

Identification of microRNAs of the Herpesvirus Family

Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grässer FA, van Dyk LF, Ho CK, Shuman S, Chien M, Russo JJ, Ju J, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zolov M, Tuschl T; *Nature Methods* 2, 269 - 276 (2005)

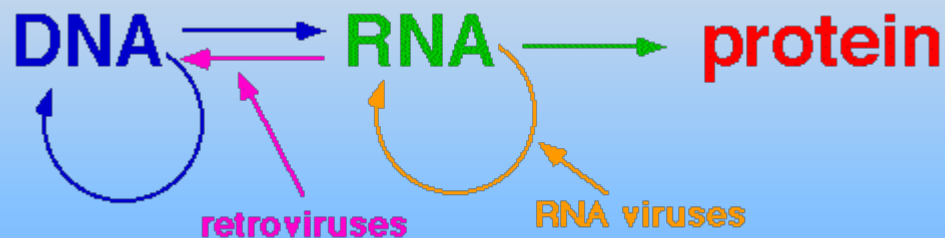
**Angelo Kontgas – Intern
Dr. Bino John, PhD – Mentor**

Paper Focus

- Identify pathogenic viral miRNA
 - Driver: miRNA's found in EBV
- Previous methods:
 - Cross-species sequence conservation
- Novel Technique:
 - Local sequence composition
 - Prediction:
 - Support Vector Machine (SVM)
 - *RNAfold*
 - Cloning Virus Infected Cells
- Results:
 - Large DNA viruses → miRNA's

Background

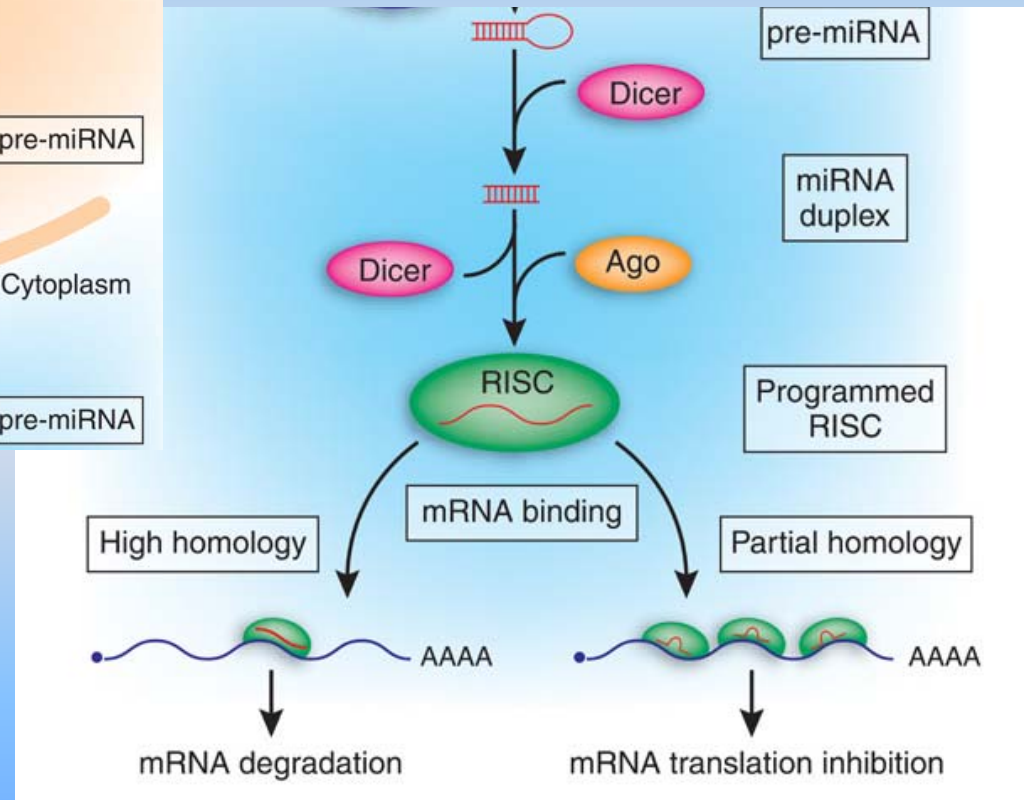
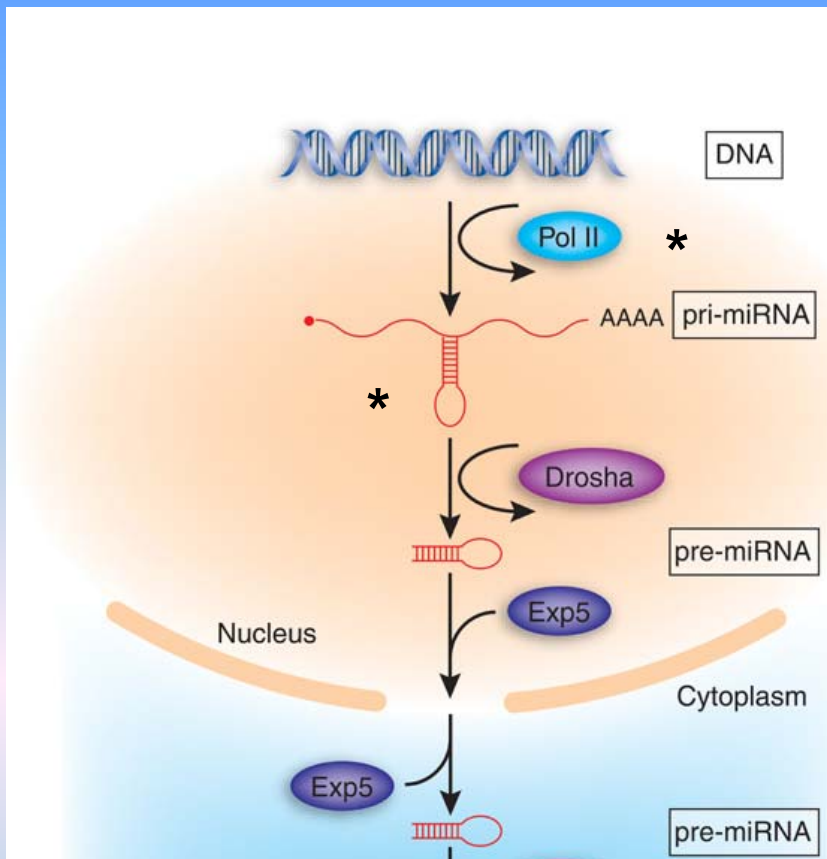
- miRNA Importance
- miRNA Biogenesis
- Computation Methods
- RNA Polymerases



