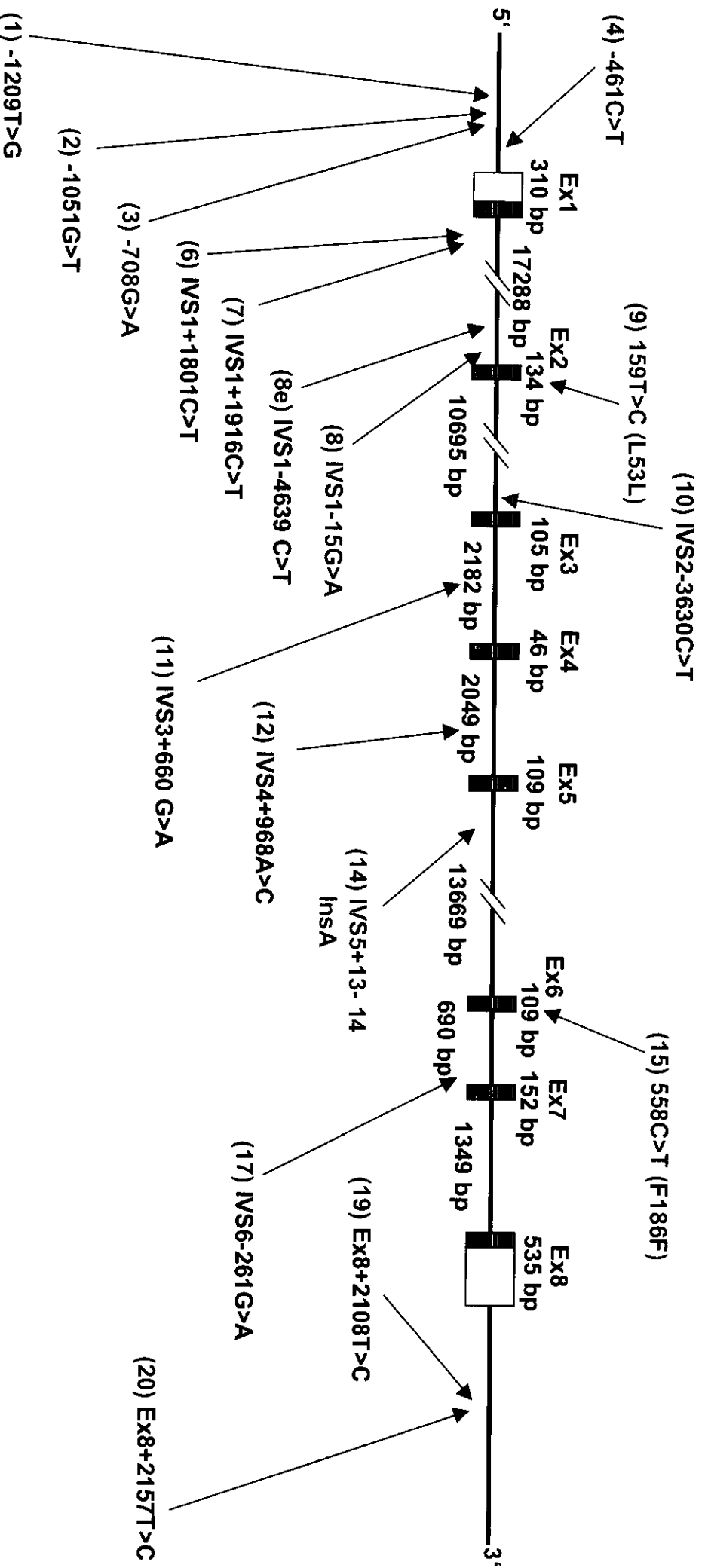


# IMPA2: 18p11.2 (UCSC 2003 Apr. NM\_014214)

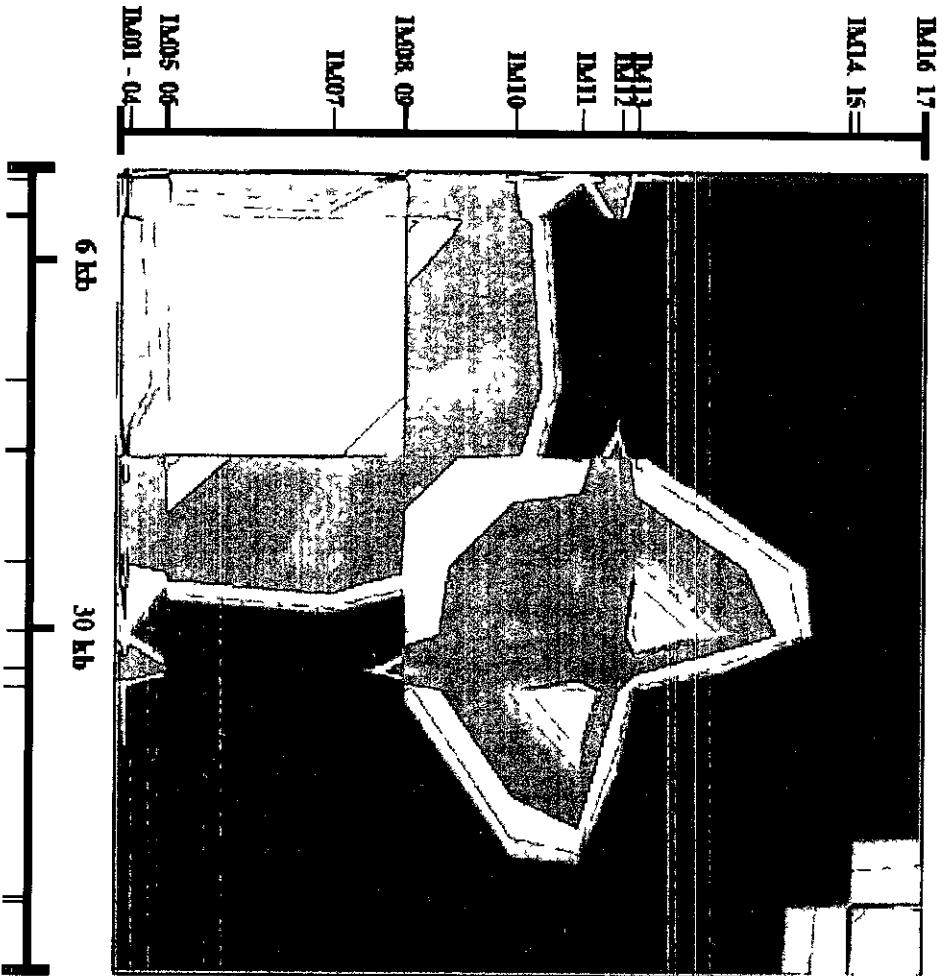
## 資料4-1 IMPA2遺伝子構造と SNPs



資料4-2

IMPA2遺伝子の  
LD構造

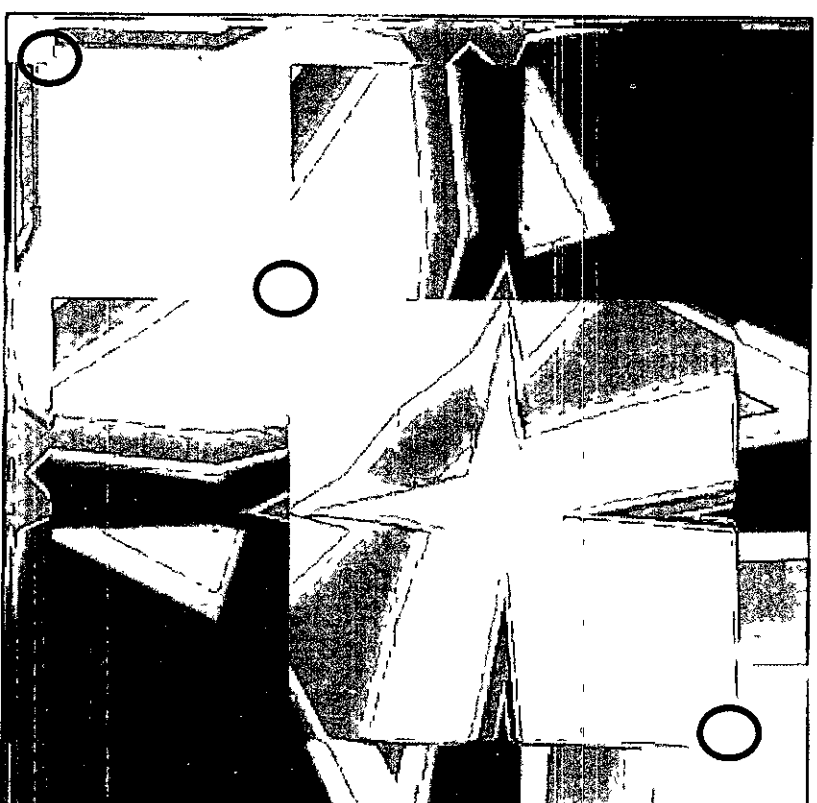
*Linkage Disequilibrium*



$r^2$

$\log_{10} r^2$

-2.00



$D'$  value

1.00

0.00

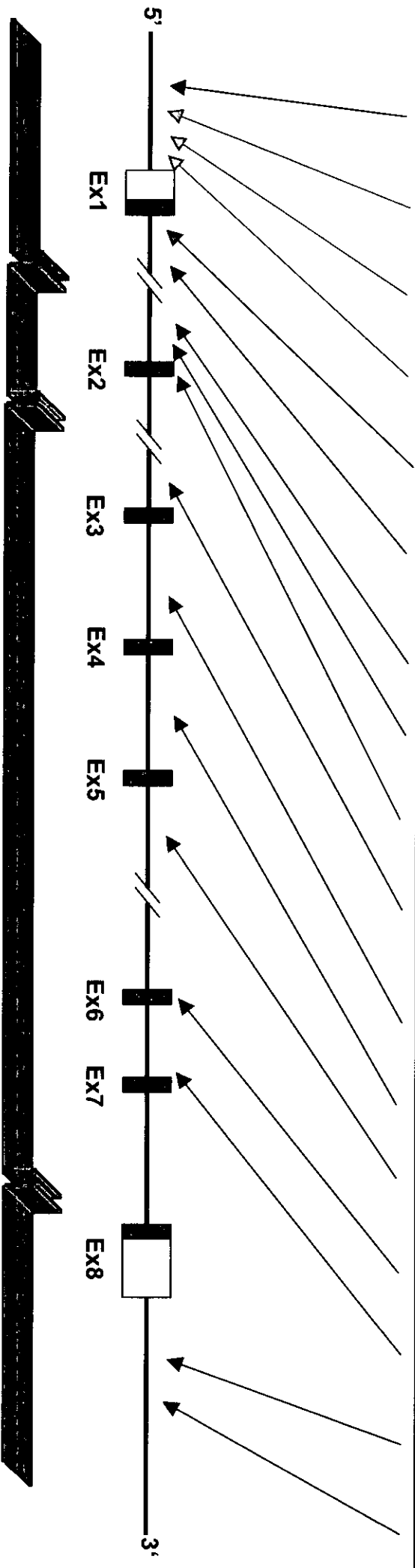


$D'$

IMPA2遺伝子の  
