

In silico Design and Evaluation of Oligonucleotide Probes



Alexander Loy



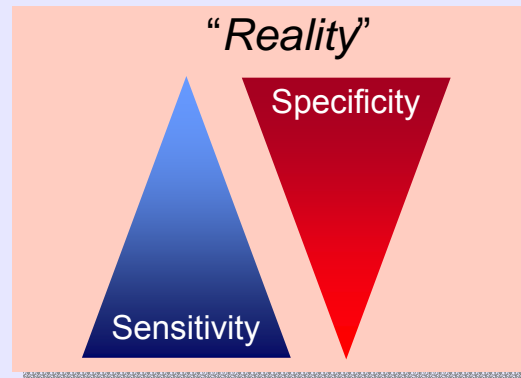
Up-to-date Sequence Databases

- **Whole-genome microarrays:**
Genome sequence database
- **Diagnostic microarrays targeting specific genes:**
Database(s) containing all publicly available sequences

Objectives of the Probe Design Procedure

“Perfect world scenario”

- **SPECIFICITY:** Set of probes being completely specific for its/their target gene(s)
- **SENSITIVITY/HOMOGENEITY:** All probes display same hybridization behavior under the same conditions

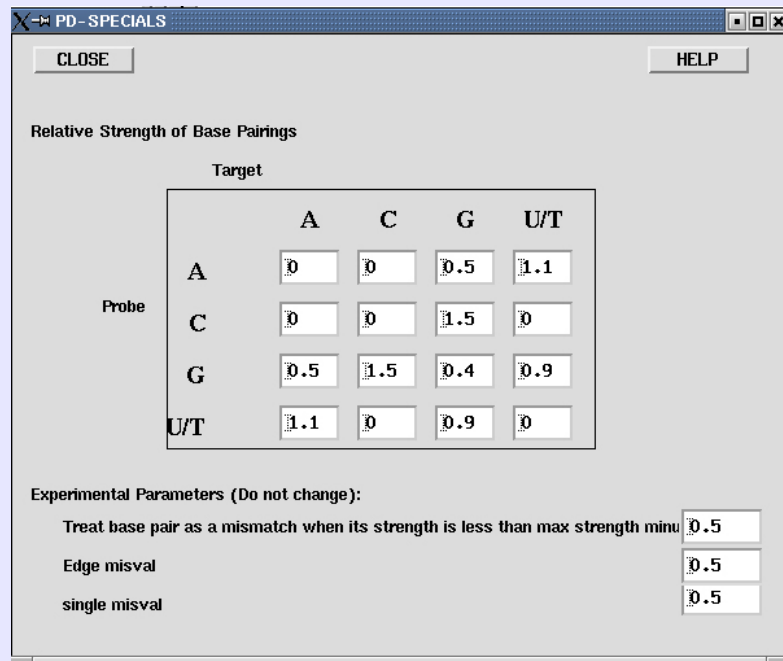


→ Simultaneous hybridization of many probes requires compromises!

Criteria for Probe Design: **Specificity**

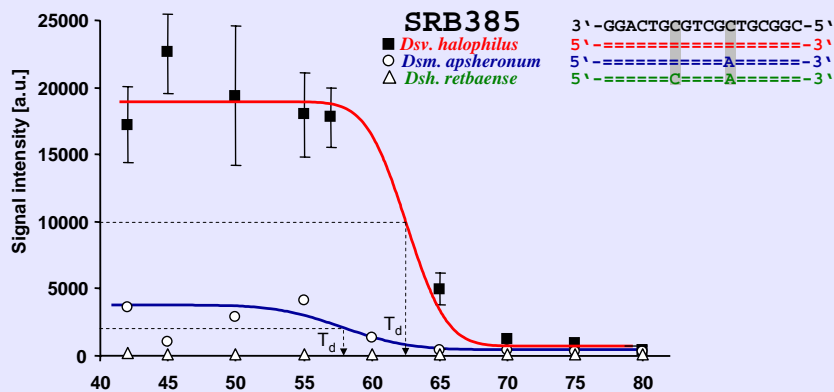
- Probe fully complementary to target sequence
- Discrimination of non-target sequences depends on number, type, and position of mismatches

