Neuraminidase activity of avian influenza neuraminidase N1 expressed in Baculovirus system

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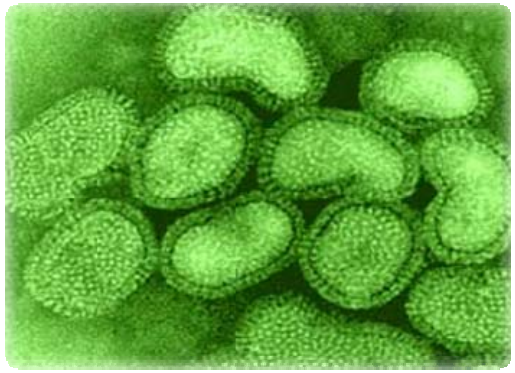
Avian Influenza Virus



- Family : *Orthomyxoviridae*
  - Genera *: Influenzavirus A*
    - Subtype : H5N1

- The cause of severe respiratory disease
- Occur naturally among birds but infections can occur in human.

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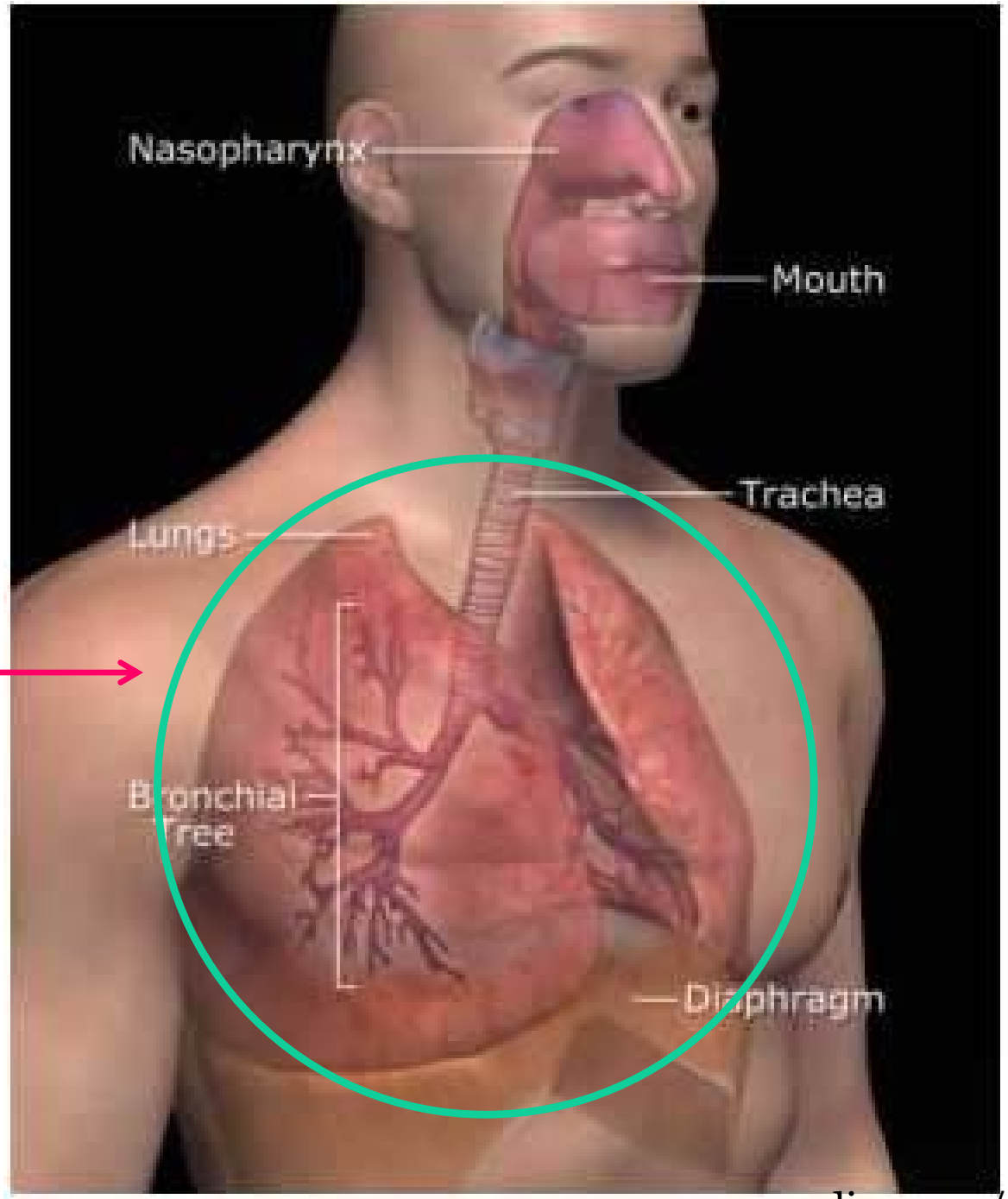
H5N1 Target

The lower respiratory tract :

Lung



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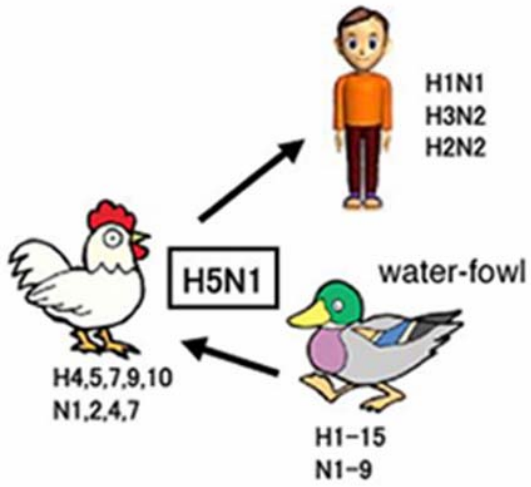
Transmission



- Direct contact
  - secretions or excretions of infected poultry
  - contaminated surface.
- Symptoms
  - Fever, cough, sore throat
  - Pneumonia, acute respiratory distress

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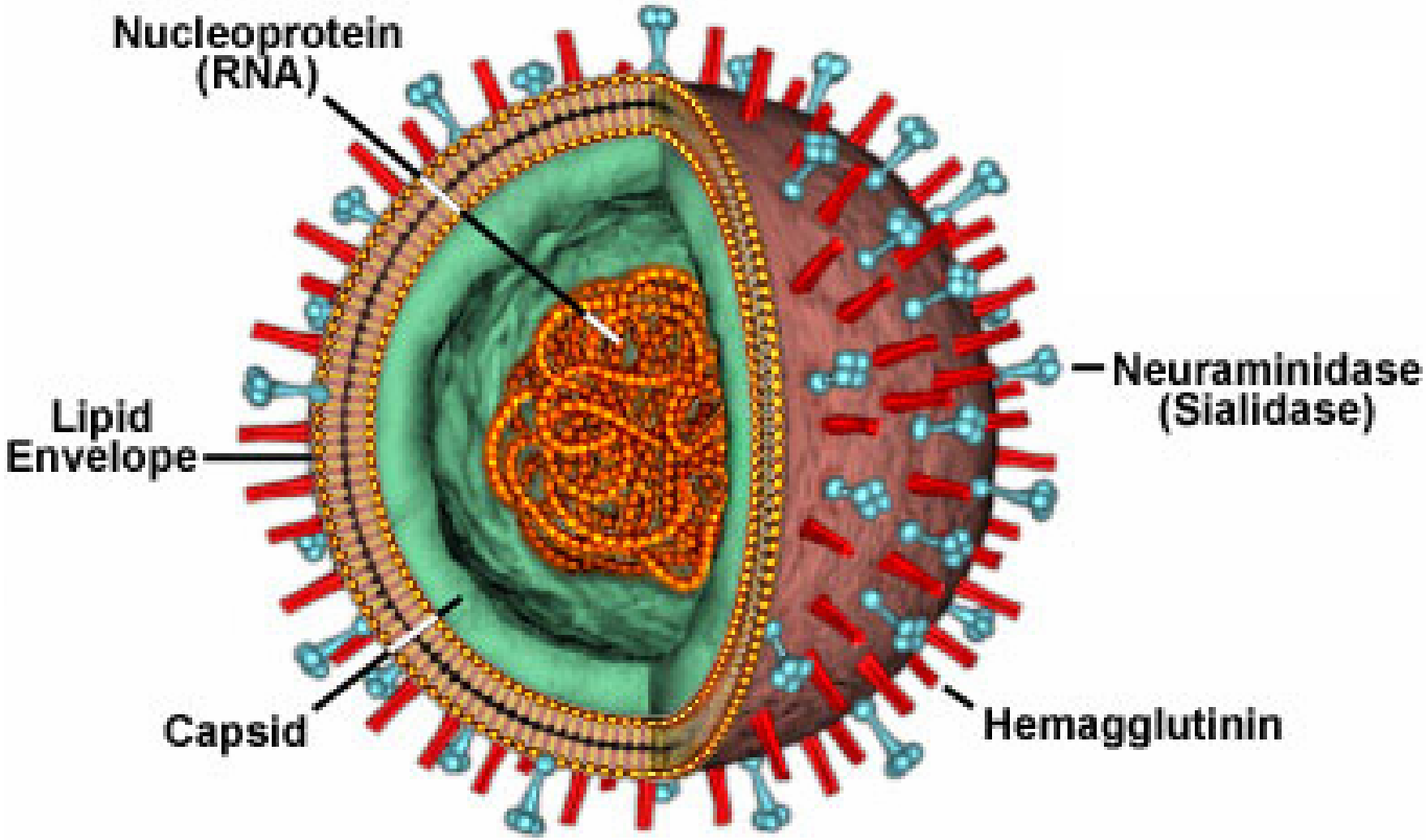
Structure



