Exome sequencing at the CCG

“NGS an der Schnittstelle von Grundlagen- und translationaler Forschung"

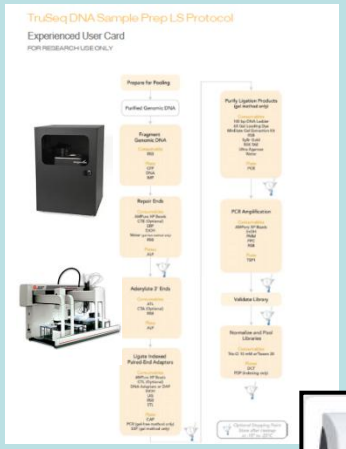
Peter Nürnberg

TMF-Workshop

Berlin, 7.12.2012

The exome sequencing pipeline

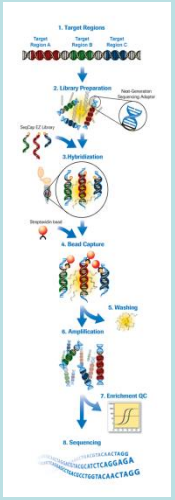
library prep



QC

80samples

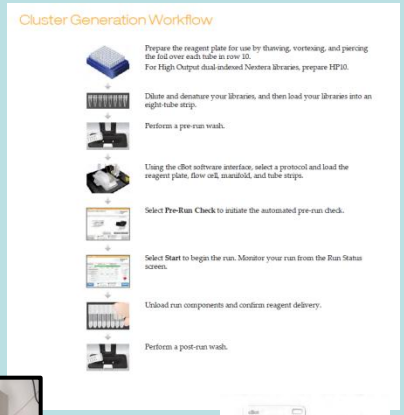
enrichment



qPCR

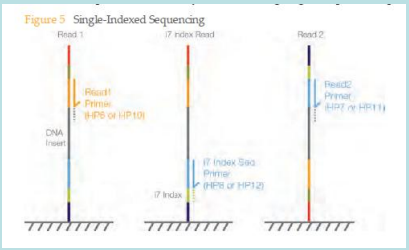
16pools

cluster generation



16 lanes
on 2 flowcells

sequencing



600Gb = raw data
7.5Gb per sample

analysis

DNA QC

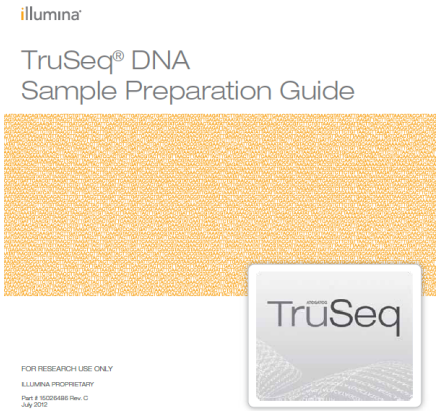
Cologne Center for Genomics

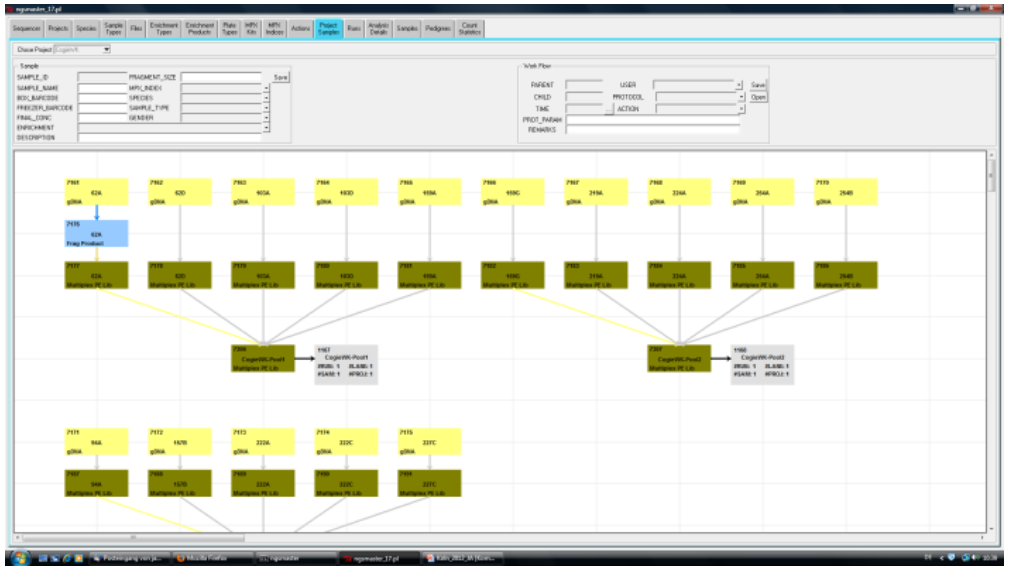
From DNA to library

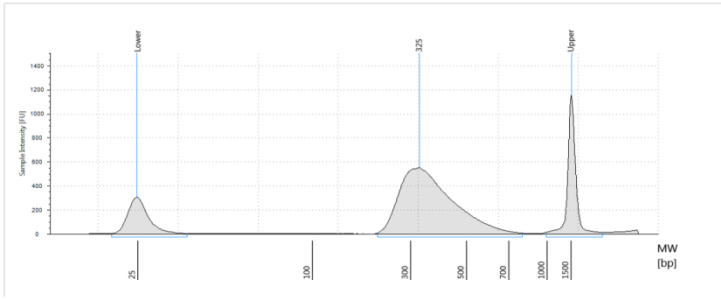
- DNA QC and input in the data base
- Library prep

(fragmentation, end repair , adenylate 3`ends,
Adapter ligation, purification, amplification, validation)

Beckman Coulter. Biomek® FXP







G01_Lib

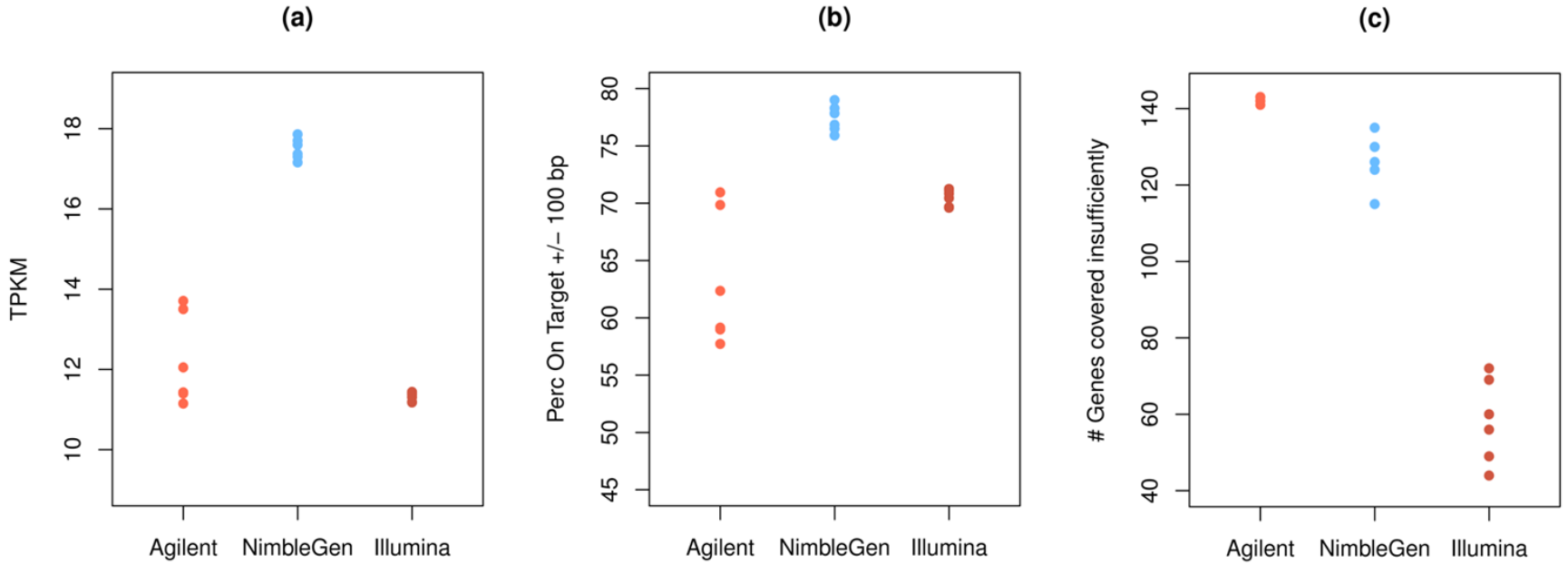
Wavelength	MW [bp]	Conc. [ng/µl]	Molarity [nmol/l]	% of Integrated Area	Peak Comment	Observations
Sample	25	4.46	271			Lower Marker
Sample	325	20.8	96.9	100.00		
Sample	1,500	6.50	6.57			Upper Marker

Enrichment: different products

company	products	Size and content	Ease of use	Performance Q1/2011	Price Q1/2011
Agilent	V1 50Mb V4 V4+UTR	38Mb 51Mb 51Mb 71Mb	x x(+) x x	(3.)	
Nimblegen	Array V2 V3	34Mb 44Mb 64Mb	x x x	(1.)	(+)
Illumina	TruSeq Nextera	62Mb	x	(2.)	

