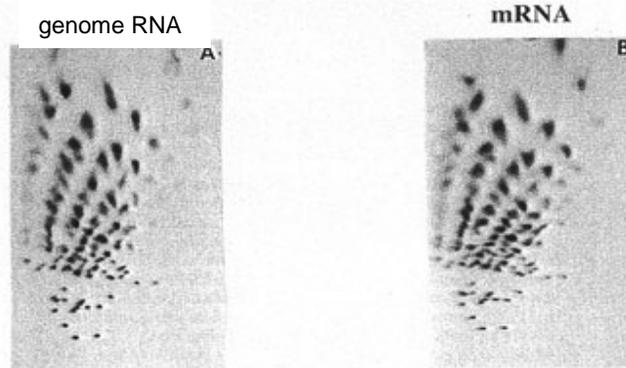


2-D fingerprint analysis of RNase T1 products

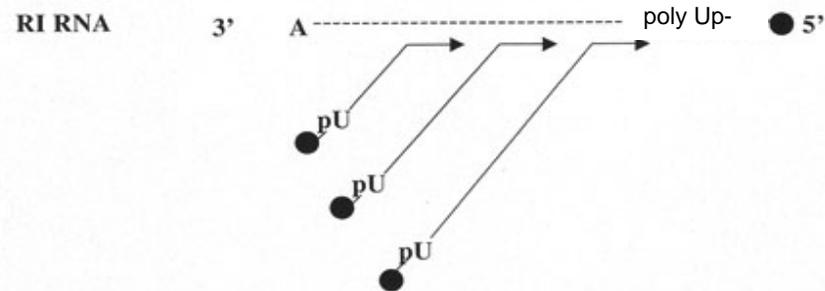


appearance of VPg (VPg : ●)

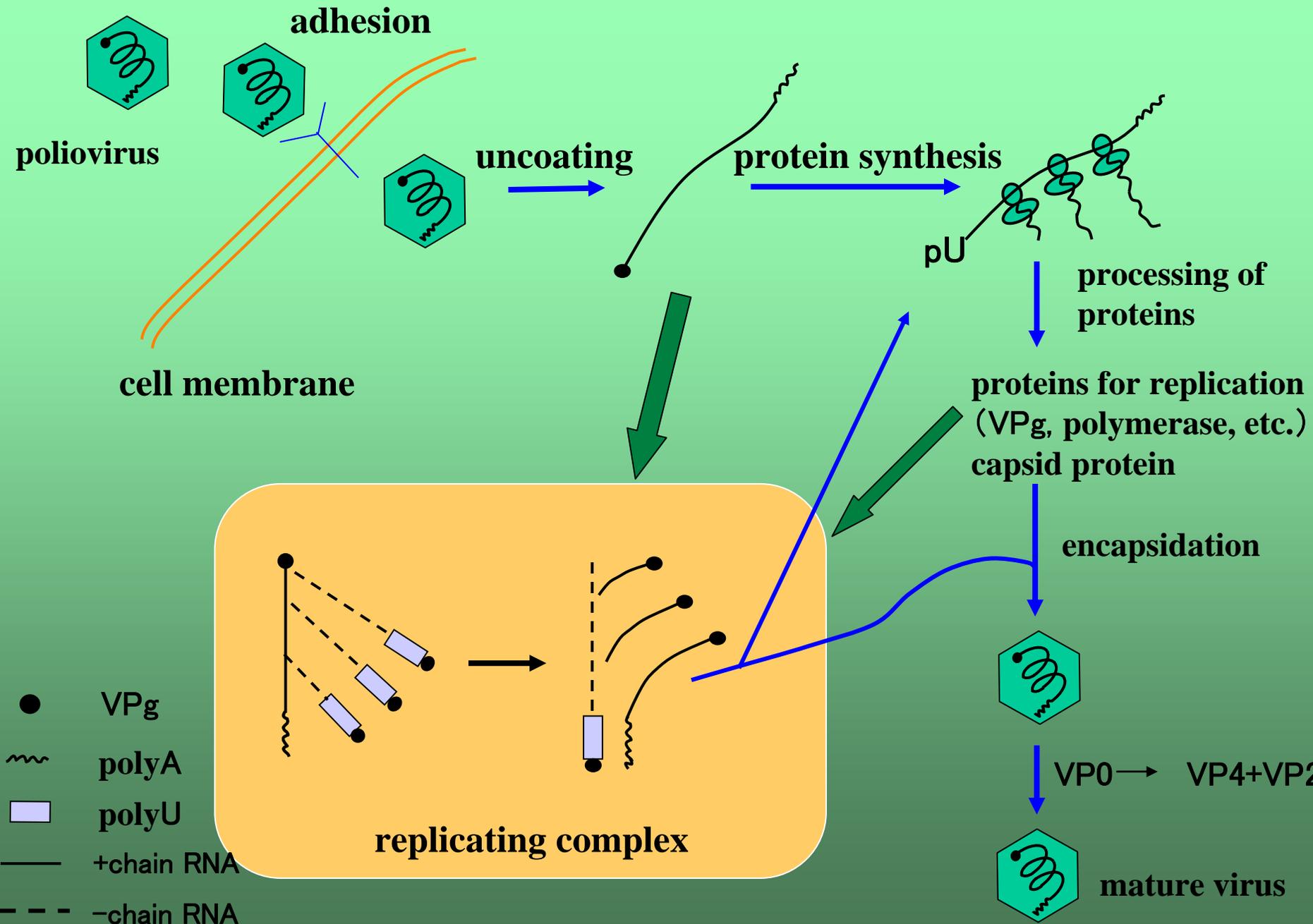
genome RNA 5' ● pU ————— poly A 3'

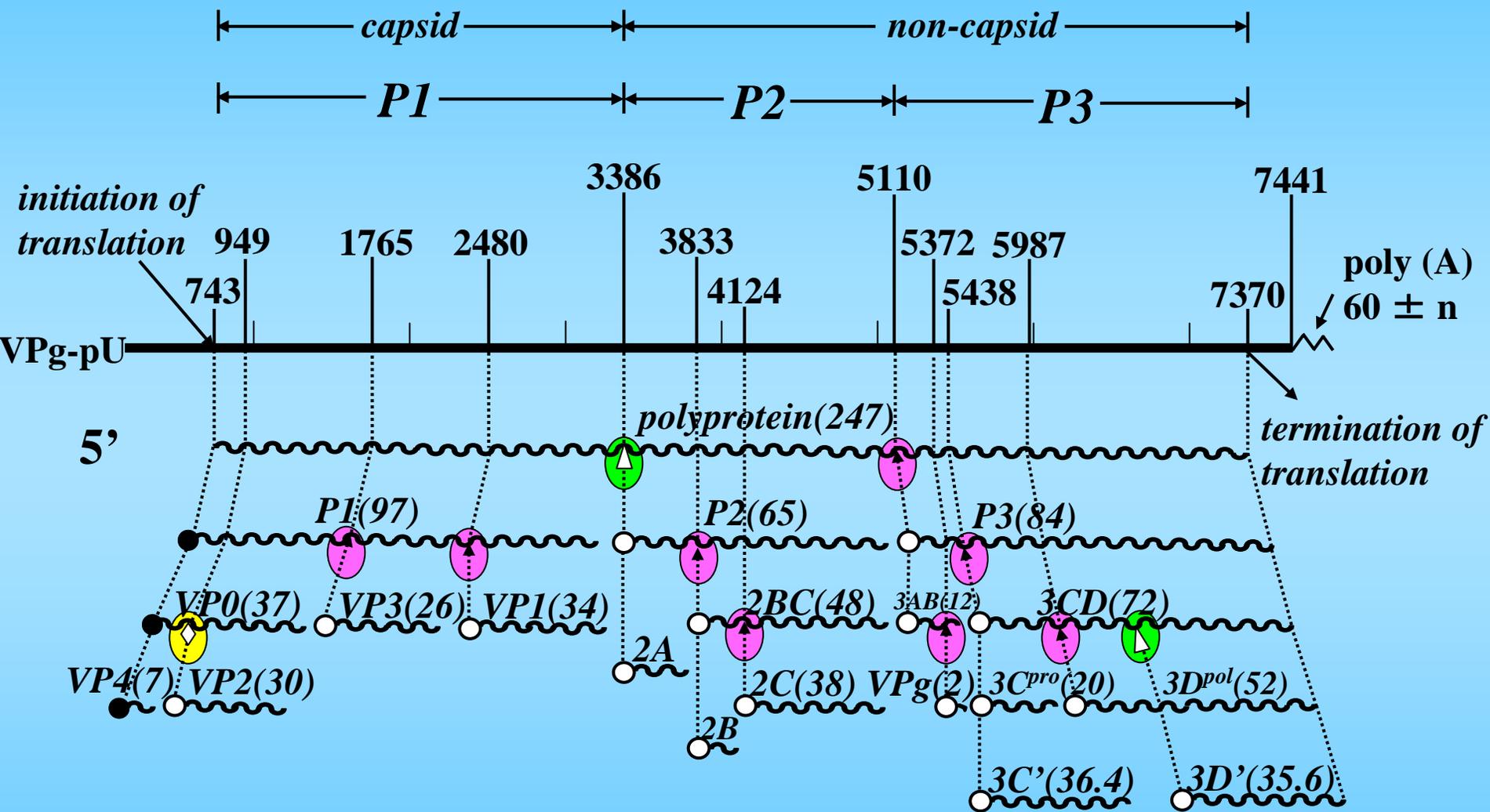
mRNA 5' pU ————— poly A 3'

RF RNA 5' ● pU ————— poly A 3'
 3' A - - - - - poly Up- ● 5'

