# DBMS-based EST Clustering and Profiling for Gene Expression Analysis

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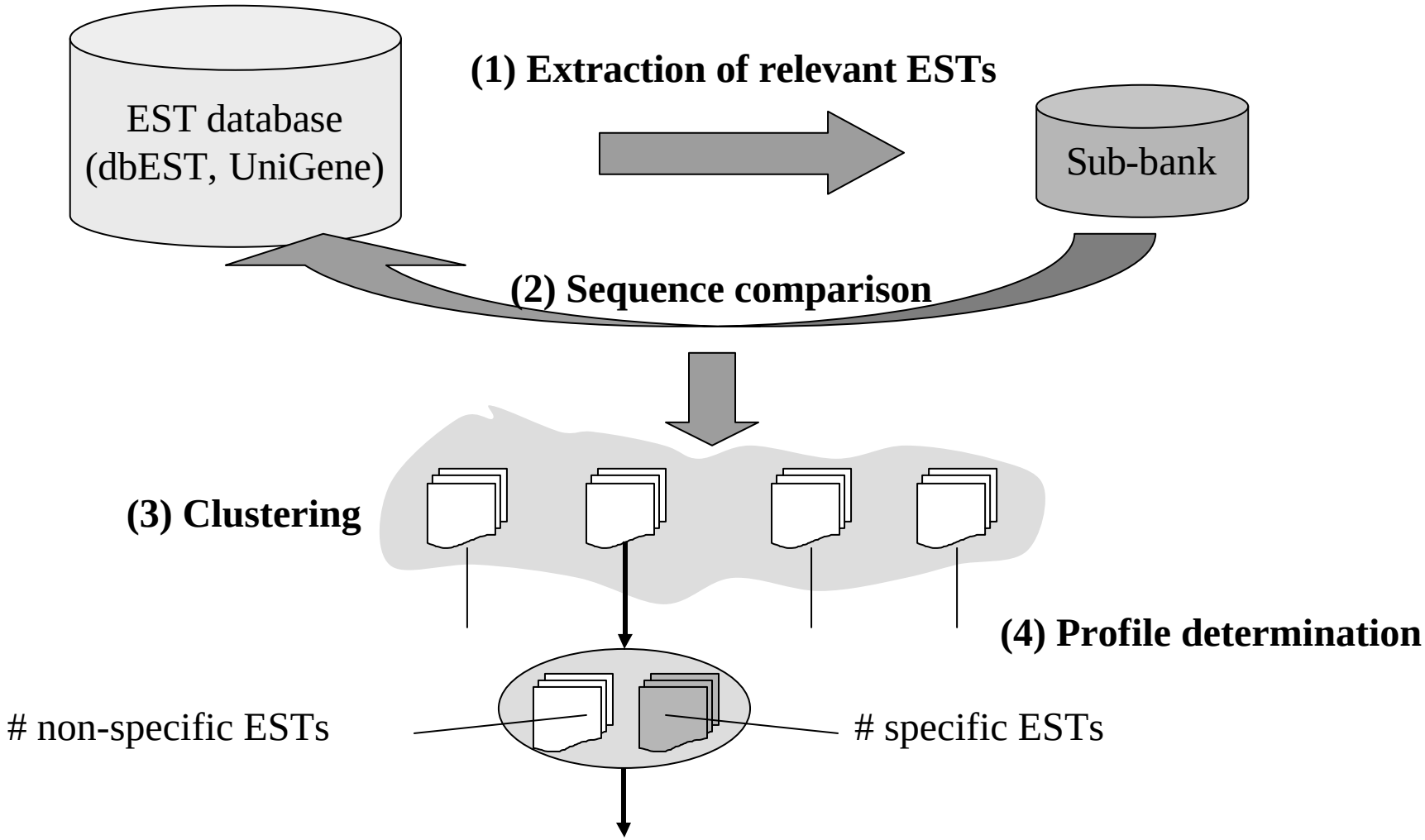
# Motivation

- Expressed Sequence Tags (EST)
  - single pass reads from randomly selected cDNA clones
  - 300-500 base pairs
  - sequences for protein coding regions: fragments of genes
- Availability of huge amounts of ESTs in public databases
  - GenBank
  - dbEST
  - UniGene, Stack, TIGR
- Steady growth due to numerous sequencing projects
  - double in size every 22 months (GenBank)

