

# Genome Editing and Engineering

Course No: BT-637



## LECTURE-8

Dr. Kusum K. Singh

Department of Biosciences and Bioengineering

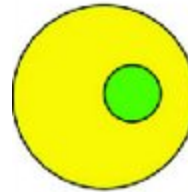
Indian Institute of Technology Guwahati

# Introduction

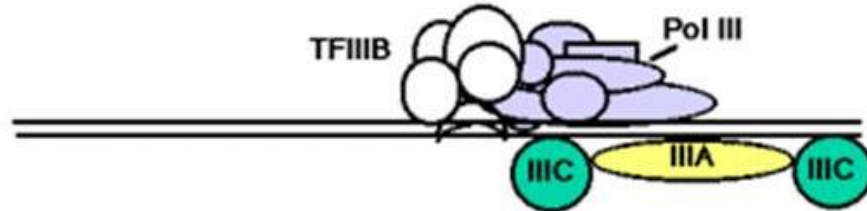
- *Xenopus laevis*



Somatic  
(body) cell



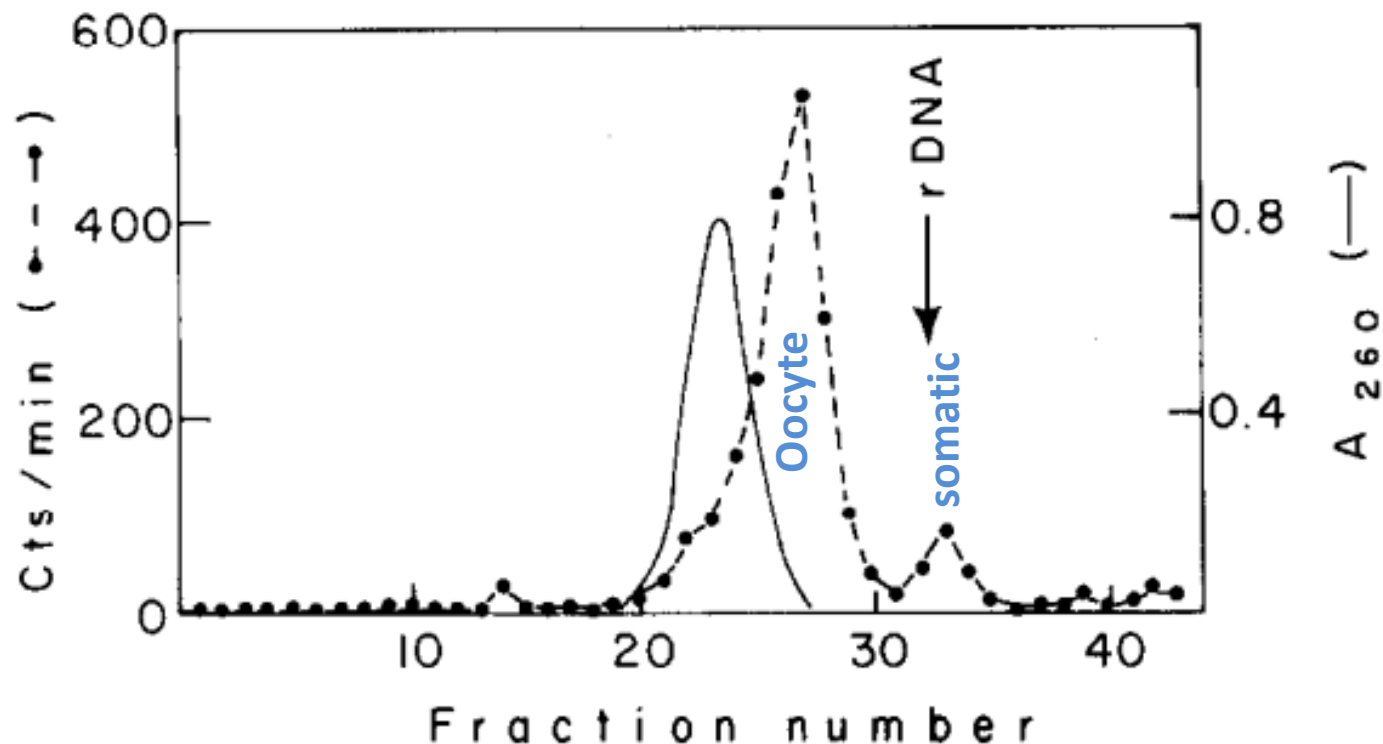
Egg cell



- Gene expression & Transcription mechanism

# Characterization of 5S RNA Genes

- By 1979: Cloning and characterization of somatic & oocyte type 5SRNA.



# Purification of RNA Polymerase III

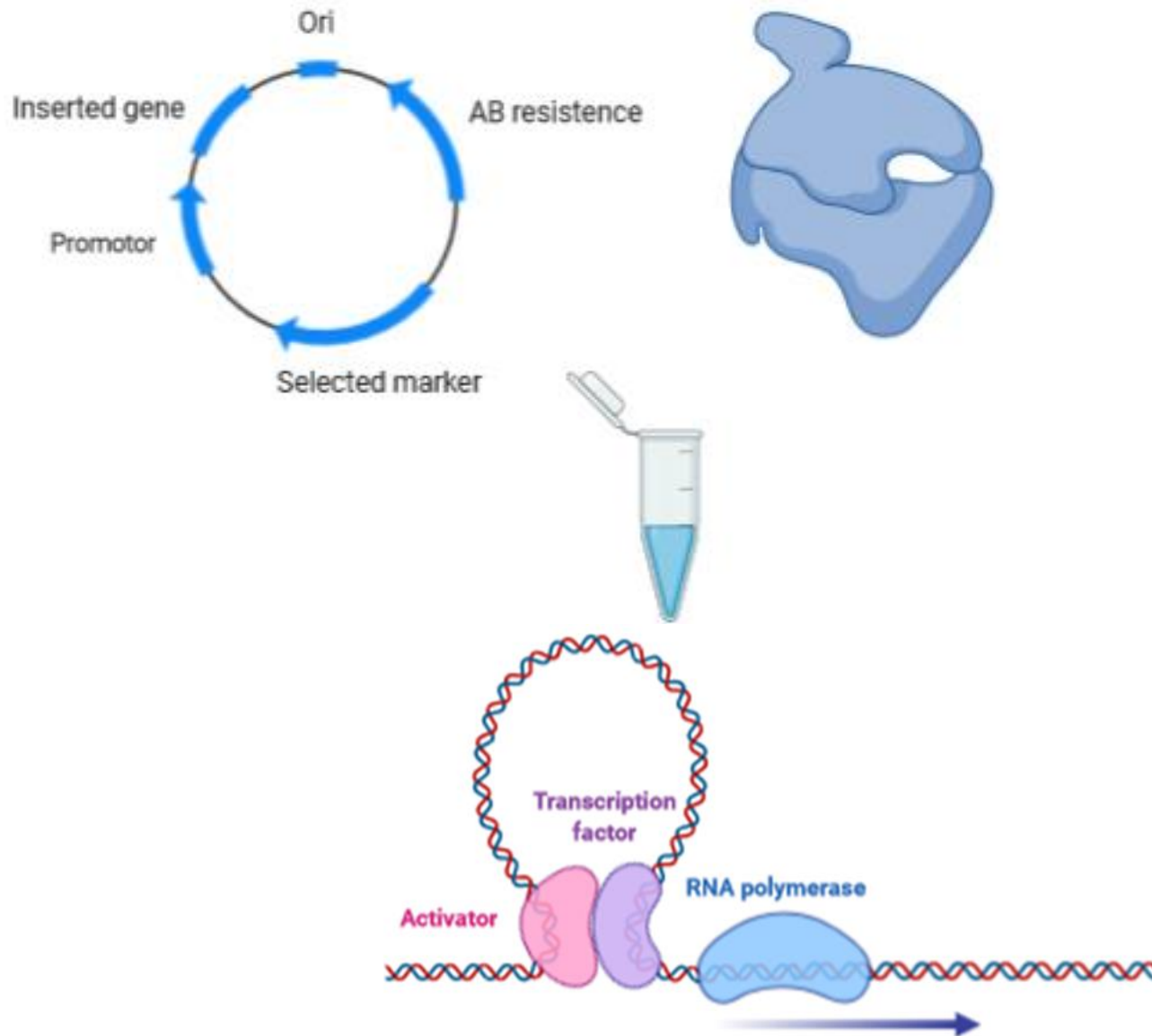
- By 1979: RNA pol III was purified and characterized

Table 1. The Purification of RNA Polymerase III from Xenopus Ovaries

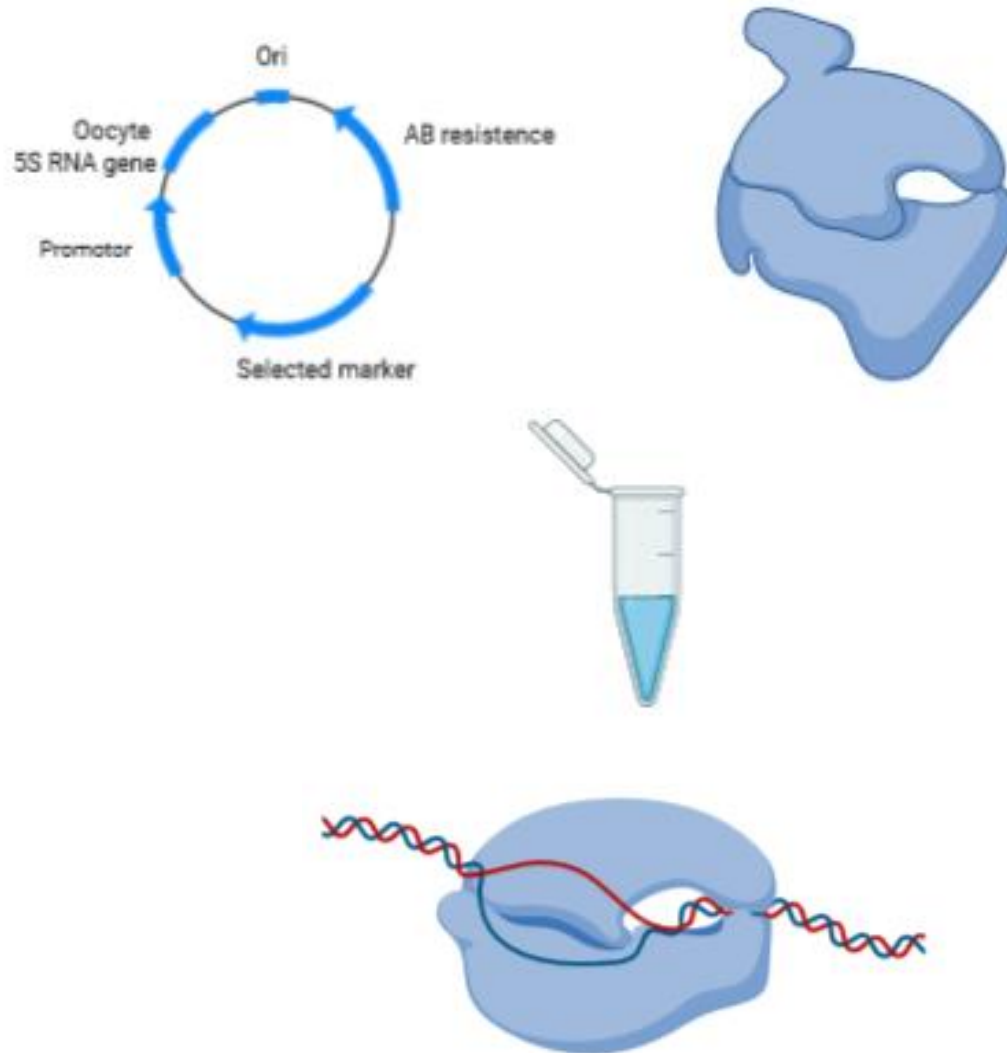
Purification Step	Total Protein (mg)	Polymerase III Units <sup>a</sup>	Yield Total (%)	Specific Activity (U/μg)
1. F4	900	170,000	100	0.2
2. DEAE Sephacel	88	160,000	96	1.8
3. Heparin Sepharose	7.6	78,000	46	10
4. DEAE Sephadex	0.6	40,000	24	67
5. Phosphocellulose	0.22	31,000	18	140
6. Sucrose gradient	0.07	21,000	12	300

<sup>a</sup> One unit of RNA polymerase is defined as the amount of enzyme that incorporates 1 pmole of UTP into RNA in 20 min at 30°C.

