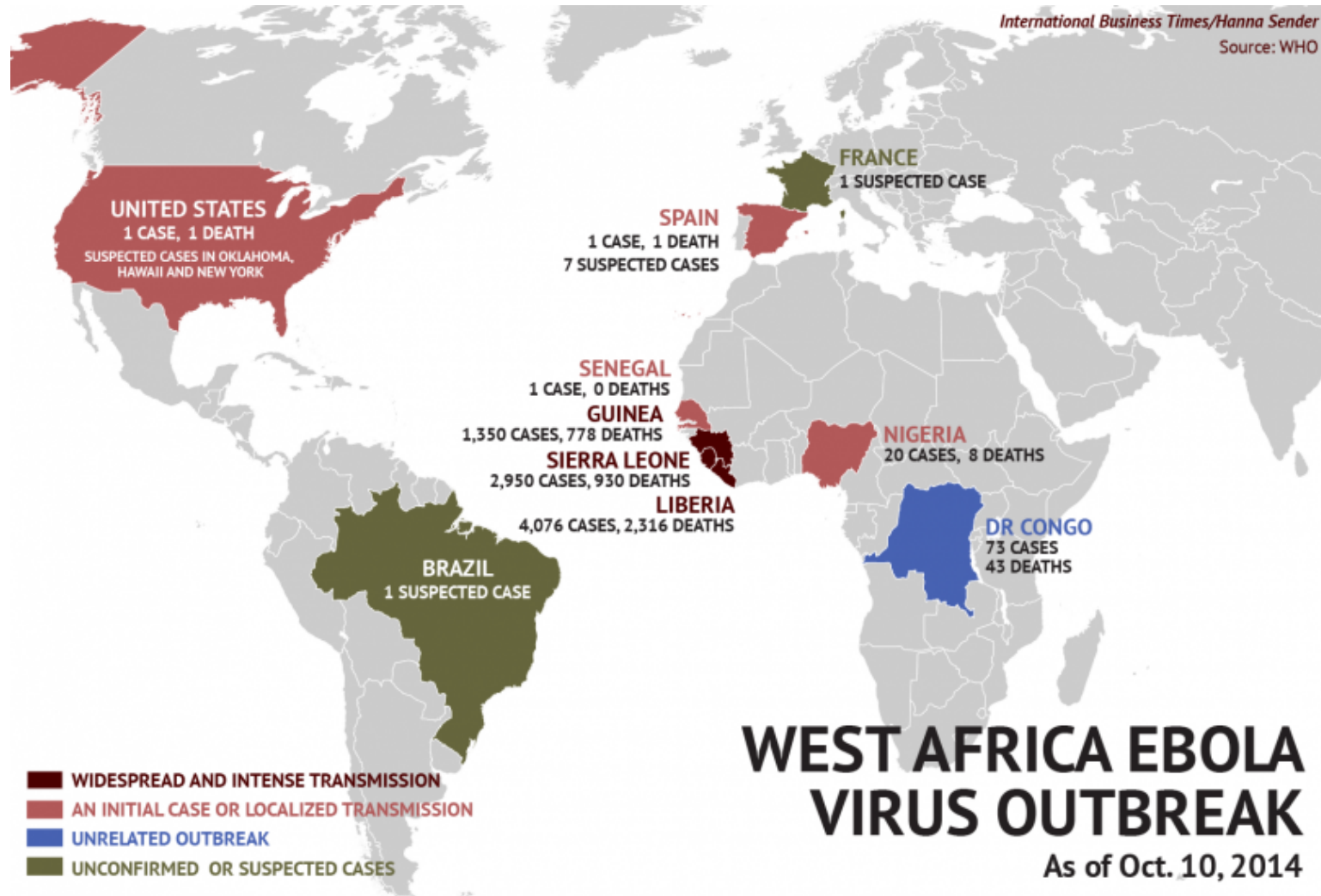


# Transcriptional regulation in Ebola Virus

## The role of VP30

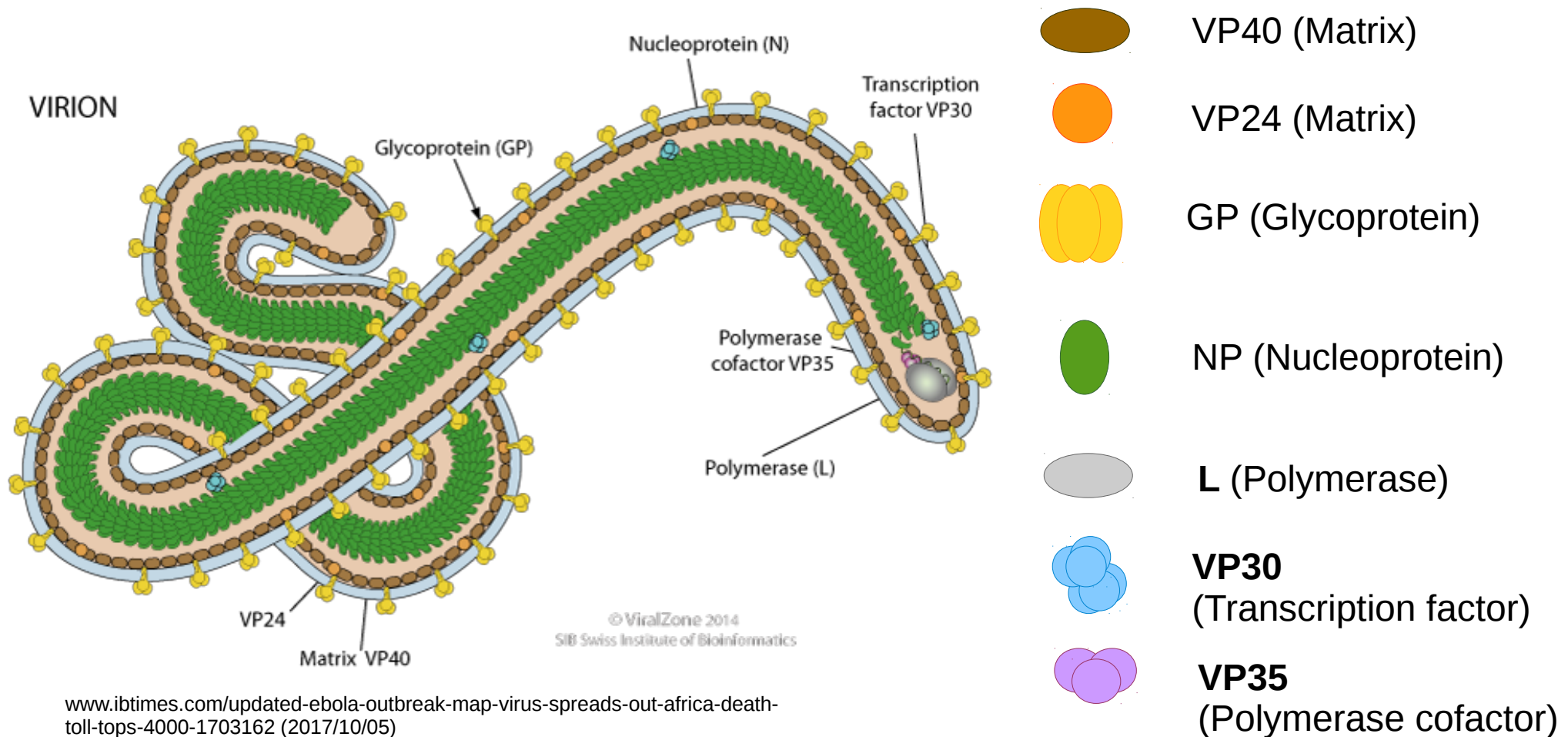
# Ebola virus



[www.ibtimes.com/updated-ebola-outbreak-map-virus-spreads-out-africa-death-toll-tops-4000-1703162](http://www.ibtimes.com/updated-ebola-outbreak-map-virus-spreads-out-africa-death-toll-tops-4000-1703162) (2018/01/26)

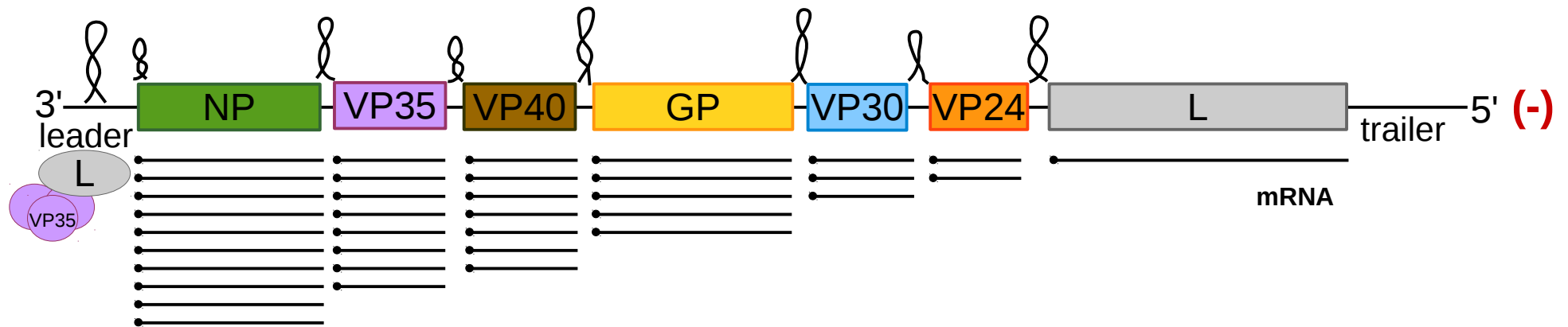
First symptoms 2 days after infection (fever, decreased liver- and kidney-function, bleeding...)  
Death after 6 – 16 days (~ 50%)

# Ebola virus



nonsegmented, filamentous, encapsidated, (-) ssRNA  
**Family: *Filoviridae*** (Marburg virus, Lloviu virus, ...)