# Gene Expression Analysis Using ESTs

- Previous work:
  - Vasmatzis et al. (1997): Discovery of three new genes specifically expressed in human prostate
  - Rosenthal et al. (1999): Investigation of genes specifically expressed in normal and tumor tissues
  - other tissues/organs: thyroid (Univ. Leipzig), retina, ...
- Main approach: *In silico* differential display - expression analysis using clustered ESTs
  - EST clusters represent candidate genes
  - existence and number (count) of ESTs of a gene: qualitative and quantitative expression level of the gene in the corresponding organ or tissue
  - sequence annotation available in databases: organism, organ, tissue, cell type, disease, clone, library, ...

# *In silico* Differential Display



# Thyroid Investigation (Univ. Leipzig)

- Identification of 2200 thyroid-related sequences from dbEST
  - 1/3 are known genes (excluded)
  - 1/3 with repetitive elements (excluded)
- 38 EST subbank-specific clusters, i.e. containing only thyroid-related sequences
  - 10 clusters with more than 3 ESTs
- PCR experiments
  - expression of 6 clusters confirmed
  - 5 clusters with higher expression in thyroid than in other tissues (brain, heart, liver, lung, muscle, testis, kidney)
  - 1 cluster with expression only in thyroid

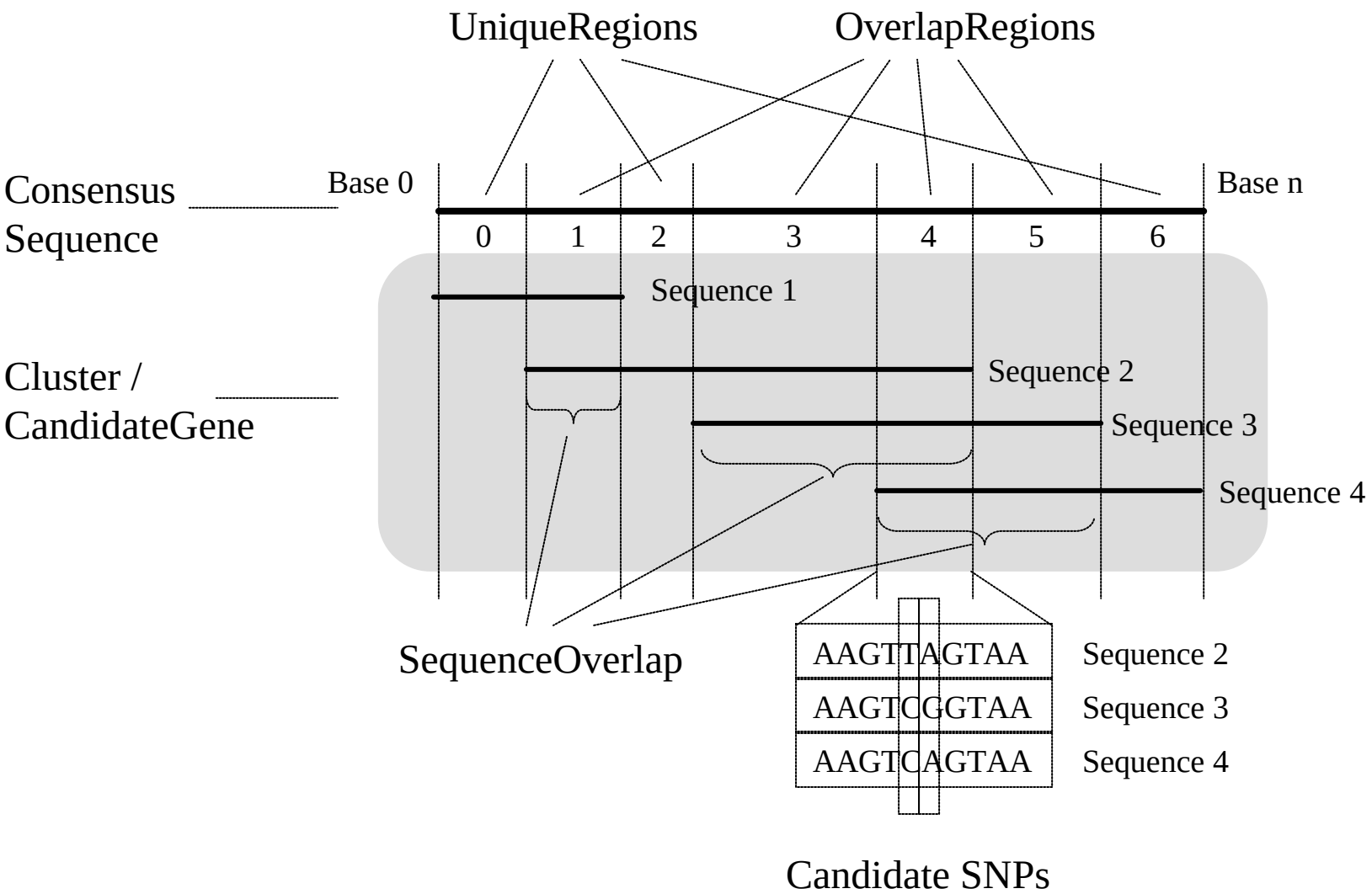
# Limitations of Previous Implementations