Mismatch Type



Location of the Mismatch

- Mismatches at the middle of the probe-target duplex are more destabilizing



- Single mismatches at the terminal or next-to-terminal position do not discriminate

Strategies for Enhancing Specificity

- Multiple probes targeting the same gene or group of sequences
- Comparisons of perfect match and mismatch probes allow the quantification and subtraction of signals caused by non-specific cross-hybridization

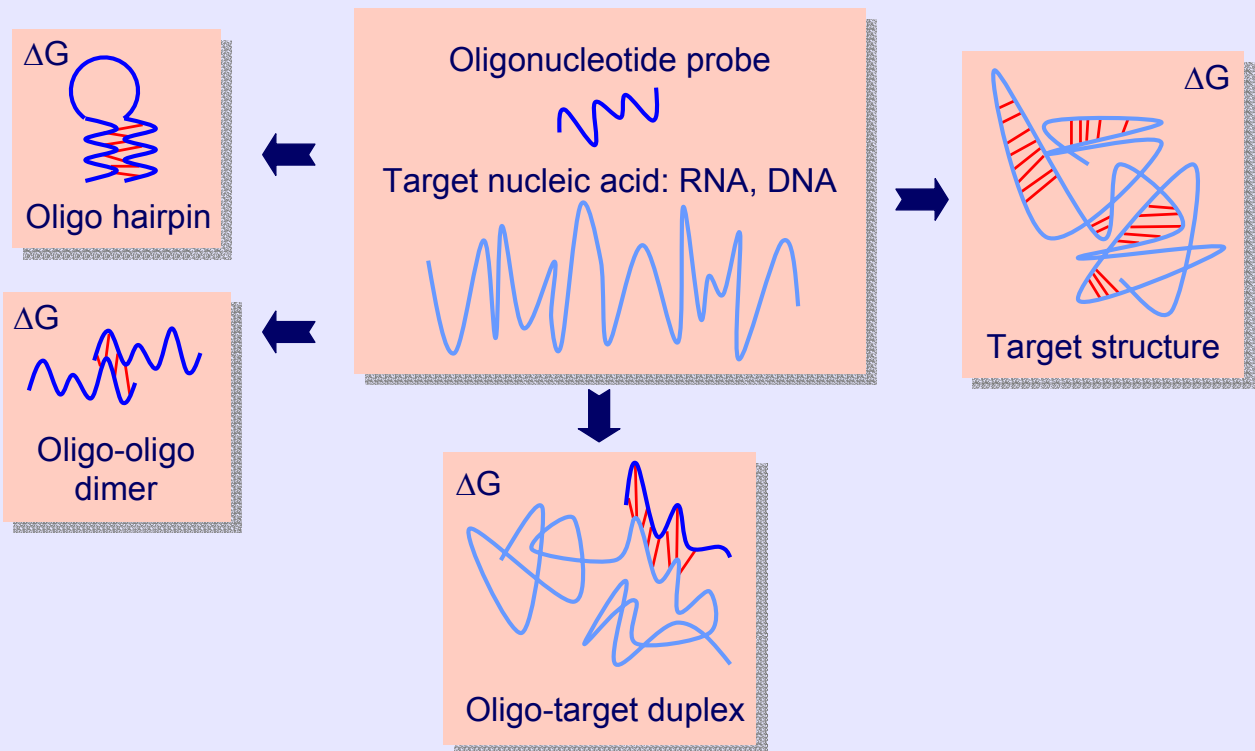
Affymetrix GeneChip Arrays®

Criteria for Probe Design: **Sensitivity**

Oligonucleotides targeting different regions of the same DNA/RNA differ in their hybridization ability

- Oligo intra- or inter-molecular structure can compete with oligo-target duplex formation
- Secondary structure of the target limits hybridization efficiency
- Duplex stability depends on base and sequence composition

Competing Probe and Target Interactions Influence Probe Yield



Sequence and Base Composition

- Probe length
- Percent G+C content
Marmur and Doty. 1962. *J. Mol. Biol.* **5**: 109-118.
- Duplex strength: DNA/DNA < DNA/RNA < RNA/RNA
- Nearest-neighbor stabilities (base stacking):
DNA at 37°C:
GC > CG > GG > GA \approx GT \approx CA > CT > AA > AT > TA
SantaLucia *et al.* 1996. *Biochemistry.* **35**: 3555-3562.

