SLIC project

tmRNA meets biosensing

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- **What's SLIC?**
- **What's going on?**
- **What's going to happen?**

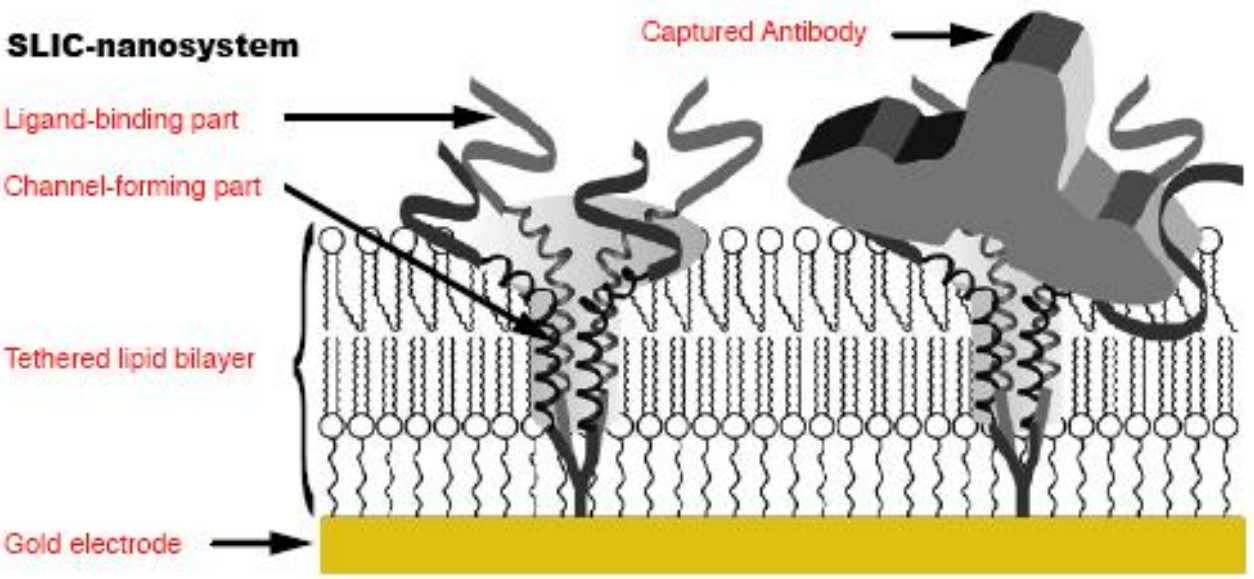
SLIC project

- **EU FP6 project**
- **SLIC cosortium develops *lab-on-a-chip* technology based biosensor for pathogen detection**

  - **Swiss Federal Institute of Technology**
    - **Ayanda Biosystems**
  - **IMTEK Albert Ludwigs Uni Freiburg**
  - **National University of Ireland, Galway**
  - **EBC workgroup**

SLIC stands for...