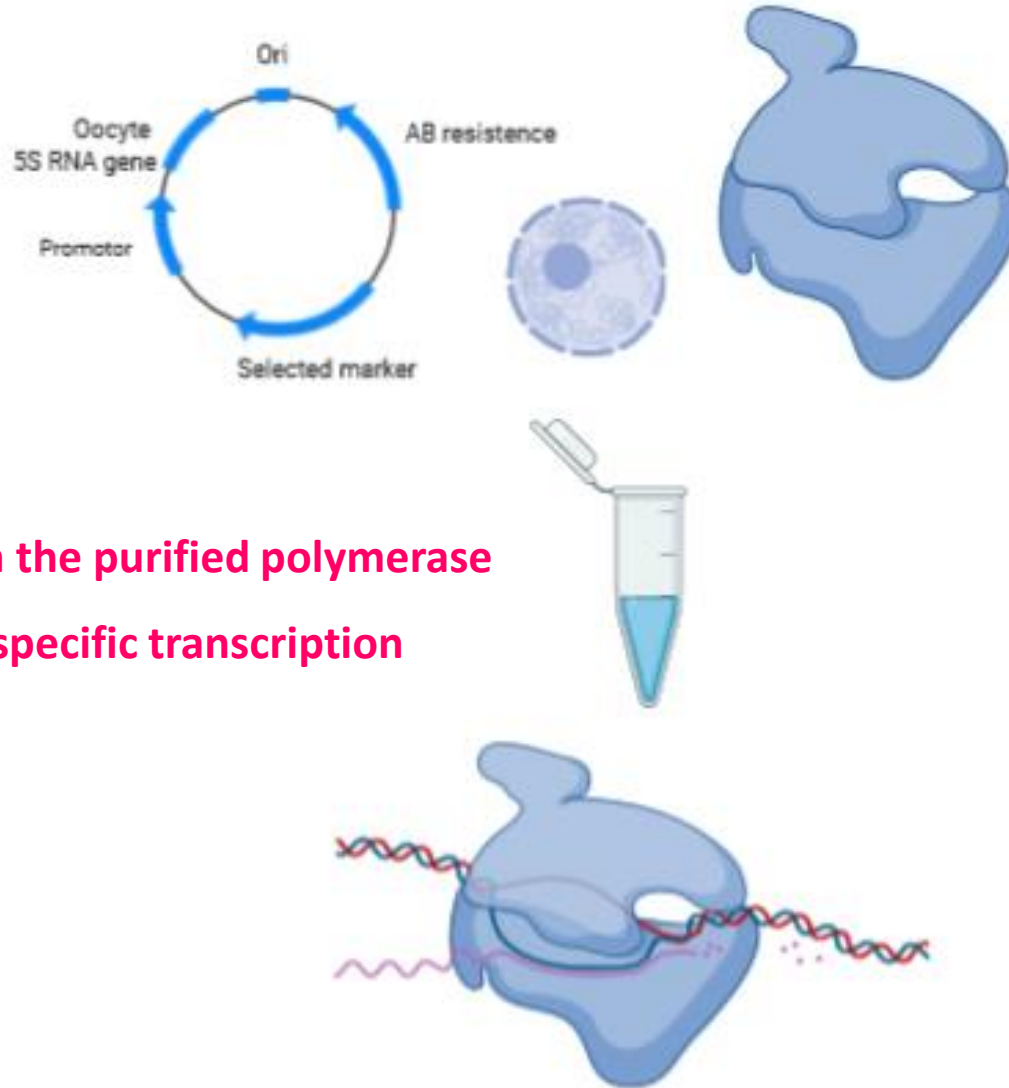
# In vitro: Gene + RNA Polymerase III



# In vitro: Gene + RNA Polymerase III



# In vitro: Gene + RNA Polymerase III



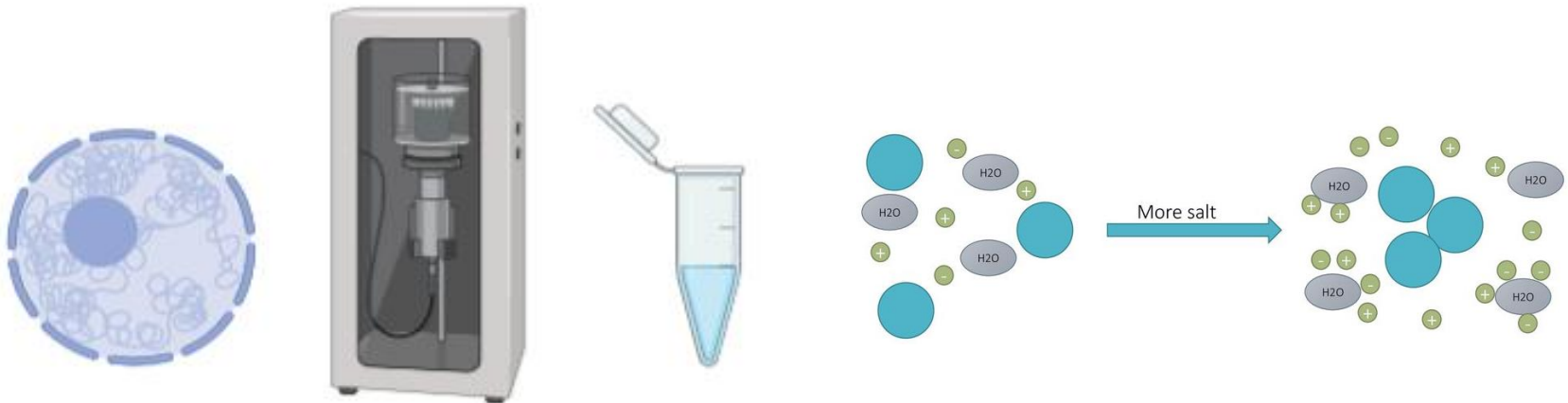
Factors other than the purified polymerase  
are necessary for specific transcription

# Specific Interaction of a Purified Transcription Factor with an Internal Control Region of 5S RNA Genes

Cell, Vol. 19, 717–728, March 1980,

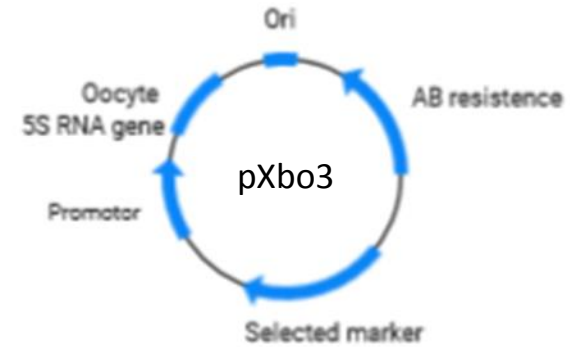
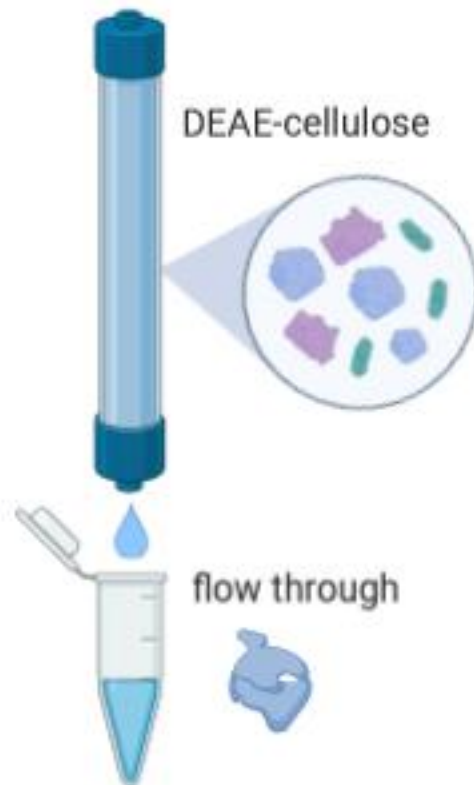
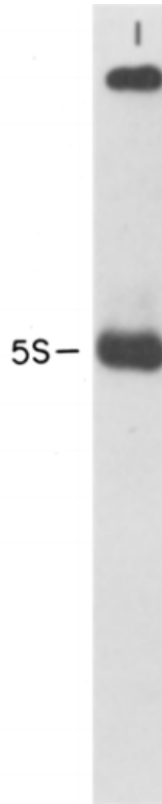
David R. Engelke,  
Sun-Yu Ng, B. S. Shastry and Robert G. Roeder

- Ng et al 1979 and Engelke 1980:
- purify “A factor” from oocytes = specific transcription of 5S gene
- Factor purification = various chromatography techniques



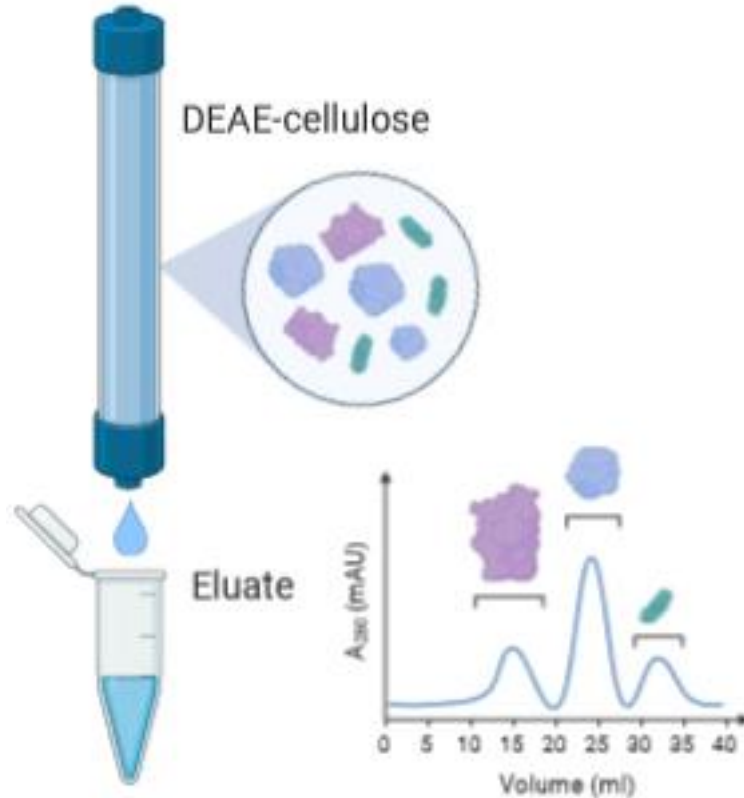
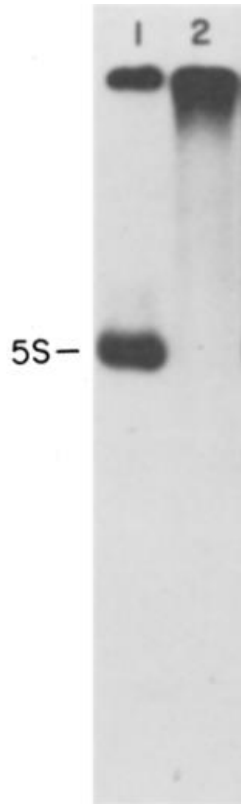