Assessing the enrichment performance in targeted resequencing experiments

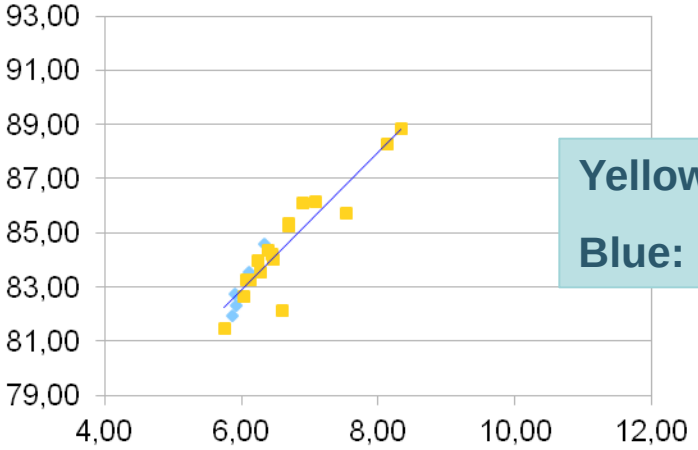
Peter Frommolt et al., Hum Mut 2011



TPKM = On-**T**arget reads **P**er **K**ilobase target region and **M**illion mappable reads

Nimblegen v2: strategy considerations

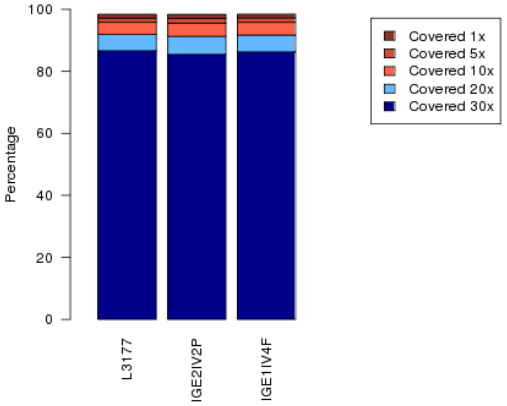
% of target > 30X



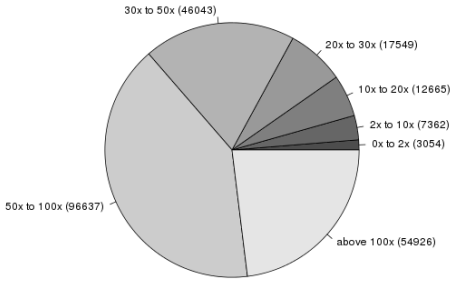
Yellow: pre-capture pooling

Blue: post-capture pooling

[Gb]

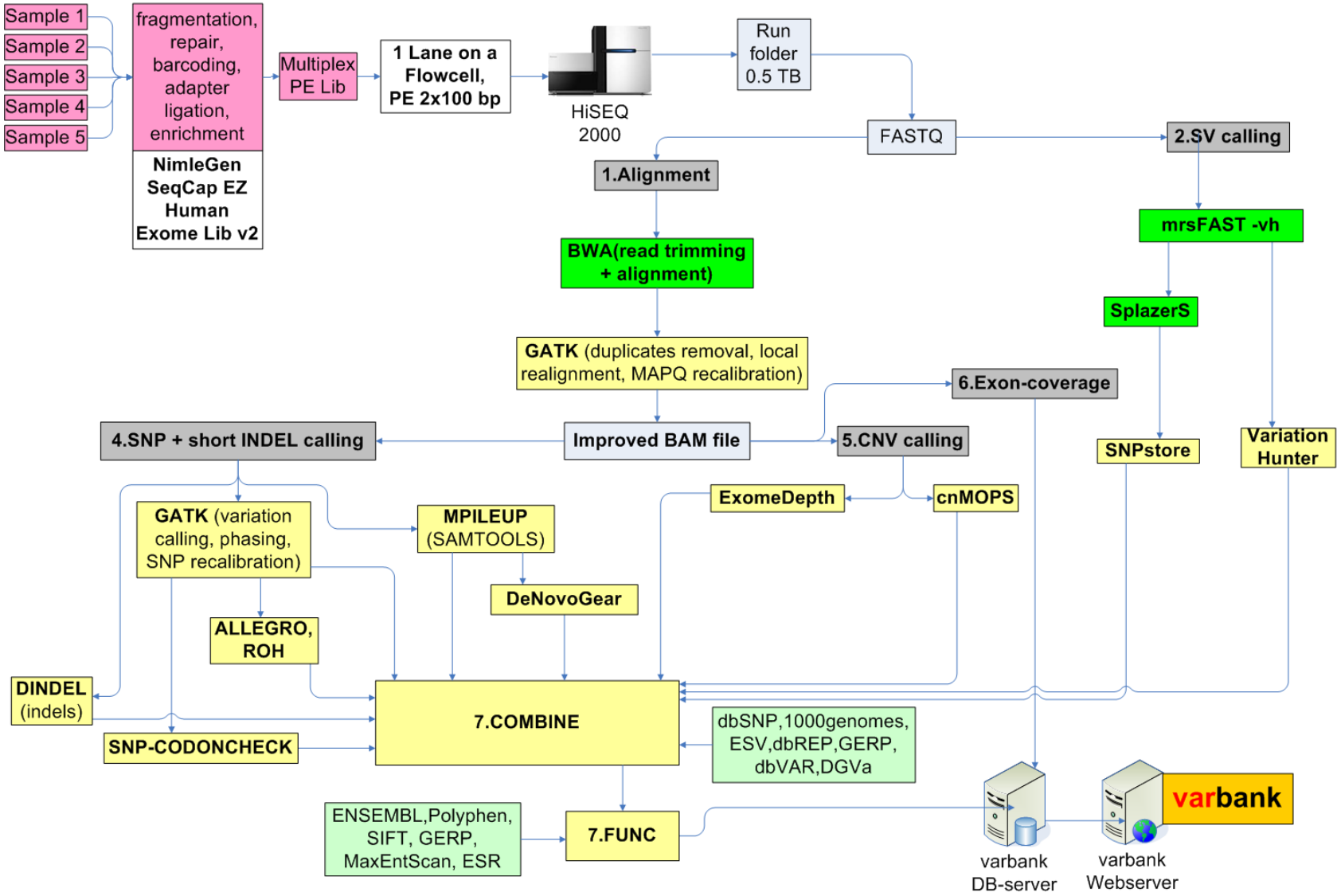


	ideal	real
Raw data	7.5Gb	5-10Gb
aligning	(all)	90-92%
Aligning to target sequence	(all)	72-78%



# Reads	64620968
# On Target ± 100 bp	44549948
Target Size (bp)	44234111
# Target Regions	238236
Coverage Mean	77.27
Coverage Std Dev	64.9
Covered 1x	97.93%
Covered 5x	96.46%
Covered 10x	94.61%
Covered 20x	89.55%
Covered 30x	82.45%
TPKM	15.59

Exome Sequencing Pipeline at the Cologne Center for Genomics



Goals for a new exome analysis pipeline

- **Establishing analysis pipeline on HPC Clusters available at the Regional Computing Center Cologne (CHEOPS/SUGI)**
- **Improving alignments**
- **Expanding the range of SNP/short indel callers**
- **Adding support for structural variations and CNVs**
- **Building up a new database and web tools to have remote access to variants, files, statistics, etc.**
- **Design the web tool to make variant browsing, filtering and interpretation as easy as possible**

11/2012
V.2.0.1

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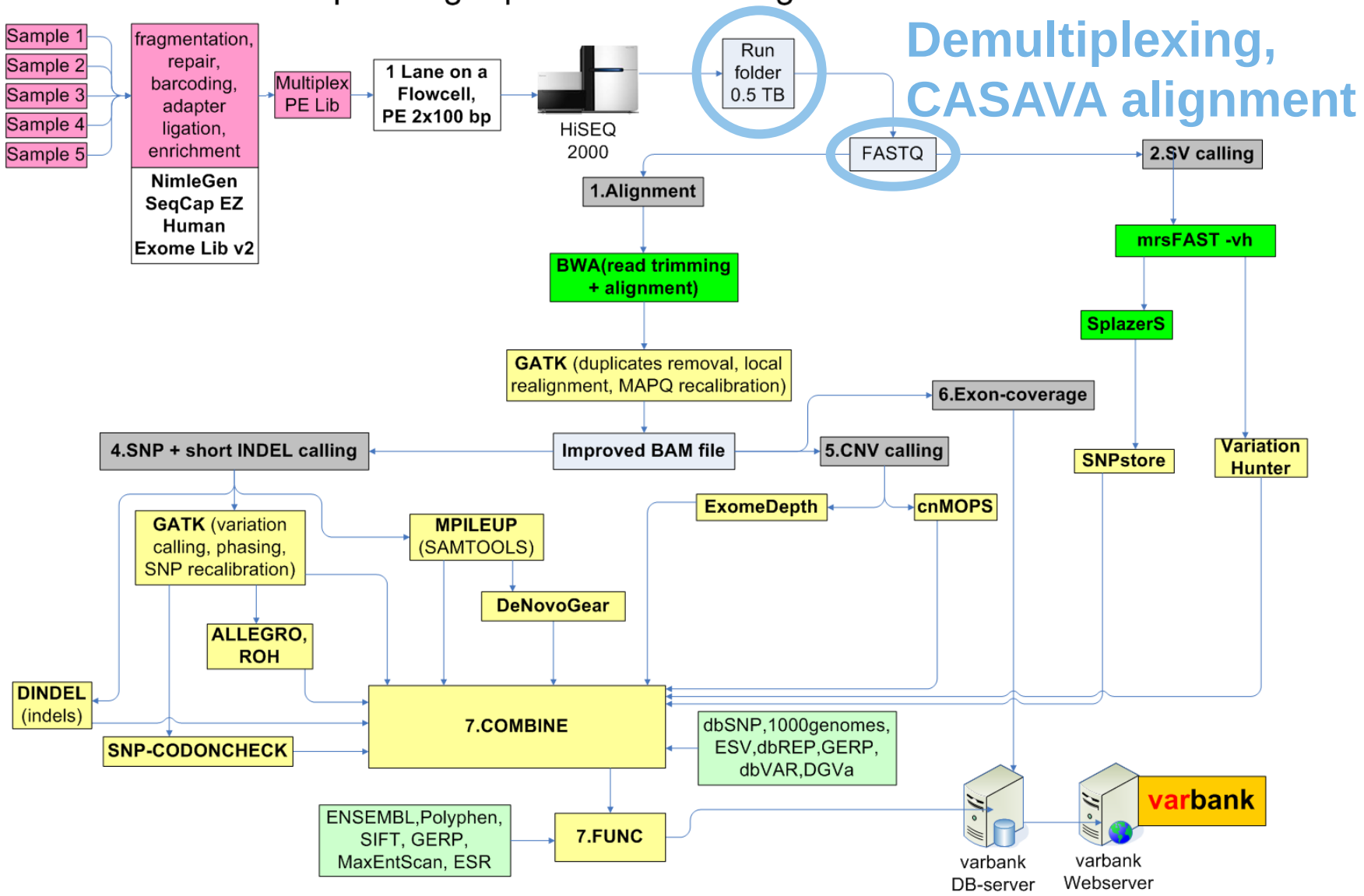