→ Melting temperature, free energy

Secondary Structure Prediction

Software for calculation of free energy [ΔG],
melting temperature [T_m], etc.

HyTher:

<http://ozone2.chem.wayne.edu/Hyther/hythermenu.html>

MFOLD:

<http://www.bioinfo.rpi.edu/applications/mfold/>

OligoAnalyzer:

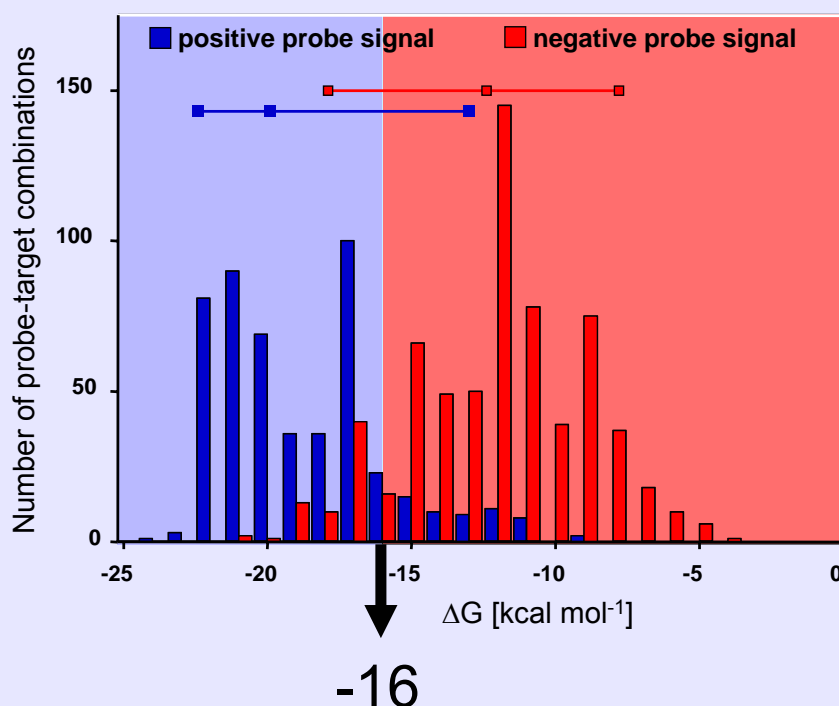
<http://biotools.idtdna.com/analyzer/oligocalc.asp>

Oligo Calculator:

<http://www.basic.northwestern.edu/biotools/oligocalc.html>

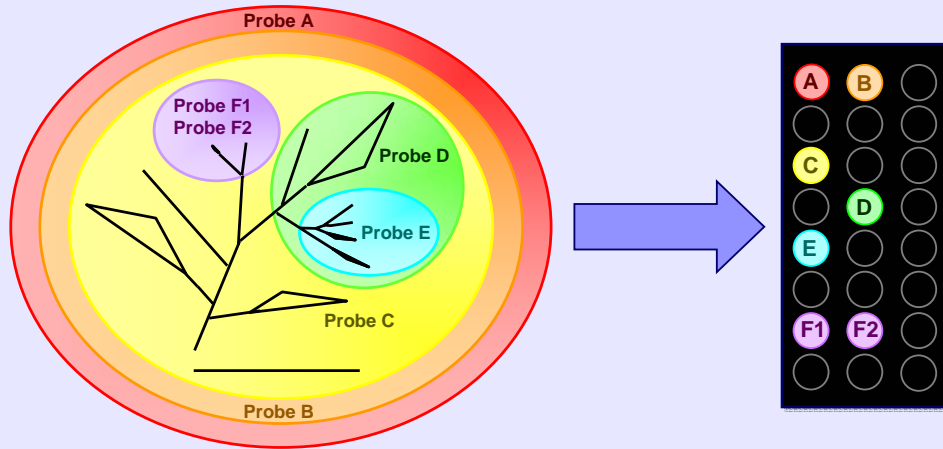
→ Algorithms, parameters for hybridization in solution!

