University Medical Center Freiburg
Department of Hematology/Oncology
Core facility II Genomics

Omics data based on Affymetrix GeneChip arrays -

from assay to analysis

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- Academic service lab for microarray technologies
- Department of Hematology & Oncology
- Affymetrix platform
- 2 Scientists, 1 MTA
- 1 Bioinformatician 50%
- 300 processed arrays in 2008 (2009: 250 until now)
- service based on collaborations

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Collaborations outside the department

- Within Freiburg:
  - AG Rospert, AG Pahl, AG Zirlik
  - AG Schueler/Oncotest
  - AG Eibel, AG Jumaa, AG Walker, AG Warnatz
- Outside Freiburg
  - AG Ch.Klein, MHH Hannover
  - A.Schaffer, NIH
  - AG B.Grimbacher, London
  - EuroGENEScan Project

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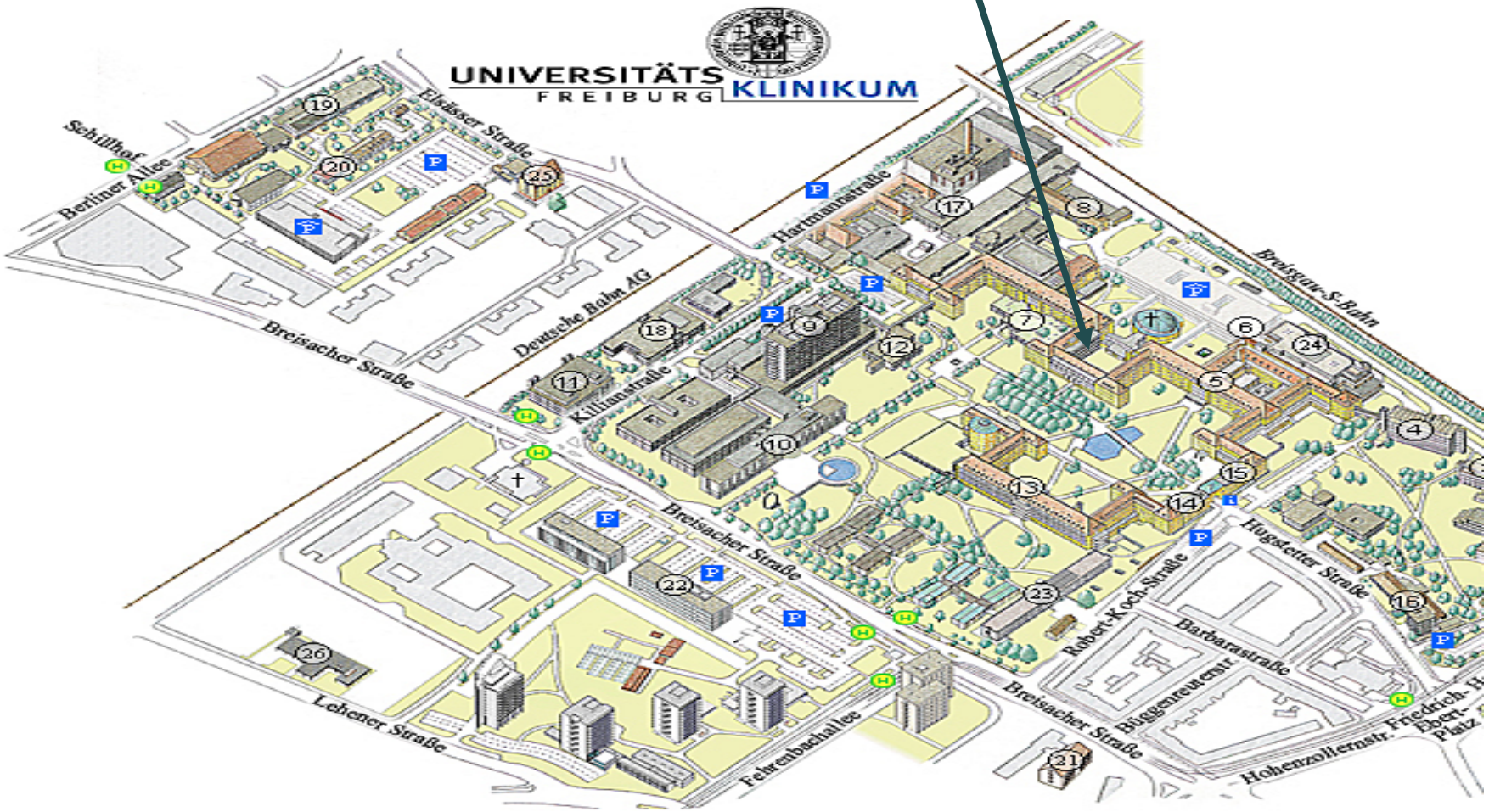