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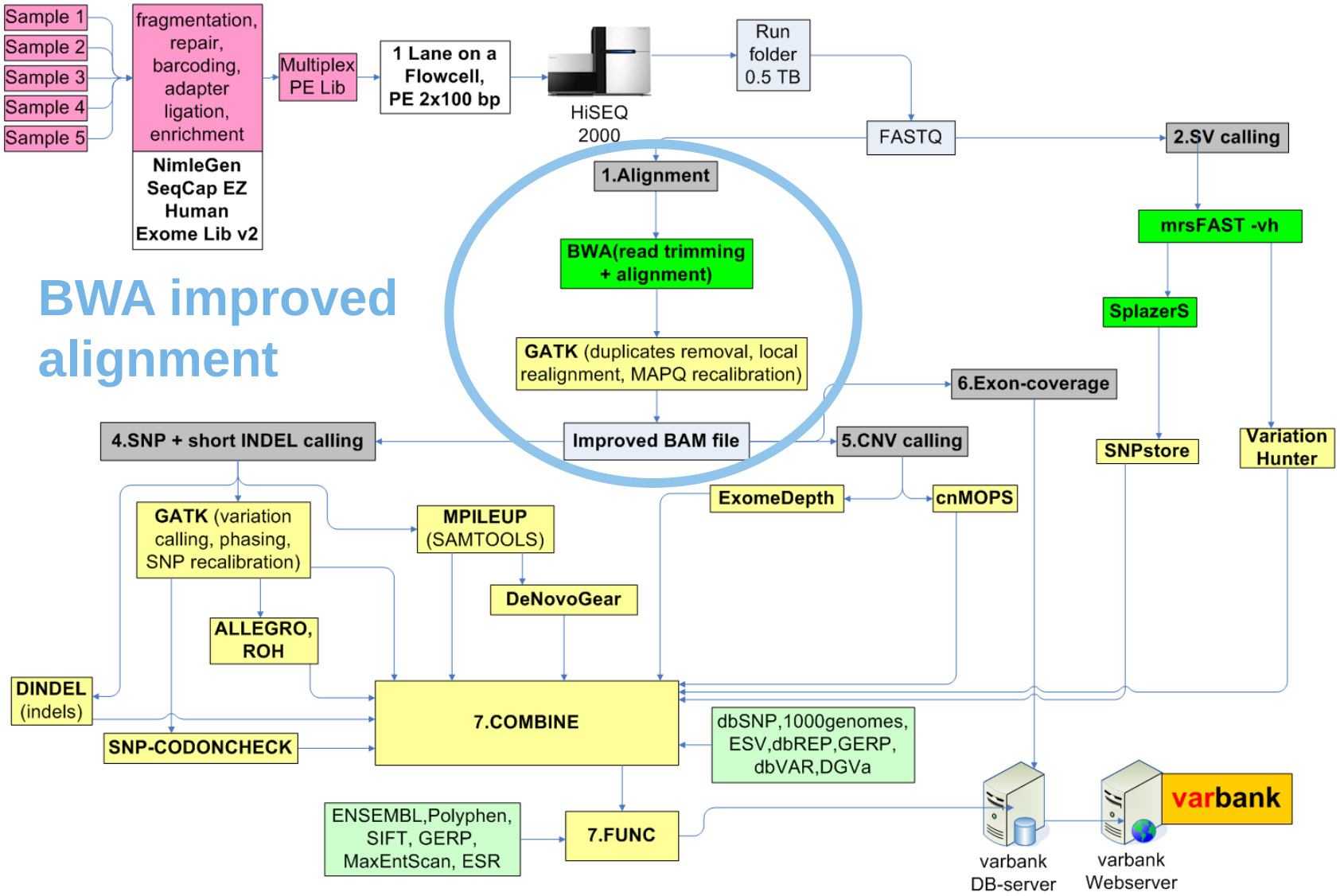
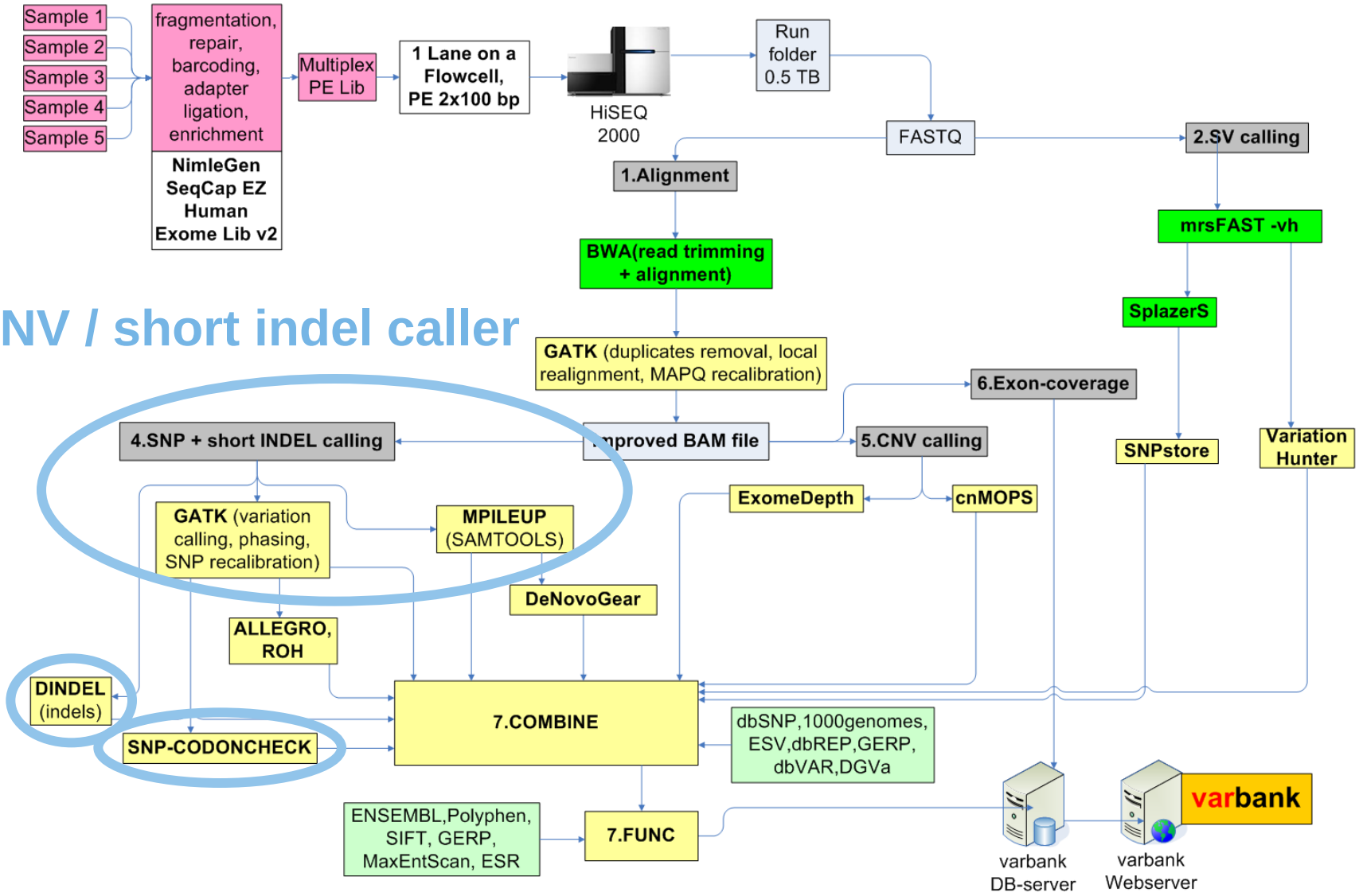


