

# Age assessment by DNA methylation on the PyroMark Q48: Comparison to Illumina MiSeq

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2018, Lisbon, Portugal

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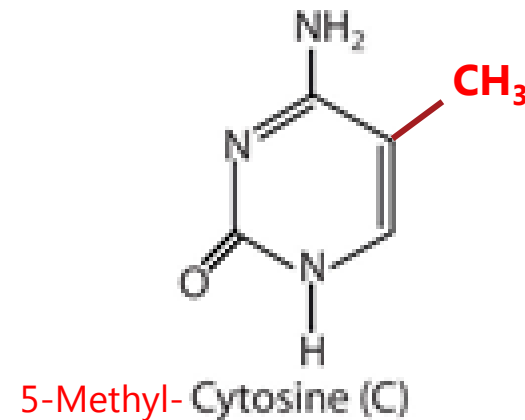
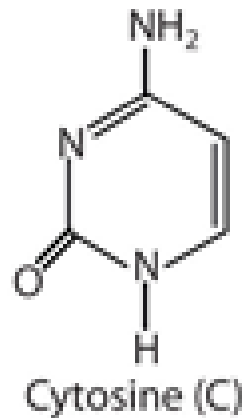


# Agenda

- Introduction to DNA methylation sequencing
- The Qiagen PyroMark Q48
- How to analyse CpG assays
- Reproducibility of CpG assays
- Comparison of DNA methylation results between the Qiagen PyroMark Q48 and the Illumina MiSeq

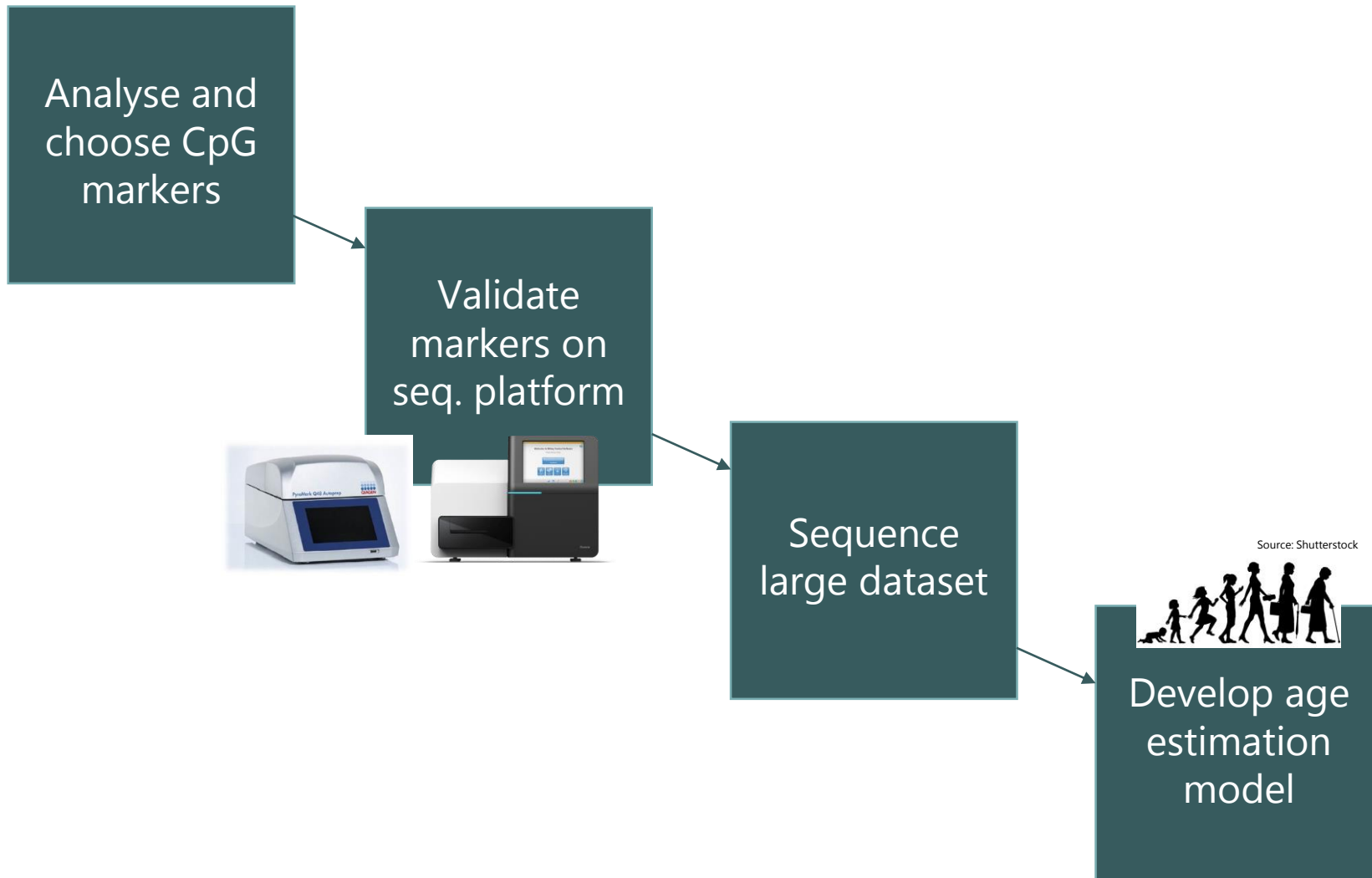
# DNA methylation

- Epigenetic marker
- Methylation of Cytosine (CpG dinucleotide)
- Regulation of transcription



Modified picture from: [https://saylordotorg.github.io/text\\_the-basics-of-general-organic-and-biological-chemistry/](https://saylordotorg.github.io/text_the-basics-of-general-organic-and-biological-chemistry/)

# Creating an age estimation model



# Sequencing protocol

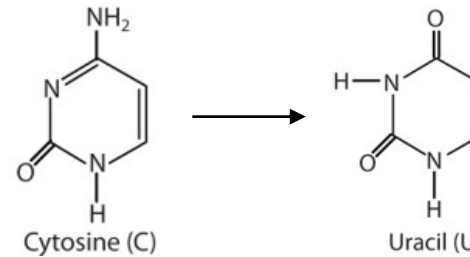


Source: Muhammed Badran

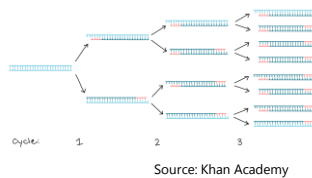


Source: Capitol Scientific

- DNA Extraction
- Bisulfite treatment

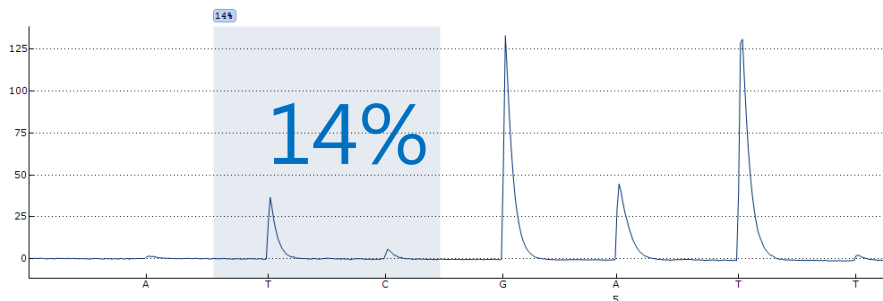


- PCR



- (Library preparation)

- Sequencing

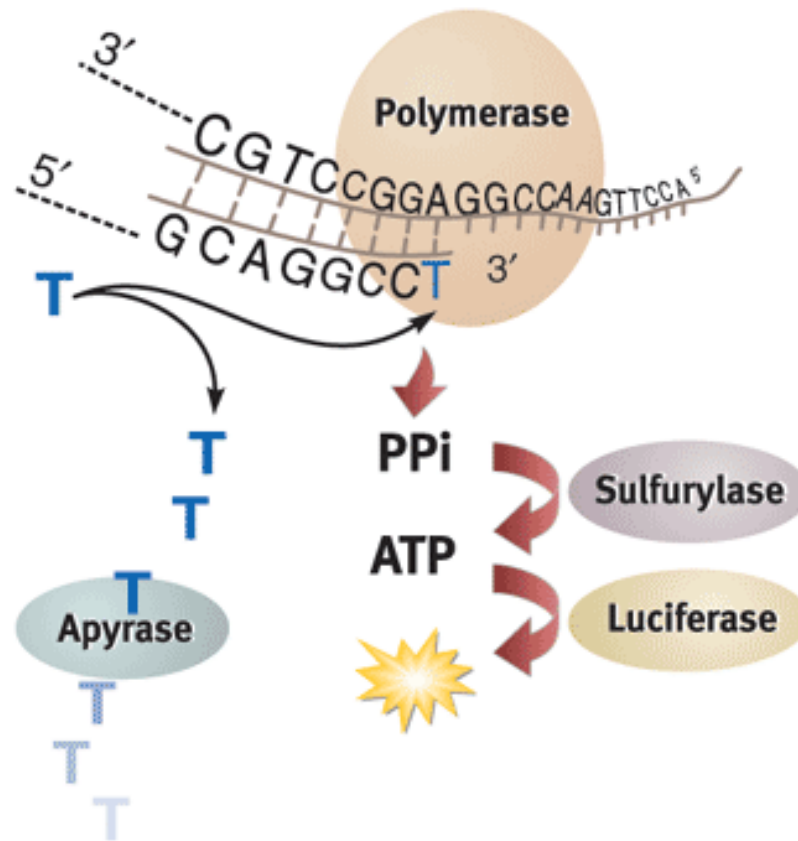


# Qiagen PyroMark Q48

- Pyrosequencing method
- 48 singleplex reactions
- Fast setup and sequencing
- No library preparation
  - Specific sequencing primer