# Ebola virus



19 kb genome

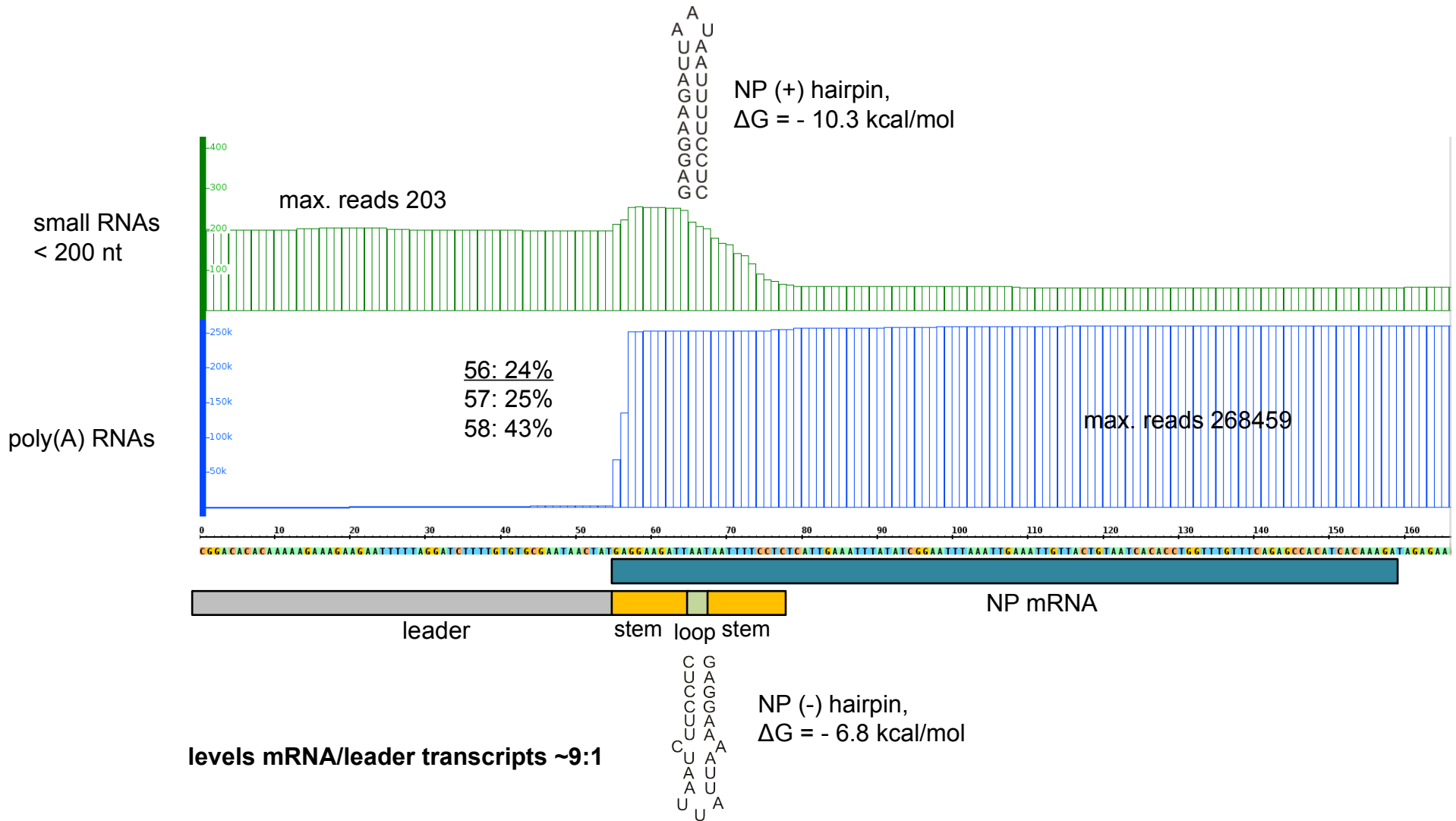
# Question

- 1.) VP30-dependence of transcription
- 2.) Influence of NP hairpin
- 3.) Potential binding sequences of VP30?

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- 1.) VP30-dependence of transcription**
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# 1.) VP30-dependence of transcription

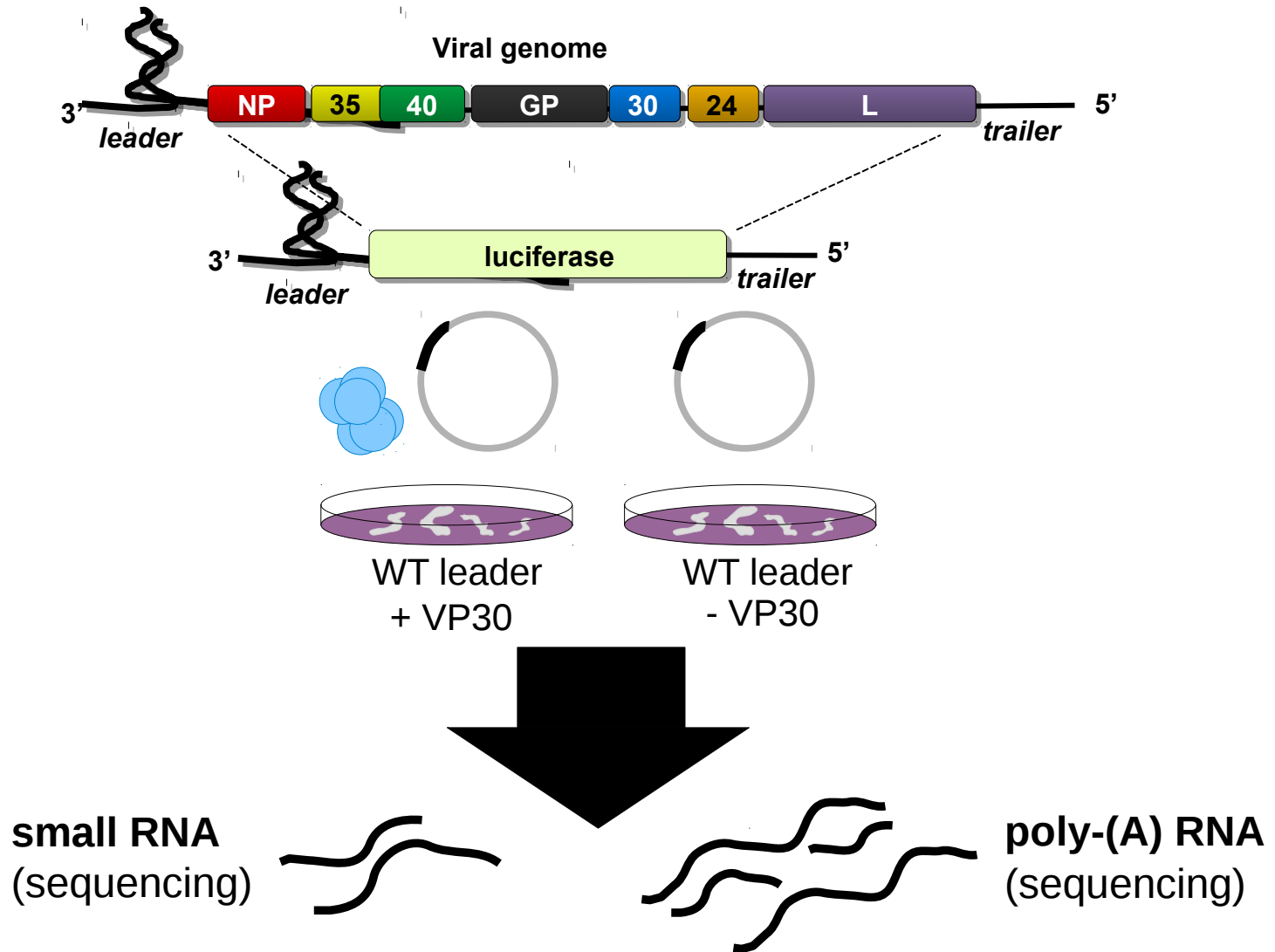


# Question

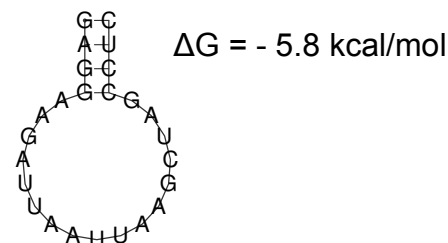
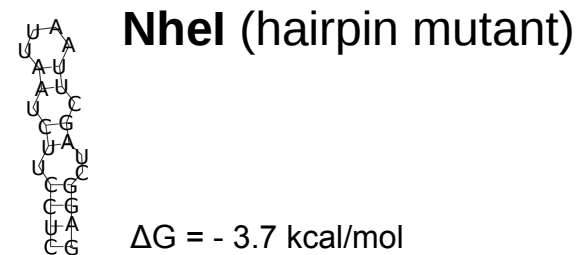
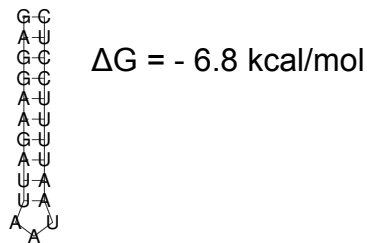
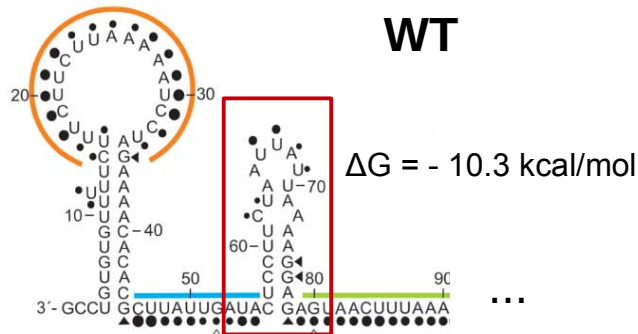
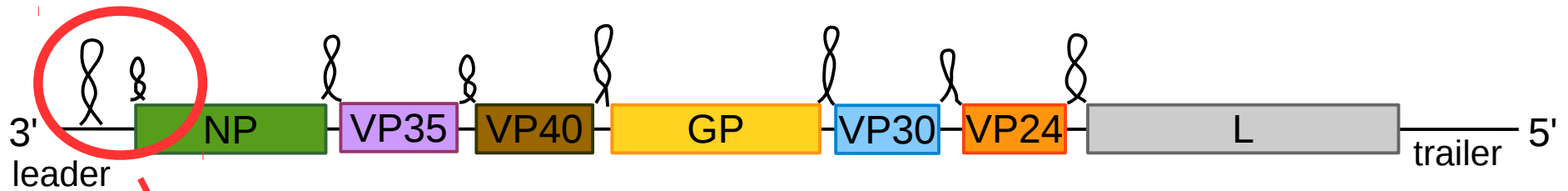
- 1.) VP30-dependence of transcription
- 2.) Influence of NP hairpin**
- 3.) Potential binding sequences of VP30?