miRNA Importance

- Regulate (multiple) gene expression
 - Early development
 - Cell proliferation and death
 - Apoptosis and fat metabolism
 - Cell differentiation
 - Brain development
 - Chronic lymphocytic leukemia
 - Colonic adenocarcinoma
 - Burkitt's lymphoma
 - Viral infection
 - Link with viral disease, neurodevelopment, and cancer
- 100's know in human; 1000's predicted
 - RNA Family Data Base (Rfam)
www.ambion.com/techlib/resources/miRNA/mirna_intro.html

miRNA Biogenesis



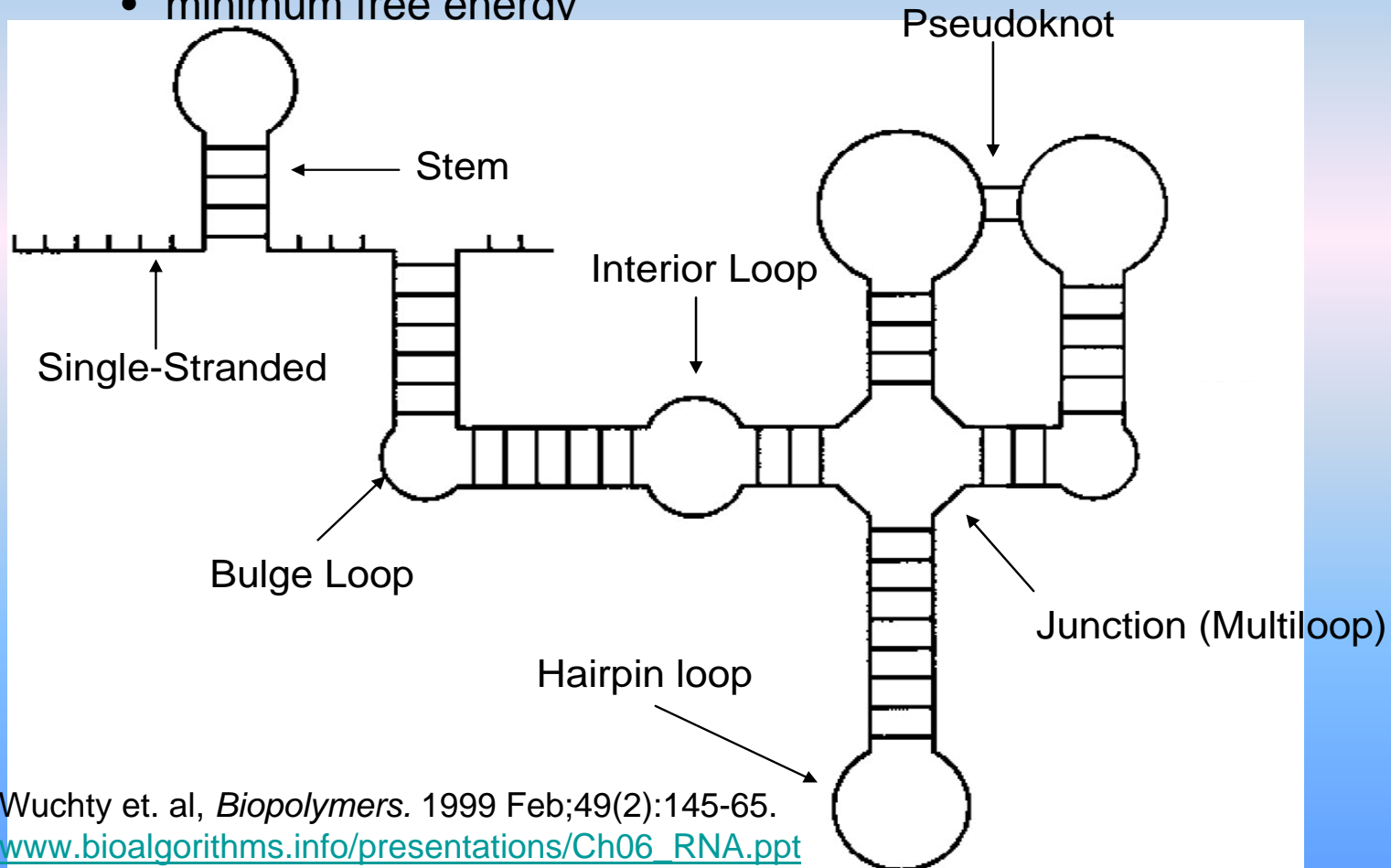
Cullen BR, *Nature Genetics* 37, 1163 - 1165 (2005)

Computation Methodologies

- SVM Methodology
 - predict new miRNA precursor
 - Vector requirements
 - Folding free energy
 - Length
 - Length longest symmetrical stem(ss)
 - Count of A, C, G, U in ss
 - A-U, G-C, G-U pair counts → minimal energy structure
 - ...
 - Trained SVM Classifier
 - + Control: = conserved stems human
 - - Control: = RNAi machinery avoids – rRNA, tRNA
 - Objective: 71% true positives, 3% false positives

Computation Methodologies

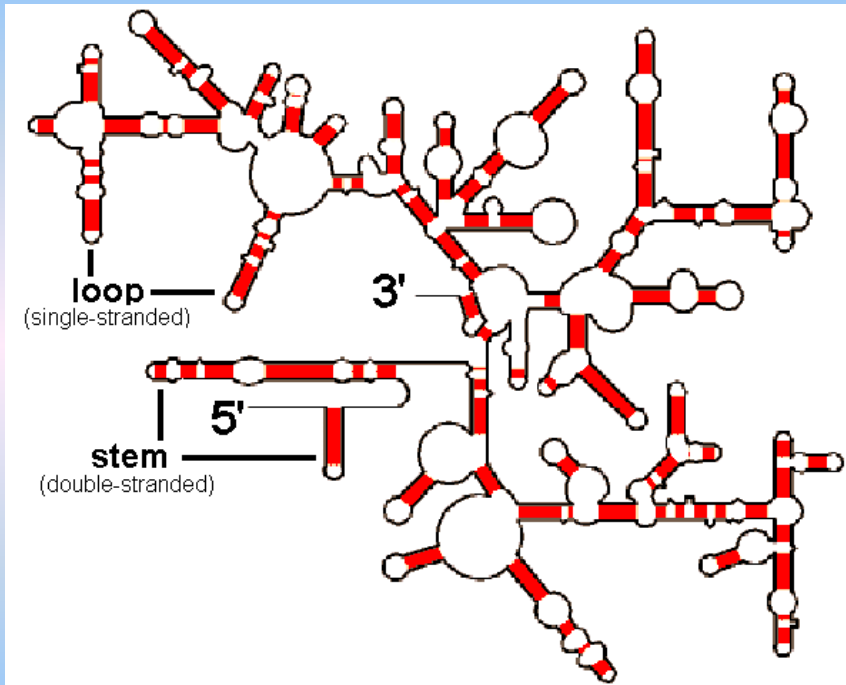
- *RNAfold* Vienna Package
 - calculate RNA secondary structures
 - minimum free energy