# Pyrosequencing

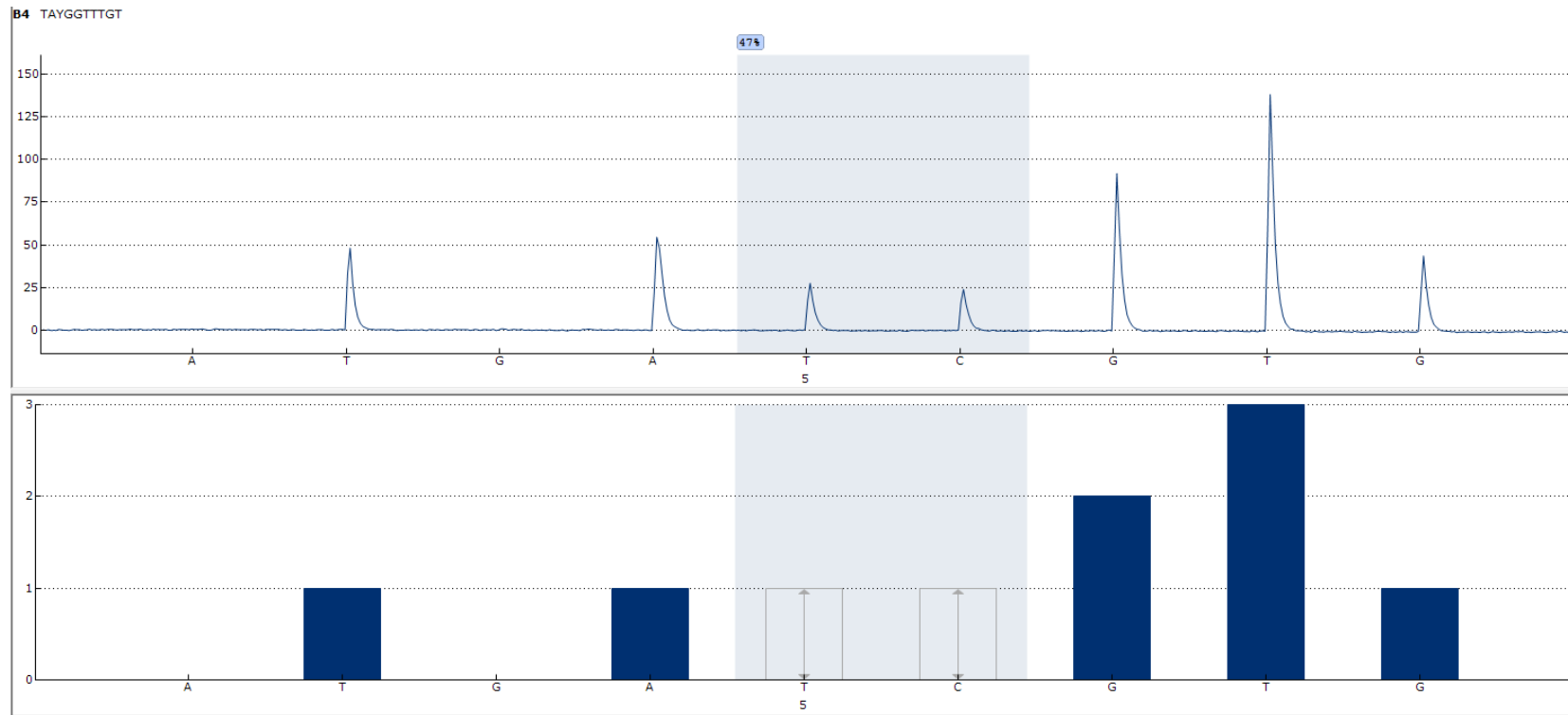


# PyroMark Q48 CpG assays results

- Assess the quality (**passed**, **check** or **failed**)
- Manually inspected all results
- Warning messages evaluated
- Sequences with errors are discarded

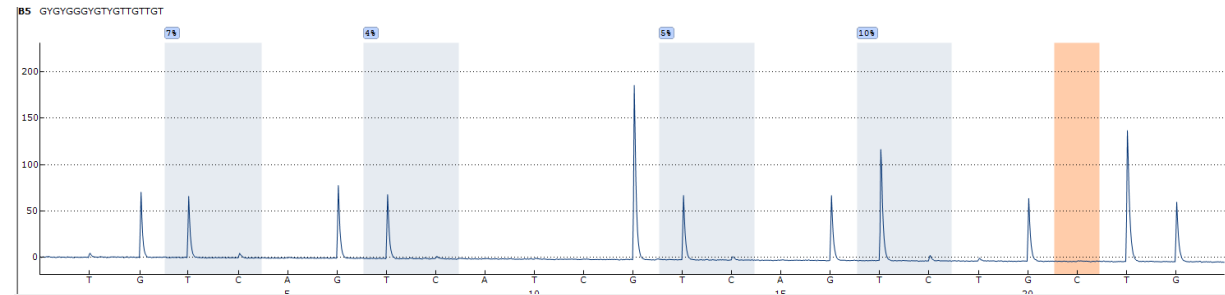


# Pyrogram example

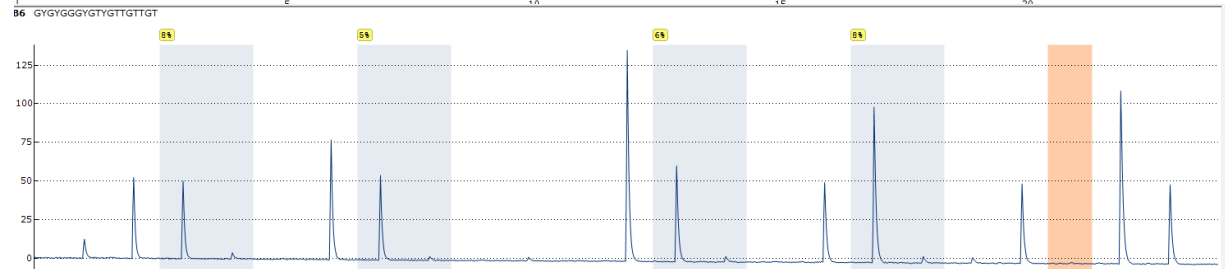


# Pyrogram qualities

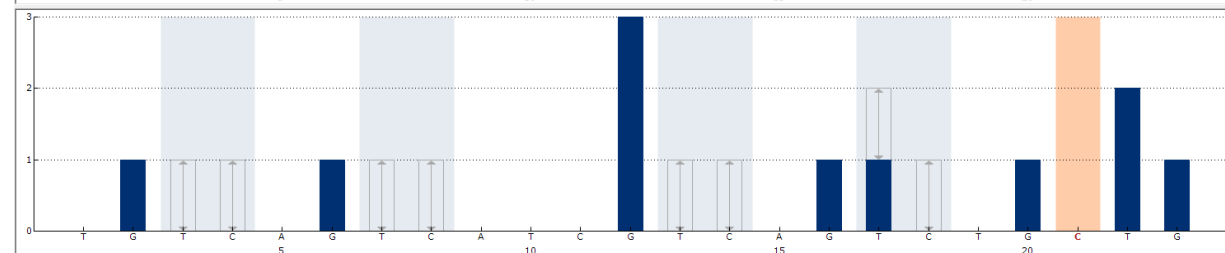
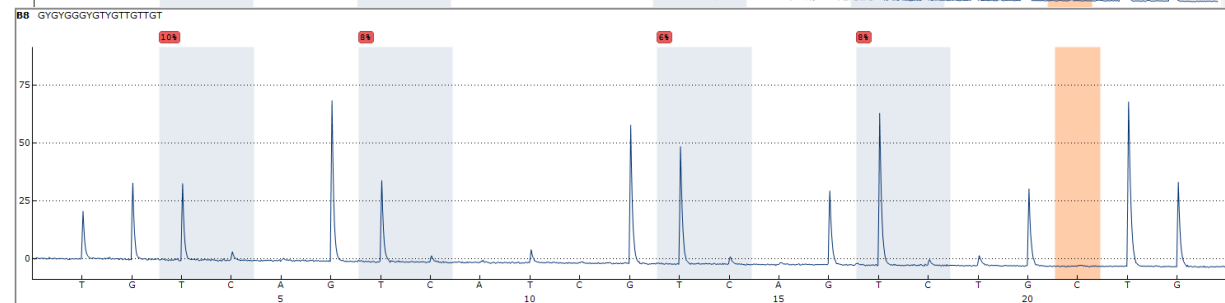
- Passed