## 2.) Influence of NP hairpin: Minigenome infected human cells

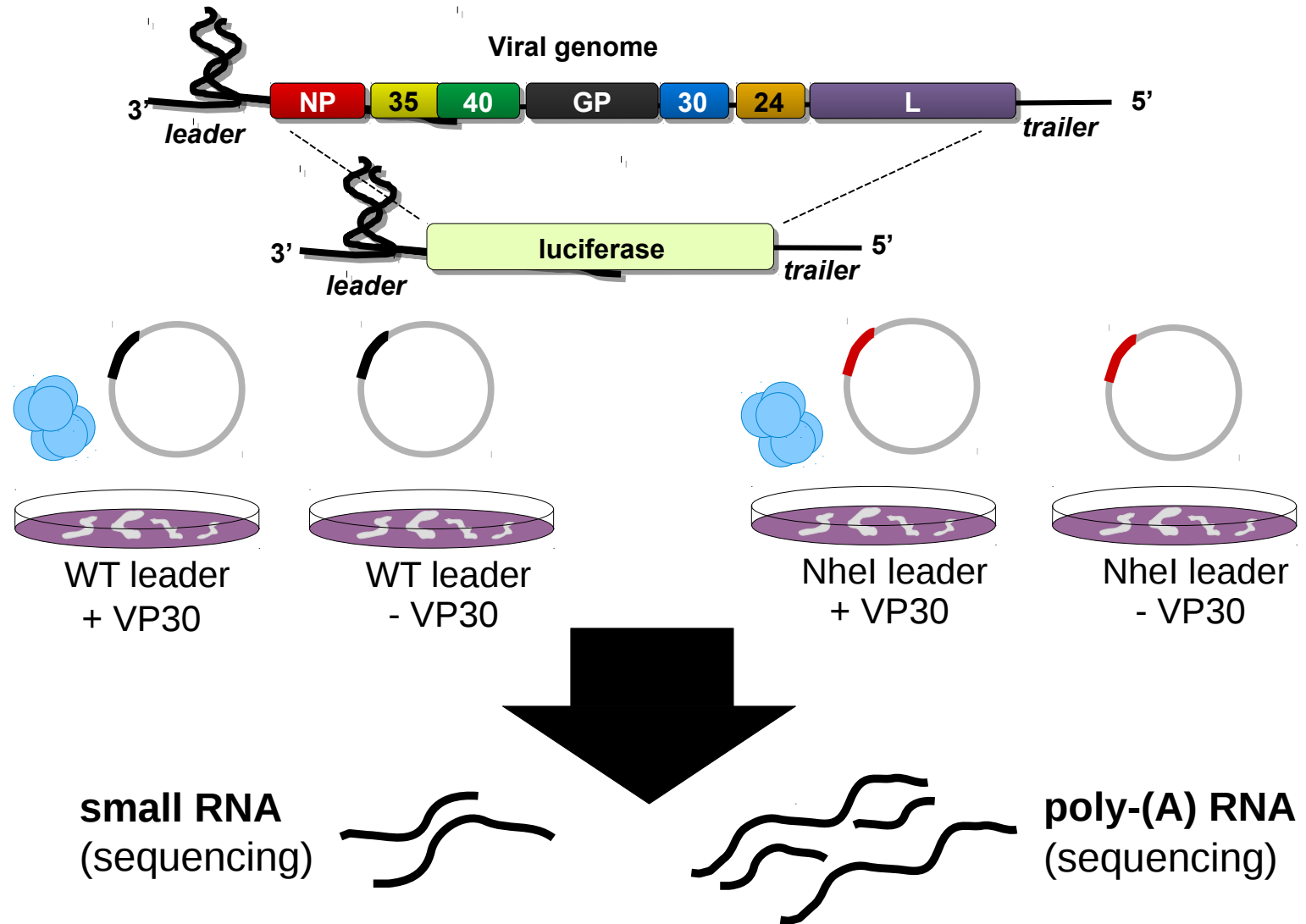


## 2.) Influence of NP hairpin

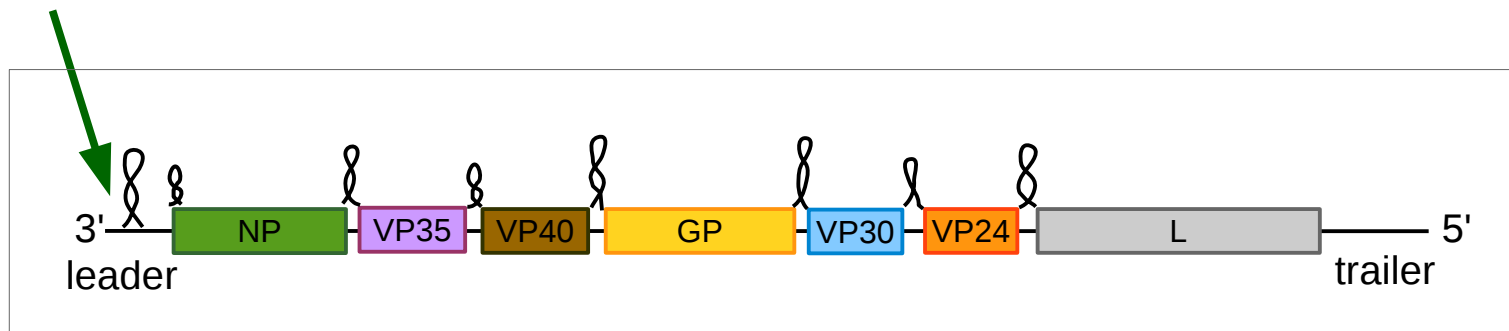
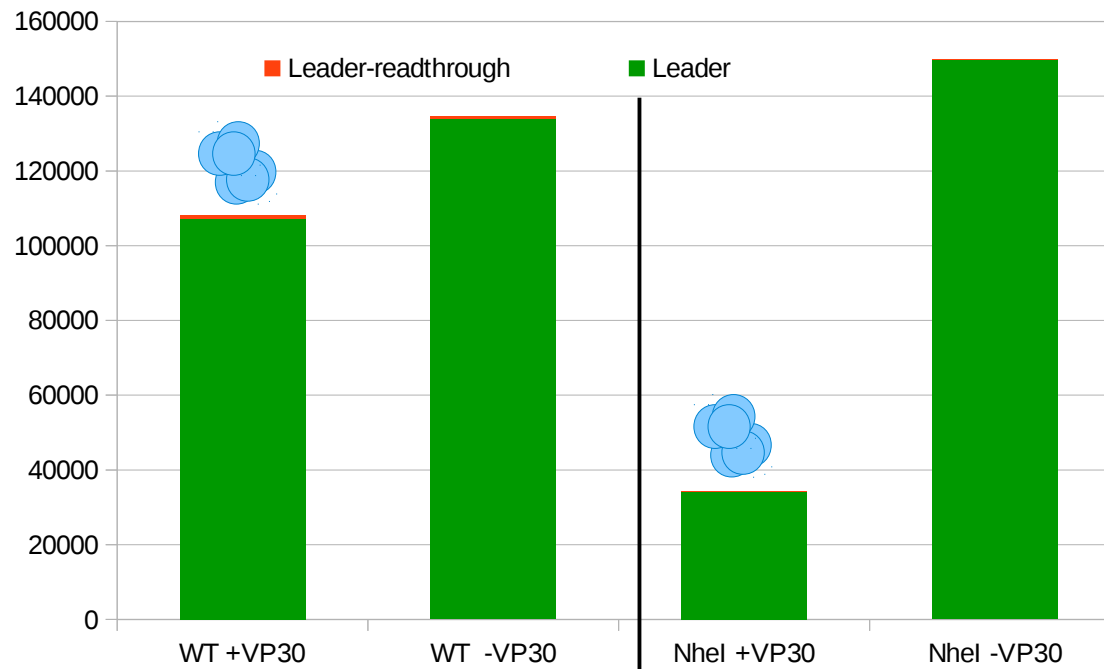


Weik *et al.*, *J. Virol.* 2005

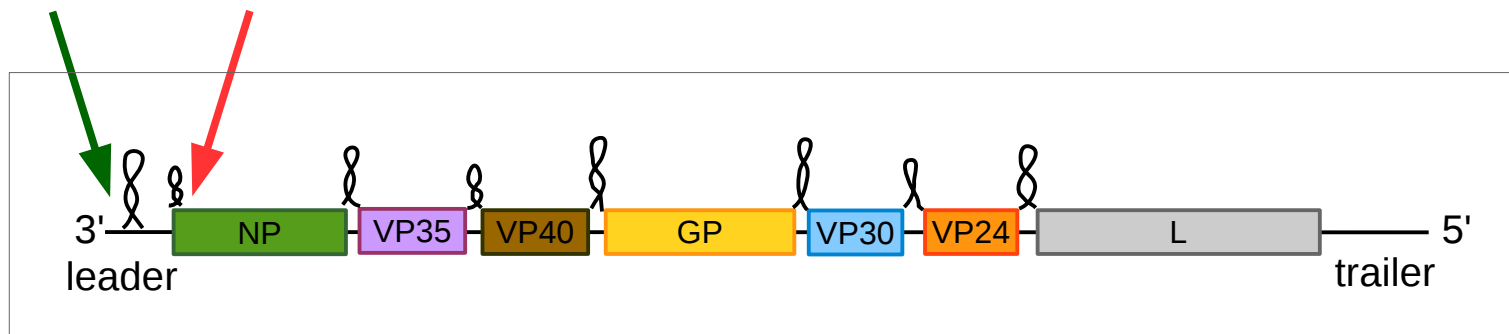
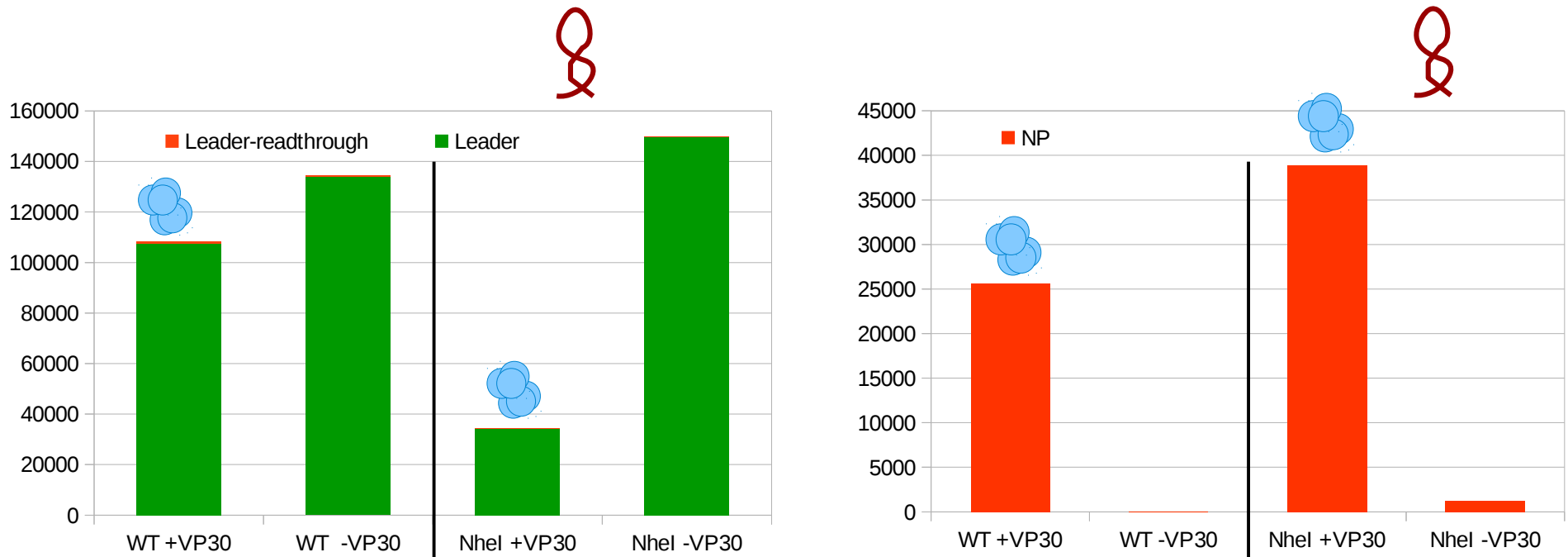
## 2.) Influence of NP hairpin: Minigenome infected human cells