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Wuchty et. al, *Biopolymers*. 1999 Feb;49(2):145-65.
www.bioalgorithms.info/presentations/Ch06_RNA.ppt

SVM Training Structures



Secondary Structure of ribosomal RNA (rRNA)

www.mun.ca/biology/scarr/rRNA_folding.html

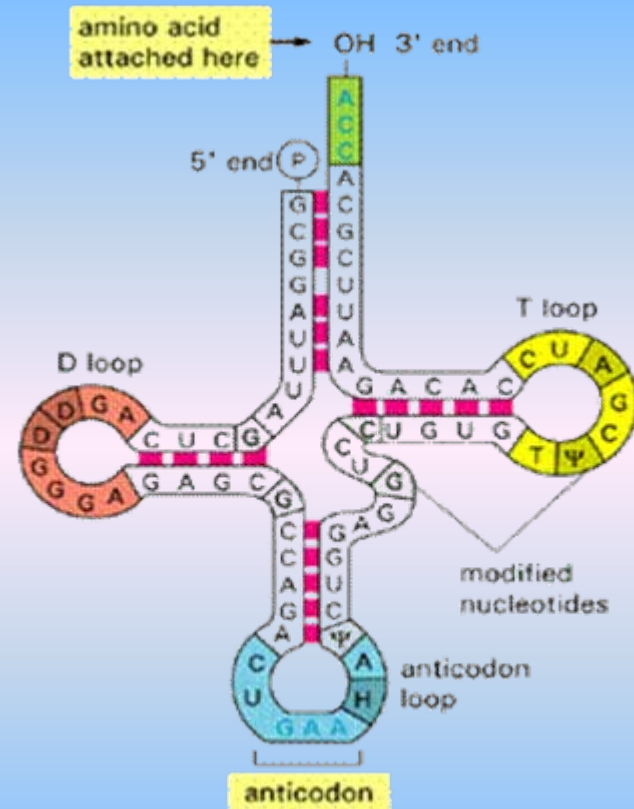


Figure 6-8. The "cloverleaf" structure of tRNA. Molecular Biology of the Cell, 3rd Ed. Part II. Molecular Genetics Chapter 6. Basic Genetic Mechanisms, RNA and Protein Synthesis

www.ncbi.nlm.nih.gov/Class/NAWBIS/Modules/RNA/images/fig_rna10.gif

RNA Polymerases

- Prokaryotes
 - single polymerase
- Eukaryotes
 - pol I → rRNA sections of ribosome
 - Located in nucleolus
 - pol II → mRNA, most snRNA
 - Located in nucleus
 - pol III → tRNA, rRNA(5S), snRNA
 - Located in nucleus (nucleolar-nucleoplasm interface)

Cloning Protocol: Confirm miRNA Predictions

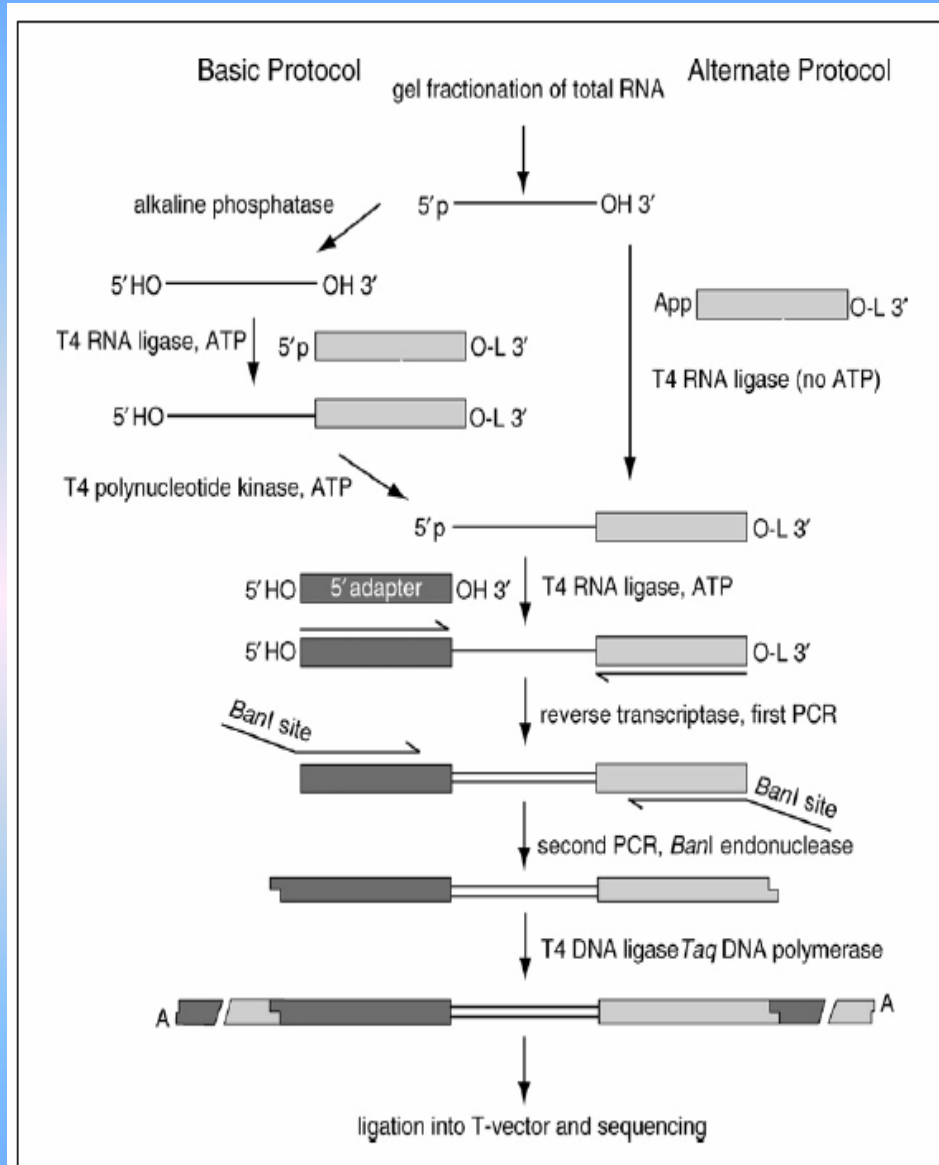
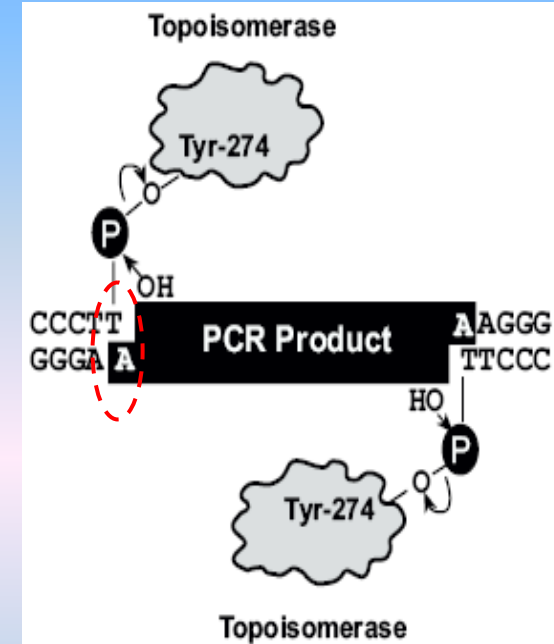


Figure 26.4.1 Schematic drawing of the Basic and Alternate Protocols for small RNA cloning.