- Largely manual process
  - manual execution of analysis steps
  - manual evaluation of results
- Small data sets in flat file-based local data management
  - small number of ESTs taken into account
  - concentration on a particular tissue or organ in the investigation
  - small spectrum of other tissues or organ for comparison
- Generation of simple, often imprecise expression profiles
  - expression profiles of EST clusters with respect to tissues and organs
- Limited analysis possibilities
  - fixed query forms
  - access to „canned“ results from investigations previously conducted under a particular focus
  - online analysis not possible

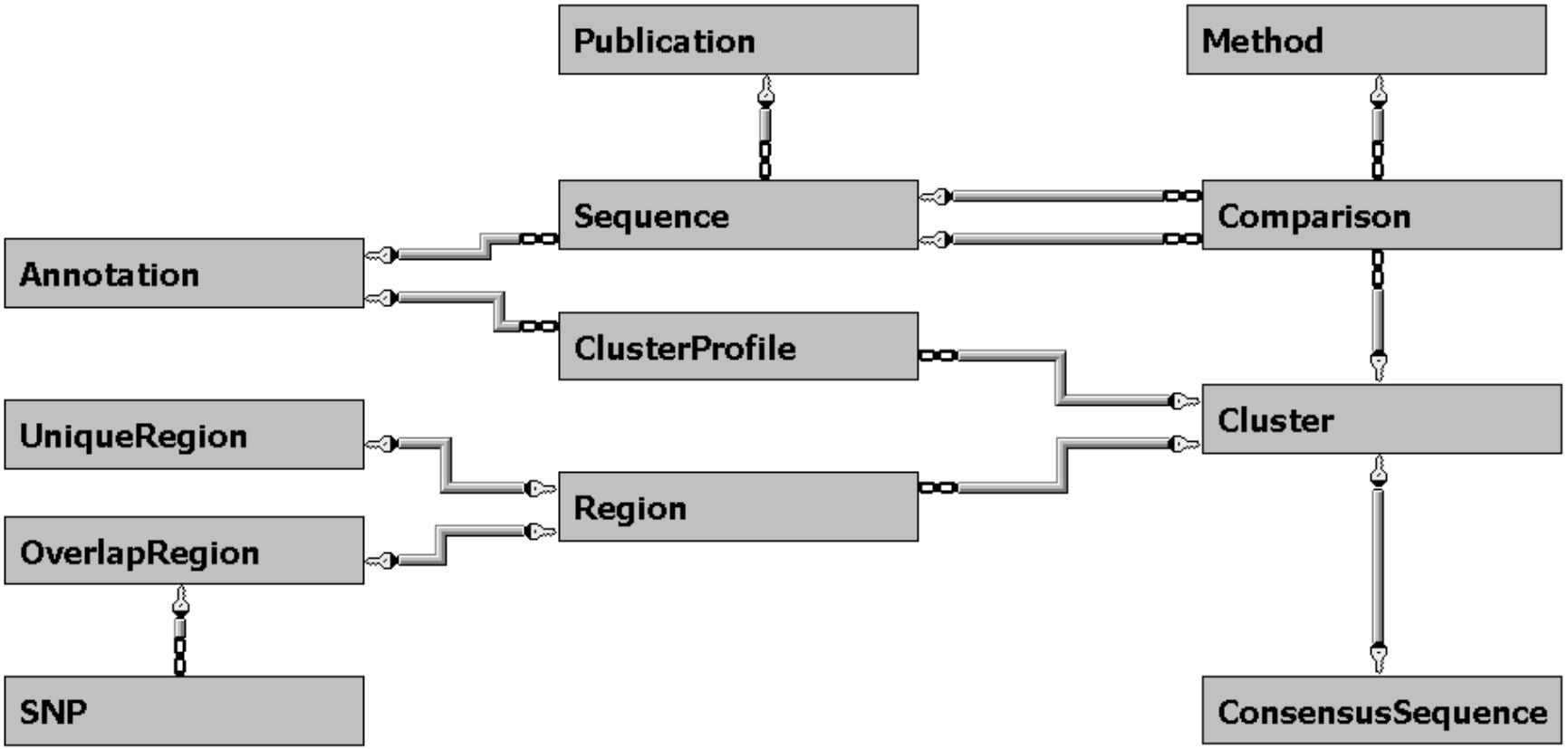
# DBMS-based Gene Expression Analysis

- Use of DBMS technologies for data management (IBM DB2, Oracle, Microsoft SQL Server, etc.)
  - huge data sets
  - flexible und declarative query languages (SQL)
  - security, performance, availability, ...
- Data integration and preparation
  - construction of a uniform data model for sequence data and analysis
  - extraction, transformation and cleaning of sequence data from different sources (public EST/cluster databases)
  - pre-computation of sequence similarities, clusters, cluster profiles to support interactive analysis
- Data analysis:
  - web-based query forms for simple queries
  - complex queries with user-friendly interfaces to SQL
  - integration of commercial tools, e.g. for data mining