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Fields of Applications

- DNA genotyping & Homozygosity Mapping
- Copy number and LOH
- CLL diagnostic panel
- Gene expression
- *Medip-Chip on tiling arrays*

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Genotyping with SNP arrays

- SNP = Single Nucleotide Polymorphism (frequency of 1% in population)
- started in 2005 with 10k arrays, subsequently upgraded to 50k/250K/SNP 6.0
- Human Genome-wide SNP 6.0 array: 1.8 million genetic markers
  - More than 906,600 SNPs (Tag SNPs , SNPs from chr. X and Y)
  - Mitochondrial SNPs, SNPs in recombination hotspots
  - **In addition**:
  - More than 946,000 copy number probes:
  - 202,000 probes targeting 5,677 CNV regions from the Toronto Database of Genomic Variants
  - 744,000 probes, evenly spaced along the genome

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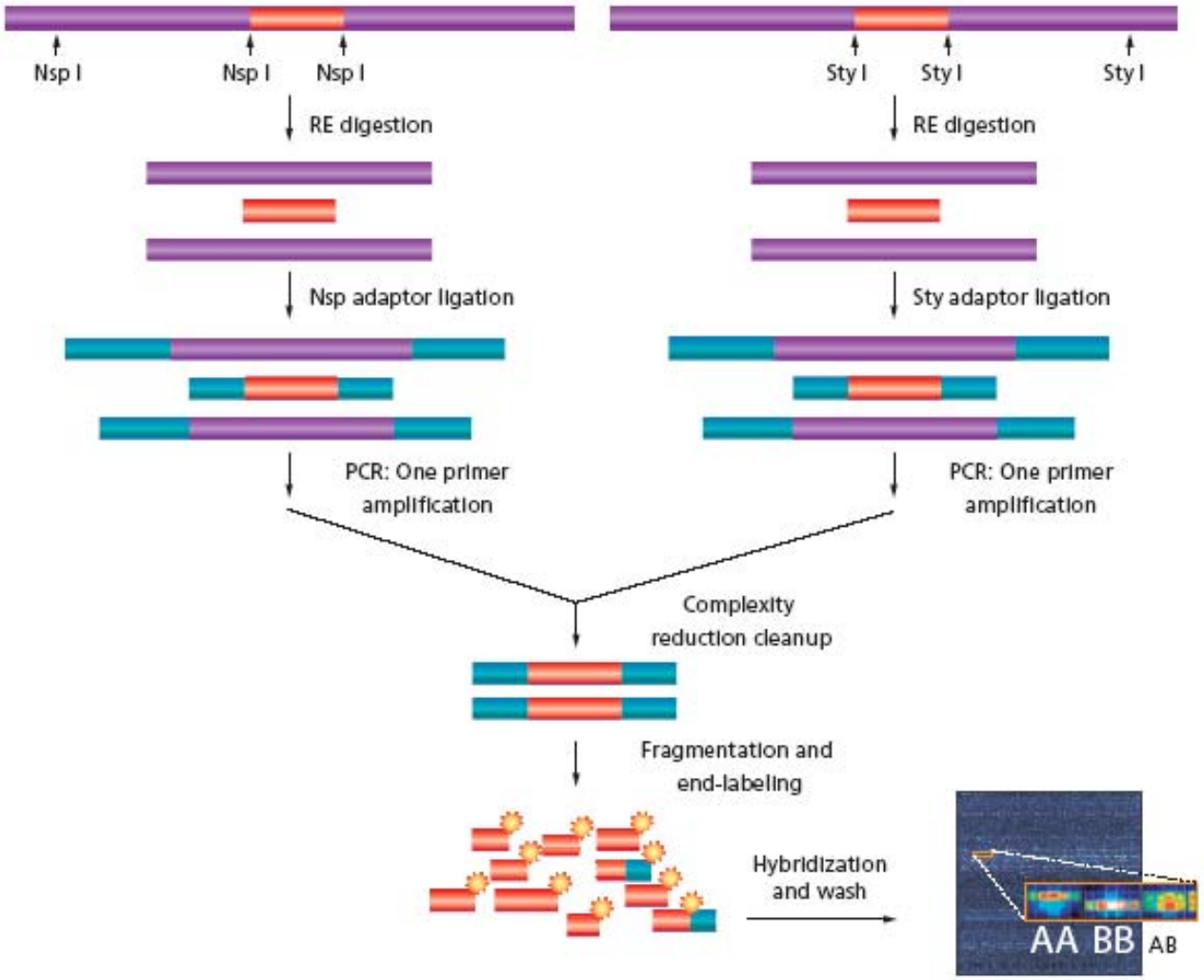
Assay workflow