# Purification of “A Factor”



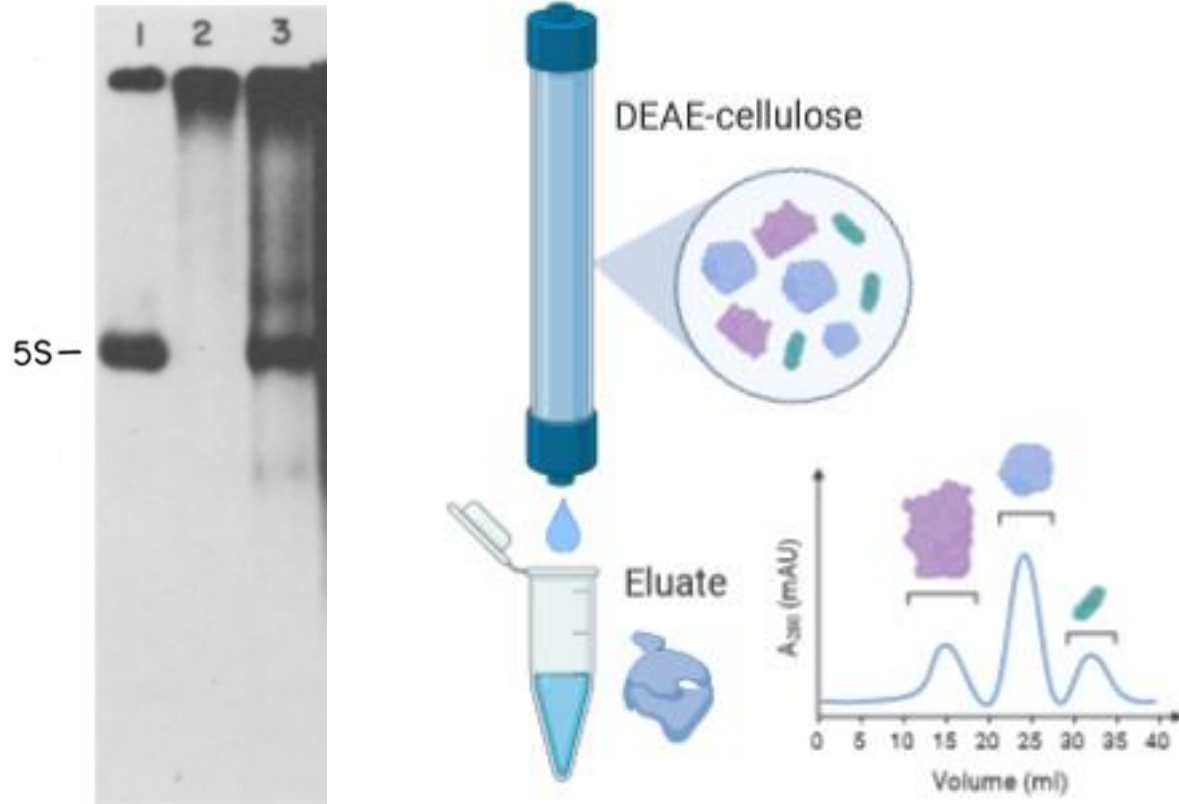
# Purification of “A Factor”

2. Nothing formed in absence of RNA polymerase



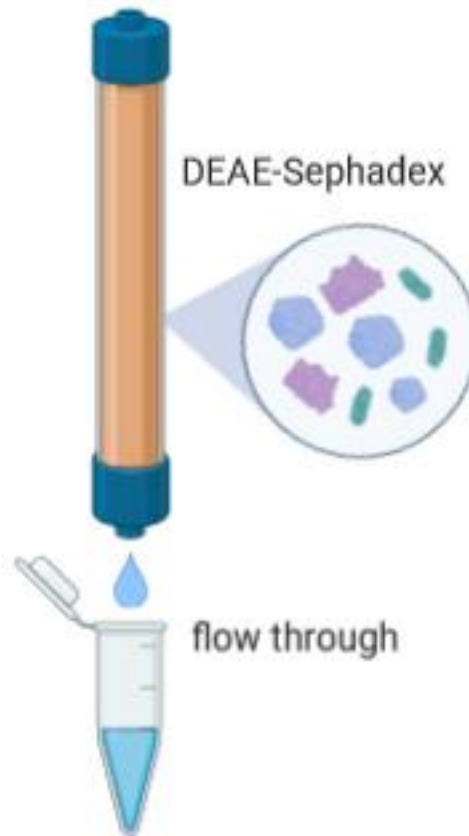
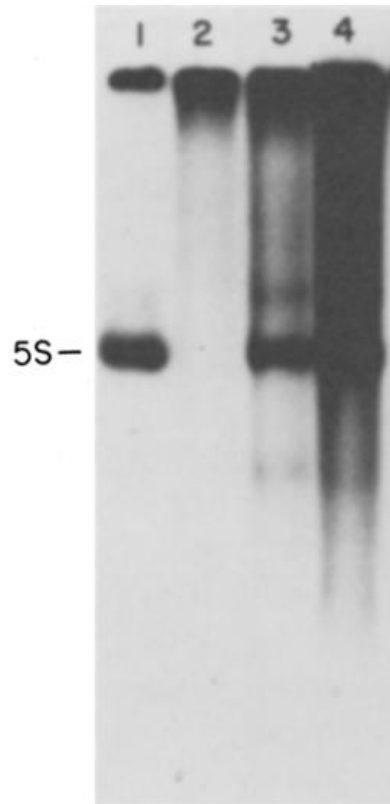
# Purification of “A Factor”

3.5sRNA formed in presence of RNA polymerase



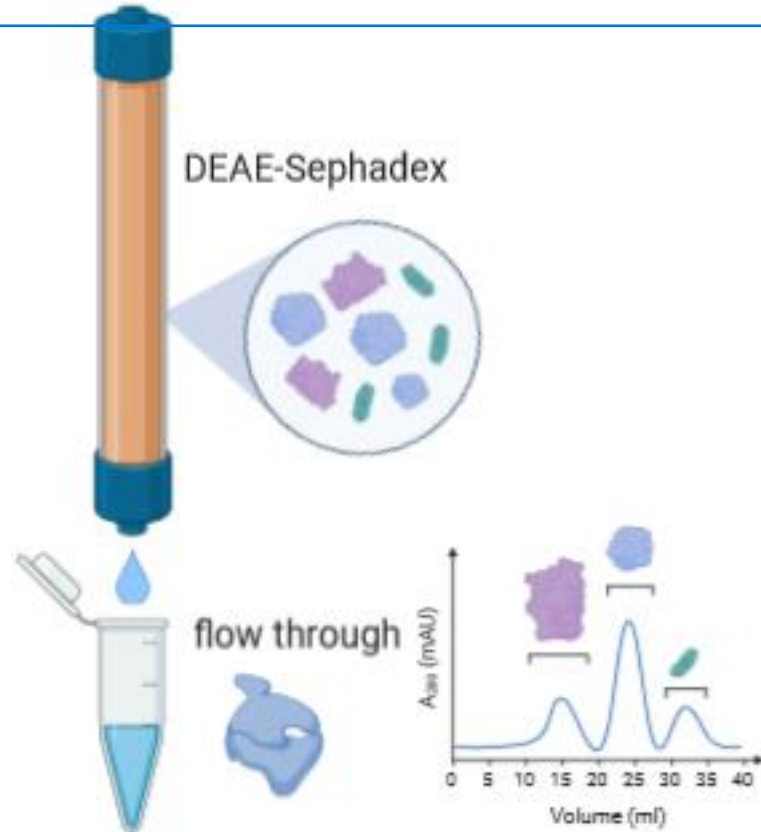
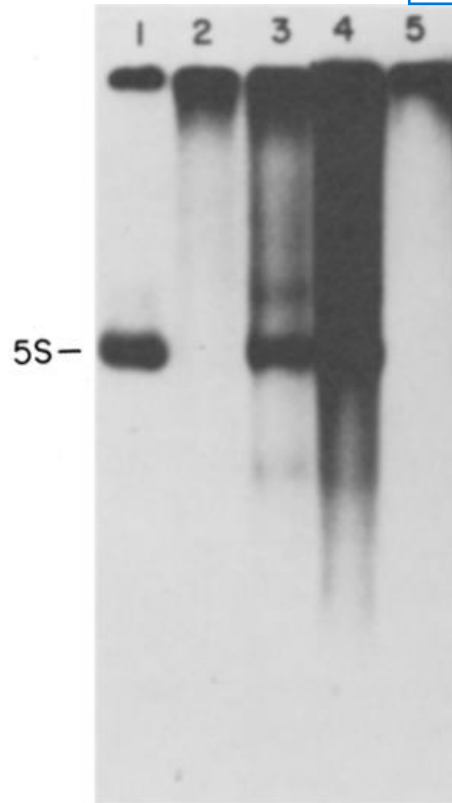
# Purification of “A Factor”

4.5sRNA and non-specific transcripts formed in absence of RNA polymerase



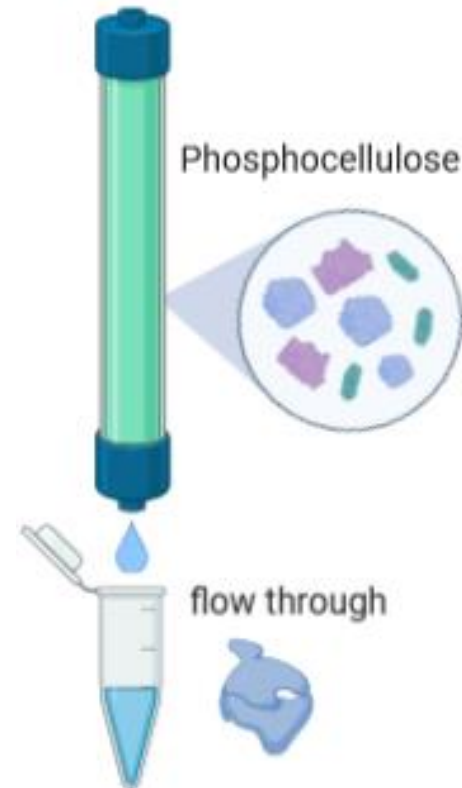
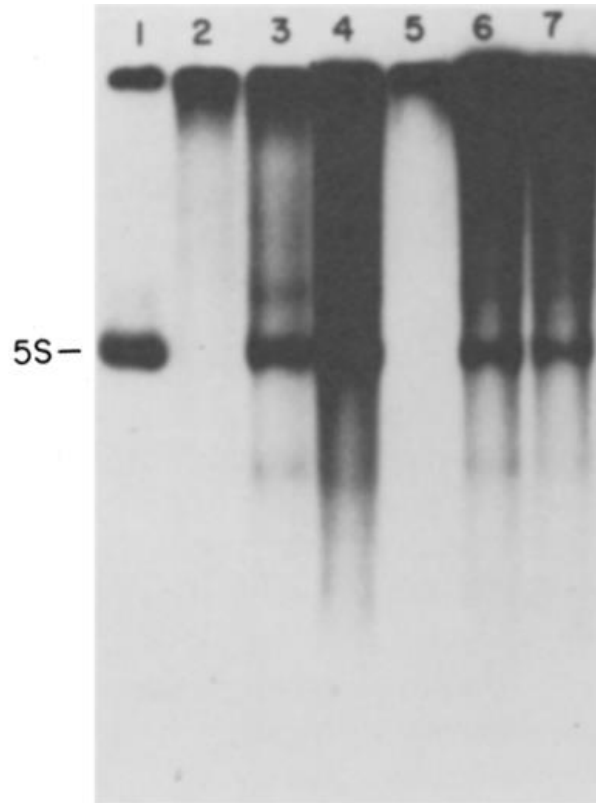
# Purification of “A Factor”