ハプロタイプ解析(1)

2 SNP-based haplotype analysis

	IM01	IM02	IM03	IM04	IM05	IM06	IM07	IM08	IM09	IM10	IM11	IM12	IM13	IM14	IM15	IM16	IM17
	0.206					0.405					0.570					0.625	
		0.022					0.592					0.508					
2SNPs			0.018					0.321		0.763			0.684			0.795	
				0.121						0.473					0.625		0.723



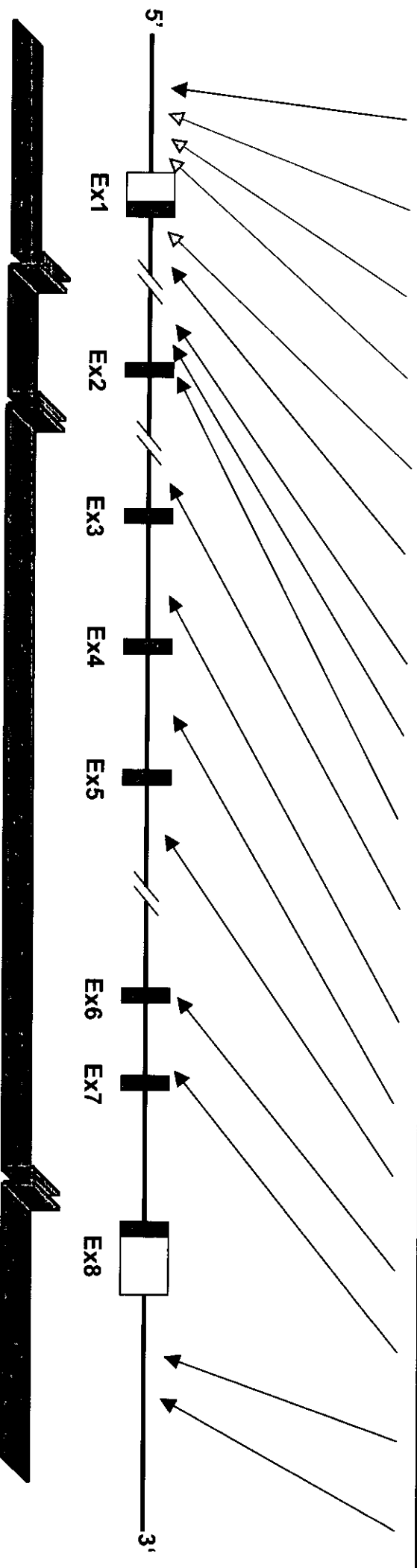
**Bipolar patients, n=494**  
**Controls, n=537**