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Analysis workflow

- Genotyping Console software (Affymetrix)
- Birdseed clustering-algorithm for genotype calling (AA or AB or BB for each SNP) using >100 samples in parallel
- Homozygosity mapping
  - Identify disease-associated regions in consanguineous families with recessive traits
  - Screening for regions with runs of homozygous SNP markers only in affected Individuals

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Copy number and LOH

- Identify genomic gains and losses with the highest resolution (SNP 6.0 array)
- Copy numbers are derived against a reference set
- no structural aberrations can be identified directly
- Genotype information enables LOH detection
  - Copy-neutral LOH, UPD
- software: Genotyping Console, Partek GS

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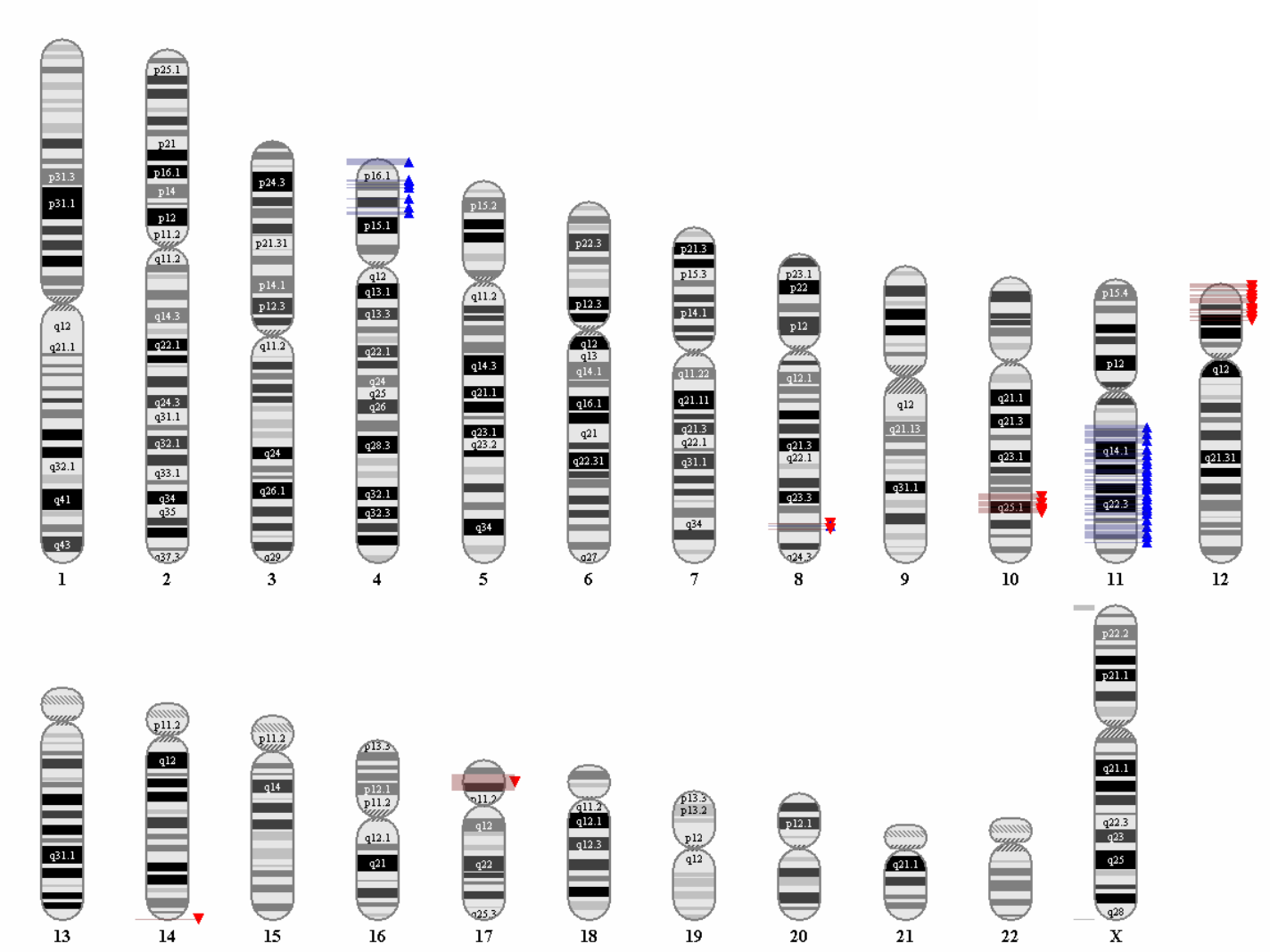
Karyoview of copy-number segmentation results



