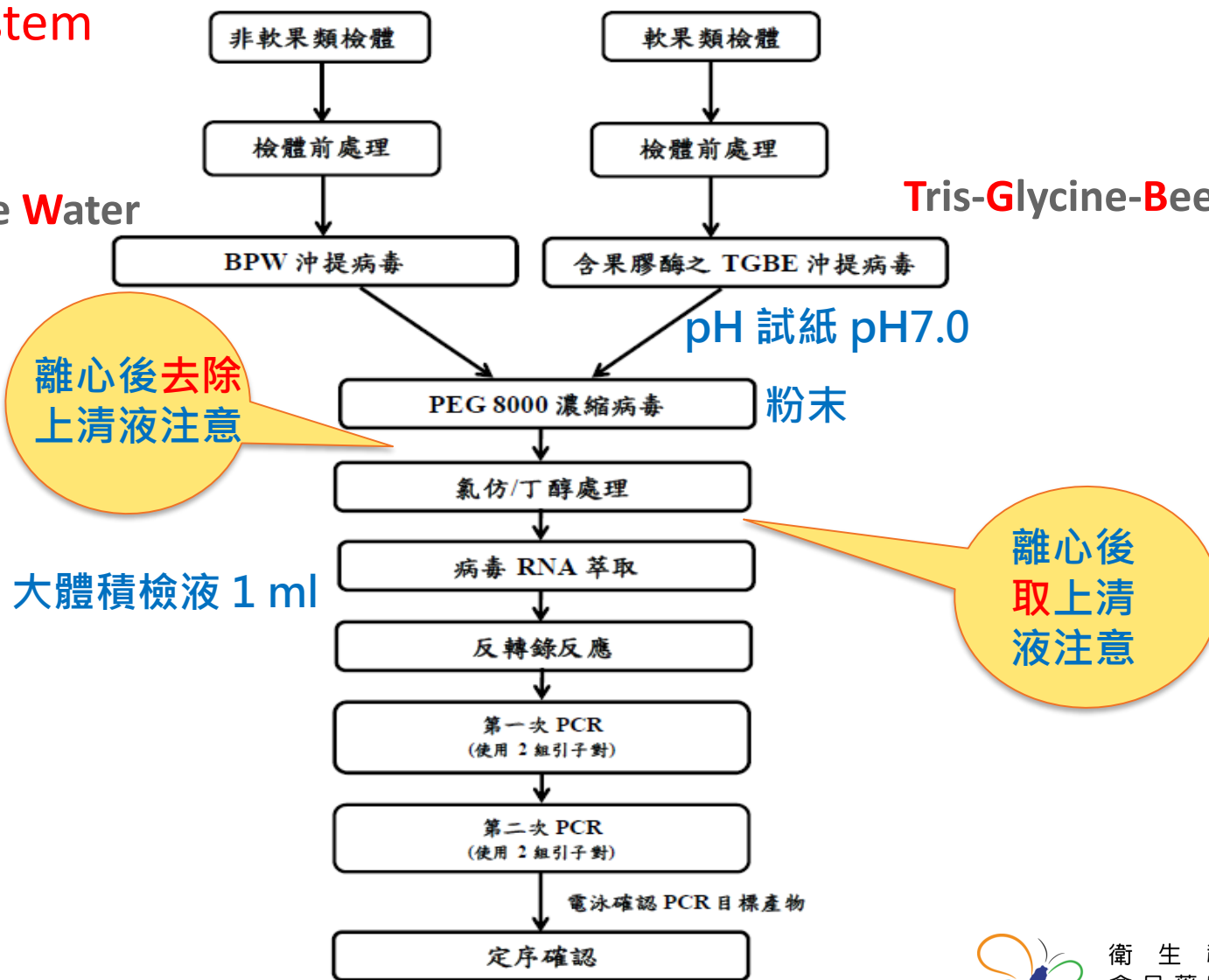
2.4.7. 離心過濾管：15 mL，篩選分子量大於 10^5 道爾頓(dalton)之物質。

病毒檢驗流程圖(蔬果類)

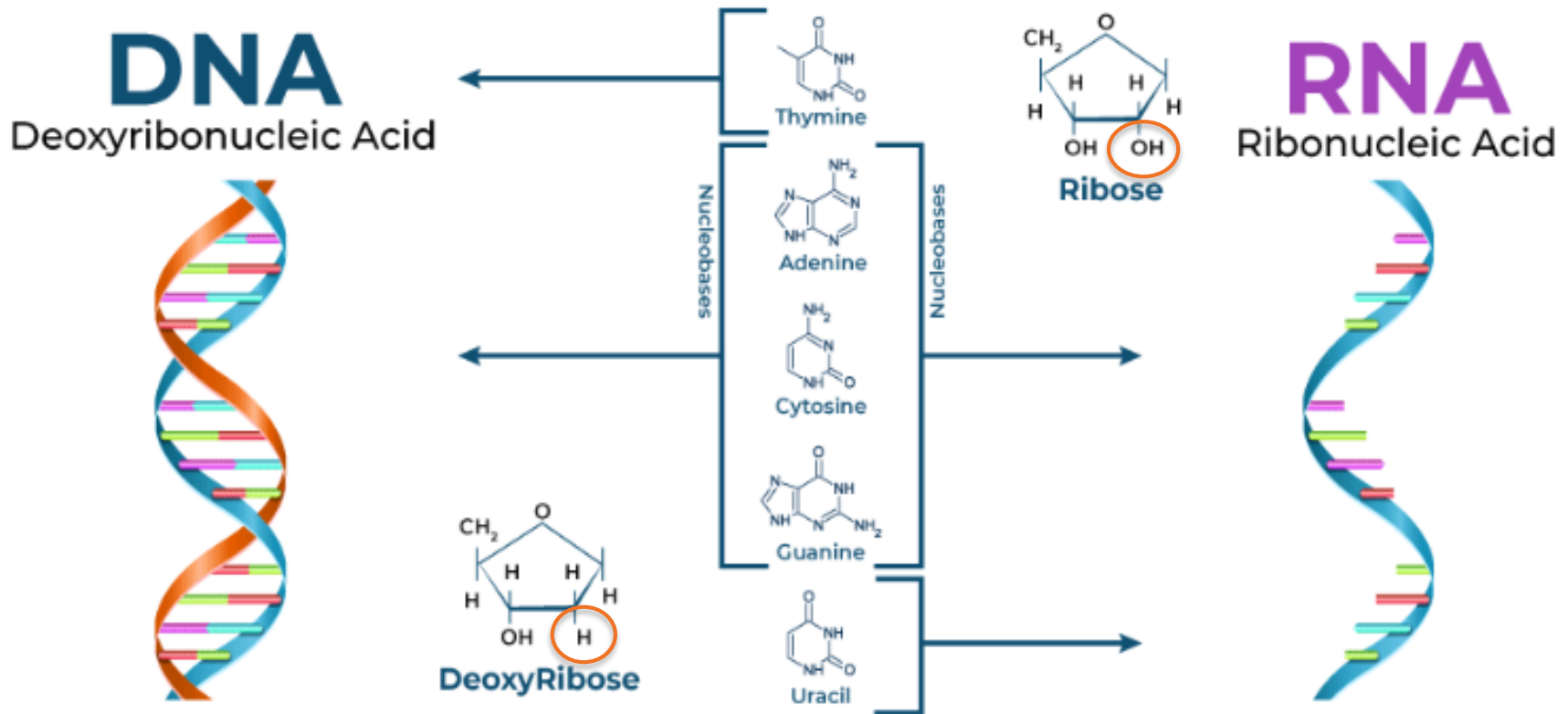
2 buffer system

Buffer Peptone Water

Tris-Glycine-Beef Extract



RNA Extraction



- ◆ RNA-single strand ,unstable
- ◆ RNase(Ribonuclease),人體汗液、唾液、皮屑含有,廣泛存在環境
- ◆ 使用RNase 移除試劑
- ◆ 檢體內源性RNase (kit 內含carrier RNA)

以DNase I 處理

2.9. 以 DNase I 處理病毒 RNA 溶液：

2.9.1. 取微量離心管，依下表配製混合液：

病毒 RNA 溶液	24.0 μ L
10 倍緩衝溶液	3.0 μ L
無菌去離子水.....	1.0 μ L
DNase I (5 U/ μ L)	2.0 μ L
總體積	30.0 μ L

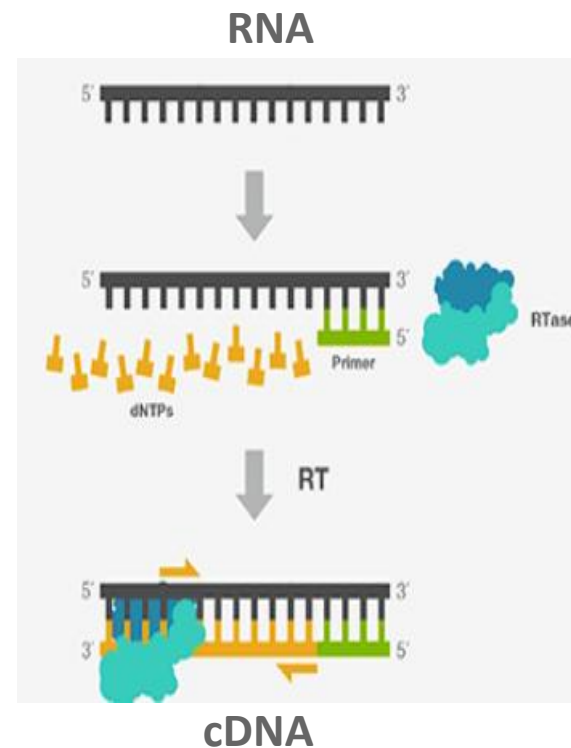
2.9.2. 混合液於 37°C 反應 30 分鐘，續以 75°C 反應 5 分鐘後，立即移置冰浴中，即為經 DNase I 處理之 RNA 溶液，供反轉錄反應用。

反轉錄反應 Reverse Transcription

2.10. 反轉錄反應：

2.10.1. 取微量離心管，依下表配製混合液：

病毒 RNA 溶液	5.0 μ L
5 倍 TBE 緩衝溶液	5.0 μ L
10 mM dNTP	4.0 μ L
25 mM 氯化鎂溶液	5.0 μ L
隨機引子(3 μ g/ μ L)	1.3 μ L
0.1 M DTT	2.5 μ L
核糖核酸水解酶抑制劑(40 U/ μ L)	1.4 μ L
反轉錄酶(200 U/ μ L)	0.8 μ L
總體積	25.0 μ L