- Synthetic Ligand-gated Ion Channel
- Swiss Federal Institute + Ayanda

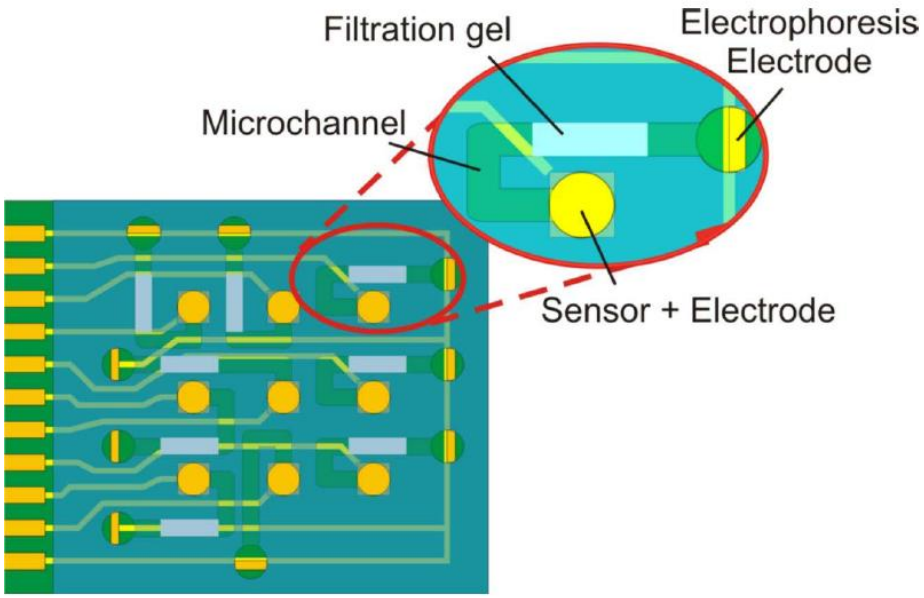


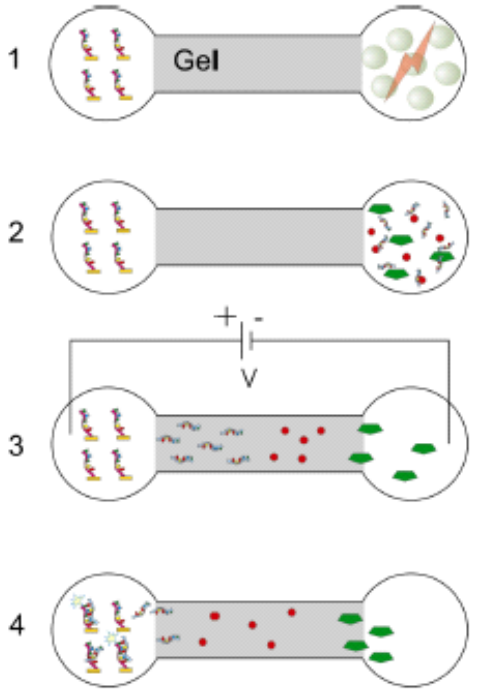
Principle

- Formation of tethered lipid bilayers of exceptionally high electrical resistance
- Detection of only a few synthetic ligand-gated ion channels (SLIC)
- Modulation of the channel activity by selective antibody binding to SLIC

Lab-on-a-Chip nanosystem

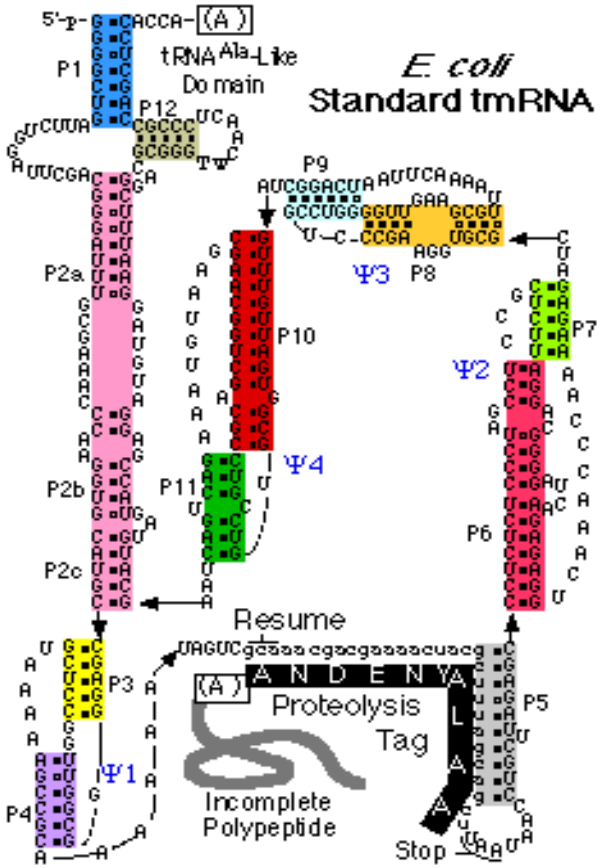
- **SLIC will be incorporated into special microfluidic device**
- **Developed IMTEK, Freiburg**





tmRNA as a target molecule

- tRNA, mRNA like regions
- structural pseudoknot regions
- Assists protein synthesis in all known bacteria
- As target
  - Highly abundant ~1000/cell
  - Species-specific core
  - Identify and distinguish different bacteria



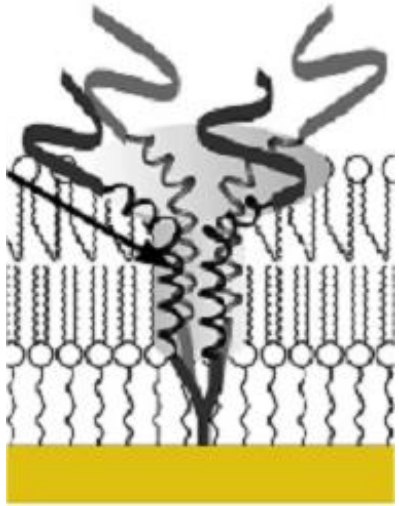
Test pathogens

- **Main subjects**
  - ***Streptococcus pneumoniae***
  - ***Haemophilus influenzae***
  - ***Mycobacterium tuberculosis***