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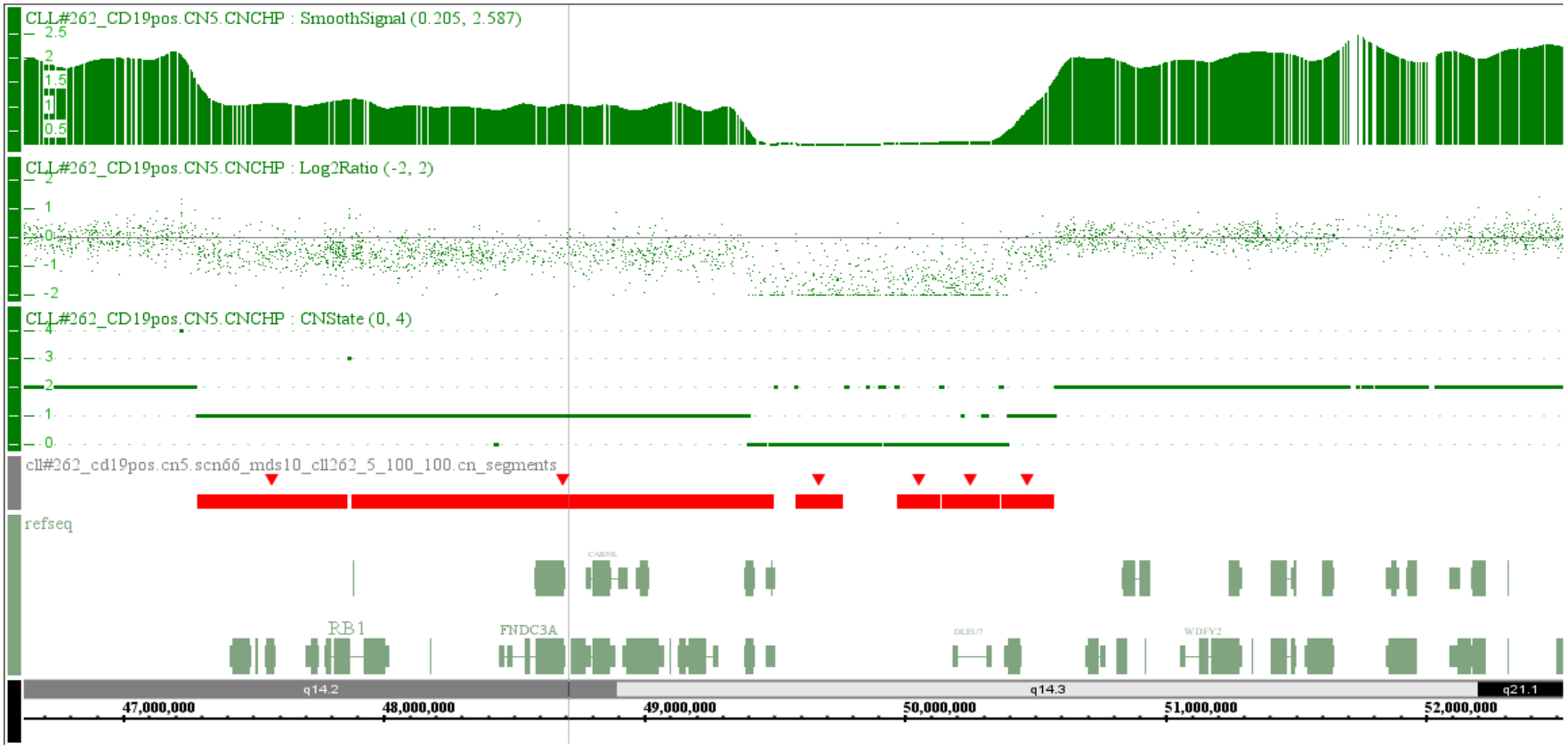
Bi-allelic deletion of chromosome 13q14



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CLL diagnostic panel

- Chronic lymphocytic leukemia (CLL) is characterized by recurrent genomic aberrations with prognostic value
- SNP arrays as tool for copy number analysis to identify gains and losses in CLL tumor cells
- CLL database with 200 genomic profiles
- around 40 new patients p.a.
- extended to other hematological malignancies (MDS, MPS)

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Project-related Publications from the Core