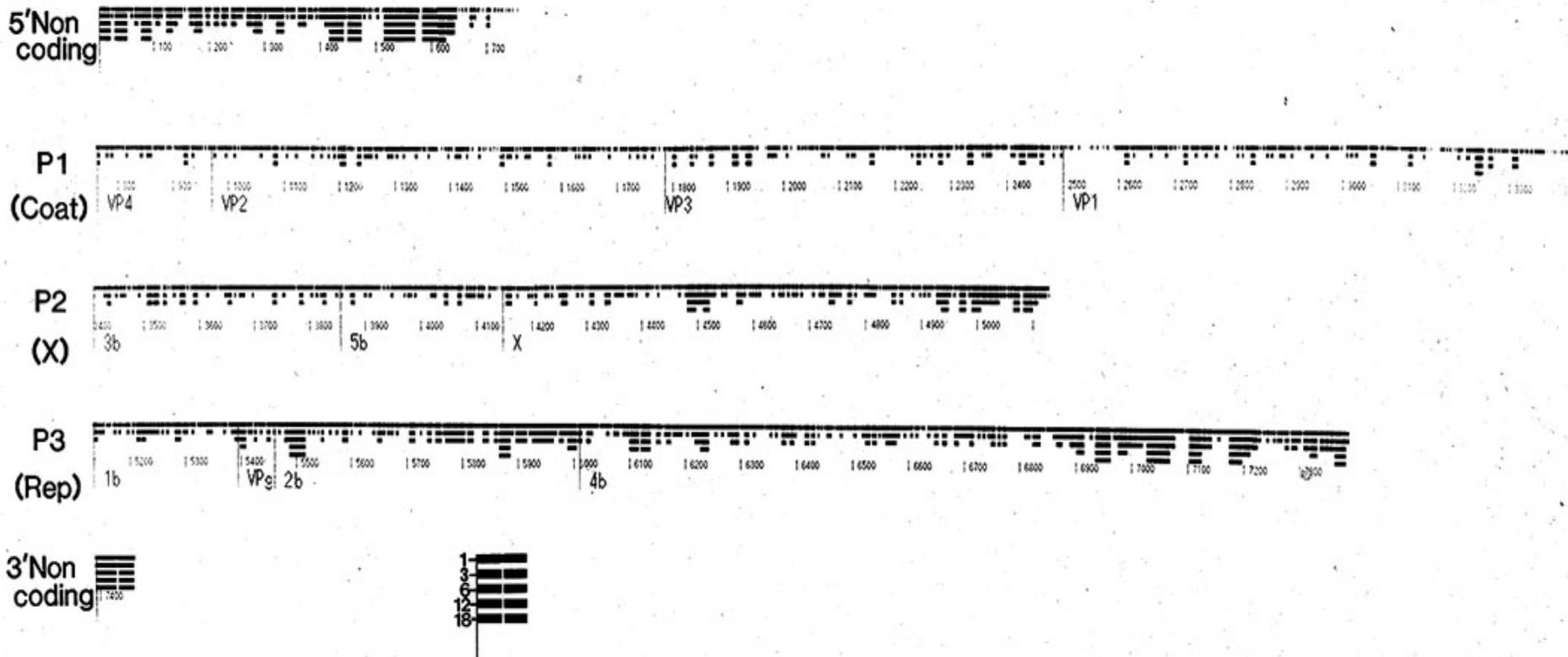


Proposition of the VPg primer theory in the beginning of RNA production





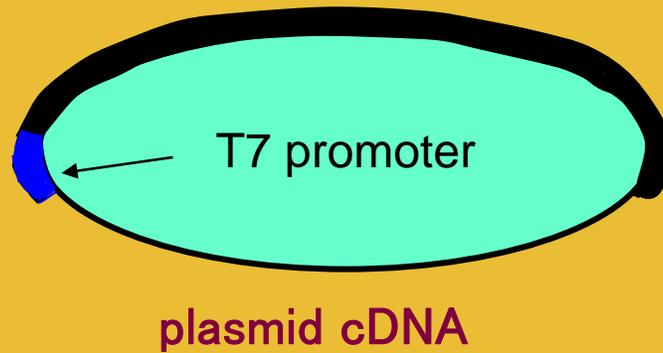
Nucleotide Sequence Homology among Three Poliovirus Serotype Genomes





infection efficiency
(pfu / μ g)

1×10^6

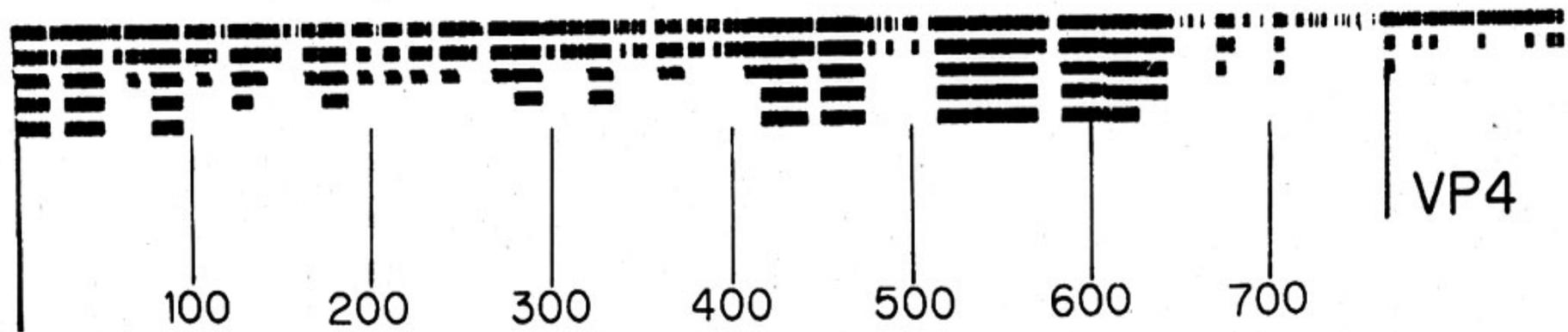


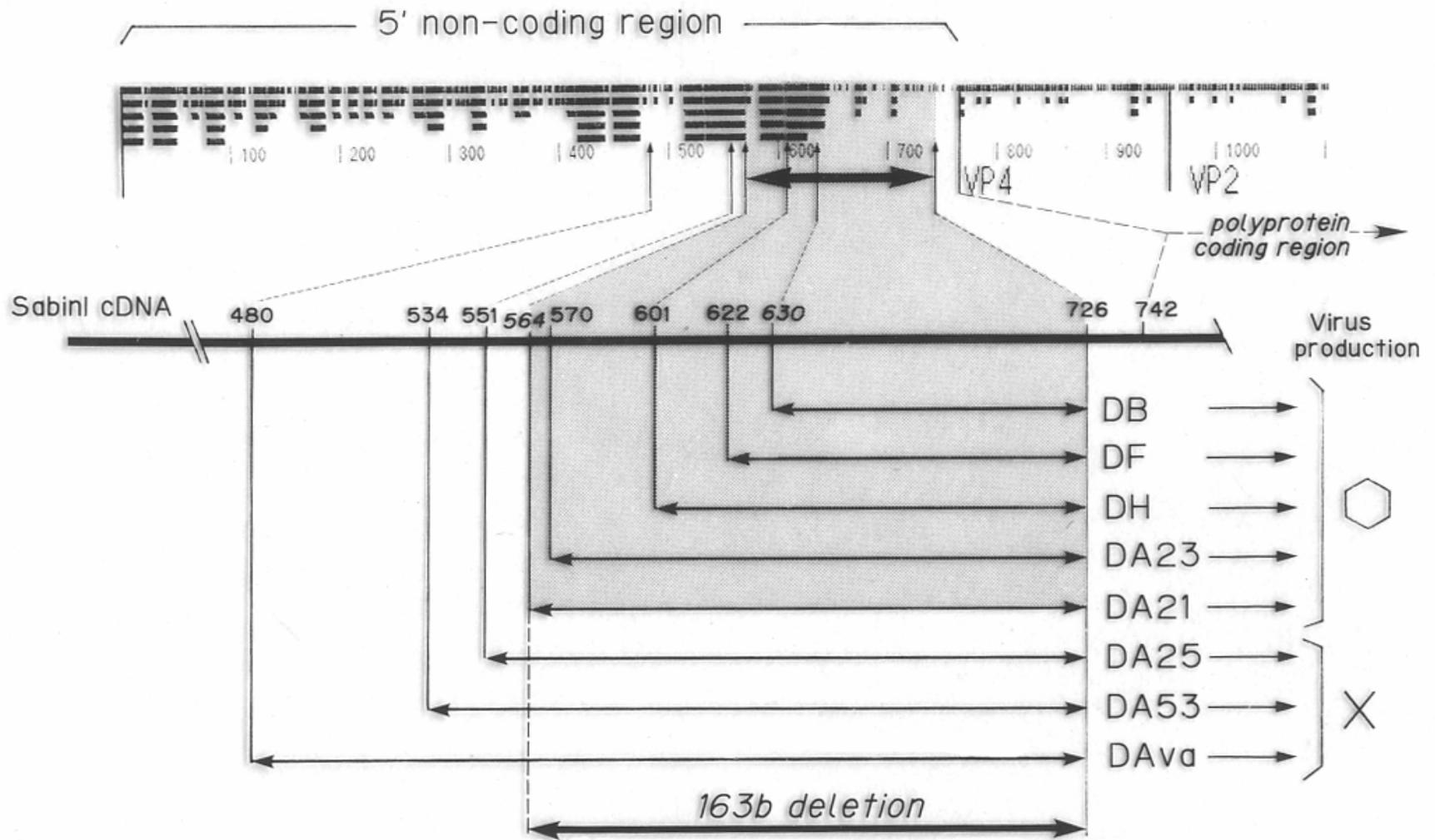
1×10^3



$1 \sim 3 \times 10^5$

5' non-coding region





5' non-coding region



Sabin1
cDNA

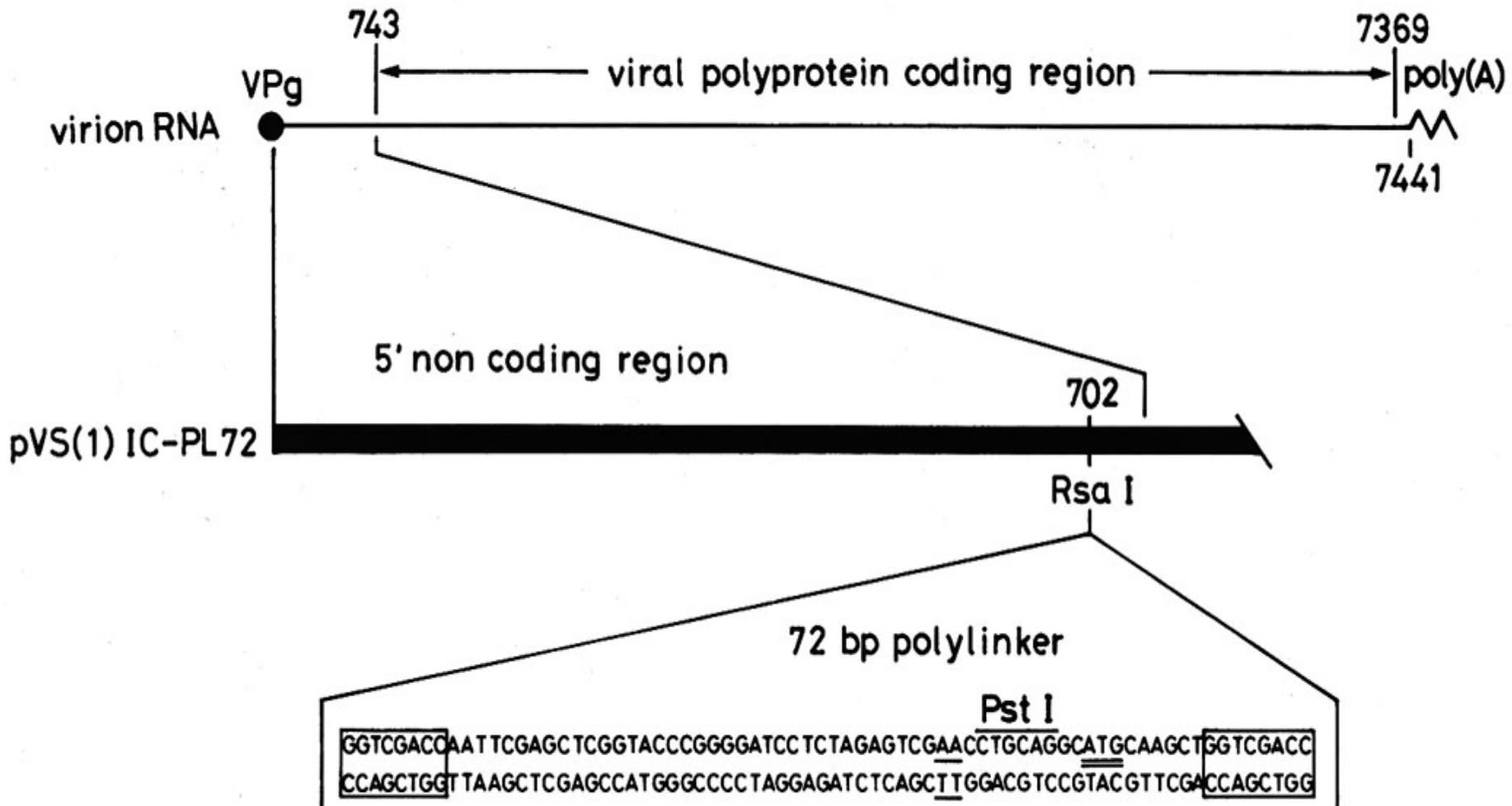
*Rsa*I
(702)