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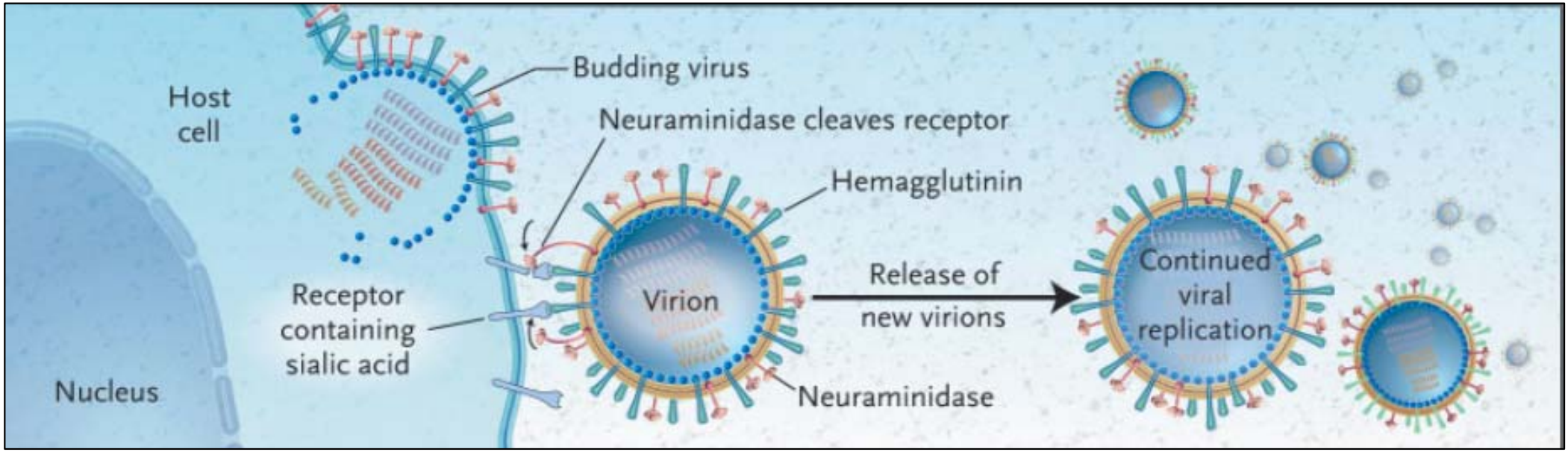
Neuraminidase(NA) gene

- Codes for neuraminidase protein
- Major surface glycoprotein
- Receptor destroying activity



N Engl J Med. 2005 Sep 29;353(13):1363-73 Neuraminidase inhibitors for influenza.

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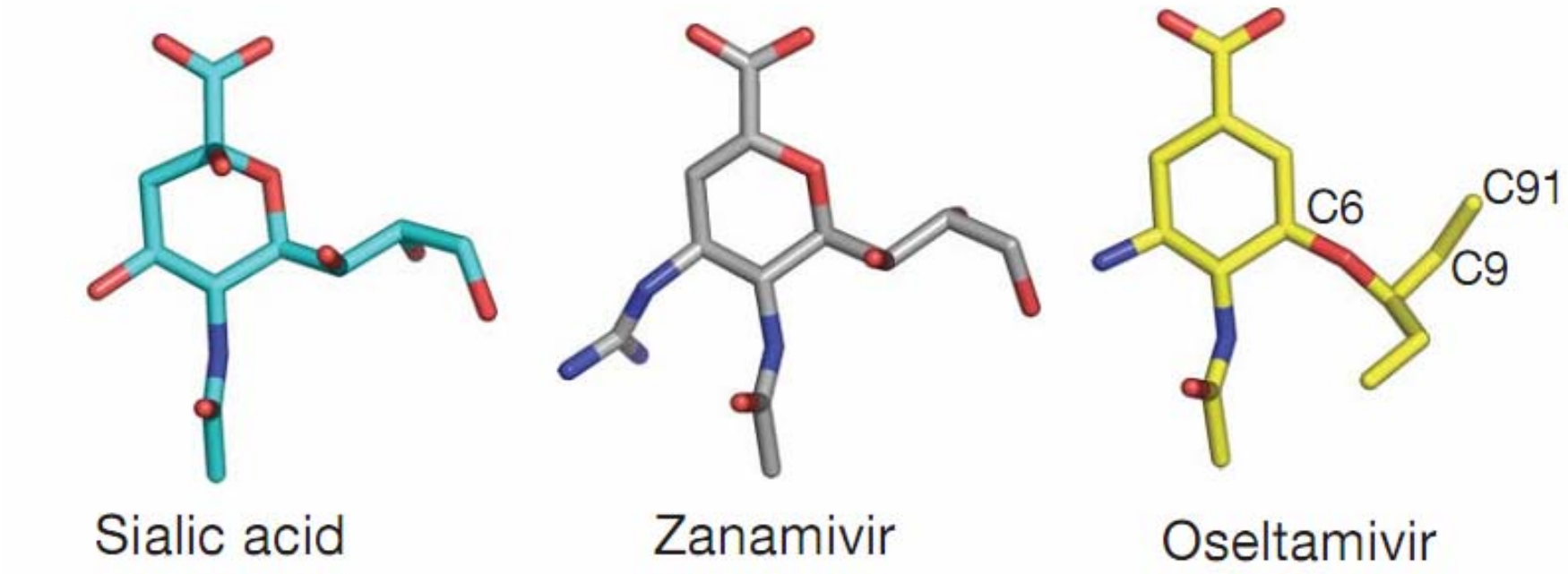
NA inhibitors



- Sialic acid analogues that have the ability to inhibit the influenza neuraminidase.

Vol 453 | 26 June 2008 | doi:10.1038/nature06956

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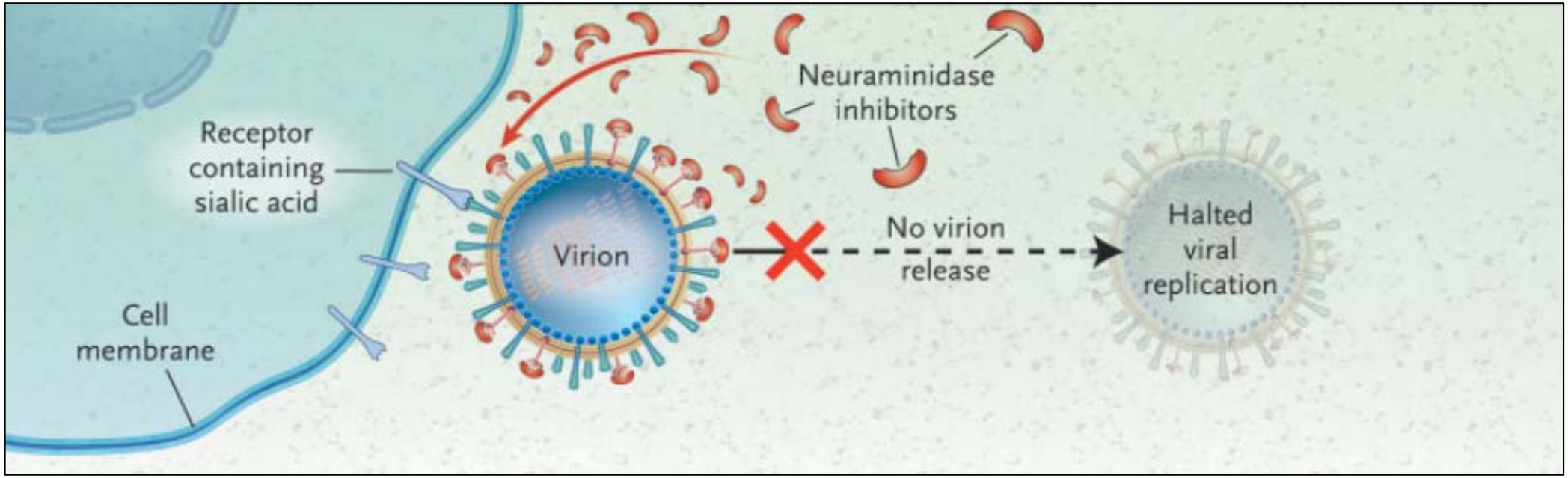
NA inhibition



- Blocking the release of newly virus particle and prevent the spread of infection in the respiratory tract.