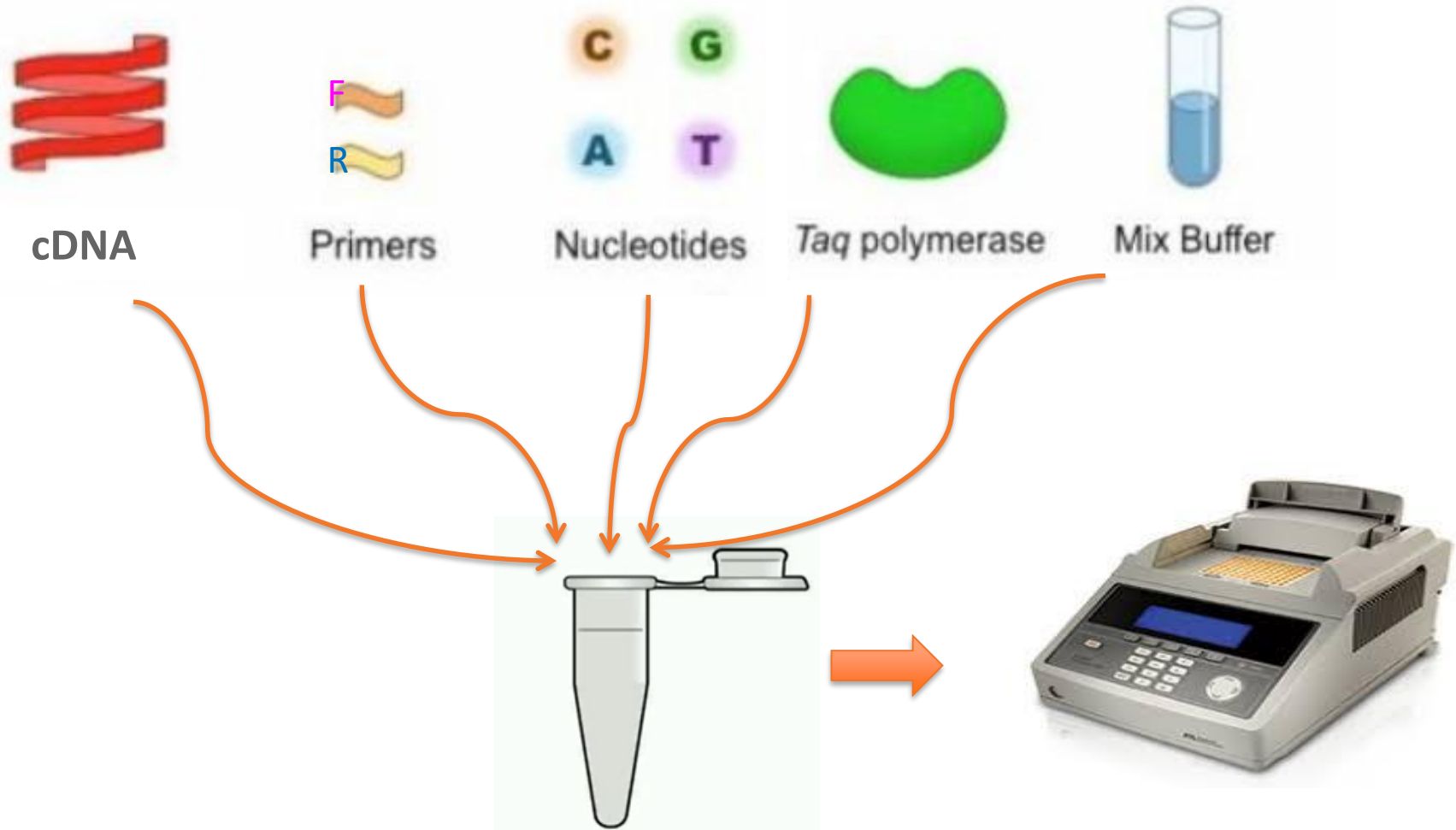


2.10.2. 混合液配製完成後，依下表條件進行反轉錄反應^(註3)

步驟	溫度(°C)	時間(min)
反轉錄	25	10
	50	50
	85	15

註3：對於同一管 RNA，應至少進行二重複反轉錄反應

聚合酶鏈反應 PCR



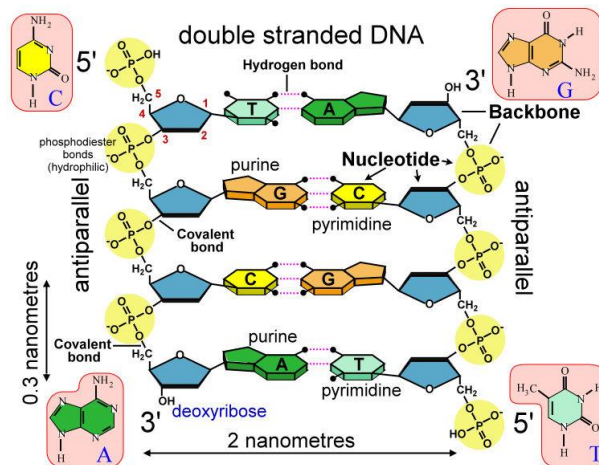
The diagram illustrates the process of DNA replication in three main stages:

- Initiation:** The original DNA double helix (green strands) is unwound. A DNA primer (red) is synthesized at the 3' end of one template strand. Nucleotides (blue) are added to the 5' end of the primer.
- Elongation:** The DNA polymerase (orange) moves along the template strand, synthesizing a new strand (blue) in the 5' to 3' direction. The template strand is labeled with 5' and 3' ends. The new strand is labeled with 3' and 5' ends. The process is labeled with a blue circle containing the number 1.
- Termination:** The DNA polymerase reaches the end of the template strand, and the new strand is released. The process is labeled with a blue circle containing the number 2.

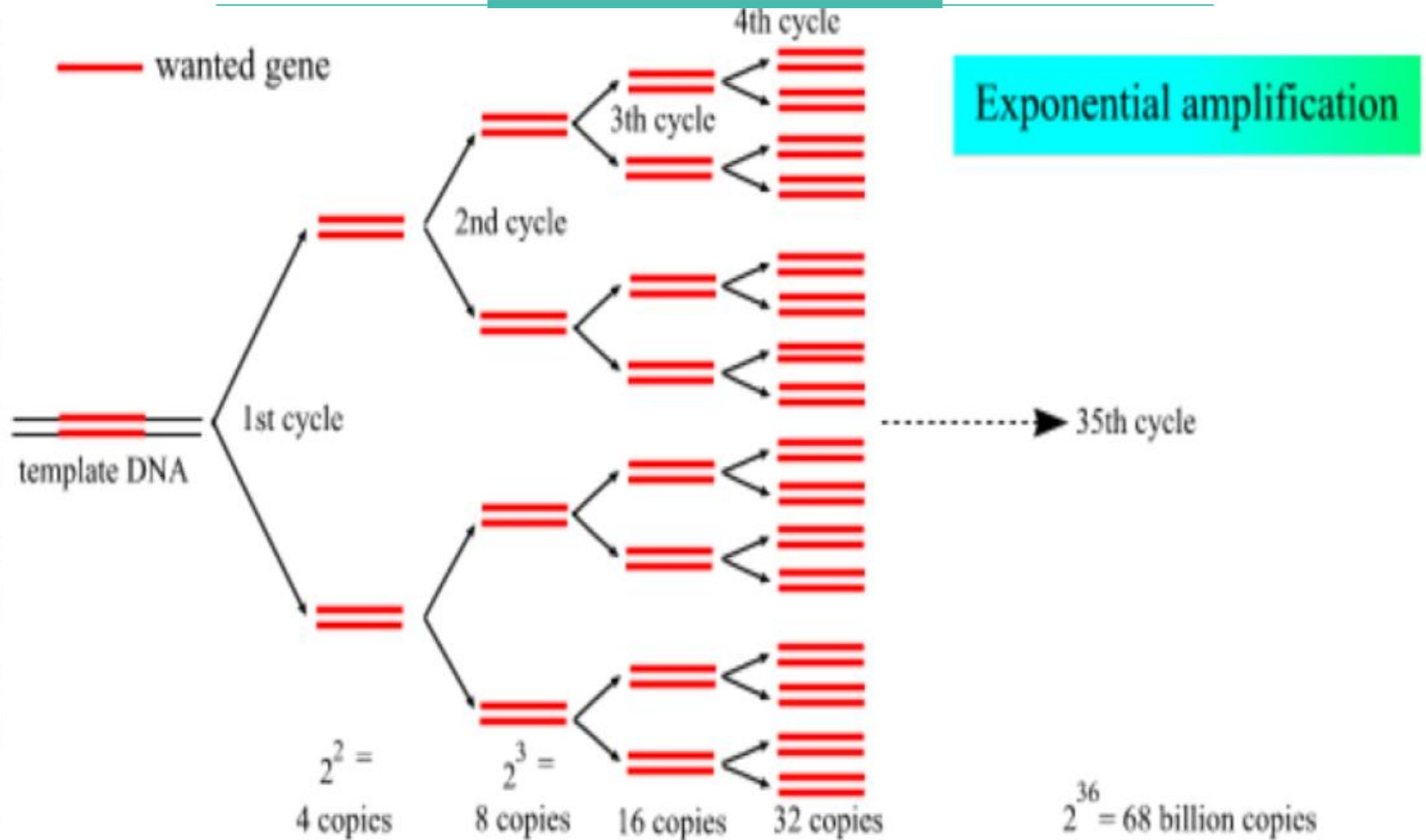
The final result is two identical DNA double helices, each consisting of one original template strand (green) and one newly synthesized strand (blue). The process is labeled with a blue circle containing the number 3.

Labels in the diagram include: original DNA to be replicated, DNA primer, nucleotide, 5', 3', R, F, double stranded DNA, Hydrogen bond, and a chemical structure of a nucleotide base (G) with its sugar and phosphate groups.

