Genome Editing and Engineering

Course No: BT-637

LECTURE-8

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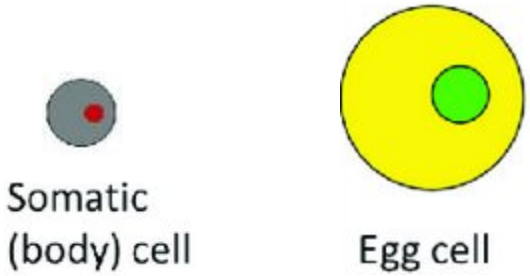
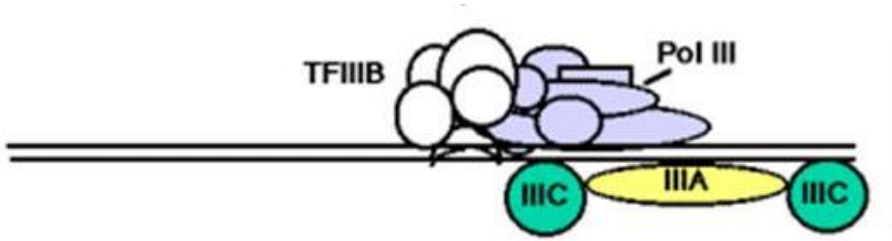
Introduction

- Xenopus laevis

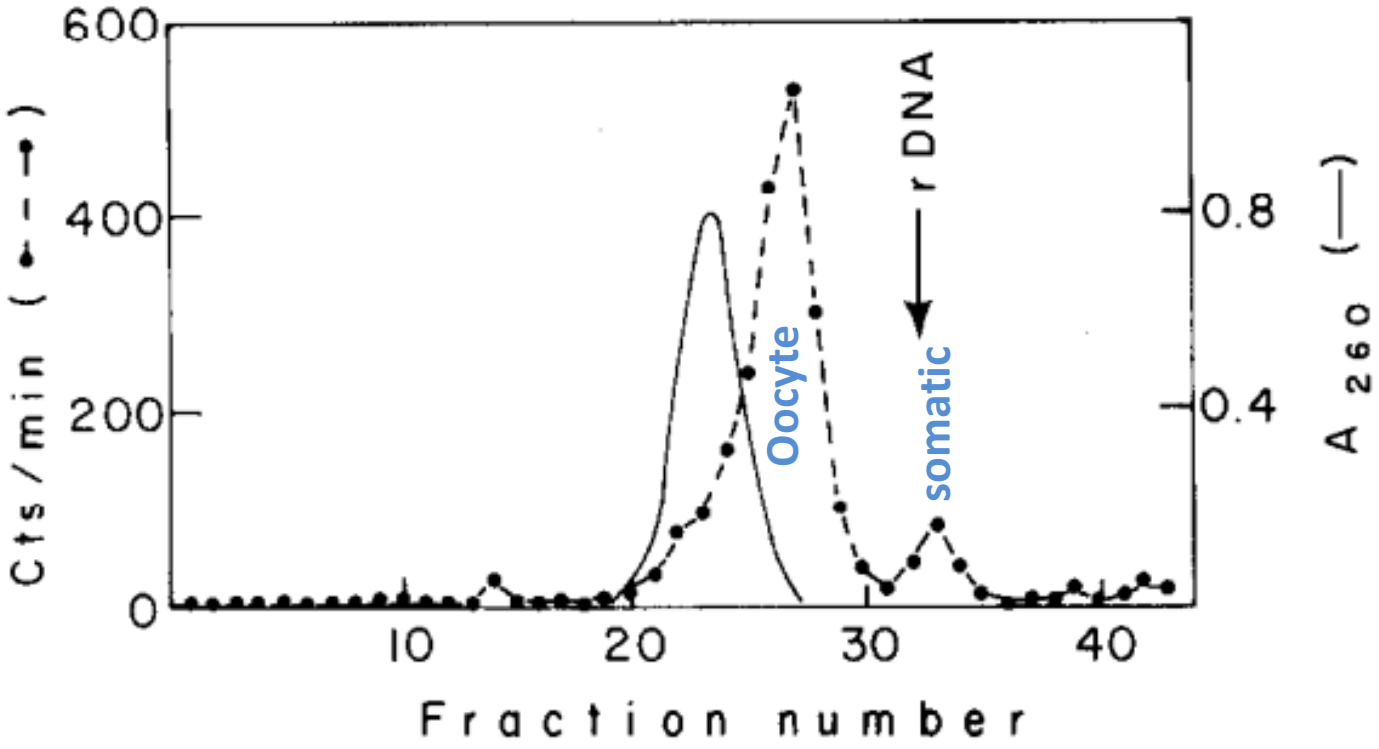




- Gene expression & Transcription mechanism

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

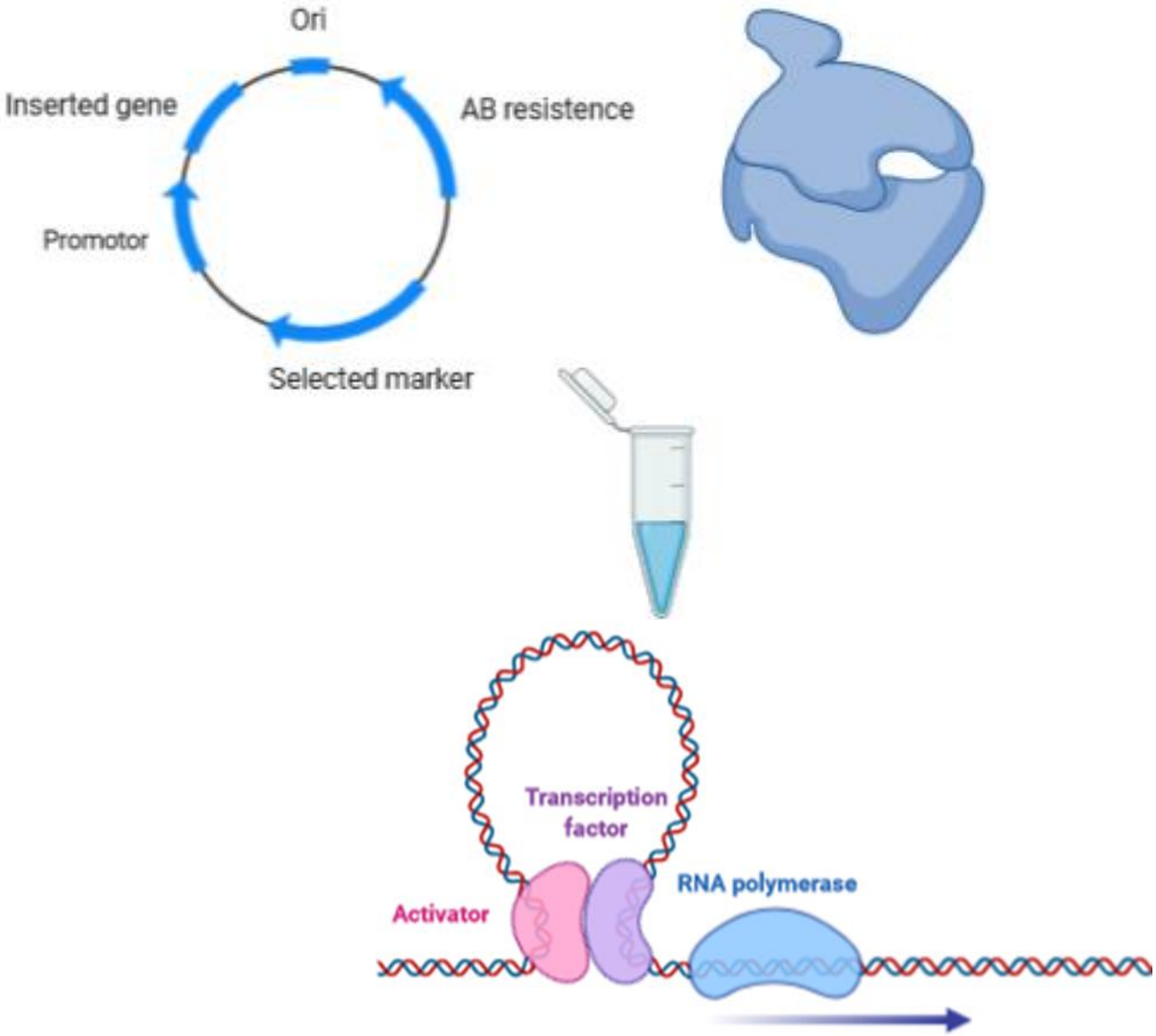
Table 1. The Purification of RNA Polymerase III from Xenopus Ovaries

Purification Step	Total Protein (mg)	Polymerase III Units[a]	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

[a] 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.

Cozzarelli et al Cell; 1983

In vitro: Gene + RNA Polymerase III



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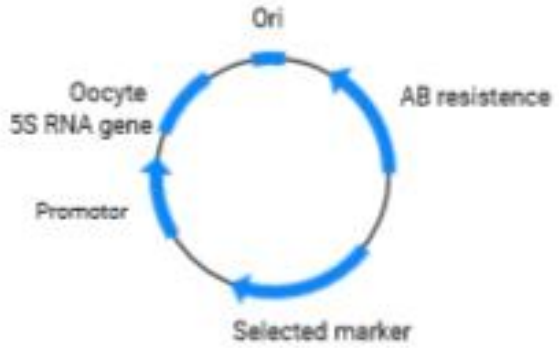
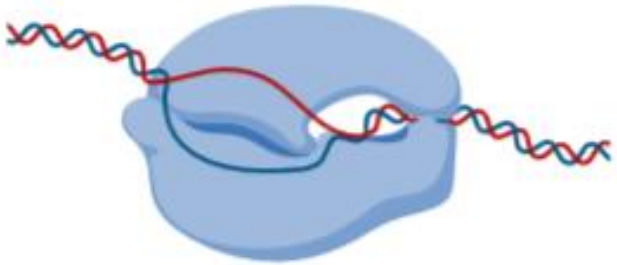
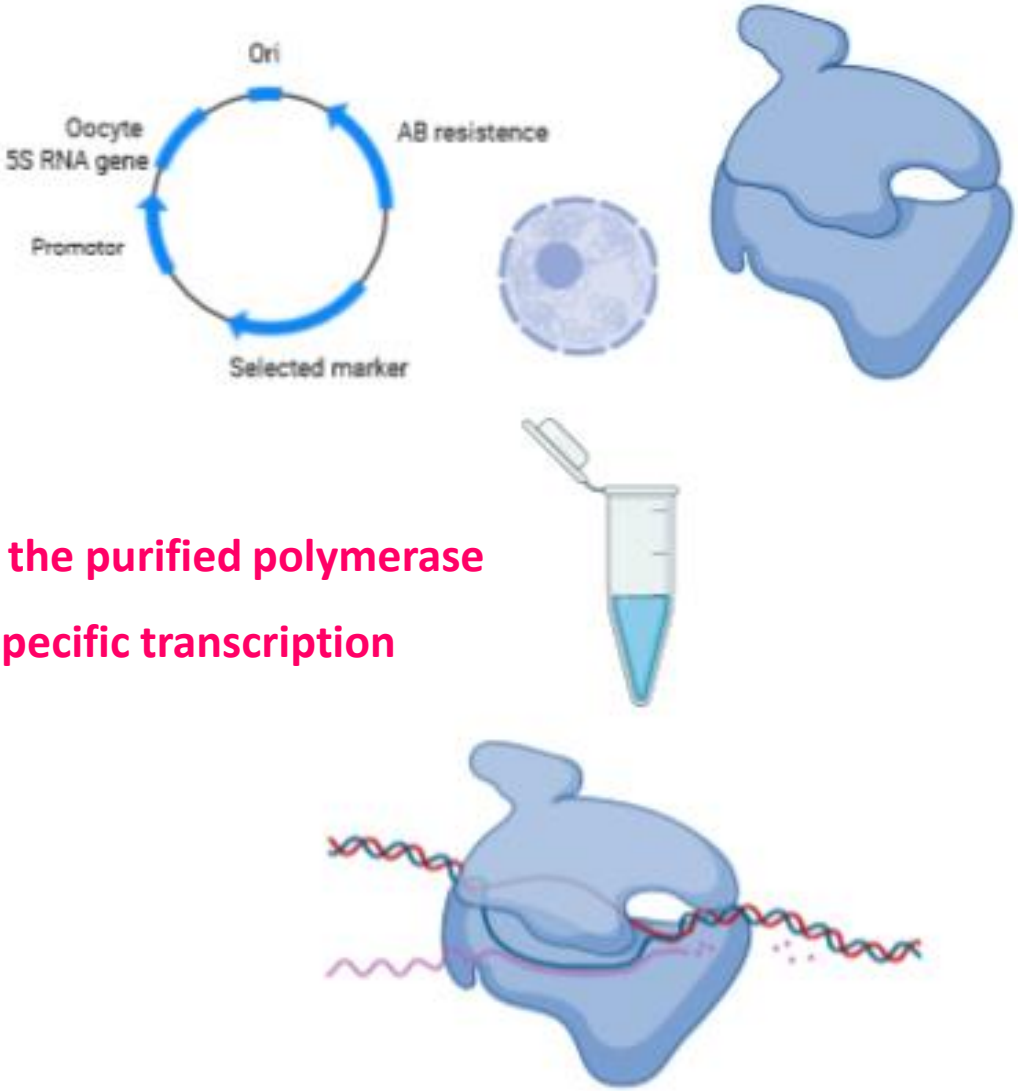