Topo-TA Cloning®
www.invitrogen.com

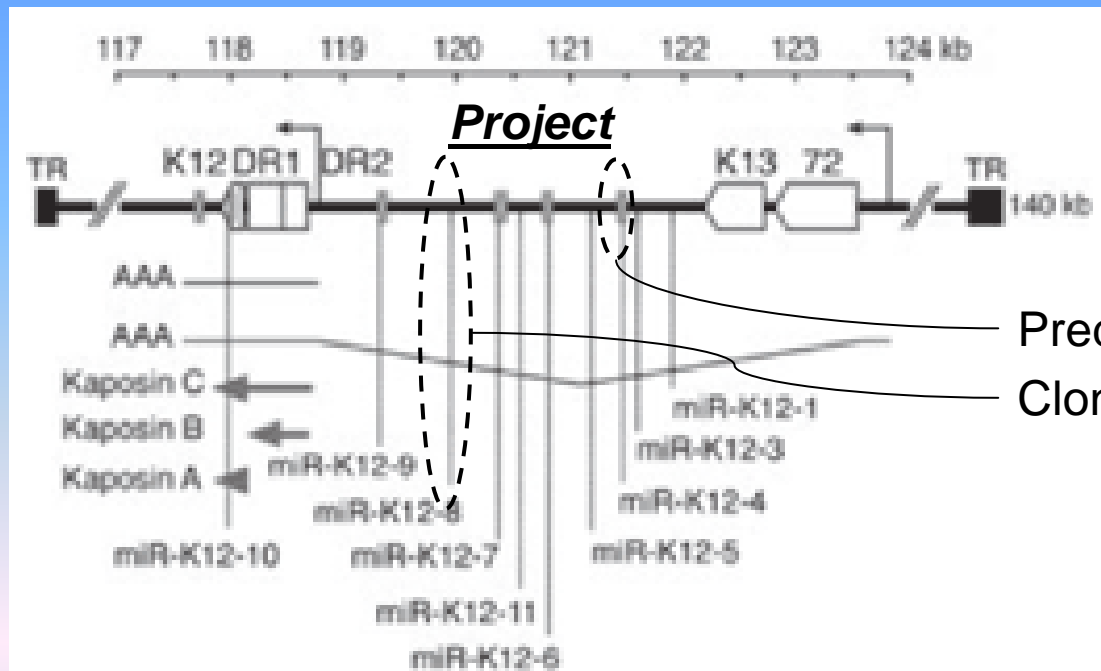
Predictions

Table 1 Summary of pre-miRNA predictions for selected human and mouse DNA and RNA viruses

Family (subfamily)	Genus	Species	Accession ^a	Prob. (%) ^b	Pred. ^c	Valid. ^d	Cloned ^e	
dsDNA viruses								
Herpesviridae (Alphaherpesvirinae)	Simplexvirus	HHV1	NC_00180699.9		8 (7)	NT	NT	
		HHV2	NC_001798100		10 (6)	NT	NT	
	Varicellovirus	HHV3	NC_00134854.7		0	NT	NT	
	(Betaherpesvirinae)	Cytomegalovirus	HHV5/HCMV	AC146907	99.9	11 (9)	5 (5)	9
		Roseolovirus	HHV6	NC_00166484.2		0	NT	NT
	(Gammaherpesvirinae)	Lymphocryptovirus	HHV7	NC_00171645.8		0	NT	NT
			HHV4/EBV	NC_00134599.0		7 (6)	2 (3)	5
Rhadinovirus		HHV8/KSHV	NC_003409100		8 (6)	5 (5)	10	
		MHV68	NC_00182697.6		4 (2)	1 (1)	9	
Poxviridae (Chordopoxvirinae)	Orthopoxvirus	Vaccinia virus	U94848	88.9	3 (3)	NT	NT	
		Variola virus	L22579	92.1	1 (1)	NT	NT	
	Molluscipoxvirus	Molluscum contagiosum virus	NC_00173199.1		5 (5)	NT	NT	
Adenoviridae	Mastadenovirus	HAVA	NC_00094240.6		1 (1)	NT	NT	
		HAVB	NC_00400146.5		1 (1)	NT	NT	
		HAVC	NC_00140543.7		0	NT	NT	
		HAVD	NC_00206767.7		3 (3)	NT	NT	
		HAVE	NC_00145436.8		0	NT	NT	
Papillomaviridae	Papillomavirus	HPV18	f	0.90	0	NT	NT	
Polyomaviridae	Polyomavirus	BK virus	NC_00153831.9		1 (1)	NT	NT	

Others: ssDNA, DNA/RNA reverse transcriptase, -ssRNA, +ssRNA ...

KSHV miRNAs



miR-K12-10b

```

5'   AG      C   C   C   UGU
    CUGG GCUUGGGG GAUA CACCA UCGUU C
    GACC UGAGCCCC CUGU GUGGU AGCGG U
        GG      C   U   U   UUG
    
```

miR-K12-9

```

5'   C   C   A   C   CGU
    GGGU UAC CAGCUGCGUA ACCC GCUG A
    CCCA AUG GUCGACGCAU UGGG CGAC A
        A   C   A   U   ACA
    
```

miR-K12-8

```

    CA C   CUA A   CC   UUUG UG
CGCG CUC CUCA CGCC GCU UC \
GCGC GAG GAGU GCGG CGA AG U
    AC A   CAGC AU   CGA- GU
    
```

miR-K12-5

```

5'   U   UA   - C U   GC   G U   AC
    GACC GGUA GU CC GGU CCUAAG G CU A
    CUGG CCGU CA GG CCG GGAUUC C GA U
    A   --   U A U   UA   A - AC
    
```

miR-K12-4

```

5'   C   AA G   C   GGL- UU
    AUAA UAGCUA CC CAGUA UCUA GCA C
    UAUU GUCGAU GG GUCAU AGAU UGU A
    A   CC A   A   ACAU UU
    
```