5. After passing through DEAE-Sephadex endogenous polymerase III abs

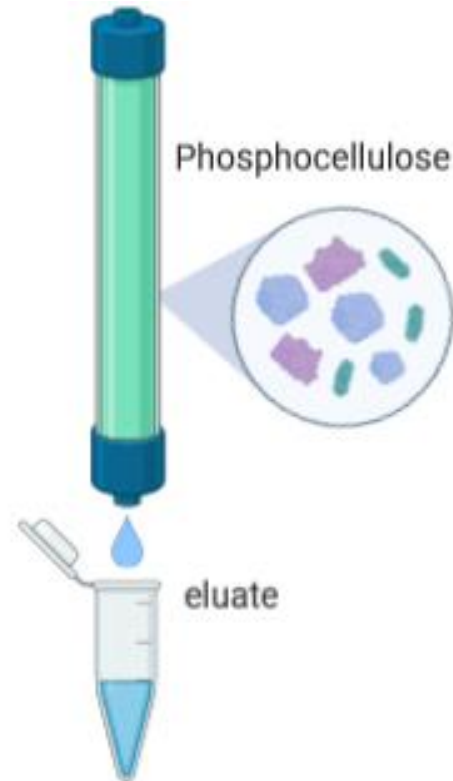
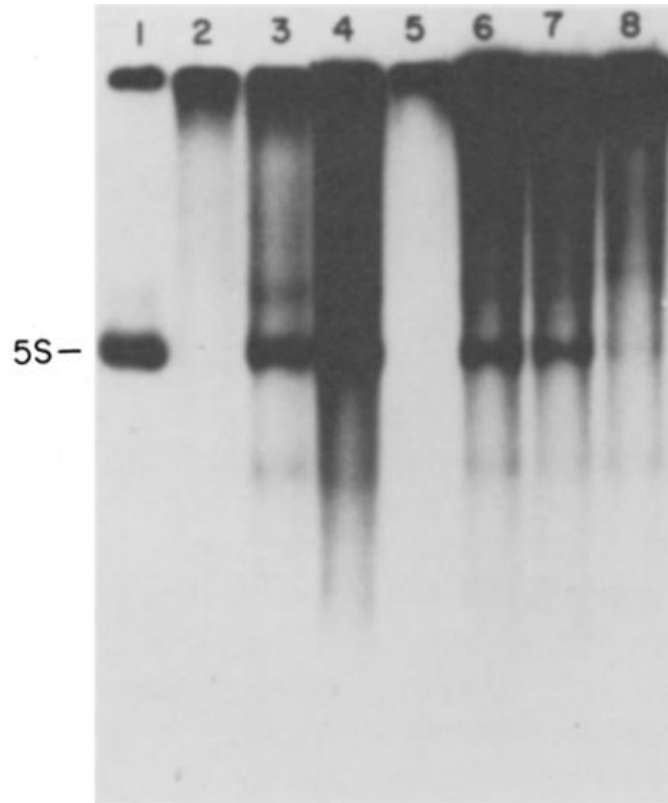


# Purification of “A Factor”

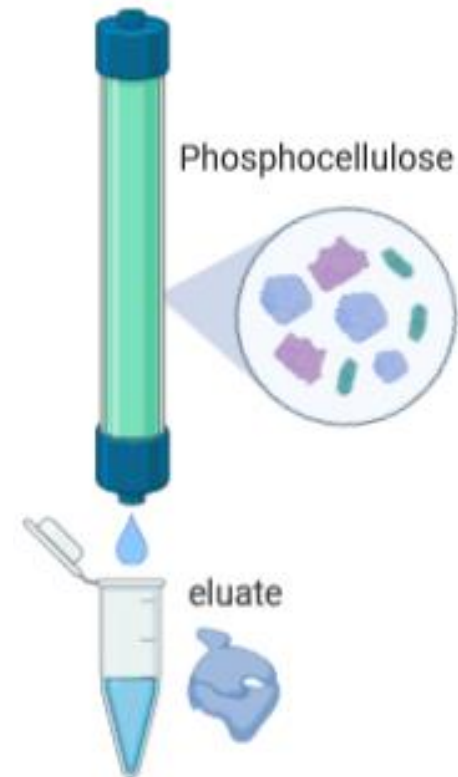
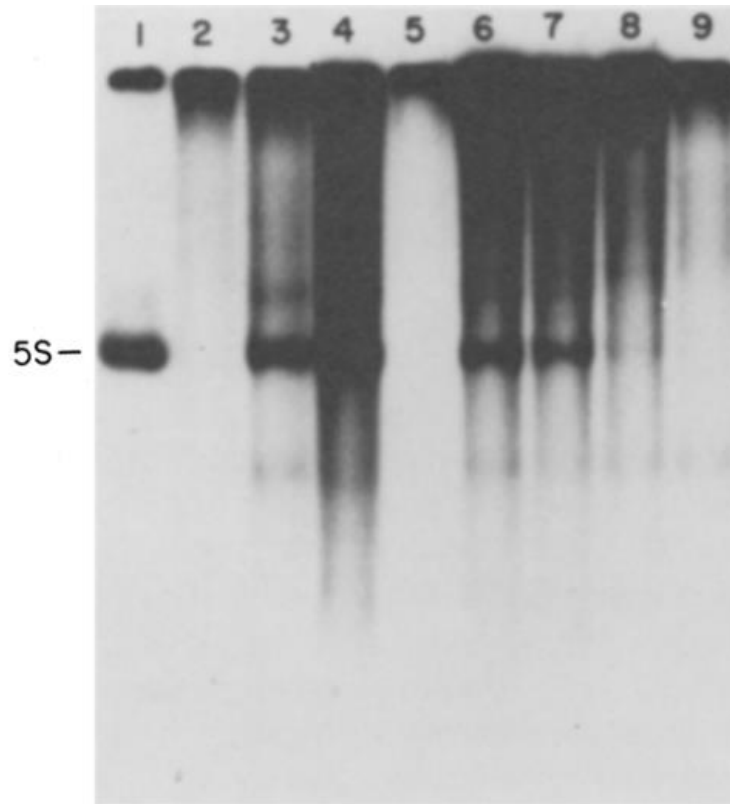
6/7. After polymerase III 5sRNA transcribed.



# Purification of “A Factor”



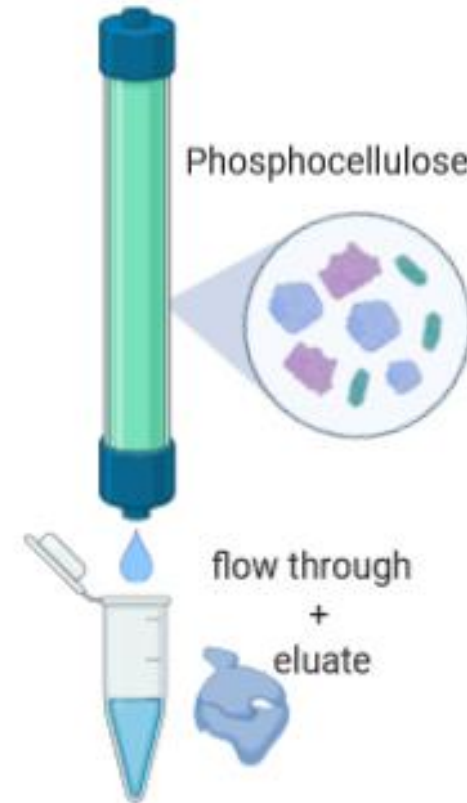
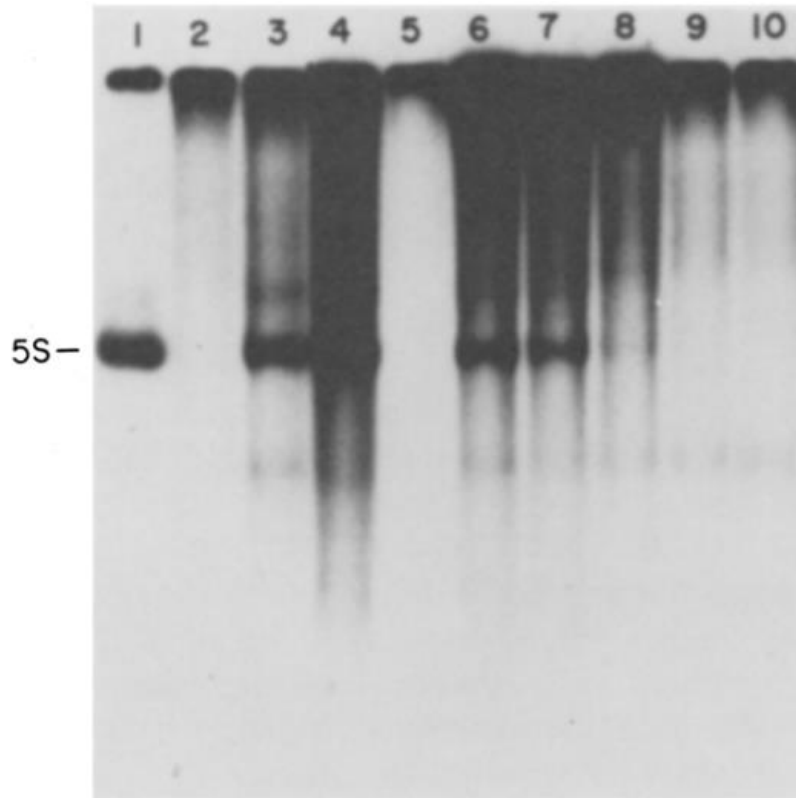
# Purification of “A Factor”





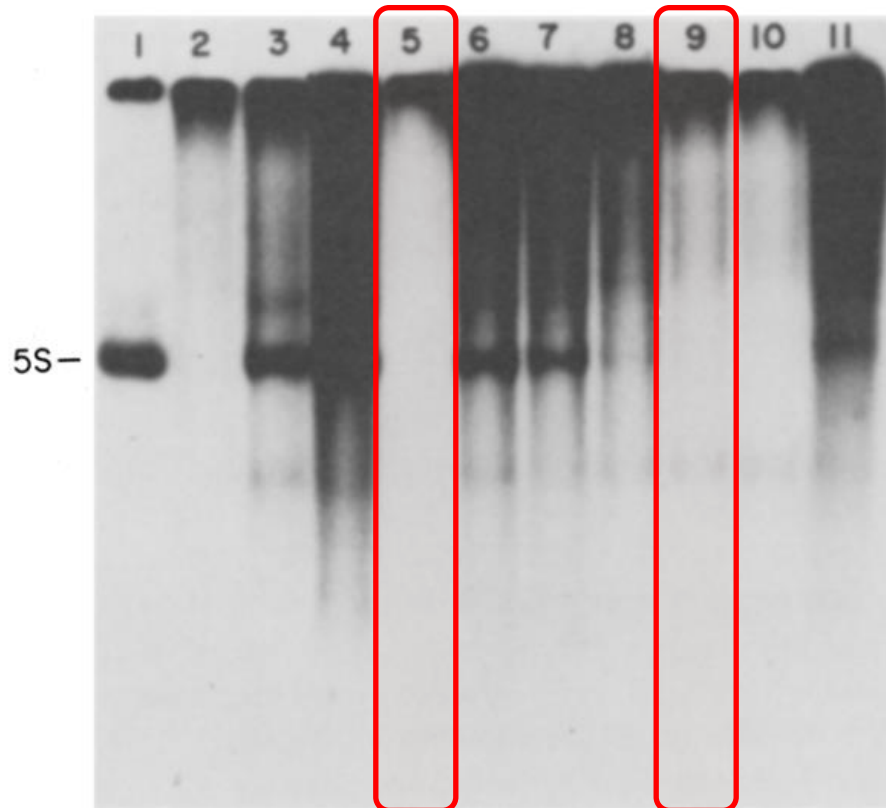
# Purification of “A Factor”