In vitro: Gene + RNA Polymerase III



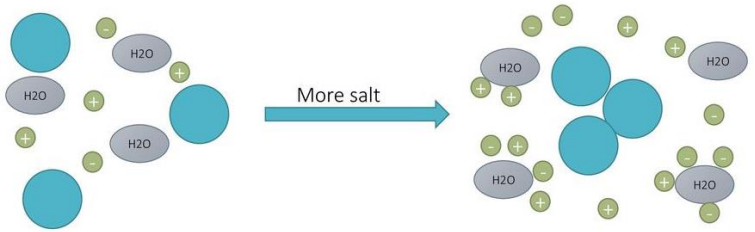
Factors other than the purified polymerase are necessary for specific transcription

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

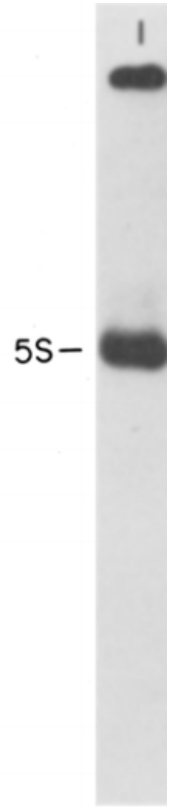
Cell, Vol. 19, 717–728, March 1980,

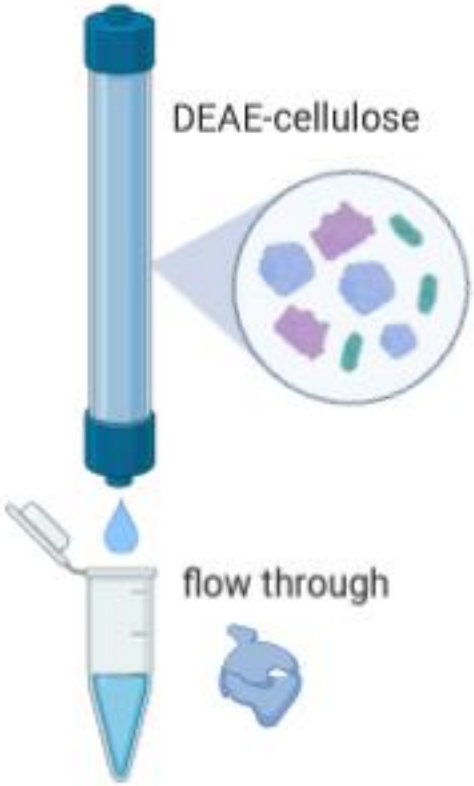
David R. Engelke,
Sun-Yu Ng, B. S. Shastry and Robert G. Roeder

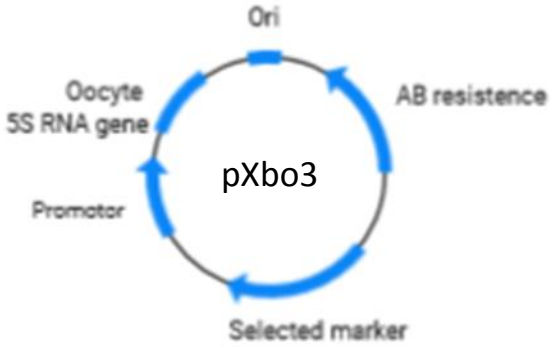
- Ng at al 1979 and Engelke 1980:
- purify "A factor" from oocytes = specific transcription of 5S gene
- Factor purification = various chromatography techniques



Purification of “A Factor”







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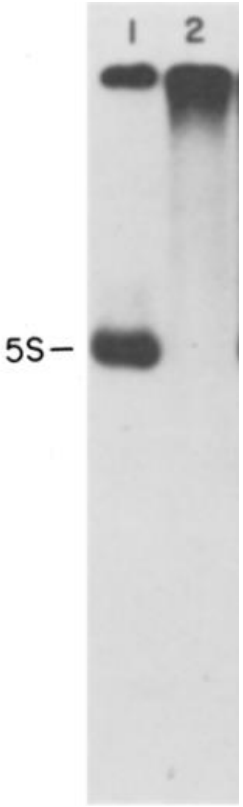

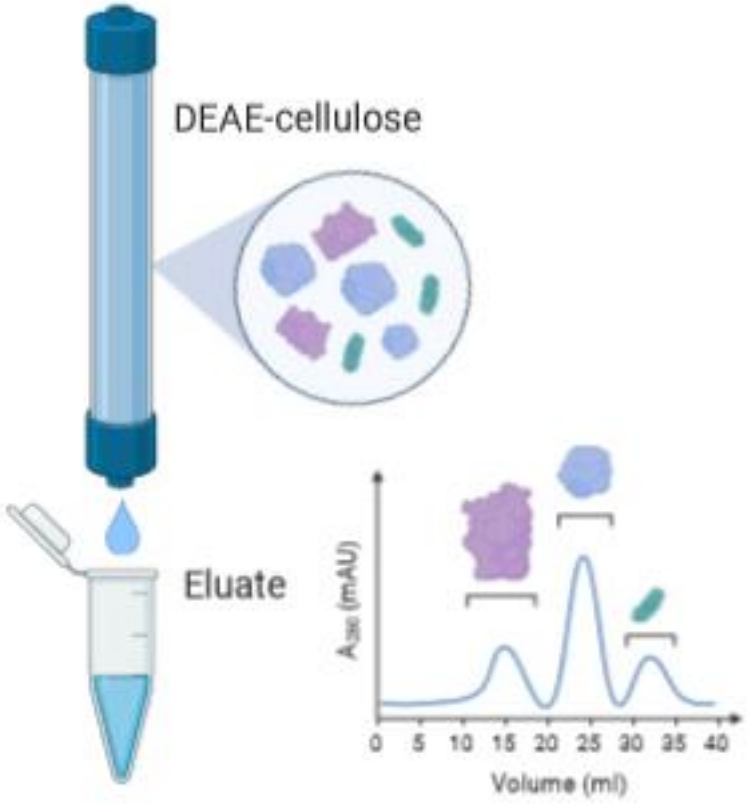

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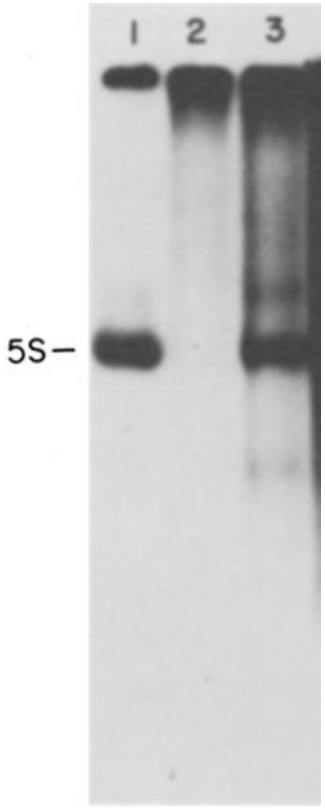

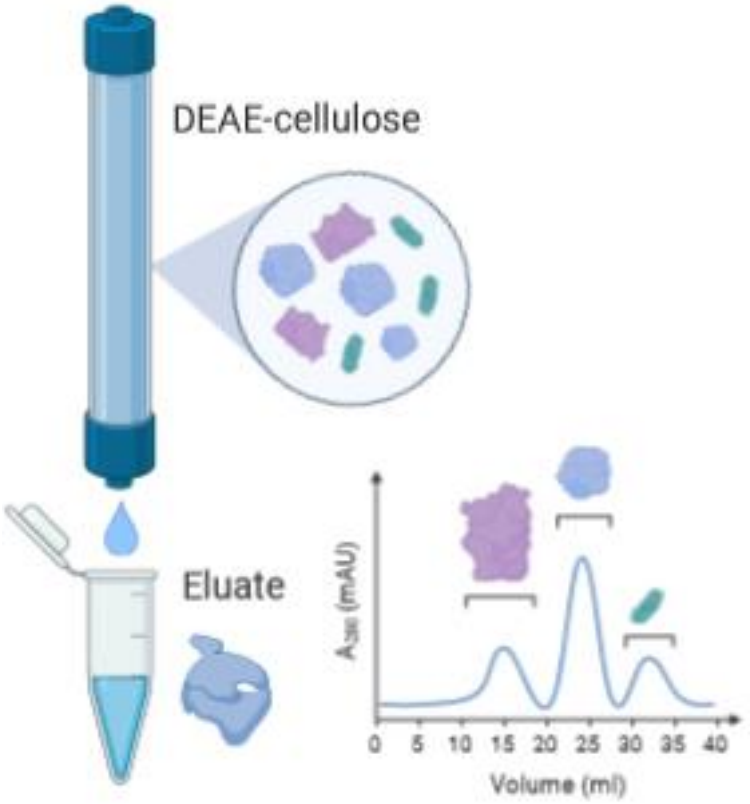

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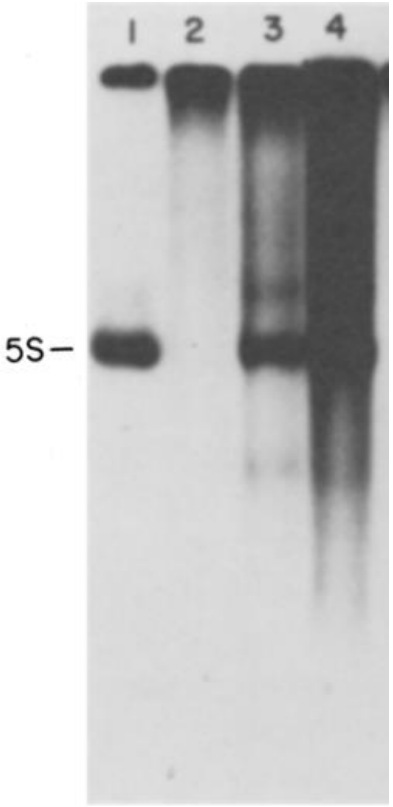