- Becker H, **Pfeifer D**, Afonso JD, Nimer SD, Veelken H, Schwabe M, Lubbert M. Two cell lines of t(8;21) acute myeloid leukemia with activating KIT exon 17 mutation: models for the 'second hit' hypothesis. **Leukemia**. 2008;22:1792-1794.
- **Pfeifer D**, Woellner C, Petersen A, Pietrogrande MC, Franco JL, Yeganeh M, Ehl S, Matamoros N, Sprecher E, Puck JM, Veelken H, Grimbacher B. The hyper-IgE syndrome is not caused by a microdeletion syndrome. **Immunogenetics**. 2007;59:913-926.
- **Pfeifer D**, Pantic M, Skatulla I, Rawluk J, Kreutz C, Martens UM, Fisch P, Timmer J, Veelken H. Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. **Blood**. 2007;109:1202-1210.
- Pop R, Conz C, Lindenberg KS, Blesson S, Schmalenberger B, Briault S, **Pfeifer D**, Scherer G. Screening of the 1 Mb SOX9 5' control region by array CGH identifies a large deletion in a case of campomelic dysplasia with XY sex reversal. **J Med Genet**. 2004;41:e47.

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3‘ expression arrays

- Affymetrix 3‘ expression arrays as standard for whole-genome expression profiling tool with more than 11.000 papers published
- multiple independent probes for each gene
- probes derived mostly from 3‘ end
- assay starts with oligo-dT priming
- Now successively replaced by new-generation Gene and Exon arrays

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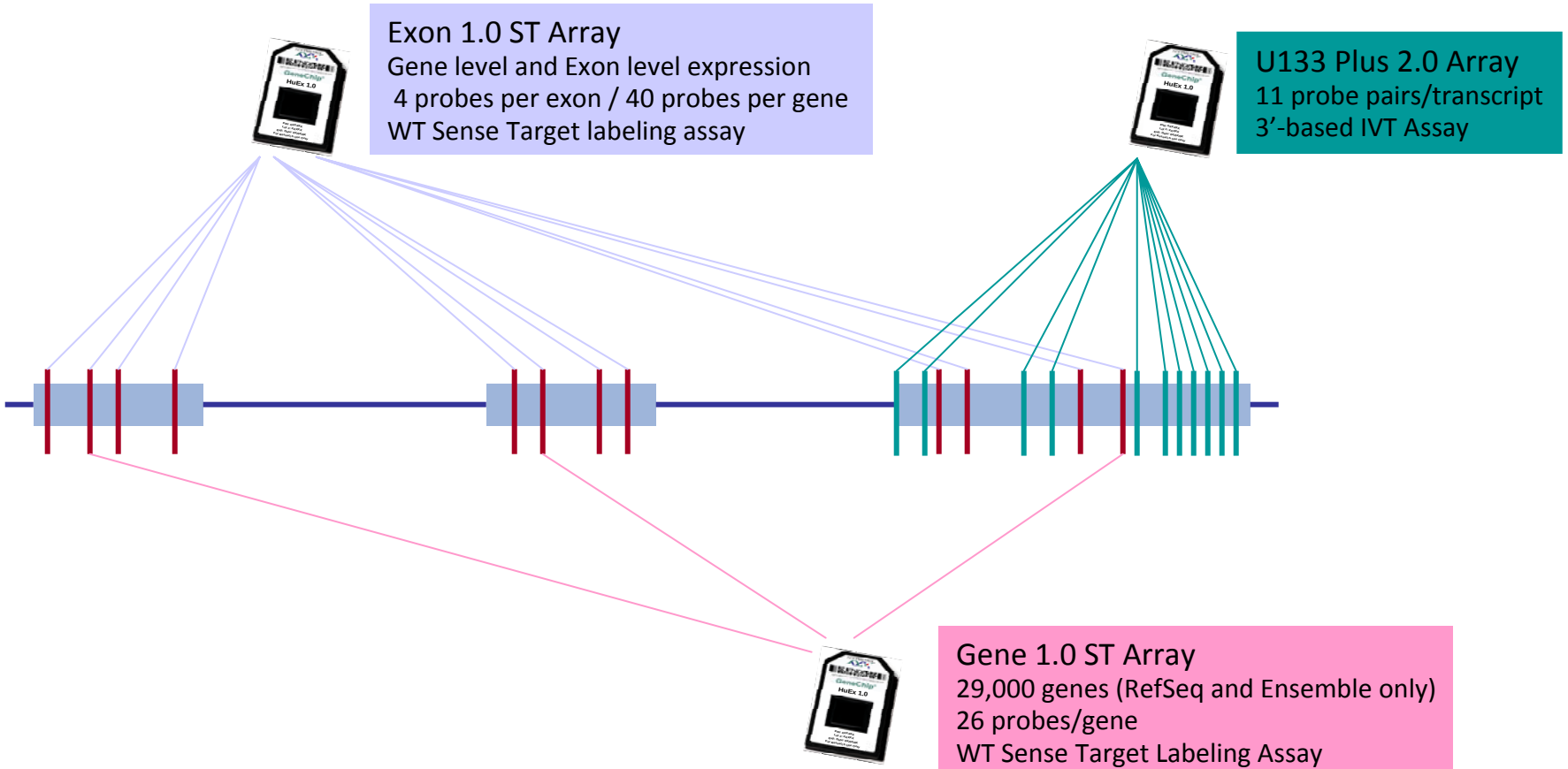
Array comparison



