KAROLINSKA
University Hospital

Karolinska Institutet

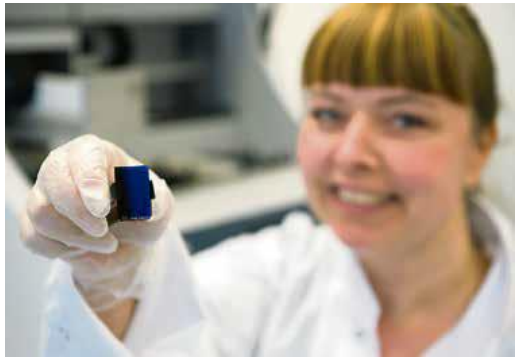
Sequenom MassArray at Karolinska Mutation Analysis Core Facility

Personnel

- Director : **Juha Kere (MD, professor)**

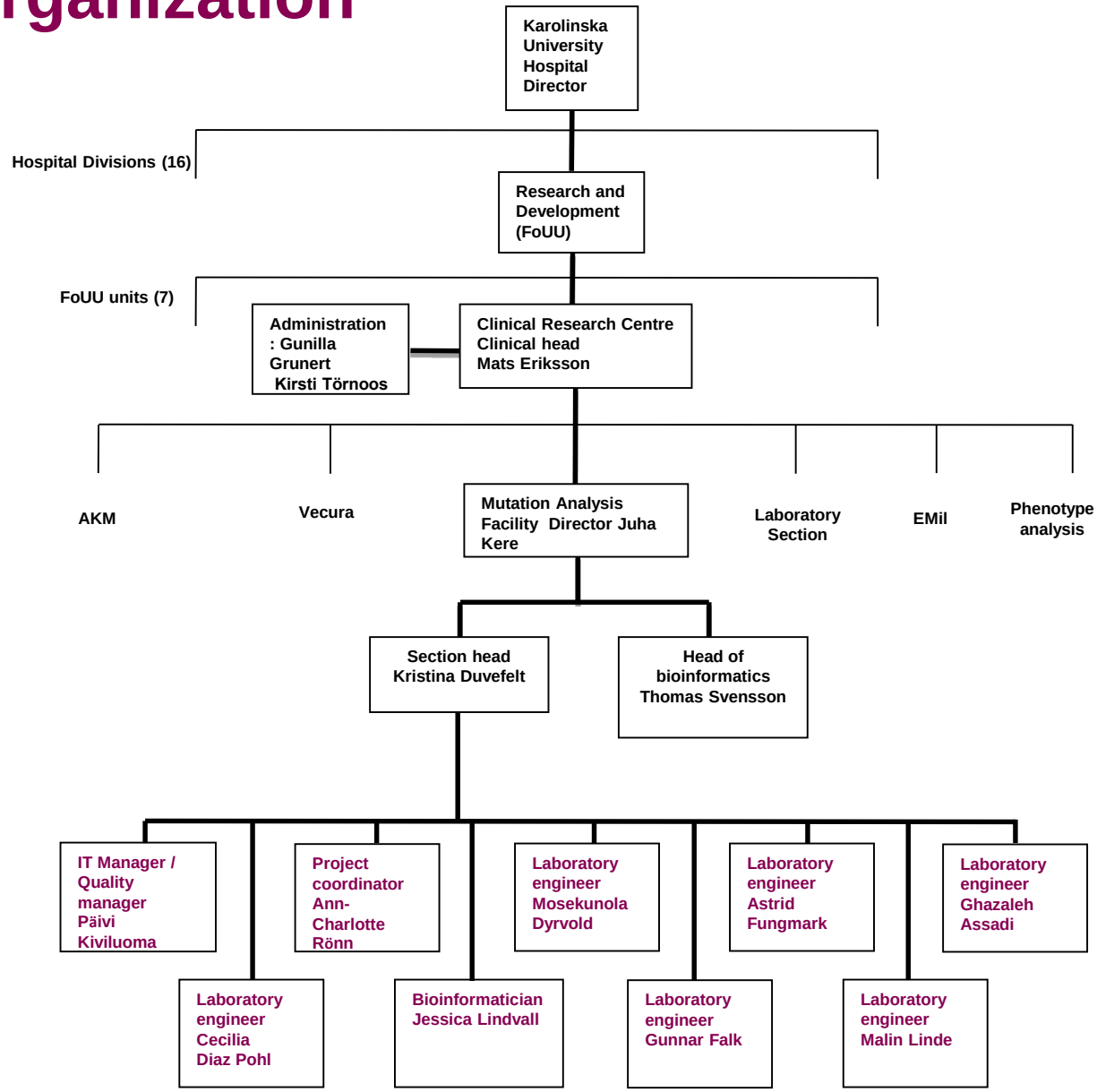
- Laboratory manager : **Kristina Duvefelt** (PhD)
- Data and Quality Manager – **Päivi Kiviluoma** (engineer)
- Project coordinator**: Ann-Charlotte Rönn** (PhD)

- Laboratory engineers
  - **Malin Linde** (MSc)
  - **Astrid Fungmark** (MSc)
  - **Mosekunola Dyrvold** (PhD)
  - **Gunnar Falk** (PhD)
  - **Cecilia Diaz Pohl** (MSc)
  - **Ghazaleh Assadi** (MSc, on leave of absence)



- Bioinformaticians
  - **Jessica Lindvall** (PhD) 50%
  - **Thomas Svensson** (PhD) 10%