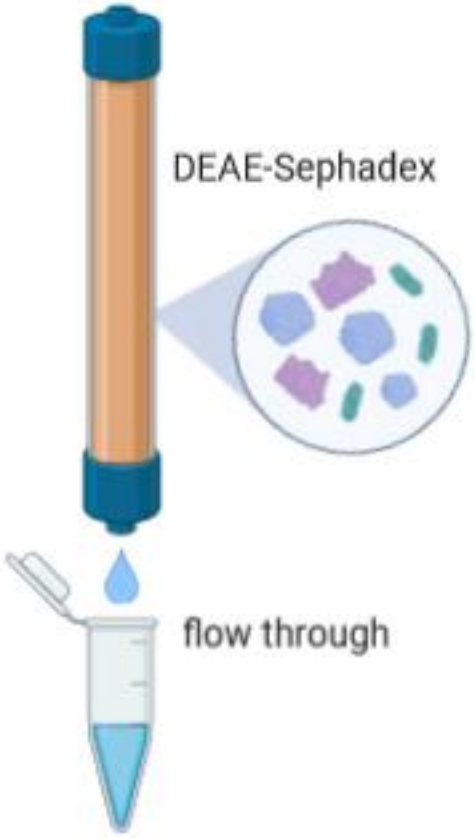

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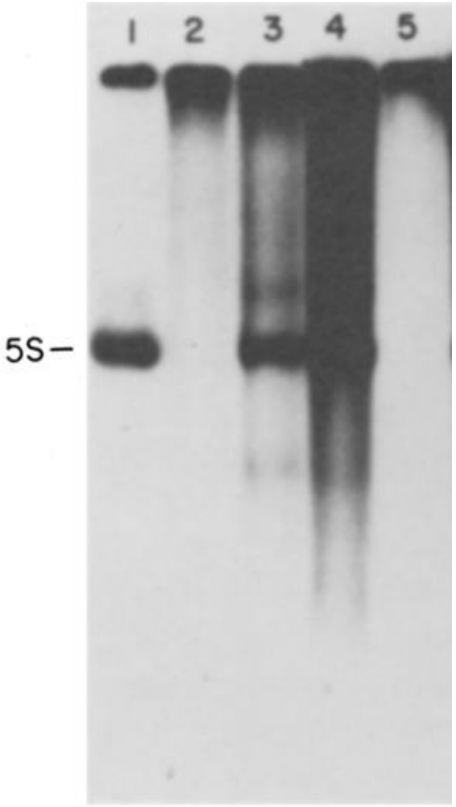

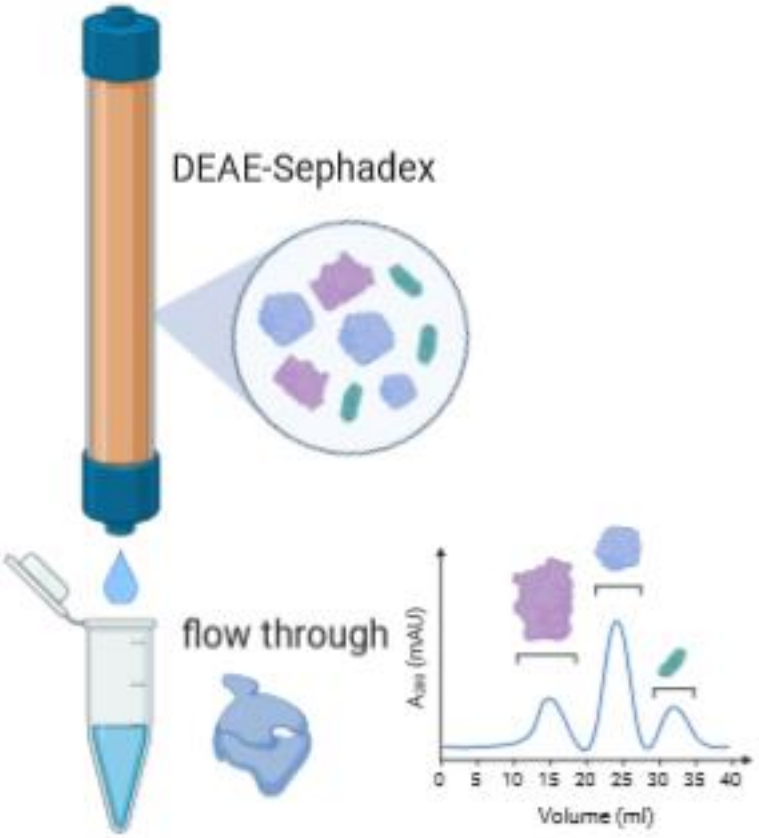

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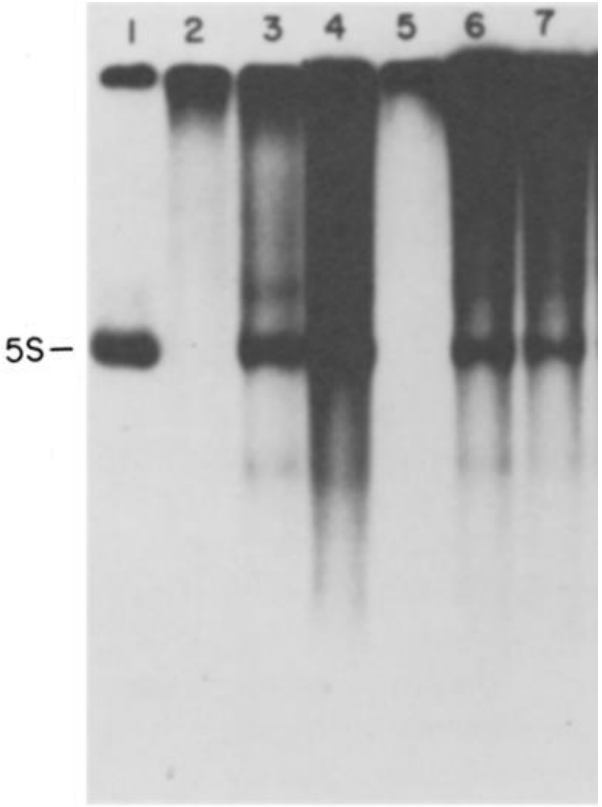

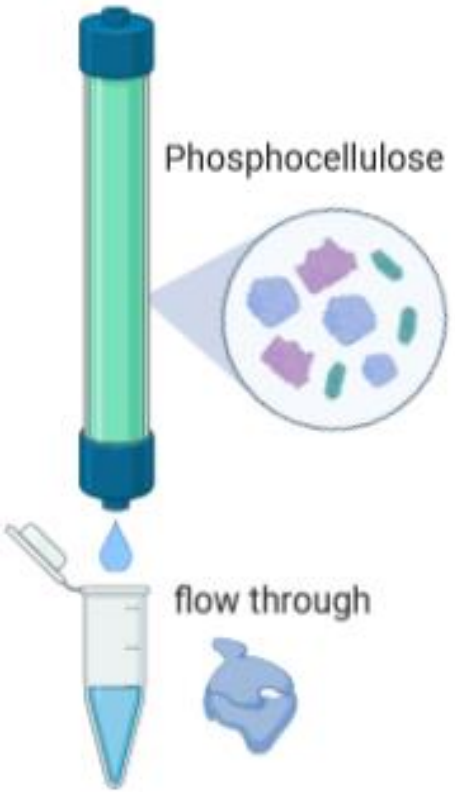

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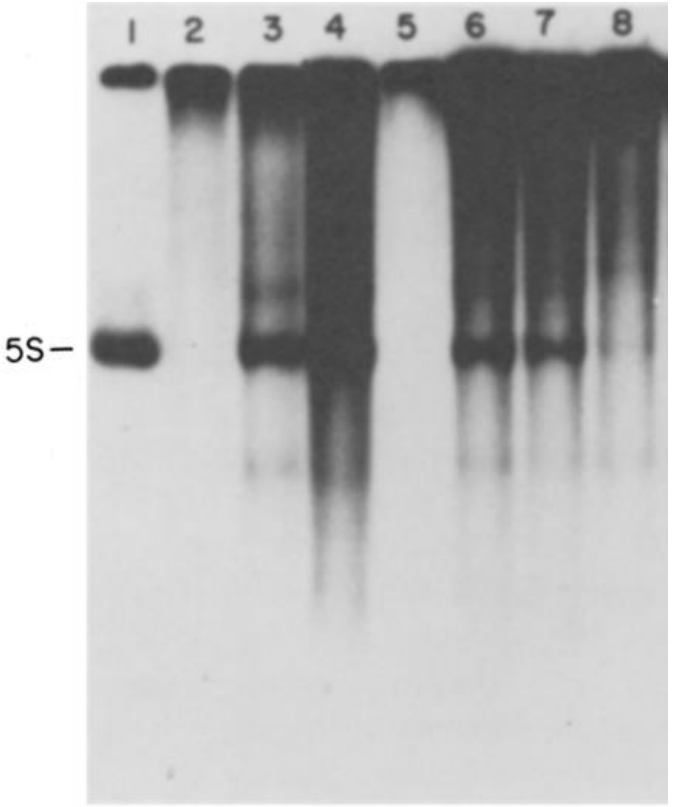

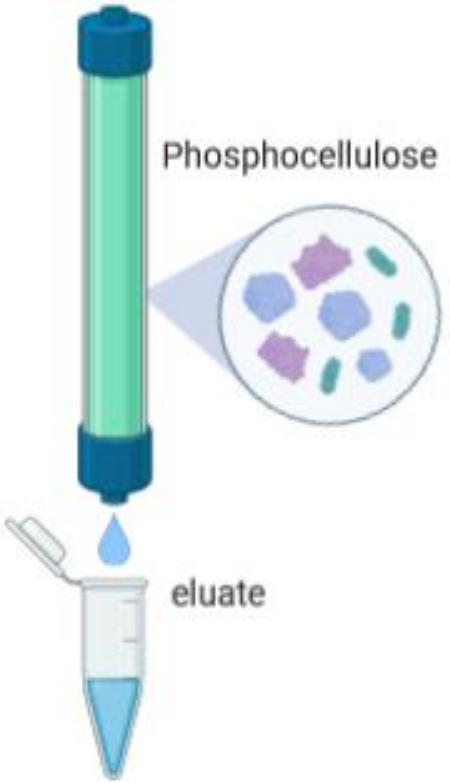

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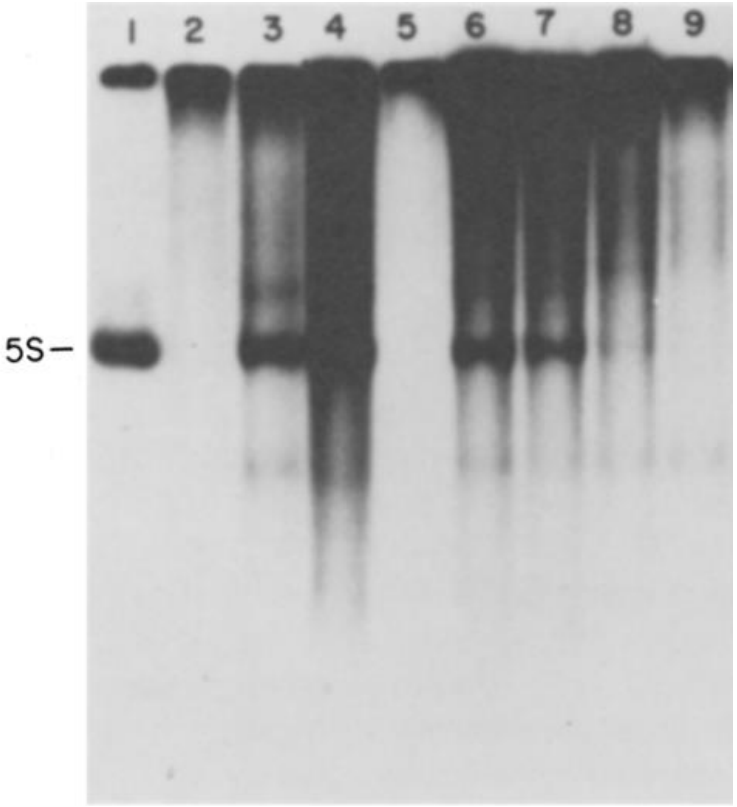