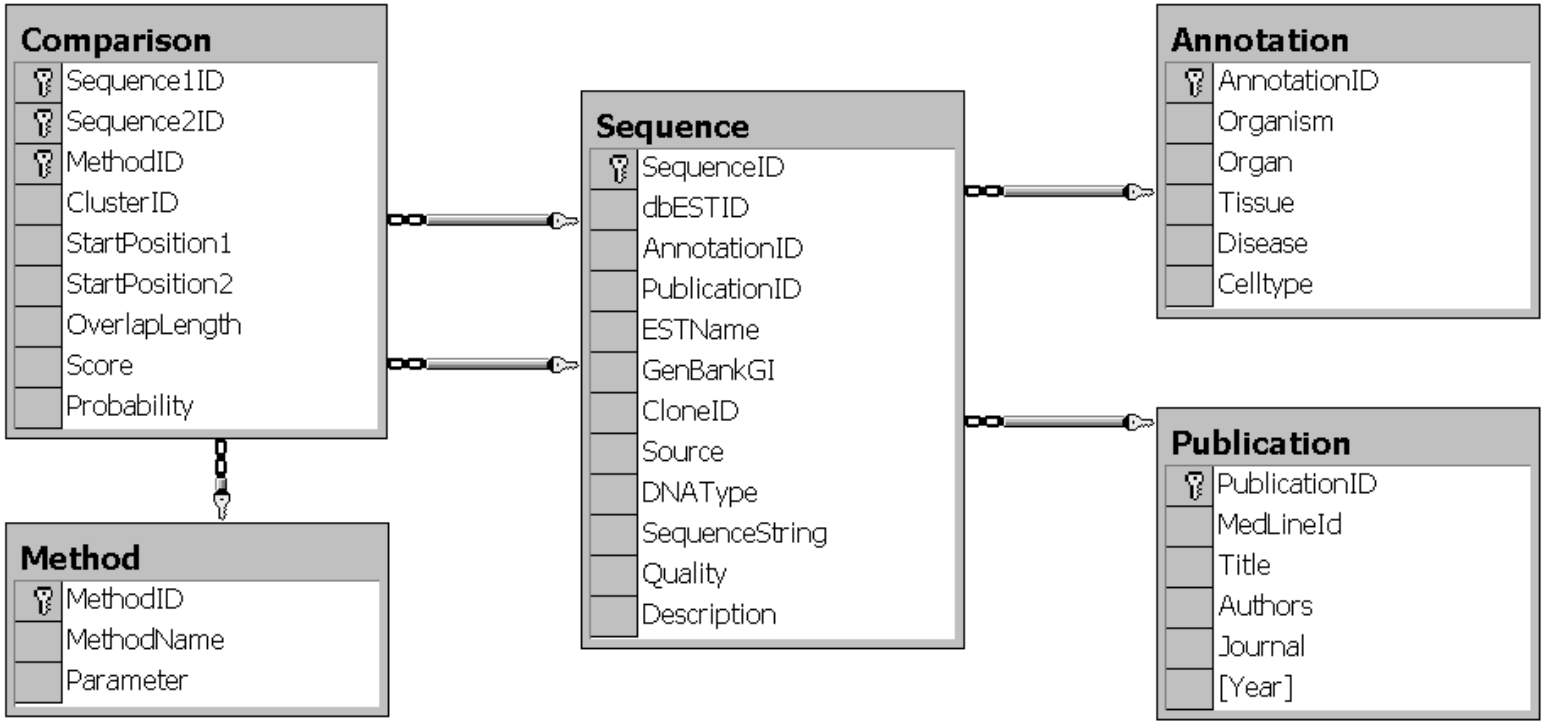
# Modeling Sequence Data



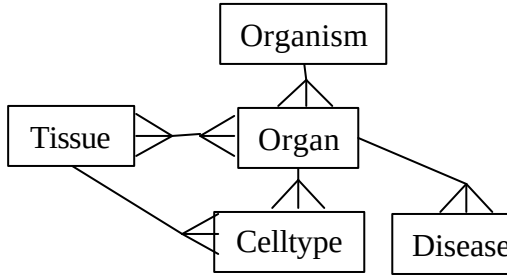
# Data Model for Sequence Data



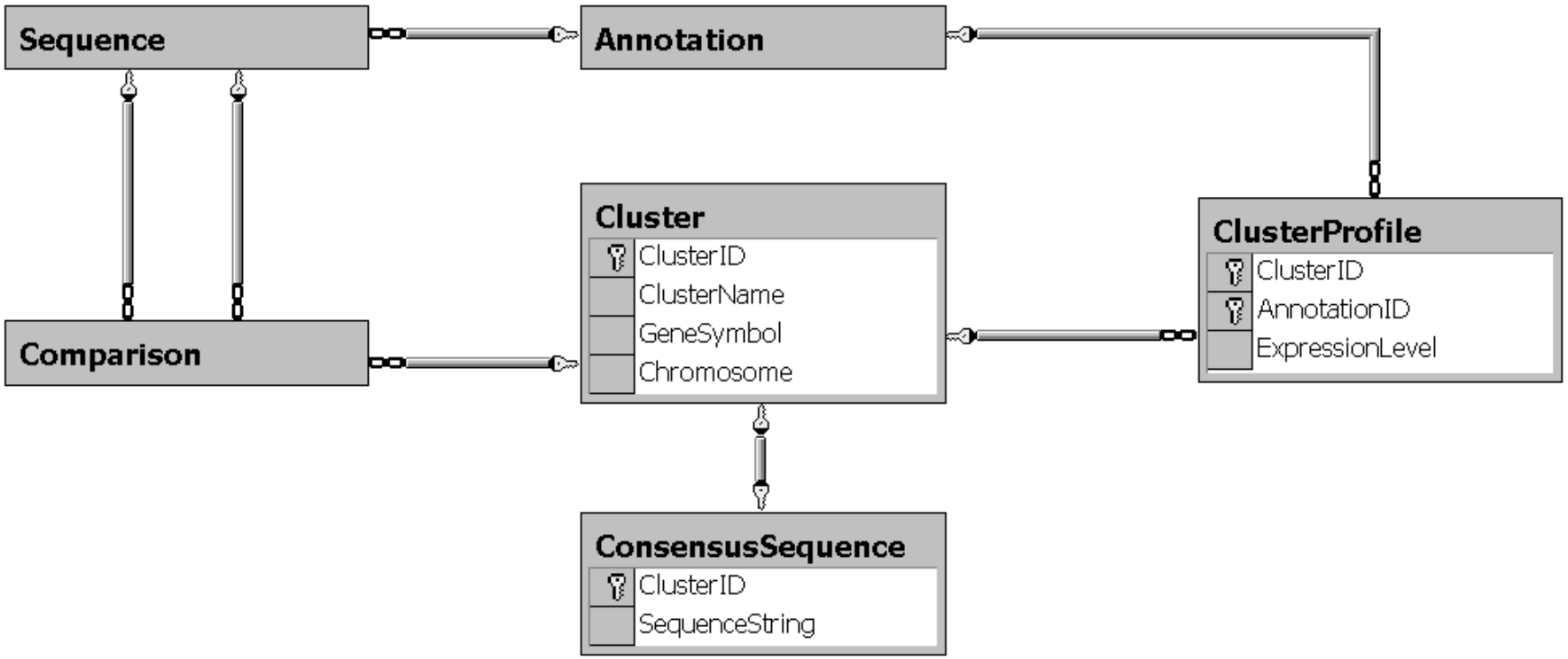
# Sequence and Sequence Similarity



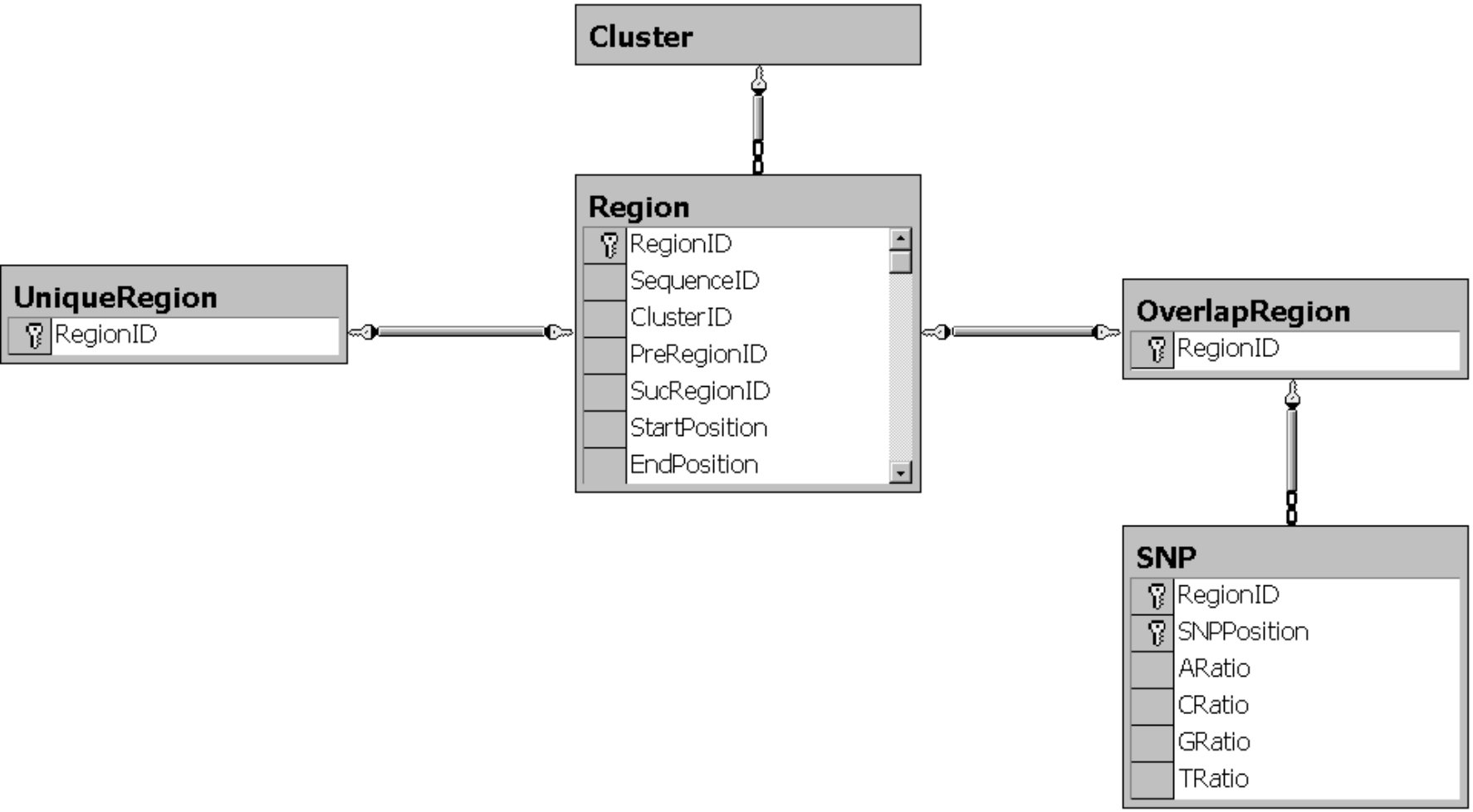

Annotation hierarchy



# Cluster and Expression Profile



# Sequence Alignment and SNP



# Data Integration and Cleaning

- Selection of relevant data sources
  - ESTs from dbEST
  - clusters from UniGene, STACK oder TIGR
- Implementation of source-specific programs (*wrappers*) for data import
  - identification of useful sequences
  - transformation of data from text files (e.g. FASTA) according to the data model
  - loading: persistent storage in the relational database
- Data cleaning and preparation
  - filter sequences with >5% Ns
  - trim leading vector sequences (sequences from automated sequencers), polyA tail