N Engl J Med. 2005 Sep 29;353(13):1363-73 Neuraminidase inhibitors for influenza.

Subtype specificity

Mutation	Obtention[a]	Select with	Subtype	Inhibitor sensitivity[b]	
				Zanamivir	Oseltamivir
Catalytic residues					
R292K	In clinic	Oseltamivir	N2		
R152K	In clinic	Zanamivir	B	R	R
Framework residues					
E119V	In clinic	Oseltamivir	N2	S	R
D198N	In clinic	Oseltamivir	B	R	R
H274Y	In clinic	Oseltamivir	N1	S	R
N294S	In clinic	Oseltamivir	N2	Nt	lowR
	In clinic	Oseltamivir	N1	Nt	lowR

[a] Mutation isolated after in vivo inhibitor treatment, rescued compared to.
[b] R for resistance and S for sensitive to the inhibitor.

Catalytic residues : direct contact to substrate
Framework residues : stabilization of the active site structure

O. Ferraris, B. Lina / Journal of Clinical Virology 41 (2008) 13–19

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- To study the enzymatic activity and sensitivity to NA inhibitor(oseltamivir carboxylate) of Neuraminidase N1.

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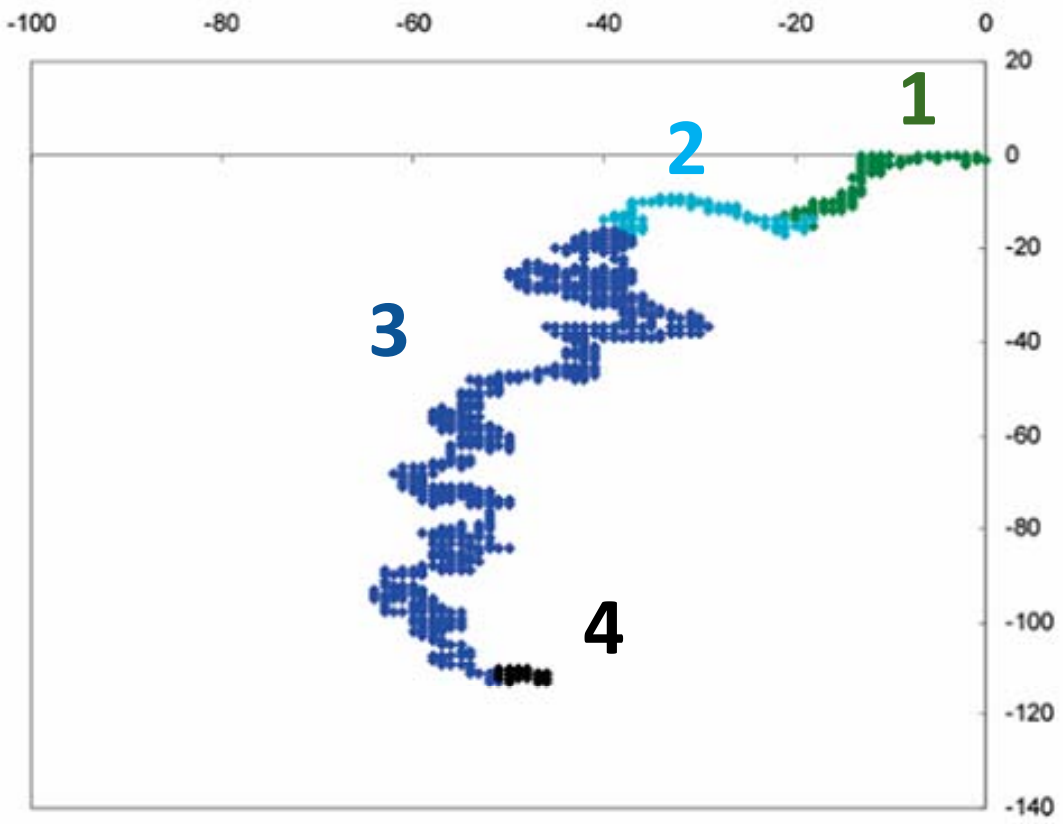
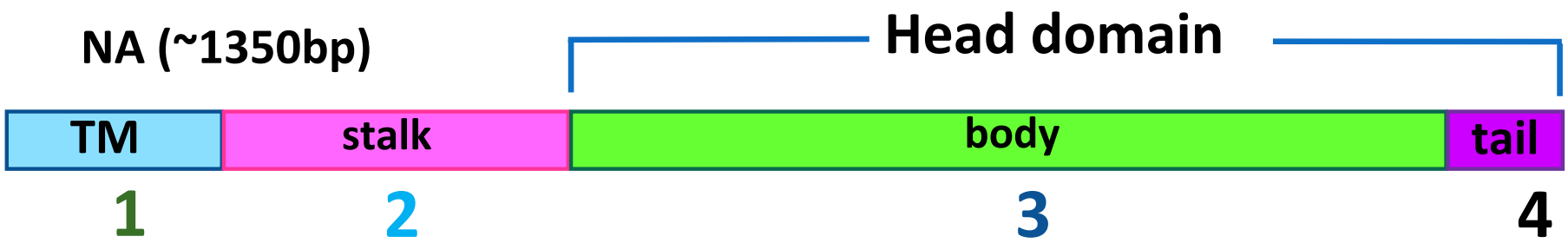
Nandy *et al.* (2007)

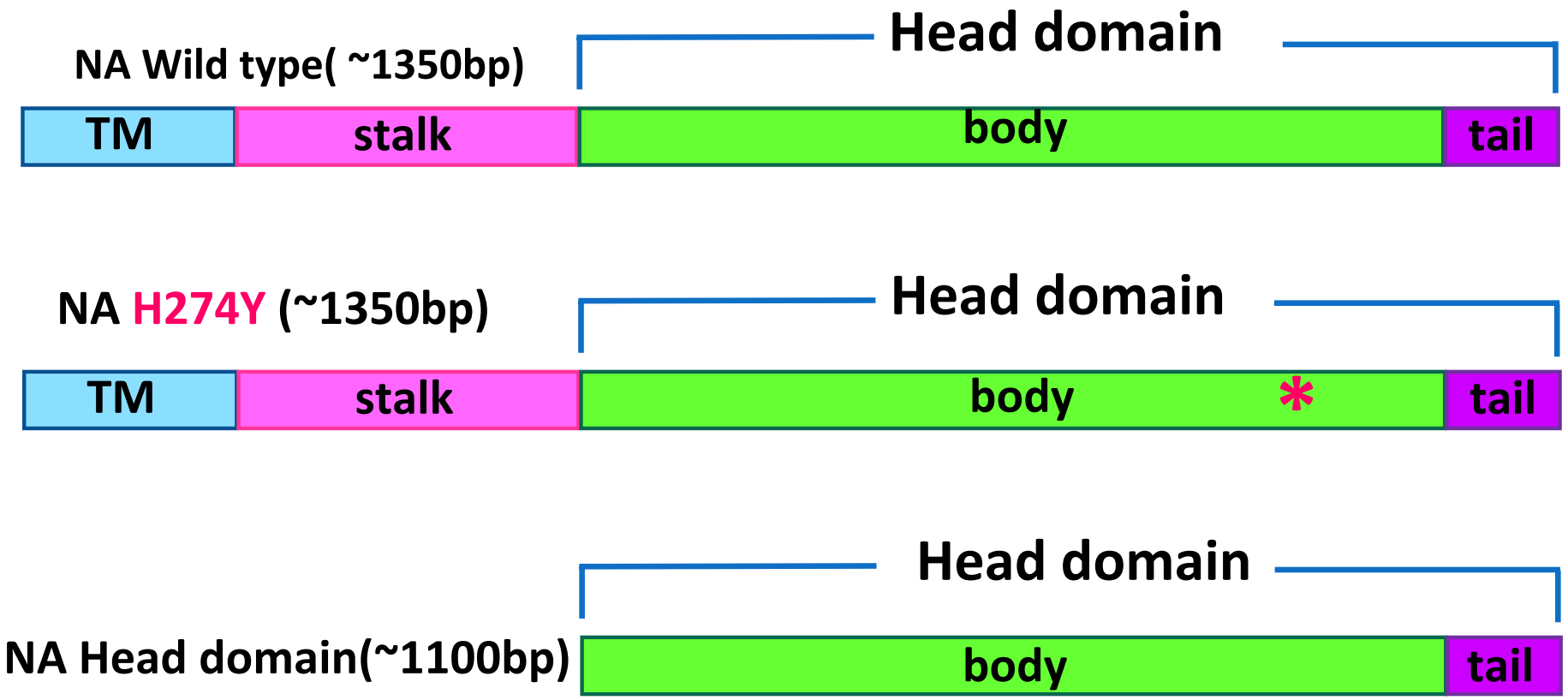


Neuraminidase H5N1 strain
A/Hanoi/30408/2005



J. Chem. Inf. Model. **2007,** *47,* 945−951

Polymerase Chain Reaction (PCR)