8/9/10. Nothing formed regardless of addition of polymerase III



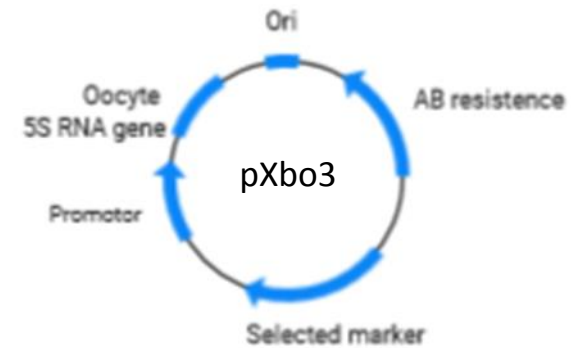
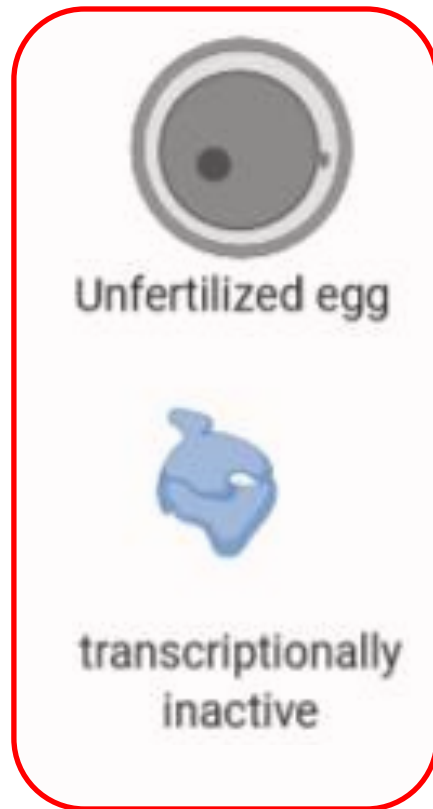
# Purification of “A Factor”

