Bacteria in Semen

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Background:

- The concept of detecting and identifying bacteria by ribosomal RNA gene sequences is about 15 years old.
- Although limited, the application of this approach to clinical specimens has revealed that most (greater than 95%) human pathogens have never been identified by laboratory culture methods.
- A well known example of this is Helicobacter pylori which was detected by molecular biology before it could be cultured.

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Why ribosomal RNA (rRNA) genes?

- All living organisms use ribosomes to synthesize proteins.
- Ribosomes have two subunits, designated by their speed of pelleting in a centrifuge:
    - 30S and 50S for bacteria -- combine to form 70S
    - 40S and 60S for mammalian cells -- combine to form 80S
- Each subunit contains both RNA and protein folded together in a very specific conformation
    - The 30S subunit contains 16S rRNA
    - The 50S subunit contains 23S and 5S rRNAs
- Some, but not all, regions of bacterial rRNAs share homology with mammalian rRNAs.

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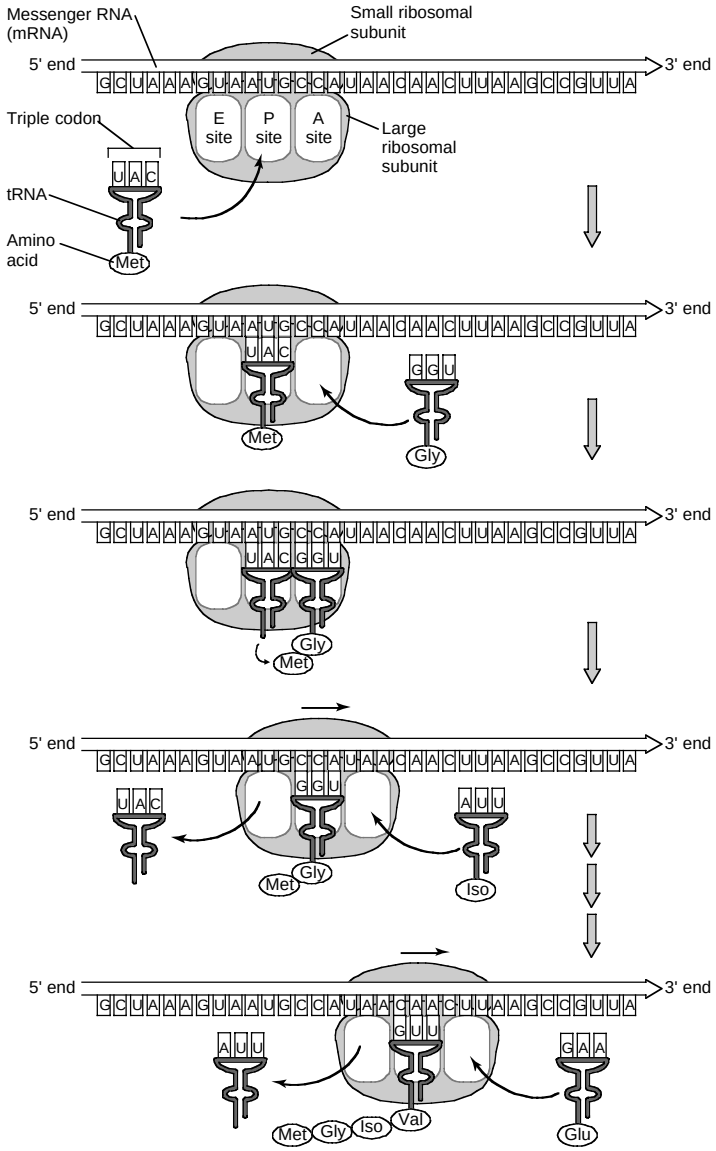
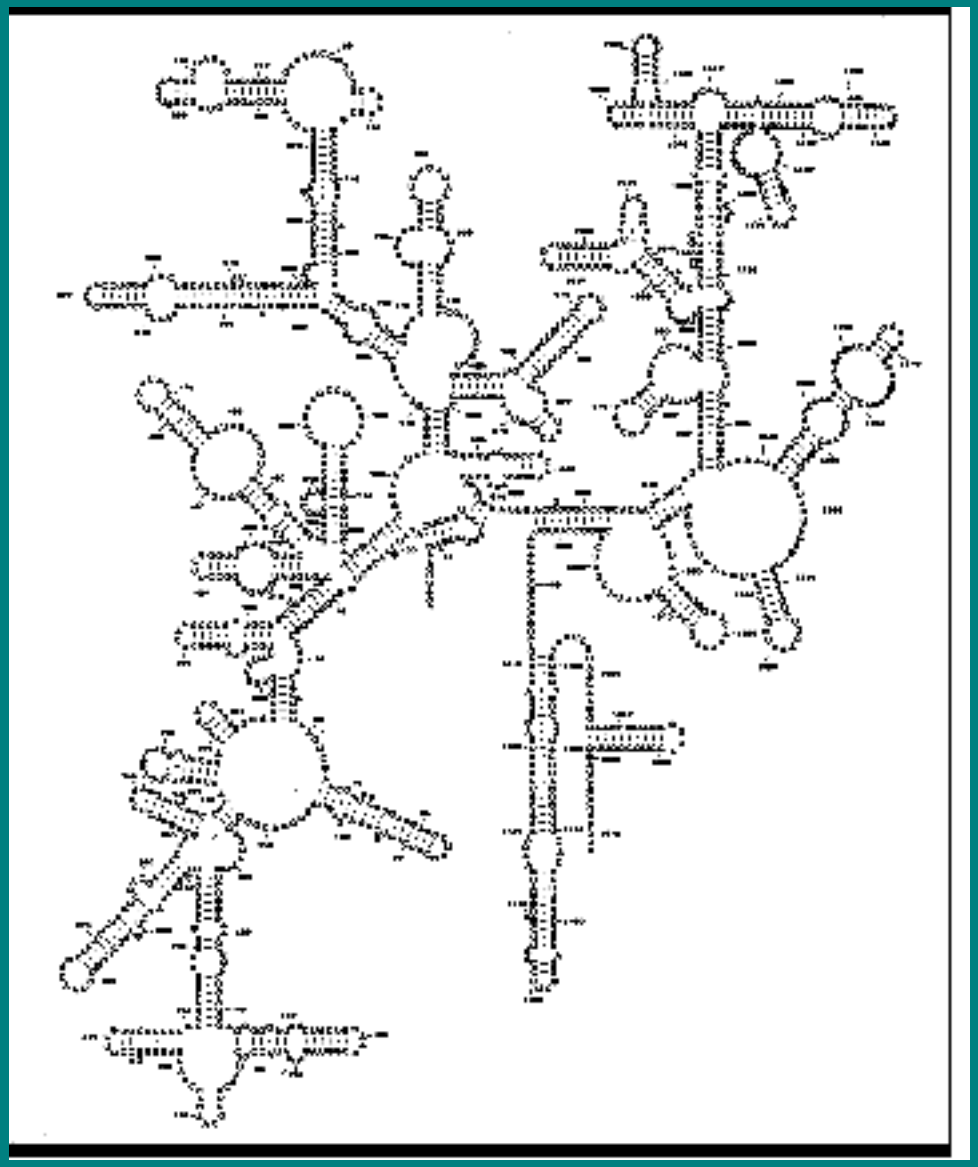
Schematic of role of ribosomes in protein synthesis:



E. Coli 16S rRNA schematic:



Ways to detect bacterial rRNA genes:

- In situ hybridization
- Amplification by Polymerase Chain Reaction (PCR)
  - Forward Primer: AACTGG…
  - Reverse Primer: AGGAGG…
- Products identified by
  - Southern Blot analysis
  - Direct gene sequencing

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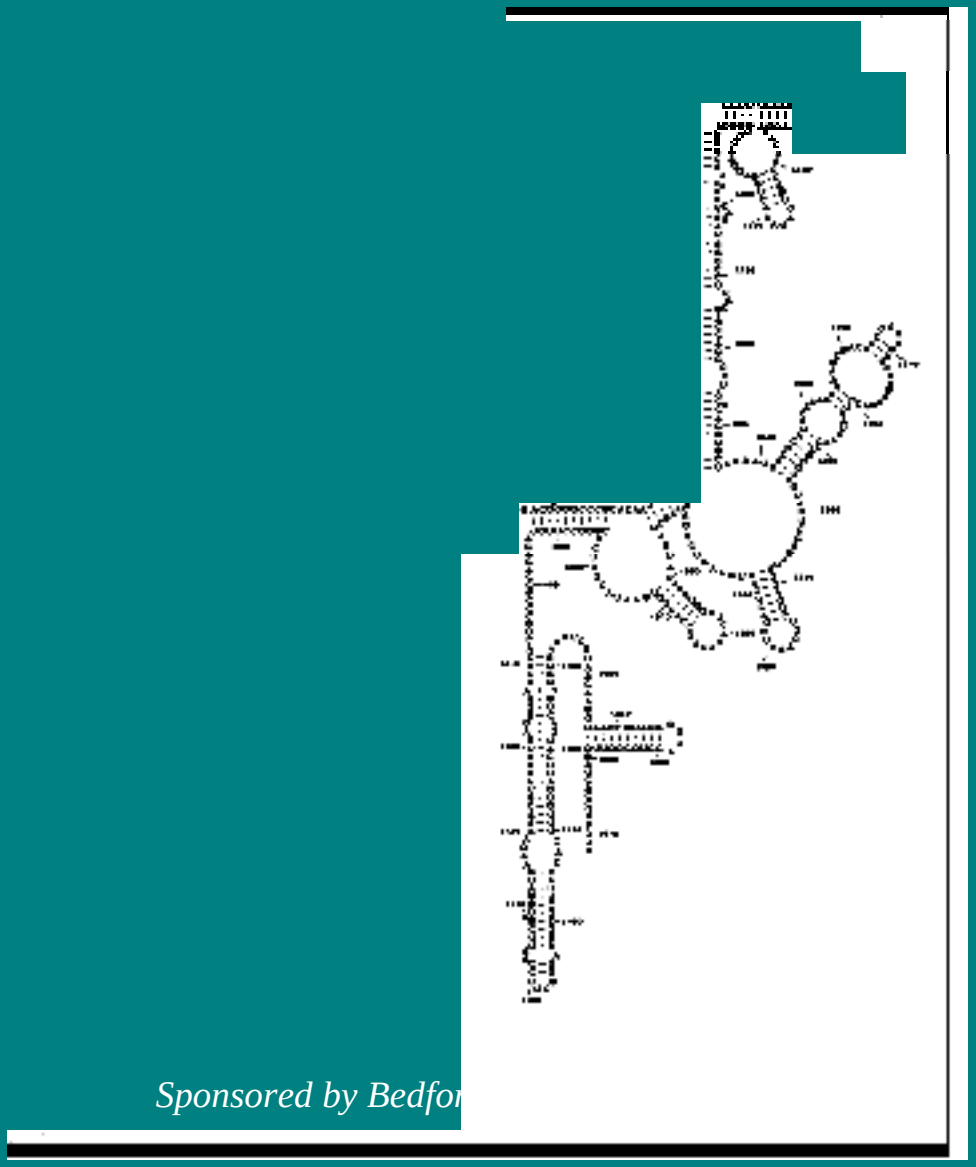
E. Coli 16S rRNA amplified:



Current Method

- Non-sperm semen DNA is isolated with a commercial column
- Start with a total semen volume of 10 to 16 microliters (approximately one loop-full)
- Subjected to 30 cycles of PCR under conditions that will detect on the order of 1000 target genes, equivalent to on the order of 300 bacteria
- Approximately equivalent to 300 colonies of bacteria on a culture plate

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Current Method (cont'd)

- PCR products are electrophoresed through an agarose gel, stained with fluorescent dye (ethidium bromide) and visualized with uv light box

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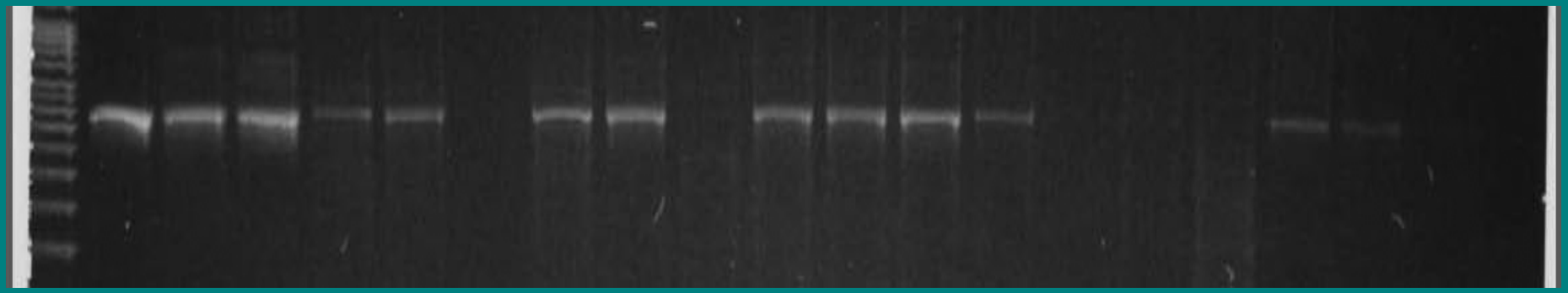
Agarose gel of 18 semen specimens, 13 positive for bacteria



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Current Method (cont'd)

- PCR products from positive reaction tubes are purified through a commercial column.
- The Forward Primer is added to a small aliquot of purified products and mailed to the gene sequencing laboratory.
- The sequences are emailed back to Bryan
- Bryan edits them in software "Sequencher" to eliminate ambiguous bases and other problems.