  - mask low complexity and repetitive regions
- Update
  - e.g. periodical import of new sequences from dbEST

# Sequence Comparison and Clustering

- Use of DBMS-proprietary functions for text comparison and text search, e.g. *like*, *score* in Oracle
  - low cost, good performance
  - inflexible: Standard-Scoring-Function for natural language and not optimized for sequences, alignment not possible
- Use of existing programs (BLAST, FASTA, ...)
  - flexible parameterization, optimized for sequences
  - operate on flat files (input and output)
- Ideal: Extension of the DBMS with an API to BLAST, etc.
  - sequence comparison and search within DBMS
- Assessing cluster quality:
  - storing only similarity scores greater than a threshold (e.g. 95% in a defined number of nucleotides) in the database
  - minimal number of ESTs per cluster
  - minimal number of ESTs per tissue/organ

# Analysis Possibilities (1)

- Support large amounts of sequence data: Broad spectrum of
  - ESTs
  - tissues, organs, etc.
  - genes
- Flexible generation of expression profiles
  - qualitative as well as quantitative expression profiles
  - more accurate expression profiles
- Integrated platform for sequence analysis
  - gene expression analysis
  - consensus sequences, sequence alignment, SNP, etc.

# Analysis Possibilities (2)

- Interactive online analysis possible due to pre-computed sequence similarities and clusters
- Support common analysis tasks:
  - discovery of new candidate genes, which are specifically expressed in a particular tissue or organ
- Support comparative analysis tasks:
  - large-scale (at genome level) correlation analysis: identification of correlated expression patterns of
    - tissues with respect to genes
    - genes with respect to tissues
  - discovery of candidate genes with similar functions, and tissues and cells with mit similar expression profiles
- Support automatic approaches

# Summary

- *In silico* differential display for expression analysis:
  - simple and effective approach for predicting (new) specifically expressed candidate genes
  - computation-intensive, time-consuming process due to large amounts of sequence data
  - currently performed largely manually
- Application of DBMS technologies
  - local DBMS-based management of all kinds of data, in particular sequence and annotation, sequence similarity and cluster
  - pre-computation of sequence similarities, clusters: performance for online analysis
  - improvement and automation of the previous *in silico* differential display approach
  - problems: integration of existing tools for sequence comparison, alignment and clustering
- Combination with other methods for expression analysis
  - Microarrays, RT-PCR, SAGE