Organization



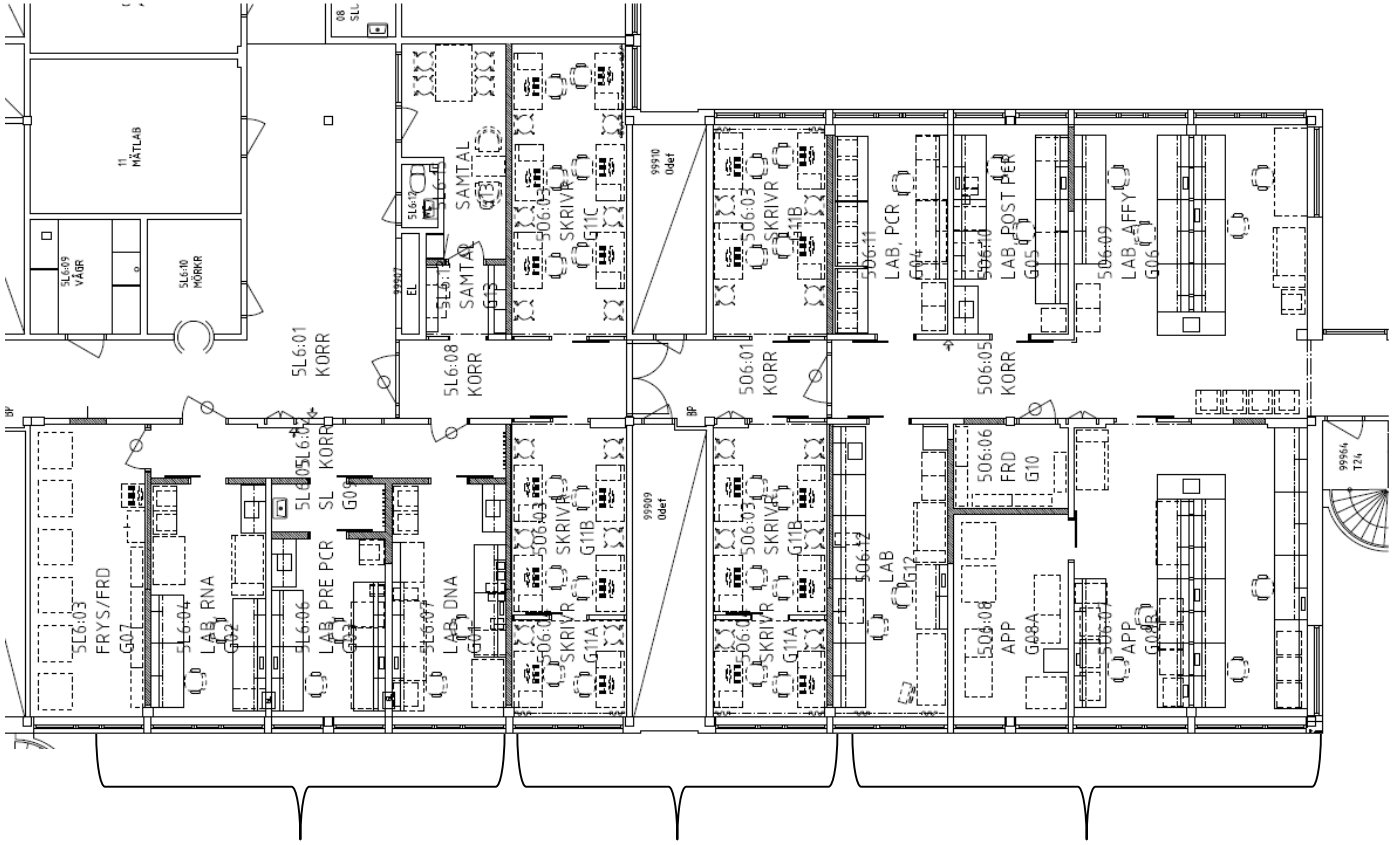
What do we do?

- **Sequenom MALDI –TOF platform** –

  - medium throughput genotyping
  - virus genotyping
  - somatic mutation analysis
  - DNA methylation analysis

- **Illumina Iscan platform-** microarray based analysis of DNA and RNA

Facility



Pre-PCR area **Writing room area** **Post-PCR area**

- Complete divsion of pre and post PCR areas
- Temperature control for best performance of instruments

Laboratory equipment

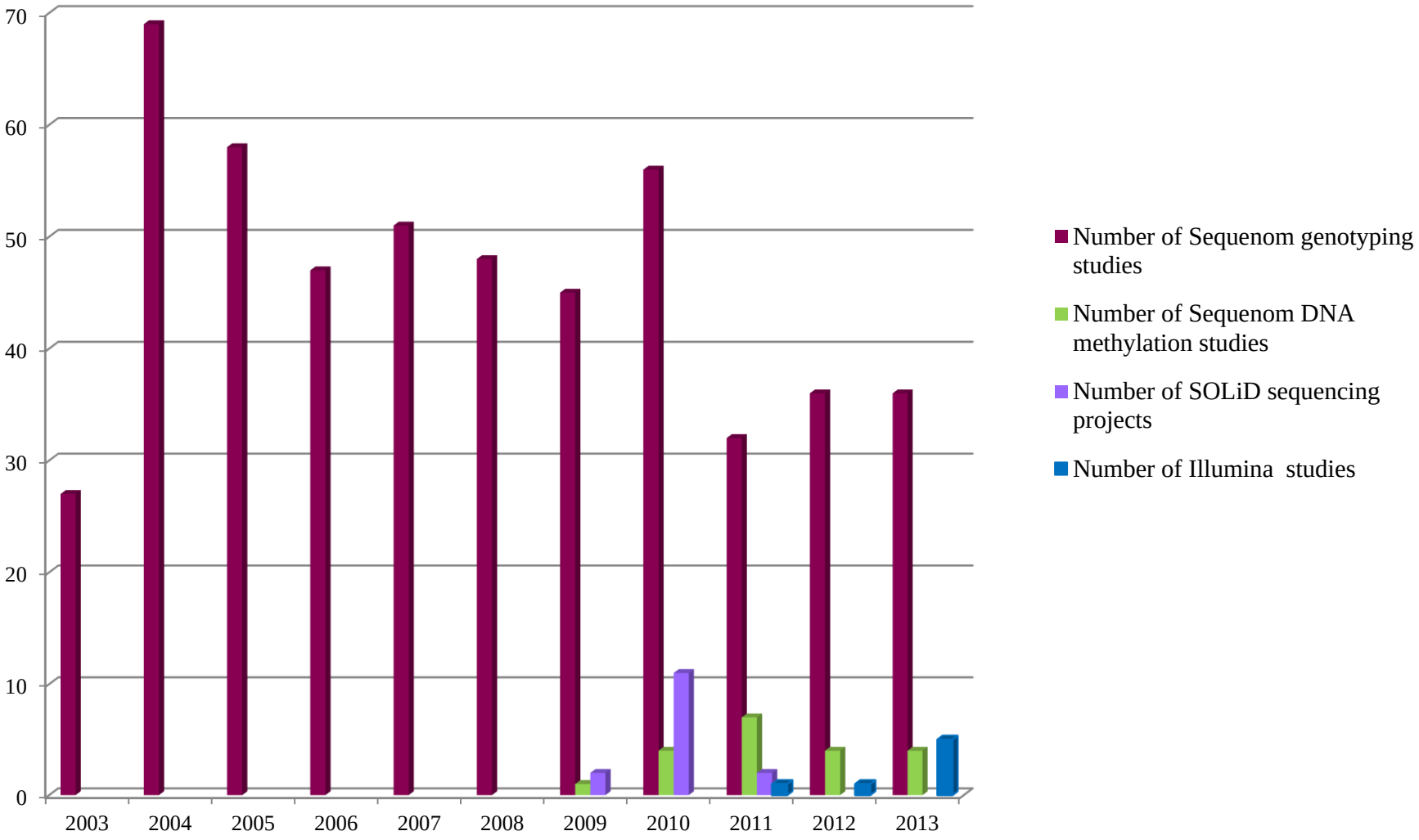
Instrument description	Number of instruments
384 well PCR machines (two blocks each)	7
96 well PCR machine	4
96-channel liquid handlers	3
24-channel microdispenser robot	1
MALDI-TOF mass spectrometer	1
Illumina IScan	1
96 well Flourimeter	1
Agilent Bioanalyzer	1