# 資料4-4

## IMPA2遺伝子の ハプロタイプ解析(2)

### 3 SNP-based haplotype analysis

	IM01	IM02	IM03	IM04	IM05	IM06	IM07	IM08	IM09	IM10	IM11	IM12	IM13	IM14	IM15	IM16	IM17
		0.087				0.498				0.748				0.893			
			0.027				0.332				0.752				0.923		
				0.019				0.649				0.572				0.890	
3SNPs					0.332				0.382				0.606				



**Bipolar patients, n=494**  
**Controls, n=537**

## IM02-03

Haplotype	Case	freq	Control	freq	chisq	p
T A	124	0.128	162	0.153	1.556	0.212
T G	317	0.329	353	0.334	0.244	0.621
G A	17	0.018	3	0.003	7.399	0.007
G G	506	0.525	540	0.510	0.816	0.366

Global P value =

0.022

## IM03-04

Haplotype	Case	freq	Control	freq	chisq	p
A T	115	0.124	164	0.156	2.720	0.099
A C	19	0.020	5	0.004	6.451	0.011
G T	306	0.329	353	0.334	0.254	0.615
G C	488	0.526	534	0.506	1.381	0.240

Global P value =

0.018

## IM04-05

Haplotype	Case	freq	Control	freq	chisq	p
T T	291	0.312	341	0.326	0.430	0.512
T C	134	0.144	179	0.171	2.778	0.096
C C	507	0.544	526	0.503	3.342	0.068

Global P value =

0.121

## Three-marker haplotype

資料4-6

IMPA2遺伝子の

ハプロタイプ解析

(4)

IM02-03-04

Haplotype	Case	freq	Control	freq	chisq	p
TAT	113	0.126	158	0.154	2.114	0.146
TGT	298	0.330	341	0.333	0.141	0.708
GAC	16	0.017	3	0.003	6.383	0.012
GGC	475	0.527	524	0.511	0.935	0.334

Global P value = 0.027

IM03-04-05

Haplotype	Case	freq	Control	freq	chisq	p
ATC	115	0.125	162	0.156	3.894	0.048
ACC	18	0.020	7	0.007	6.484	0.011
GTT	287	0.313	337	0.325	0.359	0.549
GTC	17	0.019	13	0.013	1.134	0.287
GCC	481	0.524	517	0.499	1.215	0.270

Global P value = 0.019

## Four-marker haplotype

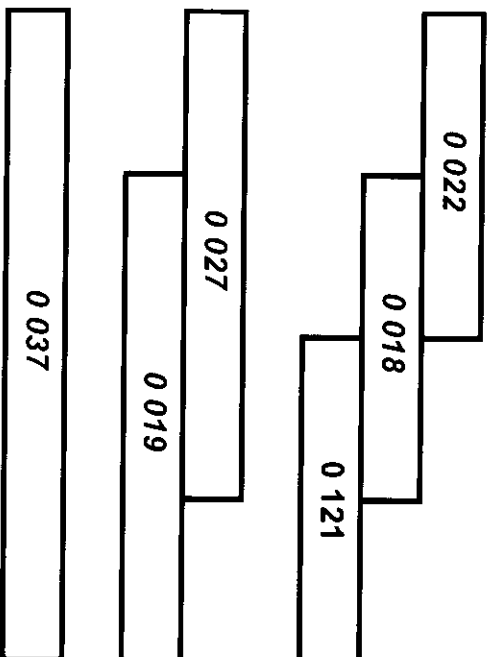
IM02-03-04-05

Haplotype	Case	freq	Control	freq	chisq	p
TATC	112	0.124	155	0.153	3.163	0.075
TGTT	283	0.314	330	0.325	0.236	0.628
TGTC	14	0.016	11	0.011	0.819	0.366
GACC	16	0.018	6	0.006	6.055	0.014
GGCC	475	0.528	514	0.506	0.917	0.338

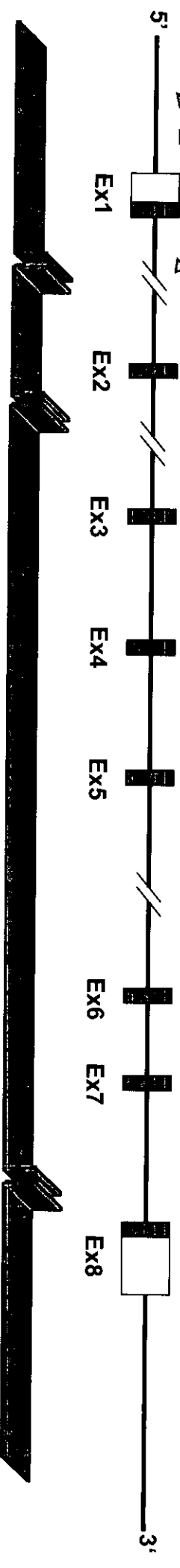
Global P value = 0.037

# Risk haplotype - Summary

	IM02	IM03	IM04	IM05
G		A	C	C



資料4-7  
 IMPA2遺伝子の  
 ハプロタイプ解析(5)