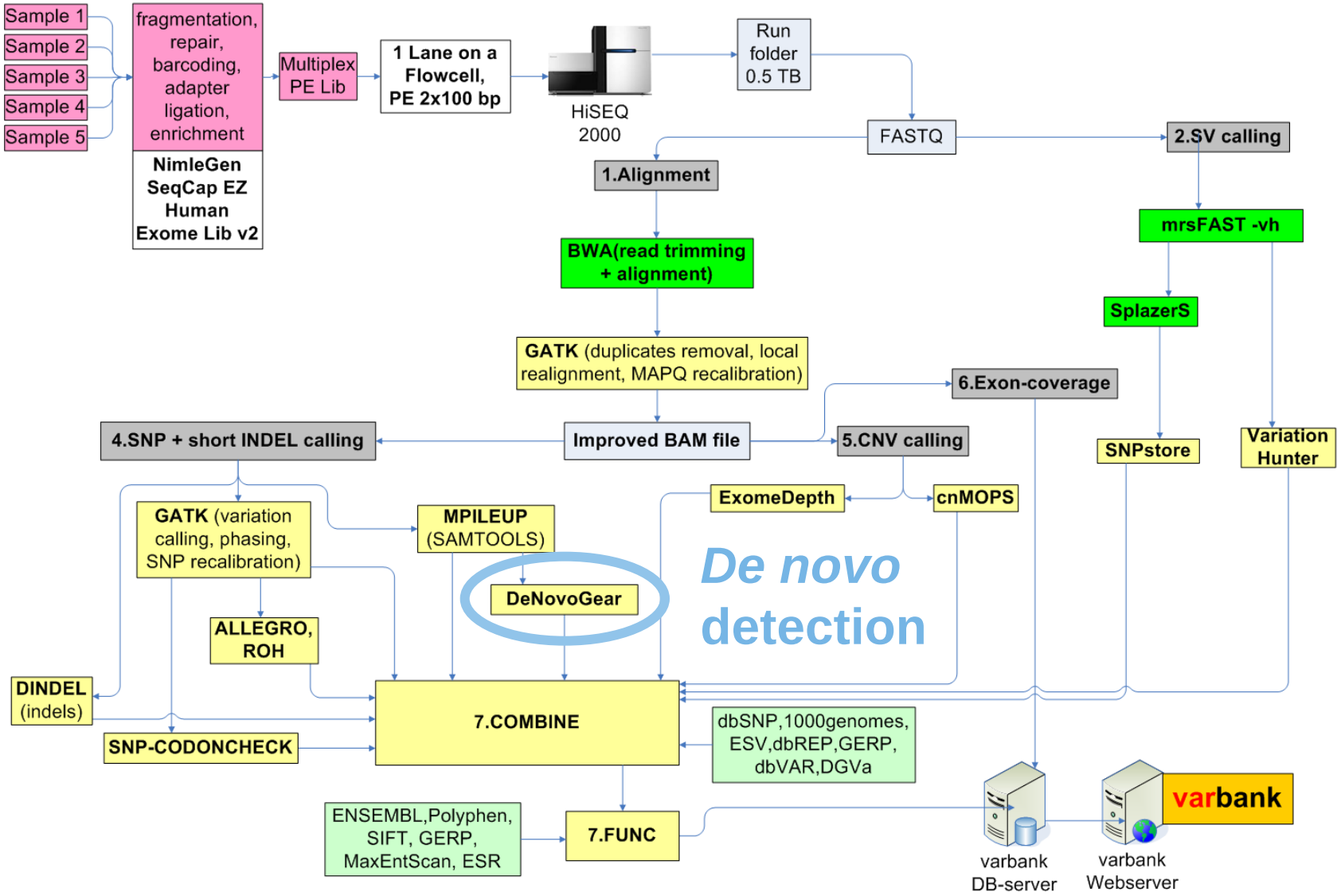
BWA improved alignment

Exome Sequencing Pipeline at the Cologne Center for Genomics



SNV / short indel caller

Exome Sequencing Pipeline at the Cologne Center for Genomics



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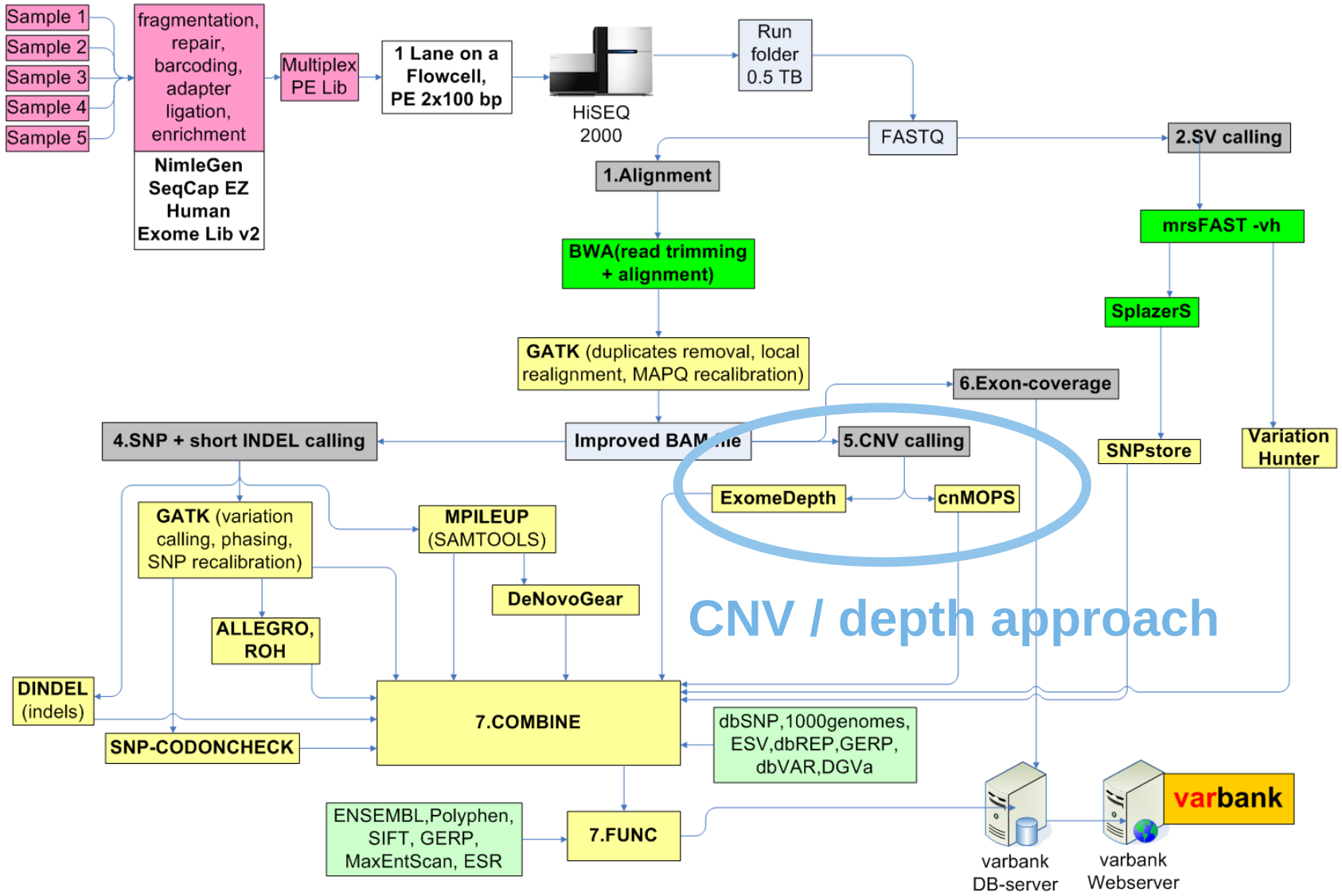