Number of samples at MAF

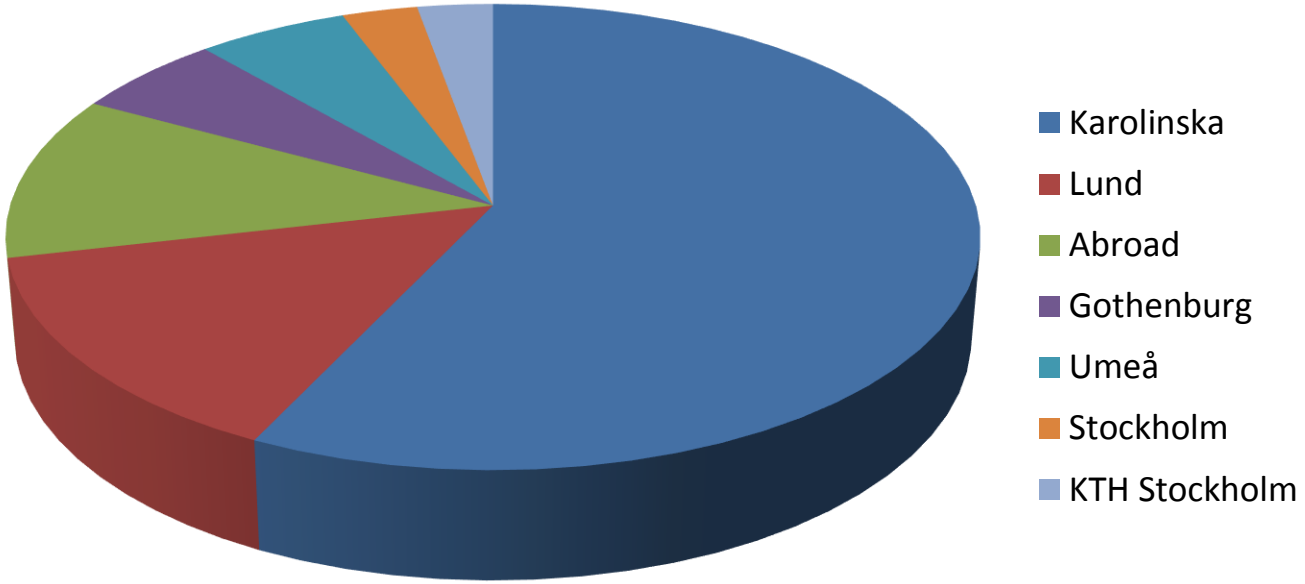
- analyzed more than 320 000 samples since 2002
- 45 658 samples 2013

- numerous cohorts are analyzed many times (different markers)

Projects at MAF



Sequenom projects 2013 - % per site



Projects 2013

- **Sequenom platform** –

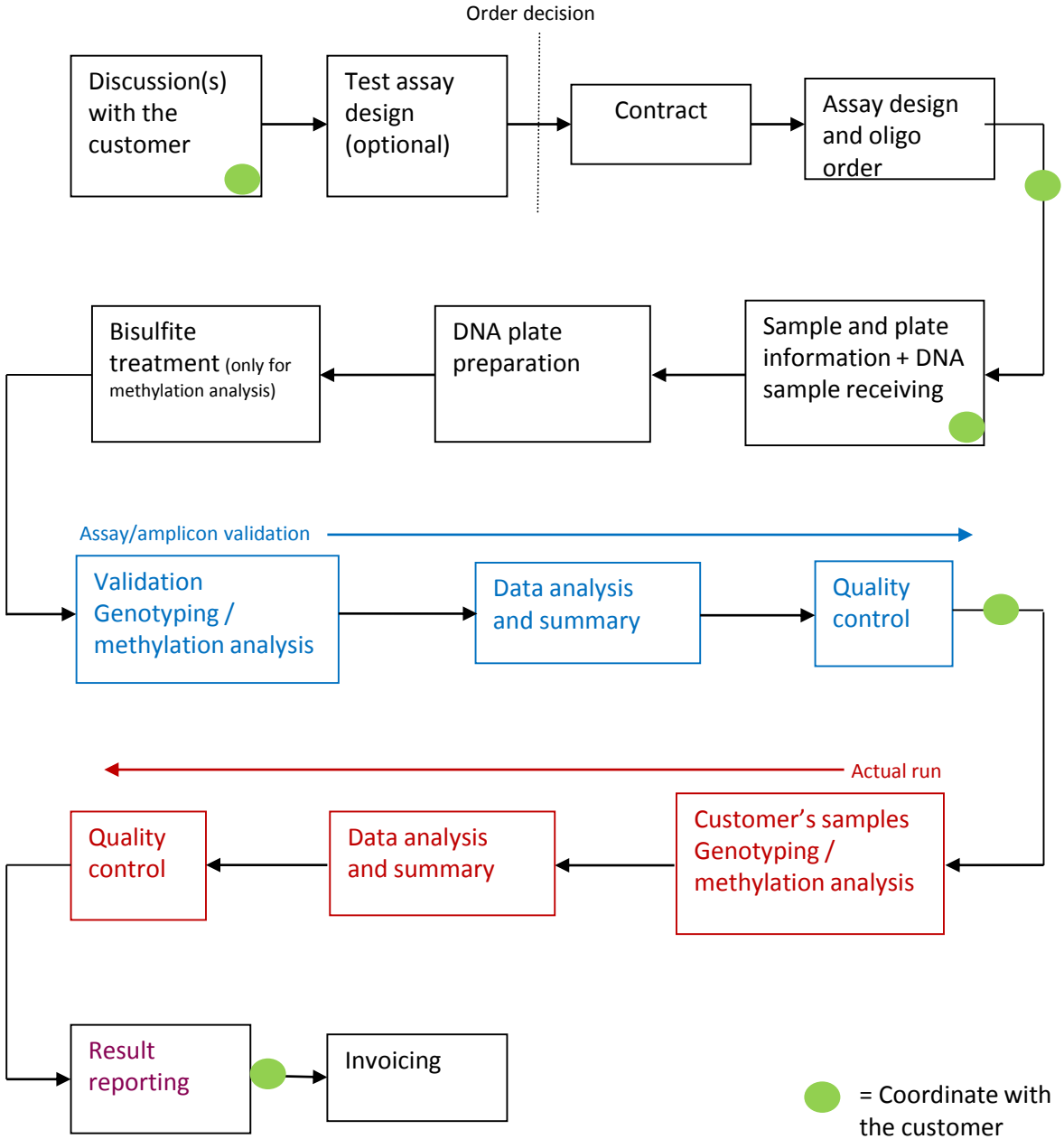
- ***24*** Genotyping (largest ~5000 samples, 95 markers)
- ***3*** Zygosity
- ***1*** Somatic mutation analysis
- ***4*** DNA methylation
- ***4*** HPV typing
- ***1*** ID panel