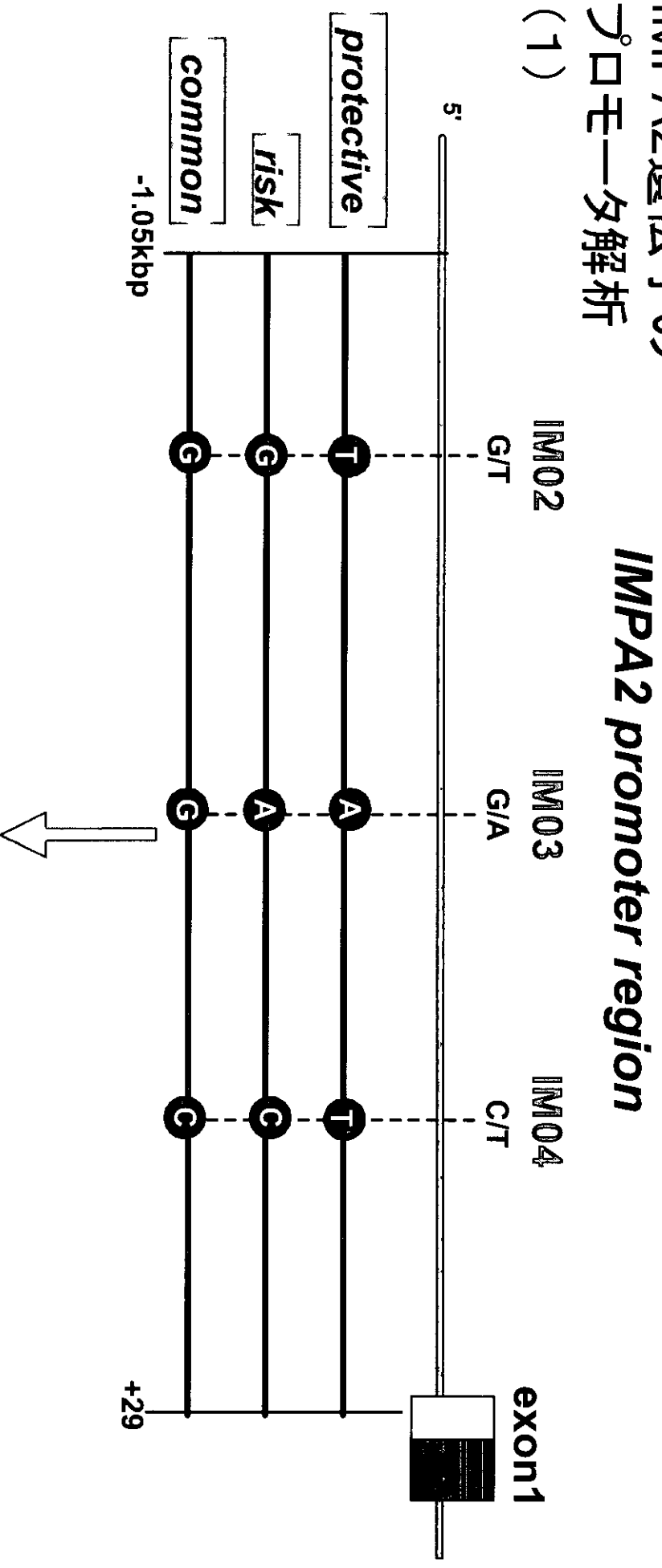
# Constructs for IMPA2 promoter assay

資料4-8

IMPA2遺伝子の

プロモーター解析

(1)



Cloned into pGL3, a Luciferase vector

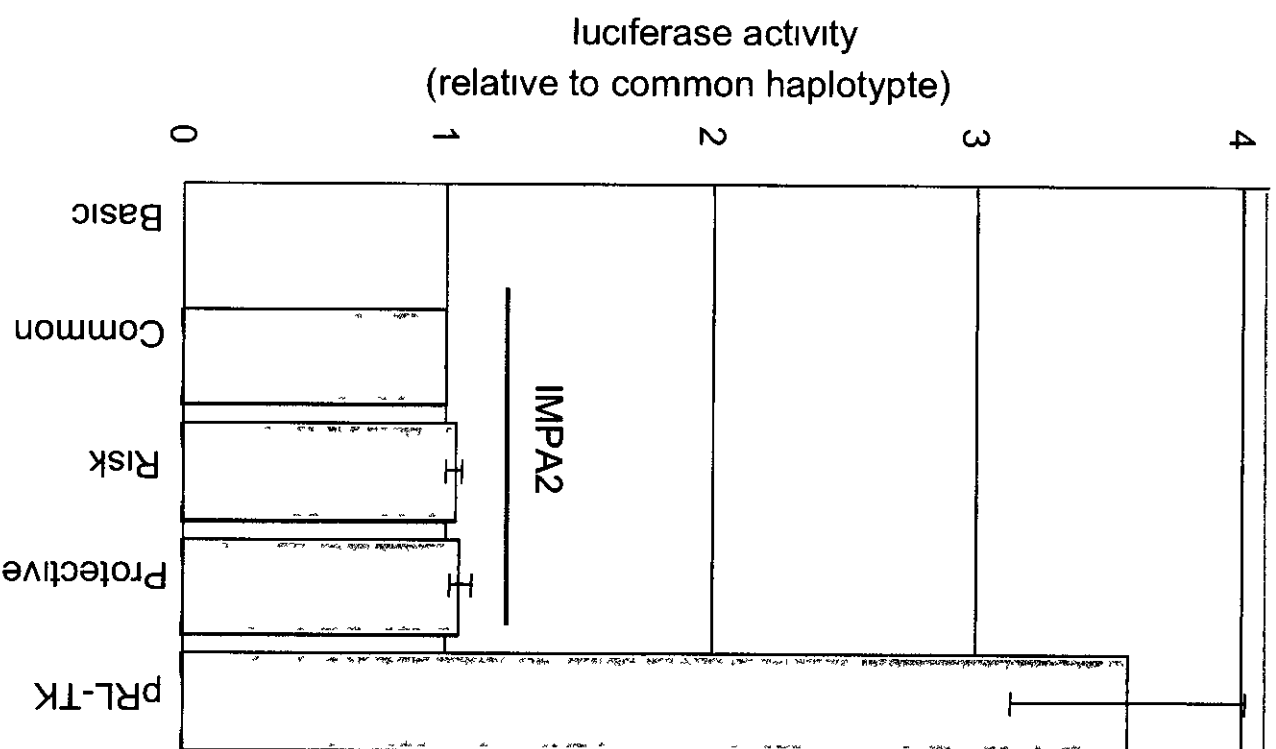


# IMPA2 promoter activity in HeLa cells

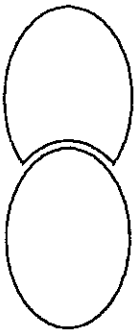
資料4-9

IMPA2遺伝子の

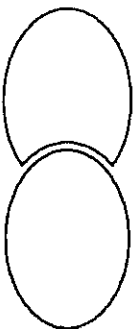
プロモータ解析(2)



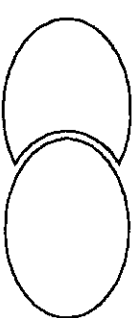
IMPA1 has been reported to form a homodimer. In order to obtain basic information on IMPA2, I tested whether IMPA2 forms a homodimer as well as a heterodimer with IMPA1 or not.



IMPA1 homodimer



IMPA2 homodimer ?



IMPA1/2 heterodimer ?

---

In order to test these, I constructed plasmids encoding tagged proteins shown below.

## 資料4-10 IMPA2遺伝子の 生化学的解析(1)

HA-tagged IMPA1



V5-tagged IMPA1



HA-tagged IMPA2



V5-tagged IMPA2



Using these constructs, I performed immunoprecipitation assay in lysate of cell transiently expressing two of these proteins.