-  **polymerase**

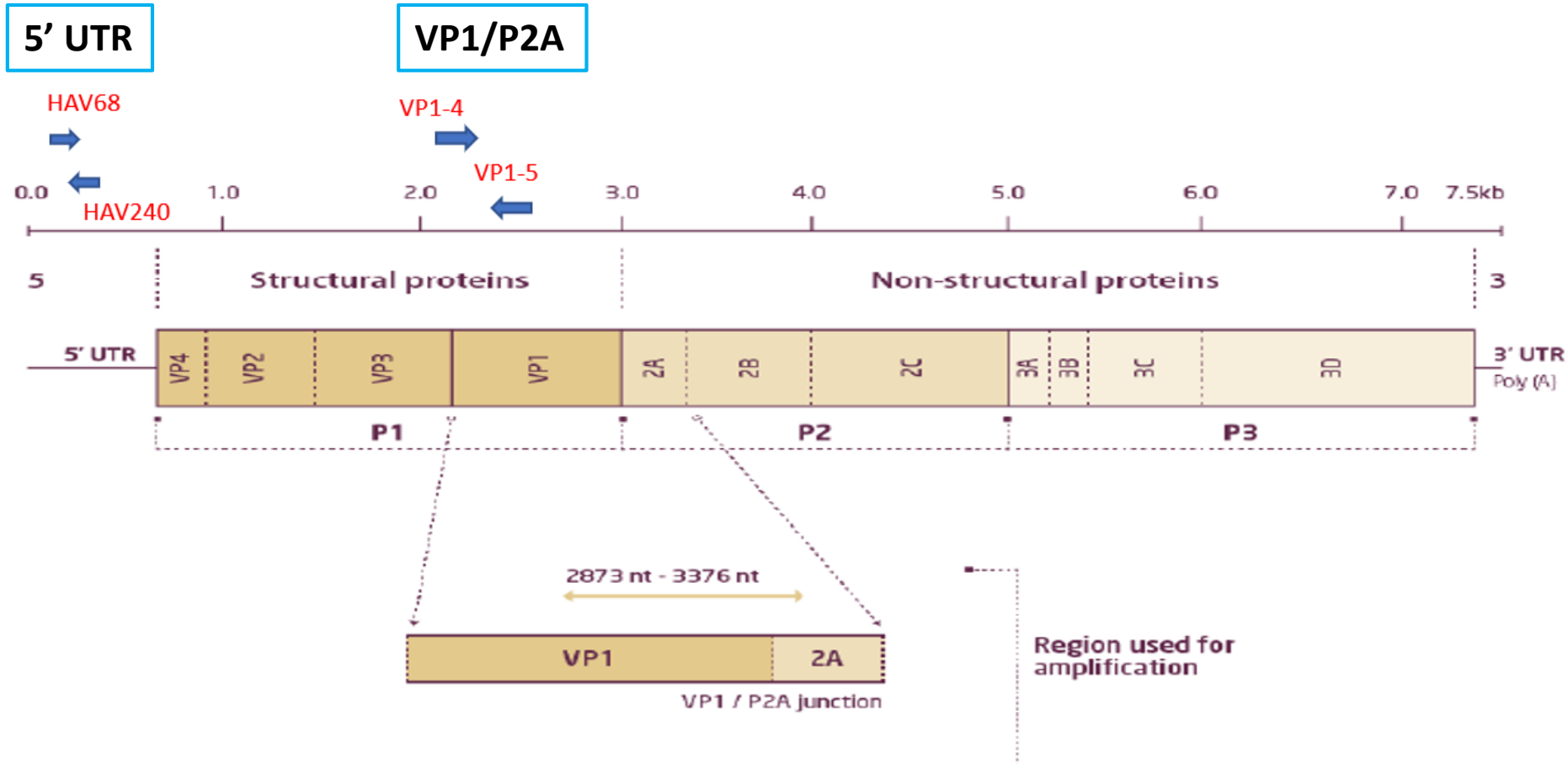


PCR-Polymerase chain reaction-2



PCR使用之引子對

HAV genome structure



PCR反應條件

2.11. 第一次聚合酶鏈反應(PCR)：

2.11.1. 取微量離心管，依下表配製第一次 PCR 混合液：

cDNA 產物	5.0 µL
10 倍 PCR 緩衝溶液(含 20 mM 氯化鎂)	5.0 µL
2.5 mM dNTP	4.0 µL
10 µM 引子 F ^(註 4)	1.0 µL
10 µM 引子 R ^(註 4)	1.0 µL
DNA 聚合酶(5 U/µL)	0.5 µL
無菌去離子水	33.5 µL
總體積	50.0 µL

2.12. 第二次 PCR：

2.12.1. 取微量離心管，依下表配製第二次 PCR 混合液：

第一次 PCR 產物之稀釋溶液 ^(註 5)	5.0 µL
10 倍 PCR 緩衝溶液(含 20 mM 氯化鎂)	5.0 µL
2.5 mM dNTP	4.0 µL
10 µM 引子 F ^(註 6)	1.0 µL
10 µM 引子 R ^(註 6)	1.0 µL
DNA 聚合酶(5 U/µL)	0.5 µL
無菌去離子水	33.5 µL
總體積	50.0 µL

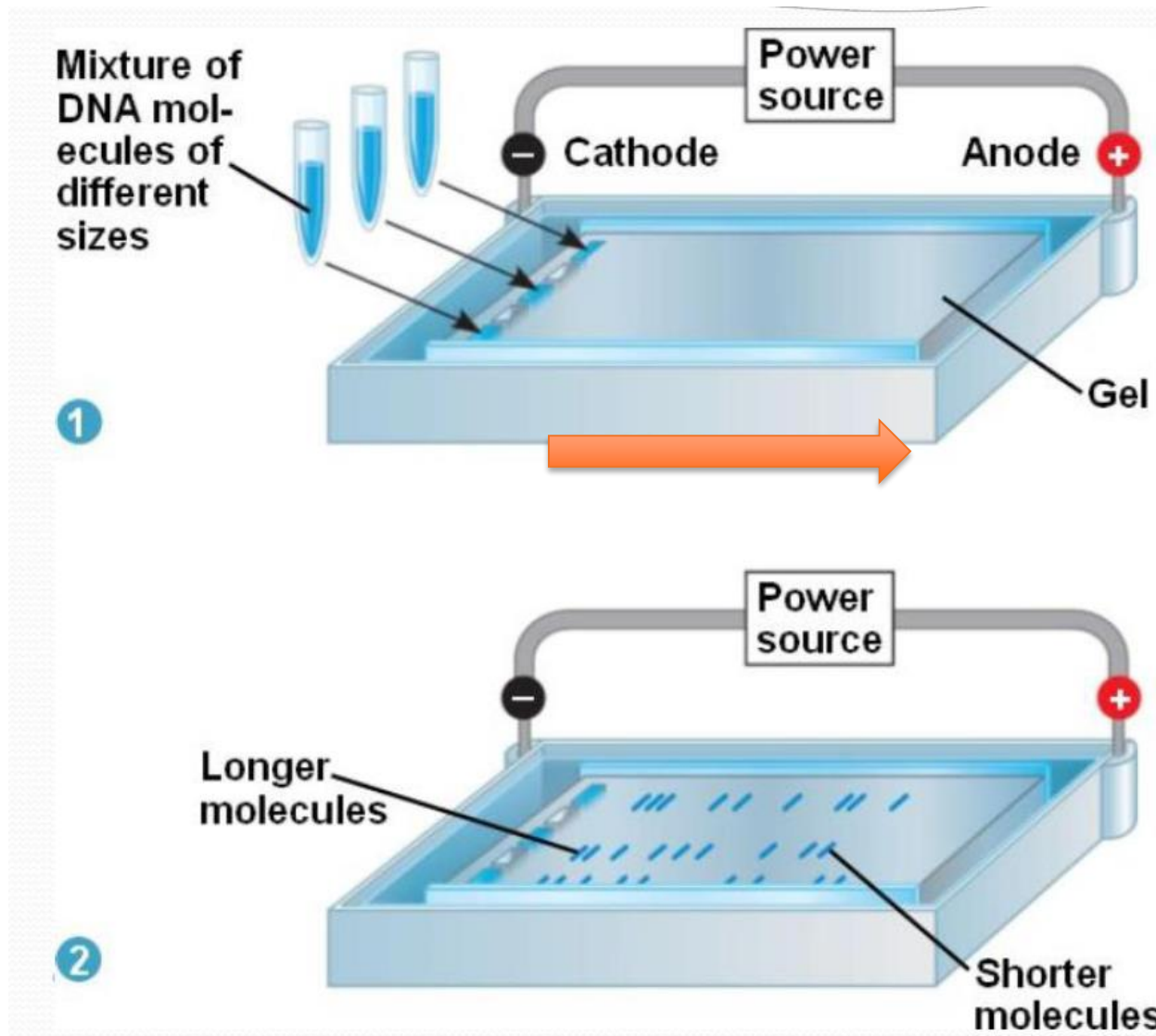
2.11.2. 混合液配製後，依下表條件進行 PCR：

步驟	溫度	時間
1.最初變性	95°C	4 min
2.變性	95°C	30 sec
3.黏接	50°C	30 sec
4.延展	72°C	1 min
步驟 2 至步驟 4，共進行 40 個循環反應。		
5.最終延展	72°C	7 min

2.12.2. 混合液配製後依下表條件進行 PCR：

步驟	溫度	時間
1.最初變性	95°C	4 min
2.變性	95°C	30 sec
3.黏接	60°C	30 sec
4.延展	72°C	1 min
步驟 2 至步驟 4，共進行 40 個循環反應。		
5.最終延展	72°C	7 min