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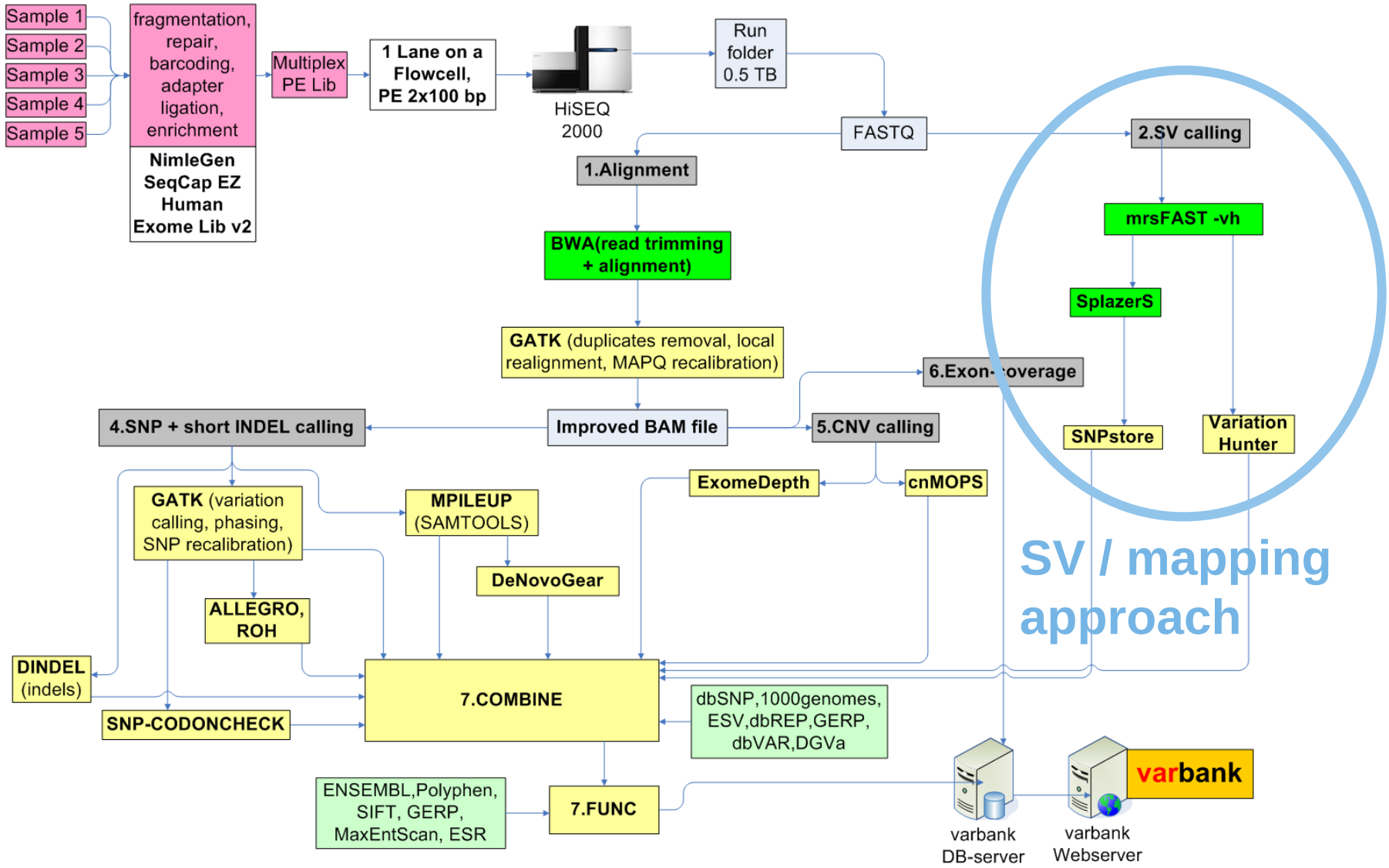
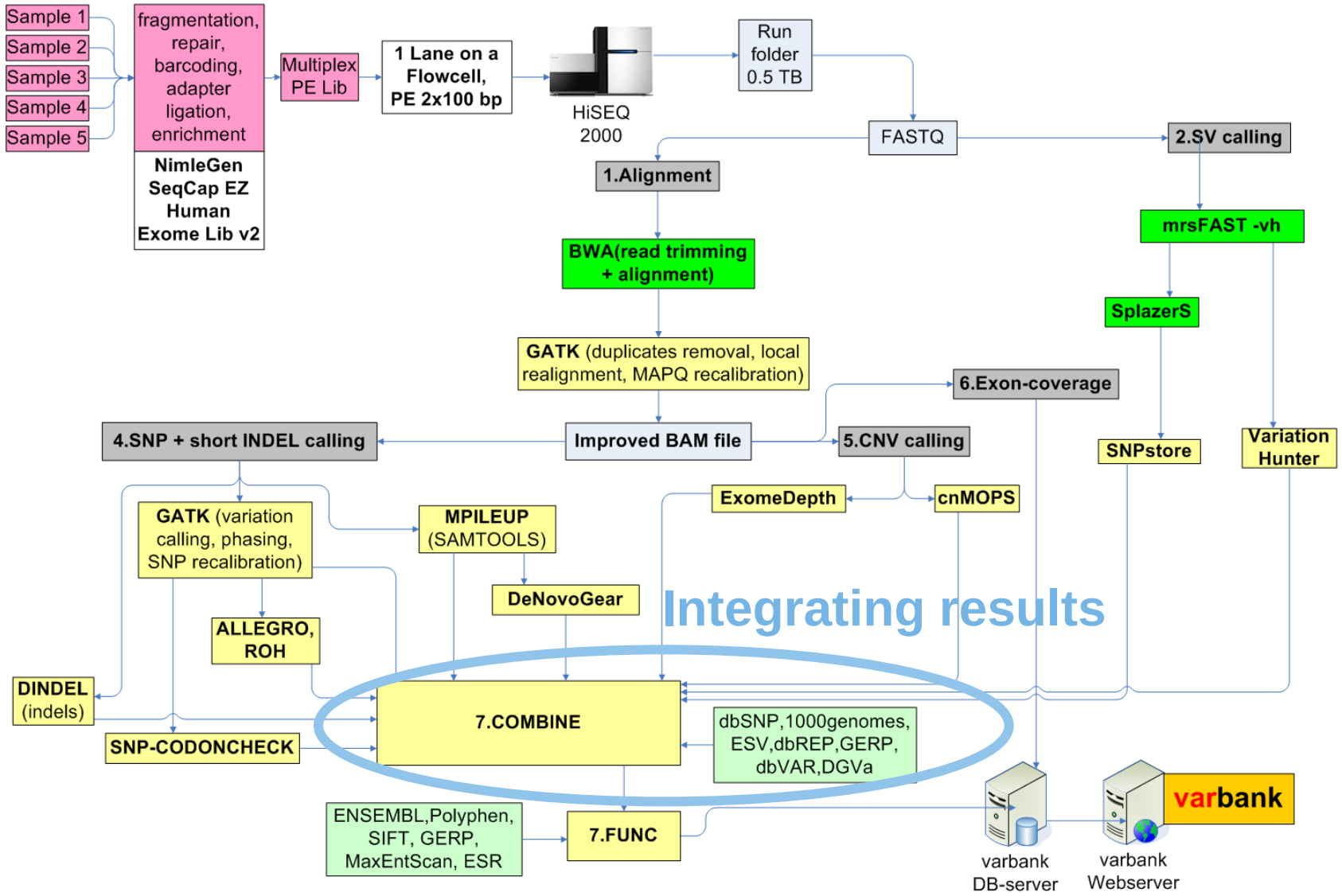


Exome Sequencing Pipeline at the Cologne Center for Genomics



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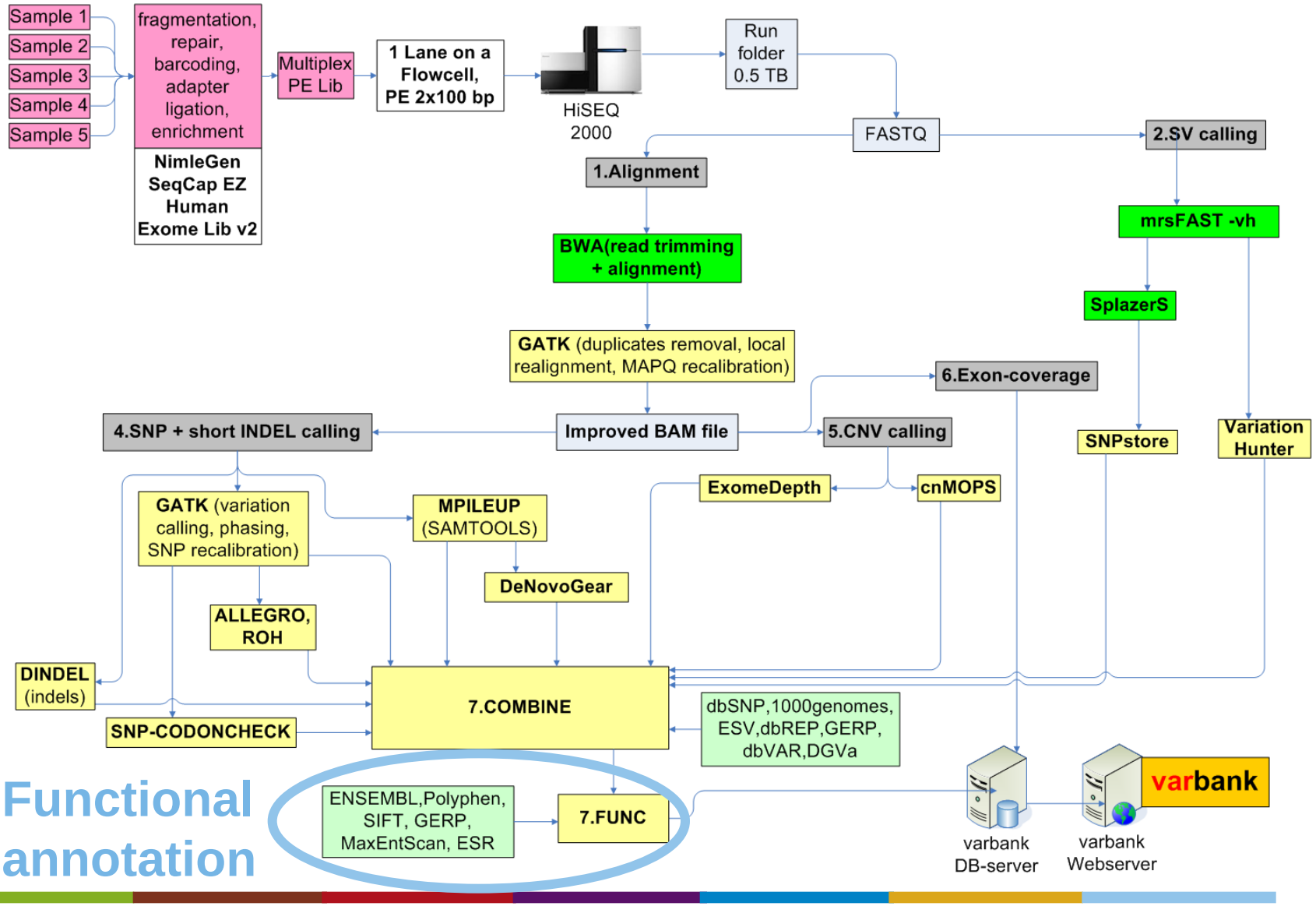