Free Energy of Positive and Negative Probe-Target Combinations



Designing rRNA-targeted Oligonucleotide Probes

Multiple Nested Probe Concept



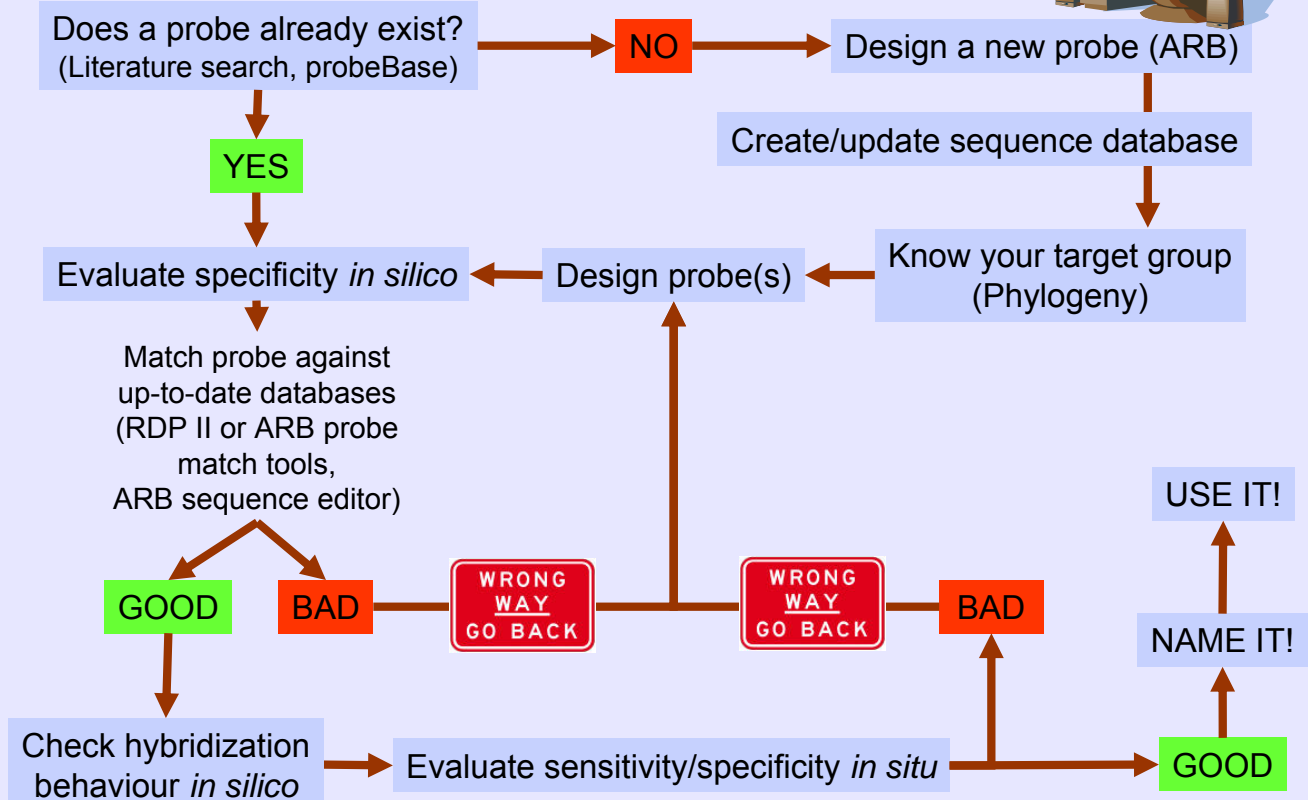
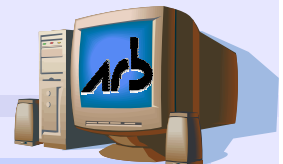
Phylogenetic Microarrays (PhyloChips) for