- **Illumina Iscan platform**
- **5** DNA Methylation project
- Pilot RNA project started

Example of Diseases

- Dyslexia
- Asthma
- Prostate cancer
- Addison
- Cushing
- Addictions
- Otitis media
- IBS/IBD/Chrons
- Alzheimer
- Obesitas
- Skolios
- Multiple Sclerosis
- Immune deficiencies
- Infertility

Sequenom process

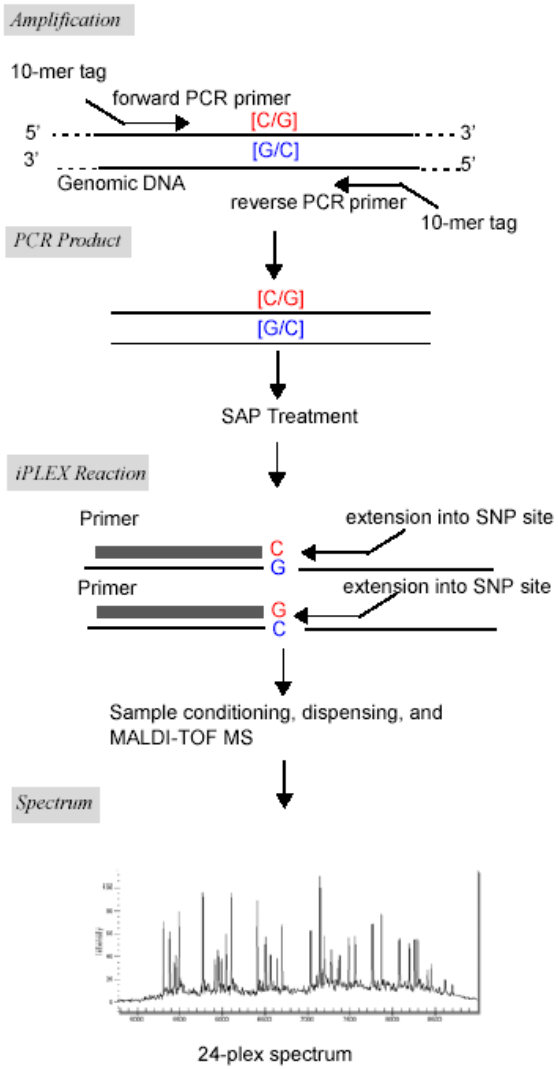


Validation analysis genotyping

- *Validation*
- 14 trios – CEPH DNA used by HapMap
- 24 other CEPH DNA
- 3 ”positive controls” – repeated an all plates during the whole project
- 6 negative controls

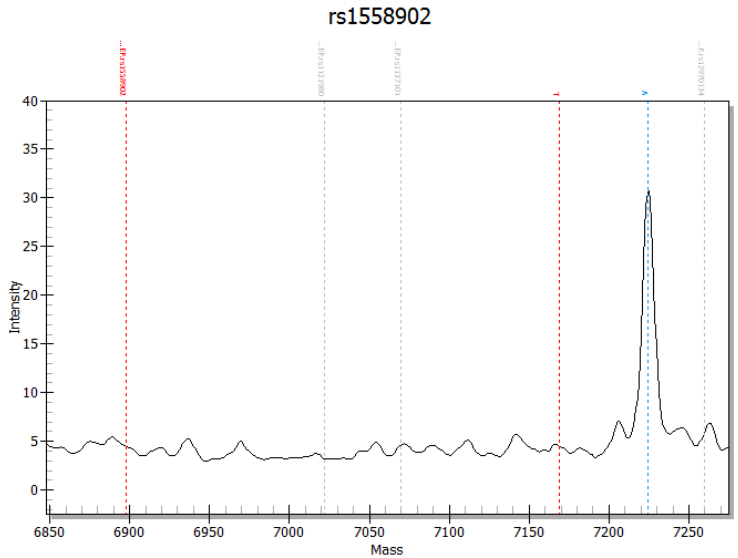
- *Analysis*
- HapMap concordance analysis
- Mendelian errors
- Concordance analysis on 17 repeated samples
- Hardy-Weinberg equilibrium on 55 unrelated individuals
- Success rate
- Negative controls