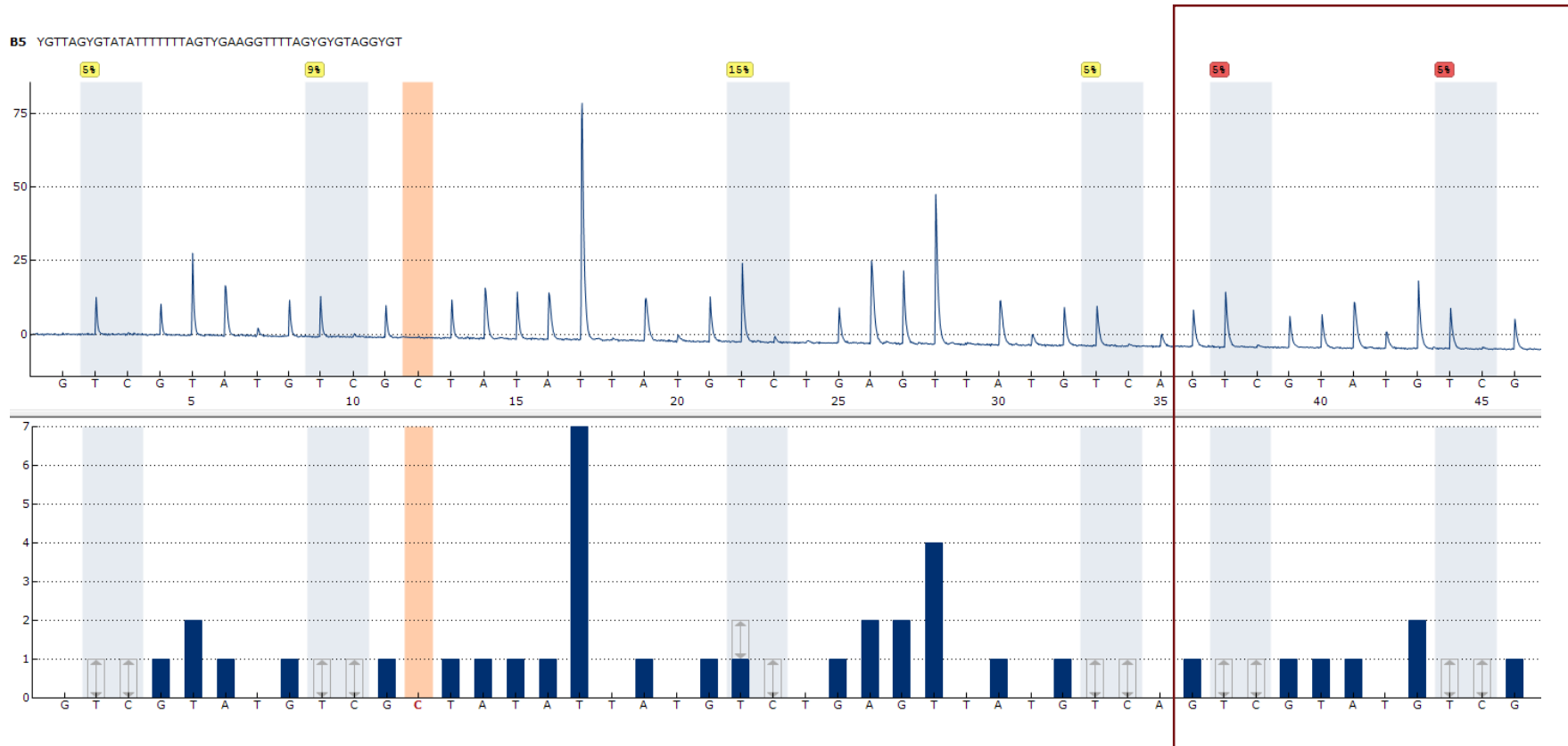
- Check



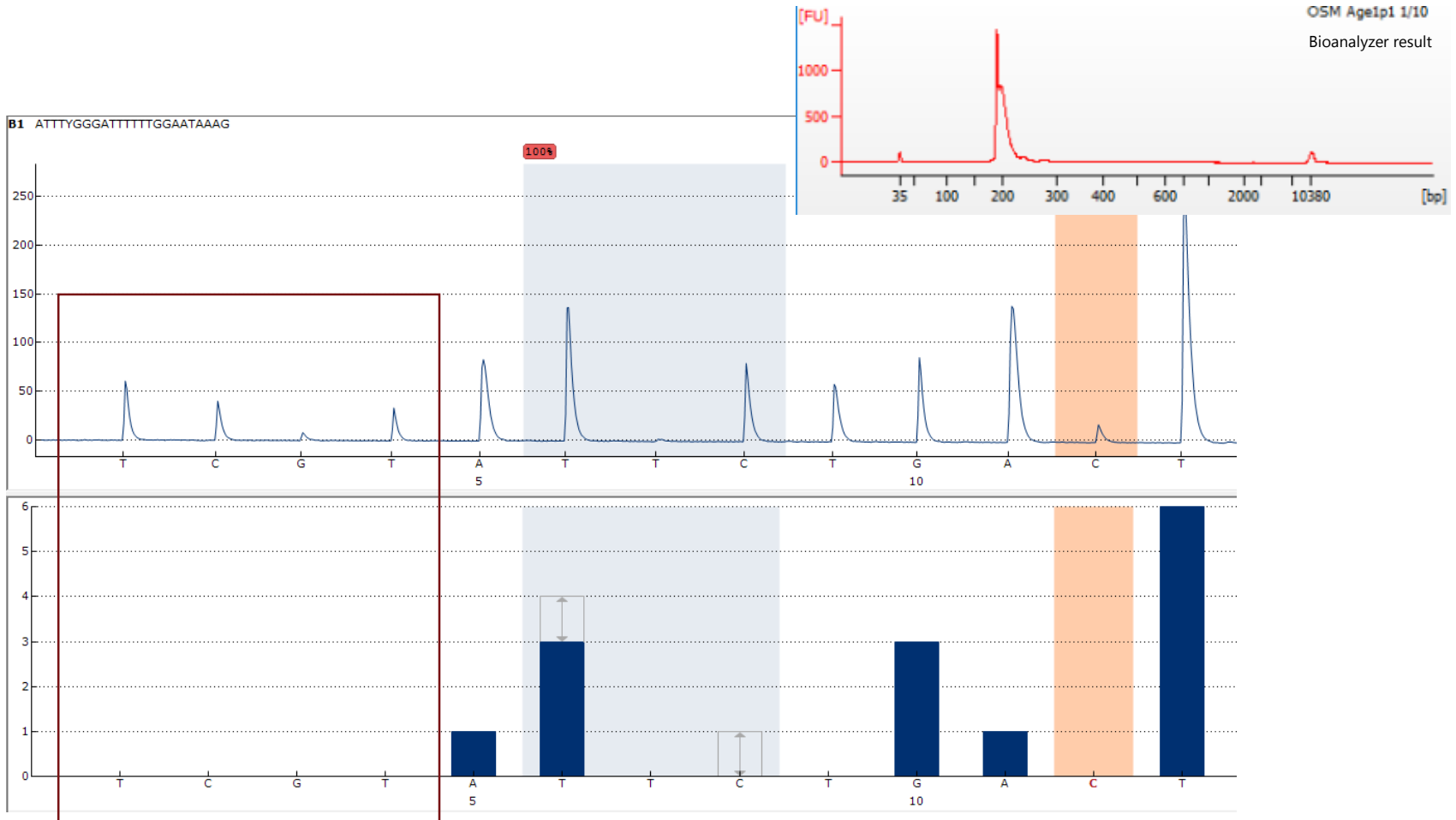
- Failed



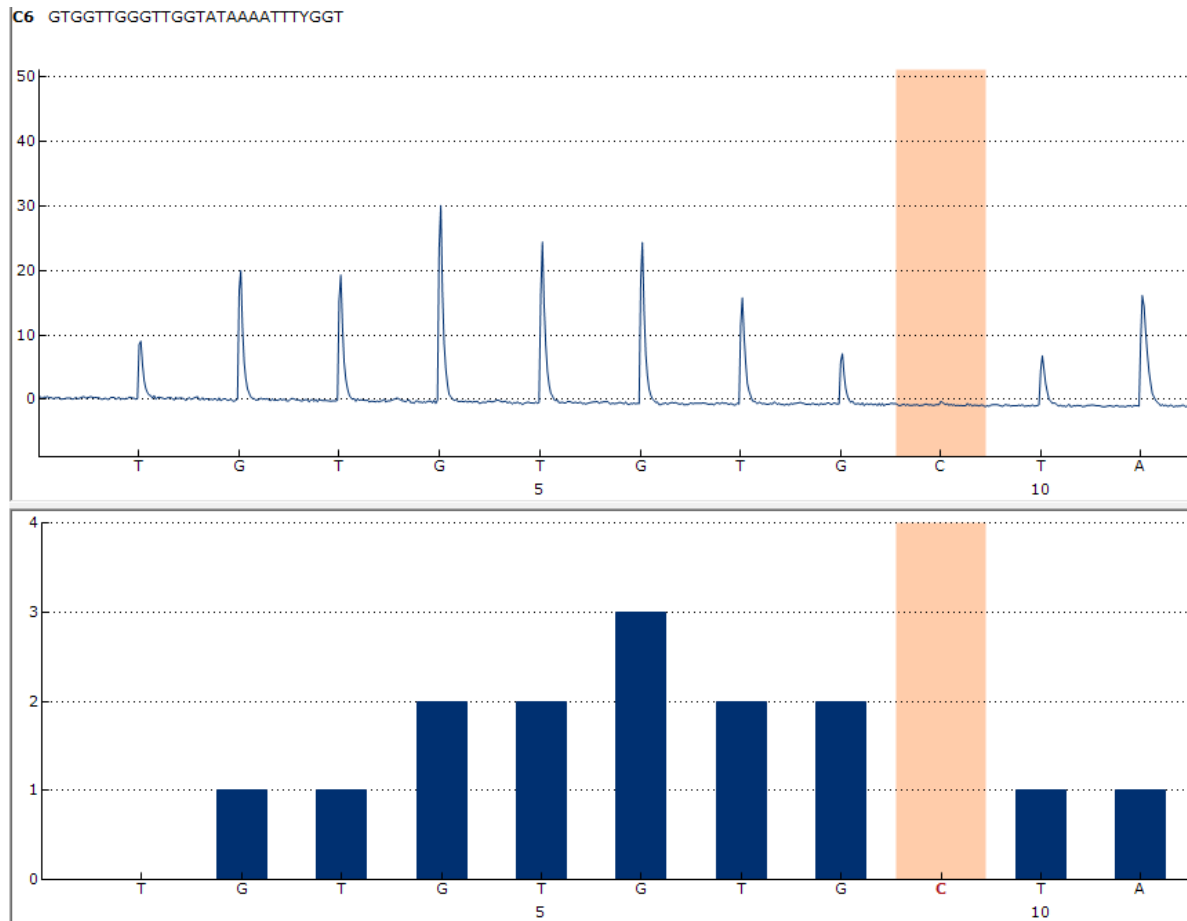
# Variable quality



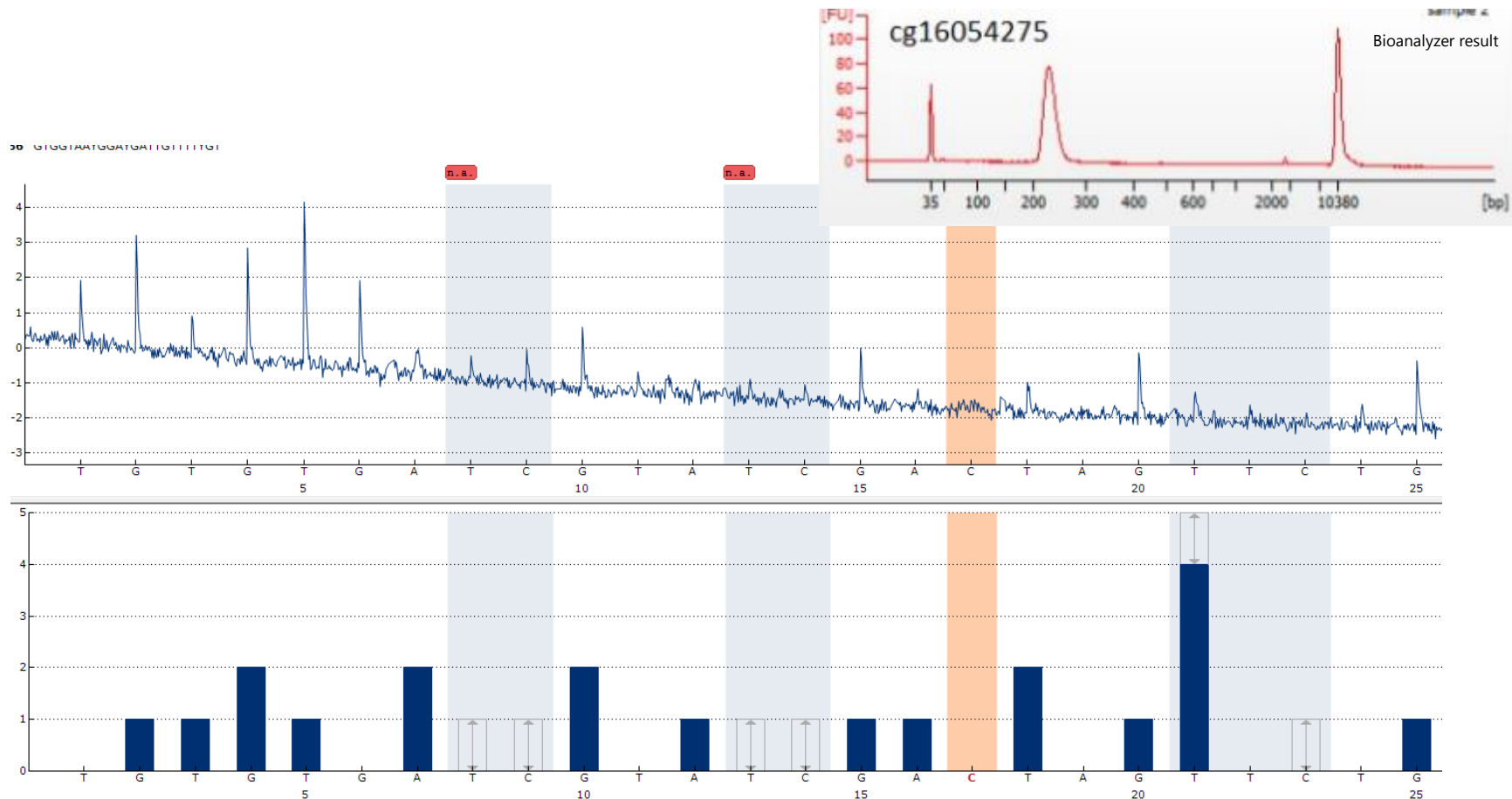