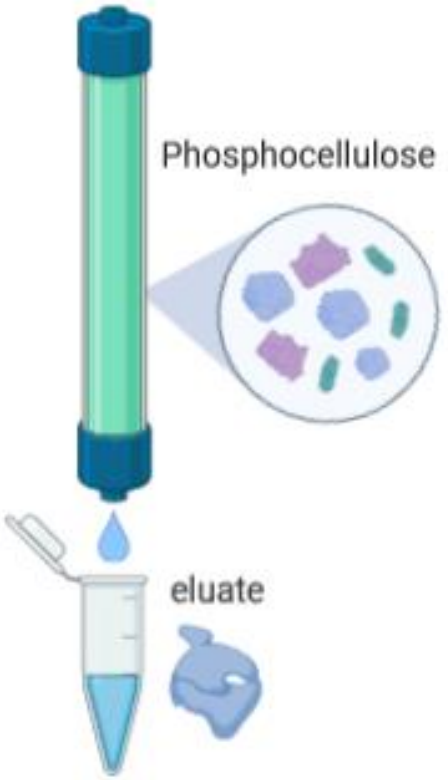

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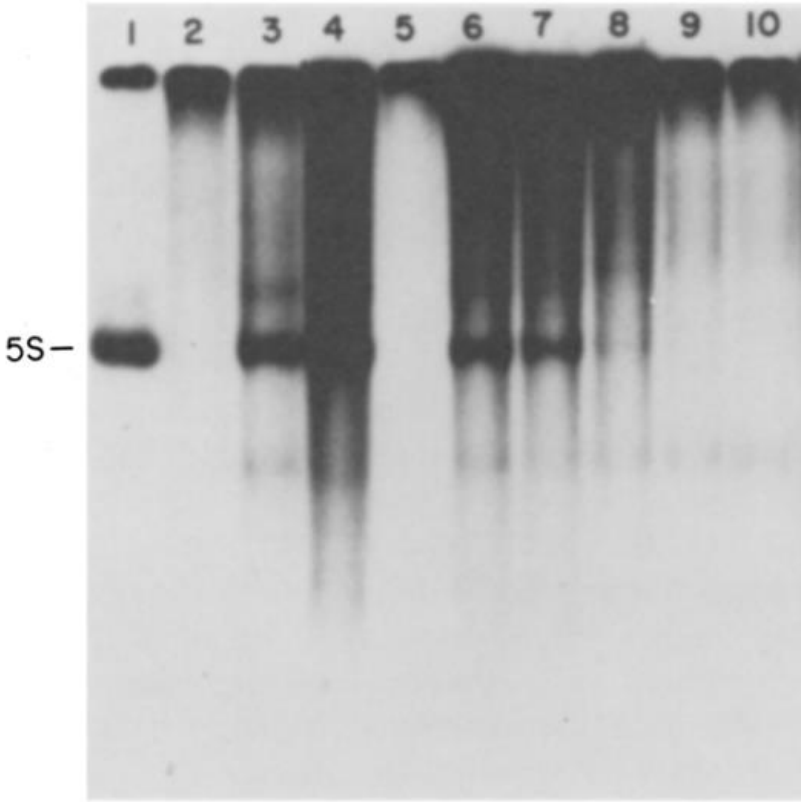

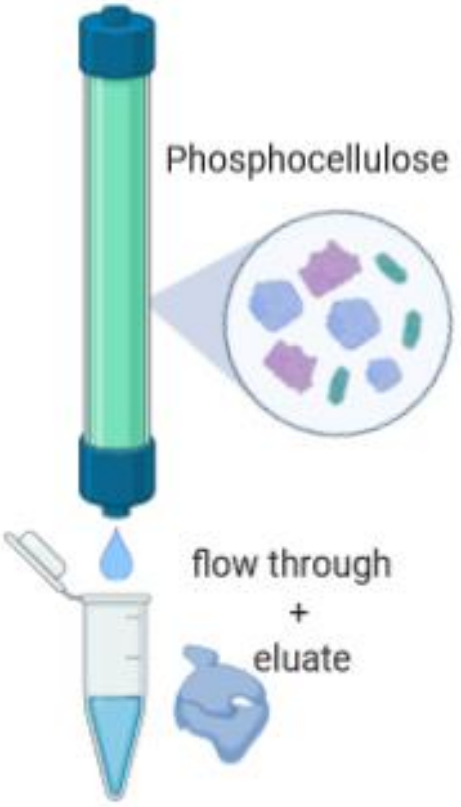

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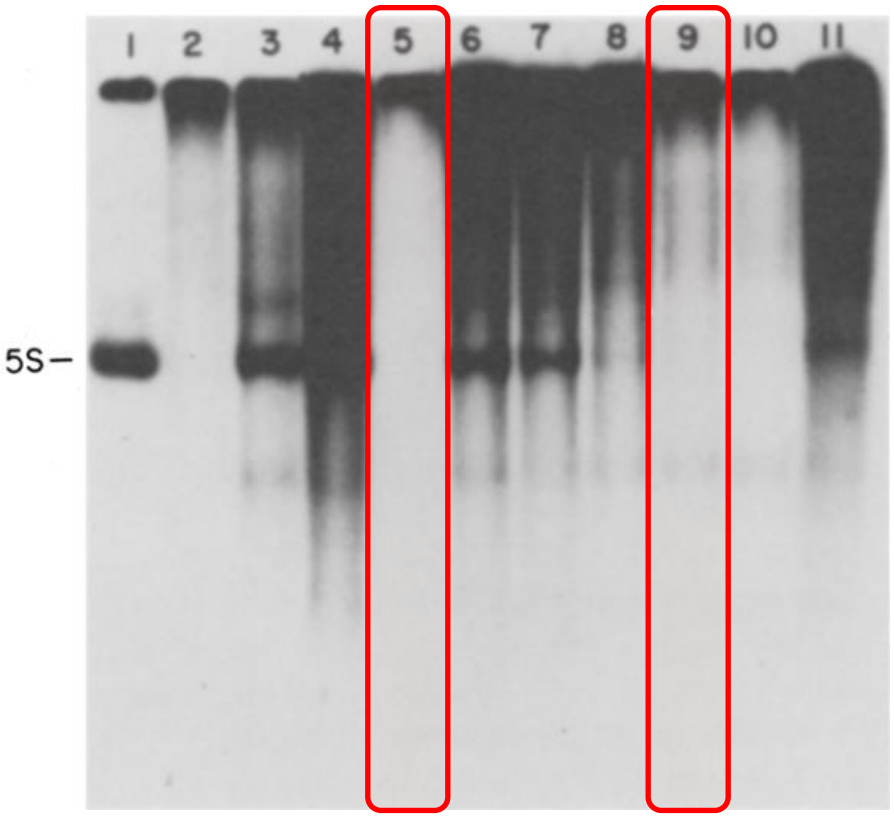
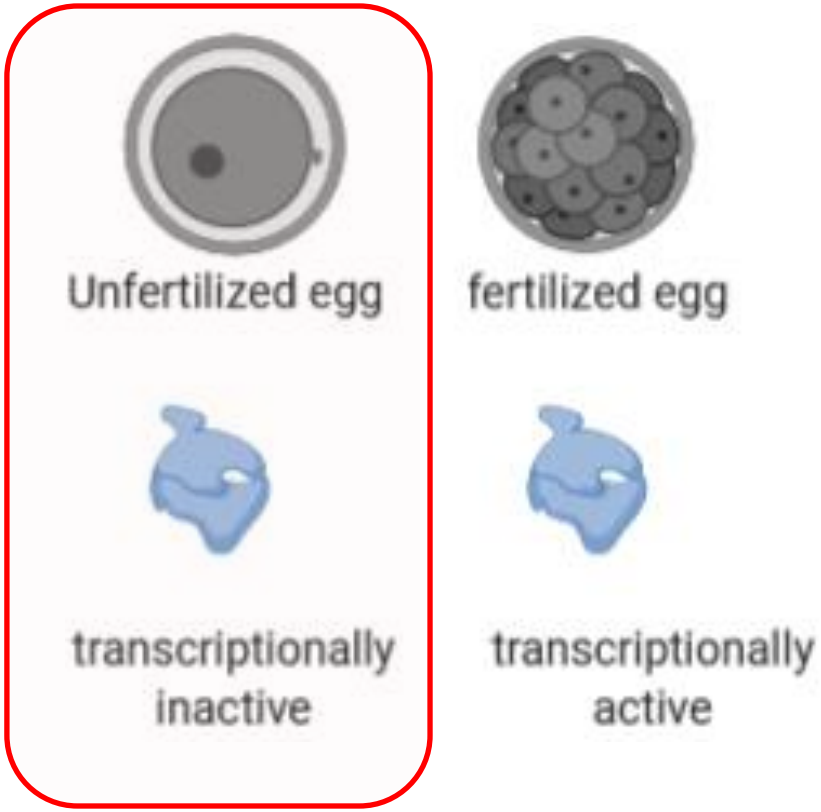
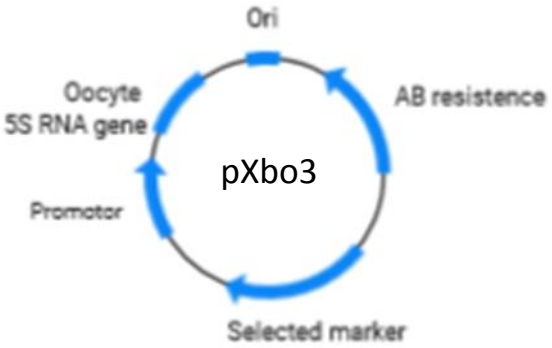


Existence of two or more components = 5S gene transcription

In vitro: unfertilized egg extract





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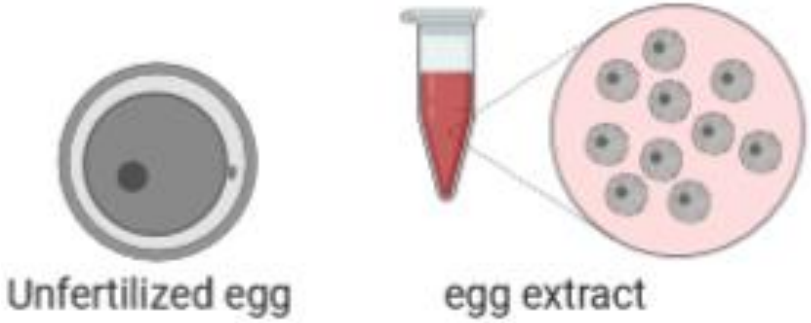