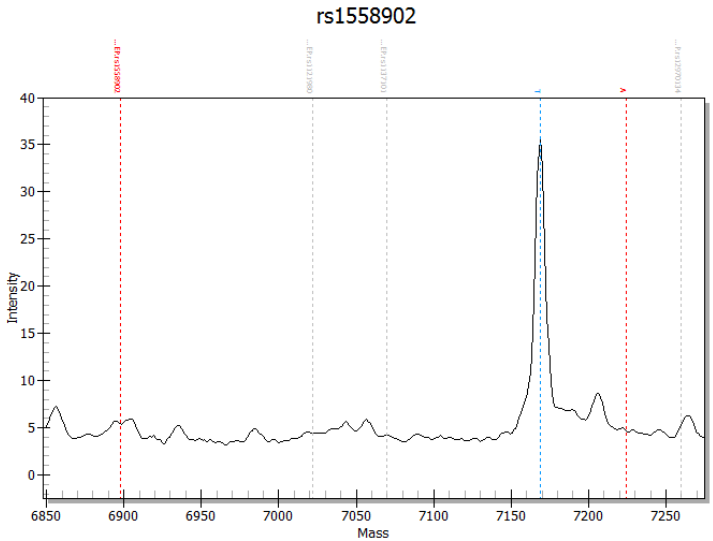
Sequenom Iplex Gold assay

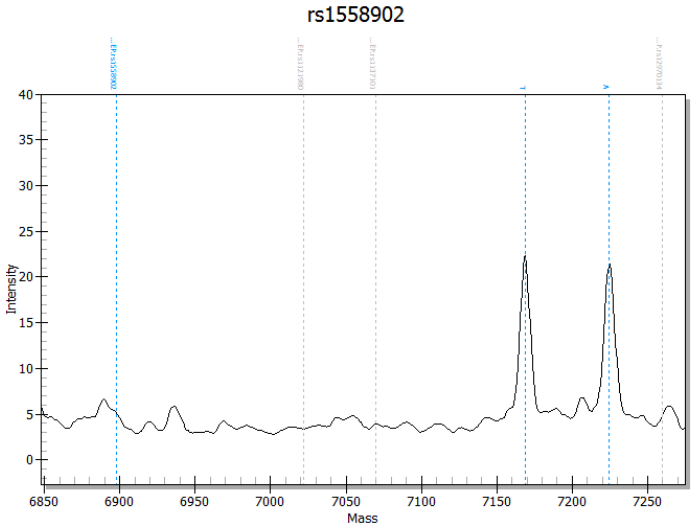


Up to 30 SNPs/pool

Sequenom


rs1558902
Intensity
Mass


rs1558902
Intensity
Mass


rs1558902
Intensity
Mass

ID panel

- In collaboration with Science for Life Stokkholm
- Unique identification of human samples
- High throughput sequencing laboratories
- Exome, transcriptome and whole genome

- 54 SNPs in two pools, three gender markers

- Turnaround – five days
- Validation and 180 samples analysed so far
- Already lead to tracing misstake in tumour – healthy tissue

ID panel

- Bi- allelic variations, excluding A-T and G-C transversions
- Synonymous variants
- Variants informative in the Swedish / Scandinavian population
- Distribution over many different chromosomes
- Analysed successfully in HapMap, CEU population
- Minor allele frequency >0,3, CEU population
- At least 140 bp from exon boundaries
- Located towards the 3' end in coding part of transcript
- Avoiding regions associated with copy number variations
- Not in repetitive sequences
- Not in MHC region
- In regions with high sequencing coverage
- In transcripts expressed in many tissues
- Markers for gender identification (ZFXY gene)

Familiar hypercholesterolemia, FH panel

- Autosomal dominant disease
- High levels of LDL cholesterol
- Premature cardiovascular events and death
- Incidence of FH: approximately 1/400 in Sweden

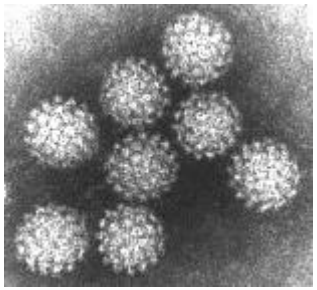
- 117 known mutations in LDLR, APO-B and PCSK-9 – validated successfully (9 pools)
- Pilot study, mutations found in 59% of cases

- No mutations ➡ sequencing
- 10 deletions, not yet validated

HemoID Beta test

- MAF one of three laboratories in beta test– you will learn more from Beatrice

Human Papillomavirus genotyping



- 16 HPV types
- 5 amplicons, 16 extension primers, one multiplex.

- Control systems
  - → WHO 2008 DNA proficency panel in each run
    - down to two copies
  - → Plasmids of all assayed types (dilution series)
    - 1000 to 10000 copies
  - → Human DNA

- 19 454 samples analyzed
- 16 000 more 2014

- Anna Söderlund Strand et al *Cancer Epidemiol Biomarkers Prev February 2013 22; 242*

Zygosity analysis

- Swedish twin registry and MAF developed
- 47 SNPs, three Iplex pools
- Can answer the important issue of zygosity in a twin pair.
- Is the pair monozygotic or dizygotic?
- Self reported zygosity is unreliable

Hannelius et al: Twin research and human genetics : 2007;10(4):604-25.

Zygosity analysis MAF

- 2006-2014: 49 studies, in total 19 378 samples
- Swedish and Norwegian Twin registry
- Usually only 3-4 % that we can not estimate zygosity on
- Analyze DNA from both saliva, blood and also from filterpaper spots
- Non normalized – works from 2,5 to 320 ng/reaction

SEQUENOM CERTIFIED SERVICE PROVIDER CSP

- Genotyping

Application Certification Criteria	**1) Design assay panel** **2) Complete wet chemistry** **3) Generate data/results** **4) Meet the following data quality measures:** **a. Min call rate of >95% for all successful assays** **b. Replicate concordance of >99.9%**

The overall result from MAF was:

- Overall success rate (28 analyzed SNPs): 85,7%
- Assay design success rate: 95,3%
- Call rate over all designed assays: 83,5%
- Call rate after filtering failed assays and samples: 98,7%
- Concordance rate (4 replicates on 23 samples and 24 assays): 100,0%

23 samples, 24 assays – Hamburg and MAF, 99, 8% concordance with Sequenom – unsecure if MAF or Hamburg was wrong...