# Peaks in surrounding sequence



# Peak height deviation

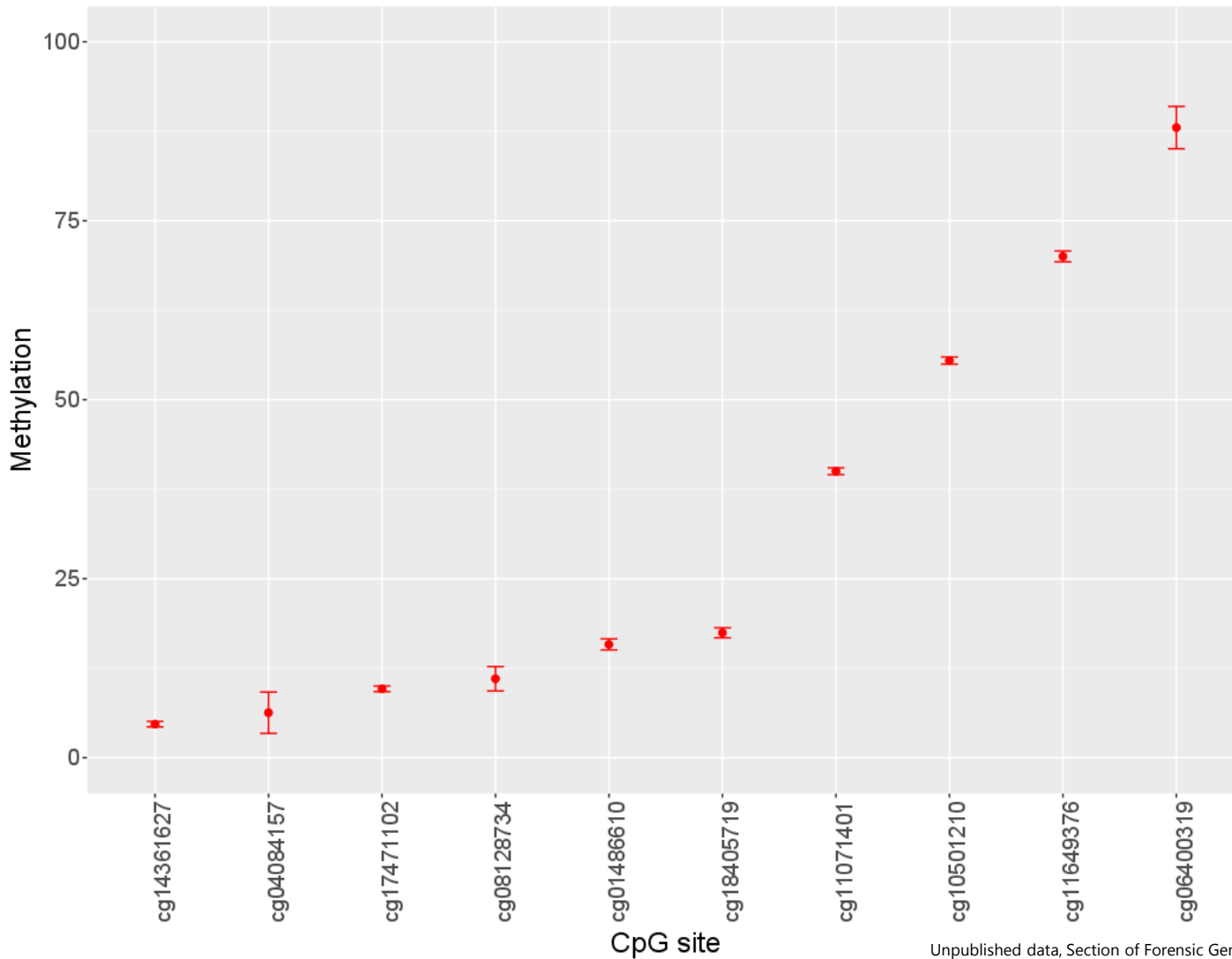


# Completely failed

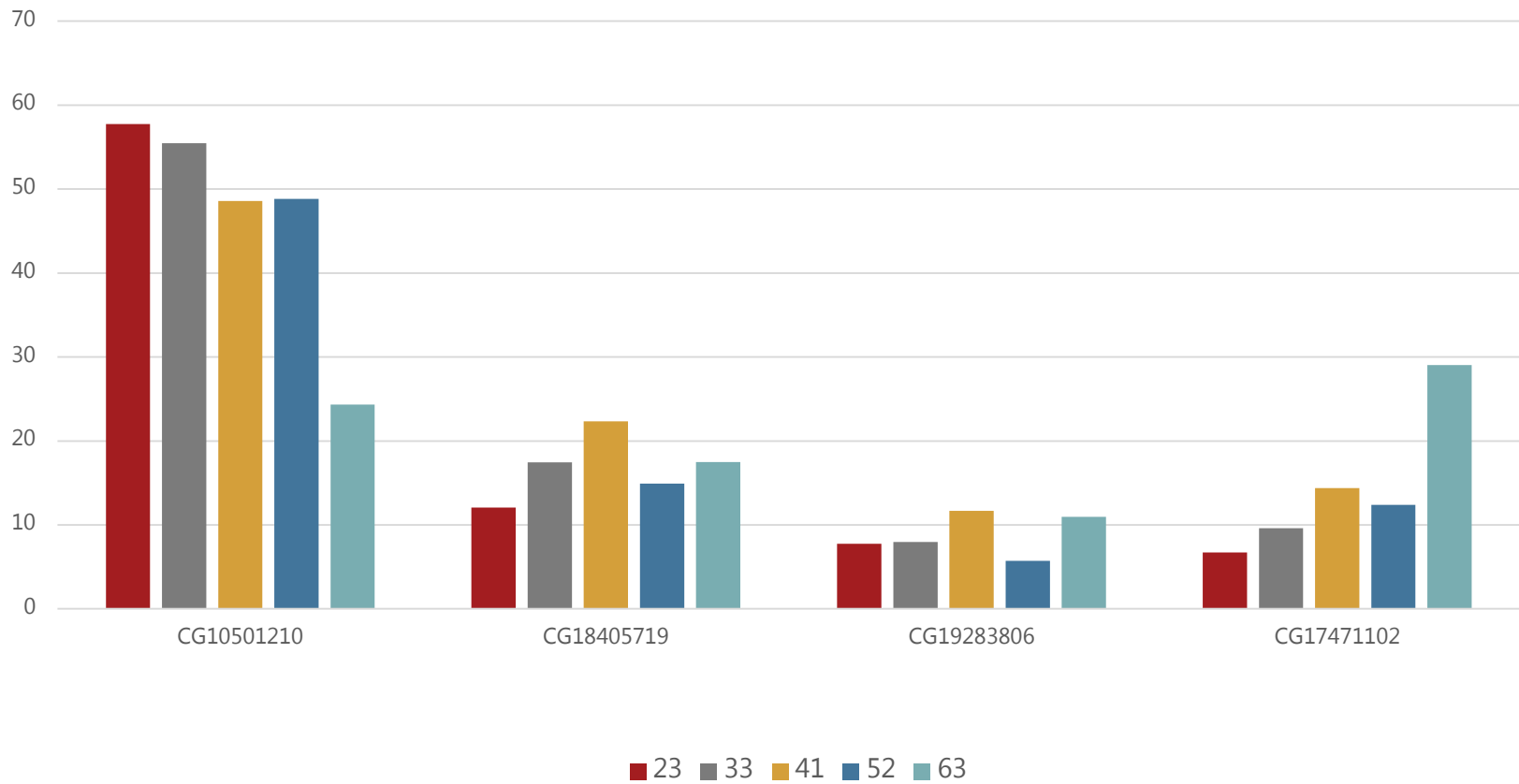


# PyroMark Q48 results

- Personal DNA methylation profile