# Coimmunoprecipitation assay of IMPA1 and IMPA2

HA-IMPA1/2 (4mg)    1   2   1   2   1   2   2   2   2   2

V5-IMPA1/2 (2mg)    -   -   1   1   1   2   2   2   -

Overexpression  
in HEK293T cells

WB anti-HA



Input

(expression of each protein)

WB:anti-V5



## 資料4-11

### IMPA2遺伝子の

### 生化学的解析(2)

WB anti-HA



Immunoprecipitate

(with anti-V5 antibody)

WB anti-V5

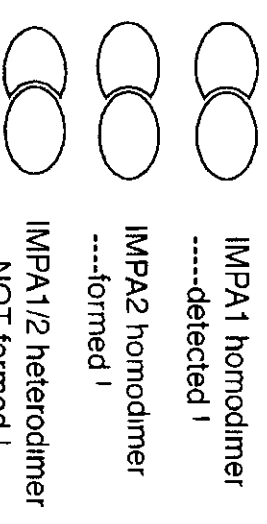


**Methods**

6cm dishes/293T transfection, CaPO4 method  
 cell lysis sonication in a buffer containing 25mM TrisHCl pH7.8  
 150mM NaCl, 1% Triton X-100, 1mM NaF 0.25M sucrose, 1mM DTT,  
 5ug/ml leupeptin, 2ug/ml aprotinin 1mM PMSF

centrifuge and transfer sup to a new tube  
 add 1ul anti-V5 monoclonal Ab preadsorbed to ~20ul protein G fast flow (Amersham)  
 2h rotation  
 wash with lysis buffi x6  
 boil in 1.5x SDS sample buffi    Western blotting

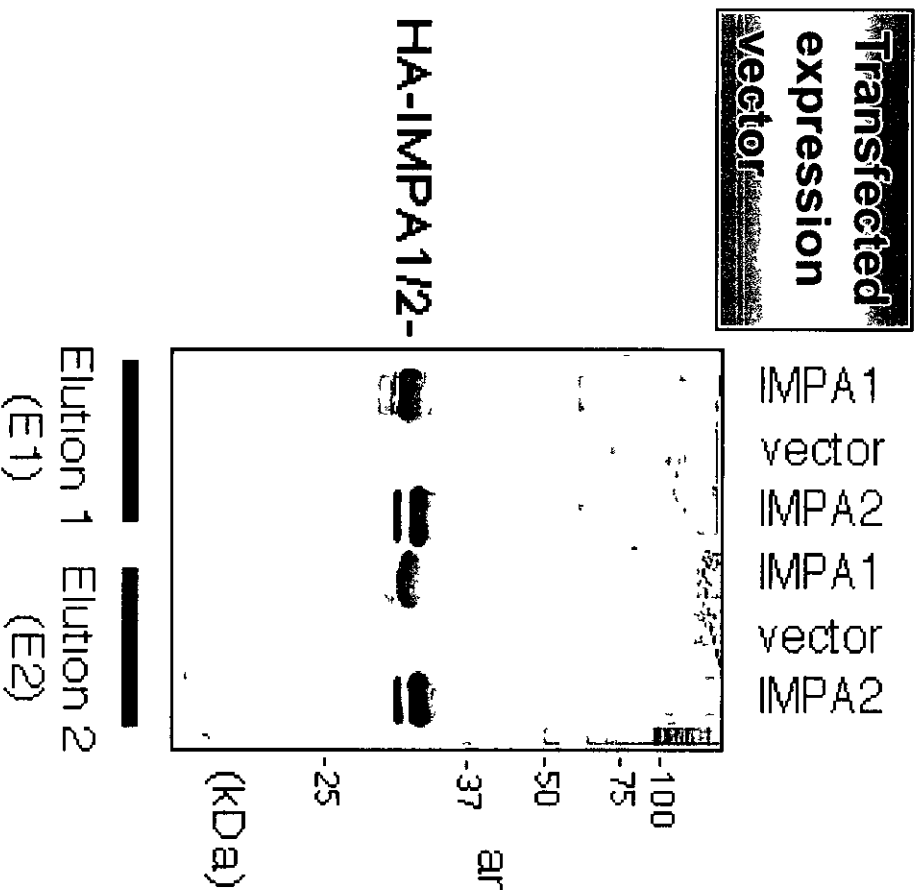
Important !



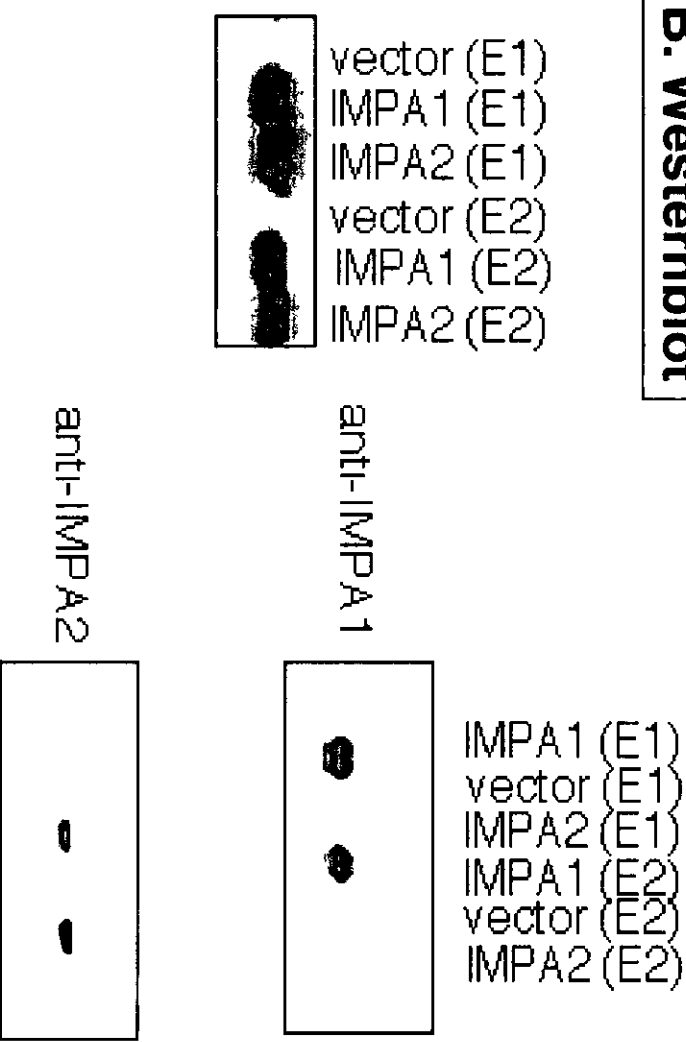
This result clearly indicates that each of IMPA1 and IMPA2 forms "homocomplex" (=homodimer?), but not "heterocomplex" (=heterodimer?).