GGTCGACC

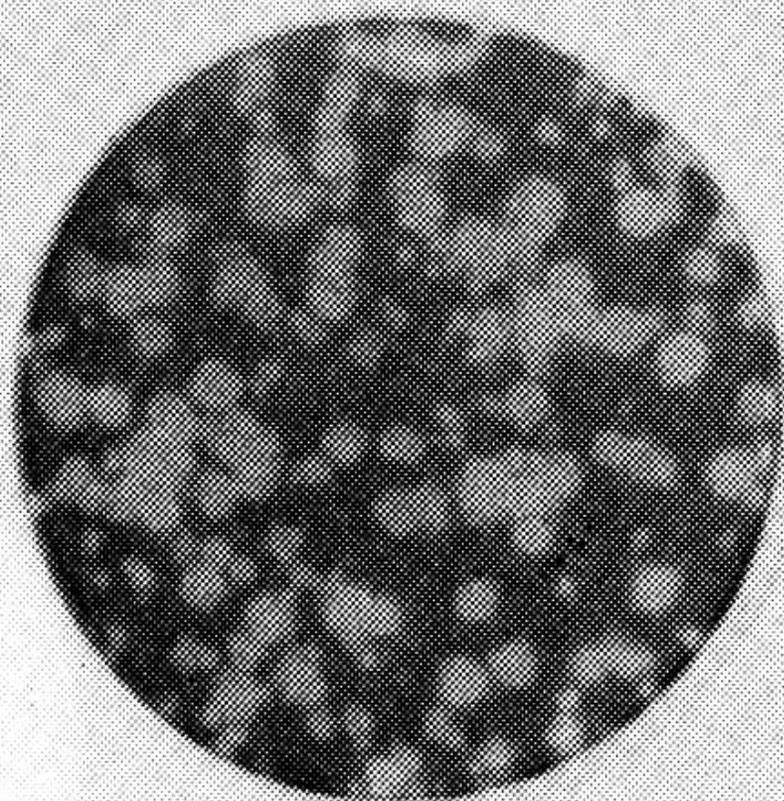
47bp
polylinker

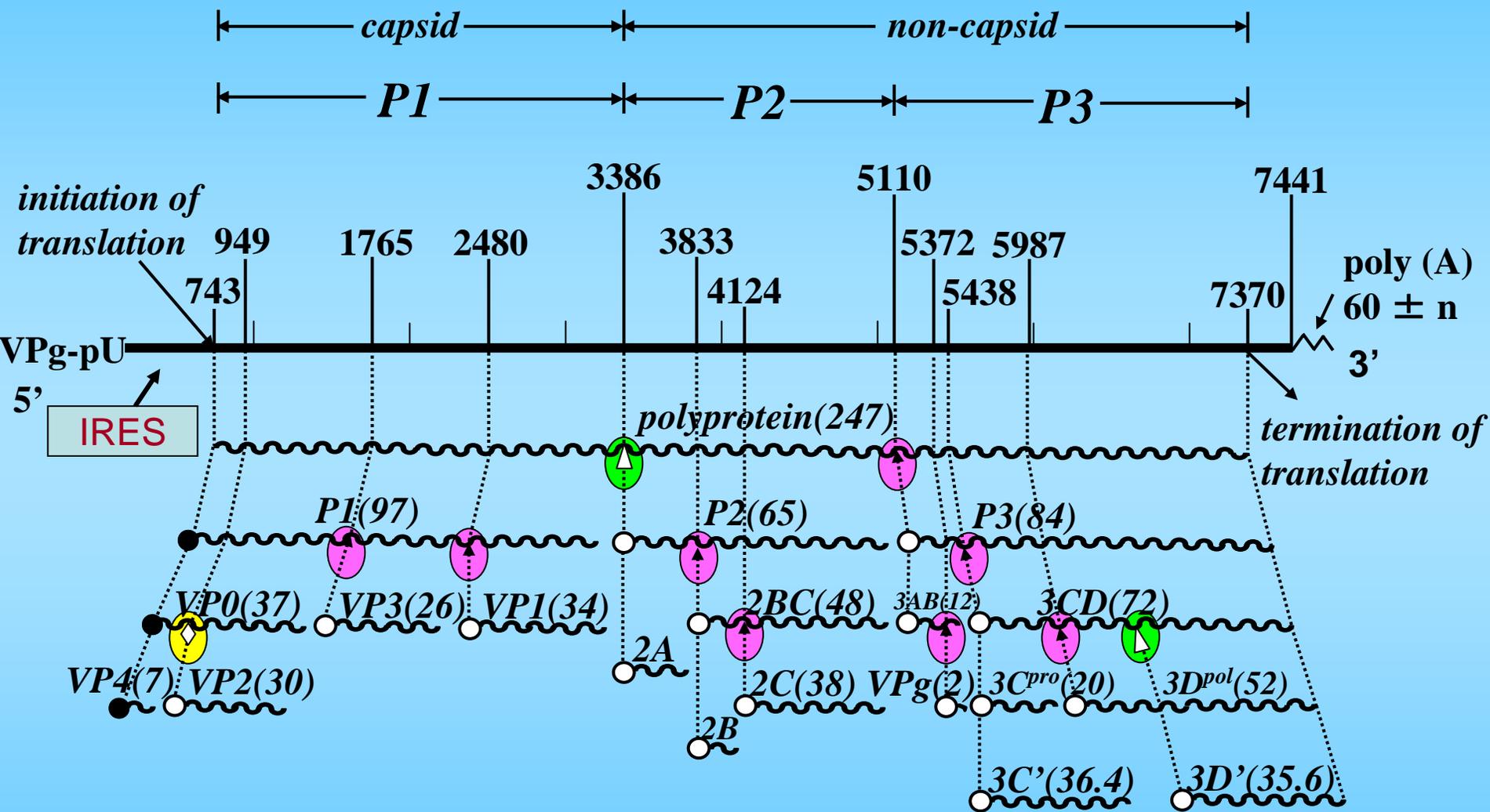
72bp
polylinker



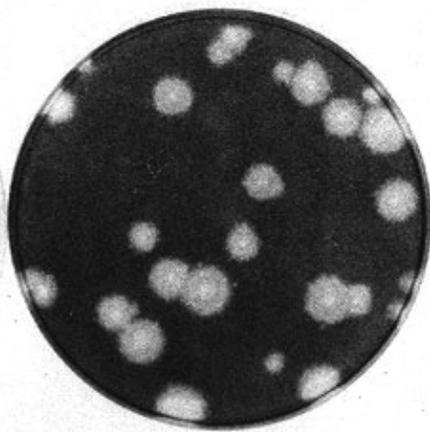
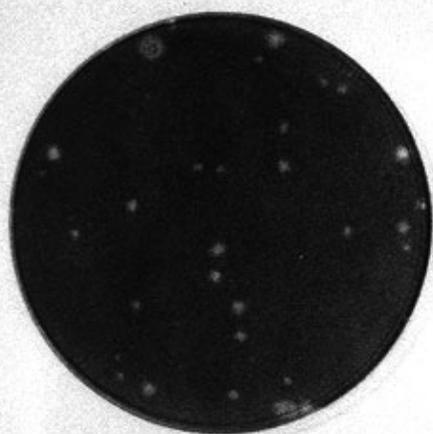