miR-K12-3

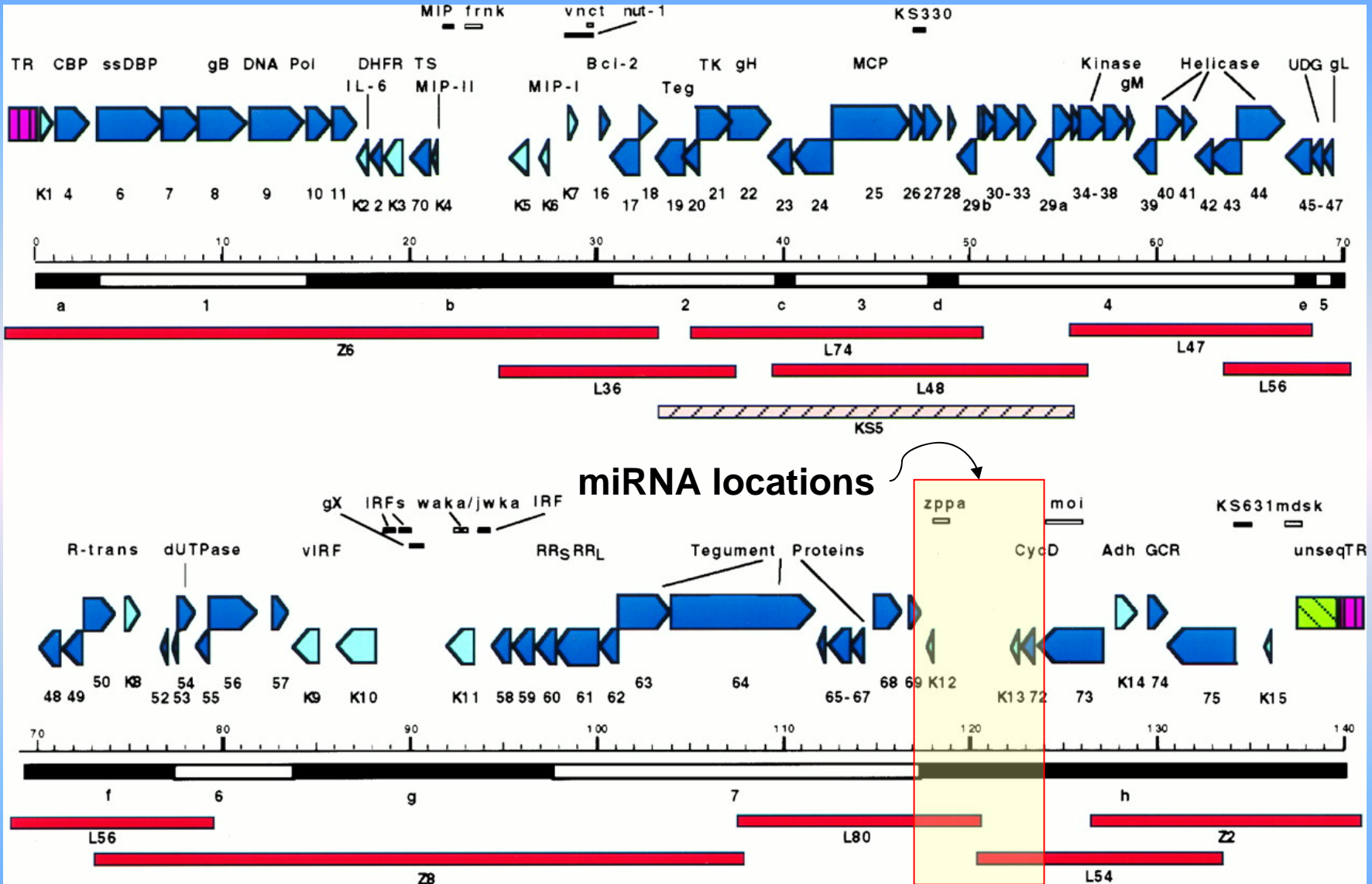
```

5'   CUA   AG G A   ---   GUG
    GG UCACAUUCUG GAC GC GC GACGU \
    CC AGUGUAAGAC CUG CG UG CUGCA U
    AC-   A- G C   CAA   AUC
    
```

Low abundance

Predominant

KSHV Genome



Russo, JJ et. al. *Proc Natl Acad Sci U S A.* 1996 Dec 10;93(25):14862-7

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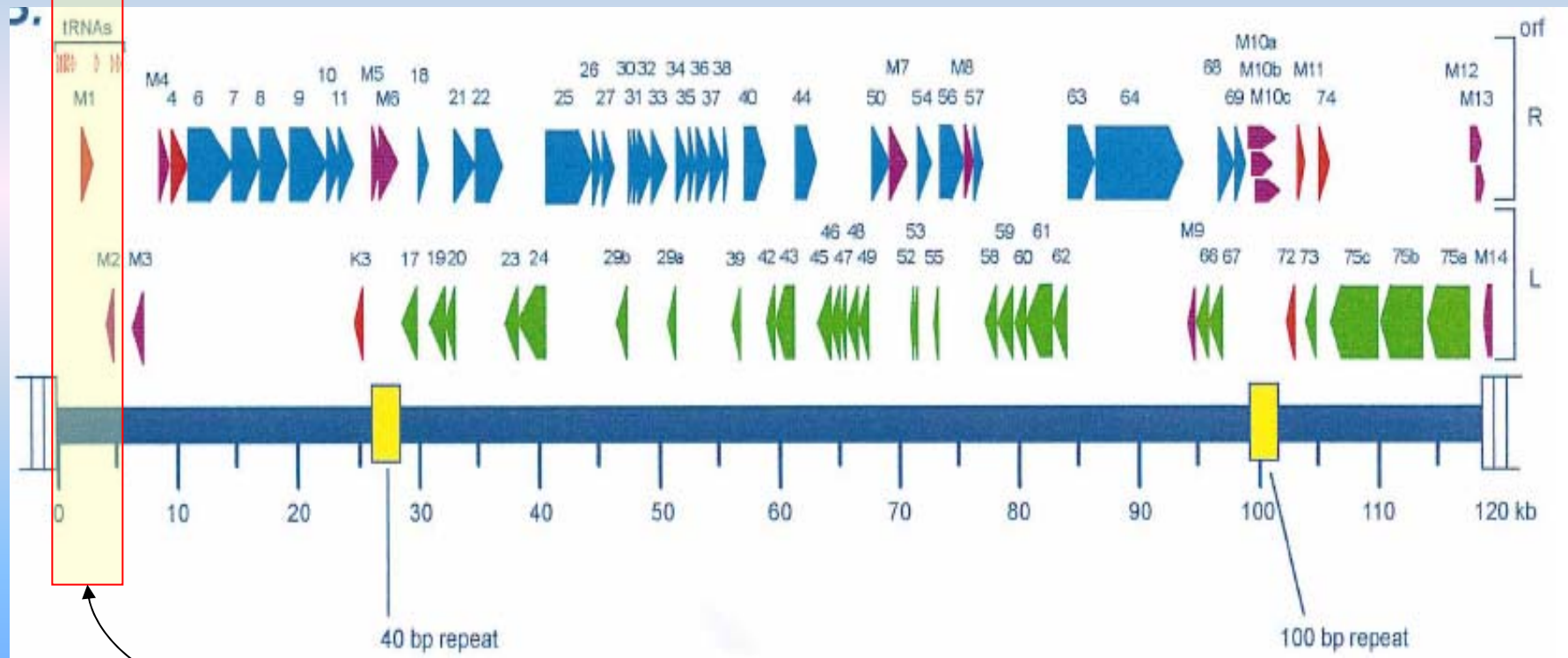
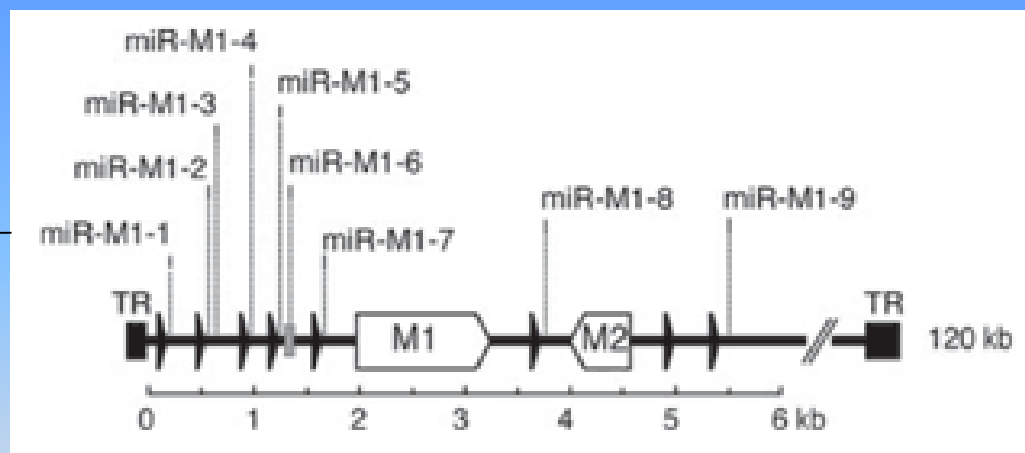
Kaposi Sarcoma