Sequenom methylation principle

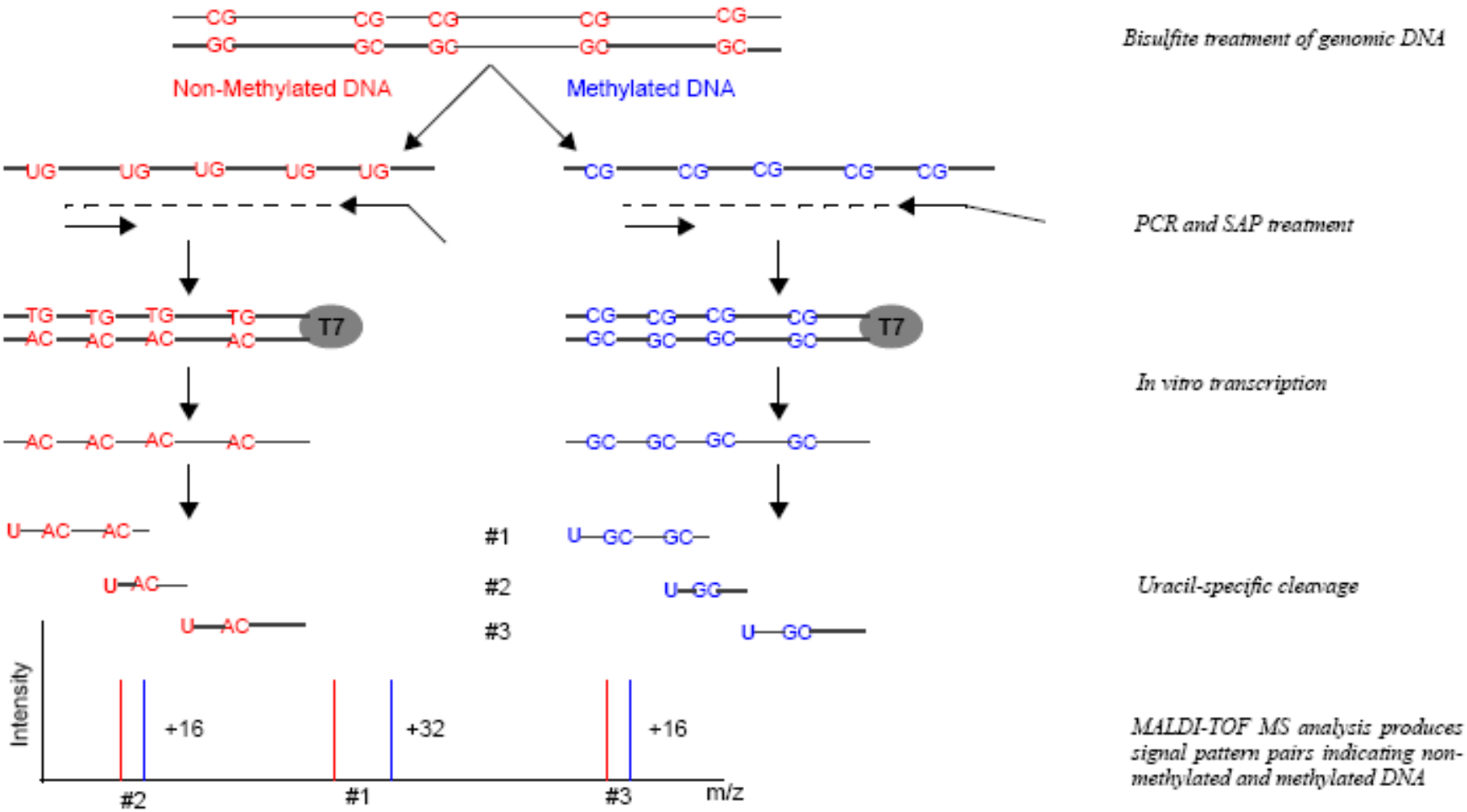
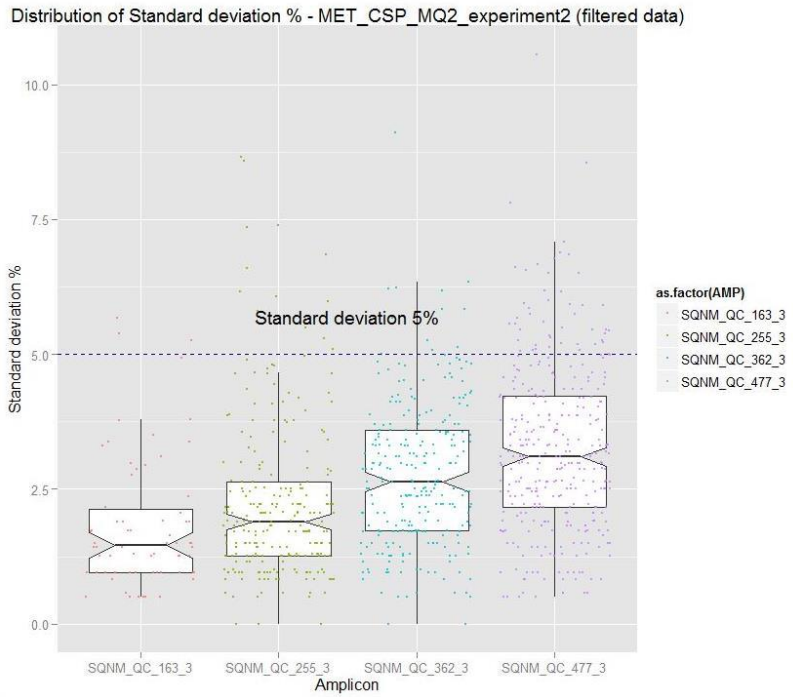