O(Sabin 1) PL72





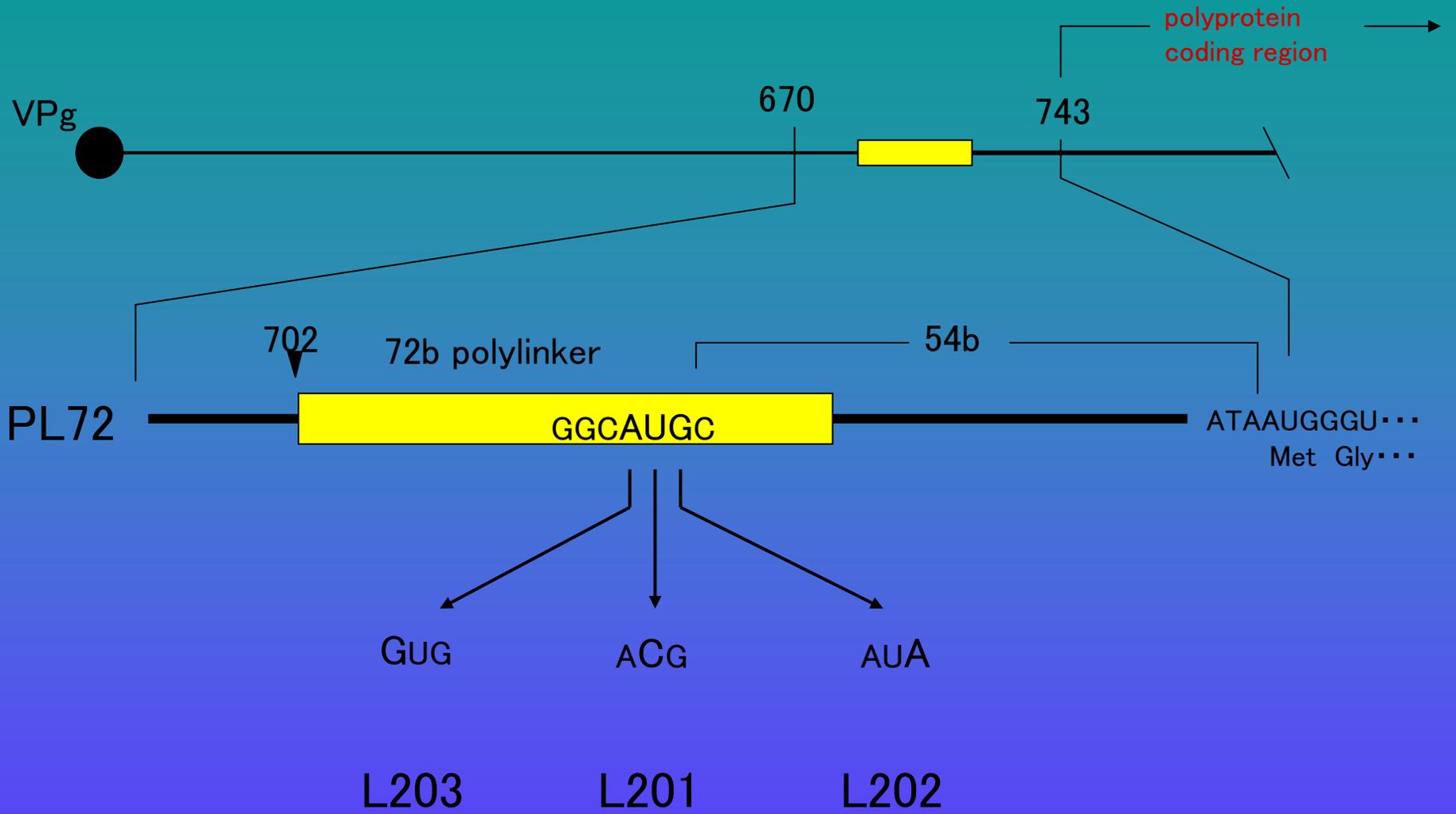
PL72 0 (Sabin 1)



L201

L202

L203



pVS(1)IC-PL72

↓ Transfection

AGMK cells in a plastic dish

↓ Virus recovery

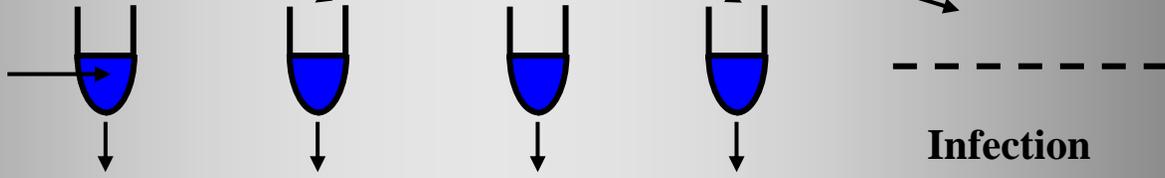
PV1(Sab)IC-PL72

↓ Infection

AGMK cells

Pick up small plaques

Medium

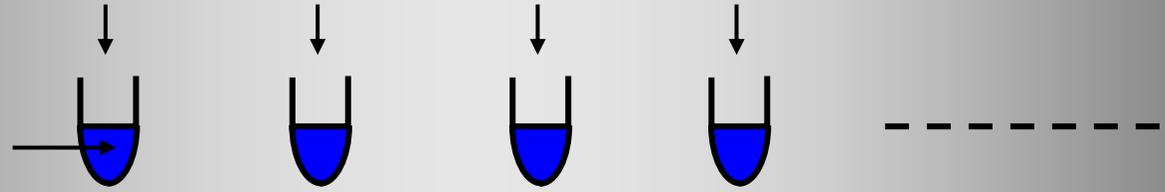


AGMK cells



Pick up only one large plaque per dish

Medium



L201

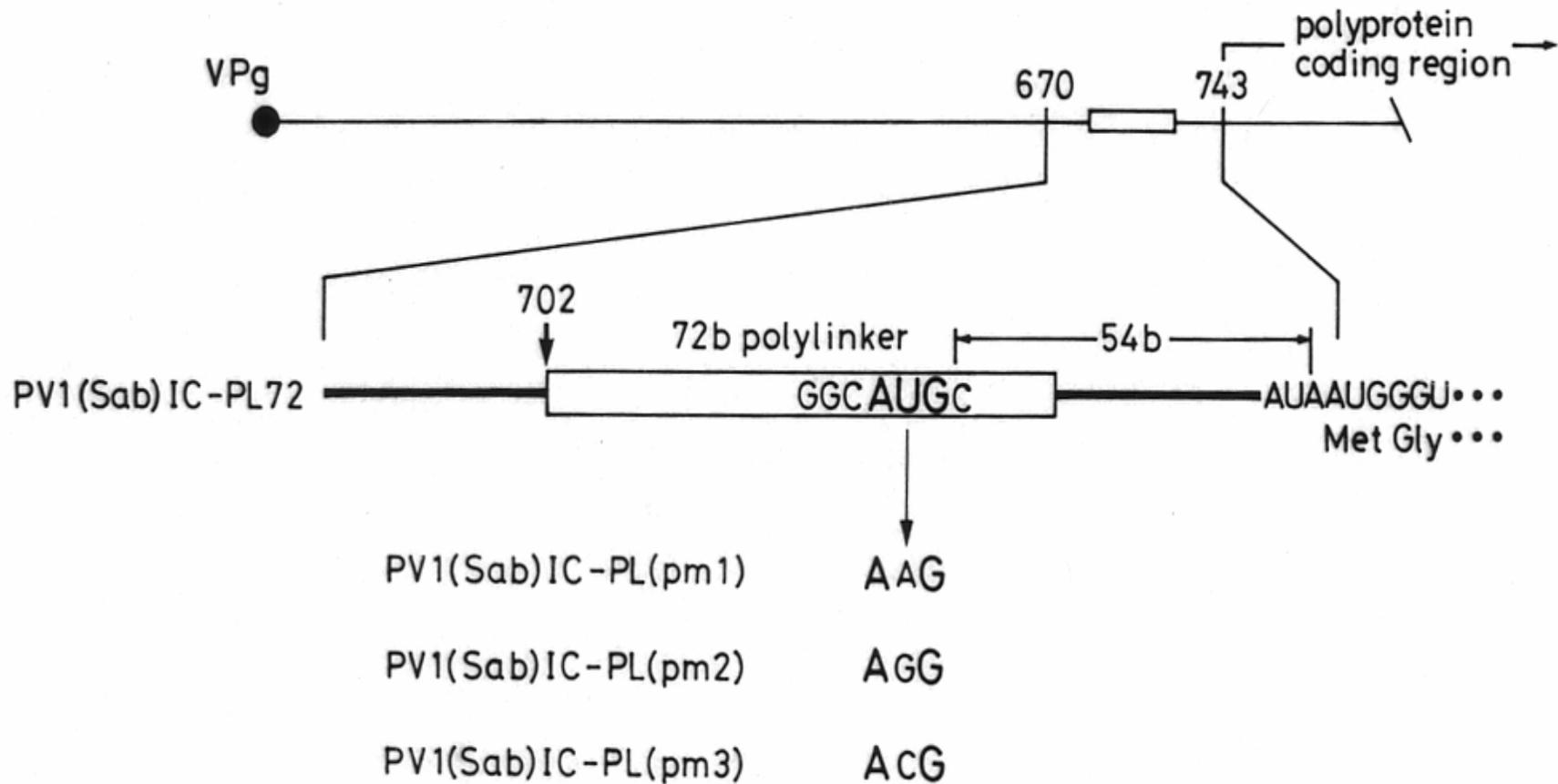
L202

L203

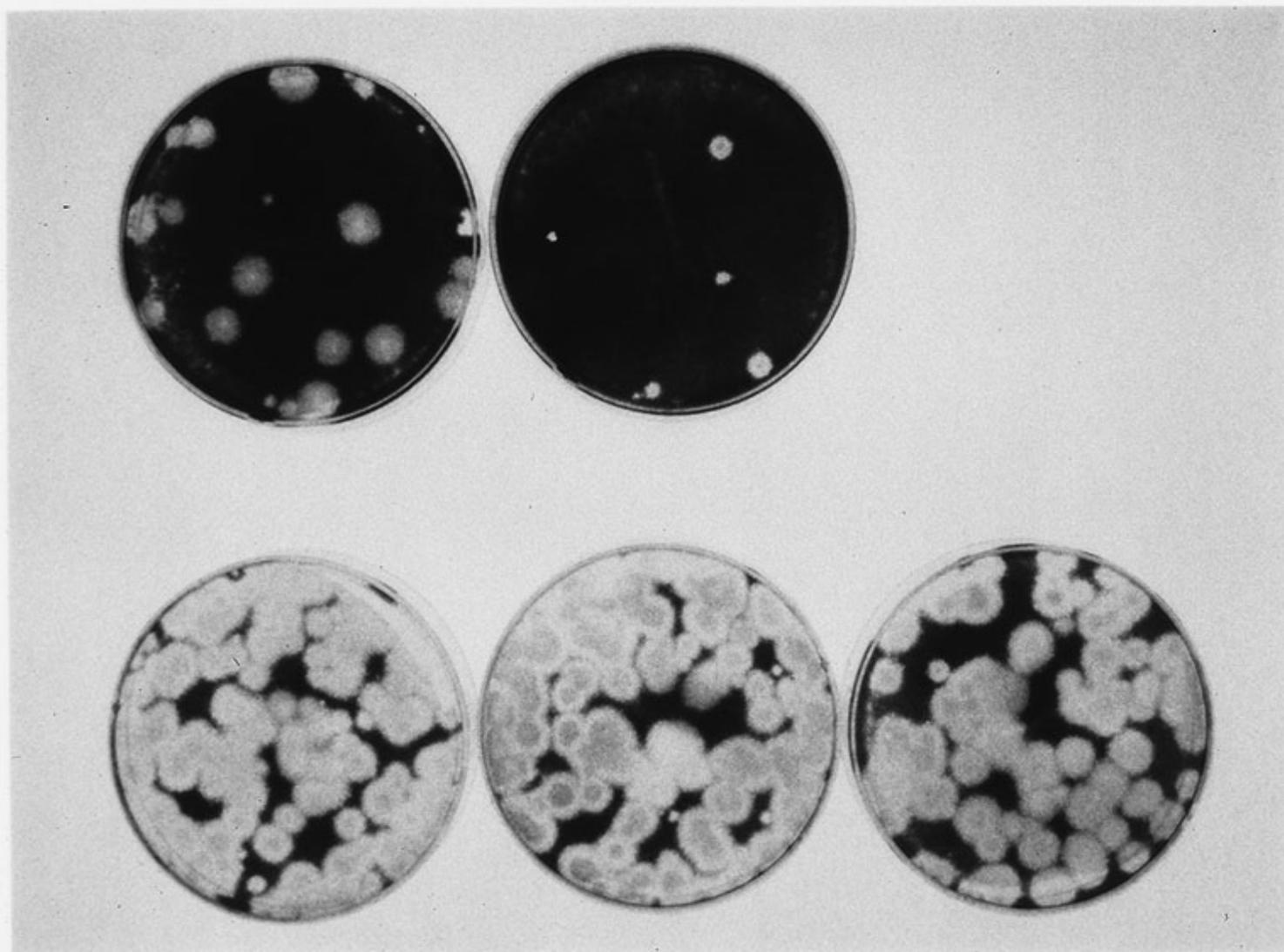
L204

Nucleotide substitutions

Initial sequence	Altered sequence			
	G	A	U	C
A	20	—	0	0
U	0	0	—	16
G	—	7	1	0



O(Sabin 1) PL72



PL(pm 1) PL(pm 2) PL(pm 3)