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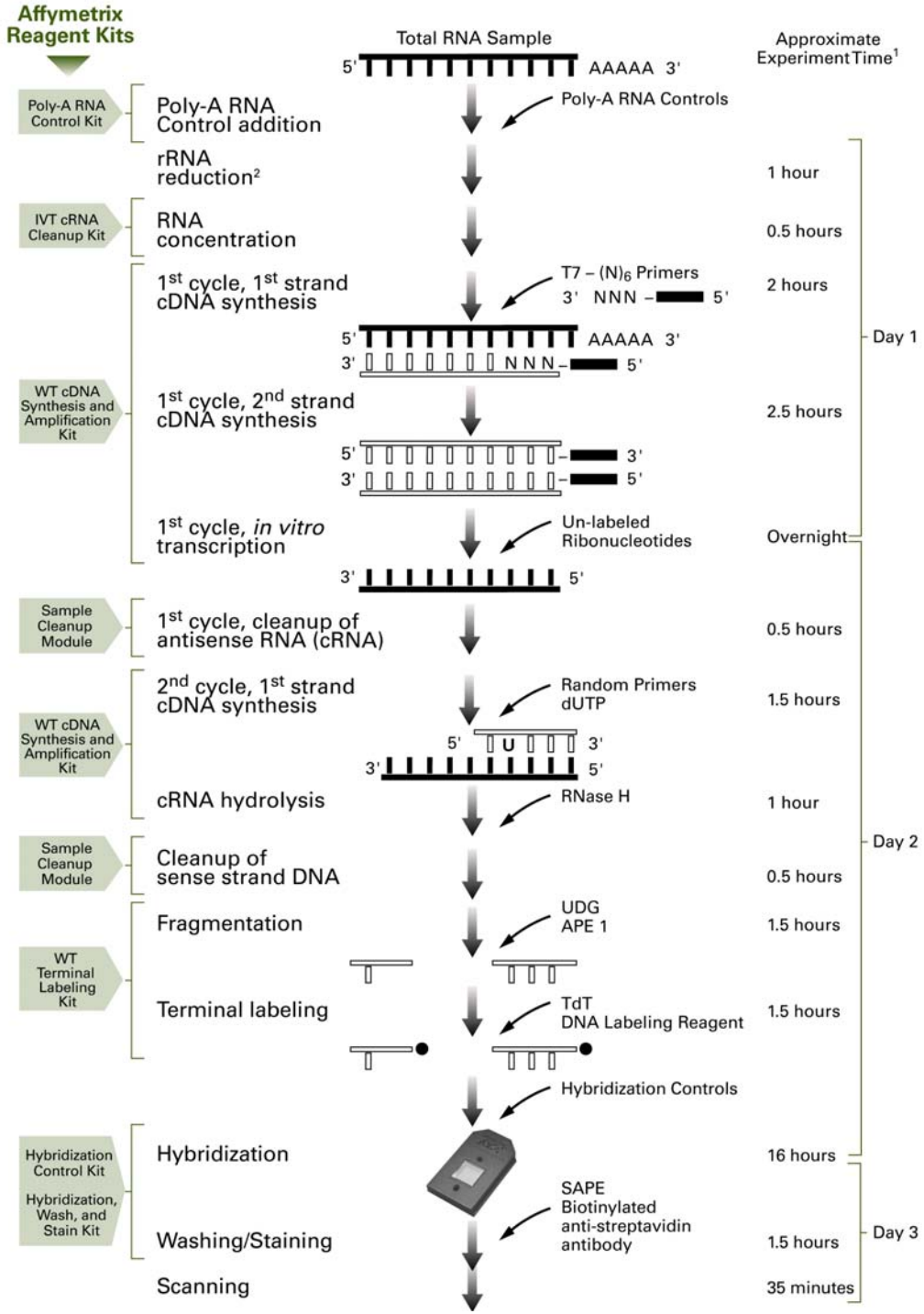
WT Assay workflow

Assay QC steps:

- RNA Integrity
- Ribominus depletion
- cRNA yield
- cDNA yield
- Fragmentation efficiency
- Labeling efficiency