Figure 5-2. Red, nodular KS lesions on the foot of a patient. Courtesy of Susan E. Krown, MD, Memorial Sloan-Kettering Cancer Center.

Volderbering, Polefsky, Walsh
Viral and Immunological Malignancies (2006)
www.bedecker.com

MHV68 Genome

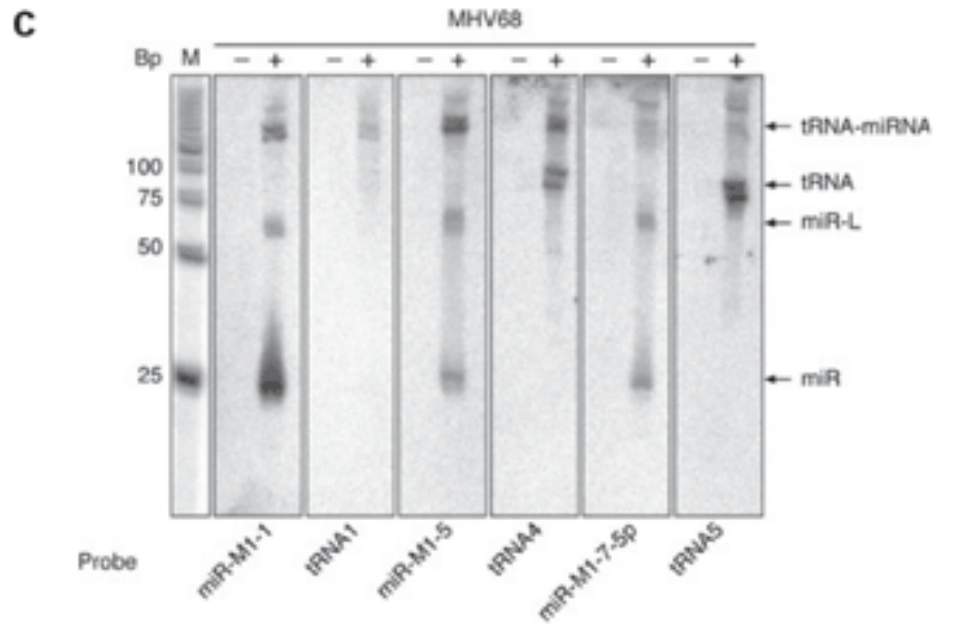
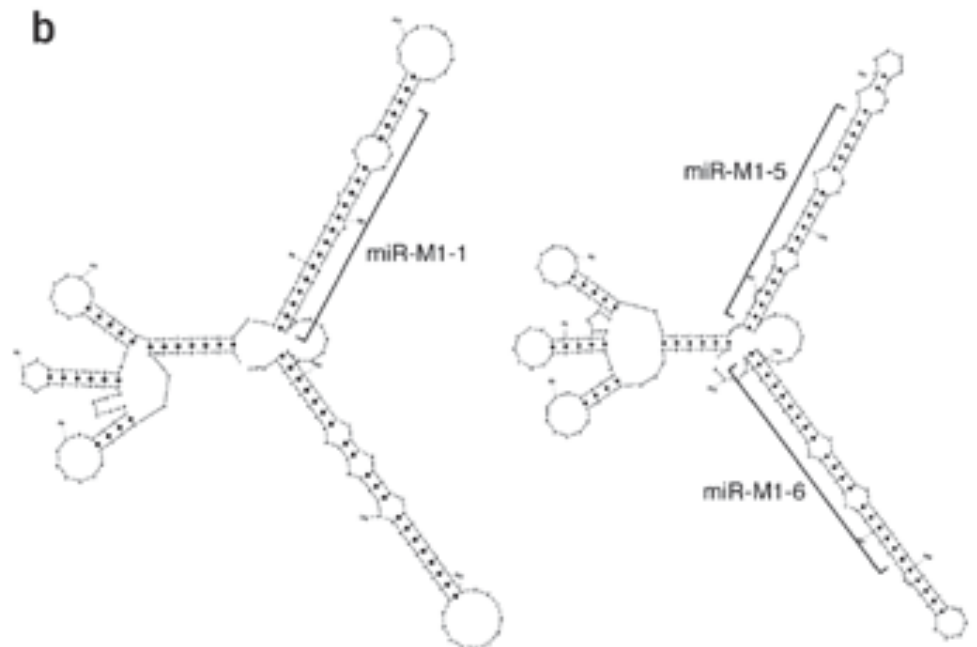
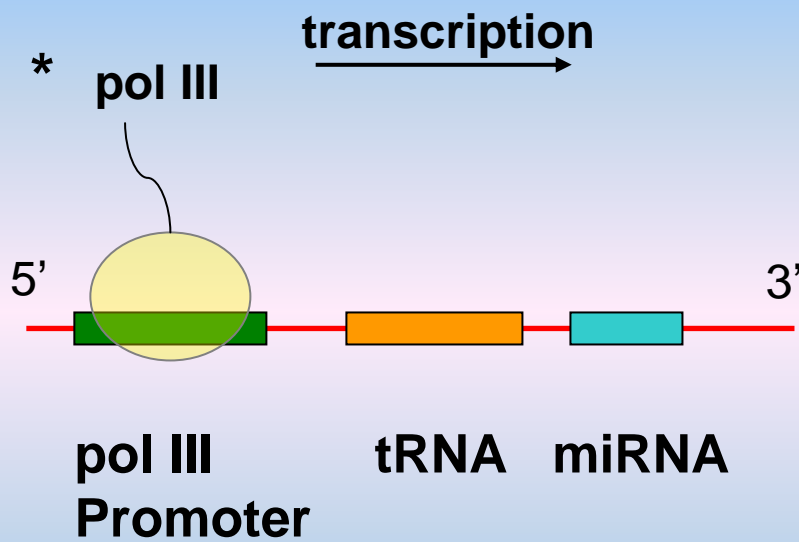