Functional annotation

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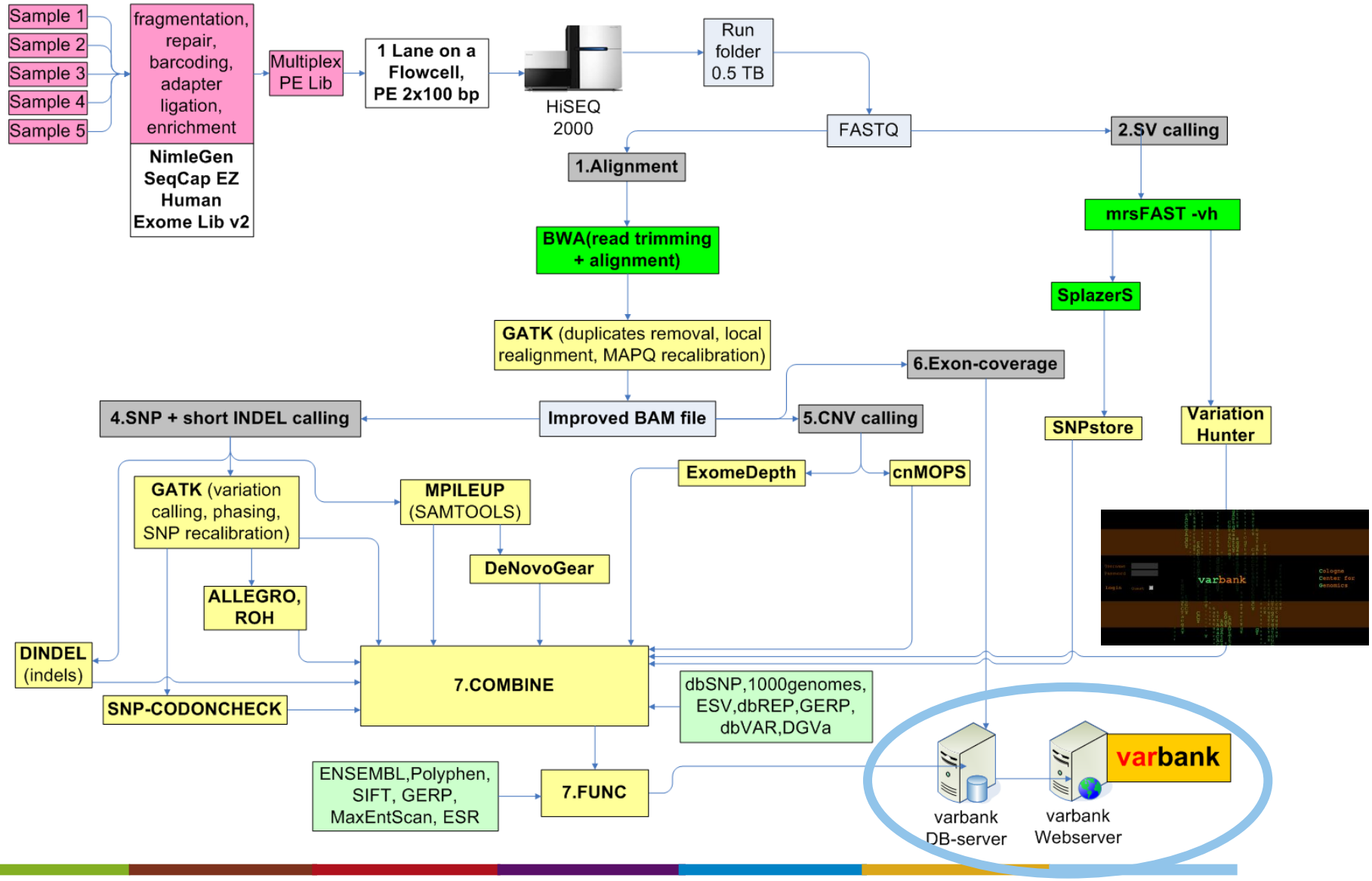

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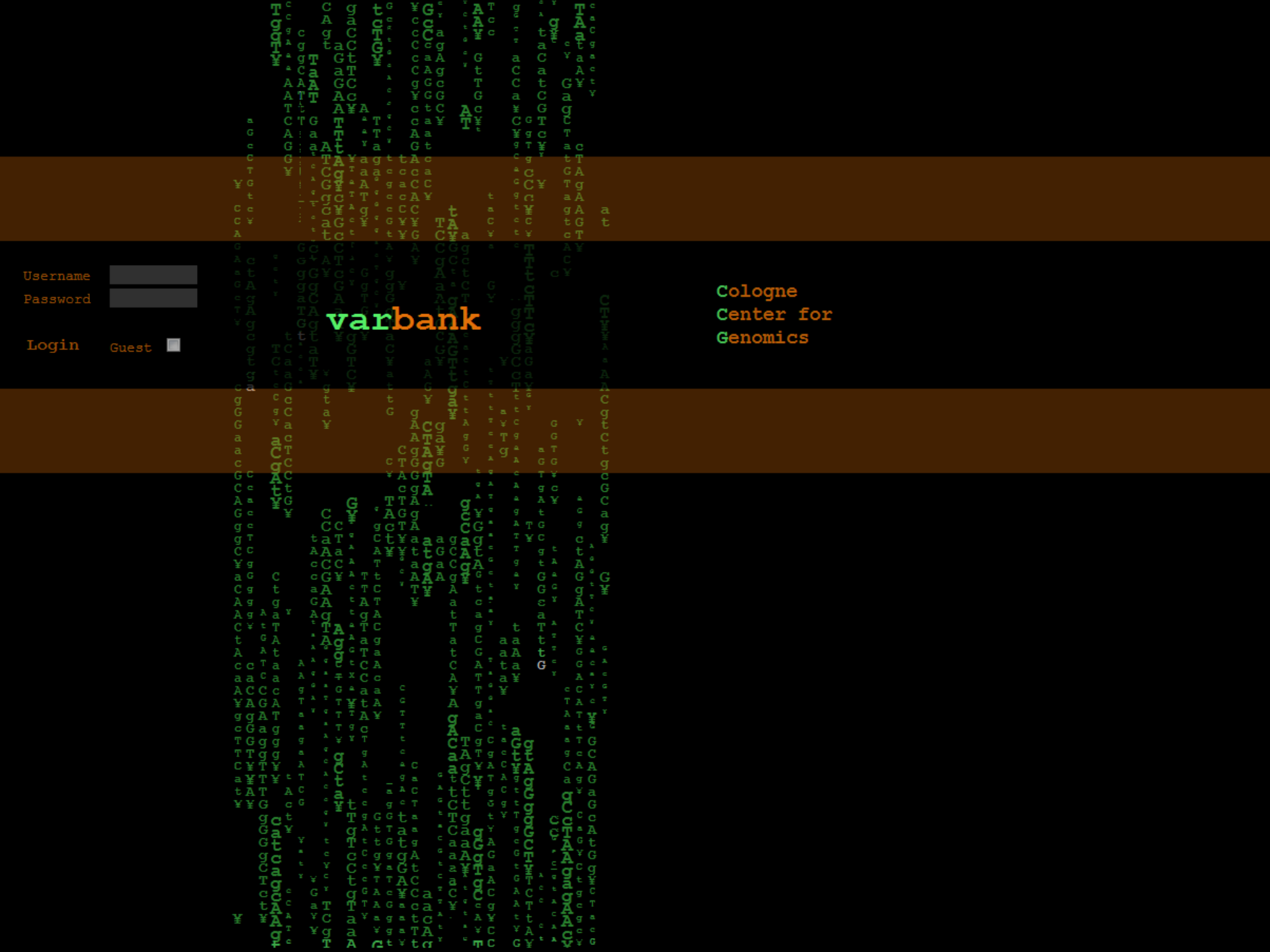
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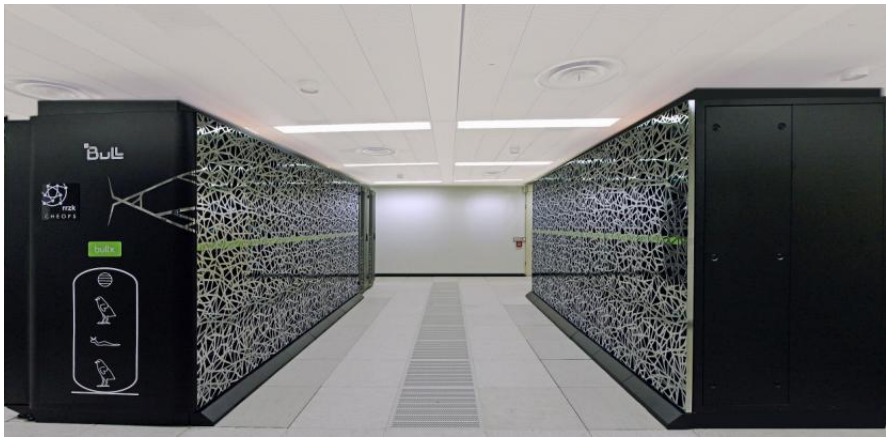