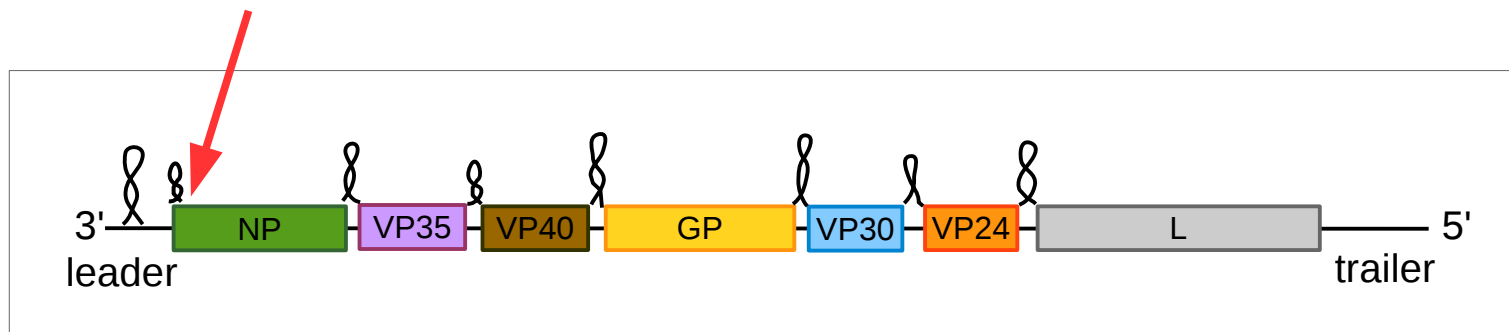
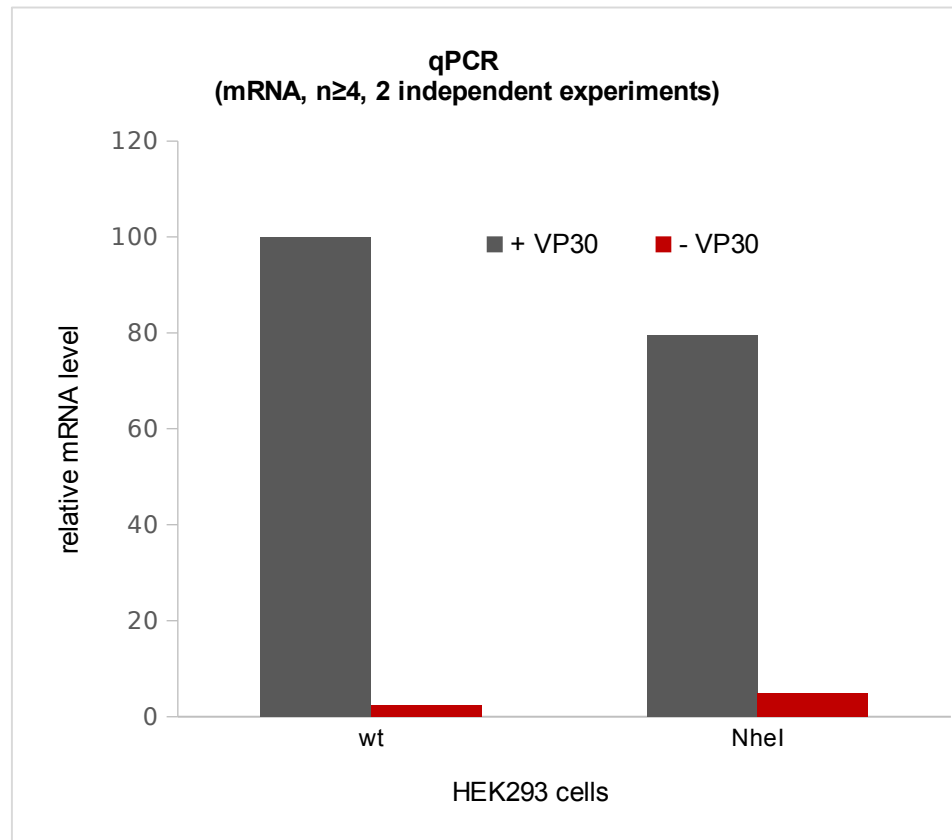
## 2.) Influence of NP hairpin



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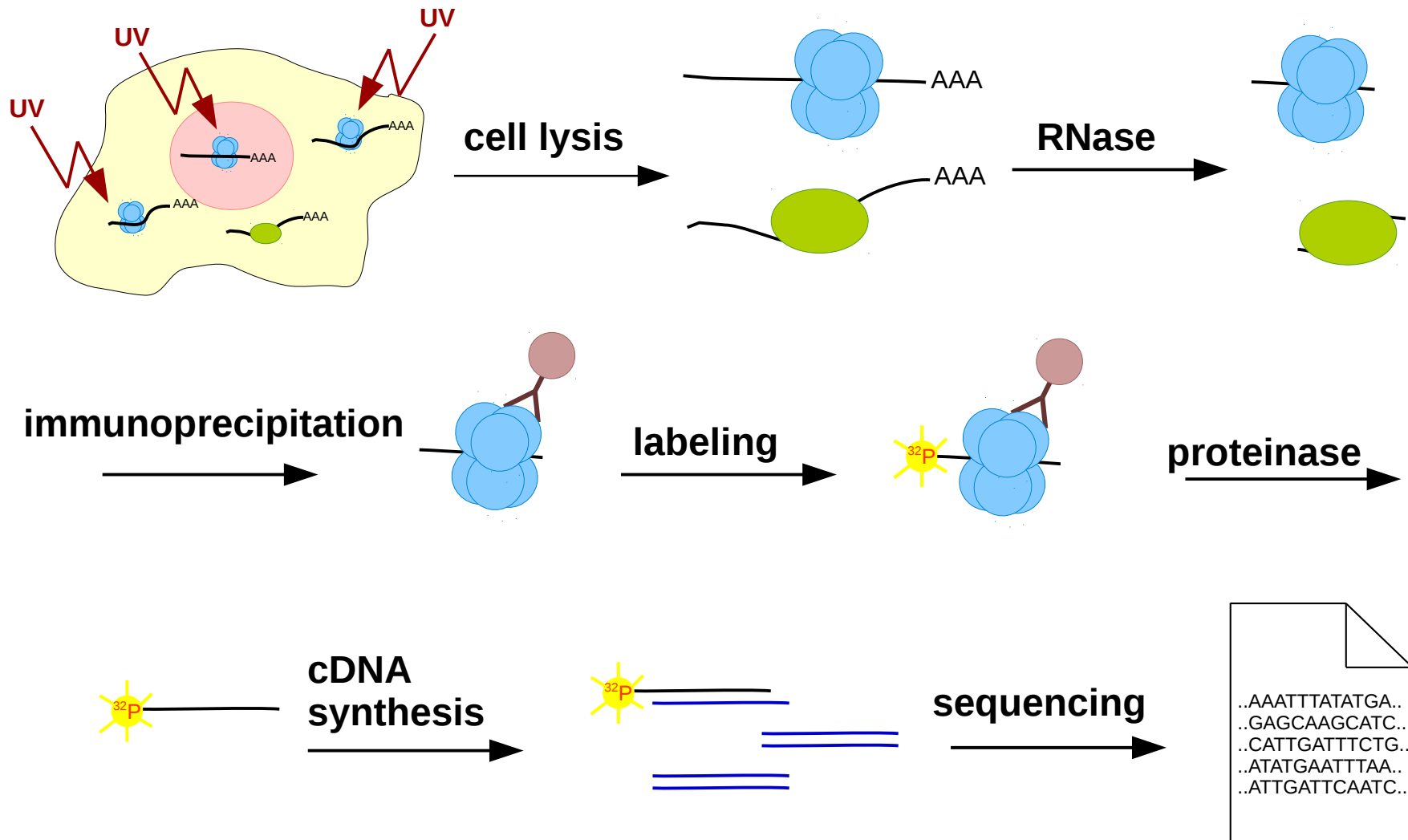
## 2.) Influence of NP hairpin



# Question

- 1.) VP30-dependence of transcription
- 2.) Influence of NP hairpin
- 3.) Binding sequences of VP30**

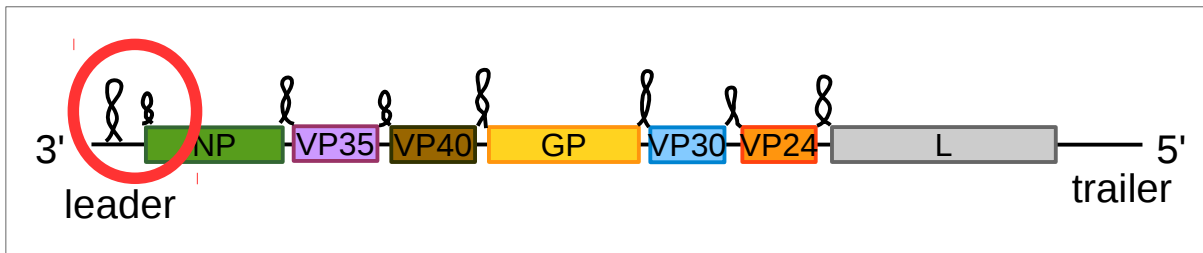
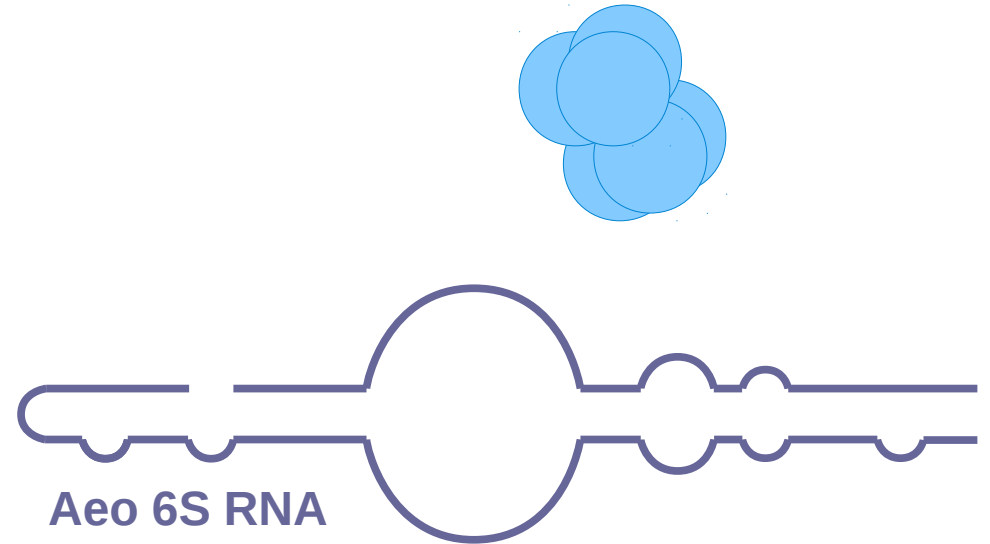
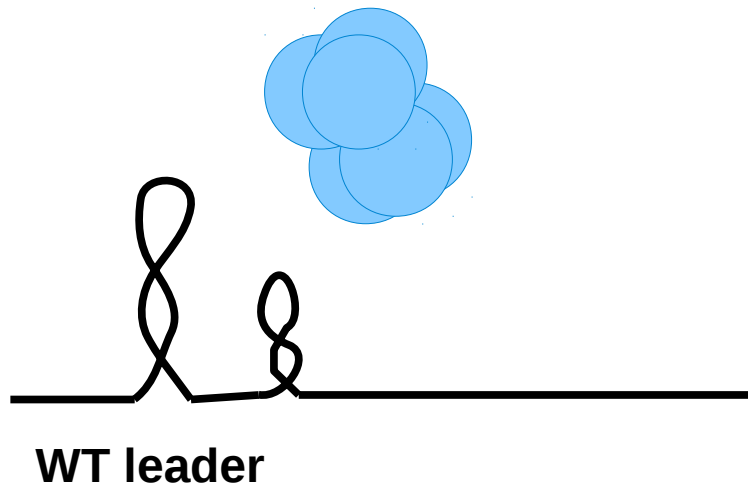
# 3.) Binding sequences of VP30 iClip