- **Control pathogens**
  - **5 *Streptococcus* family (Group ABCDG)**
  - ***Enterococcus faecium***
  - ***K.pneumoniae***
  - ***M.catarrhalis***
- **NUI Galway, National Diagnostics Centre**

tmRNA specific probes

- Estonian Biocentre
- Develop a panel of species-specific probes
  - Bioinformatics
  - Nearest-Neighbour thermodynamics based approach
  - Program SLICSel by Priit Palta
    - http://bioinfo.ut.ee/slicsel/
  - Tested against
    - Microbial genomes
    - tmRNA genes
    - Human genome

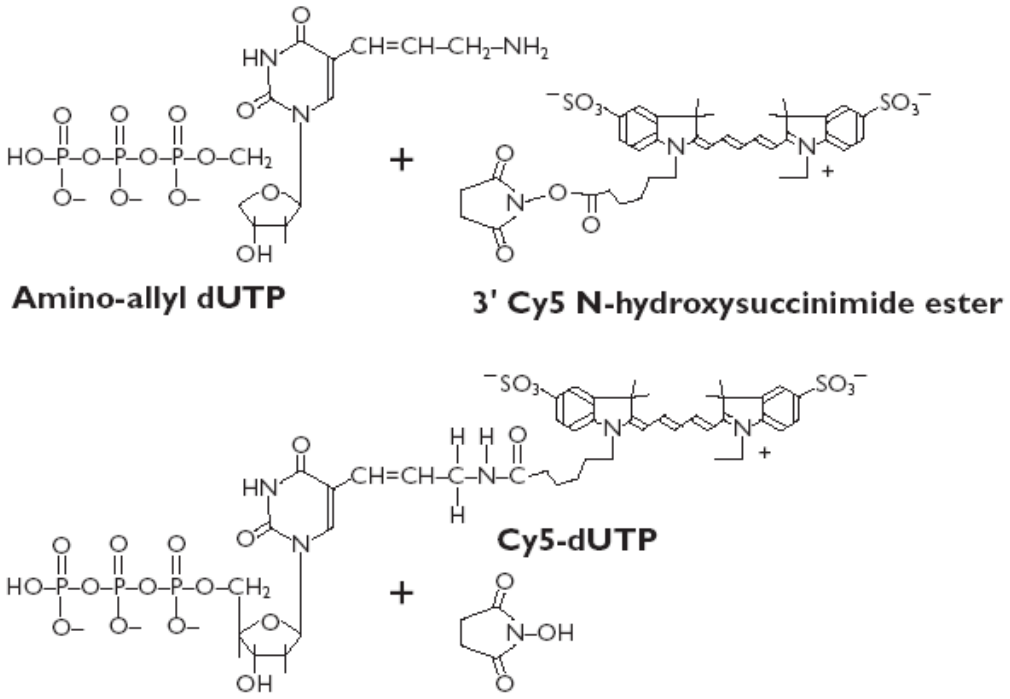


Probe testing by EBC

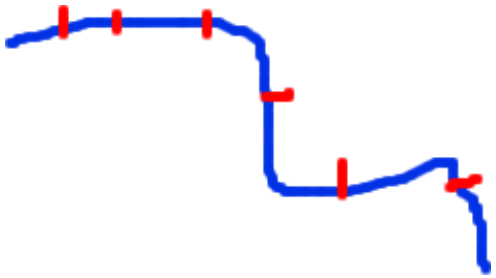
- **Microarray technology**
- **tmRNA genes cloned into vectors (NUI)**
- **Vector linearization with restrictional endonuclease (HindIII, XhoI)**
- **RNA *in vitro* transcription from the DNA template**
  - **Incorporation of aminoallyl-UTP**
  - **Labelling of the aminoallyl moiety**