Sulfate-reducing prokaryotes (Loy *et al.* 2002. *Appl. Environ. Microbiol.* **68**: 5064-5081)

the order "*Rhodocyclales*" (Loy *et al.* 2005. *Appl. Environ. Microbiol.* **in press**)

Nitrifying Bacteria (Taylor *et al.* in preparation)

The Probe Design Approach



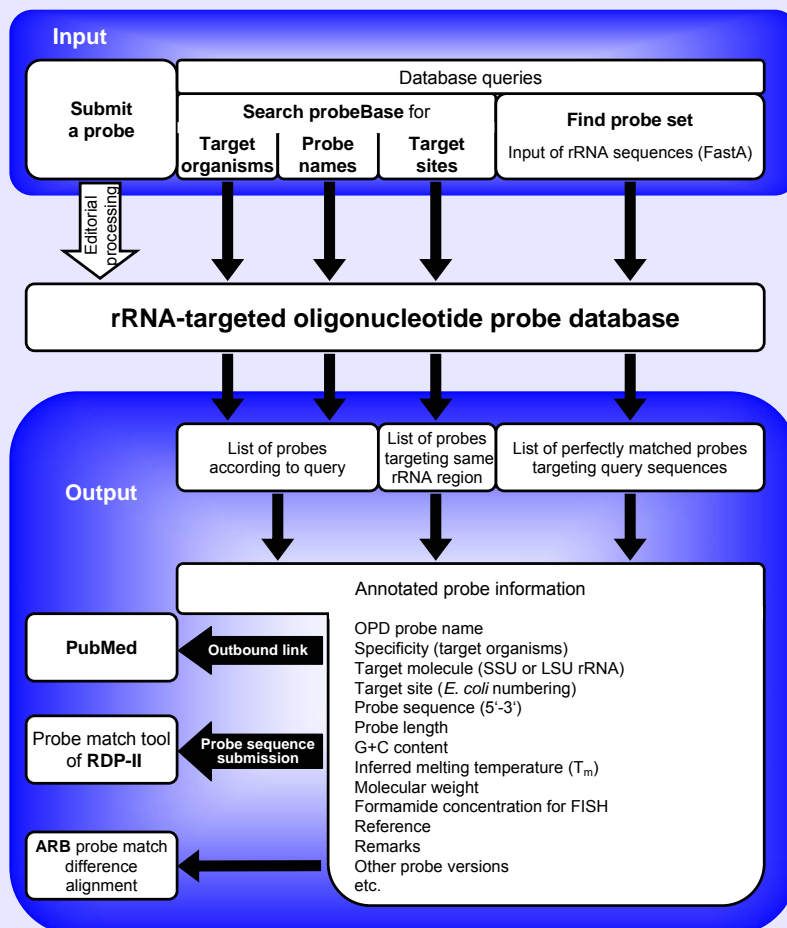
probeBase.net

an online resource for
rRNA-targeted oligonucleotide probes

<http://www.microbial-ecology.net/probebase/>

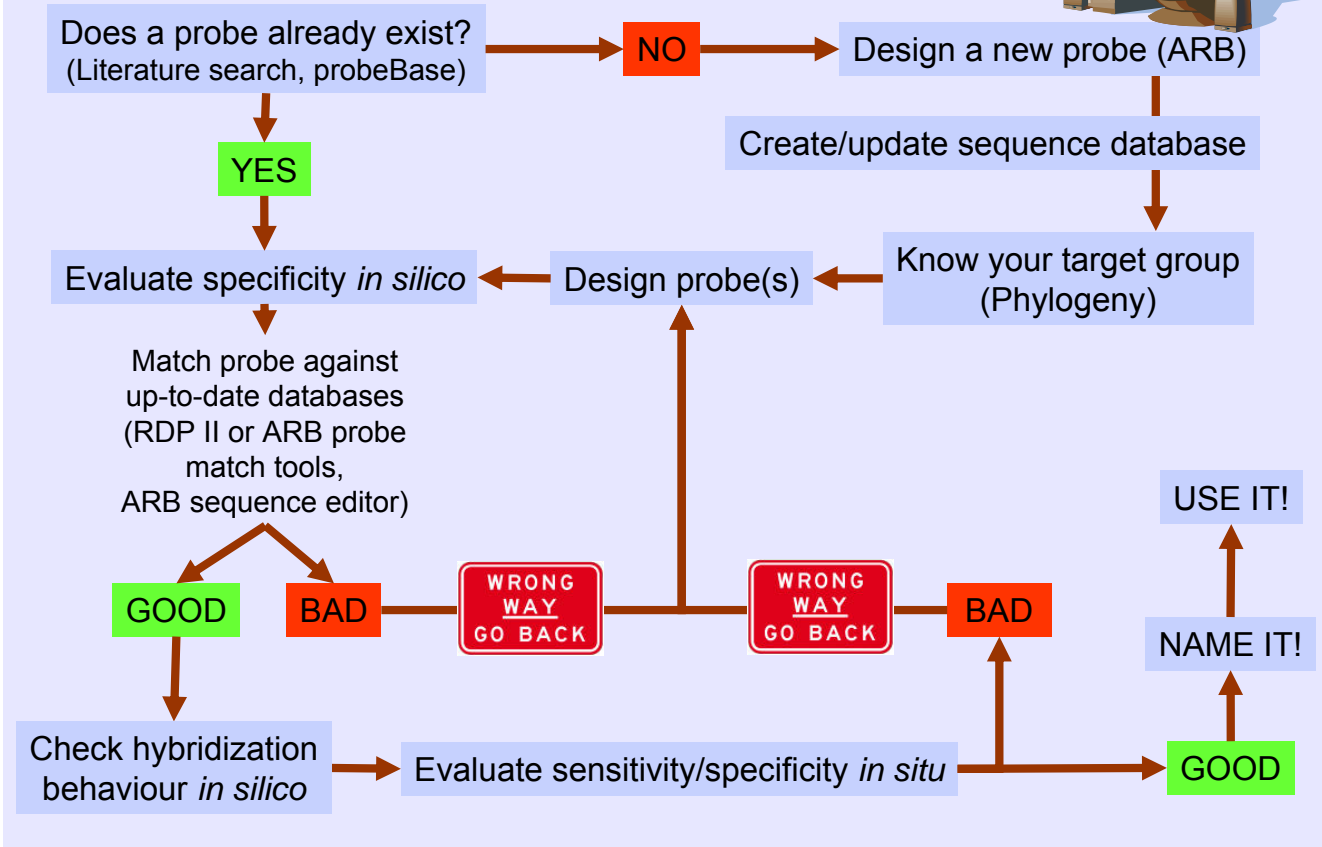
Loy *et al.* 2003. *Nucleic Acids Res.* **31**: 514-516.

probeBase.net
an online resource for
rRNA-targeted oligonucleotide probes



Concept and
Structure of
probeBase

The Probe Design Approach



Probe Design Software

Osprey: Gordon *et al.* 2004. *Nucleic Acids Res.* **32**: e133.

<http://osprey.ucalgary.ca>

OligoArray: Rouillard *et al.* 2003. *Nucleic Acids Res.* **31**: 3057-3062.

http://berry.engin.umich.edu/oligoarray2_1/

DNA-Chipdesigner: Meier *et al.* 2004. *Concurrency Computat.: Pract. Exper.* **16**: 873-893.

<http://www.bode.cs.tum.edu/~meierh/download.html>

ProbeSelect: Li *et al.* 2001. *Bioinformatics* **17**: 1067-1076

Oligodb: Mrowka *et al.* 2002. *Bioinformatics.* **18**: 1686-1687

<http://oligodb.charite.de/>

OligoWiz: Nielsen *et al.* 2003. *Nucleic Acids Res.* **31**: 3491-3496

<http://www.cbs.dtu.dk/services/OligoWiz/>

PROBESEL: Kaderali *et al.* 2002. *Bioinformatics.* **18**: 1340-1349.