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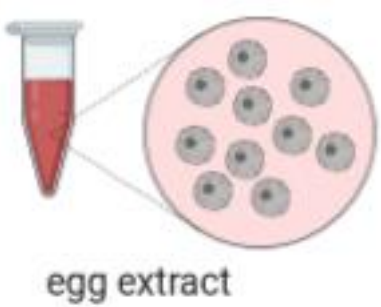

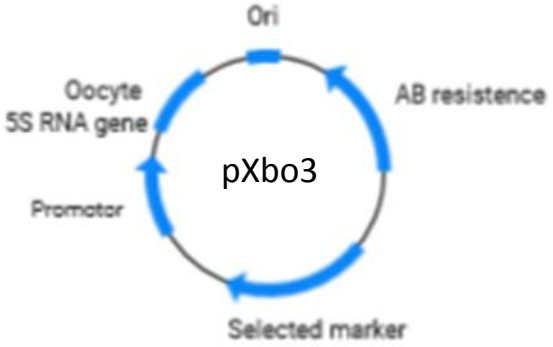

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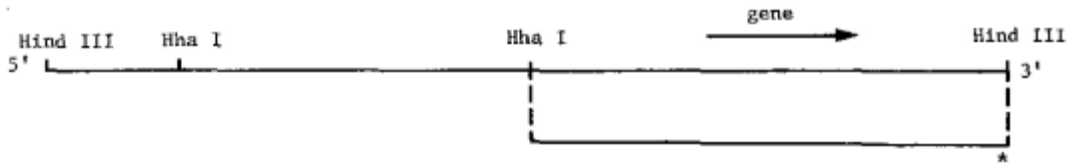

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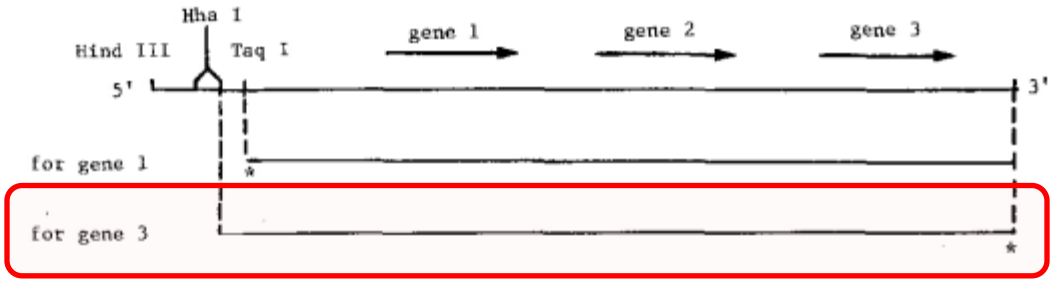

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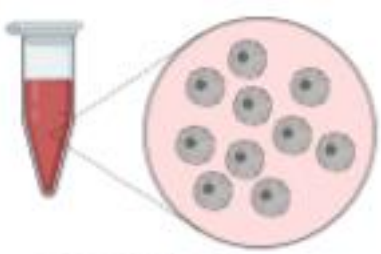

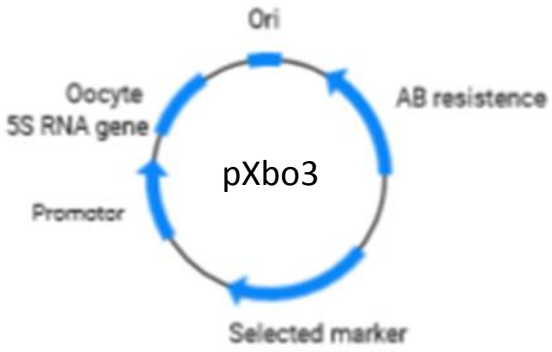

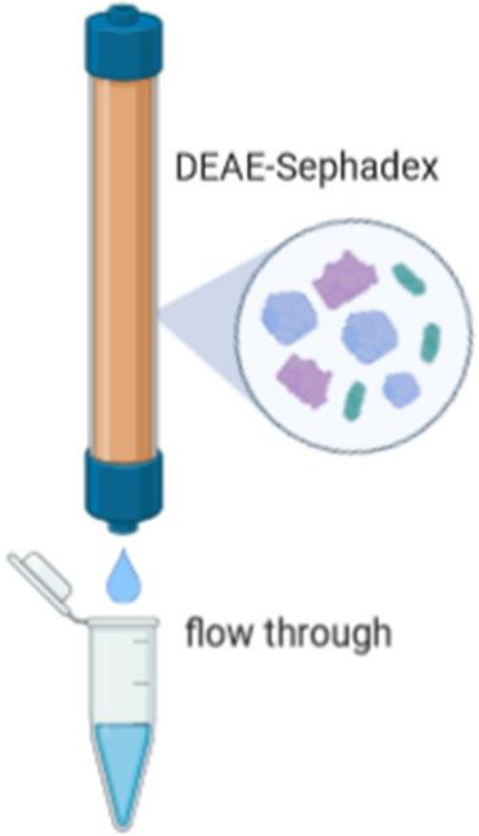

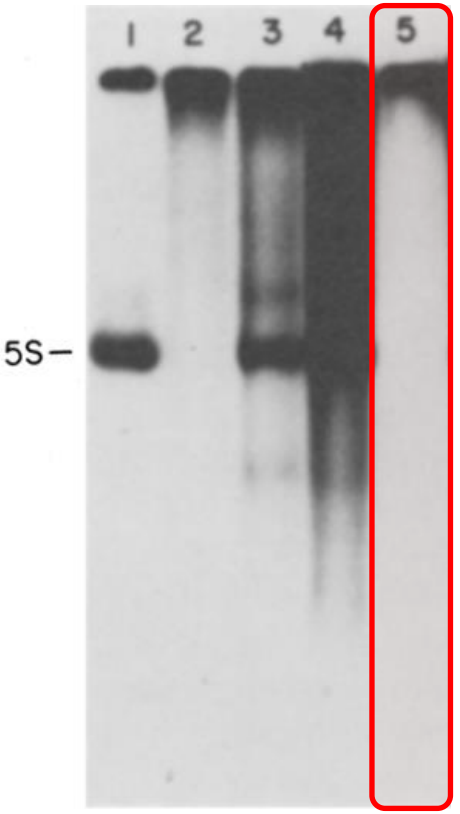

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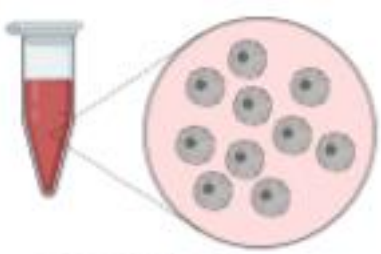
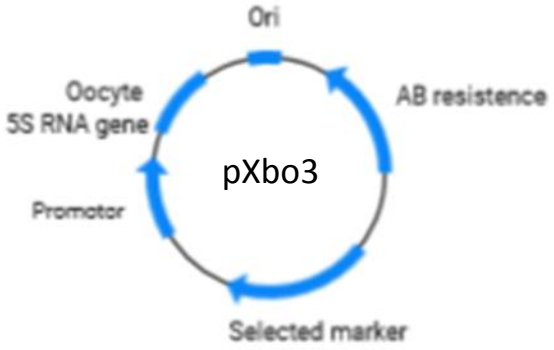
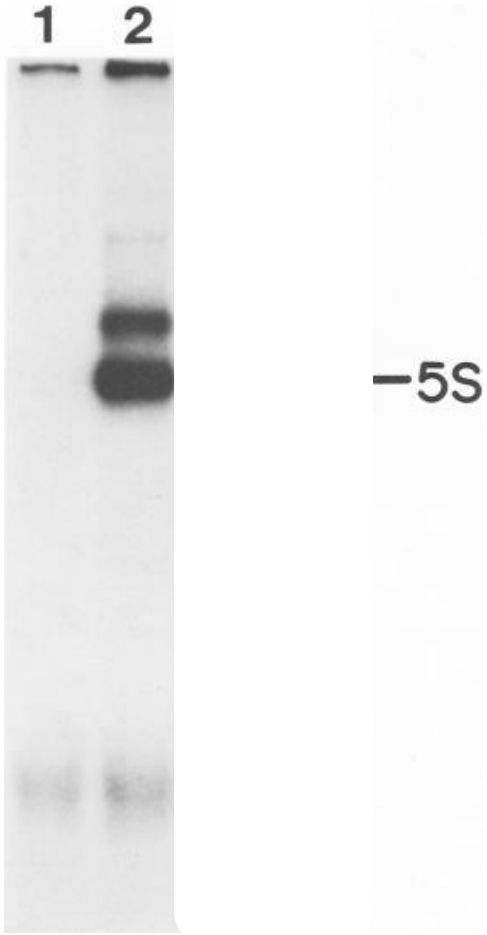


egg extract





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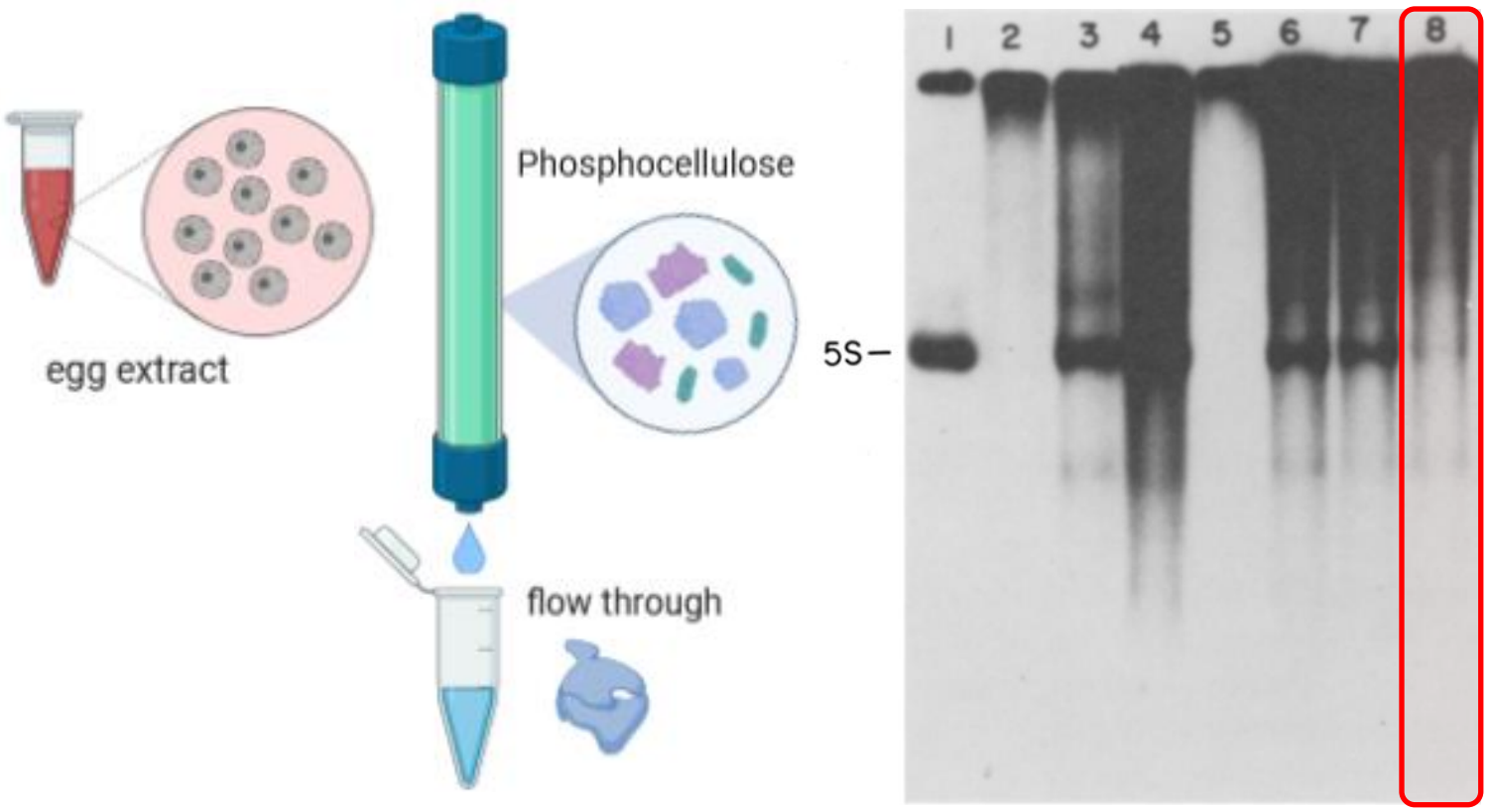