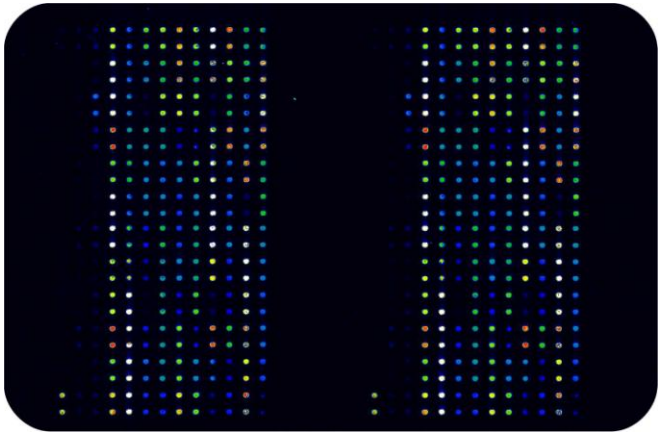
RNA labelling...

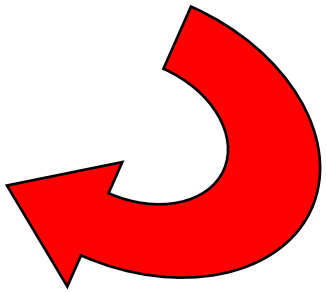
- **...with aminoreactive Cy3 dye NHS ester**