miRNA locations

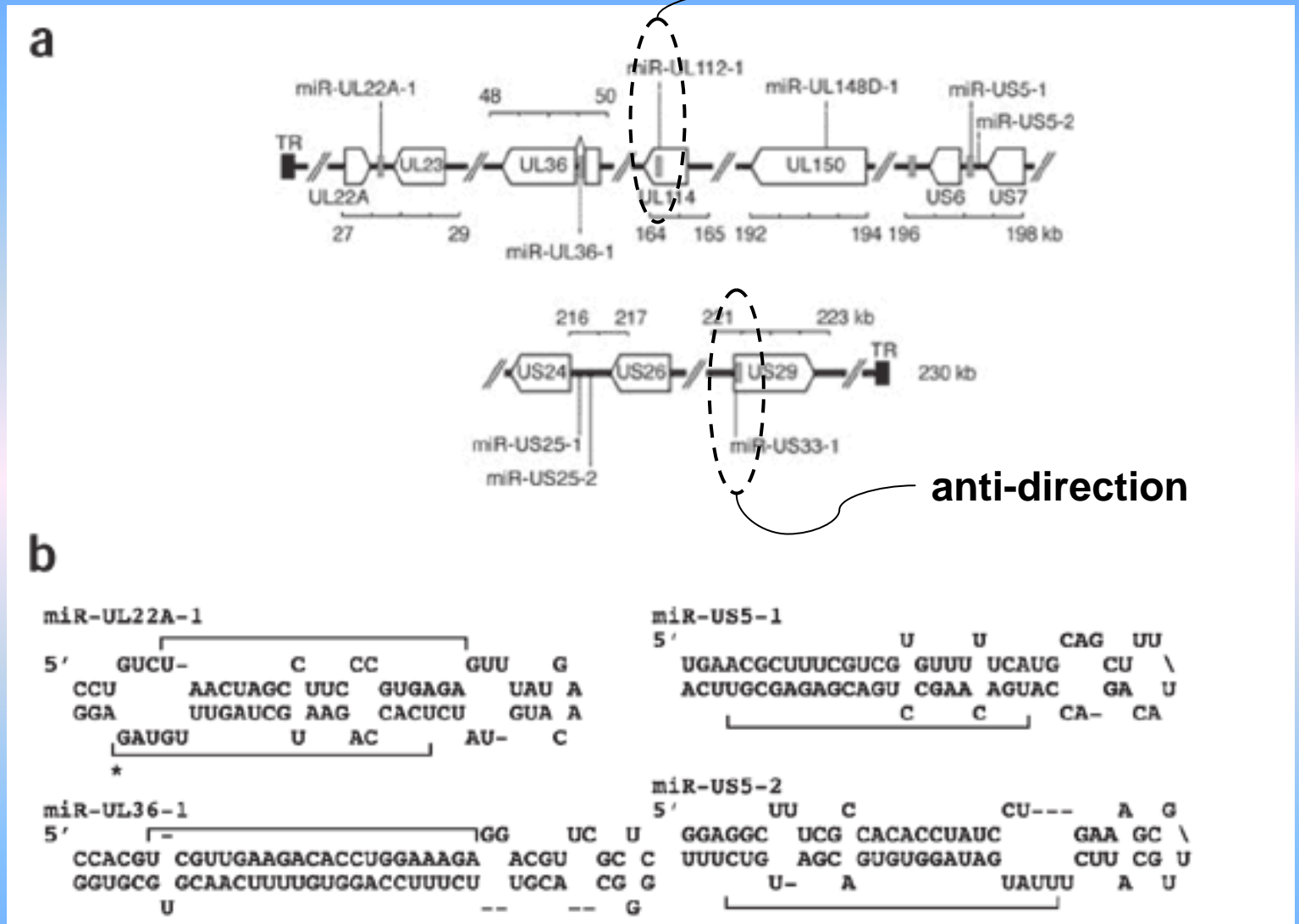
Virgin IV, et. al. *J Virol.* 1997 Aug;71(8):5894-904.