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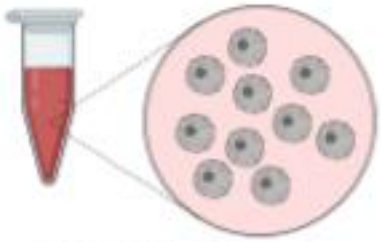

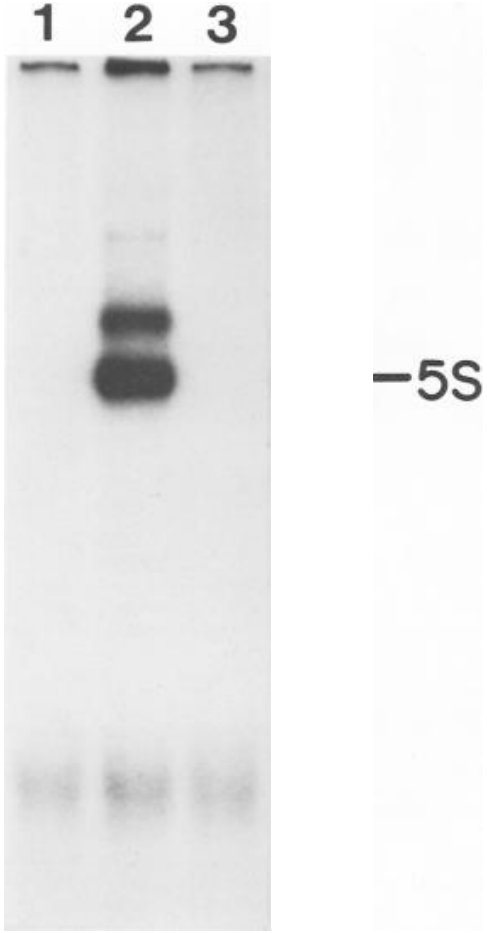

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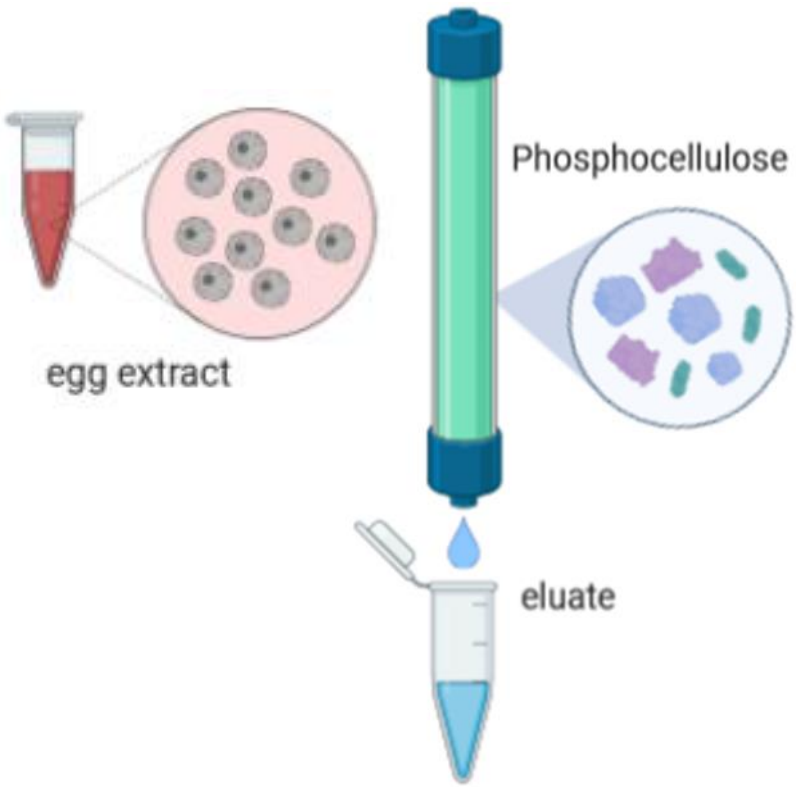

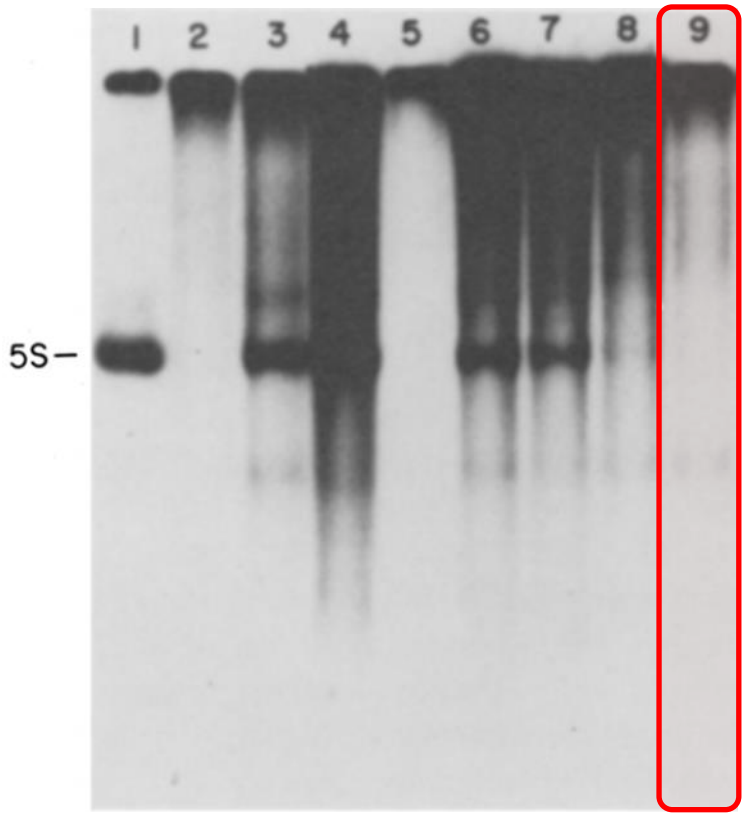

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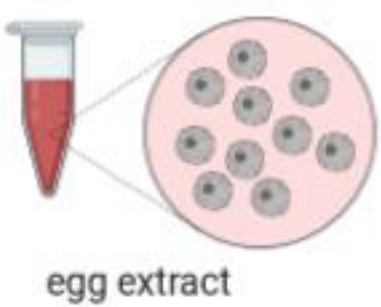
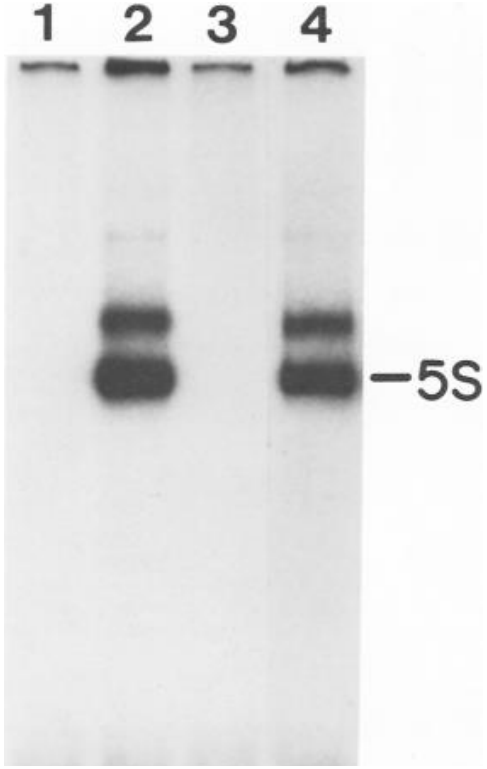
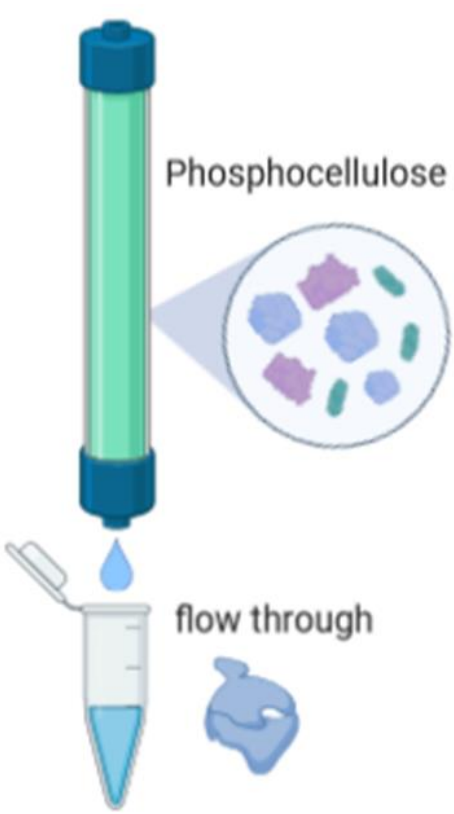


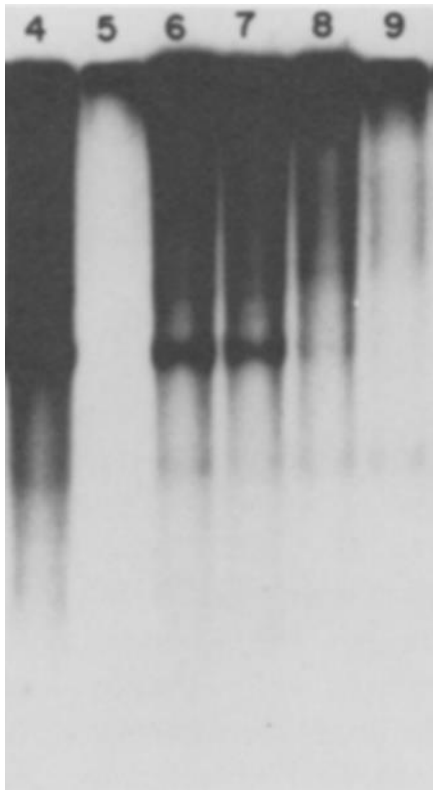


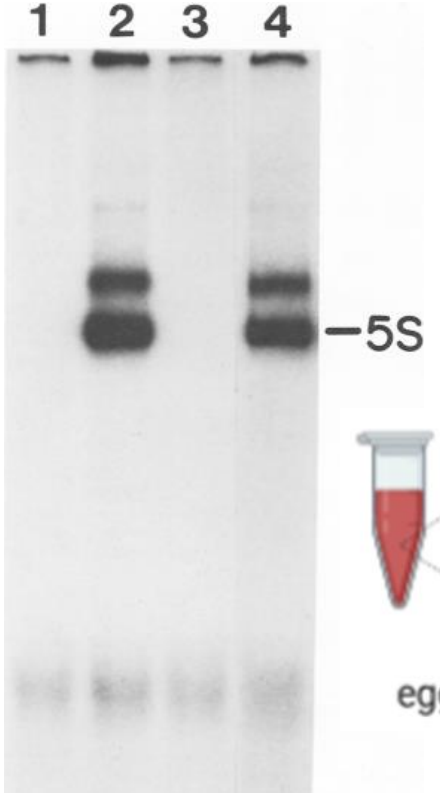
Component present : DEAE Sephadex flow through and