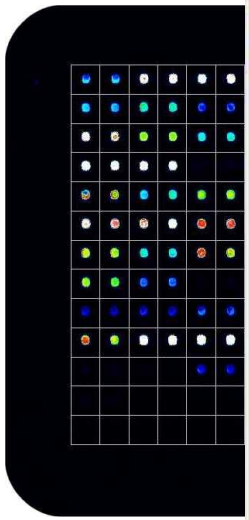
tmRNA hybridisation 4h

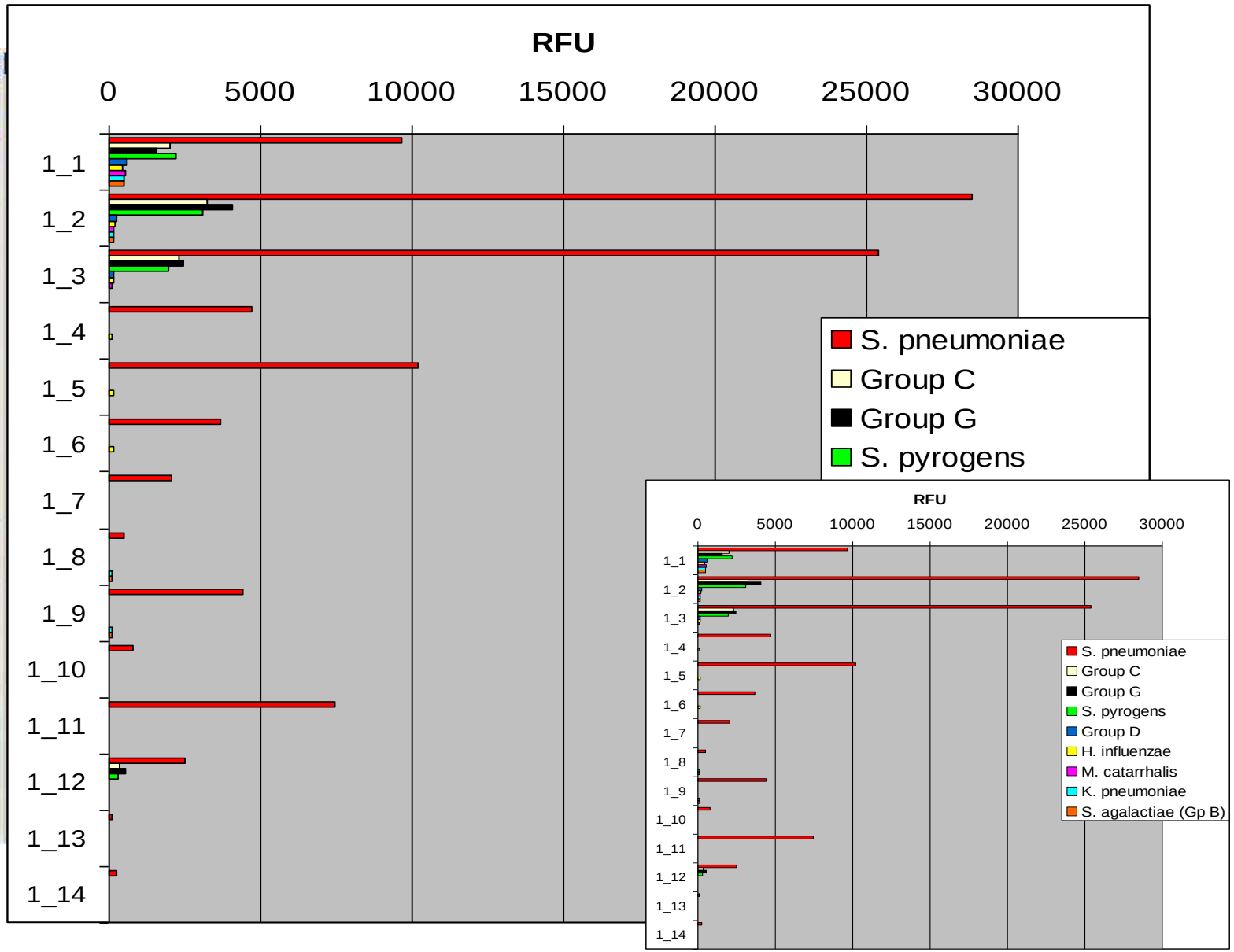






Data analysis





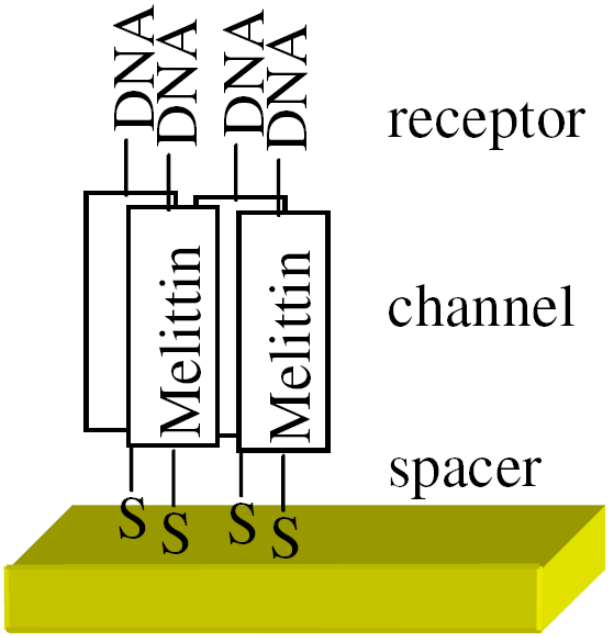
Probes tested

- 97 *Streptococcus pneumoniae*
- 5 *Haemophilus influenzae*
- *5 Mycobacterium tuberculosis*

- **Positive controls for test tmRNA-s**
  - 3 *K.pneumoniae*
  - 3 *M.catarrhalis*
  - 12 *Streptococcus* family