$$\text{Transversion} = 2.5 \times 10^{-6}$$

$$\frac{\text{Transition}}{\text{Transversion}} = 50$$

$$\text{Transition} = 1 \times 10^{-4}$$

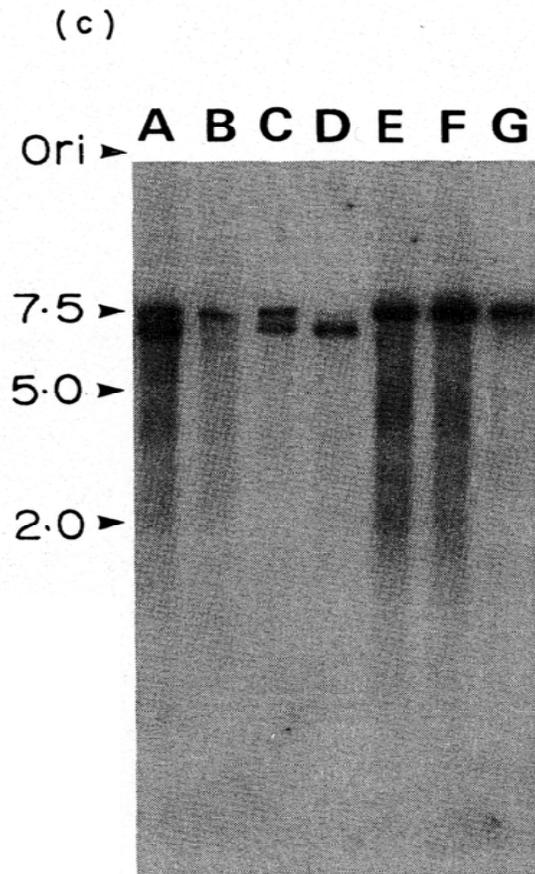
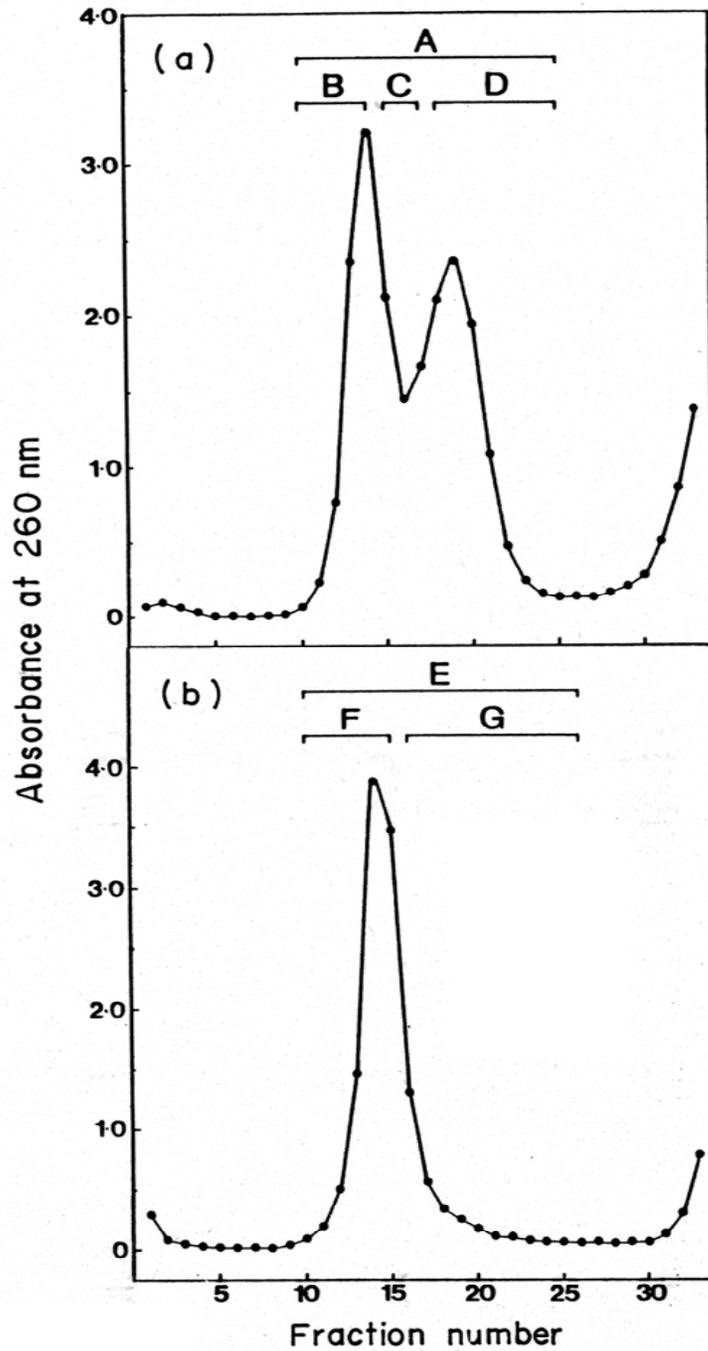
RATE OF MUTATION

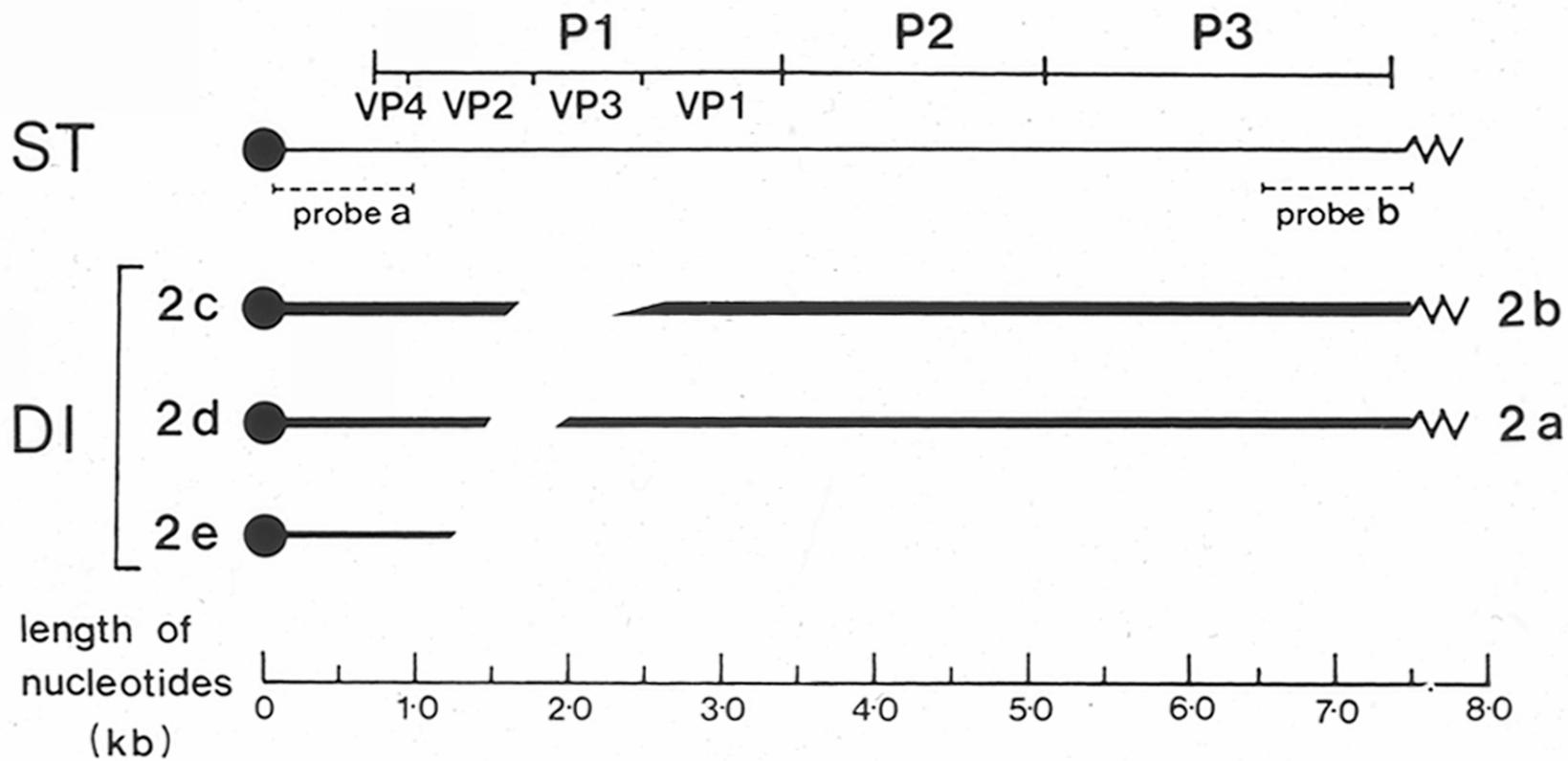
Replication of ssRNA genome ---- $10^{-3} \sim 10^{-4}$

Replication of dsDNA genome ---- $10^{-8} \sim 10^{-11}$

Holland et al. (1982) Science 215:1577

Defective Interfering (DI) Polio virus Particles





1,2

1225 2111
 --- CCU GAU GCA CTG CGG GAC AUG GGA CUC UUU UUC ACG UUU CUG UUC UGU GGA UCC AUG AUG ---
 Pro Asp Ala Leu Arg Asp Met Gly Leu Phe Phe Thr Phe Leu Phe Cys Gly Ser Met Met