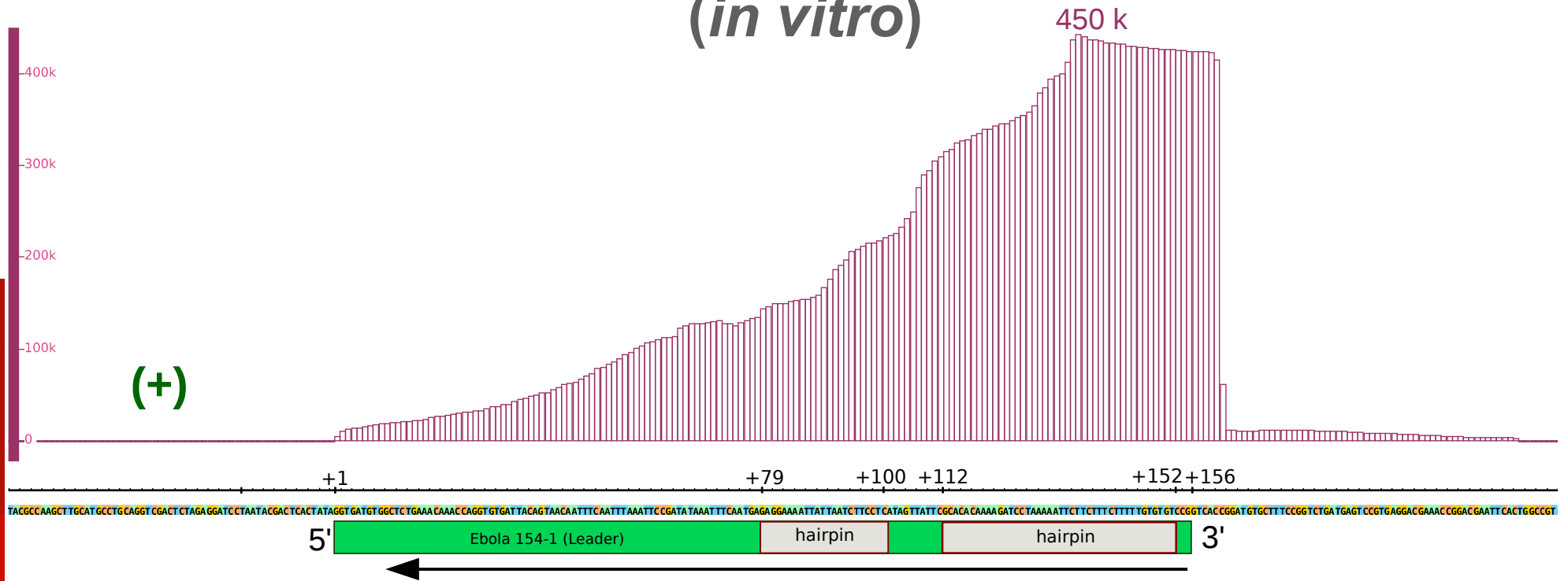


# 3.) Binding sequences of VP30 (*in vitro*)

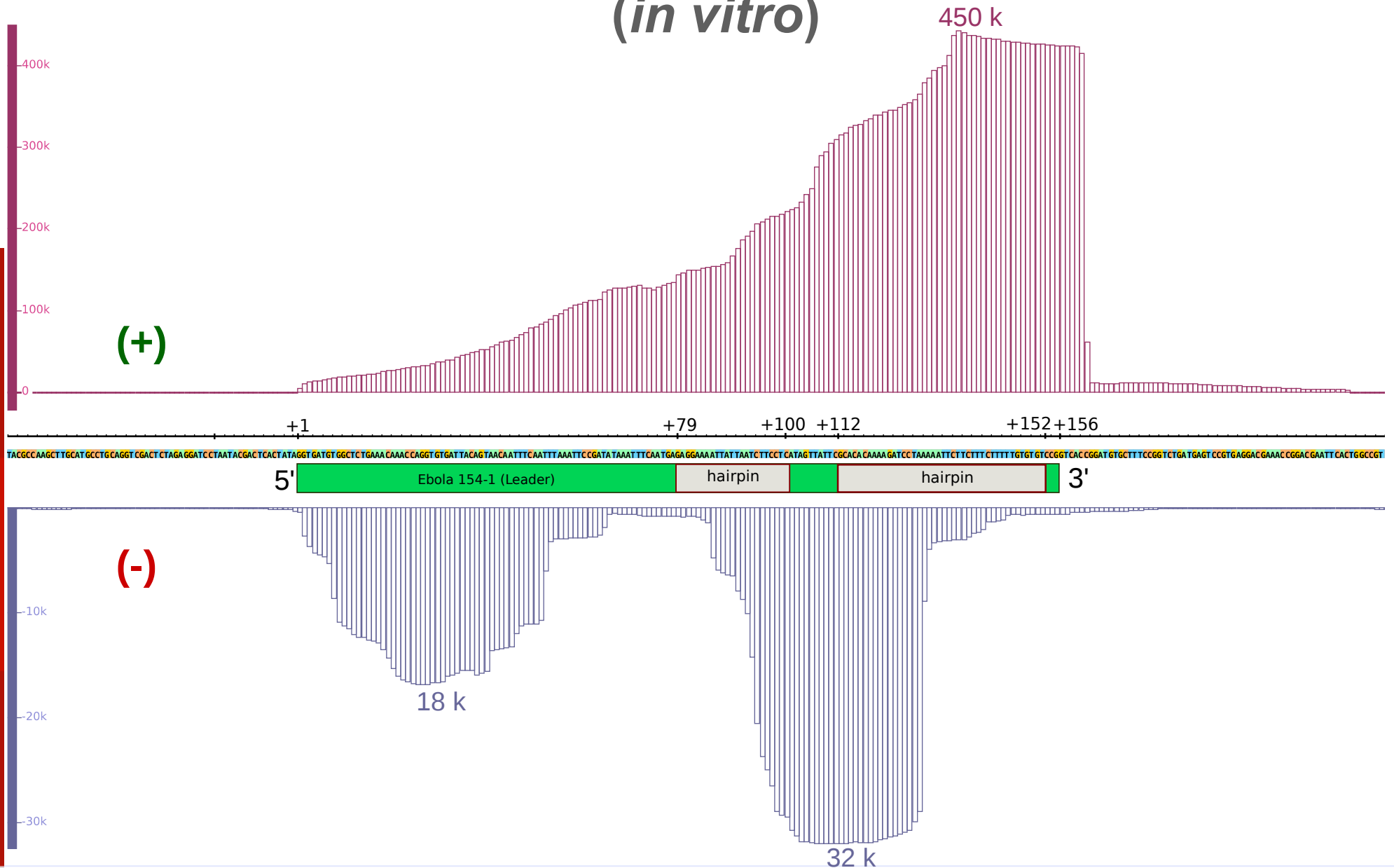
VP30 WT



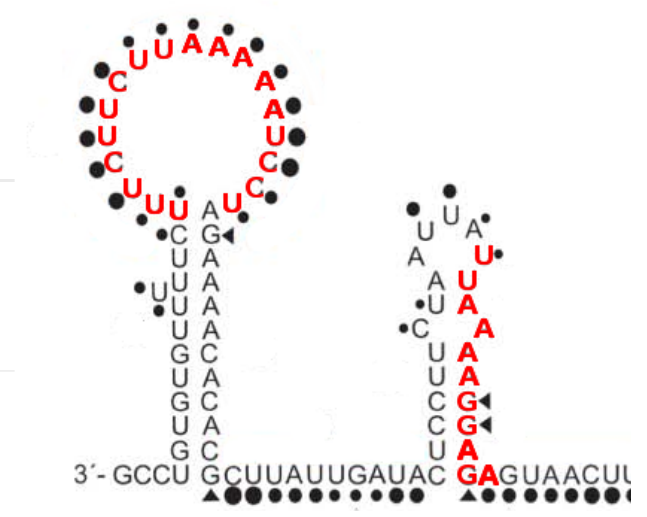
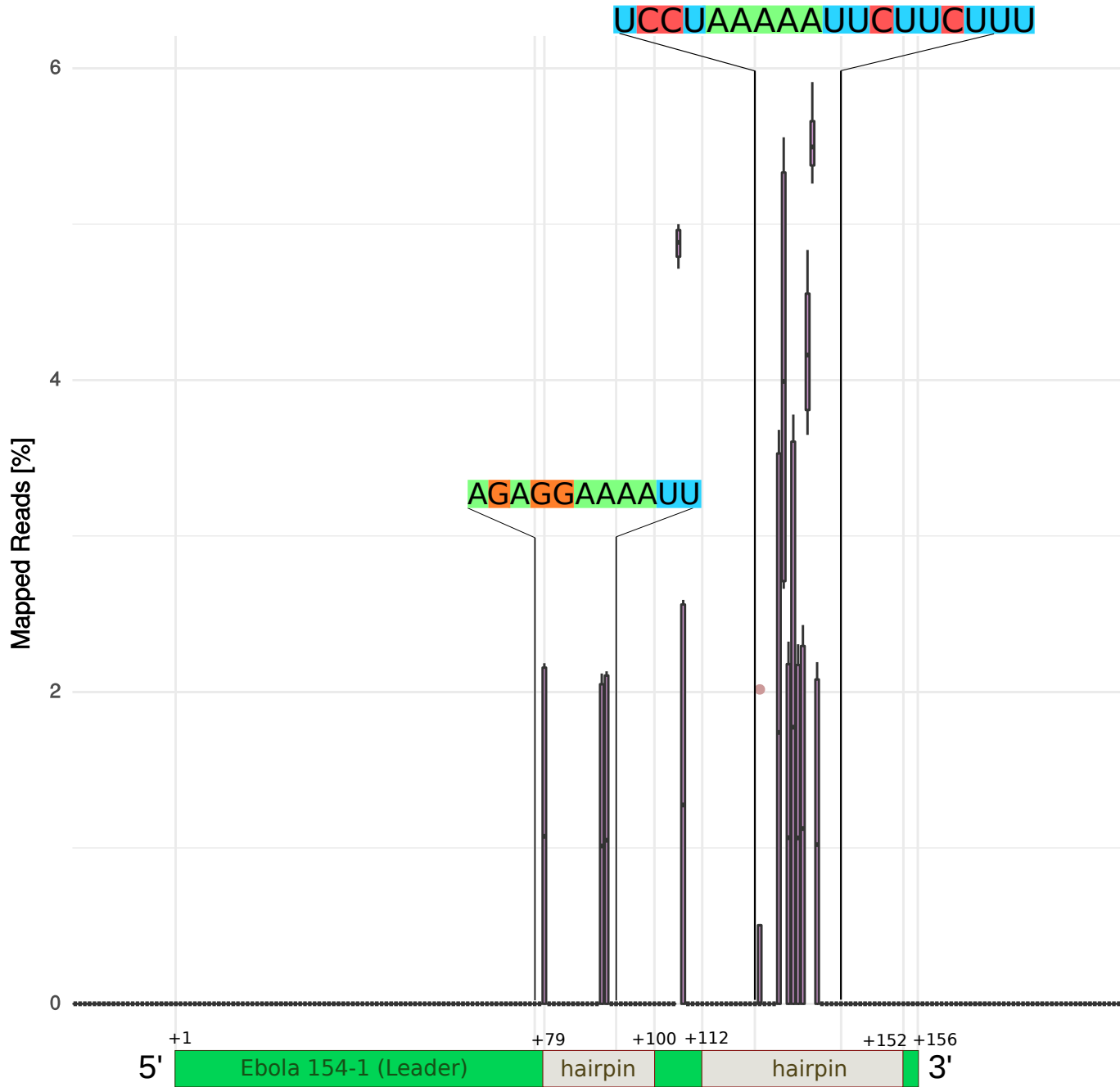
# 3.) Binding sequences of VP30 (*in vitro*)