11. When everything mixed flow through+ Eluate we got non-specific transcription.

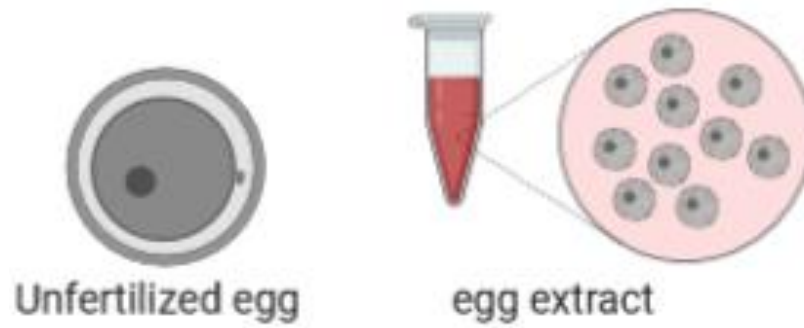


Existence of two or more components = 5S gene transcription

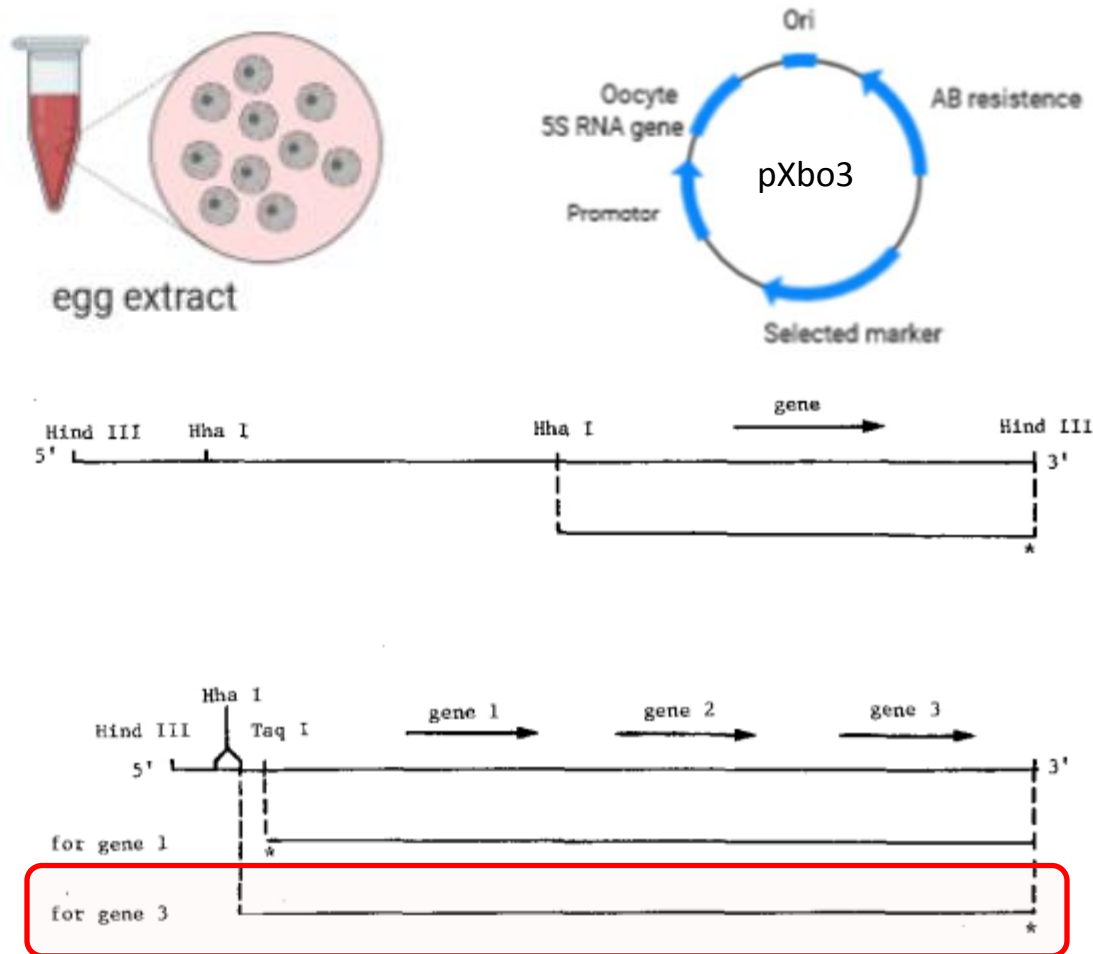
# In vitro: unfertilized egg extract



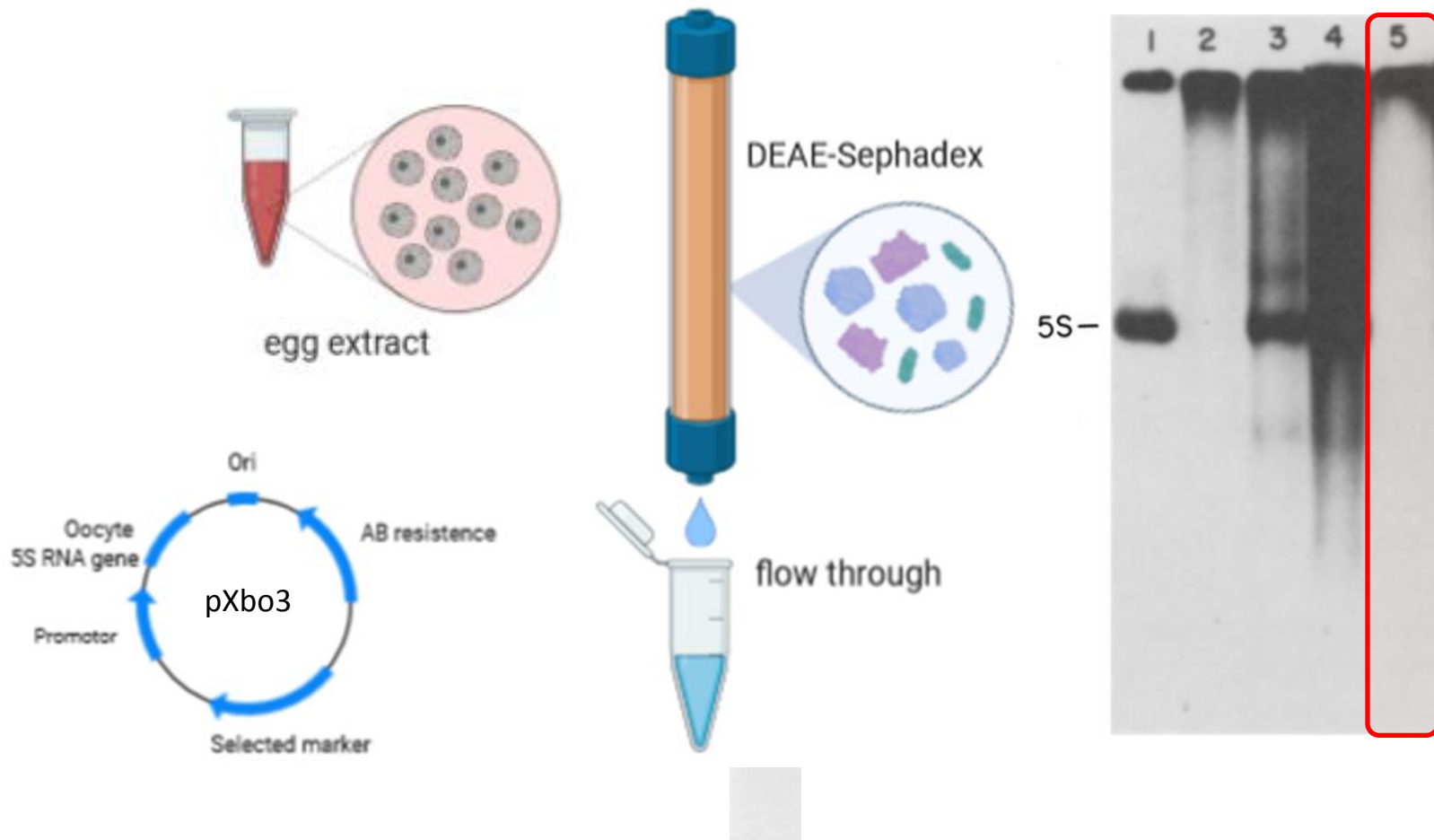
# In vitro: unfertilized egg extract



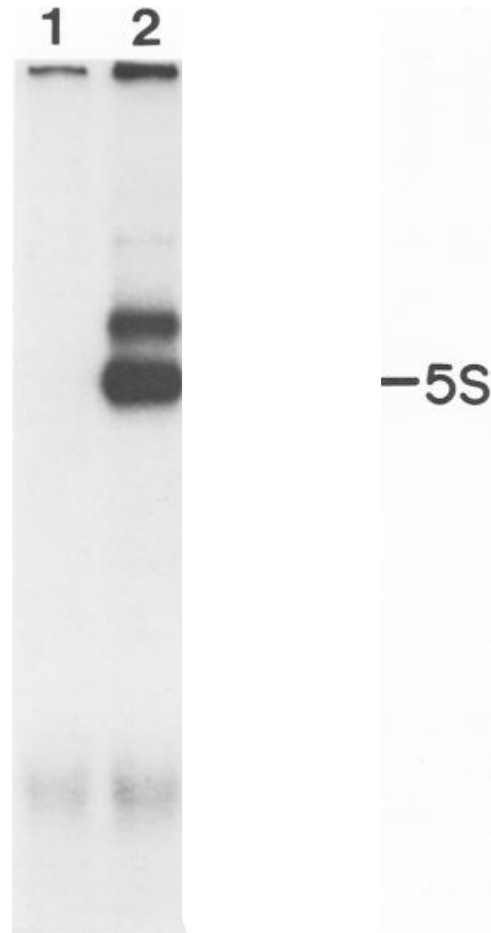
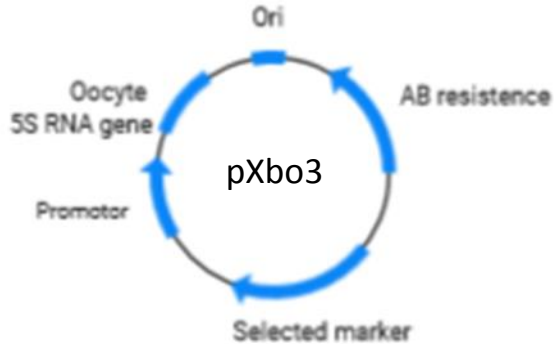
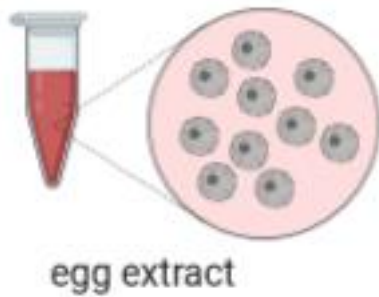
# In vitro: unfertilized egg extract



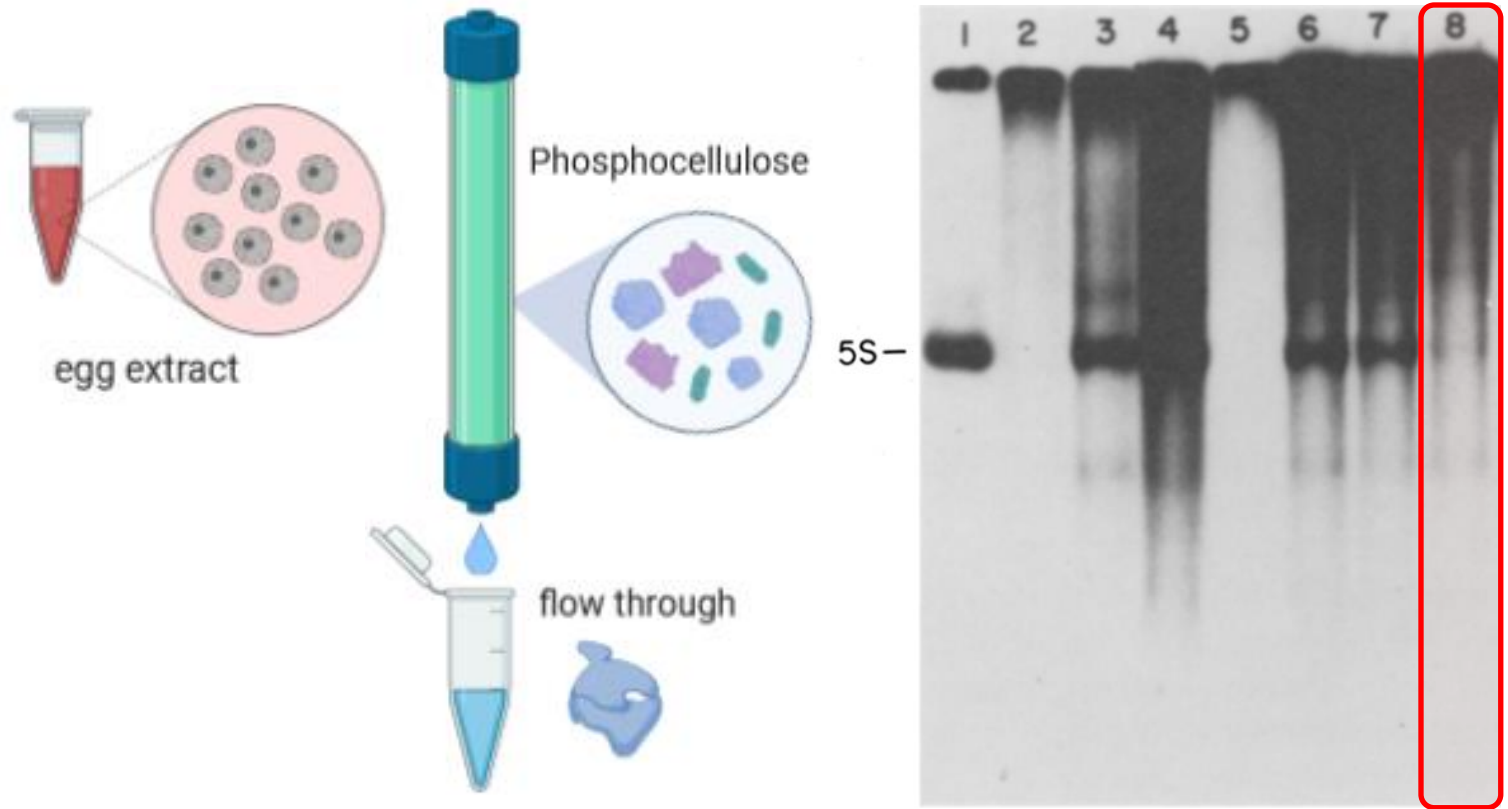
# In vitro: unfertilized egg extract



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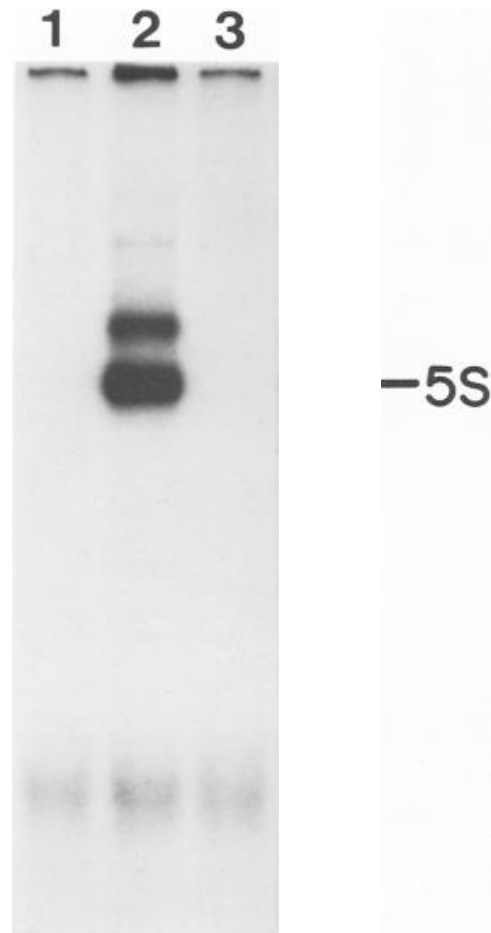
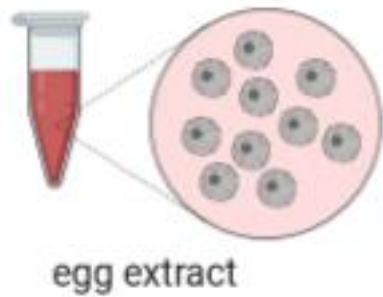