GGC CAA AAU AUG ---885 b--- GGA UCC CUG AAG
 Gly Gln Asn Met Gly Ser Leu Lys

30-1

1737 2425
 --A GCC CCU AUG UGC UGU GAG UUC AAU GGA UUC C AGC GUG CGC UUG AUG CGA GAU ACC ACA CA--
 Ala Pro Met Cys Cys Glu Phe Asn Gly Phe Ser Var Arg Leu Met Arg Asp Thr Thr

A AGA AAC AAU ACC ---687 b--- CGC UGU AAU GAC UUU
 Leu Arg Asn Ile Thr Ala Cys Asn Asp Phe

30-2

2654 2706
 -CU GGG GCC ACA AAU CGA CUA GUC CCU UCU G GC AUA CAG UCU UUC UUC GCG CGG GGU GCA U--
 Gly Ala Thr Asn Pro Leu Val Pro Ser Gly Ile Glu Ser Phe Phe Ala Arg Gly Ala

AU ACA GUG CAA ACC ---51 b--- AGG UCA GAG UCU A
 Asp Thr Val Gln Thr Arg Ser Glu Ser Ser

39

1315 2528
 --- AAC GCC UCC AAA UUC CAC CAG GGG GCA CUA ACC GUG GGG GCG GCA ACG UCU AGA GAC GCU ---
 Asn Ala Ser Lys Phe His Gln Gly Ala Leu Thr Val Gly Ala Ala Thr Ser Arg Asp Ala

GGG GUA UUC GCC ---1212b--- ACA GUC CGU GAA
 Gly Val Phe Ala Thr Val Arg Glu

209

1686 2002
 --A UUA AAU UUU GCU AGU GAG UCC UCC CCA GA C GAU CCC AUA CUC UGC CUG UCA CUC UCU CC--
 Leu Asn Phe Ala Ser Glu Ser Ser Pro Asp Asp Pro Ile Leu Cys Leu Ser Leu Ser

G AUU CCA AUC ACC ---315 b--- AAA CCA CAU ACA CA
 Glu Ile Pro Ile Thr Lys Pro His Thr Asp

25

1475 2385
 -AC CAG ACA UCA CCU GCC CGU AGG UUC UGC C AG AUG GAC AUC CUU GCU UUU GUG UCA CCG U--
 Gln Thr Ser Pro Ala Arg Arg Phe Cys Gln Met Asp Ile Leu Gly Phe Val Ser Ala

CG CUG GAU UAC CUC ---909 b--- UCG ACA CCC AGA G
 Pro Val Asp Tyr Leu Ser Thr Pro Arg Glu

16

1581 2563
 --G ACC AAC AAC UGU GCU ACA CUG GUA CUC CC A AAC ACU GAA GCC AGU GGA CCA GCA CAC UC--
 Thr Asn Asn Cys Ala Thr Leu Val Leu Pro Asn Thr Glu Ala Ser Gly Pro Ala His

U UAC GUG AAC UCC ---981 b--- AGA GAC GCU CUC CC
 Pro Tyr Val Asn Ser Arg Asp Ala Leu Pro

11

1633 2396
 --- GAU AGU AUG GUA AAG CAC AAU AAU UGG GGA CUU GGU UUU GUG UCA CCG UGU AAU GAC UUC ---
 Asp Ser Met Val Lys His Asn Asn Trp Gly Leu Gly Phe Val Ser Ala Cys Asn Asp Phe

AUU GCA AUA UUA ---762 b--- GAG AUG GAC AUC
 Ile Ala Ile Leu Glu Met Asp Ile

17

1639 2519
 --- AUG GUA AAG CAC AAU AAU UGC GGA AUU GCA GUC CGU GAA ACG GUG GGG GCG GCA ACG UCU ---
 Met Val Lys His Asn Asn Trp Gly Ile Ala Val Arg Glu Thr Val Gly Ala Ala Thr Ser

AUA UUA CCA UUG ---879 b--- AUU GAC AAC ACA
 Ile Leu Pro Leu Ile Asp Asn Thr

13,213

1662 2479
 --A AUU GCA AUA UUA CCA UUG GCC CCA UUA AA G GGG UUA GGU CAG AUG CUU GAA ACG AUG AU--
 Ile Ala Ile Leu Pro Leu Ala Pro Leu Lys Gly Leu Gly Gln Met Leu Glu Ser Met

U UUU GCU AGU GAG ---816 b--- AAA GCC CUA GCA CA
 Asn Phe Ala Ser Glu Lys Ala Leu Ala Gln

14,15

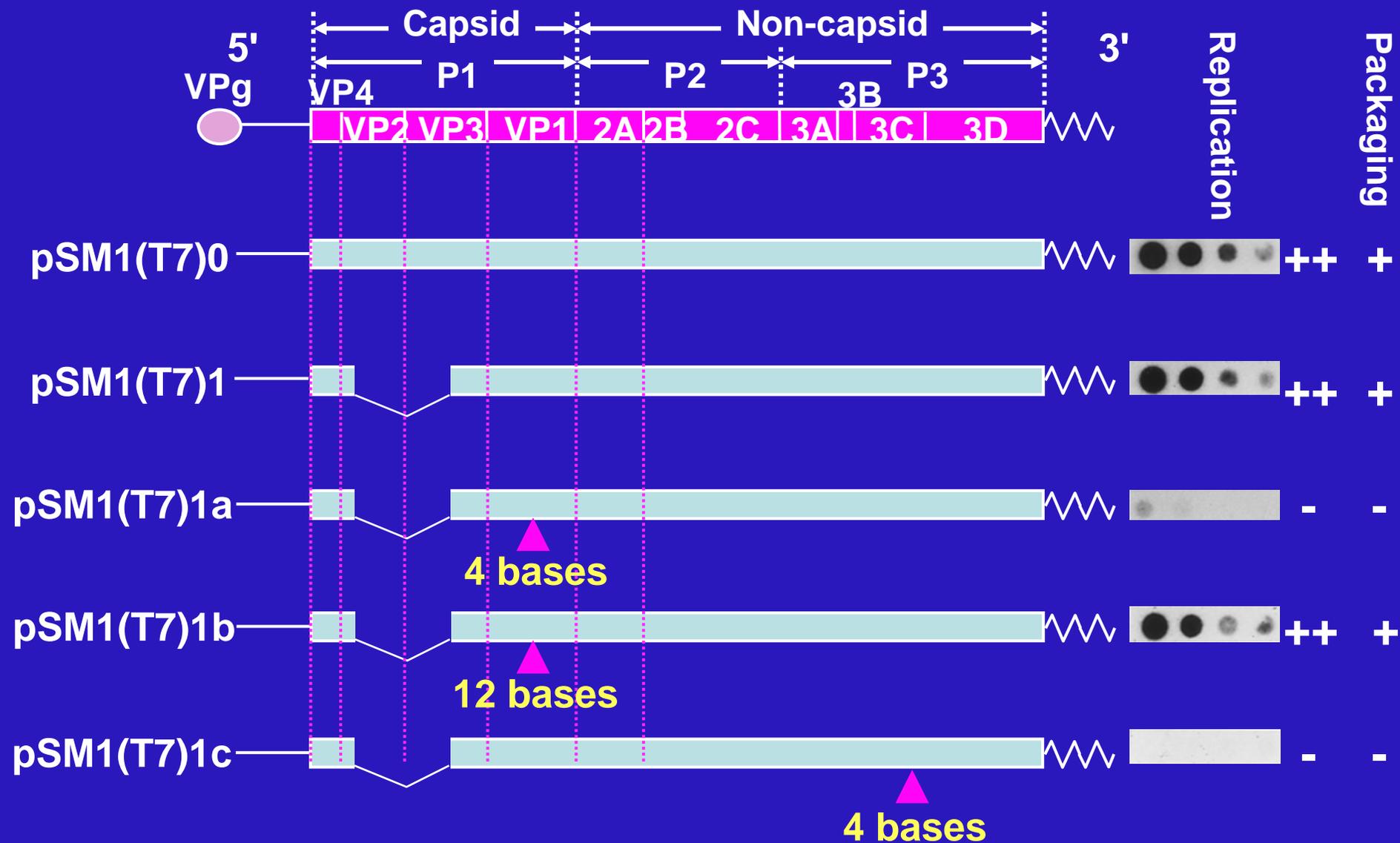
1664 2445
 -UU GCA AUA UUA CCA UUG GCC CCA UUA AAU U AU ACC ACA CAU AUA CAG CAA AAA GCG CUA G--
 Ala Ile Leu Pro Leu Ala Pro Leu Asn Tyr Thr Thr His Ile Glu Gln Lys Ala Leu

UU GCU AGU GAG UCC ---780 b--- CGC UUG AUG CGA G
 Phe Ala Ser Glu Ser Arg Leu Met Arg Asp

H

1619 2337
 -AC UCC CUC UCG AUA GAU AGU AUG GUA AAG C GC GUC UUC UAC CAA ACC AGA AUA GUC GUC C--
 Ser Leu Ser Ile Asp Ser Met Val Lys Arg Val Phe Tyr Gln Thr Arg Ile Val Val

AC AAU AAU UGG CGA ---717 b--- GGC GGA UAC AUC A
 His Asn Asn Trp Gly Gly Gly Tyr Ile Ser



Effect of in- or out-of-frame insertions

1,2

1225 2111
 --- CCU GAU GCA CTG CGG GAC AUG GGA CUC UUU UUC ACG UUU CUG UUC UGU GGA UCC AUG AUG ---
 Pro Asp Ala Leu Arg Asp Met Gly Leu Phe Phe Thr Phe Leu Phe Cys Gly Ser Met Met

GGC CAA AAU AUG ---885 b--- GGA UCC CUG AAG
 Gly Gln Asn Met Gly Ser Leu Lys

30-1

1737 2425
 --A GCC CCU AUG UGC UGU GAG UUC AAU GGA UUC C AGC GUG CGC UUG AUG CGA GAU ACC ACA CA--
 Ala Pro Met Cys Cys Glu Phe Asn Gly Phe Ser Var Arg Leu Met Arg Asp Thr Thr

A AGA AAC AAU ACC ---687 b--- CGC UGU AAU GAC UUU
 Leu Arg Asn Ile Thr Ala Cys Asn Asp Phe

30-2

2654 2706
 -CU GGG GCC ACA AAU CGA CUA GUC CCU UCU G GC AUA CAG UCU UUC UUC GCG CGG GGU GCA U--
 Gly Ala Thr Asn Pro Leu Val Pro Ser Gly Ile Glu Ser Phe Phe Ala Arg Gly Ala

AU ACA GUG CAA ACC ---51 b--- AGG UCA GAG UCU A
 Asp Thr Val Gln Thr Arg Ser Glu Ser Ser

39

1315 2528
 --- AAC GCC UCC AAA UUC CAC CAG GGG GCA CUA ACC GUG GGG GCG GCA ACG UCU AGA GAC GCU ---
 Asn Ala Ser Lys Phe His Gln Gly Ala Leu Thr Val Gly Ala Ala Thr Ser Arg Asp Ala