<http://www.zaik.uni-koeln.de/AFS/Projects/Bioinformatics/arraydesign.html>

Oliz: Chen *et al.* 2002. *BMC Bioinformatics.* **3**: 27

<http://www.utm.edu/pharmacology/otherlinks/oliz.html>

OligoPicker: Wang *et al.* 2003. *Bioinformatics* **19**: 796-802

<http://pga.mgh.harvard.edu/oligopicker/index.html>

ArrayOligoSelector: Bozdech *et al.* 2003. *Genome Biol.* **4**: R9

<http://arrayoligosel.sourceforge.net/>

Primrose: Ashelford *et al.* 2002. *Nucleic Acids Res.* **30**: 3481-349

<http://www.cf.ac.uk/biosi/research/biosoft/>

ARB probe design: Ludwig *et al.* 2004. *Nucleic Acids Res.* **32**: 1363-1371

<http://www.arb-home.de/>

What is the “right” probe?



In silico design and
evaluation of a probe set

Thorough
empirical testing



a software environment for sequence data

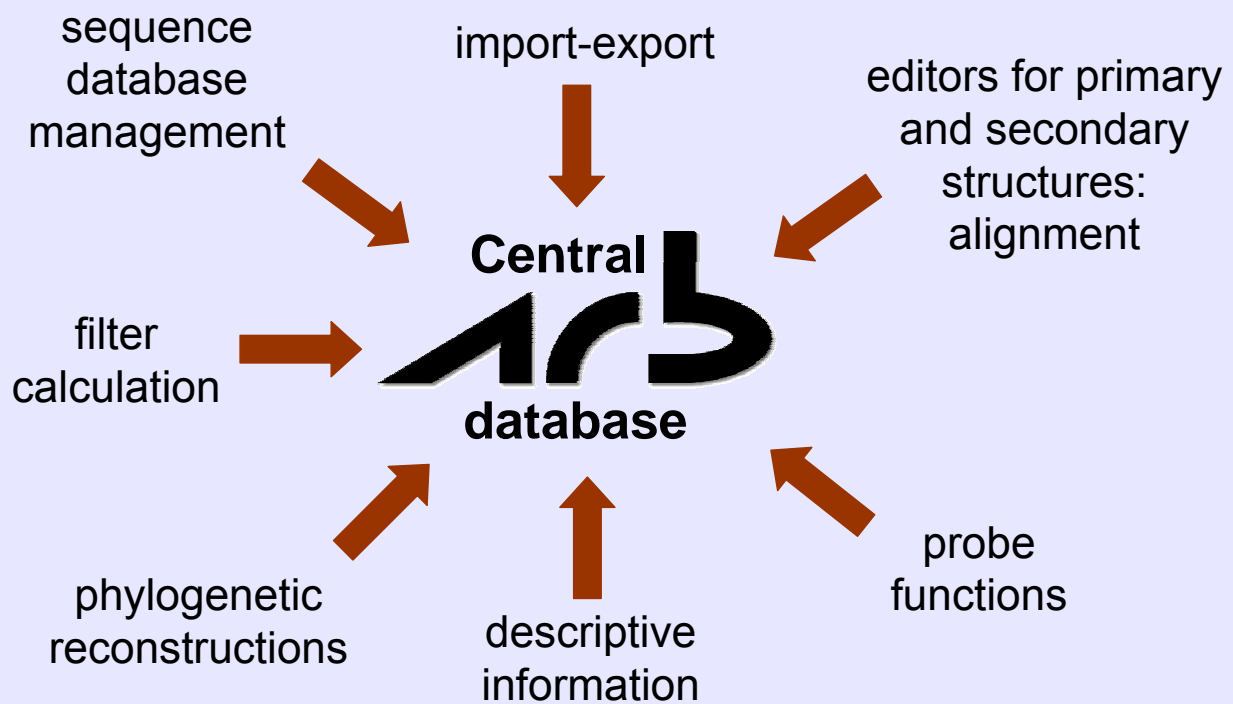
www.arb-home.de

arbor (Latin, i.e. tree)