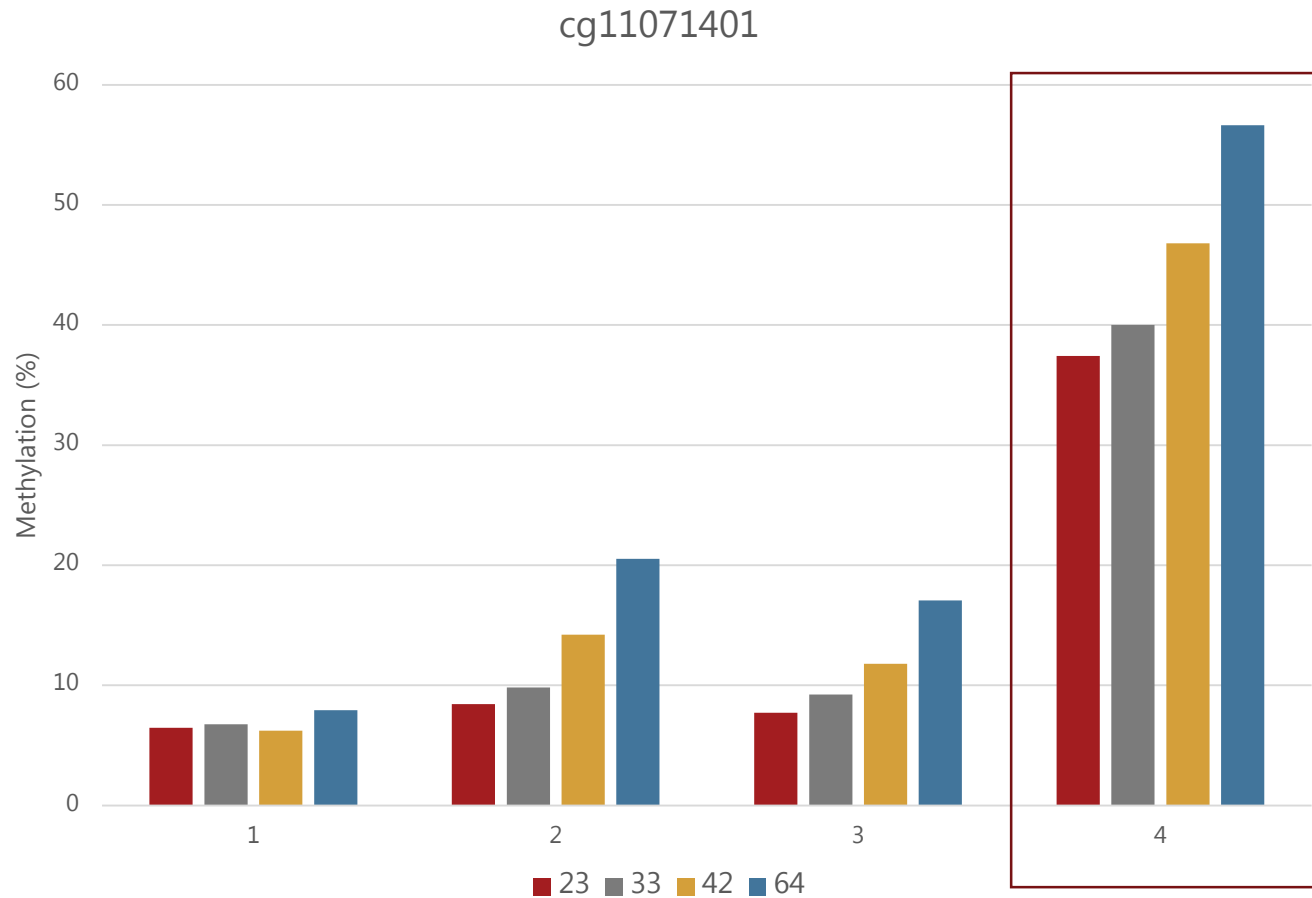


# Age association

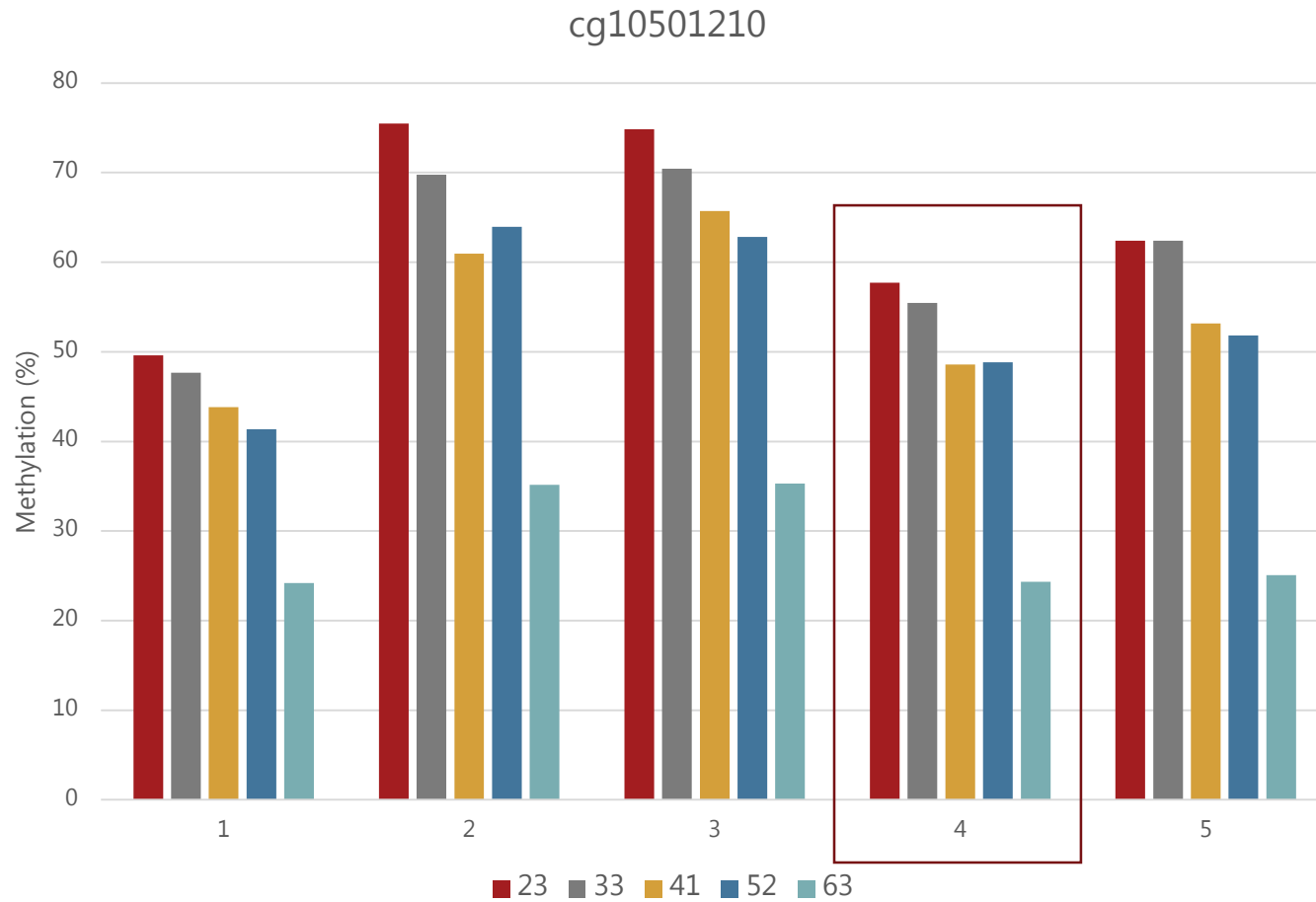




# CpGs in one sequence



# CpGs in one sequence



# Conclusion on PyroMark Q48

- Precise measurements of DNA methylation