# In vitro: unfertilized egg extract

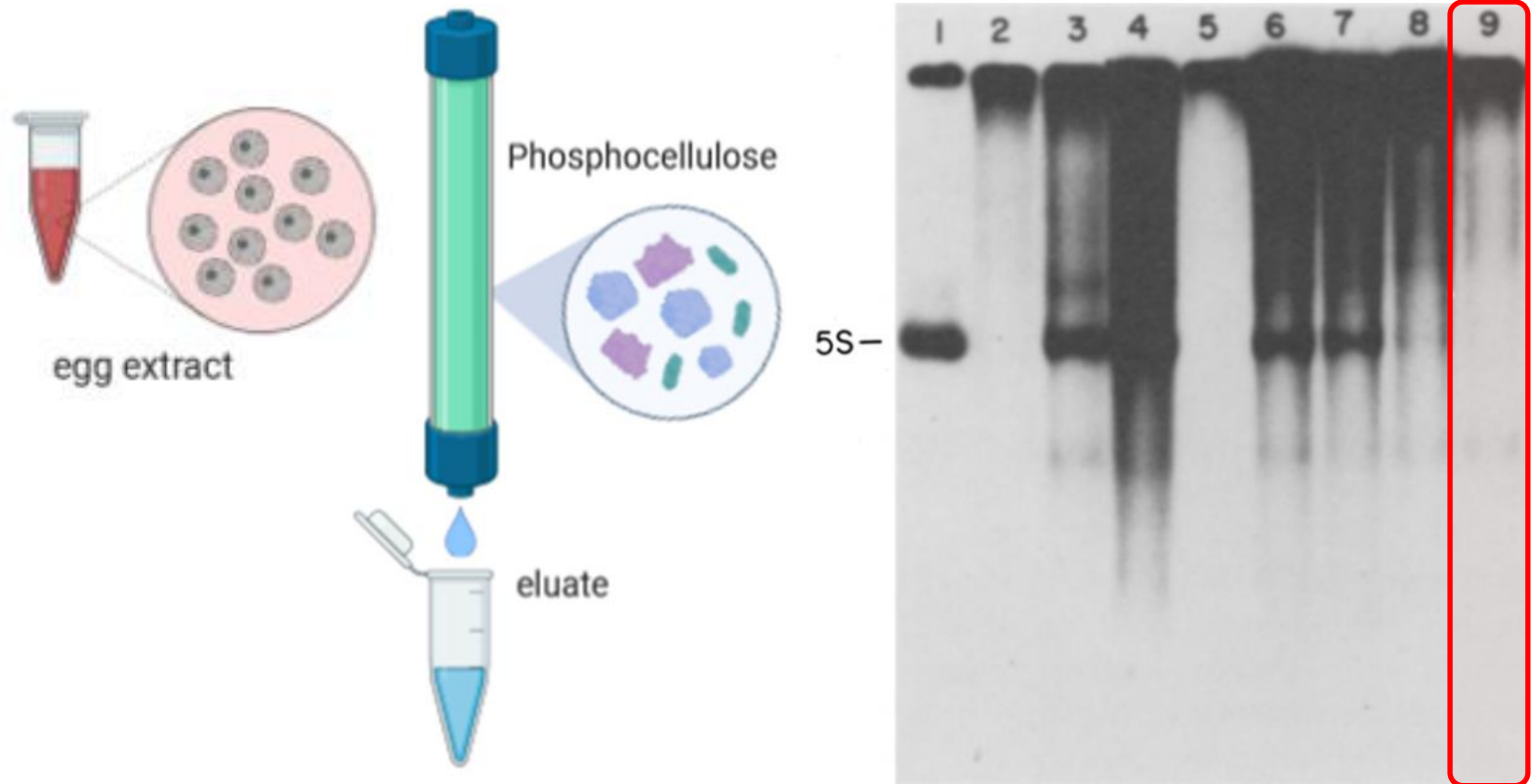




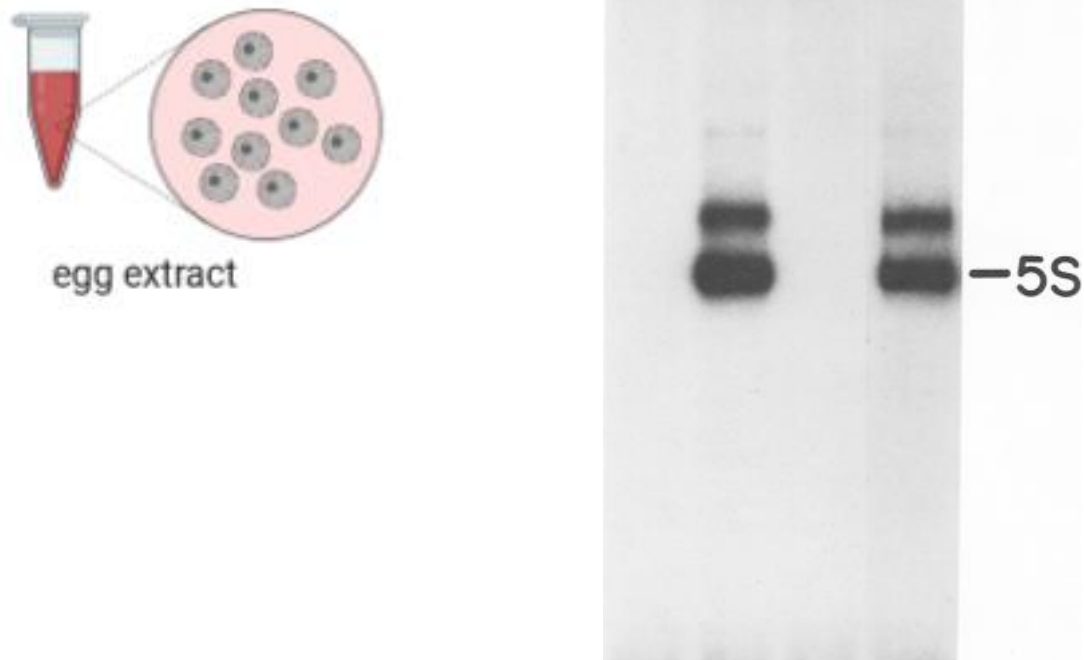
# In vitro: unfertilized egg extract



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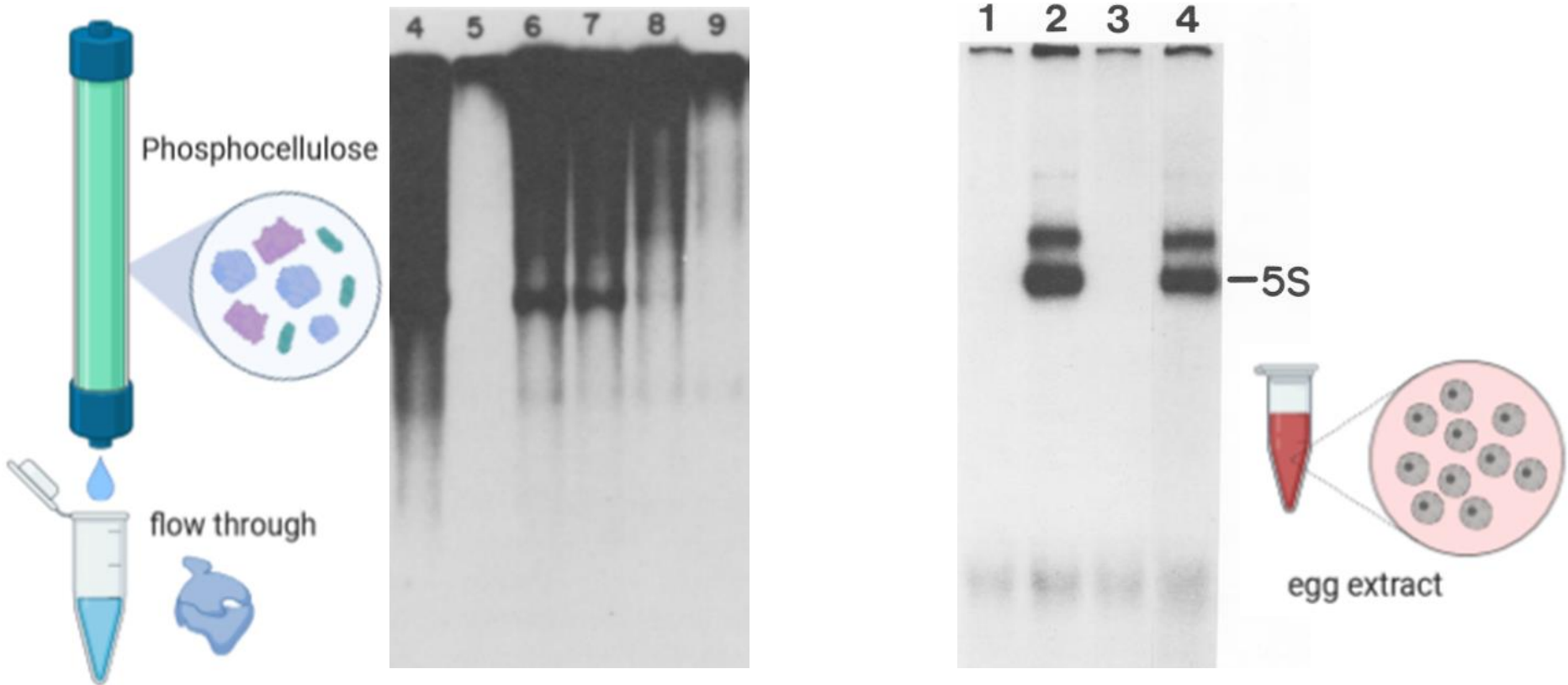
# In vitro: unfertilized egg extract



Component present : DEAE Sephadex flow through and

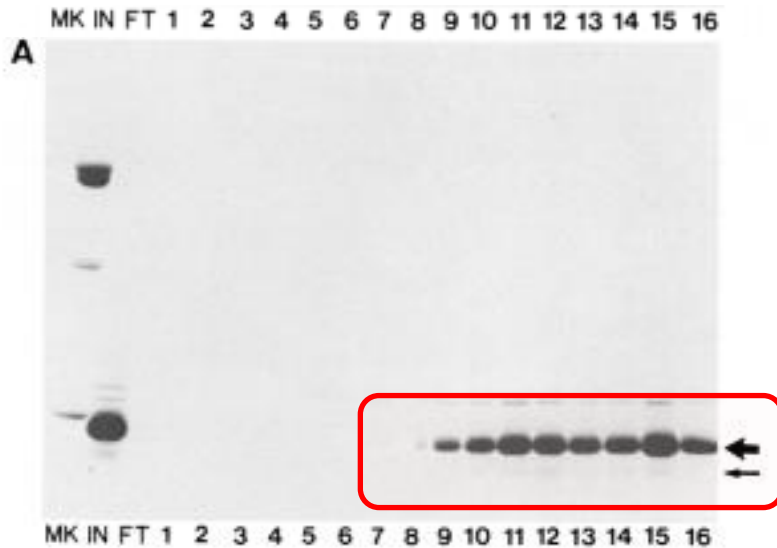
Phosphocellulose eluate is missing/inactivated in the egg extract