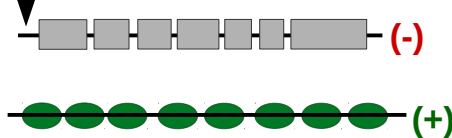
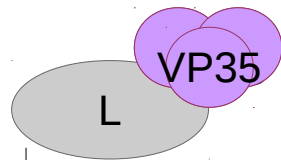
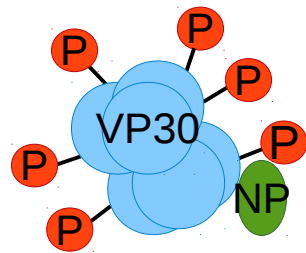
# 3.) Binding sequences of VP30 (*in vitro*)



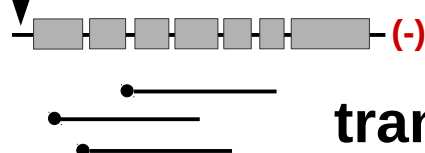
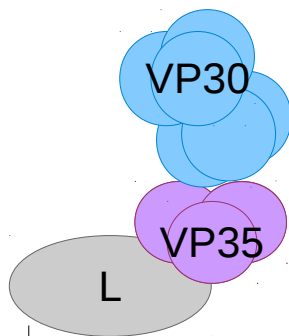
# 3.) Binding sequences of VP30 (*in vitro*)



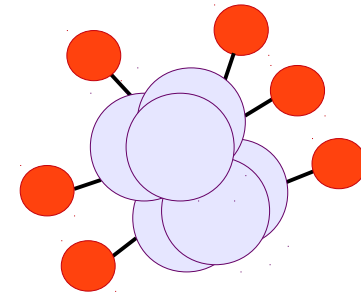
# 3.) Binding sequences of VP30 (*in vivo*)



replication

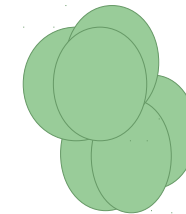


transcription



**VP30 DD mutant**  
phosphorylated

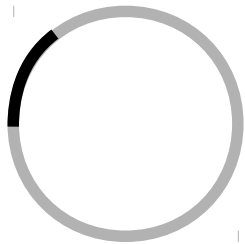
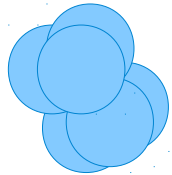
*Biedenkopf et al.*



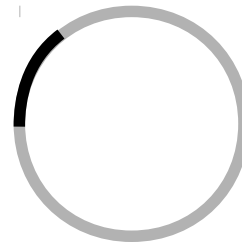
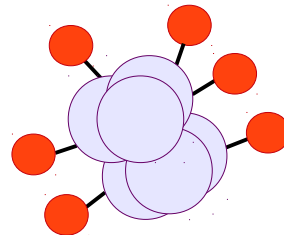
**VP30 AA mutant**  
dephosphorylated

# 3.) Binding sequences of VP30 (*in vivo*)

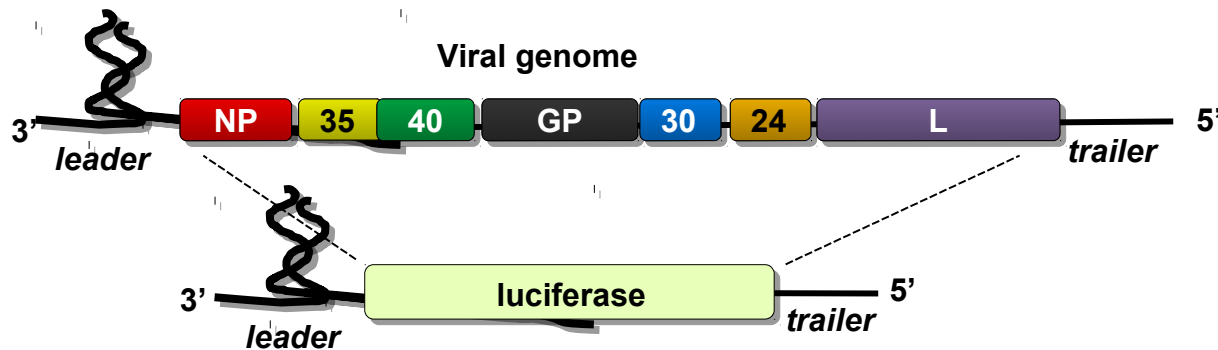
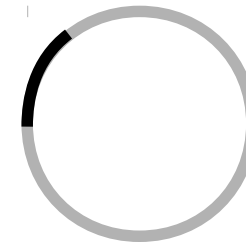
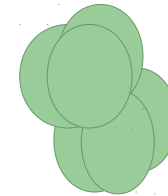
VP30 WT



VP30 DD mutant

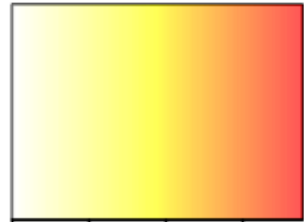


VP30 AA mutant



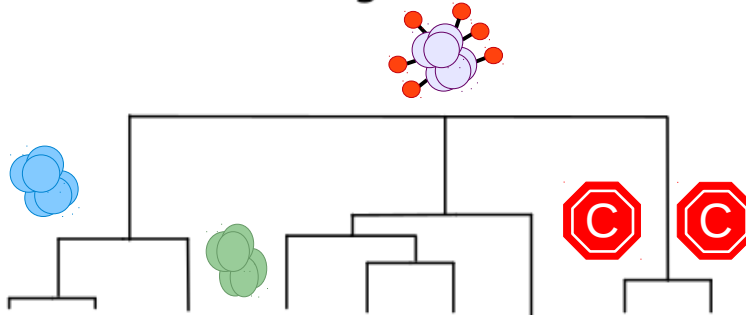
# 3.) Potential binding sequences of VP30 host transcripts