- Good for confirmation of age associated CpGs
- Need for optimization of CpG assays

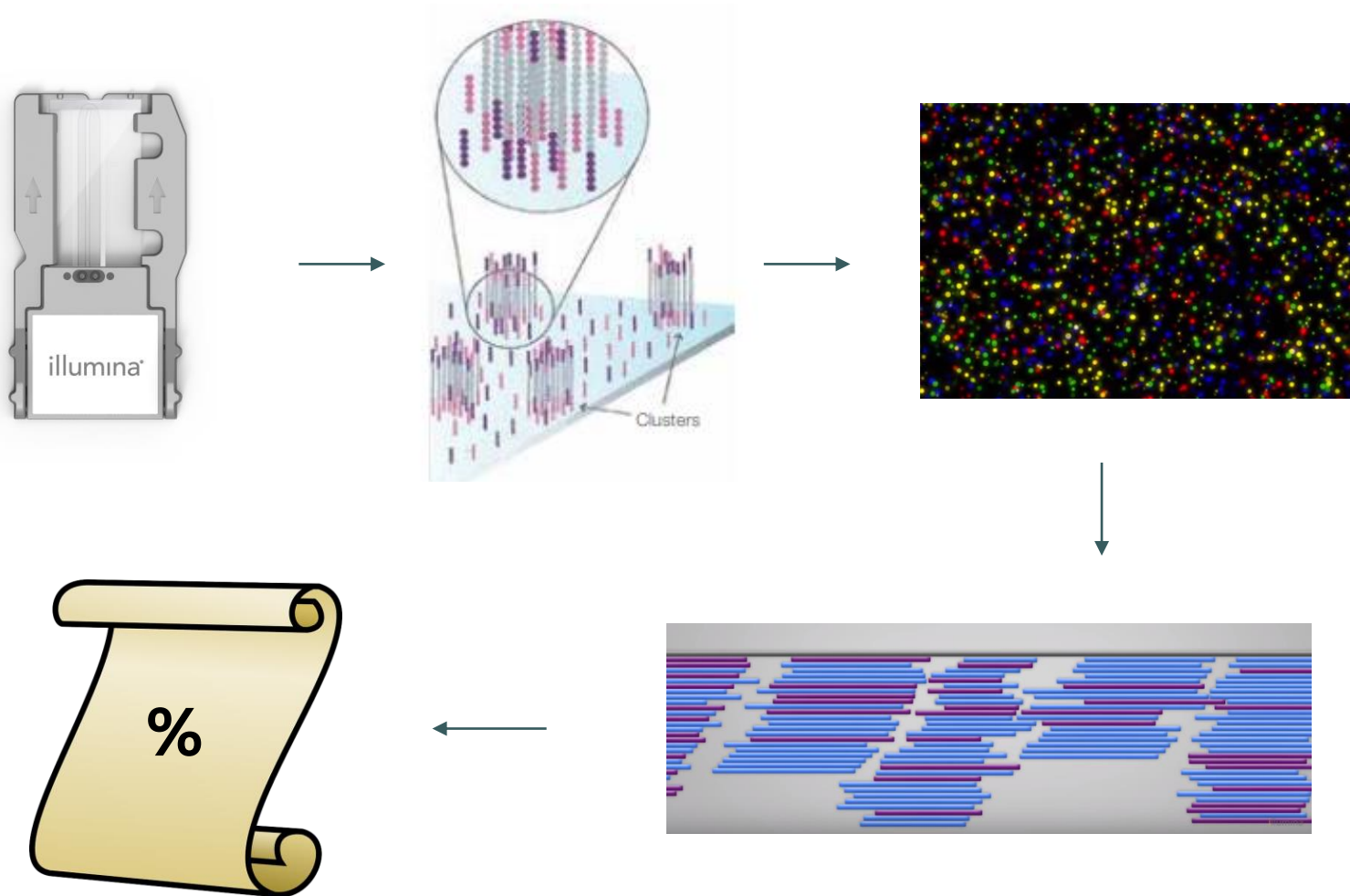


# Illumina MiSeq

- Illumina sequencing technology
- 96 multiplex reactions possible
- Library preparation