GGG GUA UUC GCC ---1212b--- ACA GUC CGU GAA
 Gly Val Phe Ala Thr Val Arg Glu

209

1686 2002
 --A UUA AAU UUU GCU AGU GAG UCC UCC CCA GA C GAU CCC AUA CUC UGC CUG UCA CUC UCU CC--
 Leu Asn Phe Ala Ser Glu Ser Ser Pro Asp Asp Pro Ile Leu Cys Leu Ser Leu Ser

G AUU CCA AUC ACC ---315 b--- AAA CCA CAU ACA GA
 Glu Ile Pro Ile Thr Lys Pro His Thr Asp

25

1475 2385
 -AC CAG ACA UCA CCU GCC CGU AGG UUC UGC C AG AUG GAC AUC CUU GCU UUU GUG UCA CCG U--
 Gln Thr Ser Pro Ala Arg Arg Phe Cys Gln Met Asp Ile Leu Gly Phe Val Ser Ala

CG CUG GAU UAC CUC ---909 b--- UCG ACA CCC AGA G
 Pro Val Asp Tyr Leu Ser Thr Pro Arg Glu

16

1581 2563
 --G ACC AAC AAC UGU GCU ACA CUG GUA CUC CC A AAC ACU GAA GCC AGU GGA CCA GCA CAC UC--
 Thr Asn Asn Cys Ala Thr Leu Val Leu Pro Asn Thr Glu Ala Ser Gly Pro Ala His

U UAC GUG AAC UCC ---981 b--- AGA GAC GCU CUC CC
 Pro Tyr Val Asn Ser Arg Asp Ala Leu Pro

11

1633 2396
 --- GAU AGU AUG GUA AAG CAC AAU AAU UGG GGA CUU GGU UUU GUG UCA CCG UGU AAU GAC UUC ---
 Asp Ser Met Val Lys His Asn Asn Trp Gly Leu Gly Phe Val Ser Ala Cys Asn Asp Phe

AUU GCA AUA UUA ---762 b--- GAG AUG GAC AUC
 Ile Ala Ile Leu Glu Met Asp Ile

17

1639 2519
 --- AUG GUA AAG CAC AAU AAU UGC GGA AUU GCA GUC CGU GAA ACG GUG GGG GCG GCA ACG UCU ---
 Met Val Lys His Asn Asn Trp Gly Ile Ala Val Arg Glu Thr Val Gly Ala Ala Thr Ser

AUA UUA CCA UUG ---879 b--- AUU GAC AAC ACA
 Ile Leu Pro Leu Ile Asp Asn Thr

13,213

1662 2479
 --A AUU GCA AUA UUA CCA UUG GCC CCA UUA AA G GGG UUA GGU CAG AUG CUU GAA AGC AUG AU--
 Ile Ala Ile Leu Pro Leu Ala Pro Leu Lys Gly Leu Gly Gln Met Leu Glu Ser Met

U UUU GCU AGU GAG ---816 b--- AAA GCC CUA GCA CA
 Asn Phe Ala Ser Glu Lys Ala Leu Ala Gln

14,15

1664 2445
 -UU GCA AUA UUA CCA UUG GCC CCA UUA AAU U AU ACC ACA CAU AUA CAG CAA AAA GCG CUA G--
 Ala Ile Leu Pro Leu Ala Pro Leu Asn Tyr Thr Thr His Ile Glu Gln Lys Ala Leu

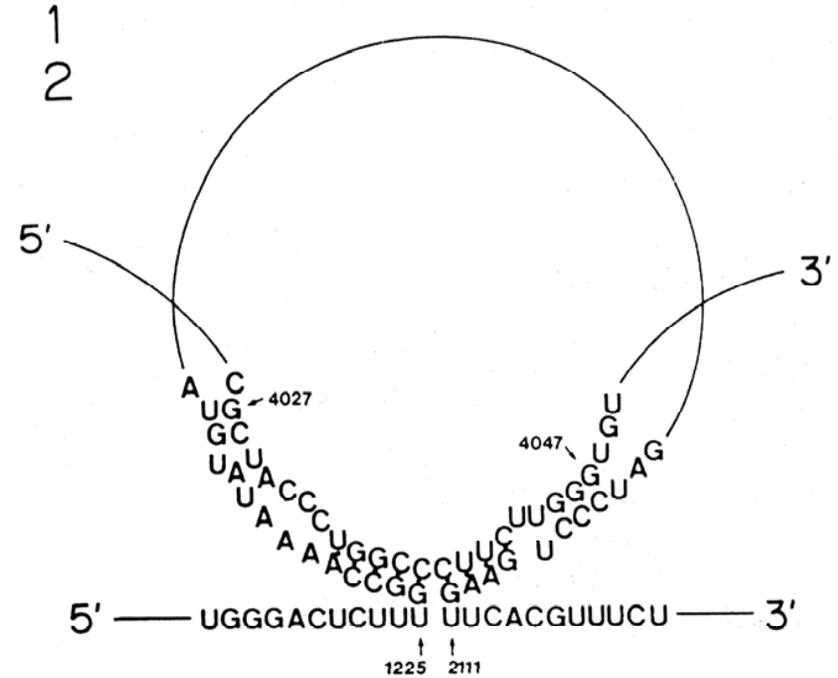
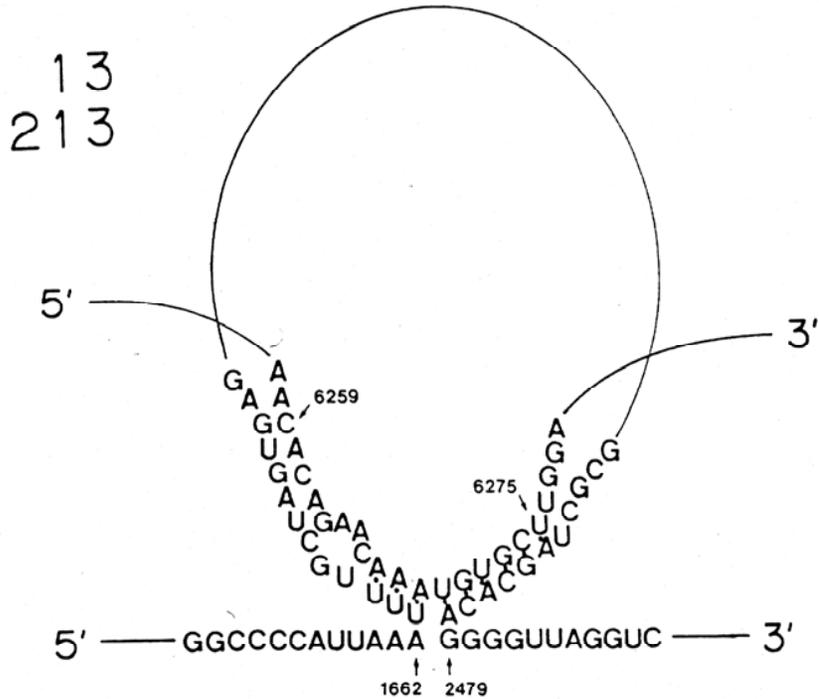
UU GCU AGU GAG UCC ---780 b--- CGC UUG AUG CGA G
 Phe Ala Ser Glu Ser Arg Leu Met Arg Asp

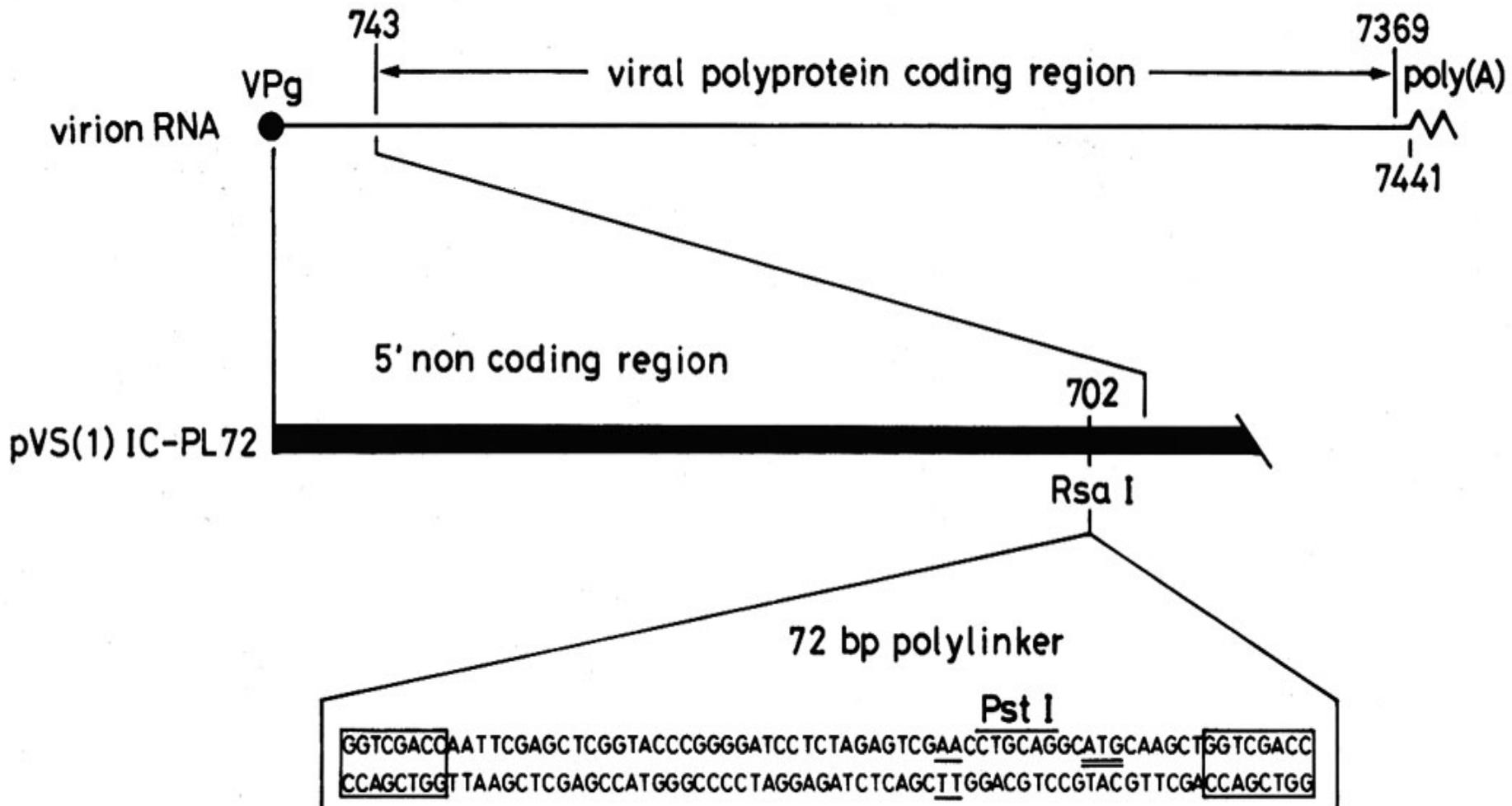
H

1619 2337
 -AC UCC CUC UCG AUA GAU AGU AUG GUA AAG C GC GUC UUC UAC CAA ACC AGA AUA GUC GUC C--
 Ser Leu Ser Ile Asp Ser Met Val Lys Arg Val Phe Tyr Gln Thr Arg Ile Val Val