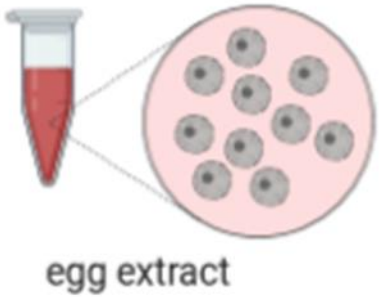
Phosphocellulose eluate is missing/inactivated in the egg extract

Complementation of 5S RNA synthesis



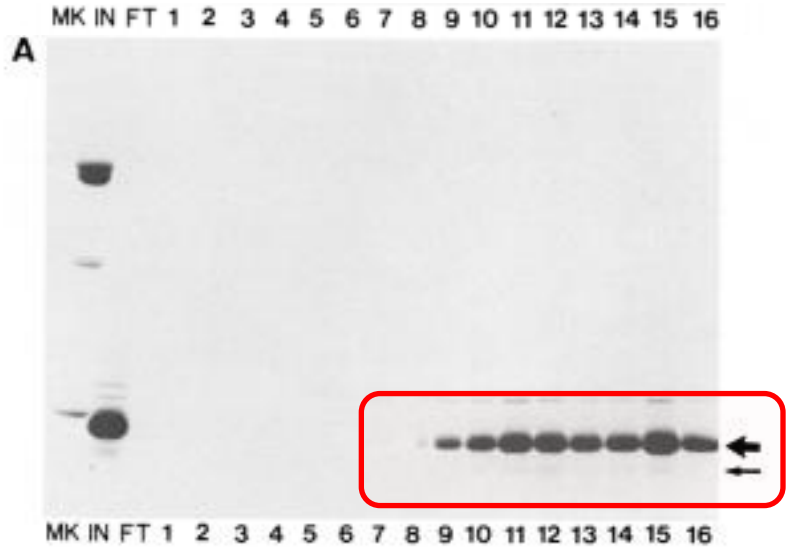




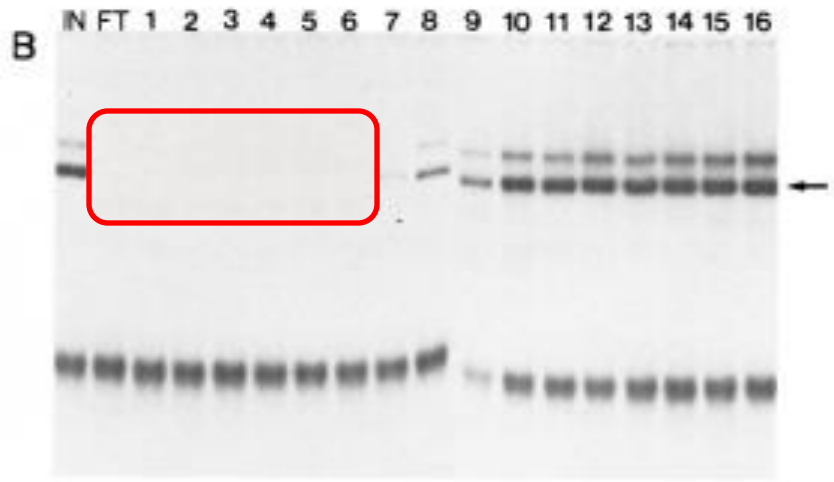


Egg extract contain component for specific transcription of 5S gene

Purification of transcription factor



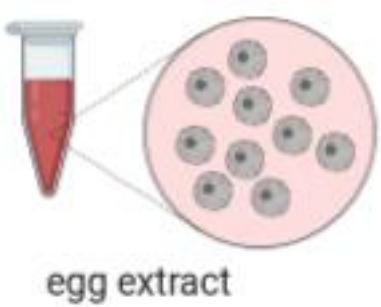
Denaturing PAGE

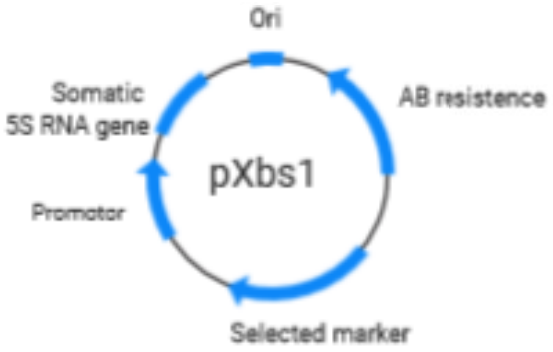


Complementation assay

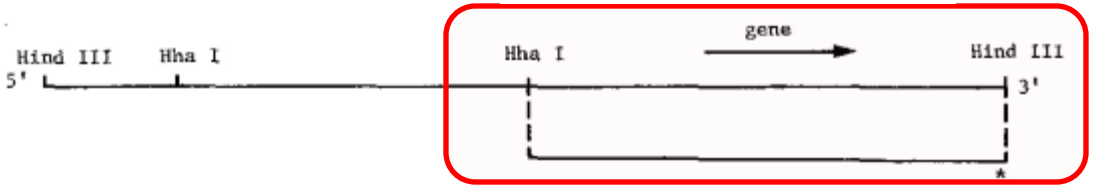
Egg extract contain component for specific transcription of 5S gene