# Illumina sequencing

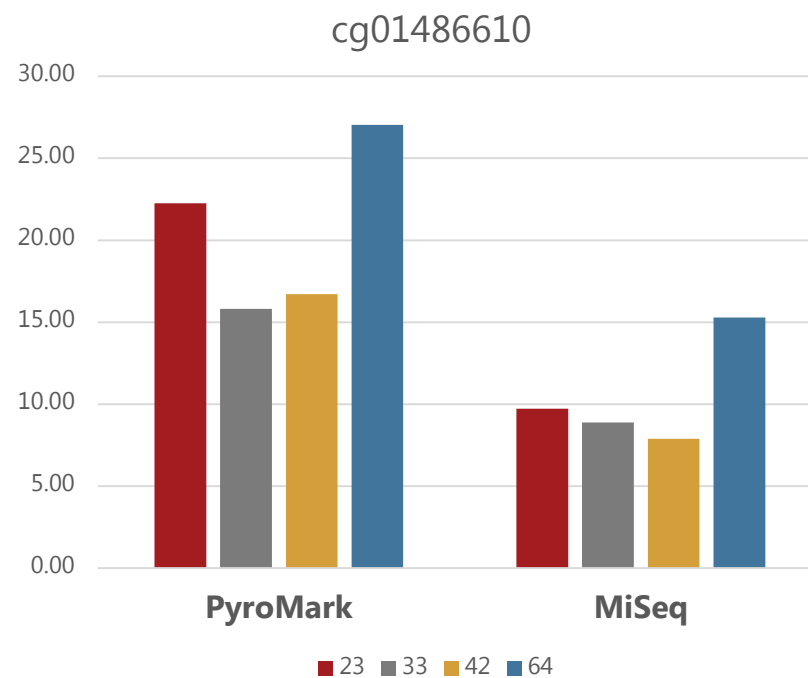
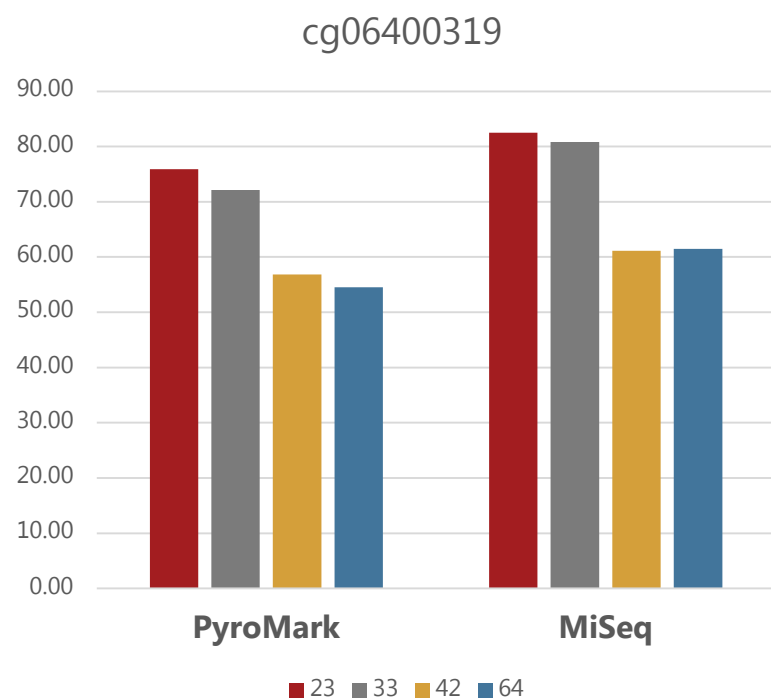