Figure 2. Overview of EpiTYPER process

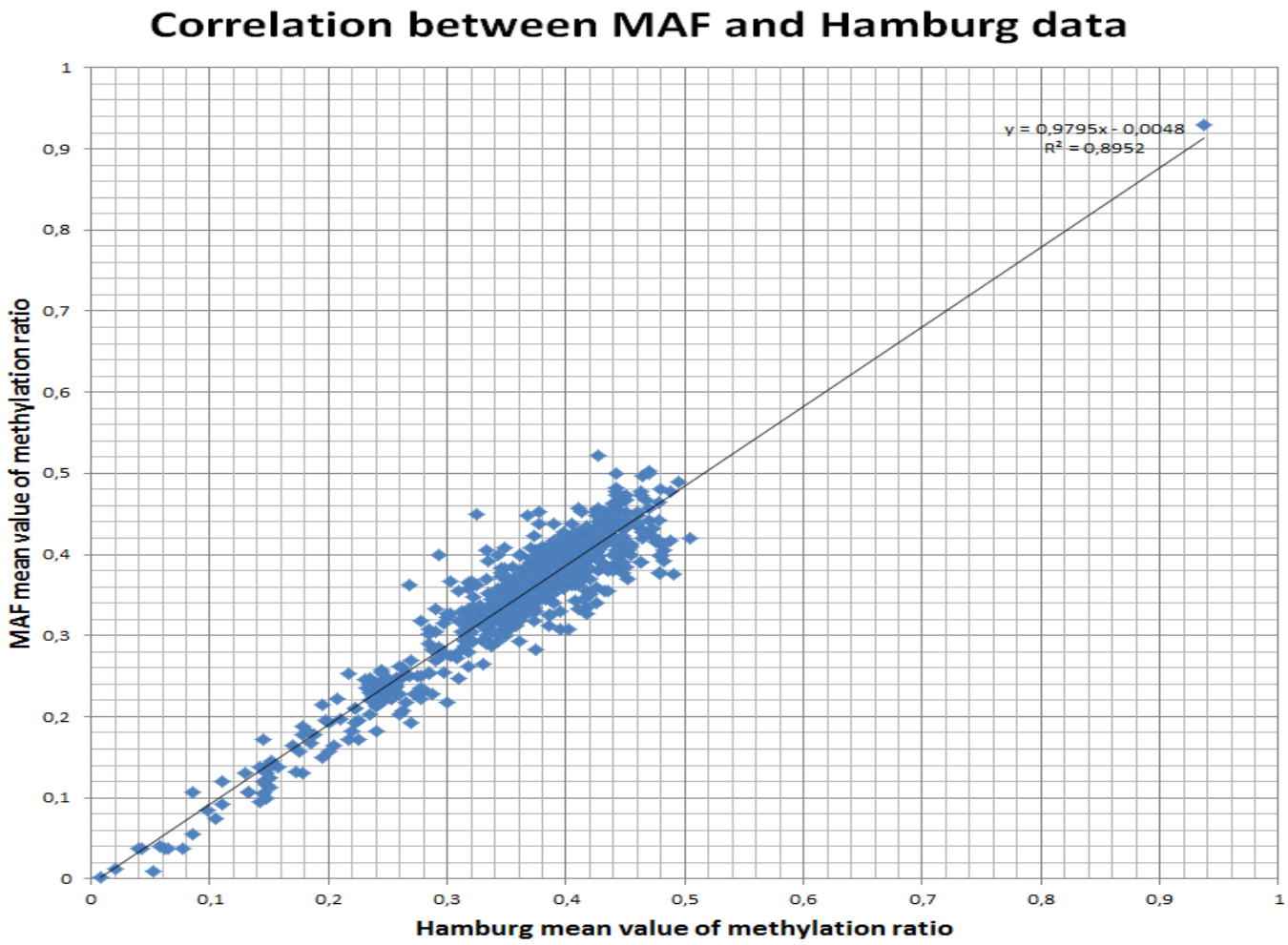
Sequenom certified Service provider

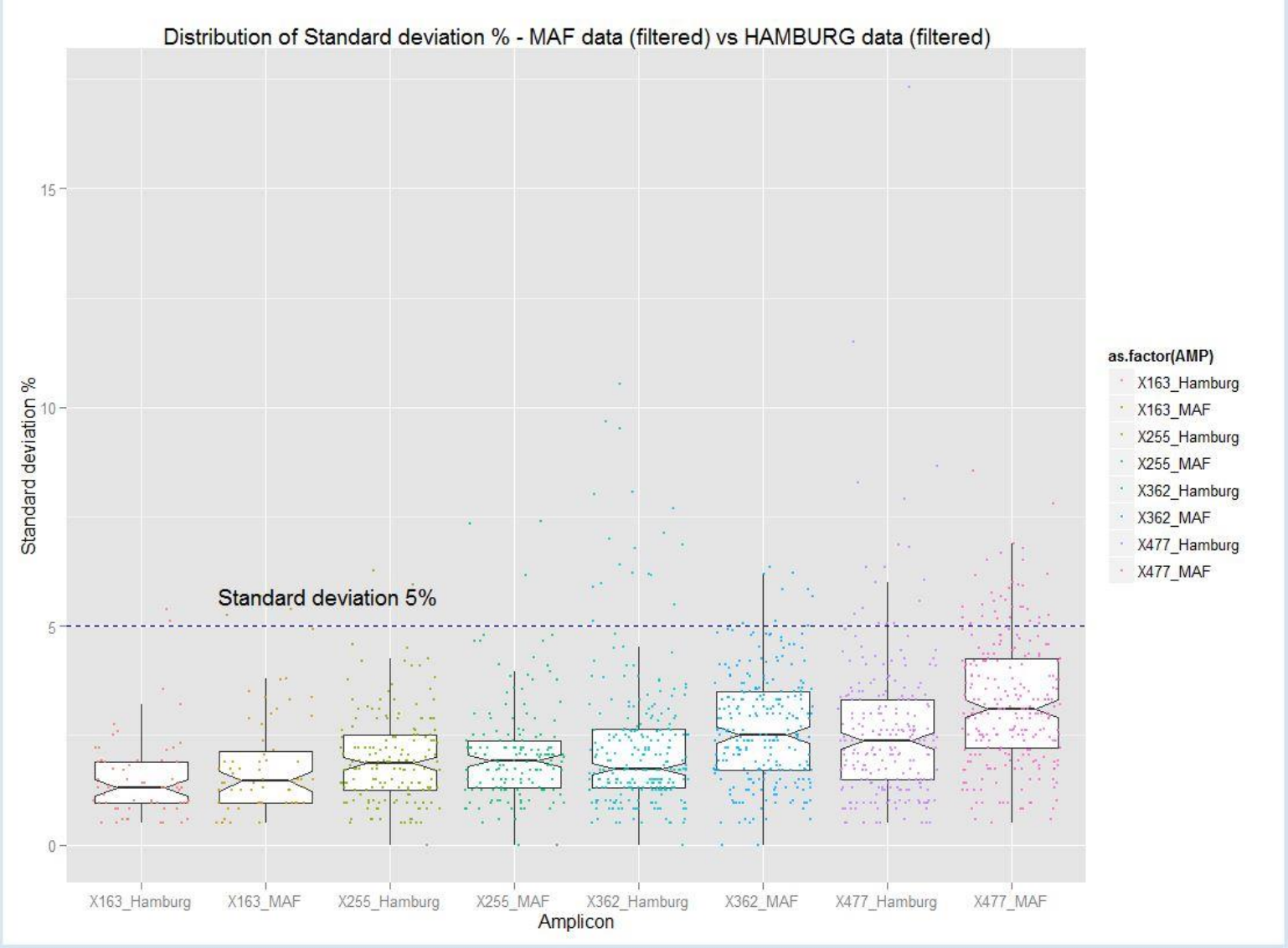
- EpiTyper DNA methylation

Assay Design Protocol	Design 16 Amplicons for Target Genes of your own choice
Chemistry Protocol	1. <u>Experiment 1:</u> Validate 16 Amplicons against 24 controls 2. <u>Experiment 1:</u> Run 4 QC Amplicons on 24 samples



Proficiency methylation




Distribution of Standard deviation % - MAF data (filtered) vs HAMBURG data (filtered)
Standard deviation %
15
10
5
0
Standard deviation 5%
X163_Hamburg
X163_MAF
X255_Hamburg
X255_MAF
X362_Hamburg
X362_MAF
X477_Hamburg
X477_MAF
Amplicon
as.factor(AMP)
X163_Hamburg
X163_MAF
X255_Hamburg
X255_MAF
X362_Hamburg
X362_MAF
X477_Hamburg
X477_MAF

Bisulfite treatment and PCR contributors to measurement of uncertainty?