電泳分析



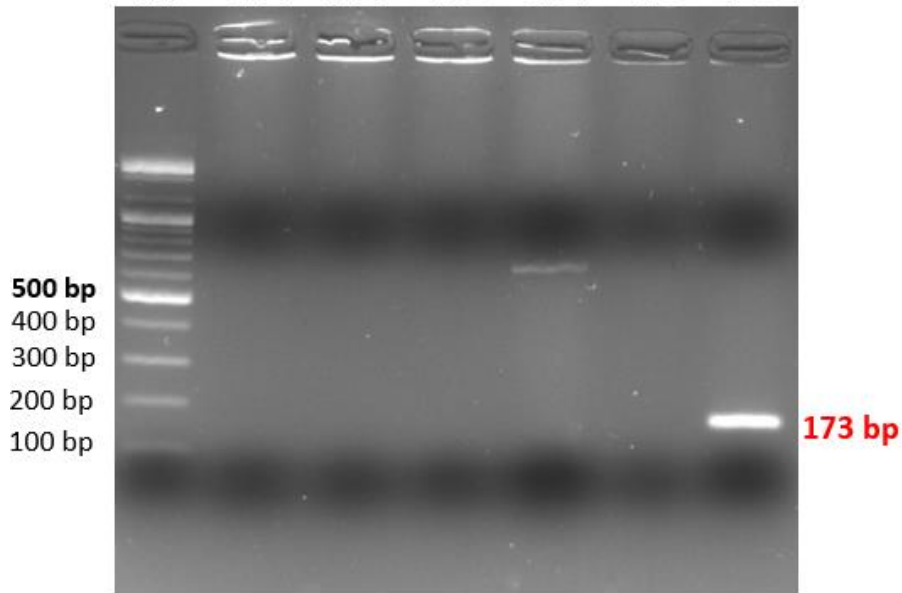
電泳分析結果

引子對 HAV68/HAV240

HAV(5'UTR)-173 bp

cDNA 1 cDNA 2

M 1-1 1-2 2-1 2-2 NC PC

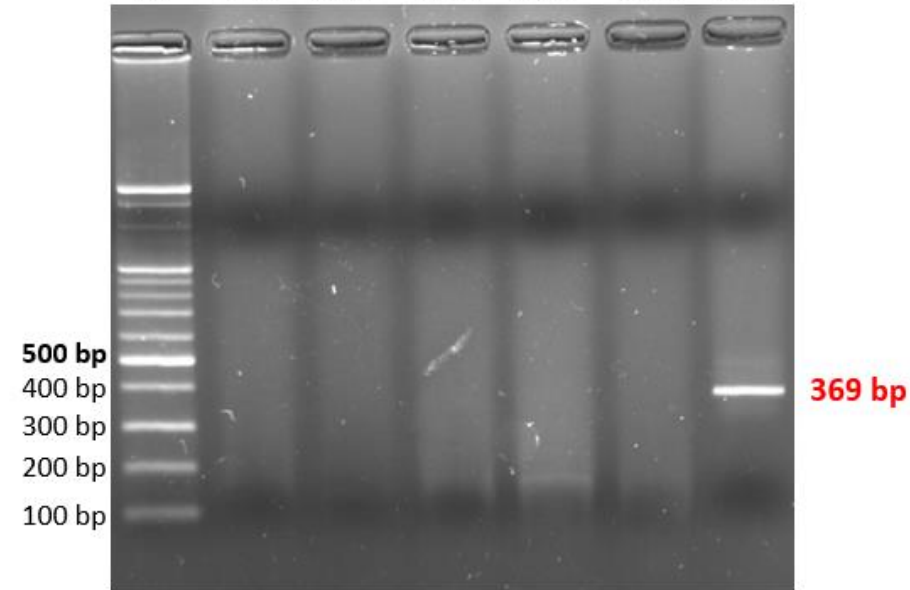


引子對 VP1-4/VP1-5

HAV (VP1/P2A)-369 bp

cDNA 1 cDNA 2

M 1-1 1-2 2-1 2-2 NC PC



定序結果



BioEdit

UTR-HAV68 (F)

(定序長度144 bp)

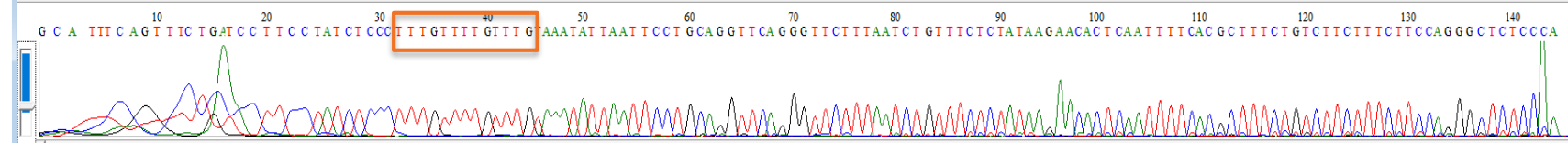
BioEdit Sequence Alignment Editor

File Edit view Zoom Horizontal Scale Accessory Application RNA Window Help



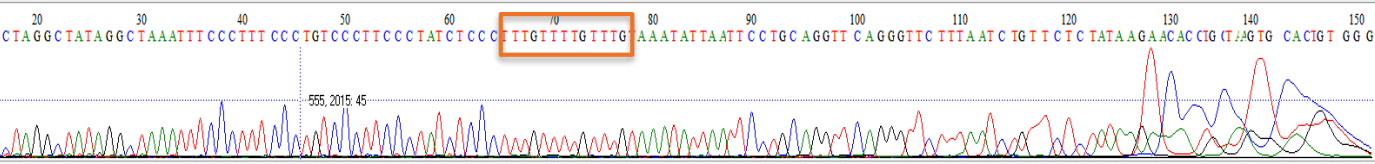
ABI Chromatogram: C:\Users\shingyuan\AppData\Local\Temp\15\Temp529781e-b201-498b-aac7-89c082aee5b0_SD230506004_230508K08D.zip\UTR-560_HAV68.ab1

Selected: none Sample: UTR-560_HAV68 File: C:\Users\shingyuan\AppData\Local\Temp\15\Temp529781e-b201-498b-aac7-89c082aee5b0_SD230506004_230508K08D.zip\UTR-560_HAV68.ab1



ABI Chromatogram: C:\Users\shingyuan\AppData\Local\Temp\15\Tempf9873d56-0ca6-4d28-af06-2e572253ef27_SD230506004_230508K08D.zip\UTR-560_HAV240.ab1

Selected: none Sample: UTR-560_HAV240 File: C:\Users\shingyuan\AppData\Local\Temp\15\Tempf9873d56-0ca6-4d28-af06-2e572253ef27_SD230506004_230508K08D.zip\UTR-560_HAV240.ab1



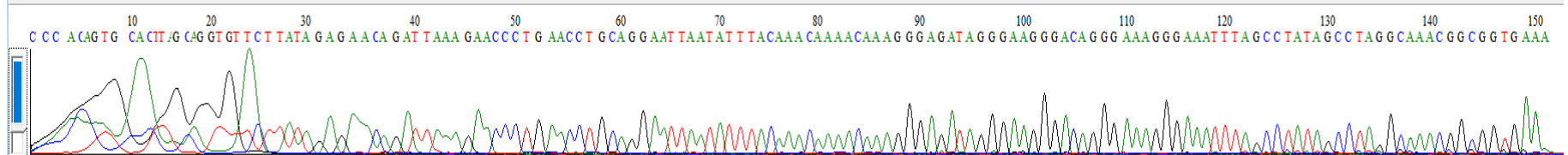
UTR-HAV240
(Reverse)

UTR-HAV240(R)

(定序長度151 bp)

ABI Chromatogram: C:\Users\shingyuan\AppData\Local\Temp\15\Tempf9873d56-0ca6-4d28-af06-2e572253ef27_SD230506004_230508K08D.zip\UTR-560_HAV240.ab1

Selected: none Sample: UTR-560_HAV240 File: C:\Users\shingyuan\AppData\Local\Temp\15\Tempf9873d56-0ca6-4d28-af06-2e572253ef27_SD230506004_230508K08D.zip\UTR-560_HAV240.ab1



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BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

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Thu, 24 Aug 2023

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Web BLAST

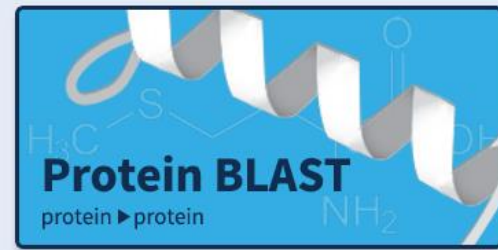


blastx

translated nucleotide ► protein

tblastn

protein ► translated nucleotide



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定序結果上傳NCBI比對

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear

Query subrange ?

From

To

ACTTCGCTATGCTCTGTTCATTGTCCCTCT
CTCCTCTAGTCTCTGTCTTGCCCCCTGCCCCCTTTGTTTCAGCCTTTCT
TCCAGGCTCTCCA

Or, upload file 未選擇任何檔案 ?

Job Title

Enter a descriptive title for your BLAST search ?

☐ Align two or more sequences ?

Choose Search Set

Database ☒ Standard databases (nr etc.): ☐ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus

New ☐ Experimental databases [Try experimental taxonomic nt databases](#)
For more info see [What are taxonomic nt databases?](#)

Nucleotide collection (nr/nt) ?

Organism Optional ☐ exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown ?

Exclude Optional ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to Optional ☐ Sequences from type material

Entrez Query Optional [YouTube](#) [Create custom database](#)

Program Selection

Optimize for ☒ Highly similar sequences (megablast)
☐ More dissimilar sequences (discontiguous megablast)
☐ Somewhat similar sequences (blastn)
Choose a BLAST algorithm ?

BLAST Search **database Nucleotide collection (nr/nt)** using **Megablast (Optimize for highly similar sequences)**
☐ Show results in a new window

UTR-HAV68 (序列長度144 bp)

Job Title	Nucleotide Sequence
RID	HR4A59AK013 Search expires on 10-04 11:57 am Download All ▾
Program	BLASTN ? Citation ▾
Database	nt See details ▾
Query ID	lcl Query_30177
Description	None
Molecule type	dna
Query Length	144
Other reports	Distance tree of results MSA viewer ?

Filter Results

Organism only top 20 will appear ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity

E value

Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len
228	228	87%	3e-55	99.21%	675
222	222	87%	1e-53	98.43%	7477

Descriptions

Graphic Summary

Alignments

Taxonomy

Sequences producing significant alignments

Download ▾

Select columns ▾

Show 100 ▾



☒ select all 100 sequences selected

[GenBank](#)

[Graphics](#)

[Distance tree of results](#)

[MSA Viewer](#)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒ Hepatitis A virus isolate G1B3-IRES 5' UTR	Hepatitis A virus	228	228	87%	3e-55	99.21%	675	FJ829475.1
☒ Hepatitis A virus isolate HAV-Arg/06 complete genome	Hepatitis A virus	222	222	87%	1e-53	98.43%	7477	HM769724.1
☒ Hepatitis A virus isolate G1B2-IRES 5' UTR	Hepatitis A virus	222	222	87%	1e-53	98.43%	675	FJ829474.1
☒ Hepatitis A virus isolate HAV12 complete genome	Hepatitis A virus	217	217	86%	6e-52	98.39%	7453	EU526089.1
☒ Hepatitis A virus gene 5' UTR partial sequence isolate: HA-JNG05-91	Hepatitis A virus	215	215	84%	2e-51	98.36%	395	AB245898.1
☒ Hepatitis A virus isolate G1B1-IRES 5' UTR	Hepatitis A virus	226	226	90%	1e-54	97.74%	675	FJ829473.1
☒ Hepatitis A virus isolate G1B4-IRES 5' UTR	Hepatitis A virus	226	226	90%	1e-54	97.73%	675	FJ829476.1
☒ Human hepatitis A virus HA18-0838 RNA complete genome	Human hepatitis A virus	217	217	87%	6e-52	97.64%	7494	LC416595.1
☒ Human hepatitis A virus HA18-1167 RNA complete genome	Human hepatitis A virus	217	217	87%	6e-52	97.64%	7494	LC435032.1