Current situation

- **Microarray panel with different species and genus specific tmRNA probes**
- **Slight delay in final SLIC prototype**



Spinoff projects with NUI, Galway

- I
  - RNA chaperones
- II
  - NASBA method

Spinoff projects

- I
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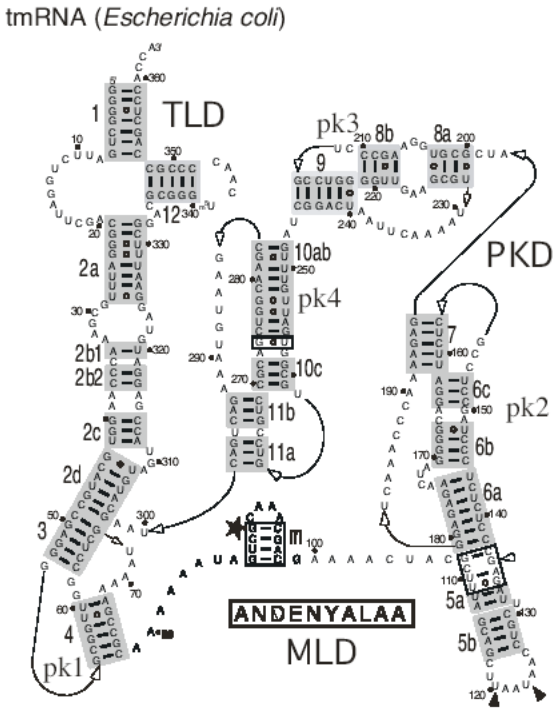
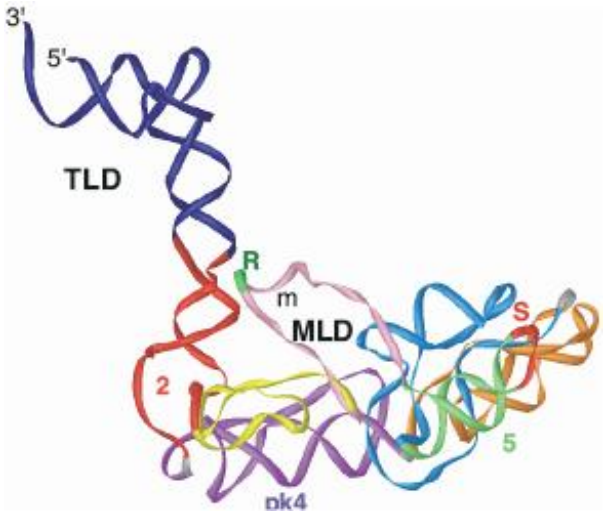
Chaperone oligonucleotides