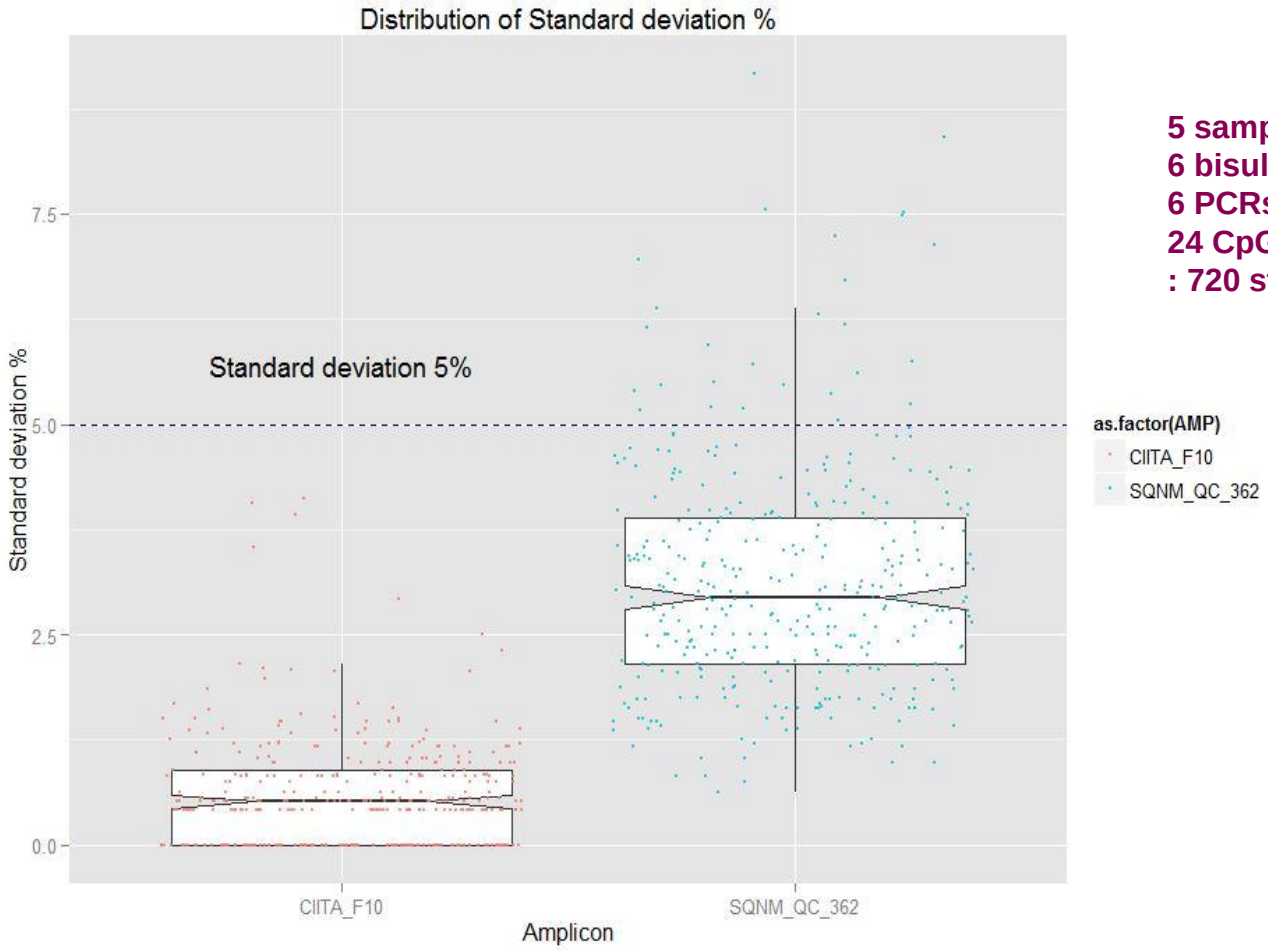
Estimate uncertainty of measurement in methylation analysis.

- 2 amplicons
- 24 CpG units
- 5 samples
- 6 bisulfite treatments/sample
- 6 PCR reactions/sample

Bisulfite treatment contribution

Sample	Number of CpG units showing **no** significant differences between bisulfite treatments (p-value < 0.05) (ANOVA)
Sample 1	23/24
Sample 2	21/24
Sample 3	22/24
Sample 4	22/24
Sample 5	23/24
Total	111/120

Distribution of standard deviation PCR



5 samples
6 bisulfite treatments
6 PCRs on bisulfite treated DNA
24 CpG analyzed
: 720 standard deviations

Measurement of uncertainty, methylation

Conclusions

- The bisulfite treatment contribution to the measurement of uncertainty is very low
- The larger contributions, but still small, are the PCRs
- All customer studies at MAF are run in duplicate PCRs to detect and show the correlation openly to the customer
- The customers are advised that standard errors of 5% are to be expected
- This evaluation of the measurement uncertainty have confirmed the previous set 5% standard deviation

Service details

- **Throughput:** ~130 000 analyses/year (Sequenom)
  ~7500 samples/year (Illumina)

- **Priorities**: First come, first served, defined on the basis of sample and information delivery date to the facility

- **Turnaround time**: Usually 8- 10 weeks/Sequenom genotyping, but differs due to the workload and project size

Quality Assurance MAF

- Accreditation according to ISO/IEC 17025
  - → Sequenom genotyping
  - → Sequenom DNA methylation analysis

- Proficiency testing- European Molecular Genetics QualityNetwork
- Sequenom certified service for genotyping and DNA methylation
- Newly renovated laboratory facilities
  - → Designed for high quality laboratory work in the genomics field

- Laboratory information management system (LIMS)
- Electronic laboratory note book system (Contur ELN)
- Document and deviation control, Centuri system

Accreditation MAF

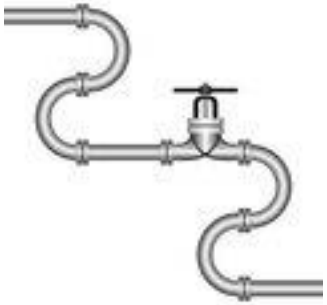
SWEDAC ACKREDITERING
1886
ISO/IEC 17025

- ISO 17025
- International standard
- Technical competence and business management

- *Directing and accounting documents*
- Directing MAF:
- Customer, Facilities, Human Resources, Information Technology, Instruments, Management, Methods, Tools, Forms.
  81 documents in total.

- *Accounting*
  → ELN, Results files, contracts (customer, suppliers), calibrations, protocols

In the pipeline



- New panels for other diseases
- Clinical samples for FH panel – research project to evaluate efficiency. Underdiagnosed in Sweden.
- ID panel – will probably be used by more sequencing facilities
- Panel of DNA from Swedish ”controls” for genotype analysis at MAF
  - 720 Individuals
  - 50 % females , 50% males

Contact information

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