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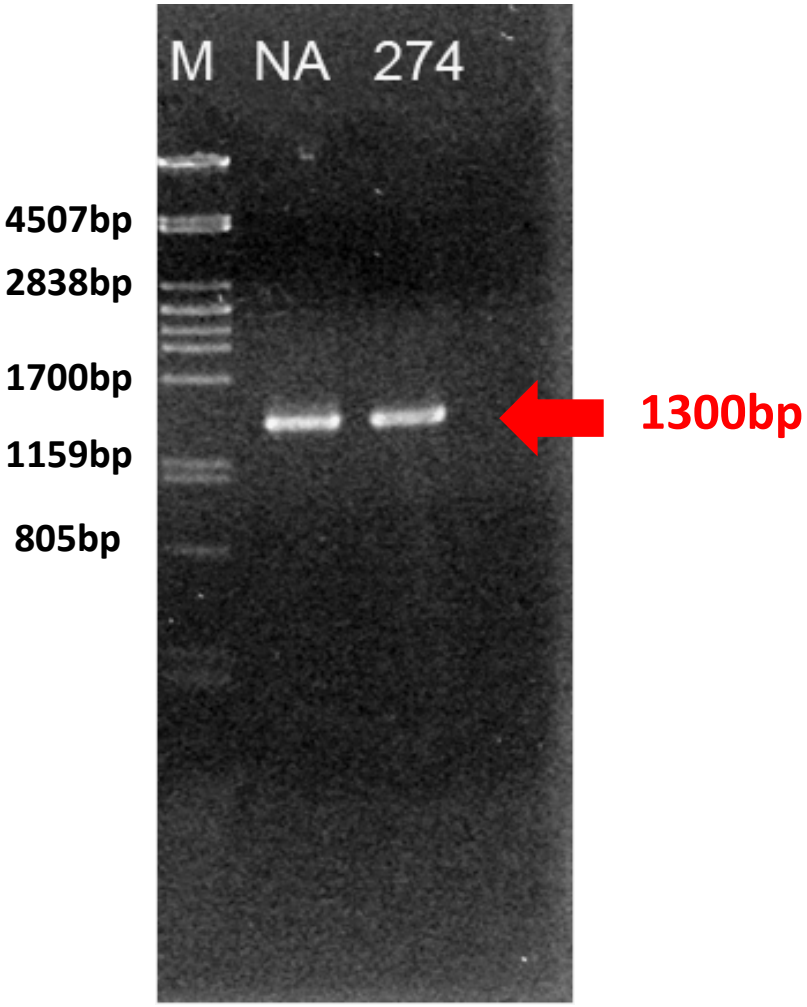
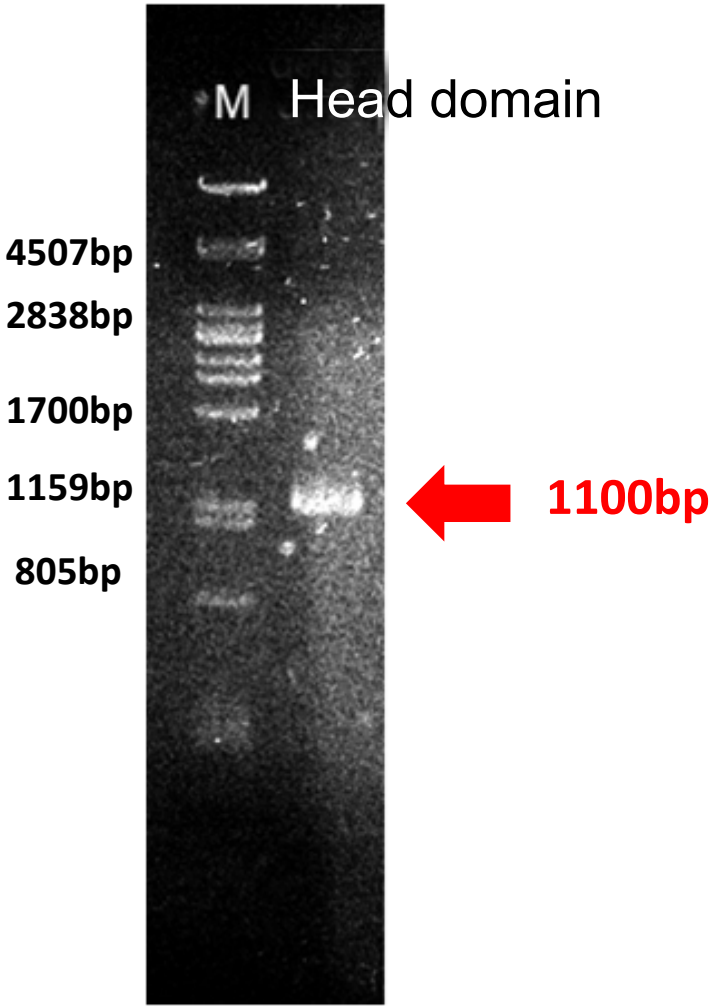
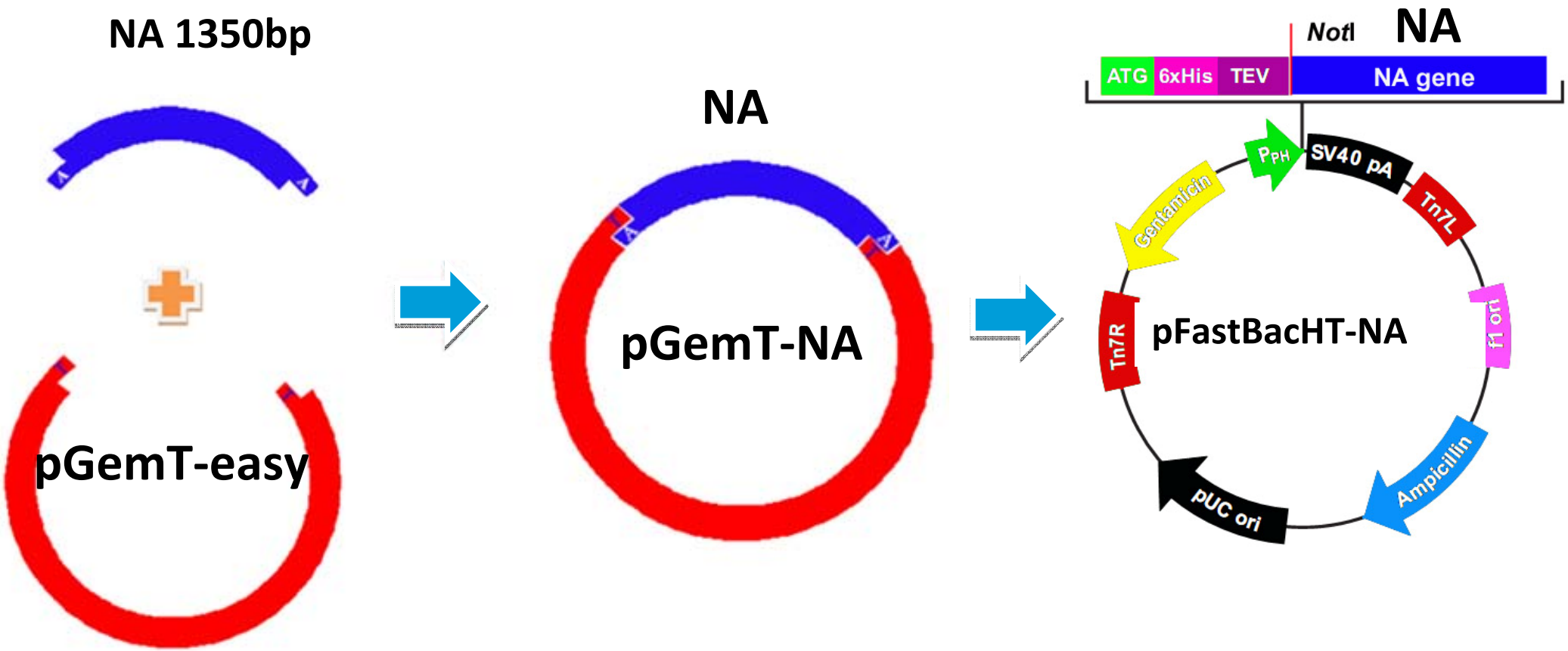
Purified final PCR product