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序列比對Alignments

Hepatitis A virus isolate G1B3-IRES 5' UTR

Sequence ID: [FJ829475.1](#) Length: 675 Number of Matches: 1

Range 1: 53 to 179 [GenBank](#) [Graphics](#)

▼ Next Match

Score	Expect	Identities	Gaps	Strand
228 bits(123)	3e-55	126/127(99%)	1/127(0%)	Plus/Plus
Query 18	CCTT-CCTATCTCCCTTTGTTTTGTTTGTAATATTAATTCTGCAGGTTCAGGGTTCTT	76		
Sbjct 53	CCTTCCCTATCTCCCTTTGTTTTGTTTGTAATATTAATTCTGCAGGTTCAGGGTTCTT	112		
Query 77	TAATCTGTTTCTCTATAAGAACACTCAATTTTCACGCTTTCTGTCTTTCTTTCCAGGG	136		
Sbjct 113	TAATCTGTTTCTCTATAAGAACACTCAATTTTCACGCTTTCTGTCTTTCTTTCCAGGG	172		
Query 137	CTCTCCC 143			
Sbjct 173	CTCTCCC 179			

Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len
228	228	87%	3e-55	99.21%	675
222	222	87%	1e-53	98.43%	7477

Hepatitis A virus isolate HAV-Arg/06, complete genome

Sequence ID: [HM769724.1](#) Length: 7477 Number of Matches: 1

Range 1: 115 to 241 [GenBank](#) [Graphics](#)

▼ Next Match

Score	Expect	Identities	Gaps	Strand
222 bits(120)	1e-53	125/127(98%)	1/127(0%)	Plus/Plus
Query 18	CCTT-CCTATCTCCCTTTGTTTTGTTTGTAATATTAATTCTGCAGGTTCAGGGTTCTT	76		
Sbjct 115	CCTTCCCTATTTCCCTTTGTTTTGTTTGTAATATTAATTCTGCAGGTTCAGGGTTCTT	174		
Query 77	TAATCTGTTTCTCTATAAGAACACTCAATTTTCACGCTTTCTGTCTTTCTTTCCAGGG	136		
Sbjct 175	TAATCTGTTTCTCTATAAGAACACTCAATTTTCACGCTTTCTGTCTTTCTTTCCAGGG	234		
Query 137	CTCTCCC 143			
Sbjct 235	CTCTCCC 241			

Query coverage= 127/144



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UTR-HAV68 (序列長度125bp)

Job Title	Nucleotide Sequence
RID	HRFEXZWZ01R Search expires on 10-04 15:08 pm Download All ▾
Program	BLASTN ? Citation ▾
Database	nt See details ▾
Query ID	lcl Query_185205
Description	None
Molecule type	dna
Query Length	125
Other reports	Distance tree of results MSA viewer ?

Filter Results

Organism *only top 20 will appear* ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to **E value** to **Query Coverage** to

[Filter](#) [Reset](#)

Descriptions

[Graphic Summary](#)[Alignments](#)[Taxonomy](#)

Sequences producing significant alignments

[Download](#) ▾[Select columns](#) ▾Show [?](#)☒ select all 100 sequences selected[GenBank](#)[Graphics](#)[Distance tree of results](#)[MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Hepatitis A virus isolate G1B3-IRES 5' UTR	Hepatovirus A	226	226	97%	8e-55	100.00%	675	FJ829475.1
☒	Hepatitis A virus isolate G1B1-IRES 5' UTR	Hepatovirus A	226	226	97%	8e-55	100.00%	675	FJ829473.1
☒	Hepatovirus A strain HepA/USA_NY/51657/2019_partial genome	Hepatovirus A	220	220	97%	4e-53	99.18%	7361	ON524431.1
☒	Hepatitis A virus isolate A3_complete genome	Hepatovirus A	220	220	97%	4e-53	99.18%	7339	KC182589.1
☒	Hepatitis A virus isolate A2_complete genome	Hepatovirus A	220	220	97%	4e-53	99.18%	7339	KC182587.1
☒	Hepatitis A virus isolate HAV-Arg/06_complete genome	Hepatovirus A	220	220	97%	4e-53	99.18%	7477	HM769724.1
☒	Hepatitis A virus isolate G1B4-IRES 5' UTR	Hepatovirus A	220	220	97%	4e-53	99.18%	675	FJ829476.1
☒	Hepatitis A virus isolate G1B2-IRES 5' UTR	Hepatovirus A	220	220	97%	4e-53	99.18%	675	FJ829474.1
☒	Hepatitis A virus gene_5' UTR_partial sequence_isolate: HA-JNG02-91	Hepatovirus A	220	220	97%	4e-53	99.18%	395	AB245895.1



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序列比對Alignments

Hepatitis A virus isolate G1B3-IRES 5' UTR

Sequence ID: [FJ829475.1](#) Length: 675 Number of Matches: 1

Range 1: 58 to 179 [GenBank](#) [Graphics](#)

▼ Next Match

Score	Expect	Identities	Gaps	Strand
226 bits(122)	8e-55	122/122(100%)	0/122(0%)	Plus/Plus
Query 3	CCTATCTCCCTTTGTTTTGTTTGTAATATTAATTCCTGCAGGTTCAAGGTTCTTTAATC	62		
Sbjct 58	CCTATCTCCCTTTGTTTTGTTTGTAATATTAATTCCTGCAGGTTCAAGGTTCTTTAATC	117		
Query 63	TGTTTCTCTATAAGAACTCAATTTTCACGCTTCTGTCTTCTTTCTTCCAGGGCTCTC	122		
Sbjct 118	TGTTTCTCTATAAGAACTCAATTTTCACGCTTCTGTCTTCTTTCTTCCAGGGCTCTC	177		
Query 123	CC 124			
Sbjct 178	CC 179			

Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
226	226	97%	8e-55	100.00%	675	FJ829475.1
220	220	97%	4e-53	99.18%	7477	HM769724.1

Query coverage= 122/125

Hepatitis A virus isolate HAV-Arg/06, complete genome

Sequence ID: [HM769724.1](#) Length: 7477 Number of Matches: 1

Range 1: 120 to 241 [GenBank](#) [Graphics](#)

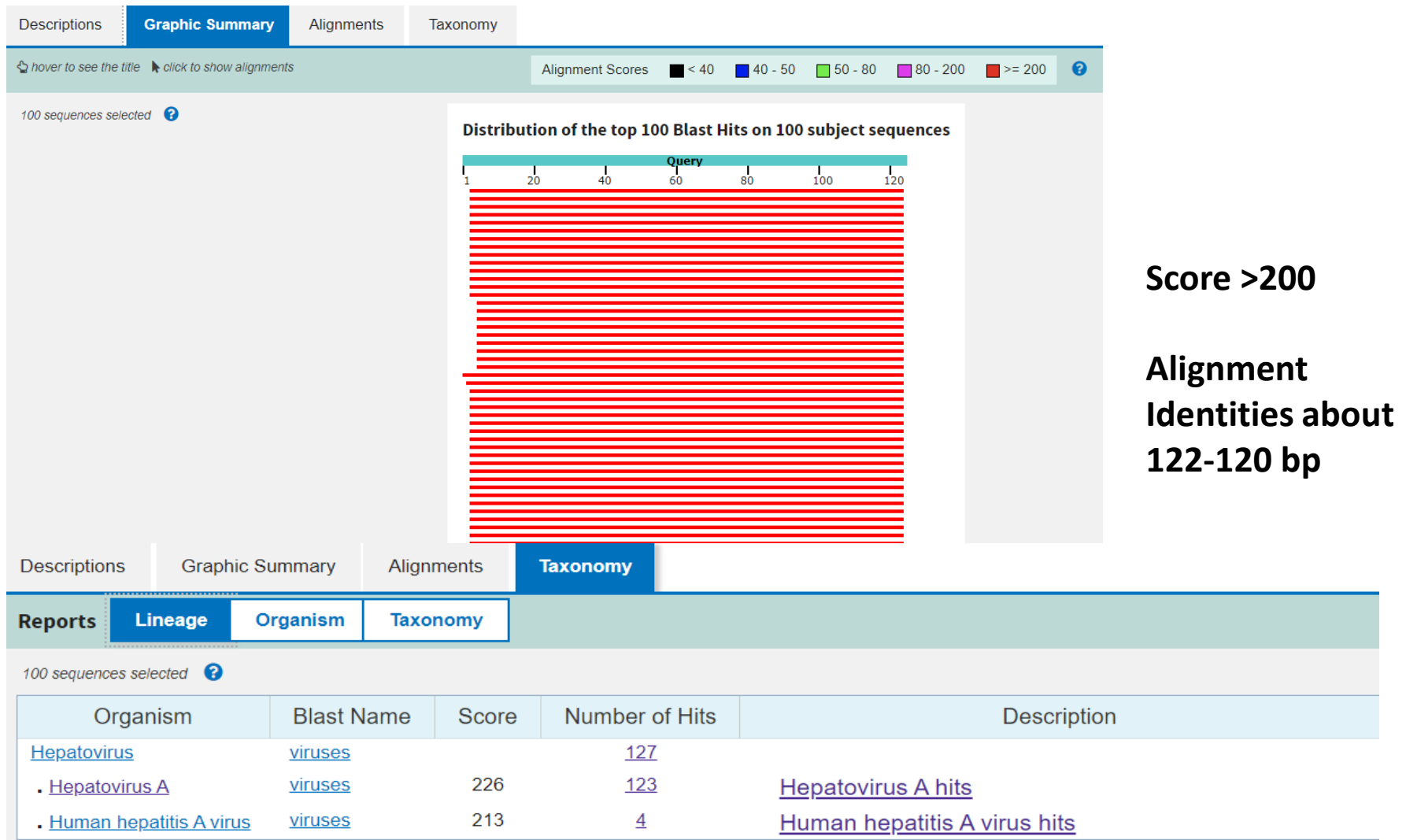
▼ Next Match

Score	Expect	Identities	Gaps	Strand
220 bits(119)	4e-53	121/122(99%)	0/122(0%)	Plus/Plus
Query 3	CCTATCTCCCTTTGTTTTGTTTGTAATATTAATTCCTGCAGGTTCAAGGTTCTTTAATC	62		
Sbjct 120	CCTATTTCCCTTTGTTTTGTTTGTAATATTAATTCCTGCAGGTTCAAGGTTCTTTAATC	179		
Query 63	TGTTTCTCTATAAGAACTCAATTTTCACGCTTCTGTCTTCTTTCTTCCAGGGCTCTC	122		
Sbjct 180	TGTTTCTCTATAAGAACTCAATTTTCACGCTTCTGTCTTCTTTCTTCCAGGGCTCTC	239		
Query 123	CC 124			
Sbjct 240	CC 241			



比對分數圖示及分類

Graphic Summary and Taxonomy



UTR-HAV240 (序列長度151bp)

Job Title	Nucleotide Sequence		
RID	HRF0KNY3016	Search expires on 10-04 15:00 pm	Download All ▾
Program	BLASTN ?	Citation ▾	
Database	nt	See details ▾	
Query ID	cl Query_409437		
Description	None		
Molecule type	dna		
Query Length	151		
Other reports	Distance tree of results	MSA viewer	?