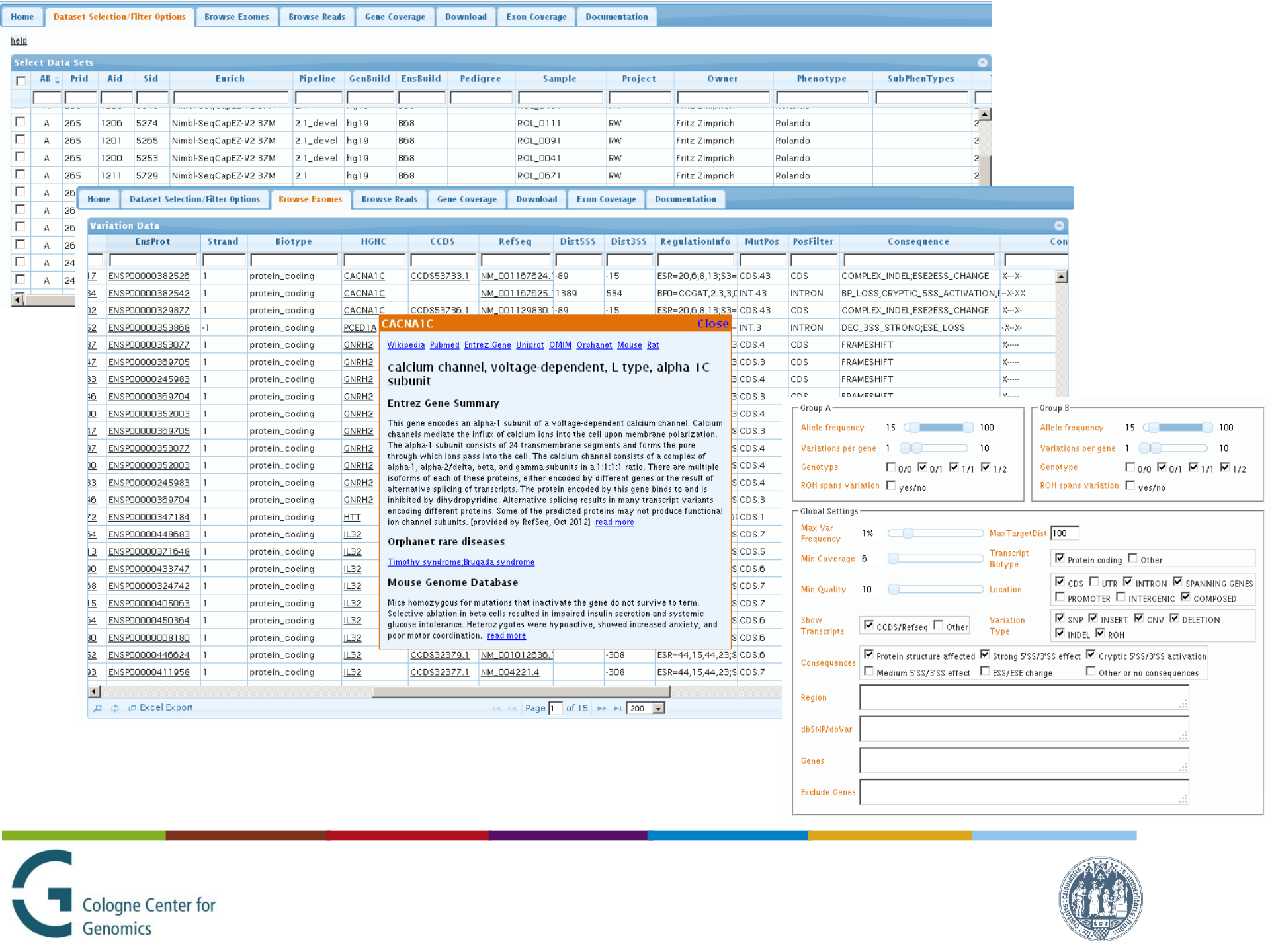
- 100 Tflop/s : in production since 2010
- 210 x 2 Nehalem EP quad-core processors 24 GB RAM
- 432 x 2 Westmere hexa-core processors 24 GB RAM
- 170 x 2 Westmere hexa-core processors 48 GB RAM
- 5 x 2 Nehalem EP quad-core processors 96 GB RAM
- 24 x 4 Nehalem EX octo-core processors 512 GB RAM
- Lustre file system, 500 TB

varbank - features

- **Repository for all relevant data files**
- **Fine-tunable access control (login, sample owner, sample sharing)**
- **User-editable for information related to pedigrees and phenotypes**
- **Variant filtering, browsing and exporting**
- **Basic read alignment viewer**
- **Gene coverage viewer**

varbank – technical implementation

- **Implementation on two VMWare servers located at the Regional Computing Center Cologne (outsourced maintanance, scalable!)**
- **Data base: Oracle 11.2 with table space partitioning**
- **Web server: Apache**
- **Web interface: perl/cgi; java script; jQuery; jQgrid; jQuery.svg; overlibmws; Matrix.js; ~ 5200 code lines**



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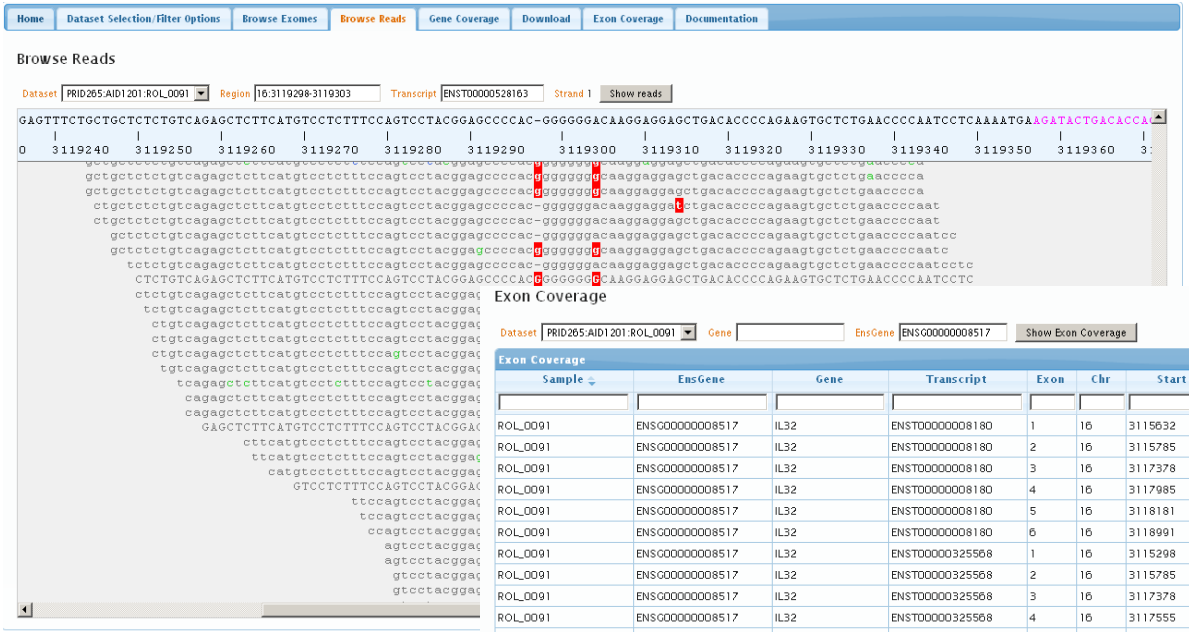