Cloning to pGemT-easy → Subcloning to pFastBacHT → Sequencing

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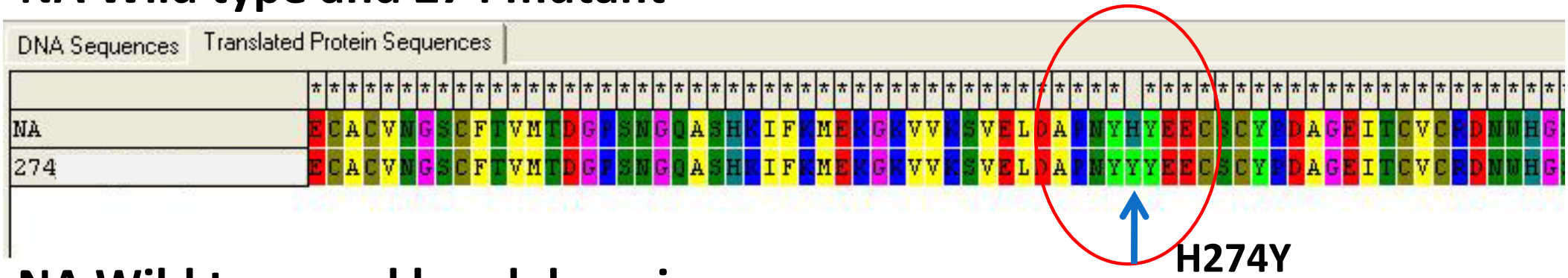
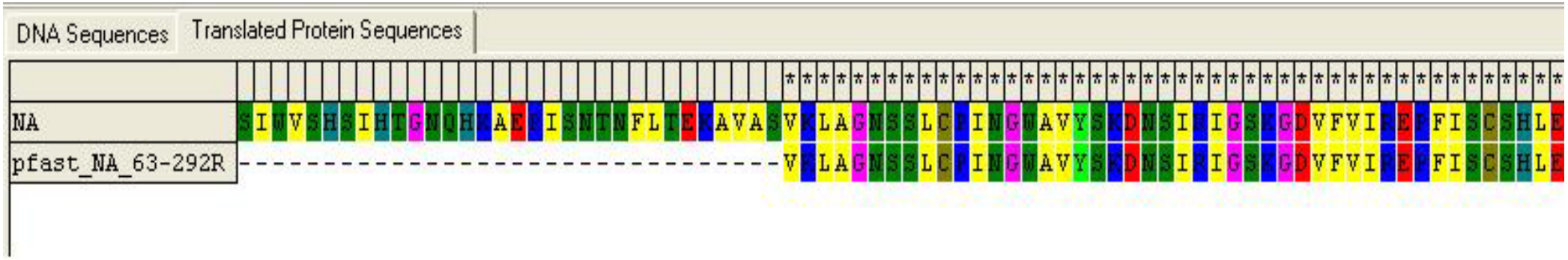
Sequencing analysis (MEGA 4)

NA Wild type and 274 mutant



NA Wild type and head domain



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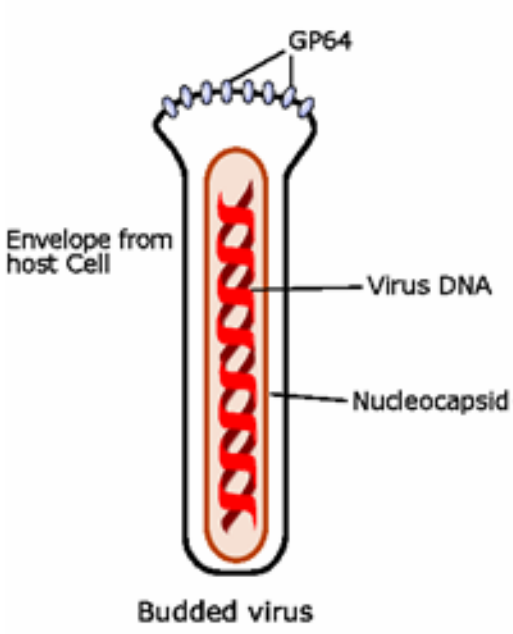
Baculovirus



- Family : *Baculoviridae*
- Rod shaped virion
- a circular, double stranded DNA genome (80 - 180 kbp)
- species-specific tropisms among insect
- Immature (larval) forms of moth species are the most common hosts

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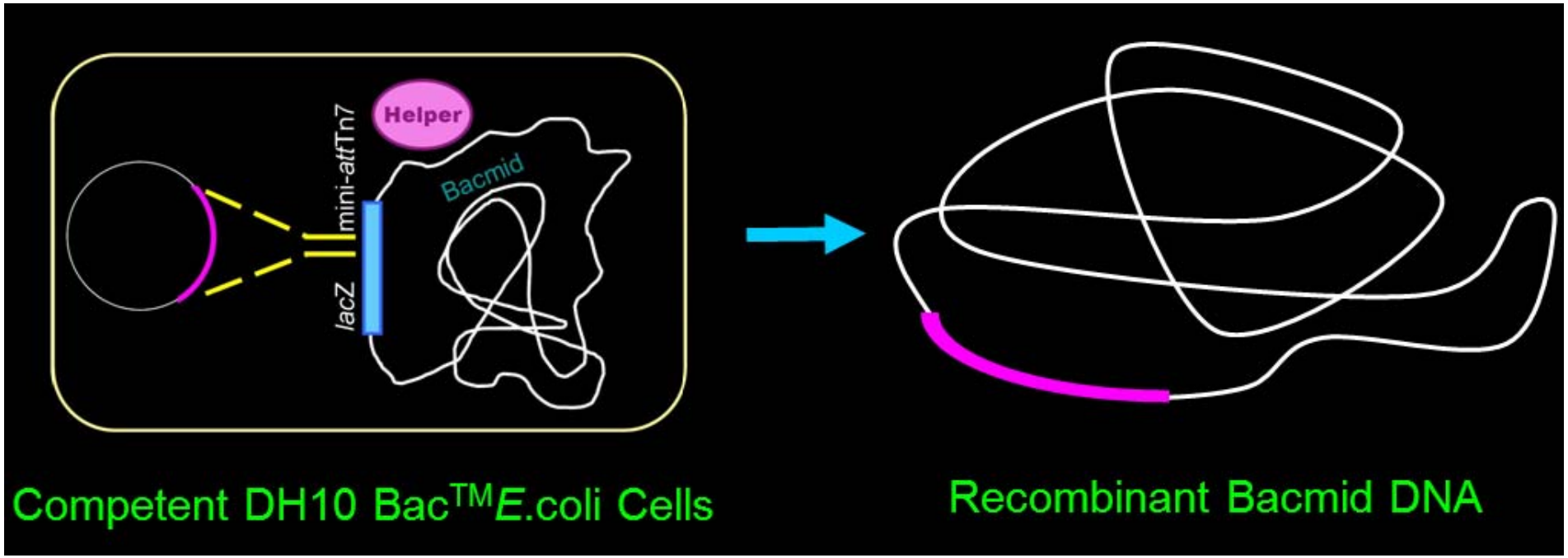
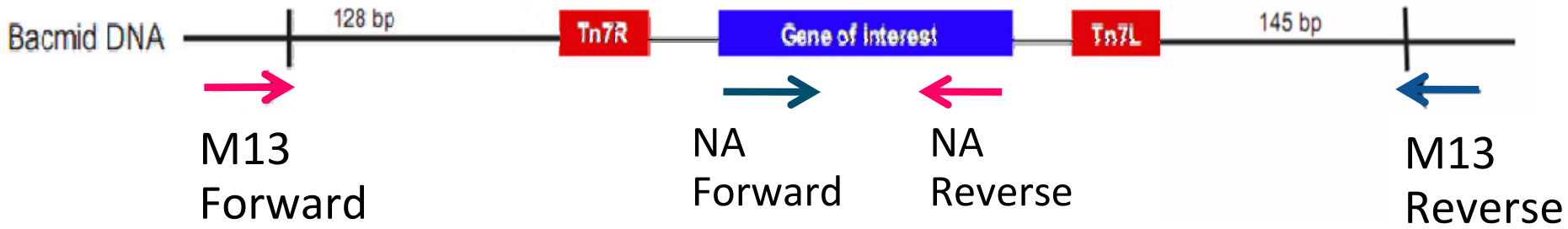
Baculovirus System

- Recombinant baculovirus vector.
  - Delivers gene of interest
- Insect cell host.
  - Produces protein product