# Comparison of DNA methylation results

## PyroMark Q48 and Illumina MiSeq

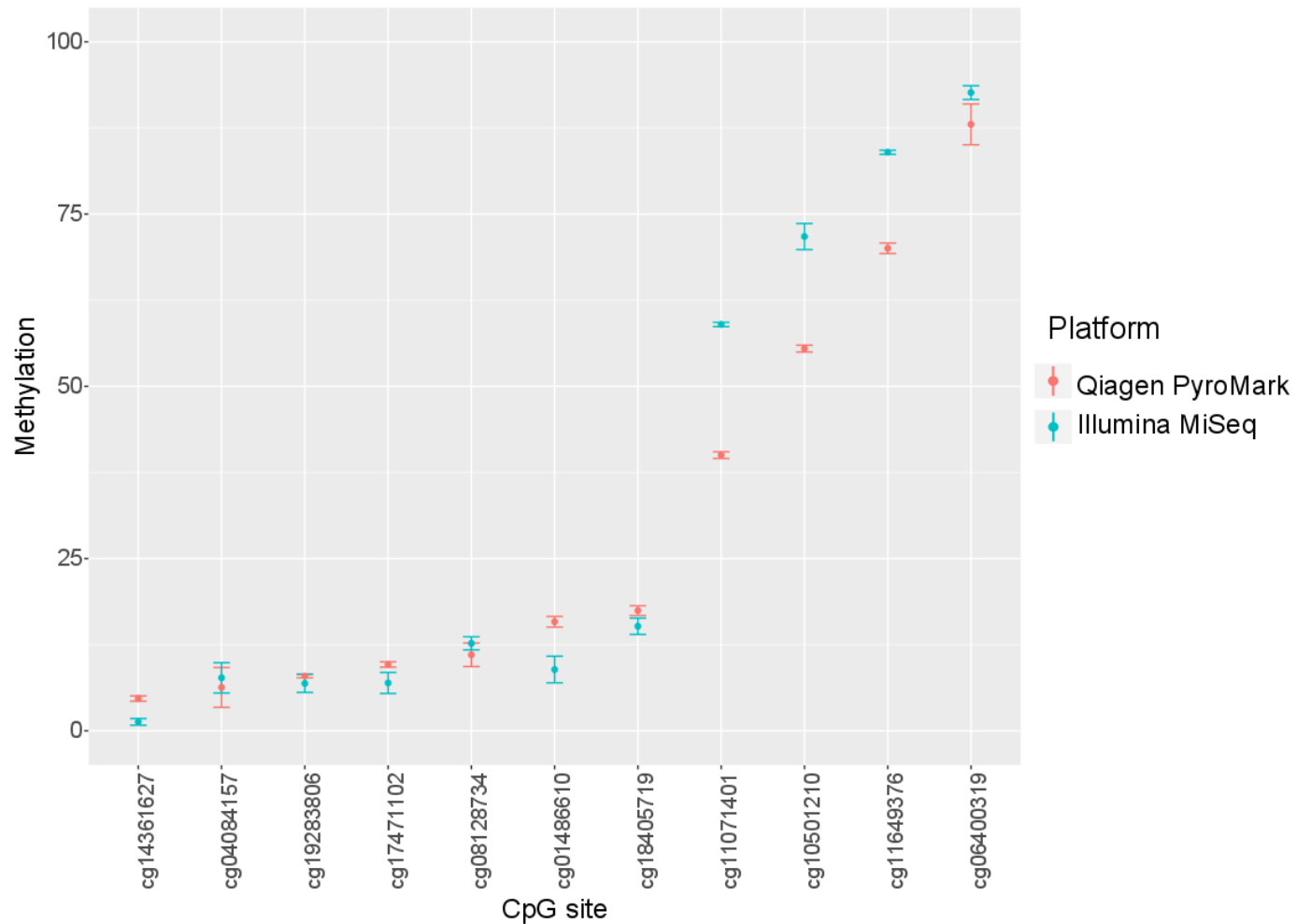


# Comparison of DNA methylation results



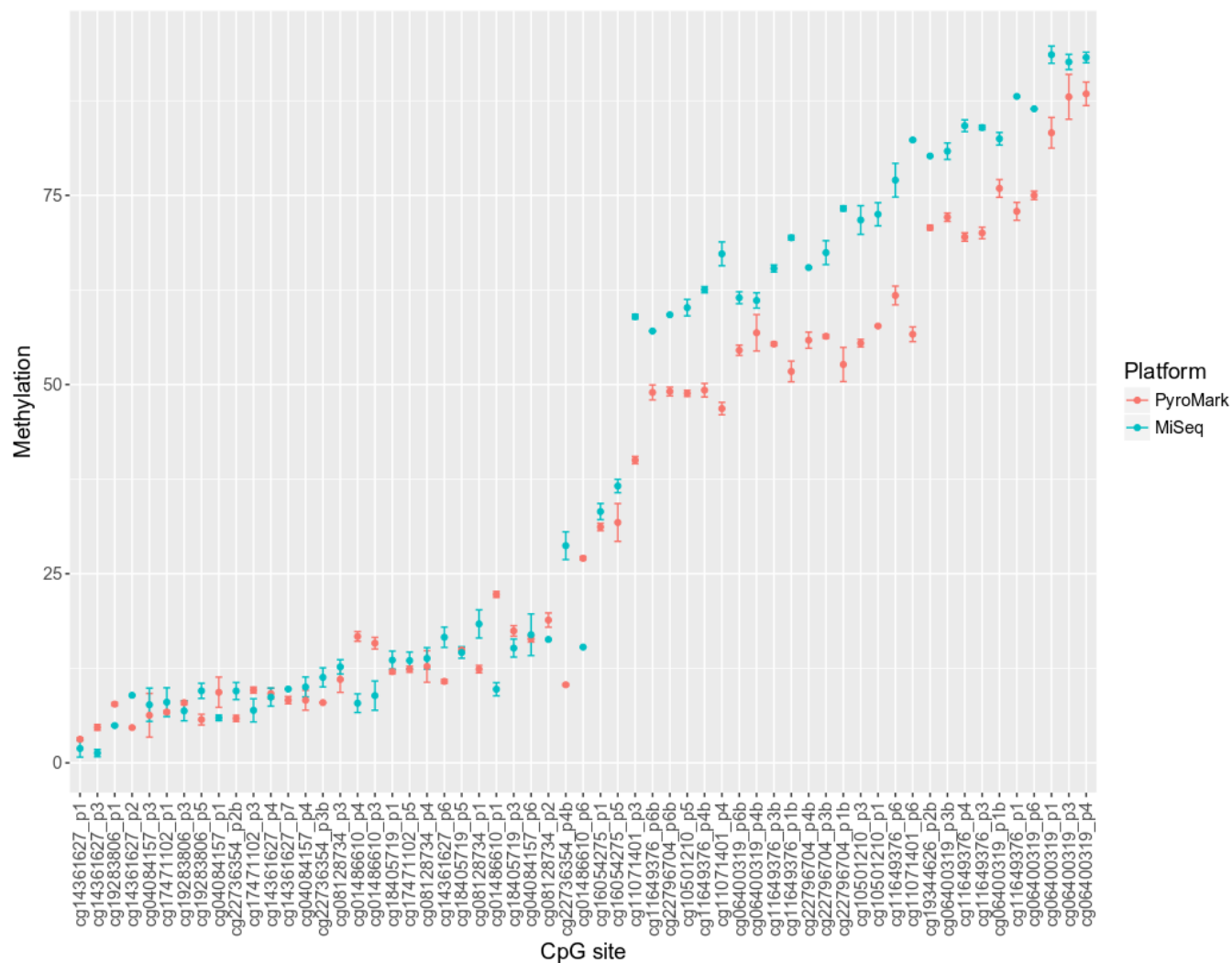
# Comparison of DNA methylation results

- Personal DNA methylation profile
- Based on buccal swab samples



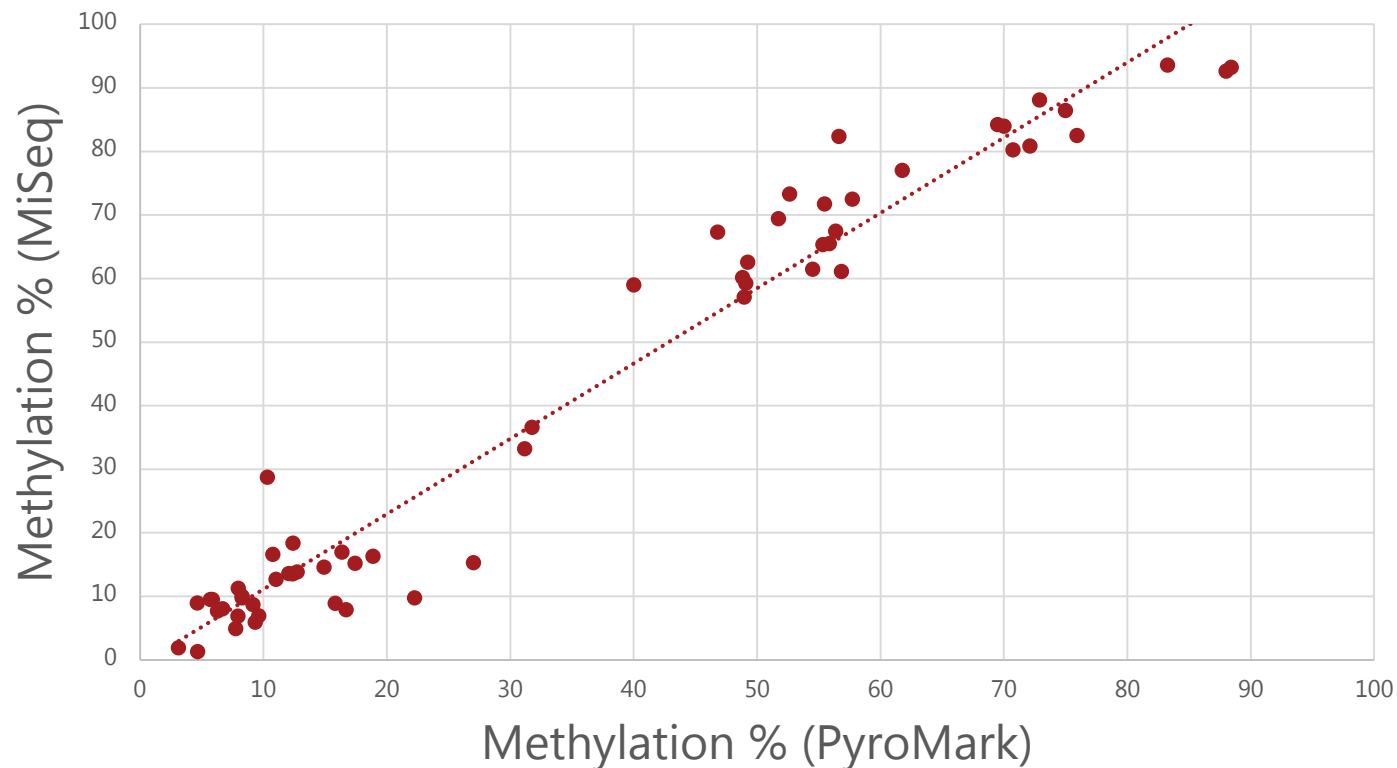


# Comparison PyroMark Q48 to Illumina MiSeq



# Comparison PyroMark Q48 to Illumina MiSeq

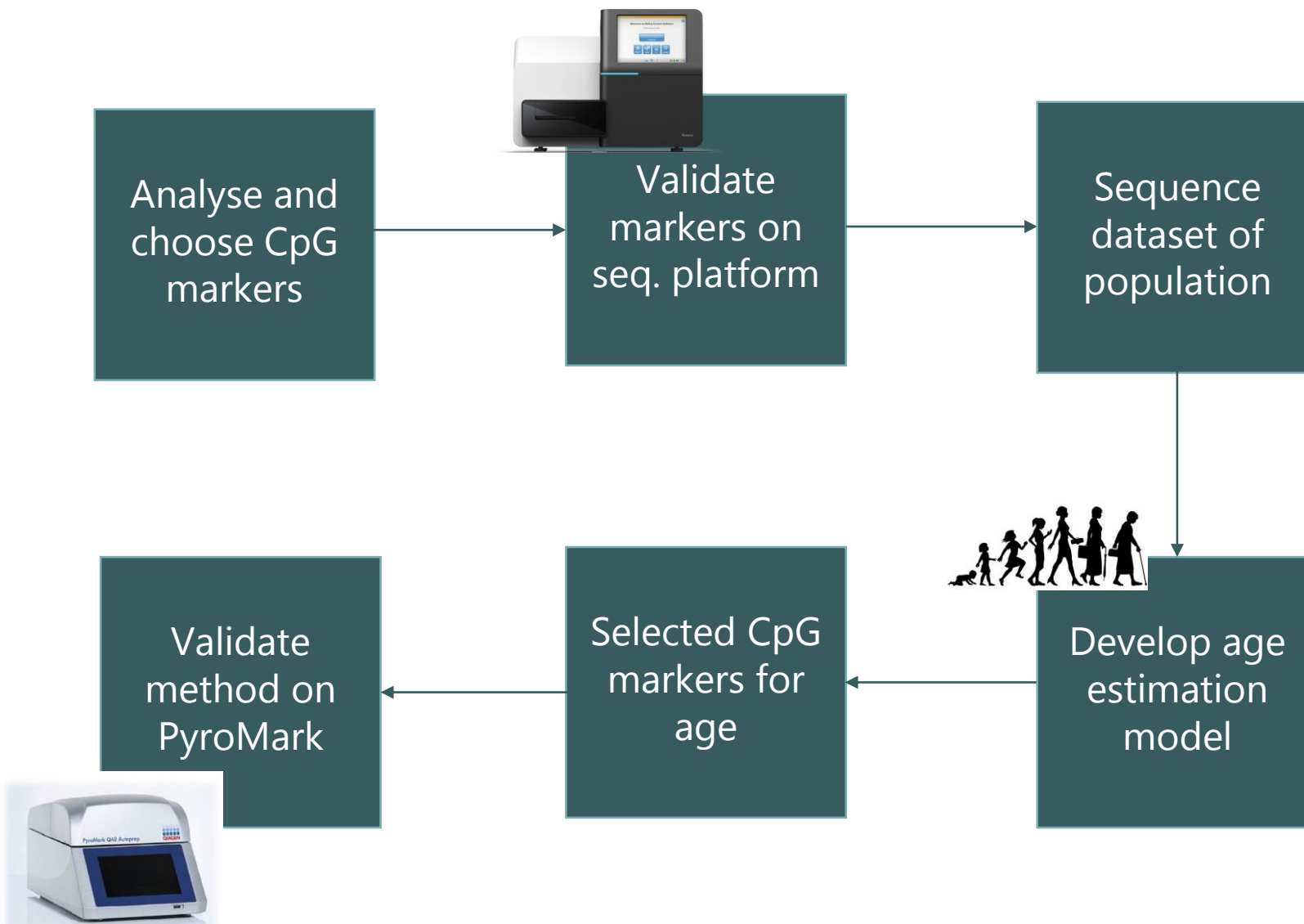
- 59 replicated measurements
- High correlation ( $R^2 = 0.958$ )
- Slope = 1.184



# Conclusion on comparison

- Both platforms are useful for analysis of DNA methylation
- DNA methylation results are reproducible between platforms
- Deviations tend to increase with higher methylation levels between platforms

# Proposed workflow



# Thanks to

- People at the Section of Forensic Genetics
- Regitze Skals
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- Olivia Meyer
- Marie-Louise Kampmann
- Claus Børsting
- Niels Morling

Thank you for listening