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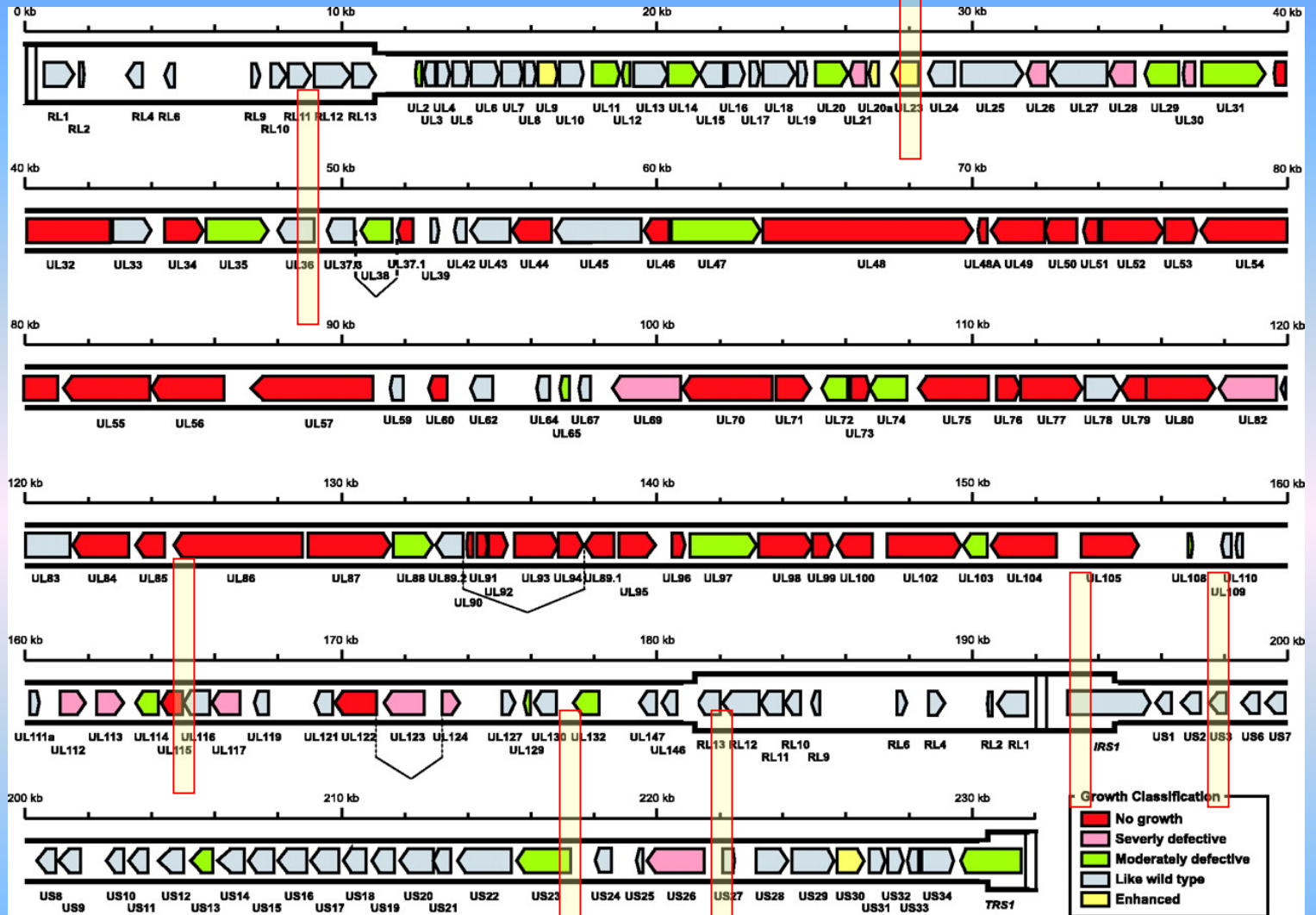
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HCMV miRNAs

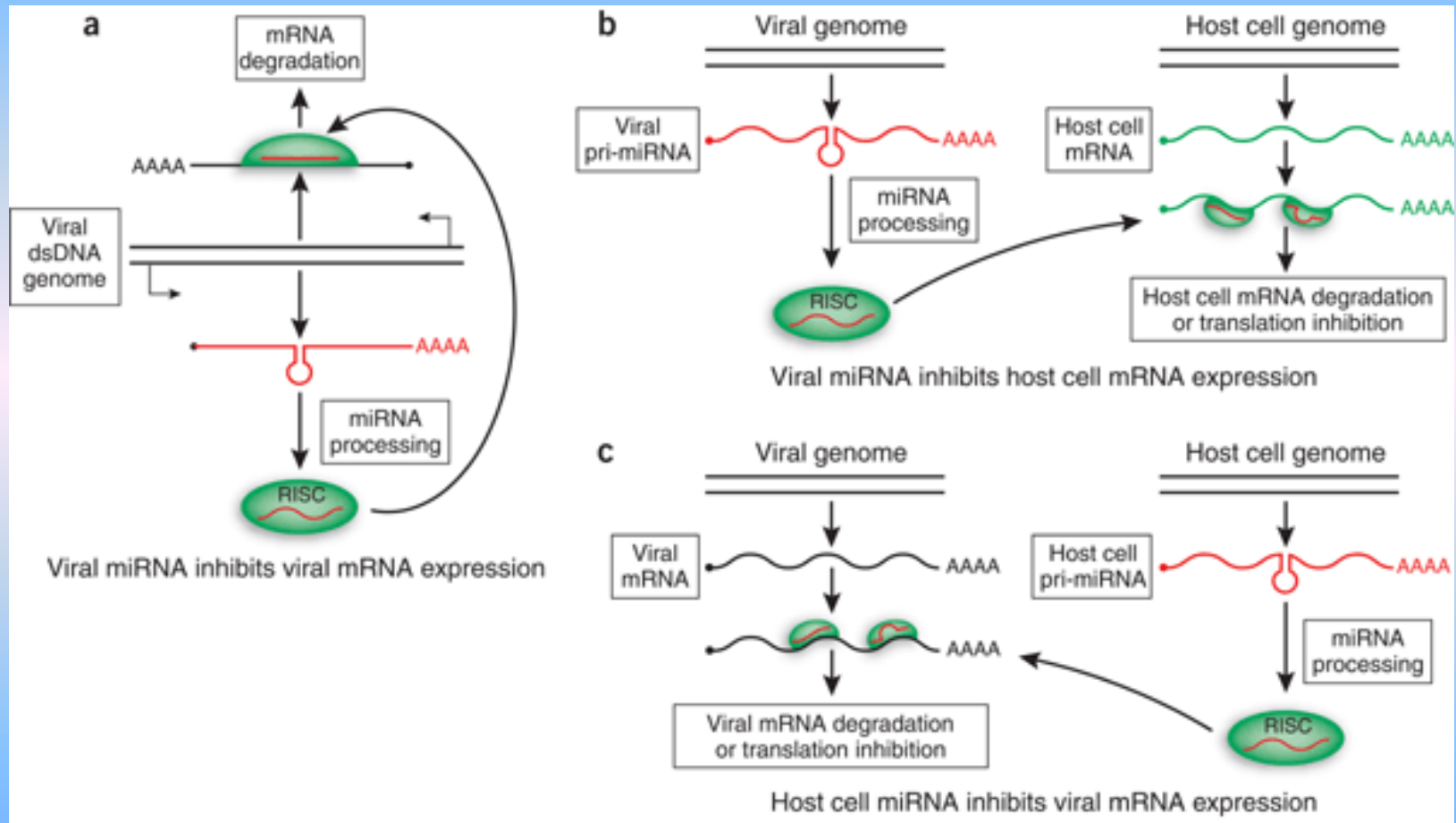


HCMV Genome



Dunn, Walter et. al. *Proc. Natl. Acad. Sci. USA* 100, 14223-14228 (2003)

microRNA actions further investigation...



Cullen BR, *Nature Genetics* 37, 1163 - 1165 (2005)

Acknowledgements

Audience

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