# Recombinant IMPA proteins

## A. Silver stain

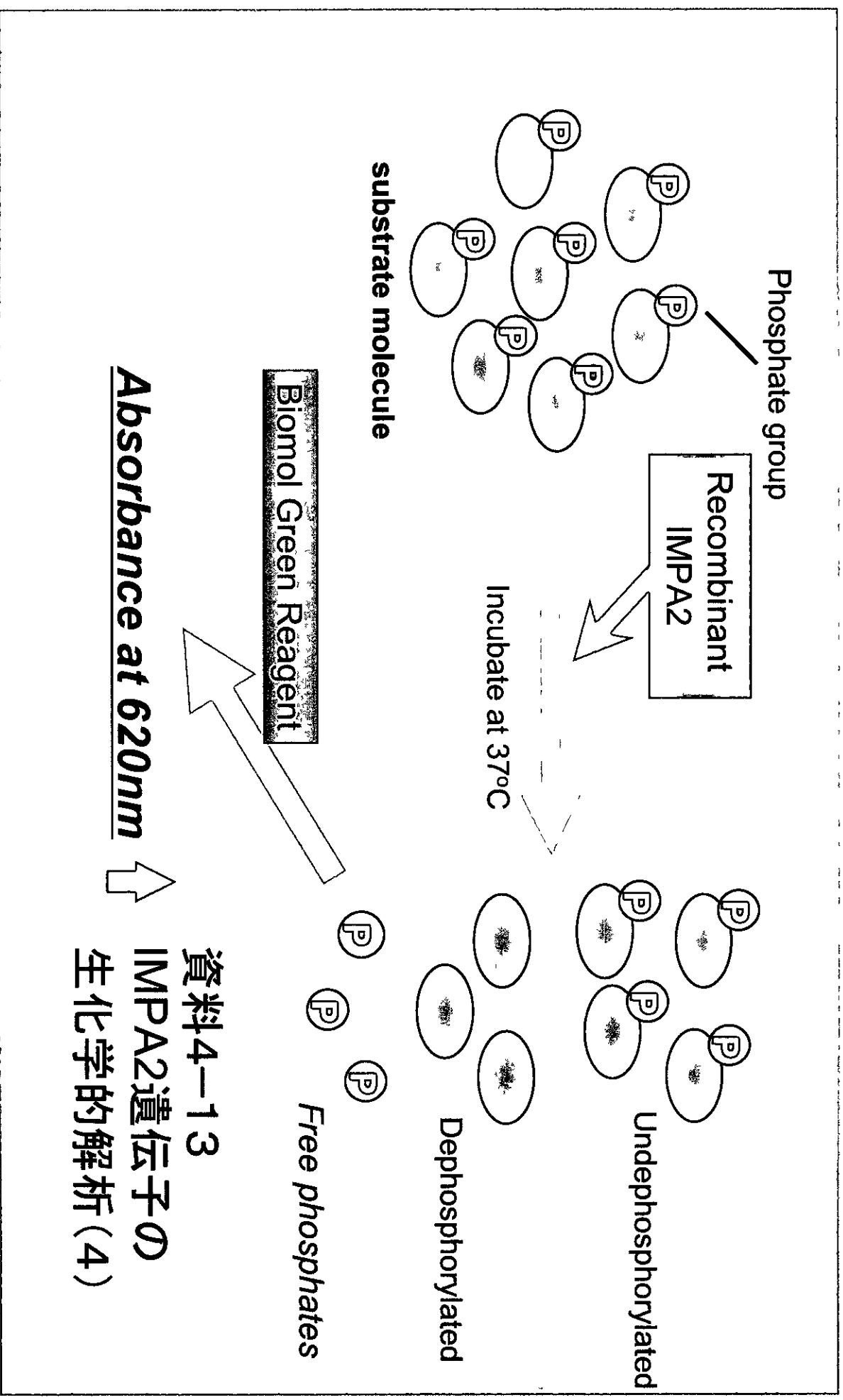


## B. Westernblot



資料4-12  
IMPA2遺伝子の  
生化学的解析(3)

# Assay system for IMPA activity

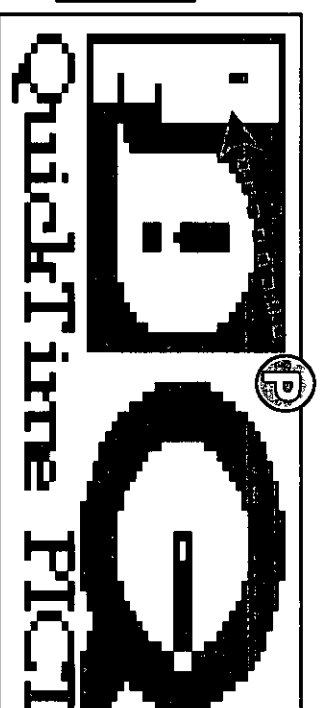


資料4-13

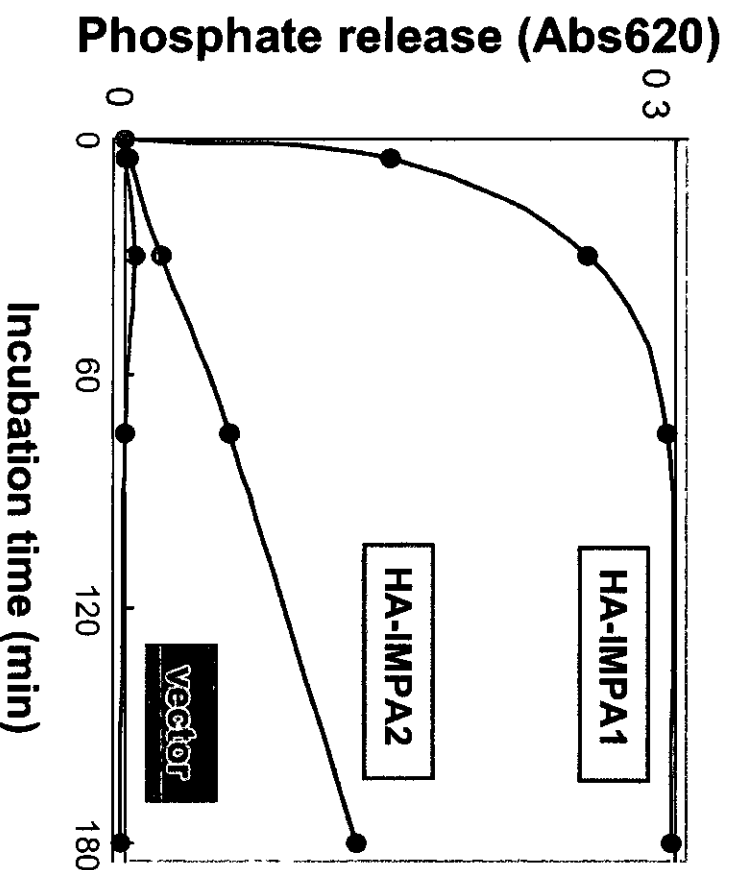
IMPA2遺伝子の  
生化学的解析(4)