# Complementation of 5S RNA synthesis

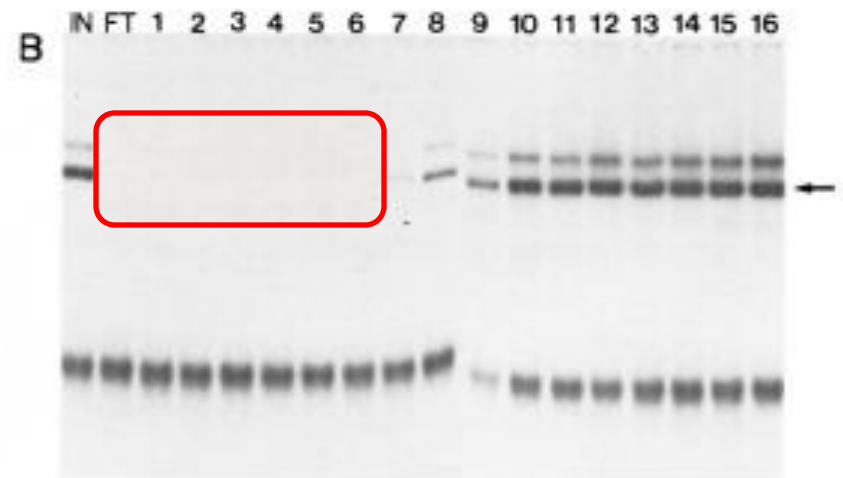


Egg extract contain component for specific transcription of 5S gene

# Purification of transcription factor



**Denaturing PAGE**



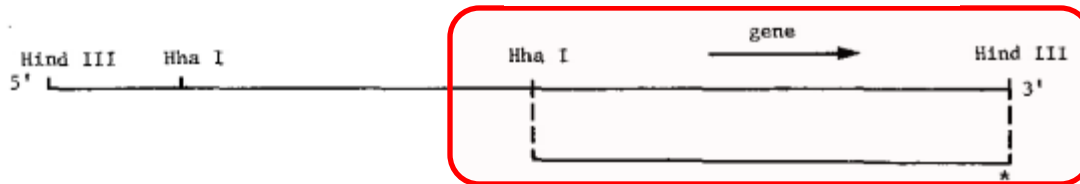
**Complementation assay**

Egg extract contain component for specific transcription of 5S gene

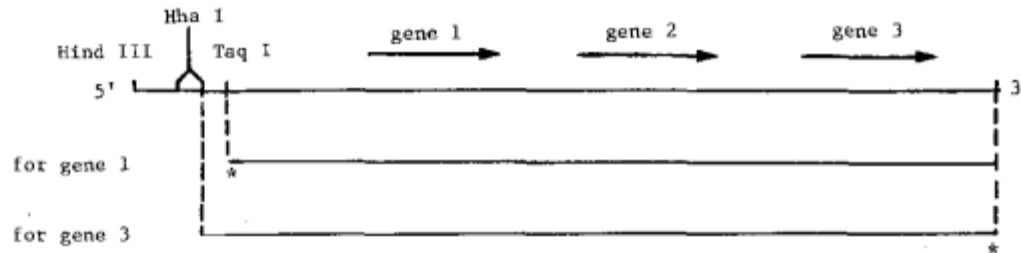
# Purified “A factor” TF pXbs1



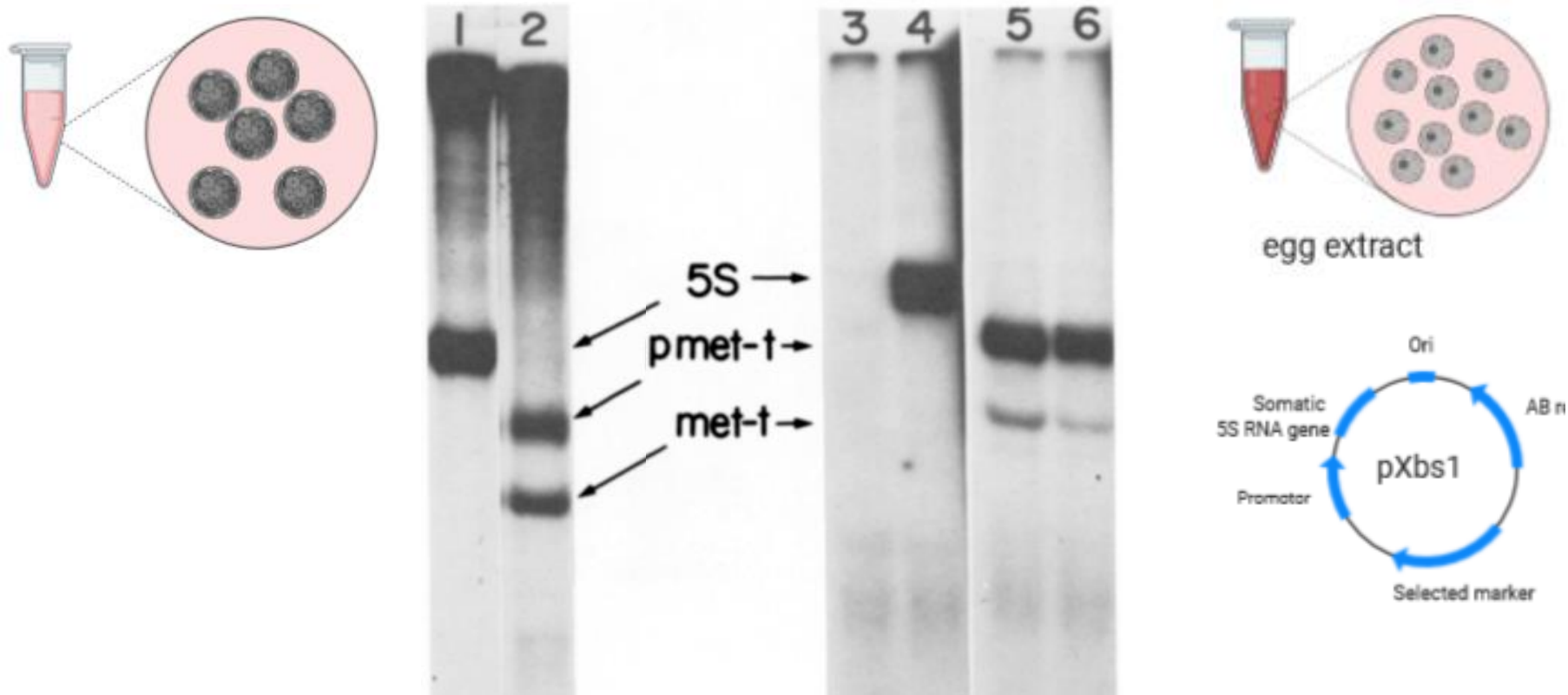
Xbs1



Xba1



# Purification of transcription factor



Purified TF is essential for somatic and oocyte 5S gene transcription

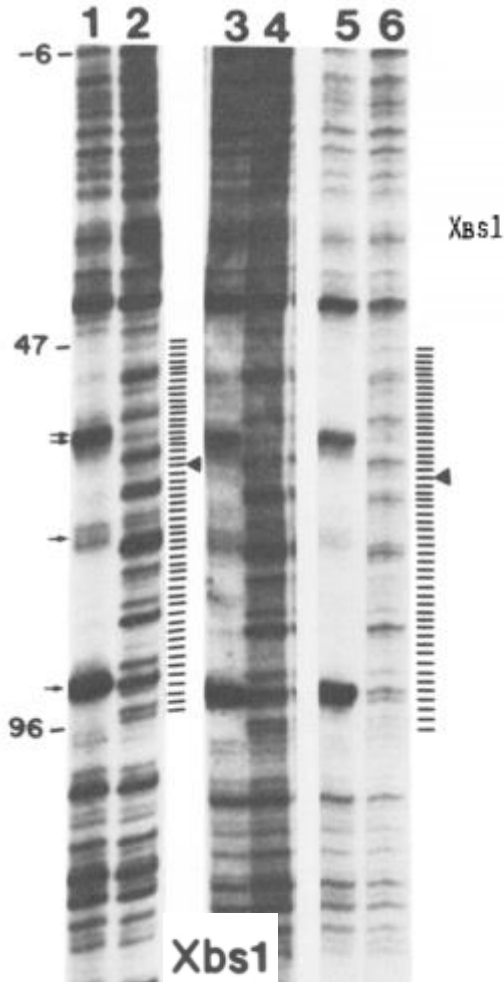
Not essential for the t-RNA gene transcription

# Foot-printing of transcription factor

Egg extract - - - - + +

Pol III - - + + + +

TF + - + - + -



50 60 70 80 90 100

GATCTCGGAAGCCAAGCAGGGTCGGGCCTGGTTAGTACTTGGATGGGAGACCGCCTGGGAATACC 3'

CTAGAGCCTTCGGTTCGTCCAGCCCGGACCAATCATGAACCTACCCTCTGGCGGACCCTTATGG 5'



# Conclusions of Lecture-8

- The transcription factor was purified
- It's specificity for 5S gene was demonstrated
- The mechanism of action = specific interaction
- Specific interaction = internal regions of 5S DNA (47-96)
- Model: Factor provides the initial DNA recognition event
- Binding, directs RNA Pol III with complex to orient for initiation.

**Questions??**

**Thank You!**

# Characterization of Two *Xenopus* Somatic 5S DNAs and One Minor Oocyte-Specific 5S DNA

560                      580                      600                      620  
 CCTGCATGGGGAGGAGCTGGGCCCCAAGAAGGCAGCACAAAGAGGAGGAAAAGTCAGCCTTGTGTTCGCCTACGGCCACACCACCTGAAA  
 640                      660                      680                      700                      720  
GTGCCCGATCTCGTCTGATCTCGGAAGCCAAGCAGGGTCGGGCCTGGTTAGTACTTGGATGGGAGACCGCCTGGGAATACCAGGTGTCGT  
 740                      760                      780                      800  
AGGCTTTTGCACTTTGCCCTTCTGAGCAGCAGGGGGCAGTCTCCTCCCTGCTTTTTCCTTCCCGCAACAGCCAGACAGCTAGCTGCCTGA  
 820                      840                      860                      880  
 TAGAGACCCCCACCCCTGTAAGGGAGCCACTCCATACGCTGAAGTGTACACCTGCGGGCCTTGCCAGGA(AAGCTT)

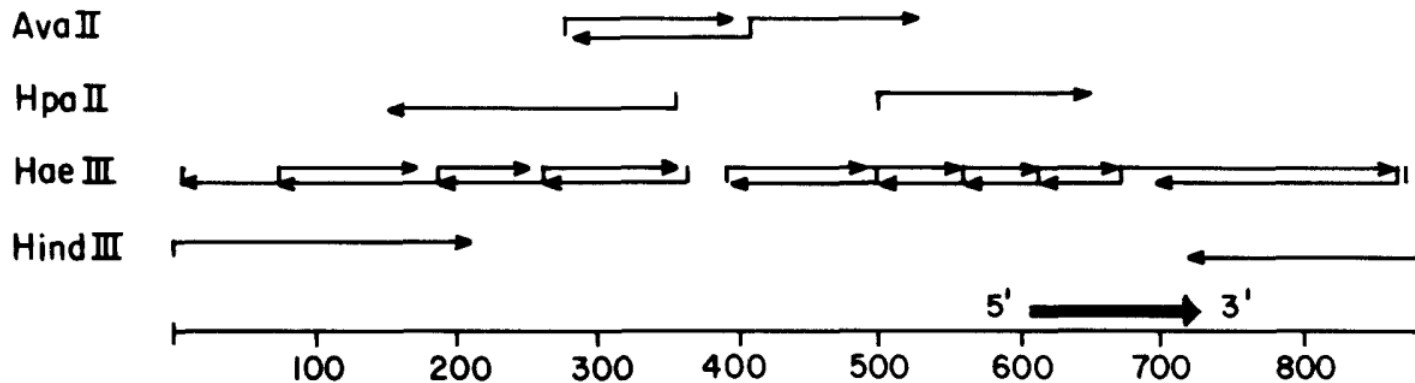


Figure 4. Nucleotide Sequence and Restriction Map of Xls11

# Characterization of Two *Xenopus* Somatic 5S DNAs and One Minor Oocyte-Specific 5S DNA

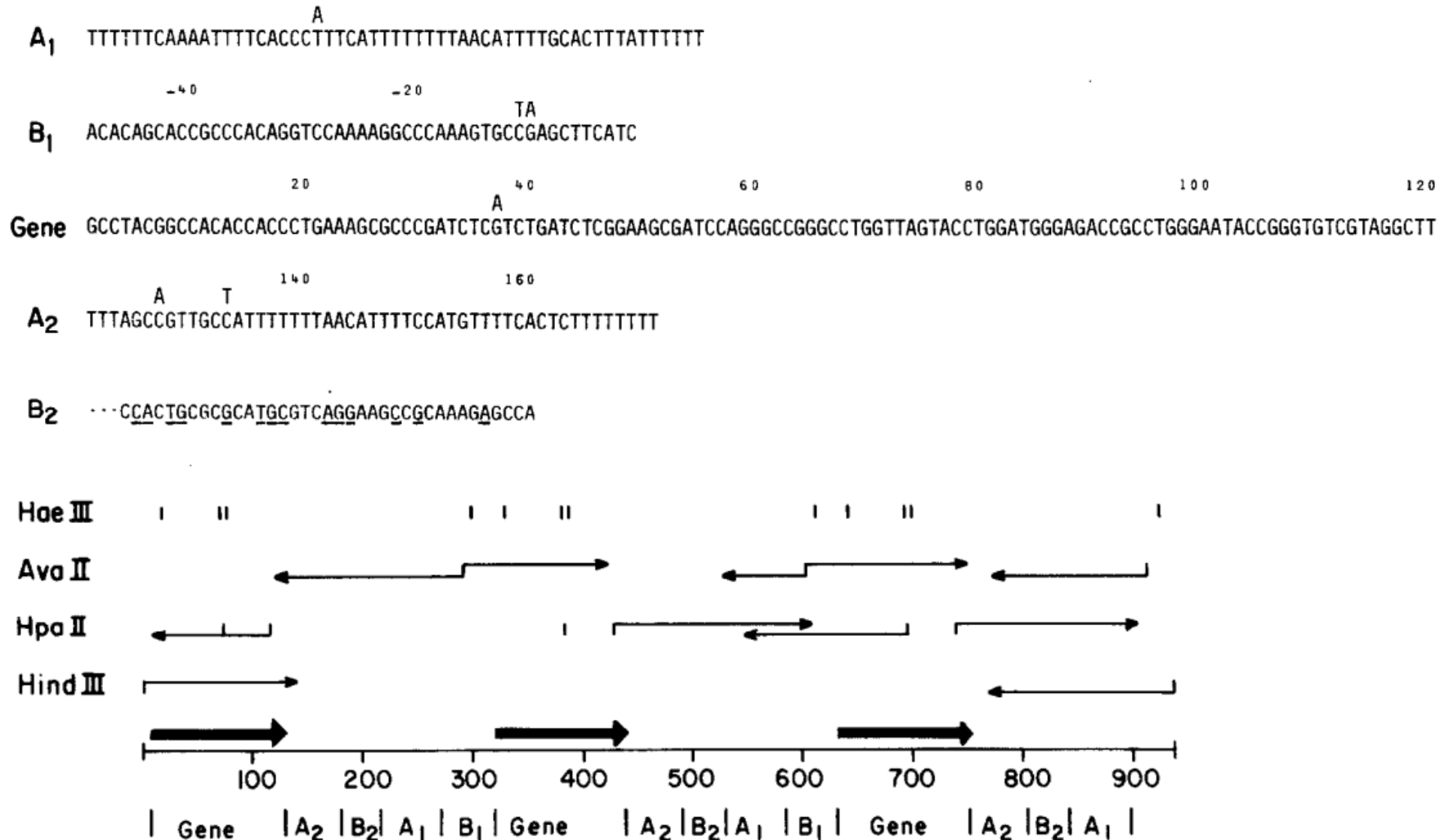


Figure 5. Nucleotide Sequence and Restriction map of Xlt1