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Assay Comparison

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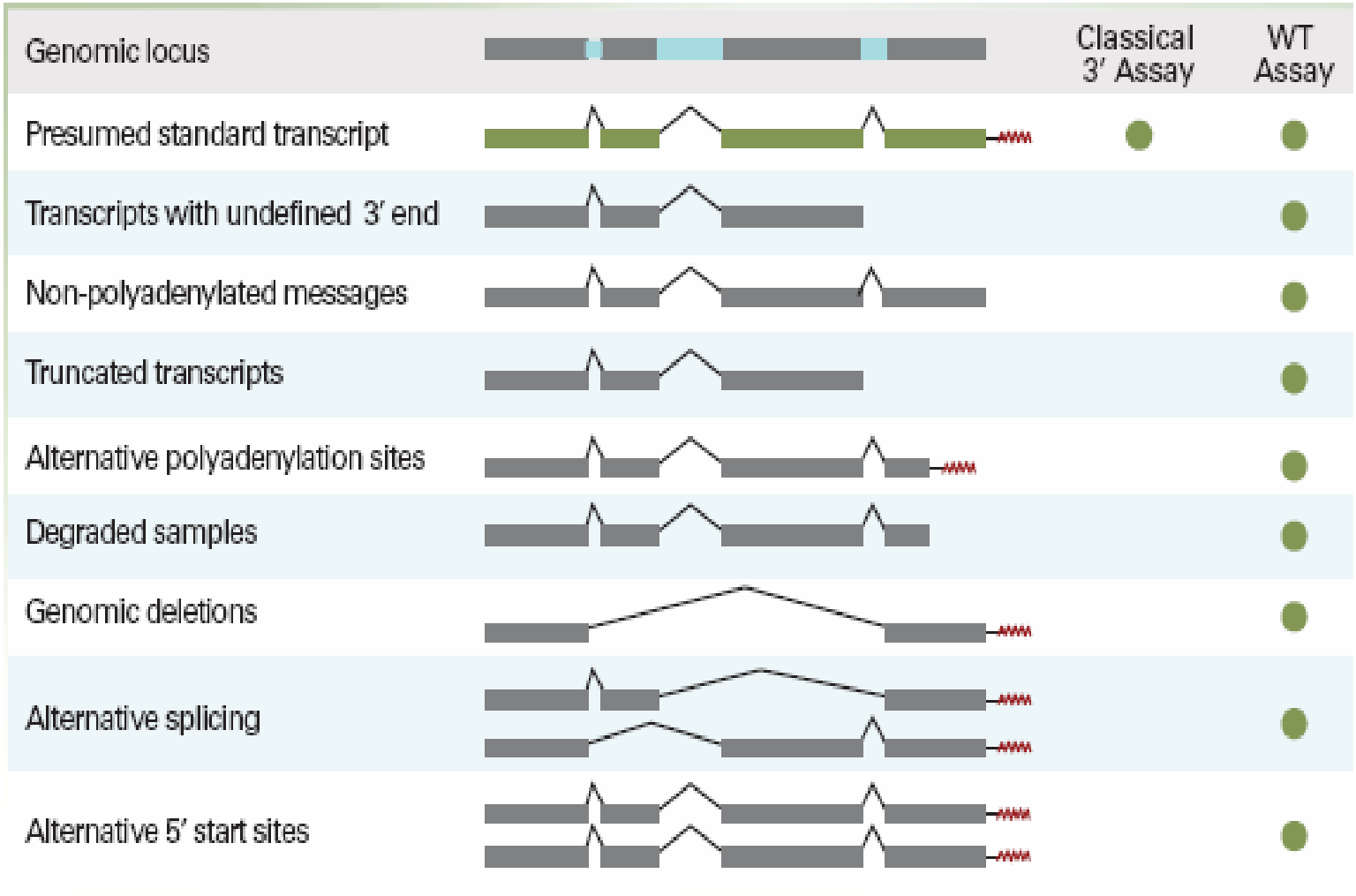

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Data Analysis

- QC in Expression Console/Affymetrix
  - monitor assay and hybridization controls
- Further data analysis in Genedata Expressionst and Partek GS
  - normalisation and summarization, data filtering
  - PCA to detect outliers
  - identify differentially expressed genes by applying statistical tests and/or fold-change analysis
  - Biological interpretation: Fisher‘s exact test with GO and KEGG pathways, GSEA
- Pathway analysis with Ariadne Pathway Studio software