- **Breaking of RNA secondary structures by hybridising to complementary regions**

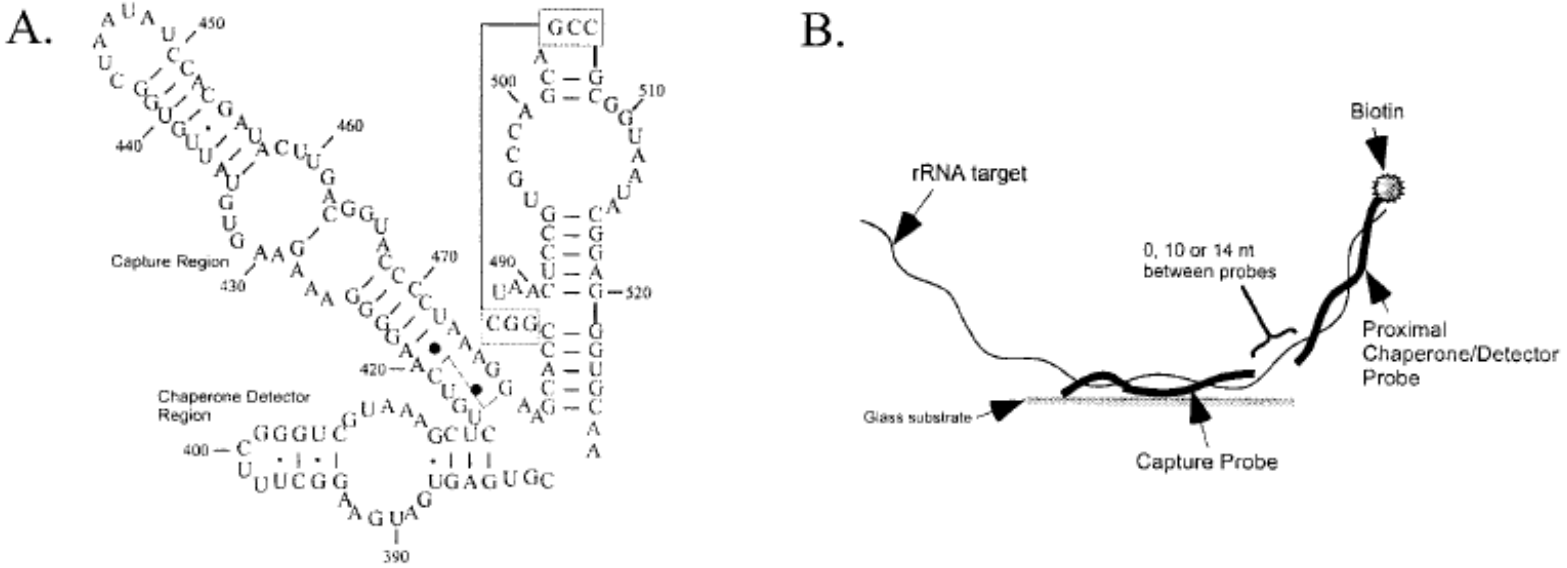


2D



3D

Chaperone principle



Chandler *et al.*,Appl. Env. Microb., 2003

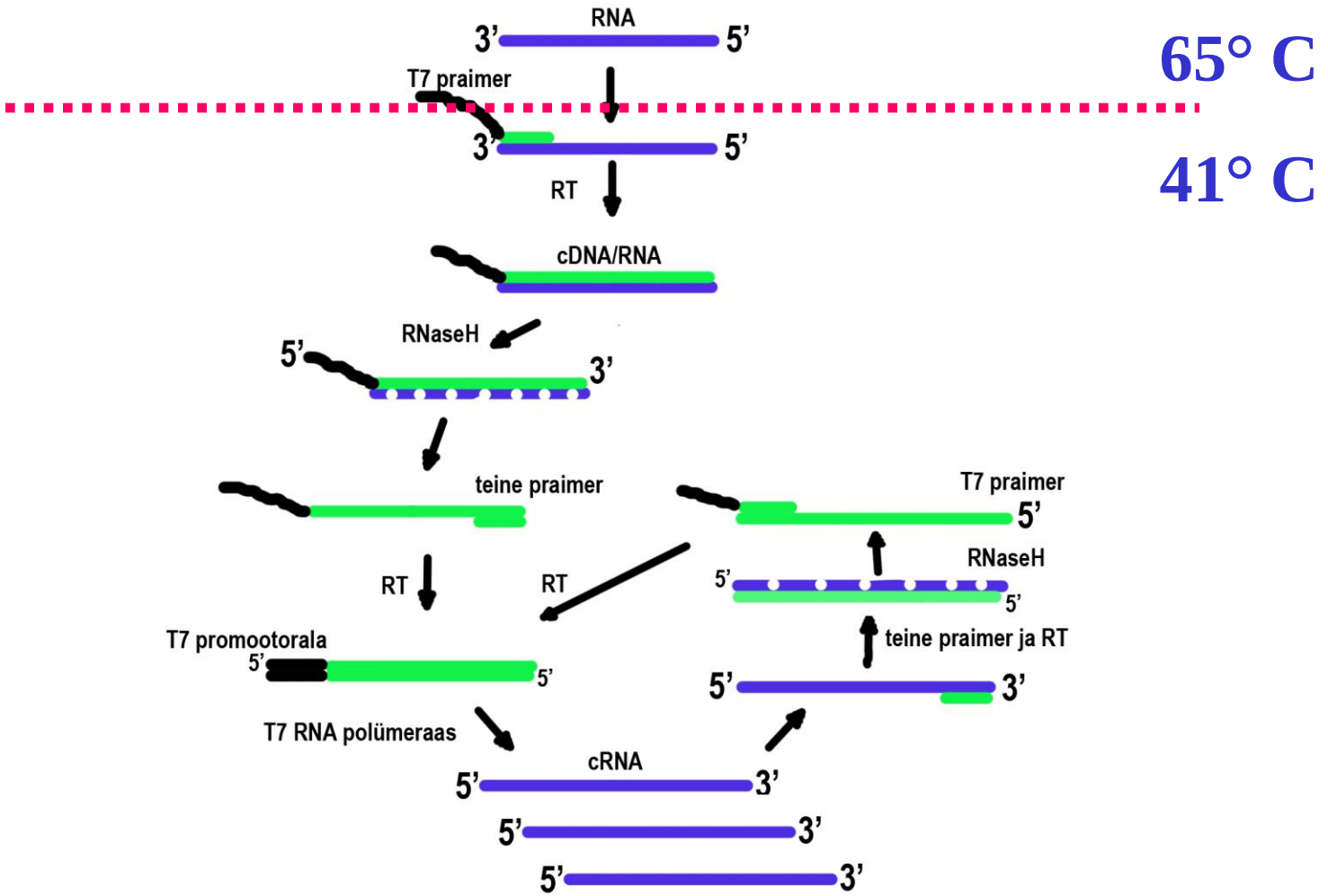
Preliminary results

- Signal intensity rises
- Chaperone excess 10 and 100X
- 6 chaperones investigated

Spinoff projects

- I
  - RNA chaperones
- II
  - NASBA method

NASBA amplification method



Advantages of NASBA

- **Quick isothermal process**
  - **Supposedly more powerful than RT-PCR**
- **Definite Amplification of RNA**
  - **DNA contamination risk ⇓**
  - **RNA shows vitality of the organism, degrades quickly in environment**