Purified “A factor” TF pXbs1

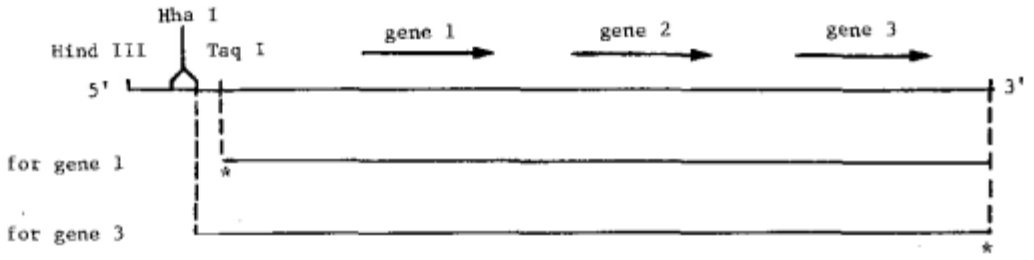




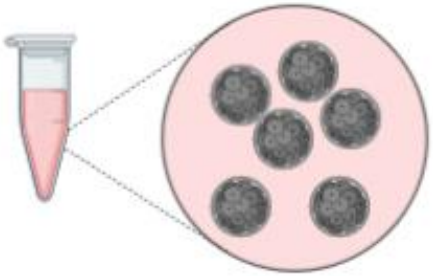
XBsl

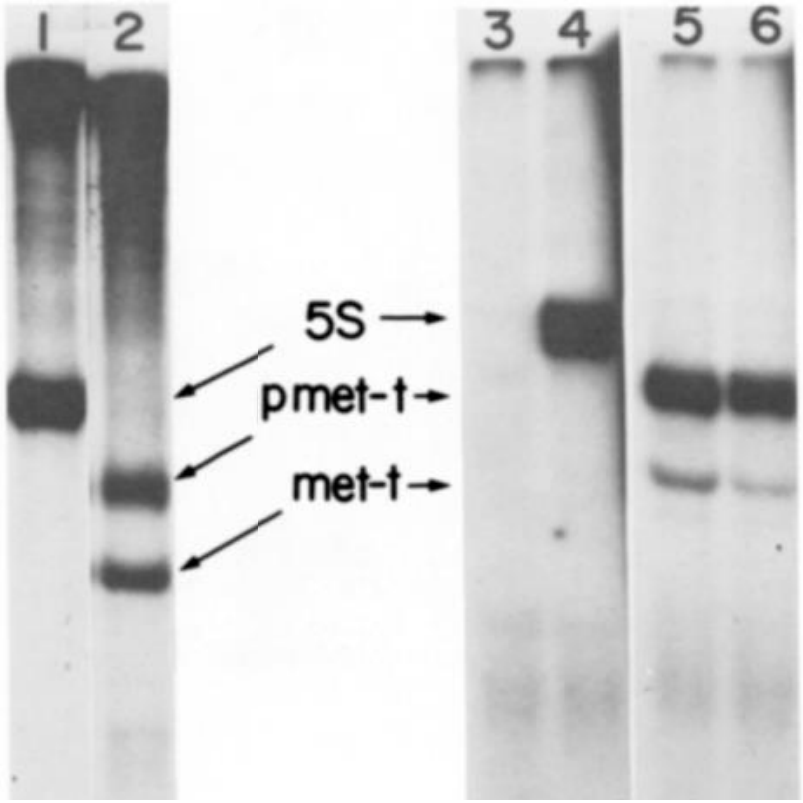


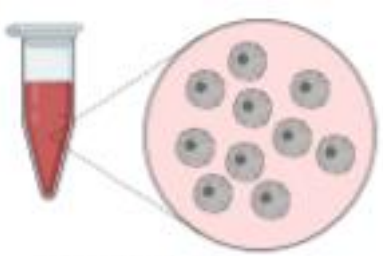
XBol



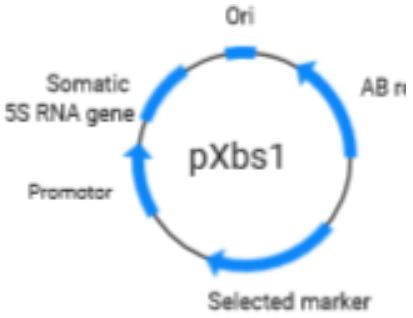
Purification of transcription factor







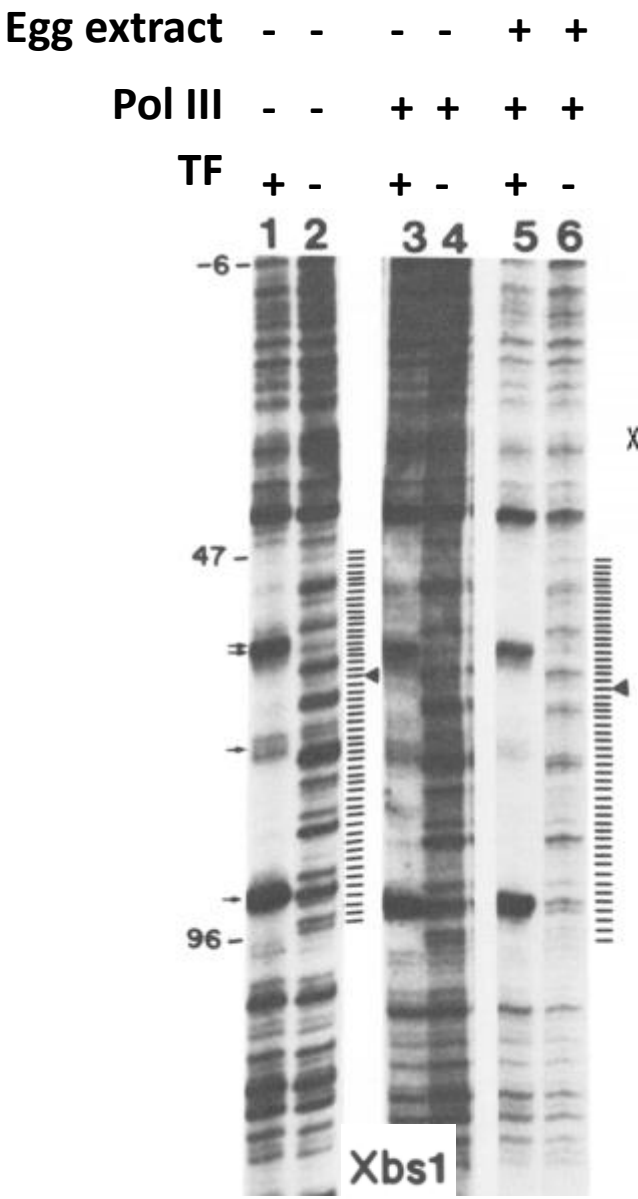
egg extract

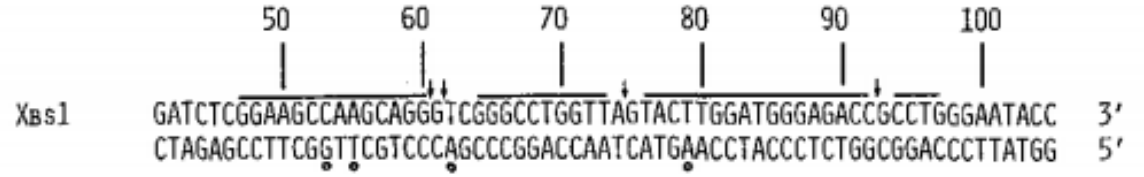


Purified TF is essential for somatic and oocyte 5S gene transcription

Not essential for the t-RNA gene transcription

Foot-printing of transcription factor





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
CCTGCATGGGGAGGAGCTGGGCCCCAAGAAGGCAGCACAAGAGGAGGAAAAGTCAGCCTTGTGTTCGCCTACGGCCACACCACCCTGAAA

      640                 660                 680                 700                 720
GTGCCCGATCTCGTCTGATCTCGGAAGCCAAGCAGGGTCGGGCCTGGTTAGTACTTGGATGGGAGACCGCCTGGGAATACCAGGTGTCGT

                740                 760                 780                 800
AGGCTTTTGCACTTTGCCCTTCTGAGCAGCAGGGGGCAGTCTCCTCCCTGCTTTTTCCTTCCCGCAACAGCCAGACAGCTAGCTGCCTGA

      820                 840                 860                 880
TAGAGACCCCCACCCCCTGTAAGGGAGCCACTCCATACGCTGAACTGTACACCTGCGGGCCTTGGCCAGGAC(AAGCTT)
```

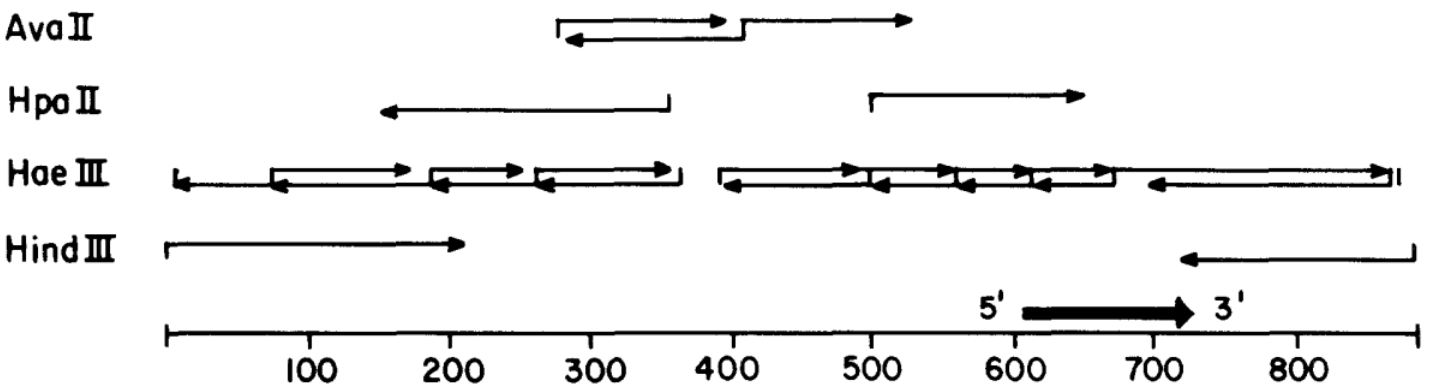


Figure 4. Nucleotide Sequence and Restriction Map of Xls11

Peterson et al Cell; 1980

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

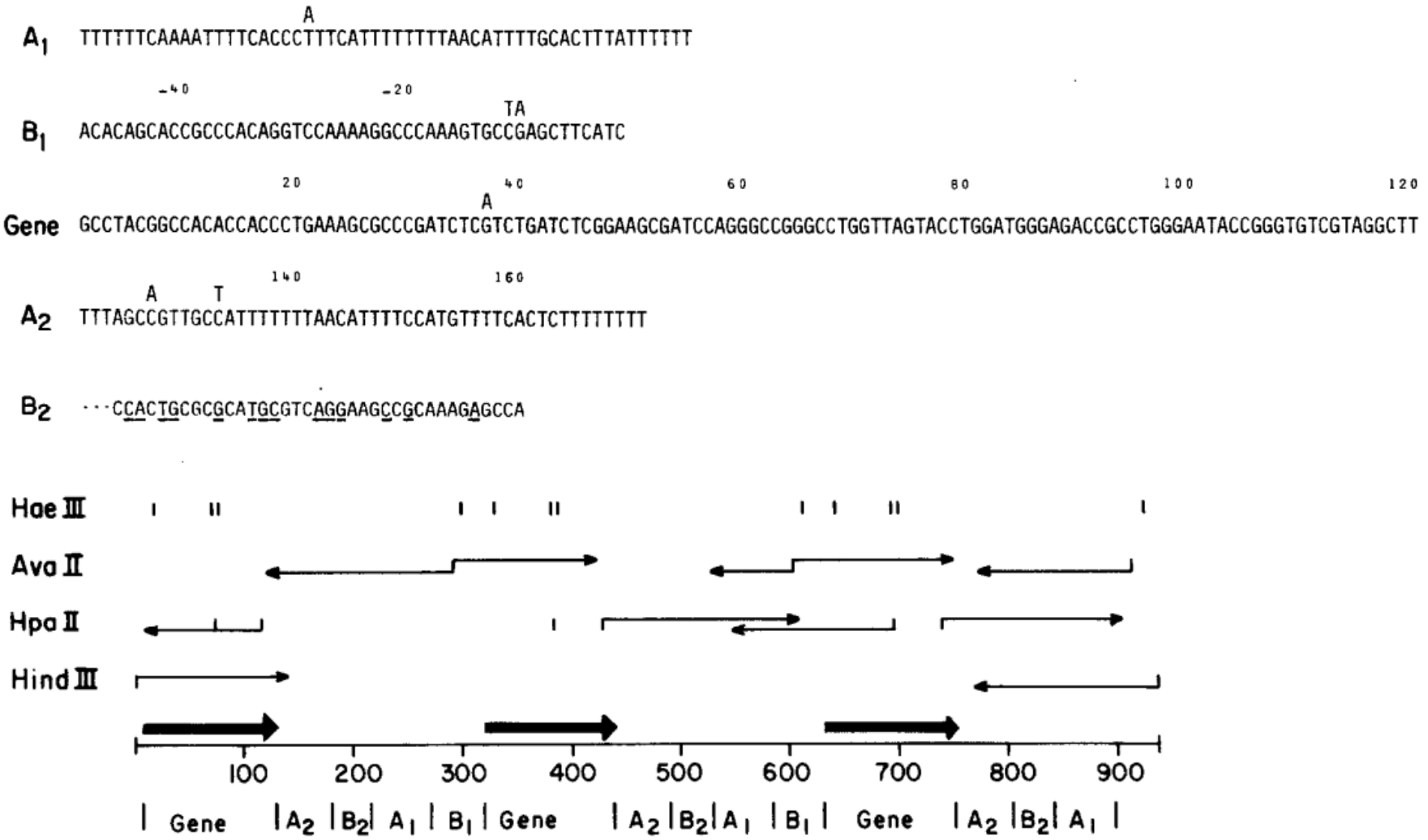
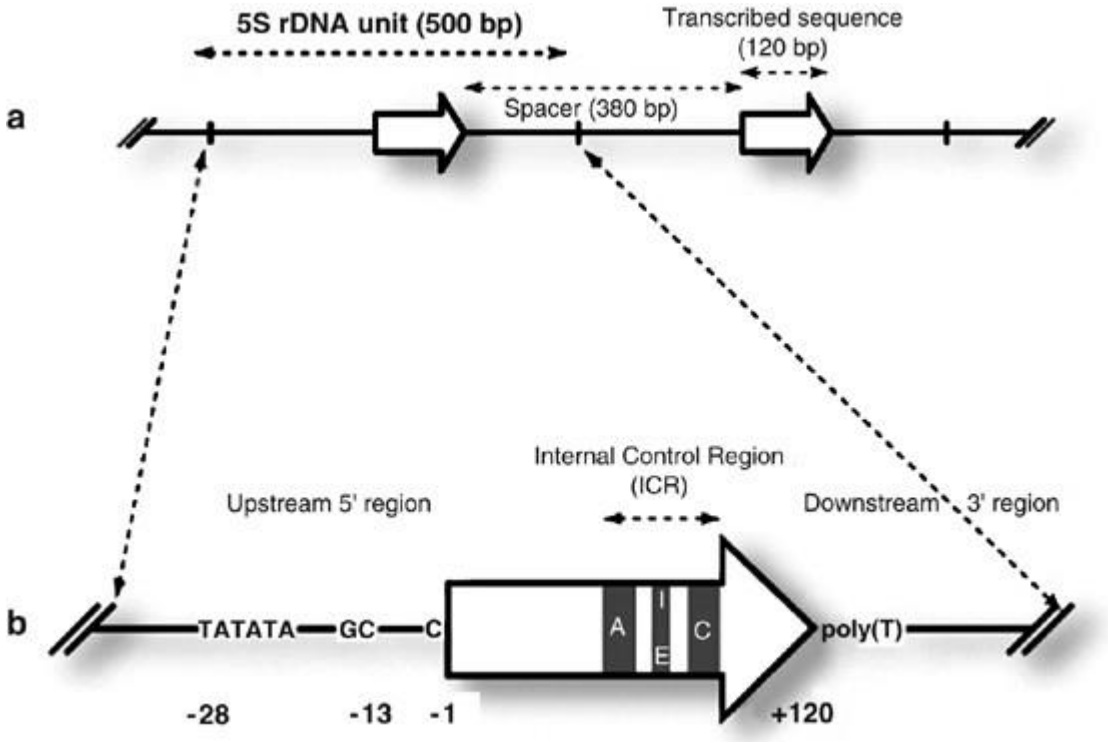


Figure 5. Nucleotide Sequence and Restriction map of Xlt1

Peterson et al Cell; 1980


5S rDNA unit (500 bp)
Transcribed sequence
(120 bp)
Spacer (380 bp)
a
Internal Control Region
(ICR)
Upstream 5' region
Downstream 3' region
b
TATATA
GC
C
A
I
E
C
poly(T)
-28
-13
-1
+120