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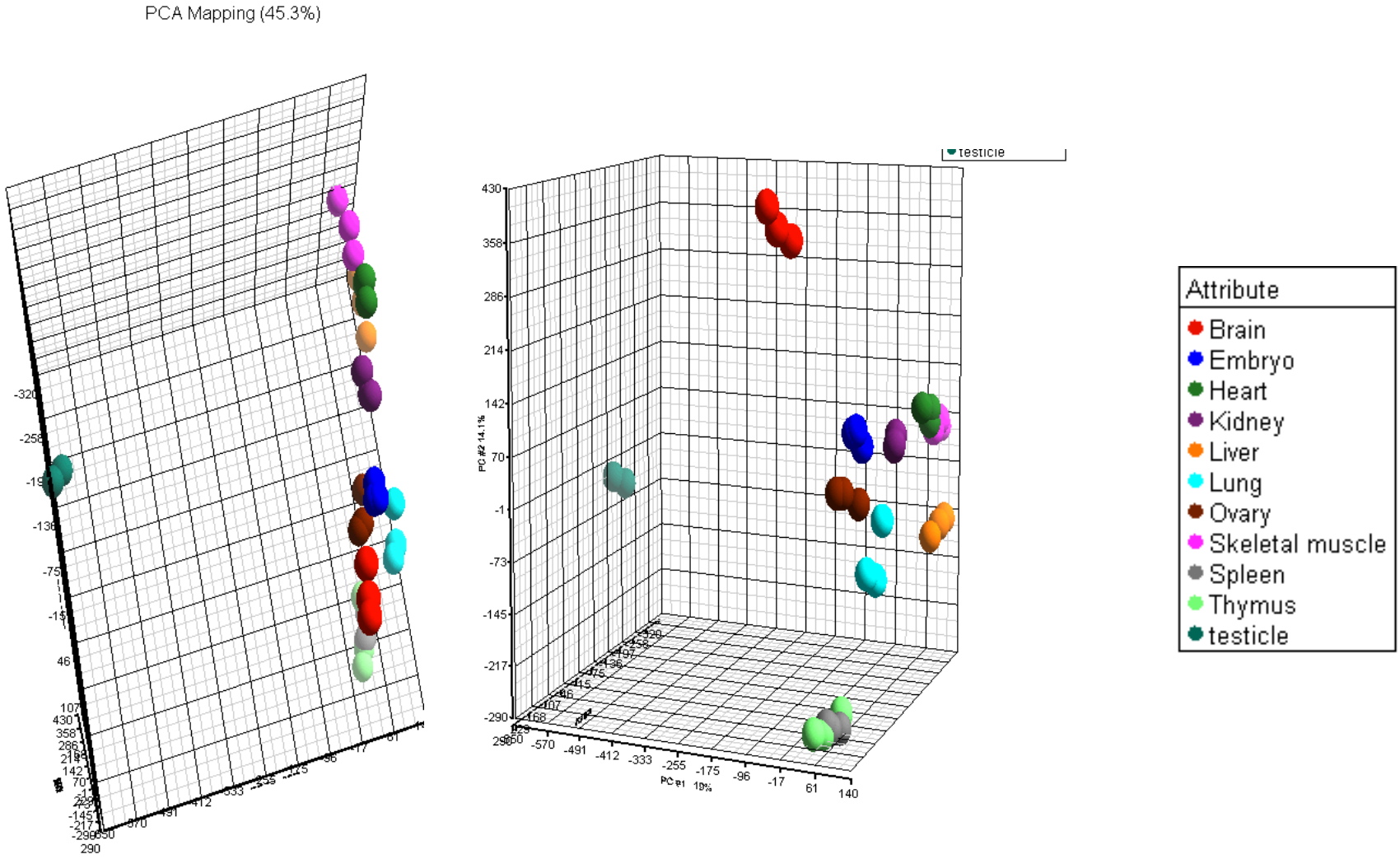
PCA for outlier detection



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- Plehwe U, Berndt U, Conz C, Chiabudini M, Fitzke E, Sickmann A, Petersen A, **Pfeifer D**, and Rospert S. The Hsp70 homolog Ssb is essential for glucose-sensing via the SNF1 kinase network. **accepted in Genes & Development** (2009).
- Buchner M, Fuchs S, Prinz G, **Pfeifer D**, Bartholomé K, Burger M, Chevalier N, Vallat L, Timmer J, Gribben J, Jumaa H, Veelken H, Dierks C, Zirlik K. Spleen Tyrosine Kinase (SYK) is Overexpressed and Represents a Potential Therapeutic Target in Chronic Lymphocytic Leukemia. **accepted in Cancer Research** (2009).
- Zimmermann S, Biniossek ML, Pantic M, **Pfeifer D**, Veelken H, and Martens UM Proteomic profiling of tumor cells after induction of telomere dysfunction. Proteomics *9*, 521-534 (2009).
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- Jager S, Jahnke A, Wilmes T, Adebahr S, Vogtle FN, Delima-Hahn E, **Pfeifer D**, Berg T, Lubbert M, Trepel M. Leukemia-targeting ligands isolated from phage-display peptide libraries. **Leukemia**.21:411-420 (2007).