Color Key




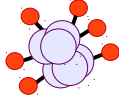
0 10 20 30  
Value


shared features of genomic islands

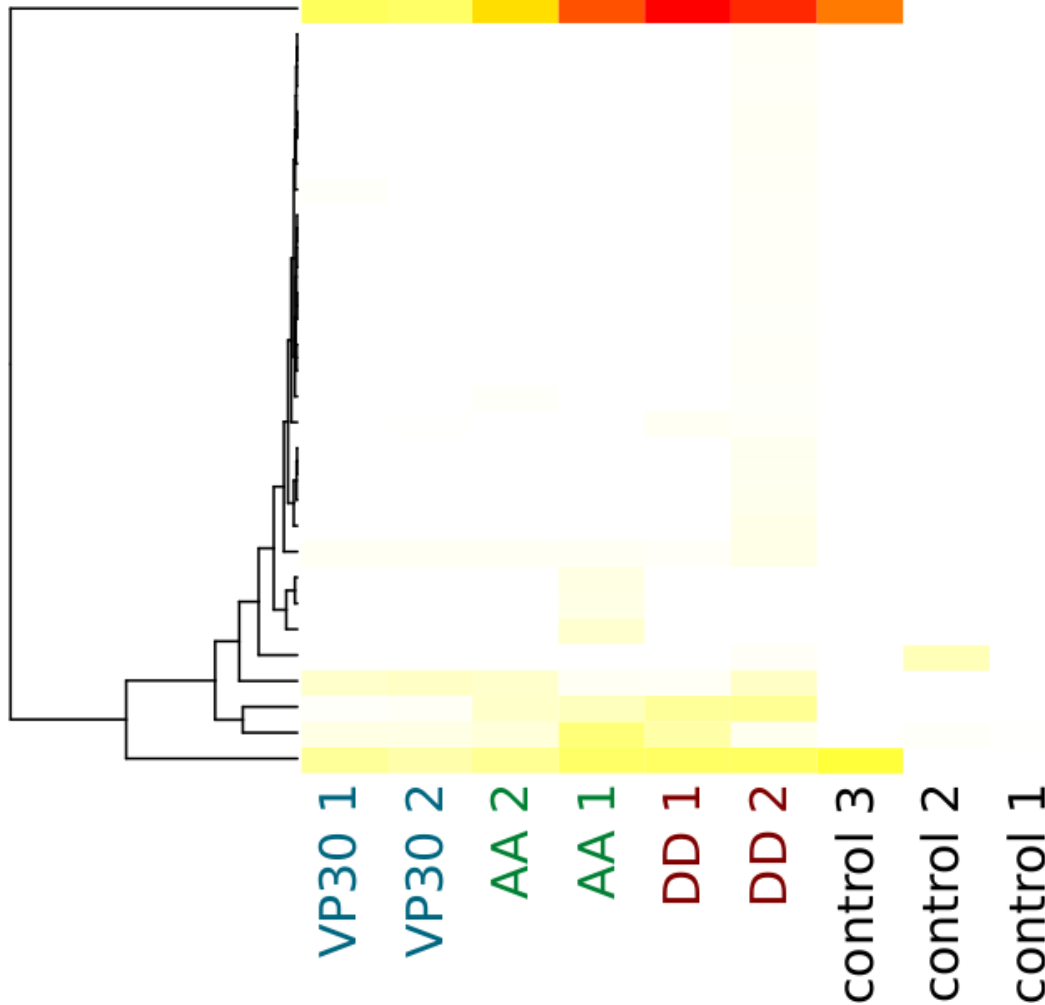


 control

 VP30 WT

 VP30 DD

 VP30 AA



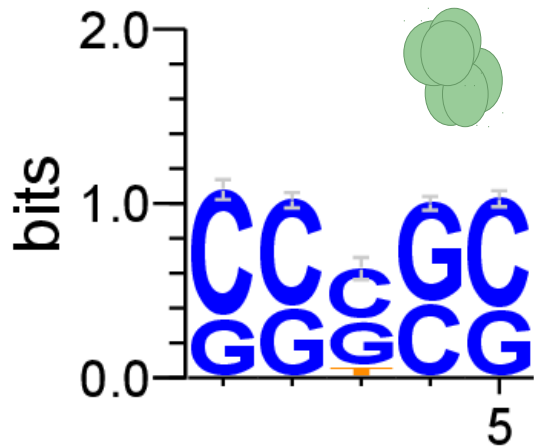
- U1 small nuclear RNA
- heterochromatin protein 1 binding protein 3 (HP1BP3)
- Itchy E3 ubiquitin protein ligase (ITCH)
- SMG1 nonsense mediated mRNA decay associated PI3K related kinase KIAA1468 (KIAA1468)
- Fanconia anemia complementation group L (FANCL)
- tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta
- transducin like enhancer of split 1 (TLE1)
- ankyrin repeat domain 13A (ANKRD13A)
- SHC adaptor protein 1 (SHC1)
- plexin B2 (PLXNB2)
- transmembrane protein 94 (TMEM94)
- dedicator of cytokinesis 4 (DOCK4)
- La ribonucleoprotein domain family member 1 (LARP1)
- protein kinase cAMP-dependent type I regulatory subunit alpha
- collagen type XXVII alpha 1 (COL27A1)
- uncharacterized LOC105369317 (LOC105369317)
- trinucleotide repeat containing 6A (TNRC6A)
- SURP and G-patch domain containing 2 (SUGP2)
- cyclin-dependent kinase 12 (CDK12)
- family with sequence similarity 208 member B (FAM208)
- WD repeat domain 74 (WDR74)
- RAD52 homolog DNA repair protein (RAD52)
- ubiquitin protein ligase E3 component n-recognin 4 (UBR4)
- DEAD/H-box helicase 11 (DDX11)
- heterogeneous nuclear ribonucleoprotein M (HNRNPM)
- U2 small nuclear RNA 1 (RNU2-1)
- ncRNA, uncharacterized (LOC15379574)
- solute carrier organic anion transporter family 5A1 (SLCO5A1)
- ncRNA, uncharacterized (LOC15379524)

VP30 1  
VP30 2  
AA 2  
AA 1  
DD 1  
DD 2  
control 3  
control 2  
control 1

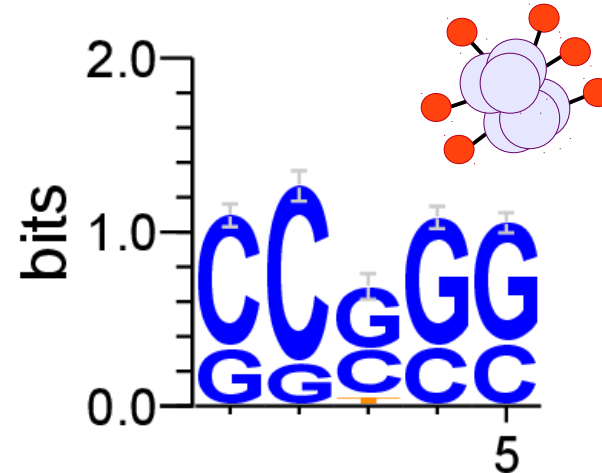
# 3.) Potential binding sequences of VP30

## Motives (enriched pentamers) (*in vivo*)

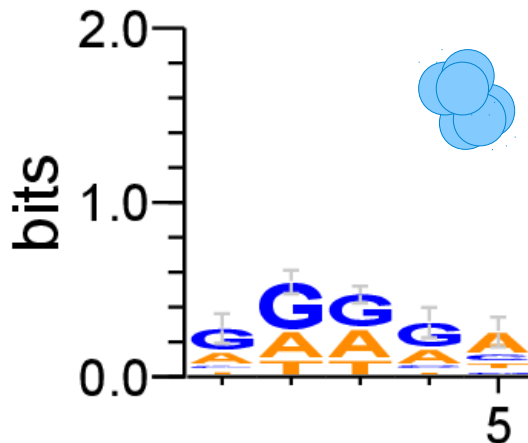
VP30 AA mutant



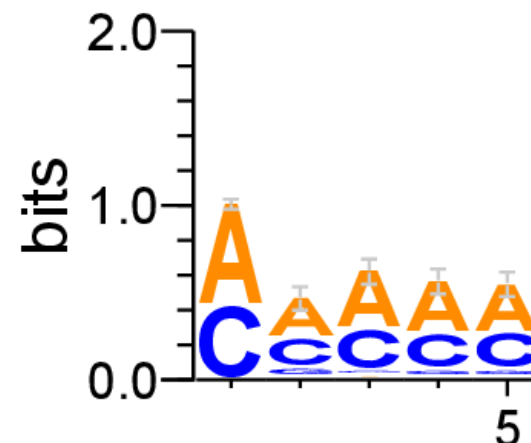
VP30 DD mutant



VP30 WT

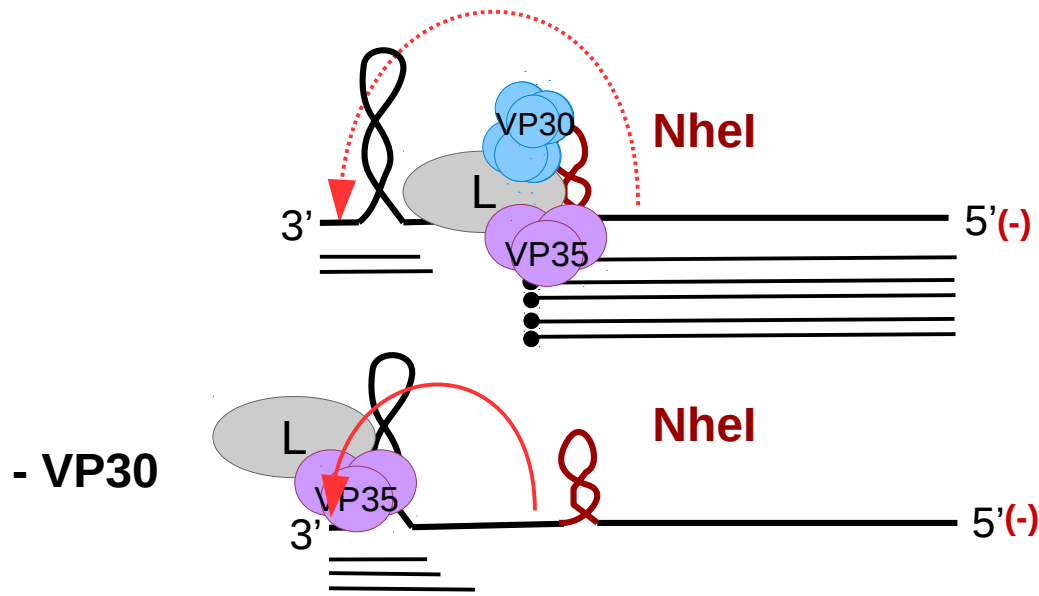
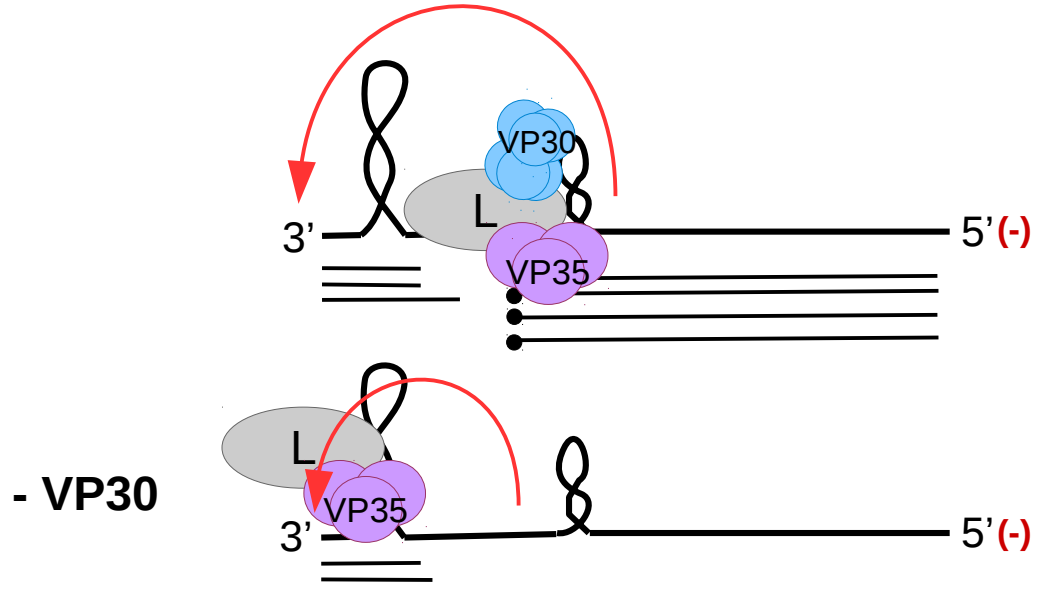


control





# Conclusion: Transcription Model



# Results in brief

## VP30:

- 1) Binds to loop region of leader and NP hairpin (*in vitro*)
- 2) Has hardly any effect on leader transcripts
- 3) Is essential for transcription of NP
- 4) Does hardly bind to viral RNA (*in vivo*)?
- 5) Might bind to host RNAs (anion transporter, uncharacterized ncRNA)

# Results in brief

## **Nhel (hairpin mutant):**

- 1) Reduces leader transcription in favor of NP transcription**
- 2) Introduces leaky NP transcription in absence of VP30**

# Conclusion

- ➔ **VP30 effects transcription transition from leader to NP, effect might mediated via host factors**
- ➔ **Weakening the NP hairpin (Nhel mutant), enhances transcription of NP**