Filter Results

Organism only top 20 will appear ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to **E value** to **Query Coverage** to

[Filter](#) [Reset](#)

Descriptions

[Graphic Summary](#)[Alignments](#)[Taxonomy](#)

Sequences producing significant alignments

[Download](#) ▾[Select columns](#) ▾Show [?](#)☒ select all 100 sequences selected[GenBank](#)[Graphics](#)[Distance tree of results](#)[MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Hepatitis A virus isolate HAV-Arg/06, complete genome	Hepatovirus A	230	230	86%	8e-56	98.47%	7477	HM769724.1
☒	Hepatitis A virus isolate G1B3-IRES 5' UTR	Hepatovirus A	230	230	86%	8e-56	98.47%	675	FJ829475.1
☒	Hepatitis A virus isolate G1B1-IRES 5' UTR	Hepatovirus A	230	230	86%	8e-56	98.47%	675	FJ829473.1
☒	Hepatovirus A strain HepA/USA_PA/42382/2019, partial genome	Hepatovirus A	224	224	86%	4e-54	97.71%	7361	ON524425.1
☒	Hepatitis A virus isolate G1B2-IRES 5' UTR	Hepatovirus A	224	224	86%	4e-54	97.71%	675	FJ829474.1
☒	Hepatitis A virus GBM/HFS RNA	Hepatovirus A	224	224	86%	4e-54	97.71%	7446	X75216.1
☒	Hepatitis A virus genome, 5'UTR	Hepatovirus A	224	224	86%	4e-54	97.71%	709	X70846.1
☒	Hepatovirus A genomic RNA, complete genome isolate: MNA06-2148	Hepatovirus A	219	219	86%	2e-52	96.95%	7492	LC049342.1



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PCR產物未比對到疑似序列

[← Edit Search](#)[Save Search](#)[Search Summary ▼](#)[? How to read this report?](#)[▶ BLAST Help Videos](#)[↶ Back to Traditional Results Page](#)

Job Title	Nucleotide Sequence
RID	HRKC77EF01R Search expires on 10-04 16:15 pm Download All ▼
Program	? Citation ▼
Database	nt See details ▼
Query ID	Icl Query_157081
Description	None
Molecule type	dna
Query Length	123
Other reports	?

Filter Results

Percent Identity

 to

E value

 to

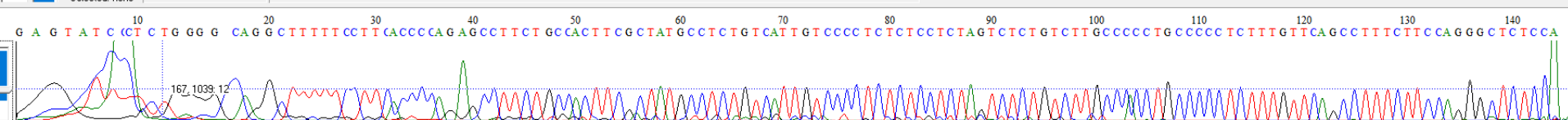
Query Coverage

 to [Filter](#)[Reset](#)

No significant similarity found. For reasons why, [click here](#)

ABI Chromatogram: C:\Users\shingyuan\AppData\Local\Temp\15\Temp7582c2ba-742b-40aa-beed-9e9b61b2ea66_SD230506004_230508K08D.zip\D-716-UTR_HAV68.ab1

Selected: none Sample: D-716-UTR_HAV68 File: C:\Users\shingyuan\AppData\Local\Temp\15\Temp7582c2ba-742b-40aa-beed-9e9b61b2ea66_SD230506004_230508K08D.zip\D-716-UTR_HAV68.ab1



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食品基質造成非專一性PCR產物

Job Title	Nucleotide Sequence
RID	HRNYMKA401R Search expires on 10-04 16:59 pm Download All ▼
Program	BLASTN ? Citation ▼
Database	nt See details ▼
Query ID	lc Query_372299
Description	None
Molecule type	dna
Query Length	137
Other reports	Distance tree of results MSA viewer ?

Filter Results

Organism only top 20 will appear ☐ exclude

Type common name, binomial, taxid or group name

+ [Add organism](#)

Percent Identity

E value

Query Coverage

to

to

to

Filter

Reset

Descriptions

Graphic Summary

Alignments

Taxonomy

Sequences producing significant alignments

Download ▼

Select columns ▼

Show 100 ▼



☒ select all 1 sequences selected

[GenBank](#)

[Graphics](#)

[Distance tree of results](#)

[MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	PREDICTED: <i>Fragaria vesca</i> subsp. <i>vesca</i> acetylornithine aminotransferase, mitochondrial-like (LOC101295...	<i>Fragaria vesca</i> s...	126	126	54%	1e-24	97.30%	1695	XM_004294056.2

PREDICTED: *Fragaria vesca* subsp. *vesca* acetylornithine aminotransferase, mitochondrial-like (LOC101295312), mRNA

Sequence ID: [XM_004294056.2](#) Length: 1695 Number of Matches: 1

Range 1: 1 to 74 [GenBank](#) [Graphics](#)

▼ [Next Match](#) ▲ [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
126 bits(68)	1e-24	72/74(97%)	0/74(0%)	Plus/Plus
Query 40	AACACAGTGTGAGCCCGTCGTTTGTATCCCTAGTTGCACAACACGAGTCATCTGTCTTC	99		
Sbjct 1	AACACAGTGTGAGCCCGCCGTTTGTATCCCTAGTTGCACATCAGCAGTCATCTGTCTTC			
Query 100	TTTTTGCTTTCTTC	113		
Sbjct 61	TTTTTGCTTTCTTC	74		

Related Information

[Gene](#) - associated gene details

[Genome Data Viewer](#) - aligned genomic context

屬： 草莓屬 *Fragaria*

種： 野草莓 *F. vesca*



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Trouble Shooting

Q: 電泳結果有非專一性條帶？

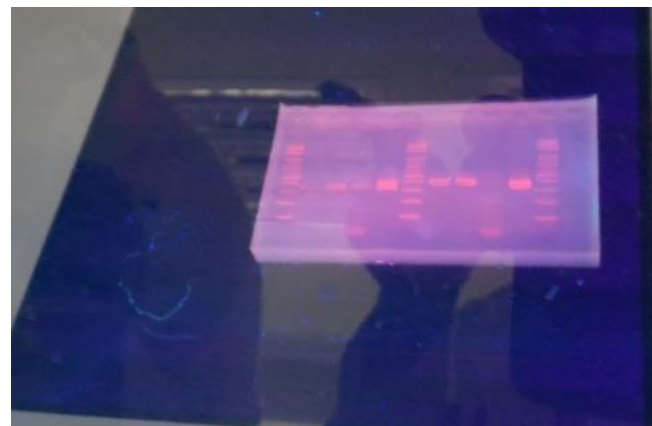
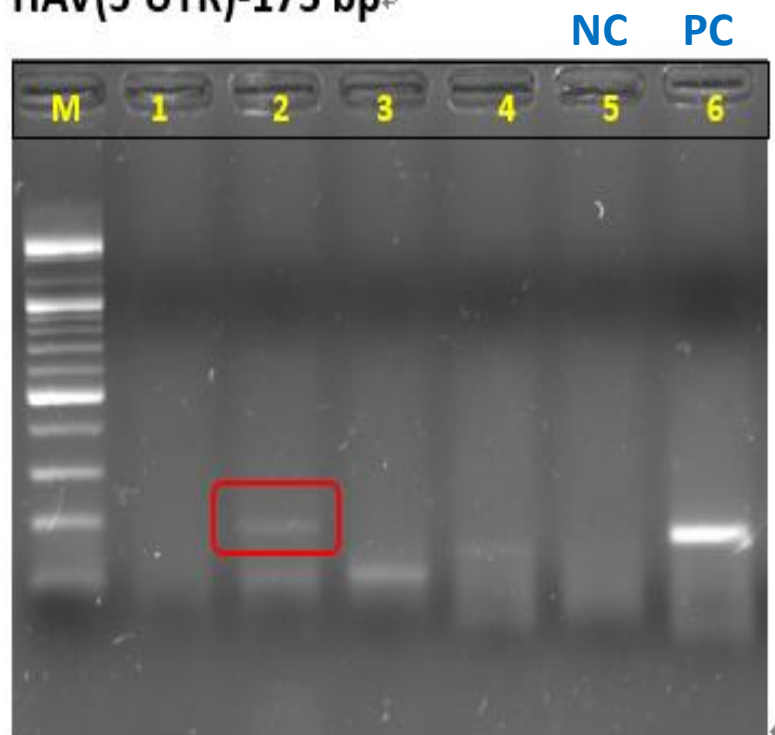
Q: 檢體只有一組引子對擴增出PCR產物？