# IMPA2 shows intrinsic inositol monophosphatase activity

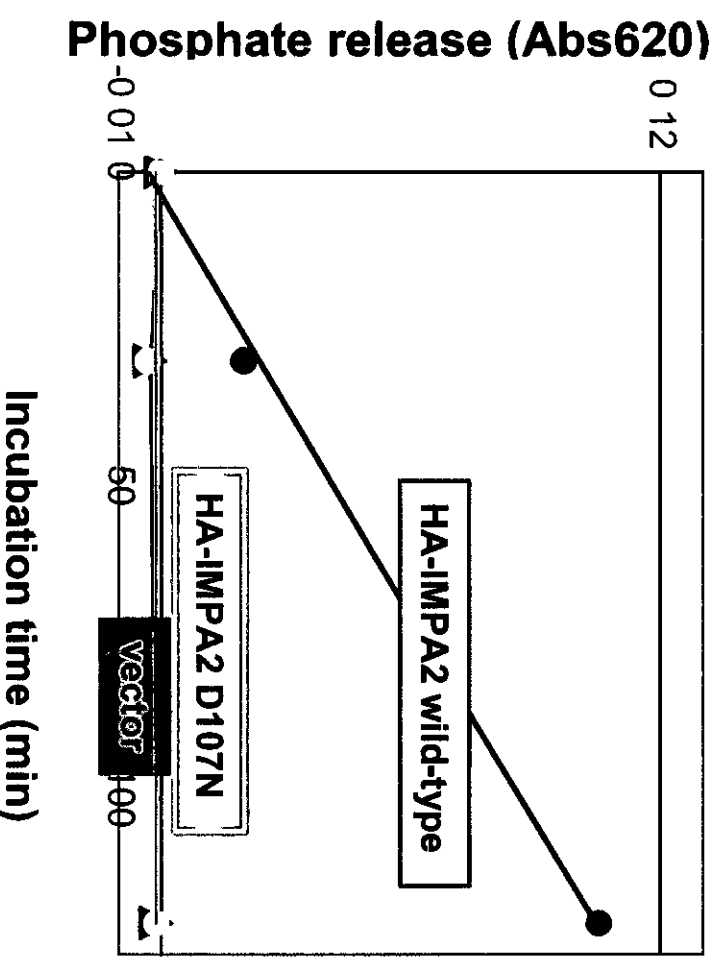
A. Chemical structure of myo-inositol and myo-inositol 1-phosphate