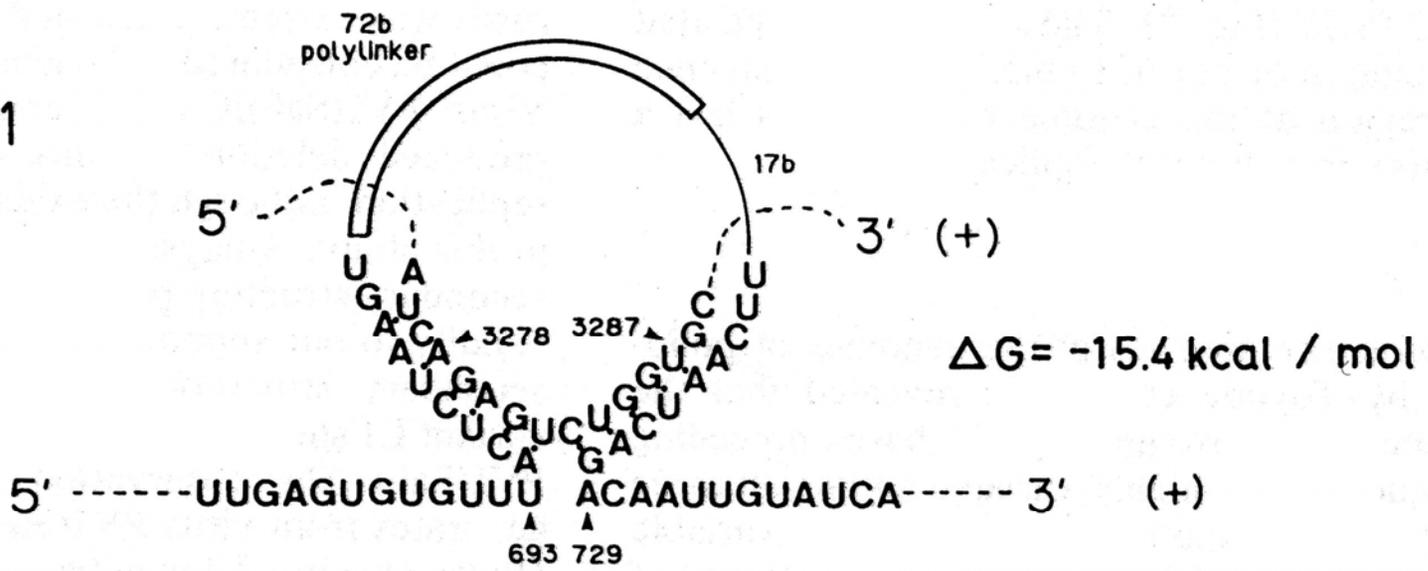
AC AAU AAU UGG CGA ---717 b--- GGC GGA UAC AUC A
 His Asn Asn Trp Gly Gly Gly Tyr Ile Ser

Supporting sequence-loop Model

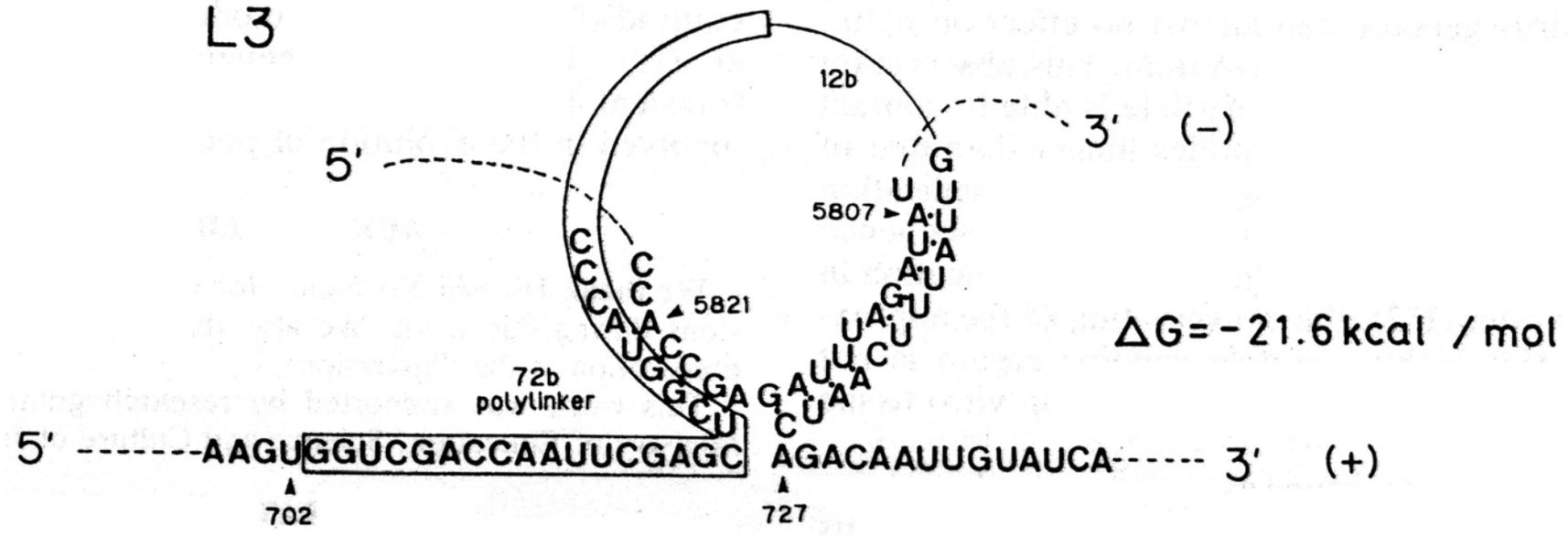




L1

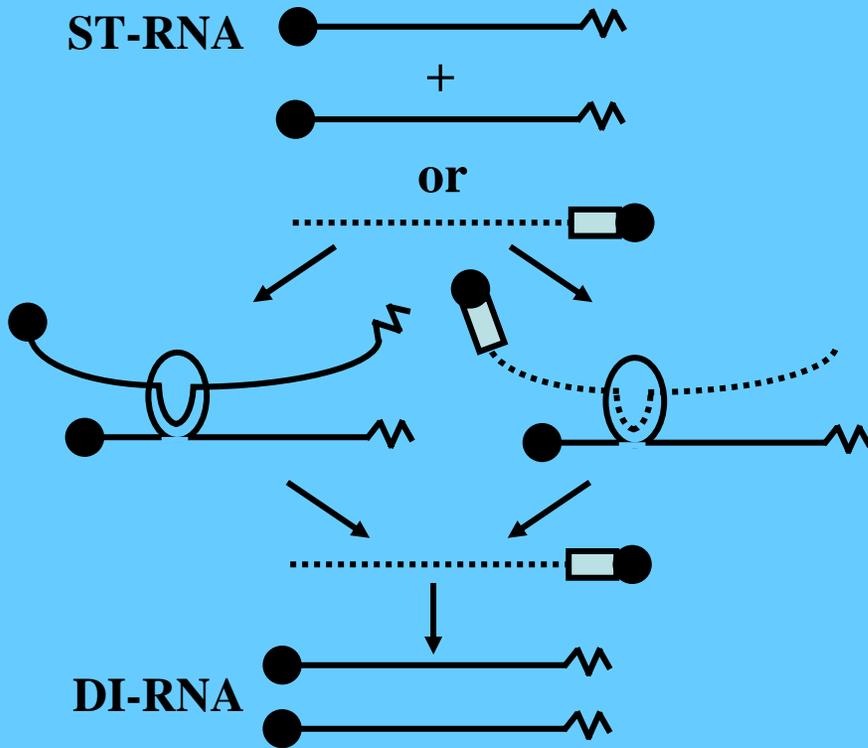


L3



RNA recombinant model “supporting a sequence-loop model”

generation of gene defective strain

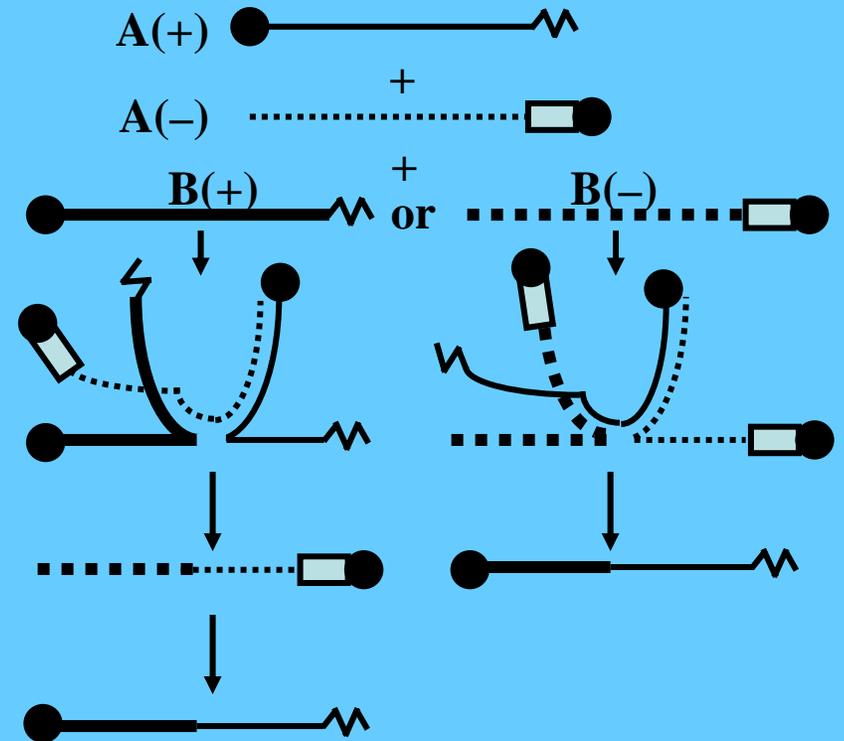


ST-RNA: RNAs of standard virus

DI-RNA: RNAs of defective viral strain

● : VPg ~ : poly A □ : poly U

genome recombination between viruses



A(+)とA(-): A strain + chain, A strain - chain

B(+)とB(-): B strain + chain, B strain - chain