Developed at the
**Lehrstuhl für Mikrobiologie and
Lehrstuhl für Rechnertechnik und
Rechnerorganisation, Parallelrechnertechnik
Technische Universität München**

Wolfgang Ludwig, Oliver Strunk, and many others

The organization of ARB



The organization of ARB (2)

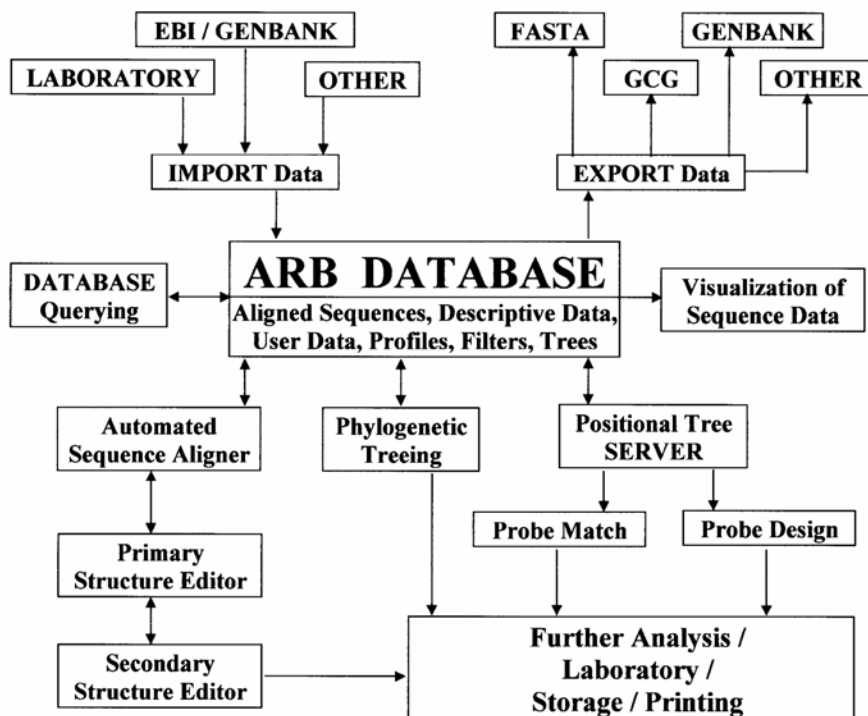


Figure 1. The interacting components and tools of the ARB software package and database.



Software implemented in ARB

- GDE
(Steven Smith, University of Illinois)
- PHYLogeny Inference Package (PHYLIP)
(Joseph Felsenstein, University of Washington)
- fastdnaML
(Joseph Felsenstein, University of Washington)
- protML-MOLPHY
(Jun Adachi and Masami Hasegawa)
- AxML
(Alexandros Stamatakis, Technical University of Munich)
- TREE-PUZZLE
(Heiko A. Schmidt, Max-Planck-Institute for Molecular Genetics)
- etc.



Where can I get ARB?

- Freely distributed over the internet:
<http://www.arb-home.de>
- Available for Linux, Unix, Apple
- Files for download
 - Installation instruction
 - ARB binaries
 - ARB installation scripts
- Databases
<http://arb-db-central.swiki.net/>



ARB documentation

www.arb-home.de

- ARB help
- FAQ
- Yahoo ARB discussion group
- Some general papers:

W. Ludwig and K.H. Schleifer, *FEMS Microbiol Rev* **15**: 155-73 (1994).

W. Ludwig et al., *Electrophoresis* **19**: 554-568 (1998).

W. Ludwig et al., *Nucleic Acids Res.* **32: 1363-1371 (2004).**



You want more?

➔ ARB workshops by W. Ludwig:
www.arb-home.de