B. IMPA2 preparation has IMPase activity

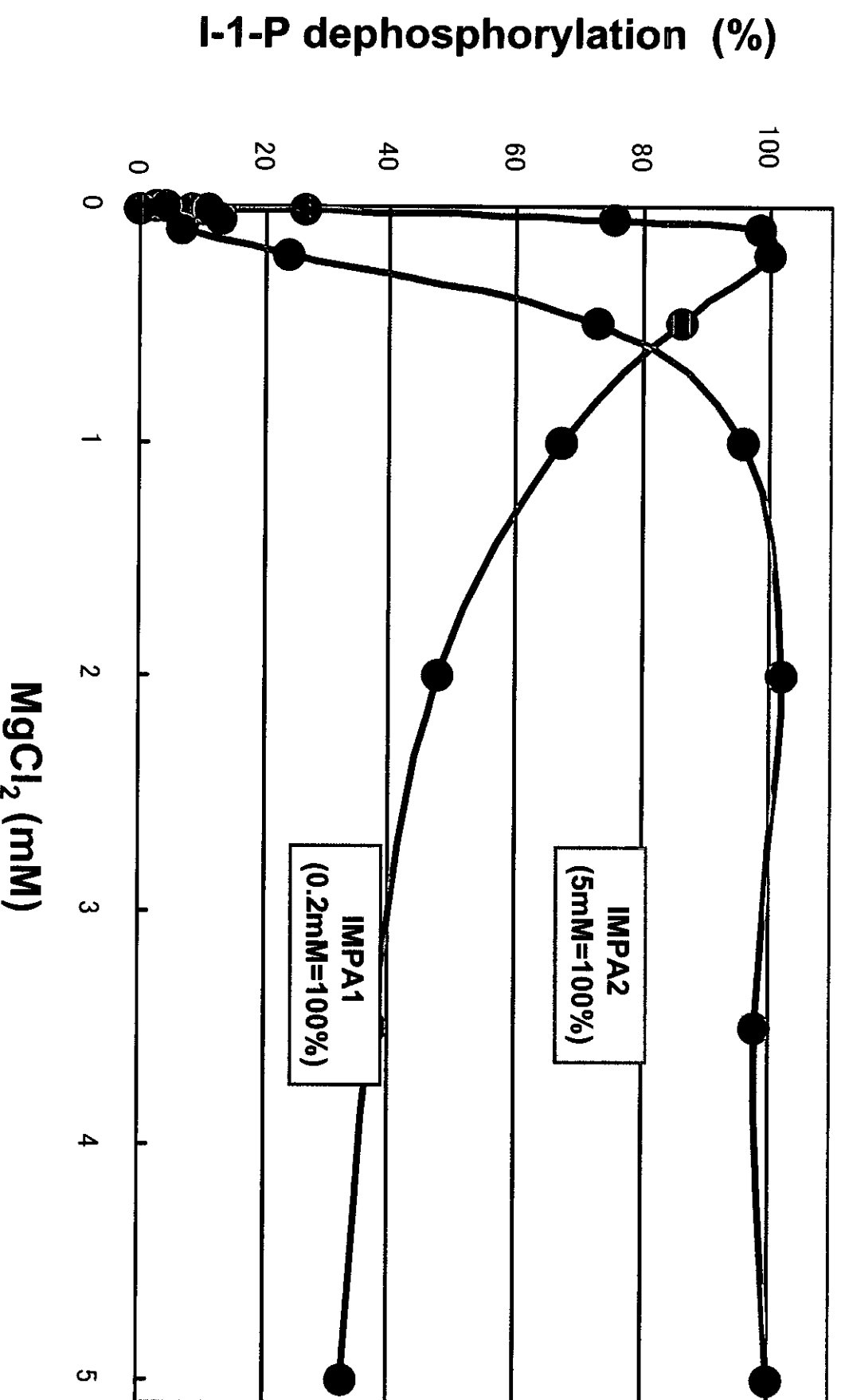


C. IMPA2 DN mutant loses the activity



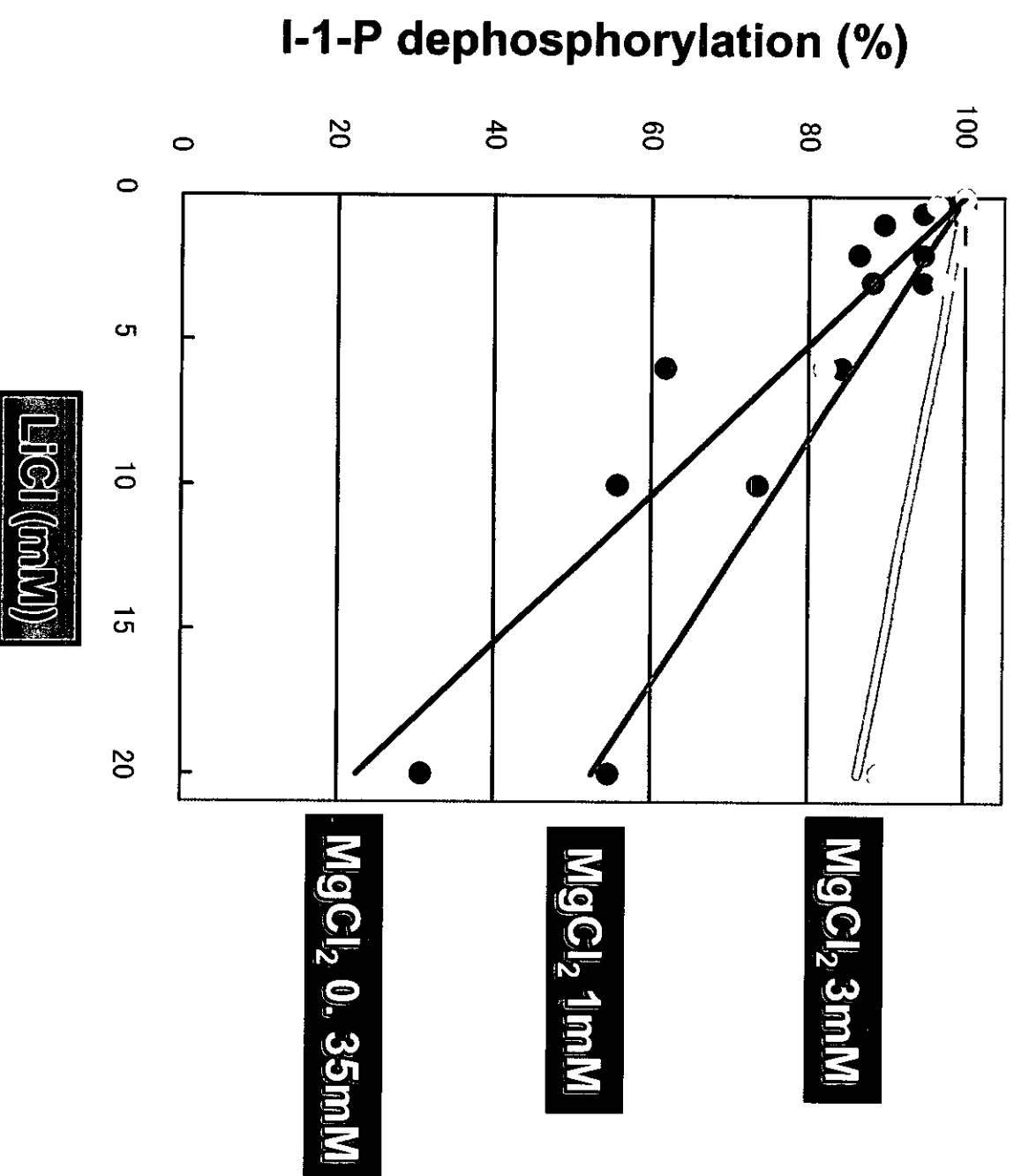
資料4-15  
IMPA2遺伝子の  
生化学的解析(6)

## Inositolmonophosphatase activity of IMPA2 is magnesium-dependent



# Lithium inhibits IMPA2 enzymatic activity

資料4-16  
IMPA2遺伝子の  
生化学的解析  
(7)





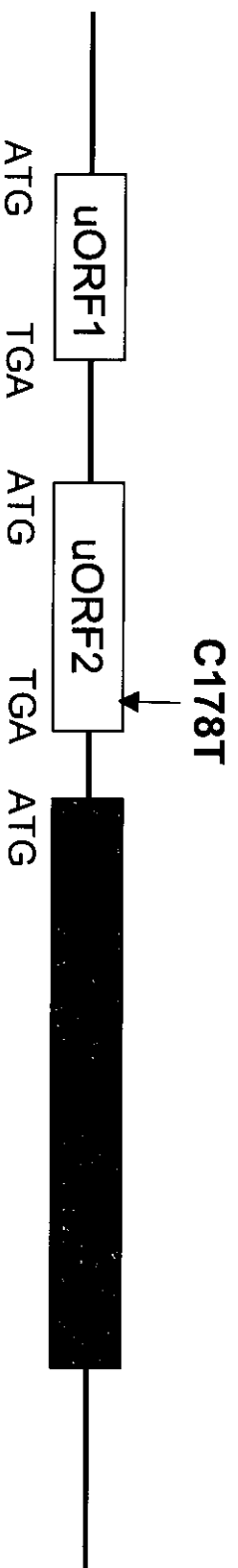
## 資料4-17

	IMPA1	IMPA2
■ Chromosomal Location	8q21.13 - q21.3	18p11.2
■ mRNA	2.3 kb	1.5 kb
■ Protein	277 a.a. (30 kd)	288 a.a. (31 kd)
homology	53.5%	
■ Catalytic activity	inositol monophosphate → inositol + orthophosphate	? probably
■ Subunit	homodimer	homo(dimer)
■ Cofactor	magnesium	probably
■ Enzyme regulation	inhibited by lithium	inhibited by lithium
■ 3D structure	known	unknown
■ Species	human/mouse/rat/bovine/ wild pig/fugu/arabidopsis/ tomato/neisseria	humany/mouse/rat

## 參考資料5-1

# HTR3A,3B genes

- ◆ Serotonin receptors, which are ligand-gated ion channels
- ◆ Mutations in uORFs of HTR3A have been recently shown to associated with mood disorder by changing expression on the translational level (*Niesler et al, 2001*)
  - C178T (P=0.00016)



- ◆ Located in the chromosomal region 11q23.1-23.2
  - Linkage region with bipolar disorder and schizophrenia  
(*Maziade et al, 1995, Detera-Wadleigh et al, 1997, Levinson et al, 1998*)

# 参考資料5-2

## 11q23.1