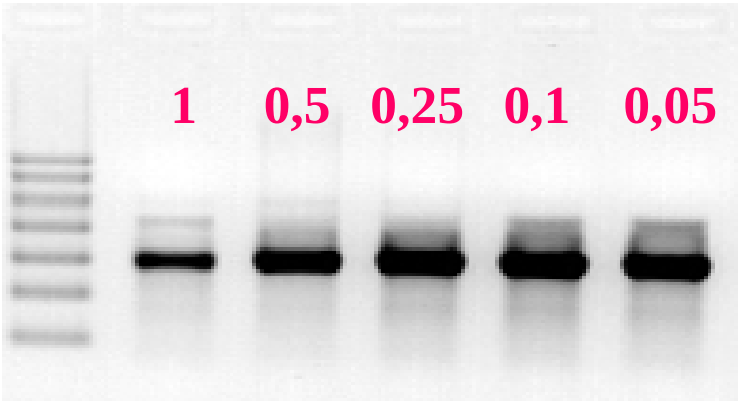
NASBA protocol

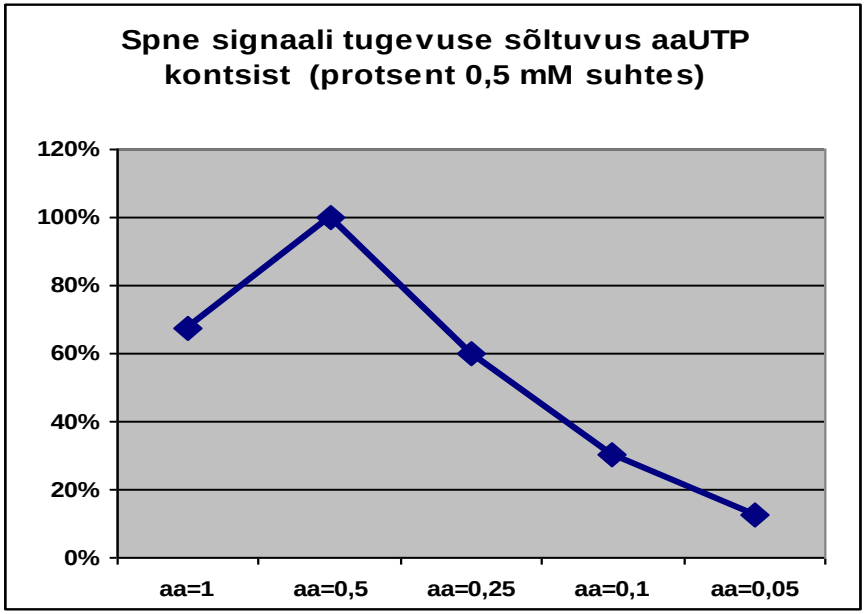
- **Only commercial kit in the world**
  - **bioMerieux**
- **Our task**
  - **Optimize protocol for microarray technology**
  - **Incorporate aminoallyl-UTPs to product**
  - **Find minimum power of our method**

BIOMÉRIEUX

Protocol optimization

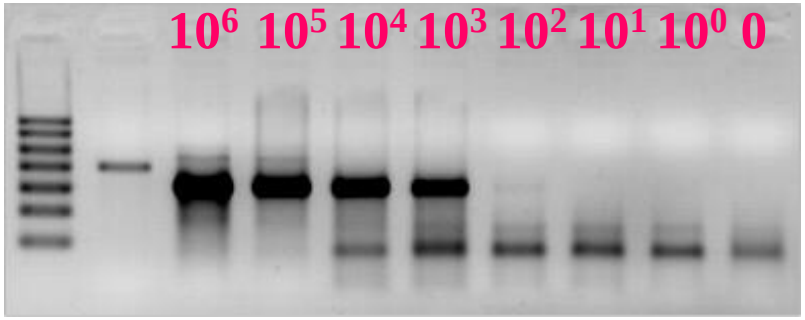
- bioMerieux doesn't say anything about the components and the concentrations
- Incorporation of aa-UTPs
  - Added different amounts of aaUTP to final reaction solution (1 => 0,05 mM)





Dilution series experiment

- **Smallest amount of RNA amplified**
  - ***S. Pneumoniae* total RNA used**
- **Dilution series**
  - **100ng => 100fg**
  - **10^6 => 1 cell**



- **Signals from 100 cell equivalent total RNA**

Future

- Hopefully SLIC-Nanosystem will be ready
  - Meeting next month
  - Clinical experiments
- Chaperones
  - Which chaperones do the best job
- NASBA
  - Optimize protocol even lower
  - Clinical experiments

Dear colleagues...

- **Biotechnology**
  - **Ants Kurg**
  - **Sven Parkel**
  - **Ott Scheler**
  - **Kadri Toome**
- **Bioinformatics**
  - **Maido Remm**
  - **Lauris Kaplinski**
  - **Priit Palta**