Transformation

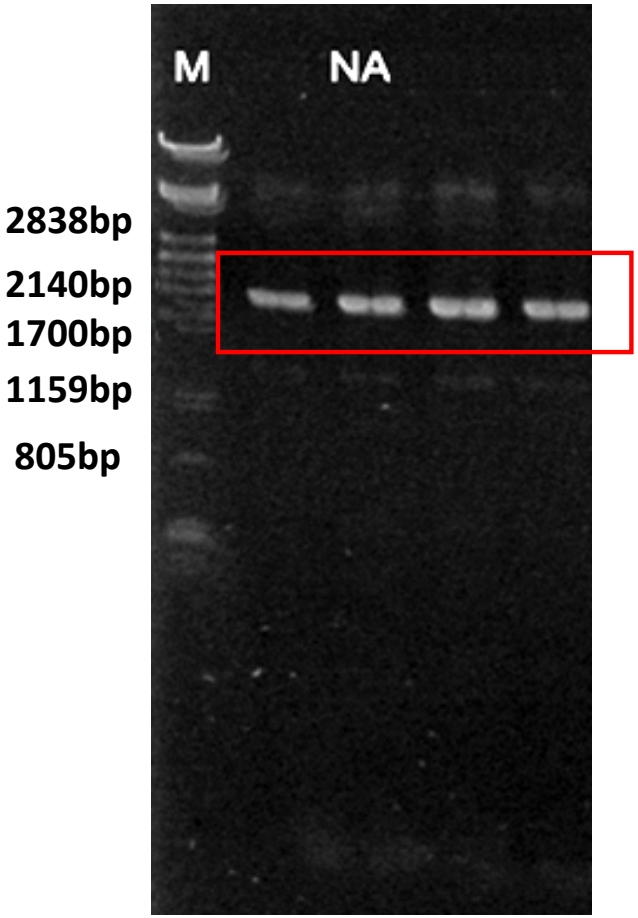


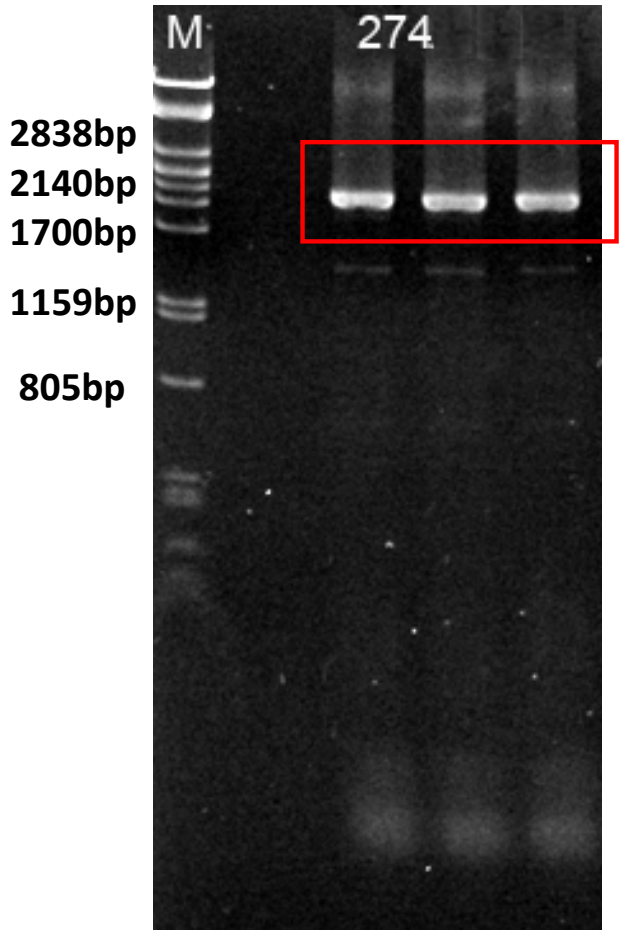
analyze by PCR technique using specific primers



Recombinant Bacmid Anaylsis

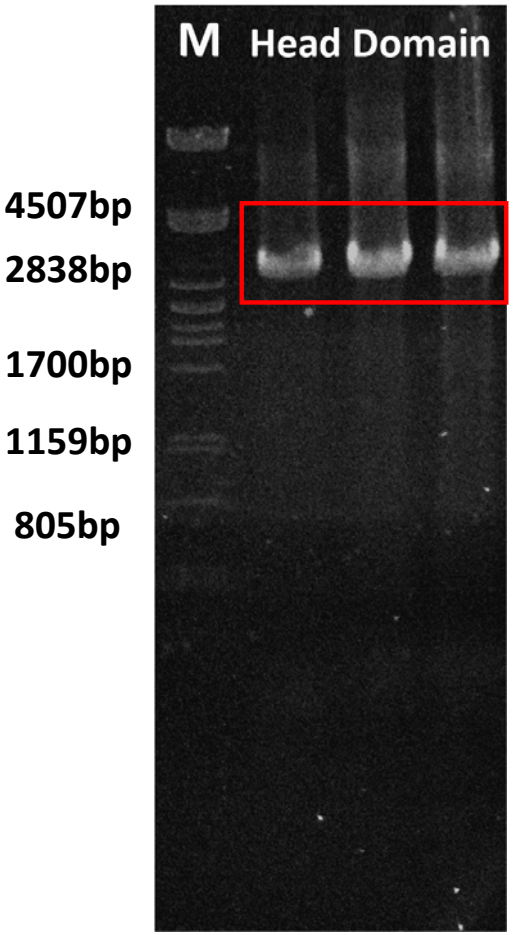
Primers :NAF-M13R





Product ~2400bp

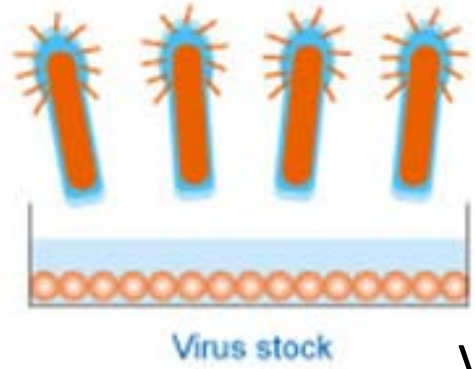
M13F-NAR

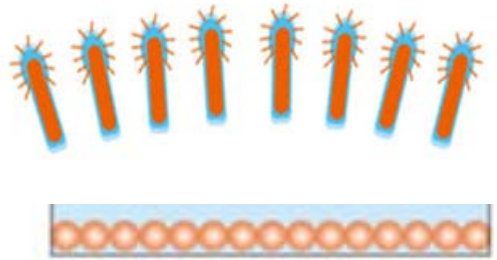


Product ~3300bp

Production of recombinant virus

Recombinant virus particles





Virus amplification

Transfection to Mimic™ cells by using Cellfectin Reagent (Invitrogen)

Recombinant Bacmid DNA extraction
by using Blood and cell culture Genomic DNA miniKit (QIAGEN)
for transfection to Mimic™ Insect Cells

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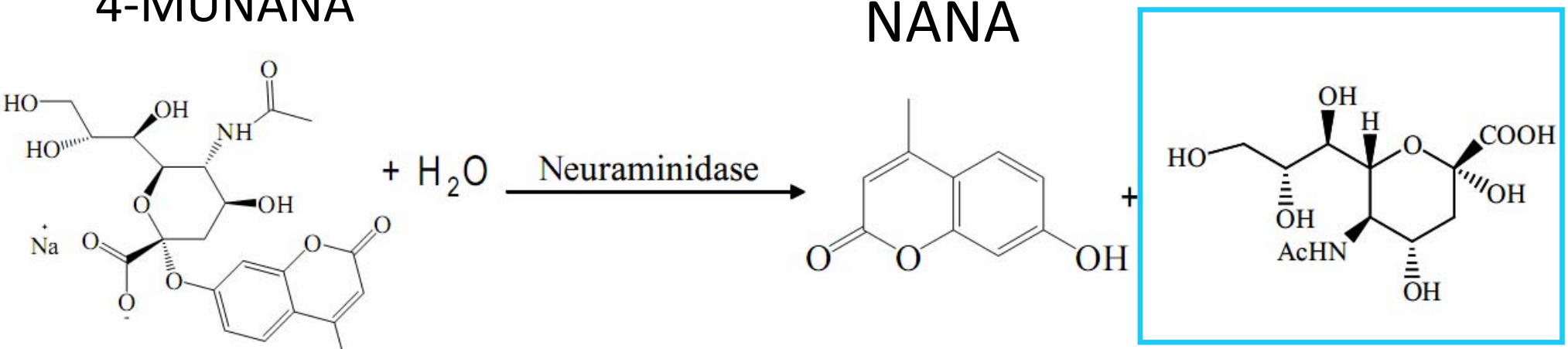
Principle of NA activity Assay

4-MUNANA

NANA



4-methylumbelliferone

4-MUNANA : 4-methylumbelliferyl-N-acetyl-α-D–neuraminic acid

NANA : N-acetylneuraminic acid

Drug Discov Ther. 2009; 3(6):260-265.