Q: 如何與正對照比對確認無汙染

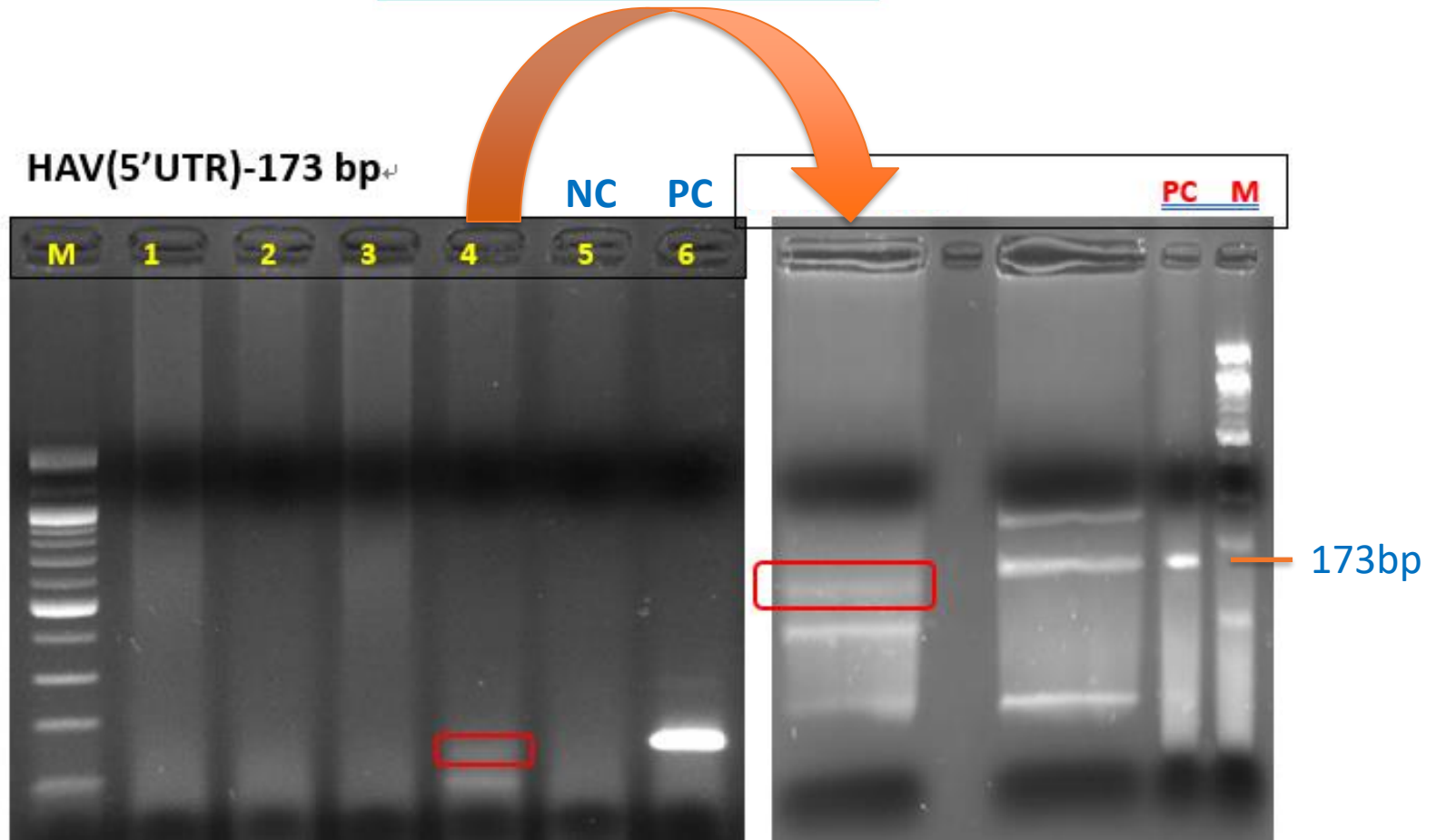
Q: 定序結果不佳

有非專一性條帶-手動切膠送定序

HAV(5'UTR)-173 bp



High percentage gel (3 %)增加解析度



條帶大小不符合，可排除

Trouble Shooting

Q: 電泳結果有非專一性條帶？

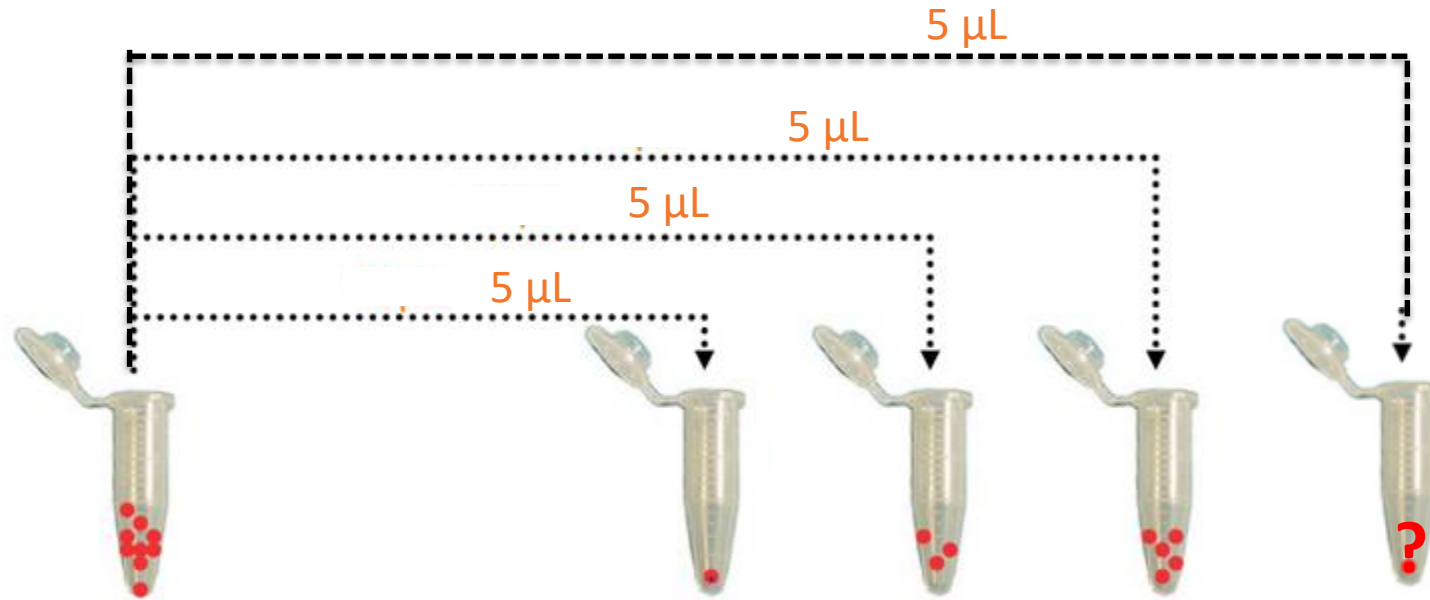
Q: 檢體只有一組引子對擴增出PCR產物？

Q: 如何與正對照比對確認無汙染

Q: 定序結果不佳

當檢體中含有A型肝炎病毒時，引子HAV68/HAV240在173 bp位置、引子VP1-4/VP1-5在369 bp位置上應各有一明顯DNA螢光帶

均勻分配的機率



9 molecules in 25 µL
(cDNA)

HAV68/HAV240

VP1-4/VP1-5

If 90 molecules in 25 µL ?

Trouble Shooting

Q: 電泳結果有非專一性條帶？

Q: 檢體只有一組引子對擴增出PCR產物？

Q: 如何與正對照比對確認無汙染

Q: 定序結果不佳

與正對照組進行比對

未知

正對照組

The image shows the NCBI BLAST search interface. It has tabs for different BLAST programs: **blastn**, blastp, blastx, tblastn, and tblastx. The **blastn** tab is selected.

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

AAGGAACTGATGTATGTCTGGATTCTCCAGGTTTCAATTCAGGAAATGTCT
CAGGTACTTTCTTTGCTAA
AACTGGATCCTCAATTGTTGTATAGCTCCACAGGTGCTTGACTCCTG
AAACATCCATTTCCCTCTG

Query subrange [?](#)

From

To

Or, upload file 未選擇任何檔案 [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

☒ Align two or more sequences [?](#)

Enter Subject Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

CAGGACTGGATGAGCGTGTAGGATTCTCCAGGTTTCAATTCAGGAAATGTC
TCAGGTACTTTCTTTGCTAA
AACTGGATCCTCAATTGTTGTATAGCTCCACAGGTGCTTGACTCCTG
AAACATCCATTTCCCTCTG

Subject subrange [?](#)

From

To

Or, upload file 未選擇任何檔案 [?](#)

Program Selection

Optimize for

☒ Highly similar sequences (megablast)

☐ More dissimilar sequences (discontiguous megablast)

☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm [?](#)

BLAST

Search **nucleotide sequence** using **Megablast (Optimize for highly similar sequences)**

☐ Show results in a new window

Annotations: A pink arrow points to the query sequence input field. A green arrow points to the 'Align two or more sequences' checkbox. A pink arrow points to the subject sequence input field. A pink arrow points to the 'BLAST' button.

序列比對Alignments

Job Title	Nucleotide Sequence
RID	HRWRBUJU114 Search expires on 10-04 18:54 pm Download All ▼
Program	Blast 2 sequences Citation ▼
Query ID	lcl Query_45117 (dna)
Query Descr	None
Query Length	342
Subject ID	lcl Query_45119 (dna)
Subject Descr	None
Subject Length	342
Other reports	MSA viewer ?

Filter Results

Percent Identity	E value	Query Coverage
to	to	to
Filter		Reset

Descriptions		Graphic Summary	Alignments	Dot Plot
Sequences producing significant alignments				
Download ▼ Select columns ▼ Show 100 ?				
☒ select all 1 sequences selected				
Description		Scientific Name		
☒ None provided				
Range 1: 21 to 342 Graphics		▼ Next Match ▲		
Score	Expect	Identities	Gaps	Strand
584 bits(316)	1e-171	321/323(99%)	1/323(0%)	Plus/Plus
Query 20	GGATTCTCCAGGTTTCAATTCAGGAAATGCTCAGGTACTTTCTTTGCTAAACTGGATC	79		
Sbjct 21	GGATTCTCCAGGTTTC-ATTCAGGAAATGCTCAGGTACTTTCTTTGCTAAACTGGATC	79		
Query 80	CTCAATTGTTGTGATAGCTCCACAGGTGCTTGACTCCTGAAACATCCATTTCCCTCT	139		
Sbjct 80	CTCAATTGTTGTGATAGCTCCACAGGTGCTTGACTCCTGAAACATCCATTTCCCTCT	139		
Query 140	GTTAGCTTTTCCTTTCAAATCTTTTCATGGTGTGTATACCAACTTGGGGATCTGGAACATT	199		
Sbjct 140	GTTAGCTTTTCCTTTCAAATCTTTTCATGGTGTGTATACCAACTTGGGGATCTGGAACATT	199		
Query 200	CTGTTCTGTAGAAACTGTTGTTGAAAAACCTCCAGAATCATCTCCAACCTGTGTAGTAAC	259		
Sbjct 200	CTGTTCTGTAGAAACTGTTGTTGAAAAACCTCCAGAATCATCTCCAACCTGTGTAGTAAC	259		
Query 260	ATCCATAGCATGATAAAGAGGAGCAAAACATTCCTCAAGTTAATTGCTGAAAGATAAACATT	319		
Sbjct 260	ATCCATAGCATGATAAAGAGGAGCAAAACATTCCTCAAGTTAATTGCTGAAAGATAAACATT	319		
Query 320	CACTCTGACATGGCAAGCAACGA	342		
Sbjct 320	CACTCTGACATGGGAAGCAACGA	342		