# Acknowledgments

**Marcus Lechner**

**Roland Hartmann**

Simone Bach

Julia Schlereth

Dennis Streng

**Thorsten Hain**

**Stephan Becker**

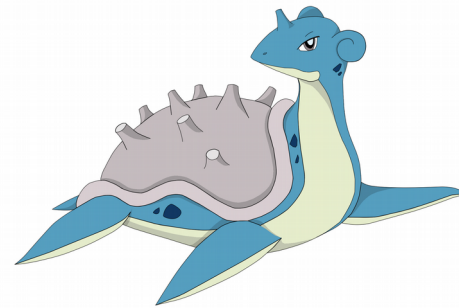
Nadine Biedenkopf



Philipps



Universität  
Marburg



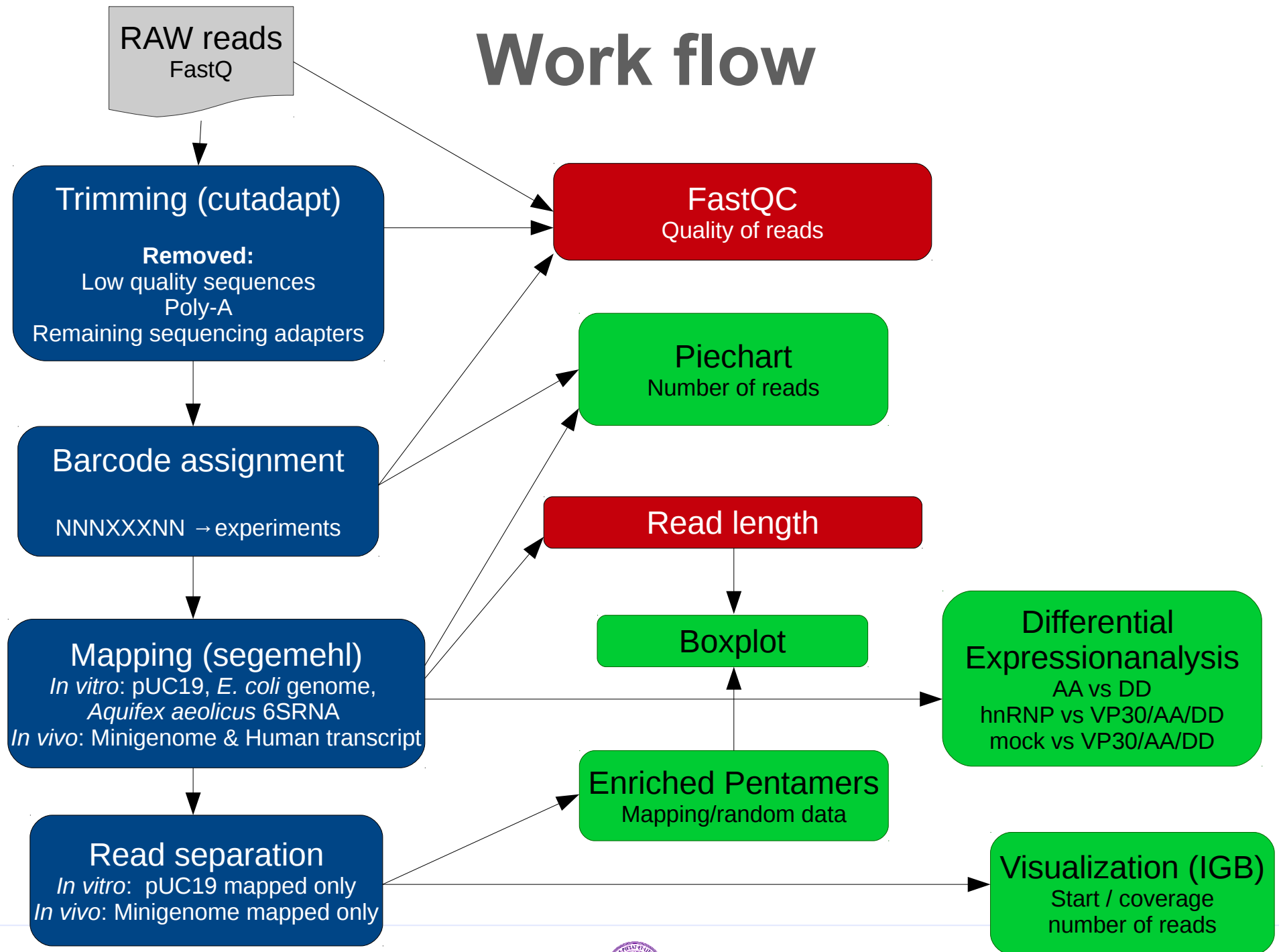




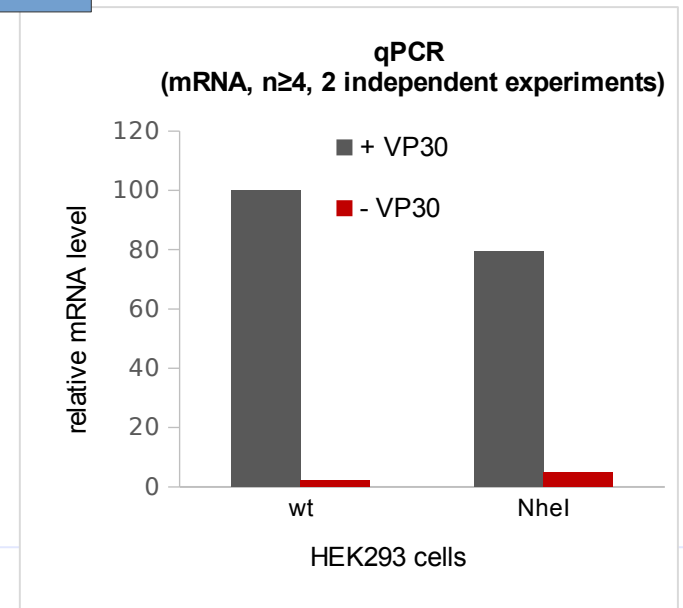
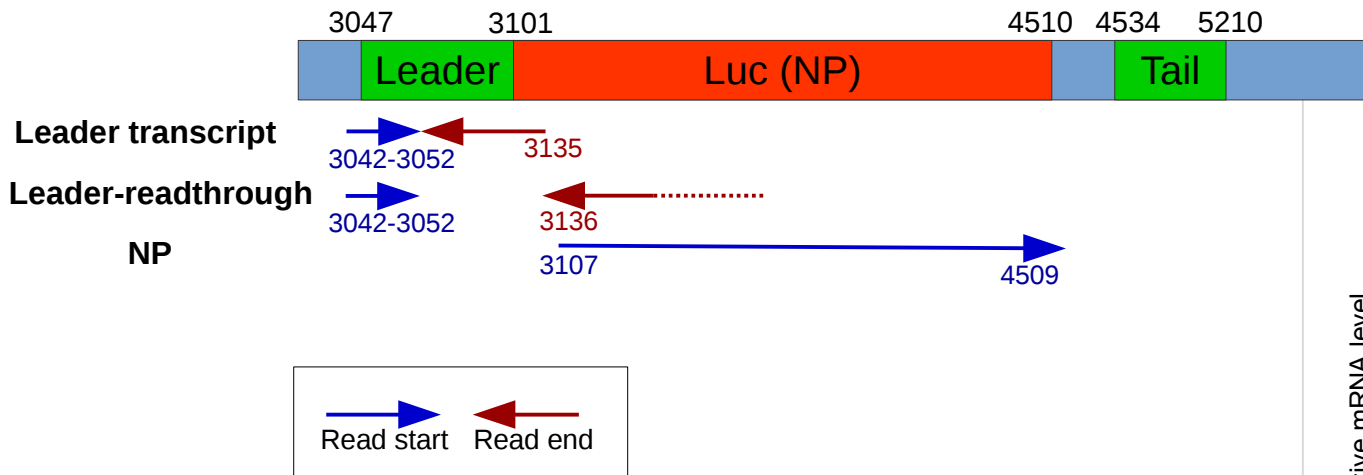
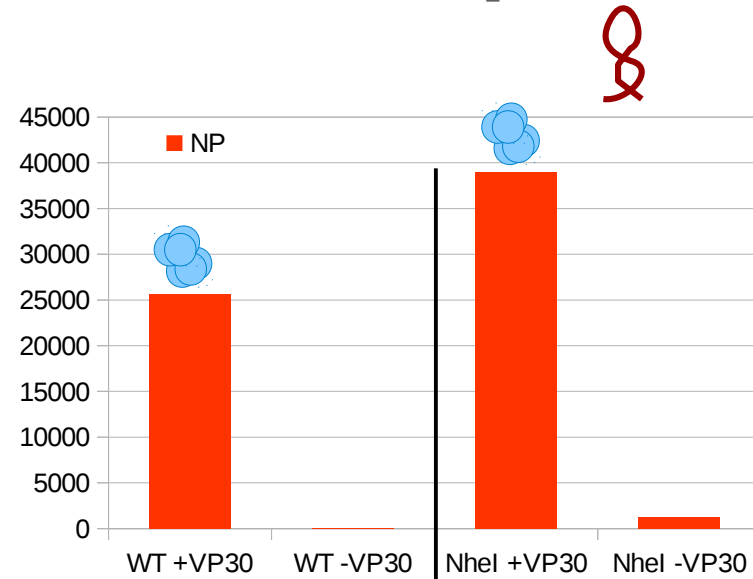
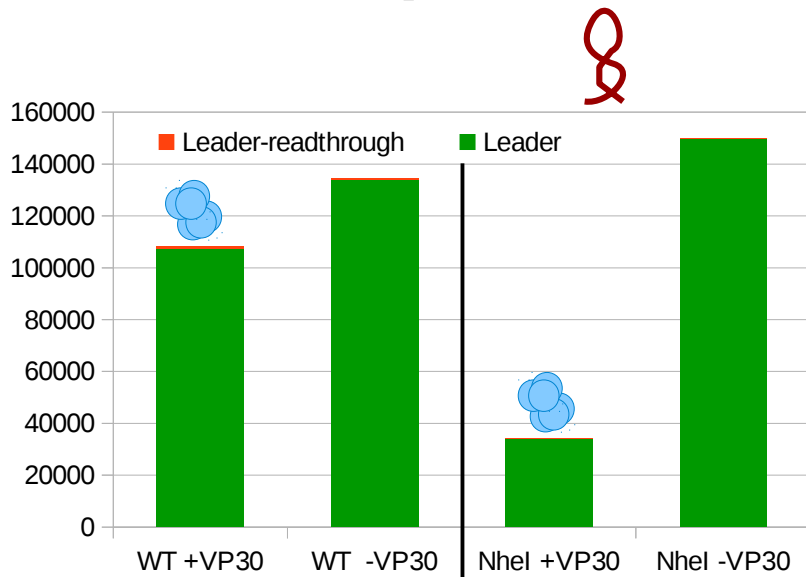




# Work flow



# 2.) Influence of NP hairpin



# 3.) Binding sequences of VP30

## iClip