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NA enzymatic activity Assay

Mix 4-MUNANA and NA in 96 well plate and incubate at 37°c for 30 min.

Add stop solution (1M glycine in 25%EtOH pH 10.7)

Measure fluorescence at 355 and 460 nm

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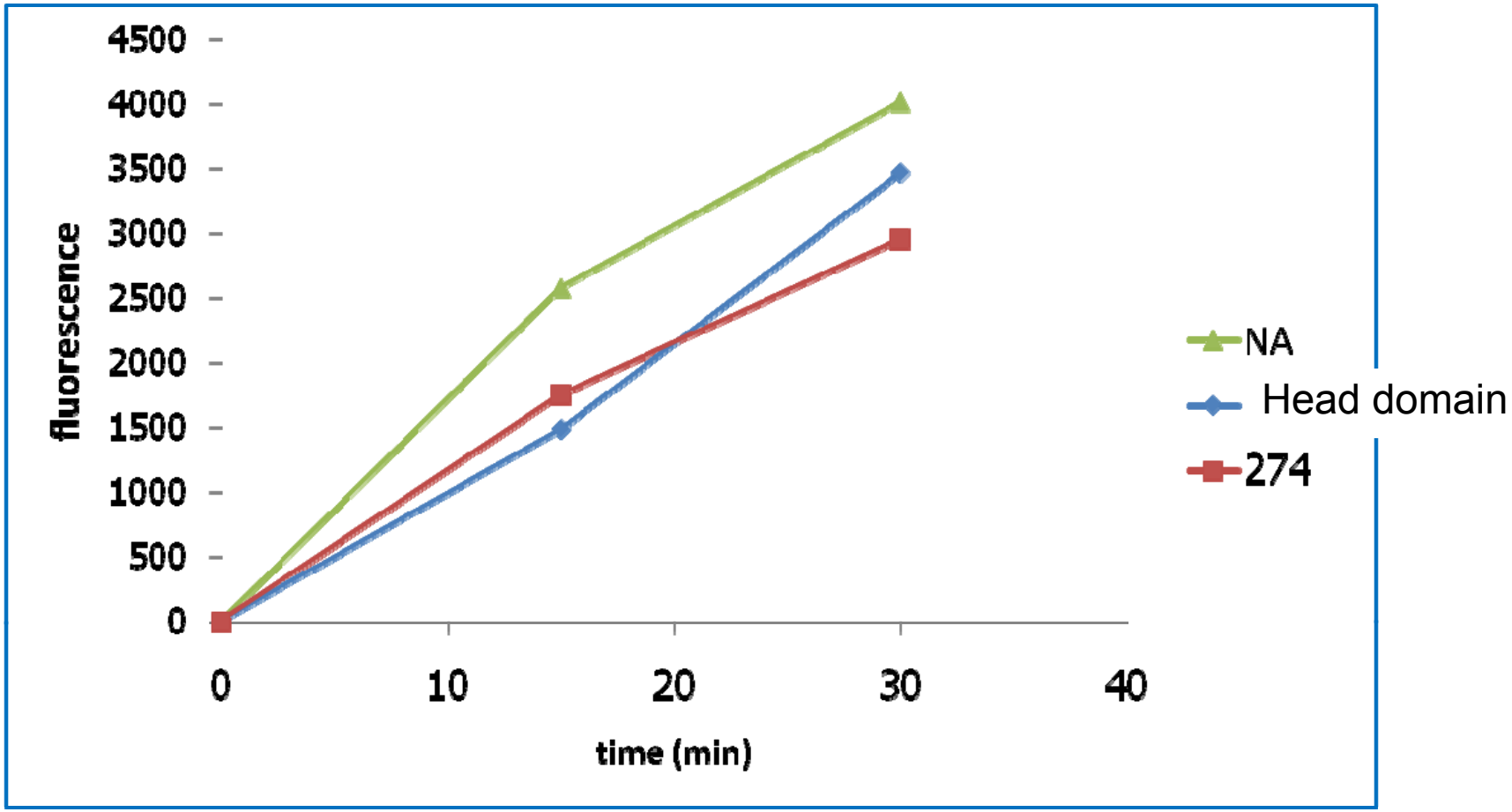
NA activity



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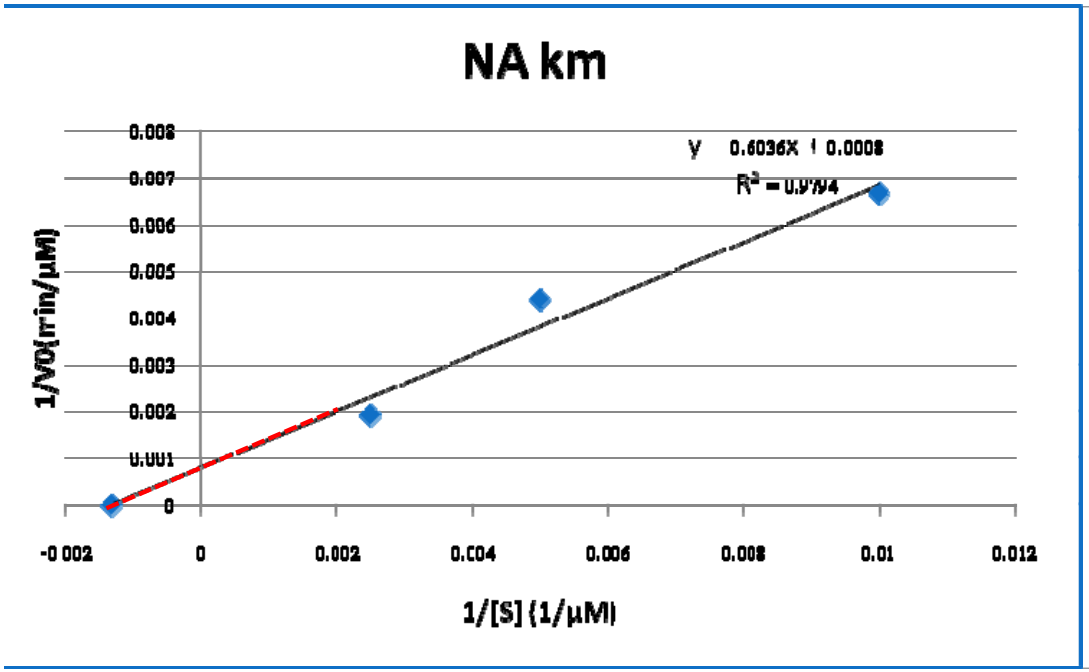