Trouble Shooting

Q: 電泳結果有非專一性條帶？

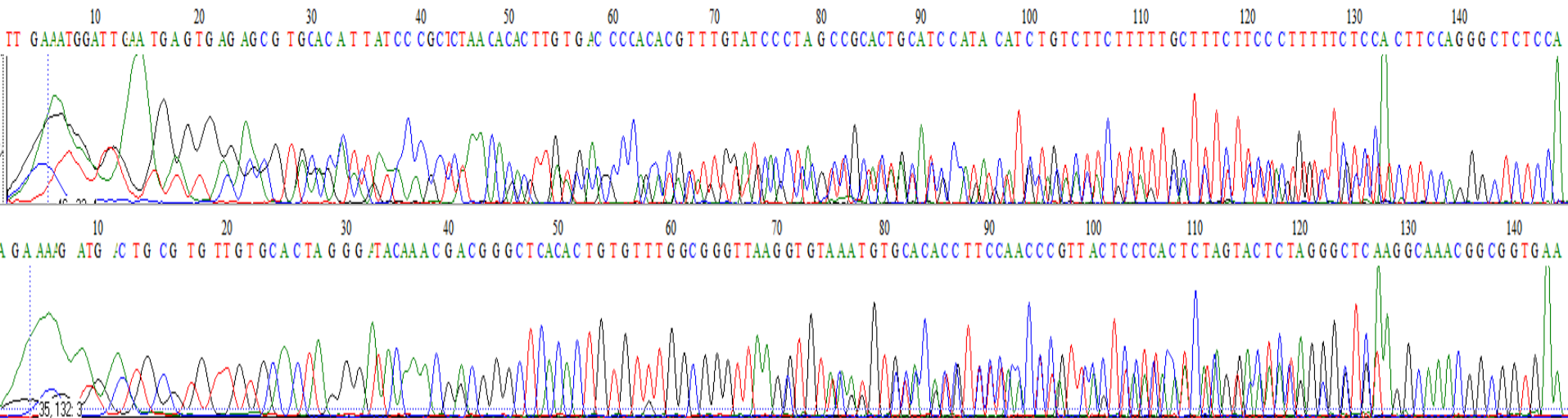
Q: 檢體只有一組引子對擴增出PCR產物？

Q: 如何與正對照比對確認無汙染

Q: 定序結果不佳

定序結果-訊號雜亂

定序結果	可能原因	建議事項
(2) 訊號雜亂 [▽]	(2-1) Primer 專一性不佳 [▽]	(2-1a) 重新設計 Primer(最適 Tm-50°C~55°C) 或更換 Primer [▽]
[▽]	(2-2) DNA 濃度不足 [▽]	(2-2a) 提高 DNA 濃度 [▽]
[▽]	(2-3) Primer degraded [▽] (N-1, N-2...) [▽]	(2-3a) 確認 primer 保存方式或更換使用 fresh primer [▽]
[▽]	(2-4) Multiple templates [▽]	(2-4a) 非單一產物請重新純化分離單一 template [▽]
[▽]	[▽]	(2-4b) 請重新純化單一菌落 [▽]
[▽]	(2-5) Multiple priming sites [▽]	(2-5a) 更換其他具有單一黏合位點的 primer [▽]



食品中檢測食因性病毒之困難

目標物稀少

- 極少量的病毒存在食品中即可能引起食品中毒，且許多重要的食因性病毒無法藉由細胞培養或培養困難，造成待測目標稀少的問題

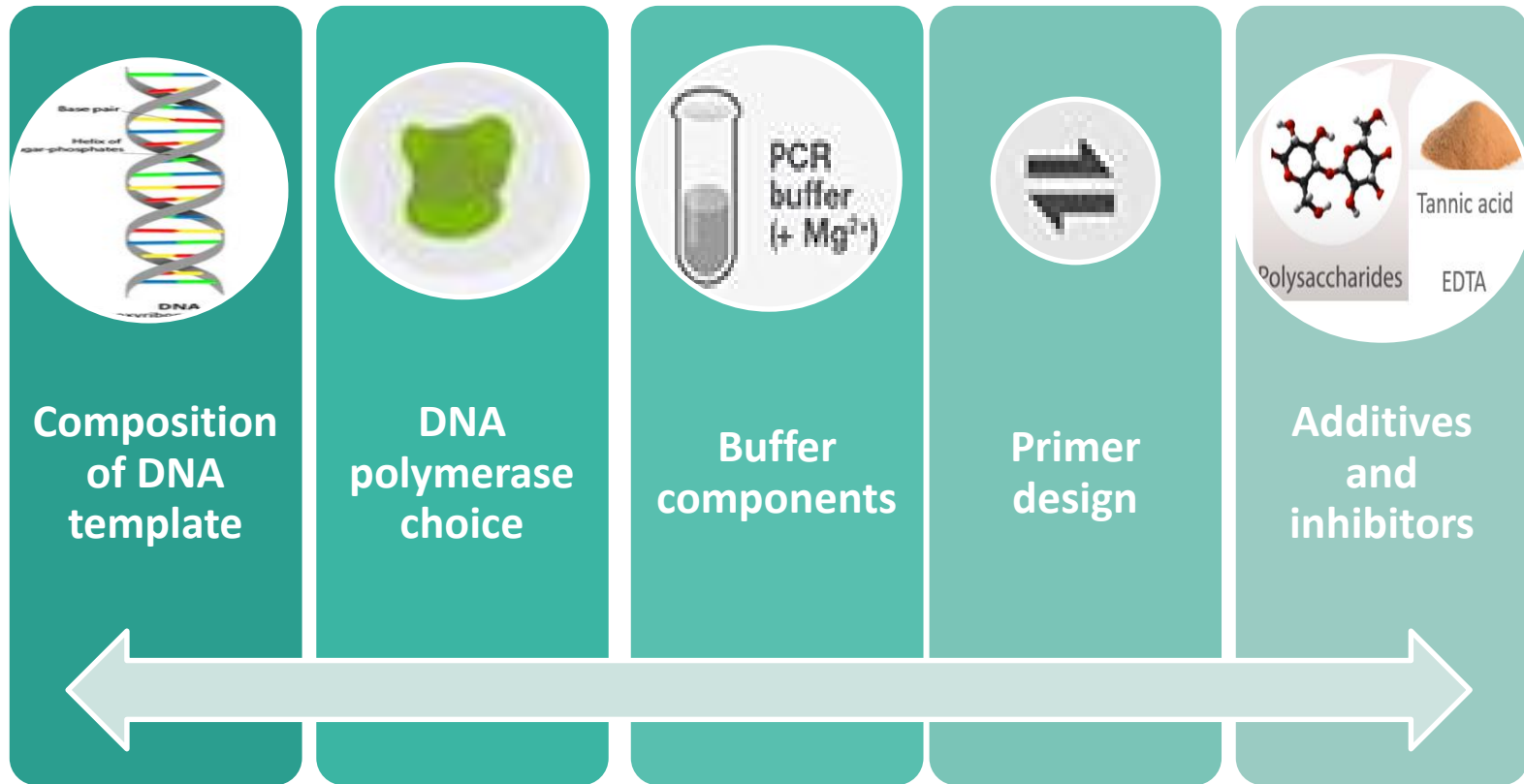
回收率

- 食品種類繁雜，相較於臨床檢體前處理不易
- 前處理為病毒回收率之最大關鍵點
- 食品中常有抑制因子抑制後續分生方法檢驗反應

病毒基因變異

- 病毒基因體序列變異性大，以單一引子對或探針檢測可能發生偽陰性

Important factors in PCR



操作注意事項

- ◆ 生安操作台(BSC)
- ◆ 消毒劑 (Virkon /Micro-Chem Plus)
- ◆ 水很重要
- ◆ 水 & 試劑要分裝且避免重複解凍
- ◆ 手避免從反應盤上空飛過
- ◆ 一定要做正/負控制組
- ◆ 每個反應最好做2-3重複
- ◆ 最好用 filter tip
- ◆ 最好用一套獨立pippelman
- ◆ 電泳區與試劑配製區分開(設備區分)

近期案例分享

媒體報導

好市多冷凍莓果檢出A型肝炎病毒案

好市多莓果再檢出A肝！「科克蘭冷凍草莓」流入市面了



4/30-5/3擴大後市場抽驗結果(好市多)

- 食藥署至好市多門市抽驗1件「**Kirkland Signature**科克蘭冷凍草莓」檢出**A肝病毒陽性**。



- 產地：墨西哥
- 有效日期：2024/08/05
- 規格：2.72 kg/包
- 進貨量：1,546包
- 庫存量：33包

邊境

後市場

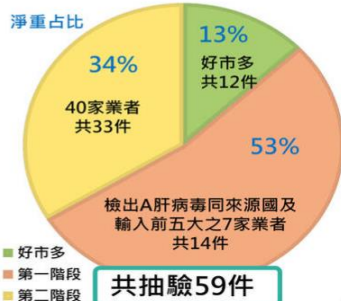
好市多公司

- 112年4月29日起，採**100%逐批**監視查驗，檢驗A肝病毒。
- 112年5月3日至112年6月2日**暫停**受理查驗申請。

其他業者

- 112年4月28日起，採**10%一般抽批**查驗，檢驗A肝病毒。

5/5**全面**要求好市多**回收**效期內之科克蘭冷凍莓果



軟果類檢體前處理

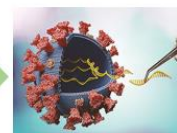
病毒沖提



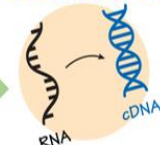
病毒濃縮



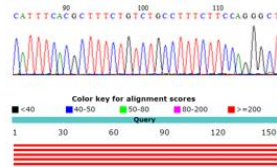
萃取RNA病毒核酸



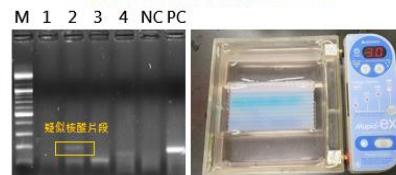
反轉錄病毒RNA為cDNA



基因定序/資料庫比對/產出報告



電泳偵測標的核酸片段



第一次PCR反應
第二次PCR反應



衛福部公告檢驗方法：食品中微生物之檢驗方法 - A型肝炎病毒之檢驗(MOHWM0027.00)



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敬請指教



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