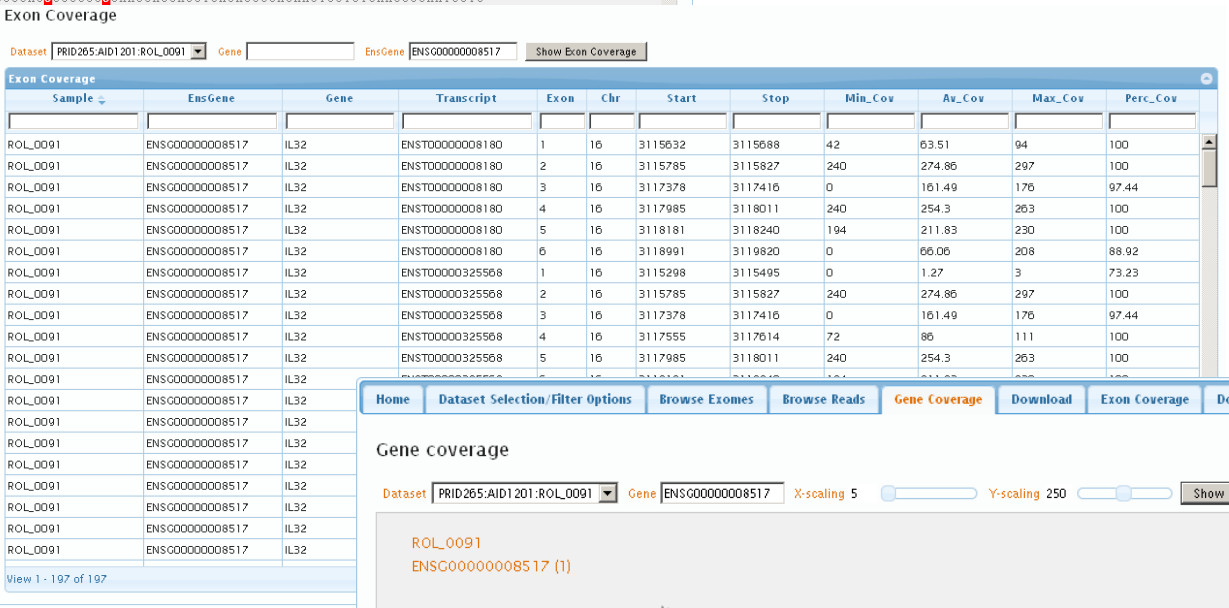

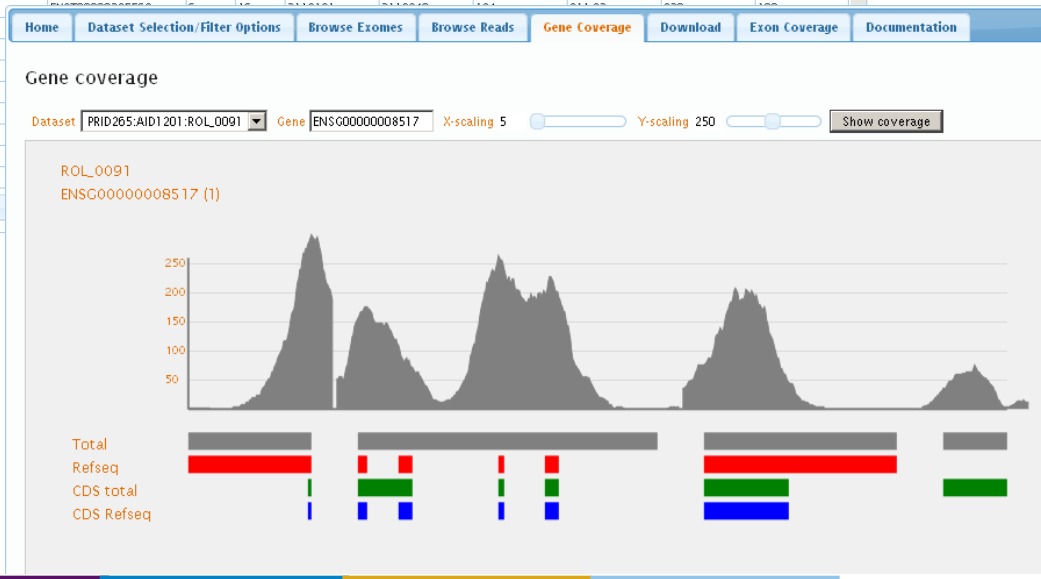

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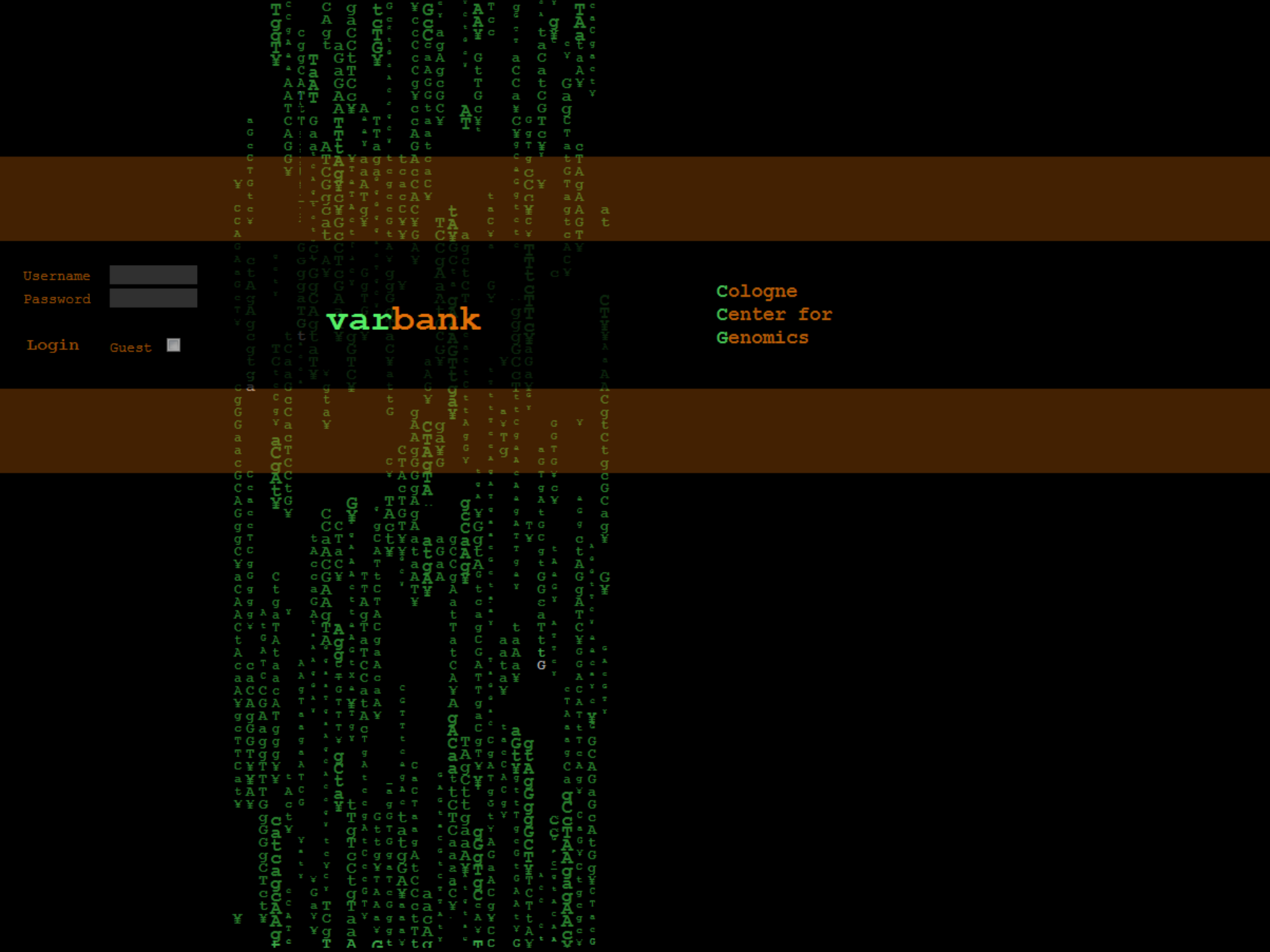
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Conclusion

WES is extremely successful for disease variant identification in Mendelian diseases → clinical geneticists and pediatricians ask for diagnostic exome-seq!

- **few exomes of affected individuals in consanguineous pedigrees**
- **3 or more individuals (affected and unaffected) in dominant families (combination with linkage)**
- **parents/offspring trios for *de-novo* mutations**

CCG's contribution:
>1.500 exomes so far,
(1/3 monogenic, 1/3 cancer, 1/3 complex diseases)

Acknowledgments/Responsibilities

Lab & Planing

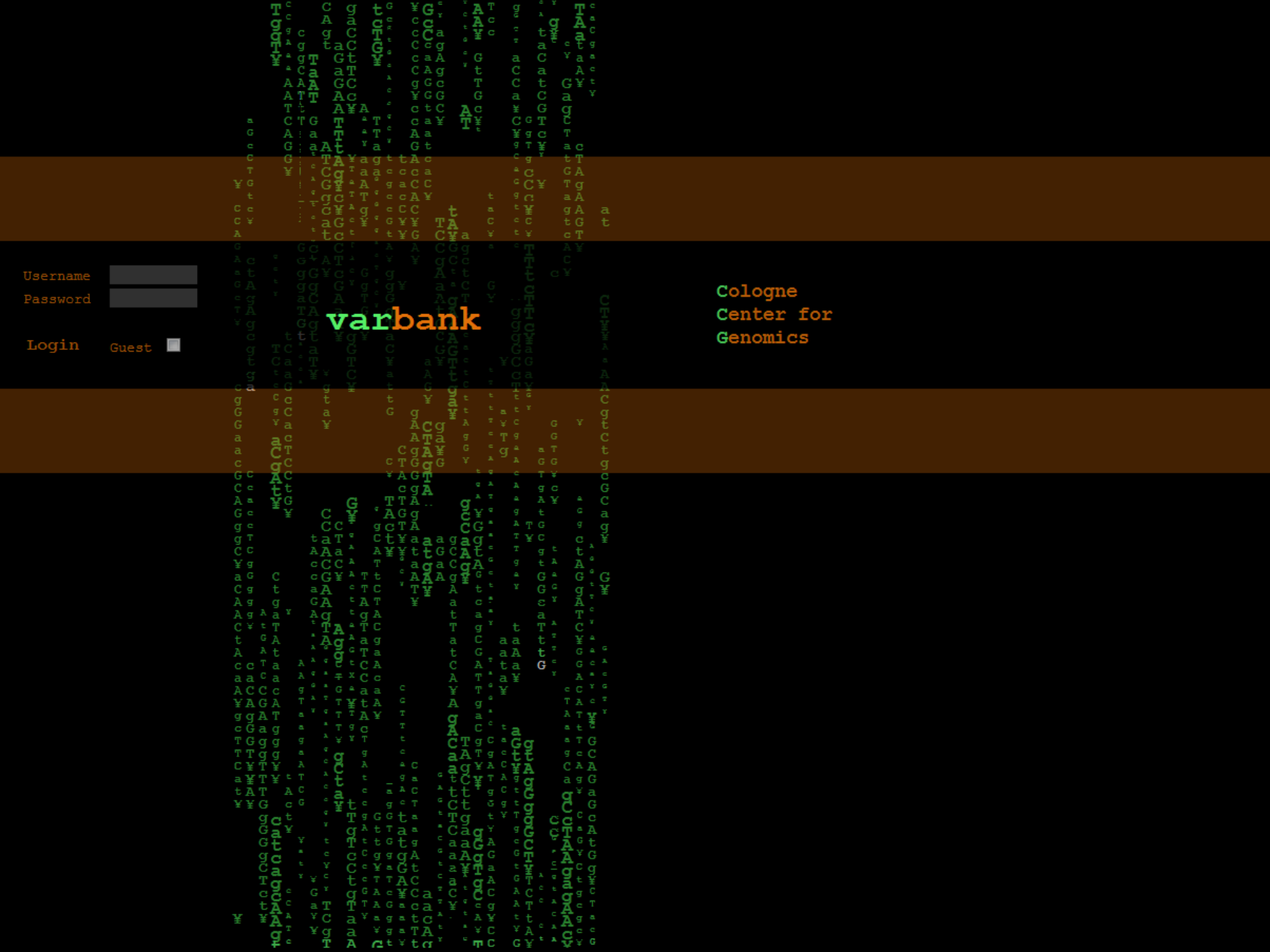
- **Janine Altmüller**
  (project managment)
- **Christian Becker**
  (library prep, sequencing)
- **Elisabeth Kirst**
  (library prep, sequencing)
- **Marek Franitza**
  (library prep, sequencing)

Computing Center

- **Viktor Achter**
  (HPC administration)
- **Ulrich Lang**
  (resource management)

Bioinformatics

- **Susanne Motameny**
  (HPC exome pipeline, CNV)
- **Peter Frommolt**
  (HPC, NGSrich, Dindel, Tumor/Transcriptome analysis)
- **Kamel Jabbari**
  (SV, CoGIE project data analysis)
- **Amit Kawala**
  (BWA Alignment)
- **Holger Thiele**
  (pipeline planning, data integration, functional annotation, trios, database, web front end)
- **Wilfried Gunia**
  (server administration, deNovo@HPC)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