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Current Method (cont'd)

- The edited sequences are then
  - Submitted to BLAST search for identification
  - Compared with other sequences from semen specimens

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Important: this method avoids cloning

History:

- Two reports appeared in 1996:
  - One from Keith Jarvi's lab in Toronto
    - 30 infertility semen specimen
      - 8 positive by routine bacterial culture
      - 20 positive by PCR
      - PCR products cloned and sequenced
    - Most organisms not identified in GenBank
    - Found several species of Peptostreptococcus, Streptococcus and Cornybacterium

History (cont'd):

- Second 1996 report from John Krieger's lab at the U of W:
  - Biopsy study of 135 men with chronic prostatitis
  - 77% of biopsies were positive for bacterial DNA
  - Cloned products from 10 patients had no matches in GenBank
  - Only one third of men with bacteria-positive biopsies exhibited leukocytes in expressed prostatic secretions

History (cont'd):

- Two subsequent biopsy studies from Krieger lab in 1998 and 2000:
  - Bacteria species still not in GenBank, but some related to Staphylococcus epidermis
  - Only 20% of cancer biopsies (107 men) positive for bacteria sequences
  - These two studies used to define two groups of bacteria, "prostate A" and "prostate B"

History (cont'd):

- Another biopsy study in 2000 by Anthony Schaeffer's lab:
  - Detected no bacterial gene sequences from 18 organ donors biopsied under sterile conditions
  - Two patients with BPH had bacteria-positive biopsies
  - Six of seven patients with prostate cancer had bacteria-positive biopsies

Concluded no "normal flora" in the prostate

History (cont'd):

- A study in 1999 of expressed prostatic secretions (EPS) by Norman Pace's lab:
  - 17 men with chronic, refractory prostatitis
    - 8 EPS positive for bacteria by culture
    - 11 positive for bacteria by PCR
    - 4 positive men did not respond to antibiotic therapy
  - 8 controls with no prostatitis symptoms
    - 6 positive for bacteria by PCR
  - Prostatitis specimens many more types of bacteria and usually included Cornybacteria

Where we are:

- Conducting three series of patient specimens:
  - Infertility
  - HIV infected
  - Prostatitis
- Results to date only from Infertility series

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Where we are:

- Data to date from 18 semen specimens from 15 men
  - 12 (67%) are positive for bacteria
  - Only four positively identified in GenBank
    - Staphylococcus (2), Lactobacillus (1), Cornybacterium (1)
    - Some maybe related to Peptostreptococcus
    - Some not identified because a mixture of organisms
    - No E. coli detected
  - No correlation with semen leukocyte count

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SEMEN BACTERIA EXPERIMENTS

Total Numbers (millions)

	Sperm	NSCs	Hle+	LC+	Bacteria
1	0	0.9	0.2	0.1	Streptococcus
2	202	6.5	2	0.1	Staph epid
3	129	44	18	1.7	pos, P
4	53	3.9	0.7	0.1	pos,mix
5	51	3	0.1	<0.1	pos, P
6	81	2	<0.1	<0.1	pos,P
7					-
8	200	150	118	62	-
9	244	79	9	44	-
10	123	36	12	5.4	-
11	50	9.6	6.9	2.1	Staph epid,hem,hom
12	193	7	3	0.8	pos, Pepso
13	181	1.7	0.5	0.1	pos, mix, enteric
14					-
15	340	21	19	7	pos, aminomonas
16					-
17	60	3.8	0.1	0.8	cornybacterium
18	23	2	0.6	0.2	Lactobacillus crispatus

Where we are (cont'd):

Our data basically agree with previous studies

Multiple specimens from two men:

- Two positive, third undetectable after antibiotic therapy
- One positive, second negative after therapy
- Both a mix of bacteria

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What we intend to do:

- Continue to study prostatitis patients
- Develop a faster way to identify the bacteria
  - Gene chip technology
  - Need to know identity of organisms
- Begin to study prostate cancer patients
  - Method easily adapted to prostate cancer markers

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