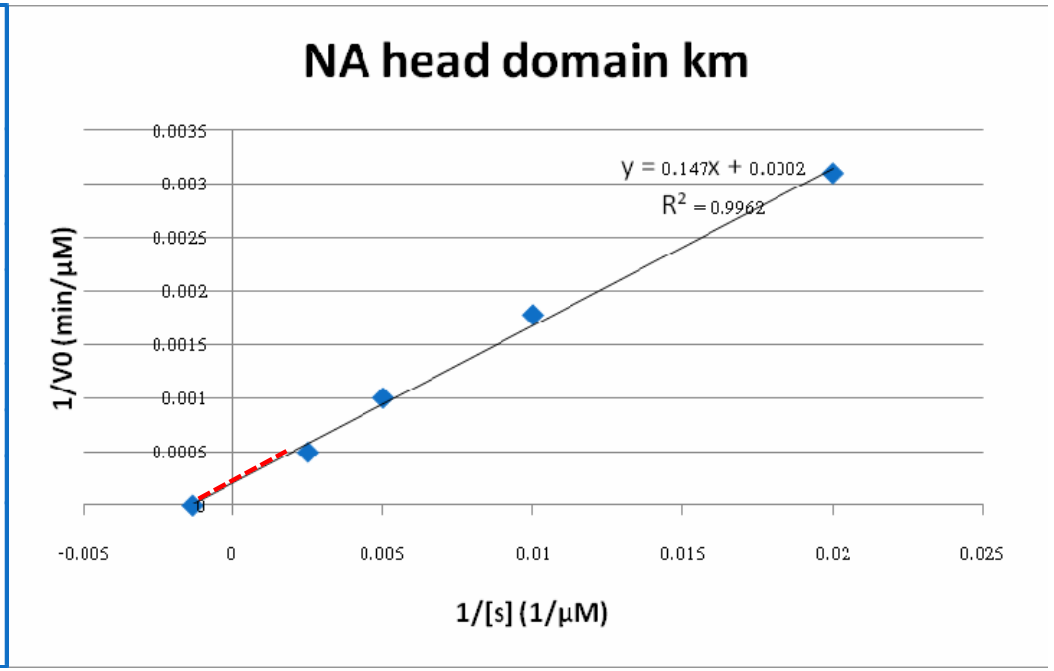

km = 751 µM

km = 731 µM

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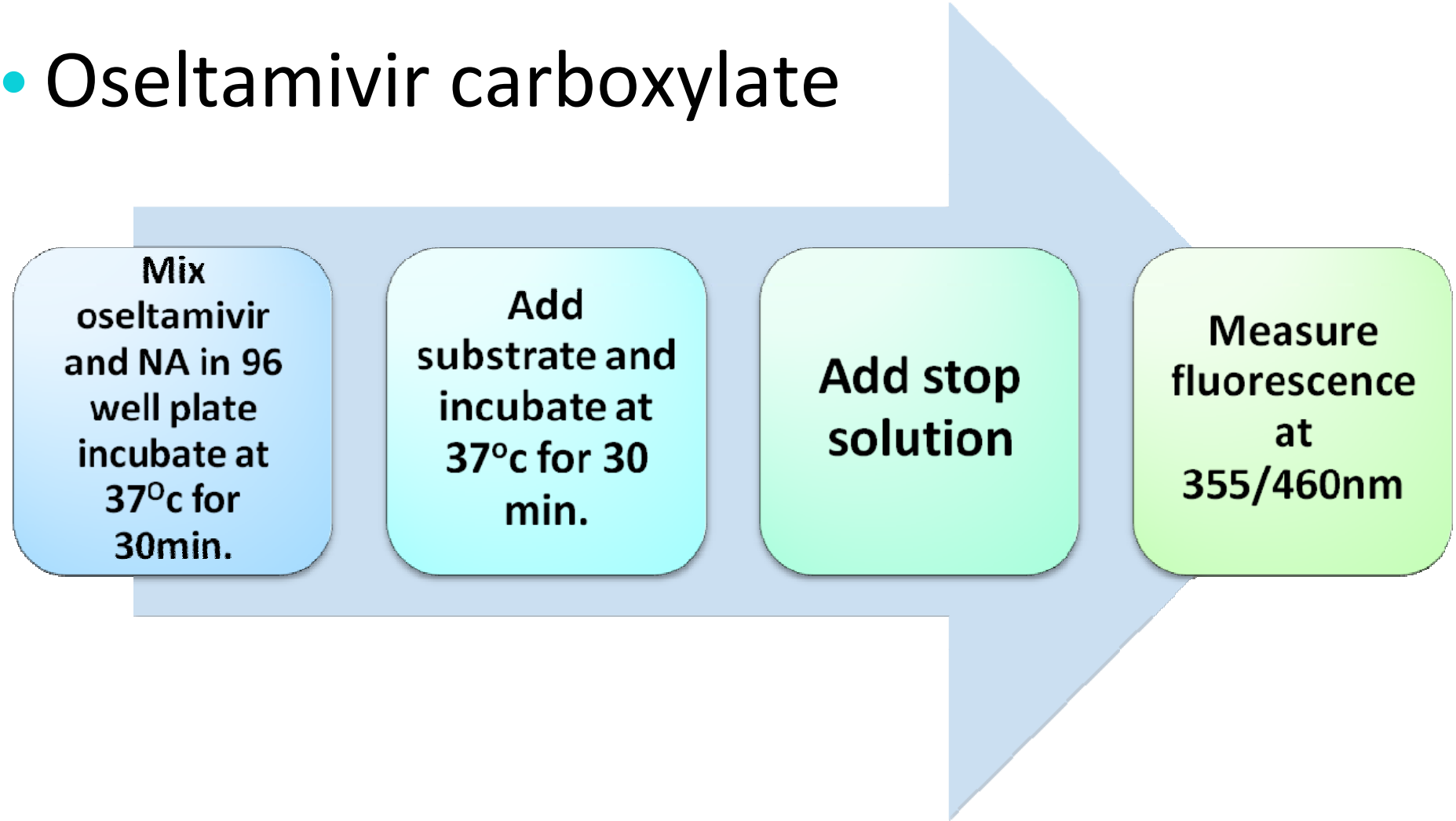
NA inhibition Assay

- Oseltamivir carboxylate



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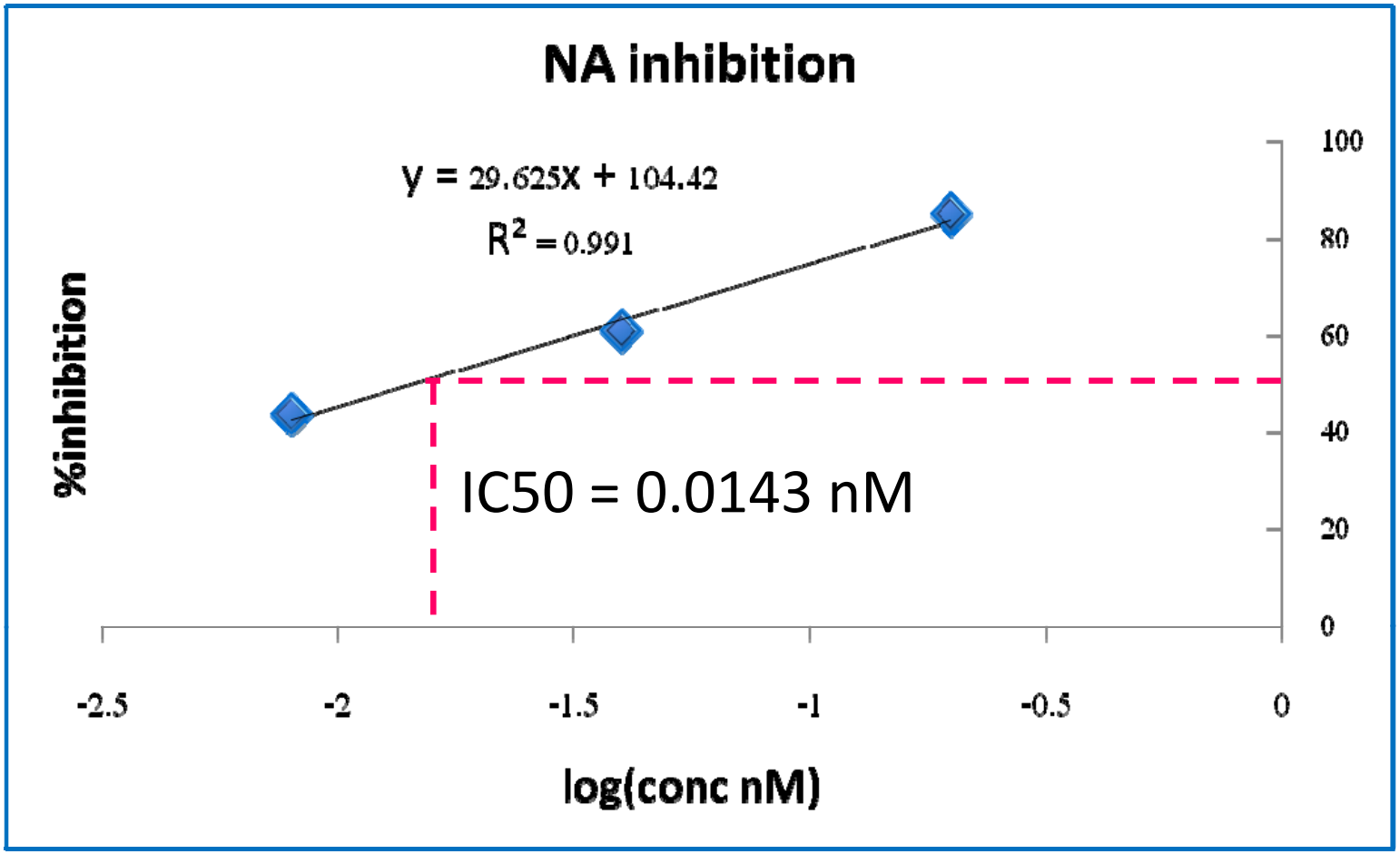

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Conclusions

- NA activity of NA Wild type was higher than that of NA 274 mutant and NA head domain
- NA Wild type is expressed at high level than another clones.
- Deletion of 63 amino acid can reduced NA activity, so this deleted region may affect the enzyme properties.

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Conclusions

- Mutation at framework residue H274Y can reduce the NA activity.
- Km of NA Wild type was slightly higher than the NA head domain which indicated the binding properties of enzyme.
- According to IC50 value, neuraminidase gene constructed in this experiment is sensitive to oseltamivir carboxylate.

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Future studies

- Enzyme kinetic of mutant clone
- There should be studies for susceptibilities to oseltamivir of the head domain and mutant clones.
- The recombinant protein should be purify and studies for NA activity.

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Acknowledgements

- Special thanks to :

- Assistant Professor Dr. Saowakon Paca-Uccaralertkun, Department of Microbiology, Mahidol University

Thank you for your attention

All questions and suggestions are welcome