

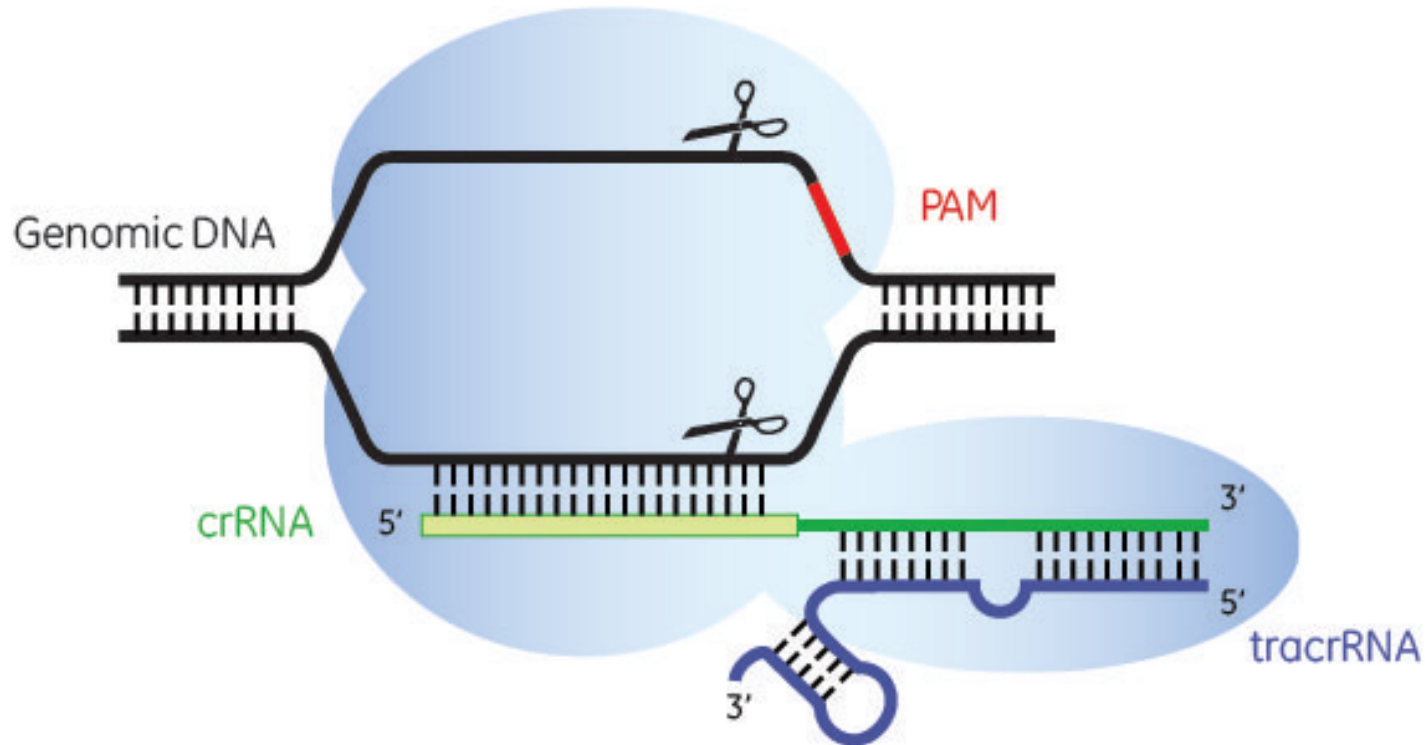


# Repeat Expansion Genotyping With PacBio No-Amp & Repeat-Analysis

Brett Bowman  
January 17<sup>th</sup>, 2018

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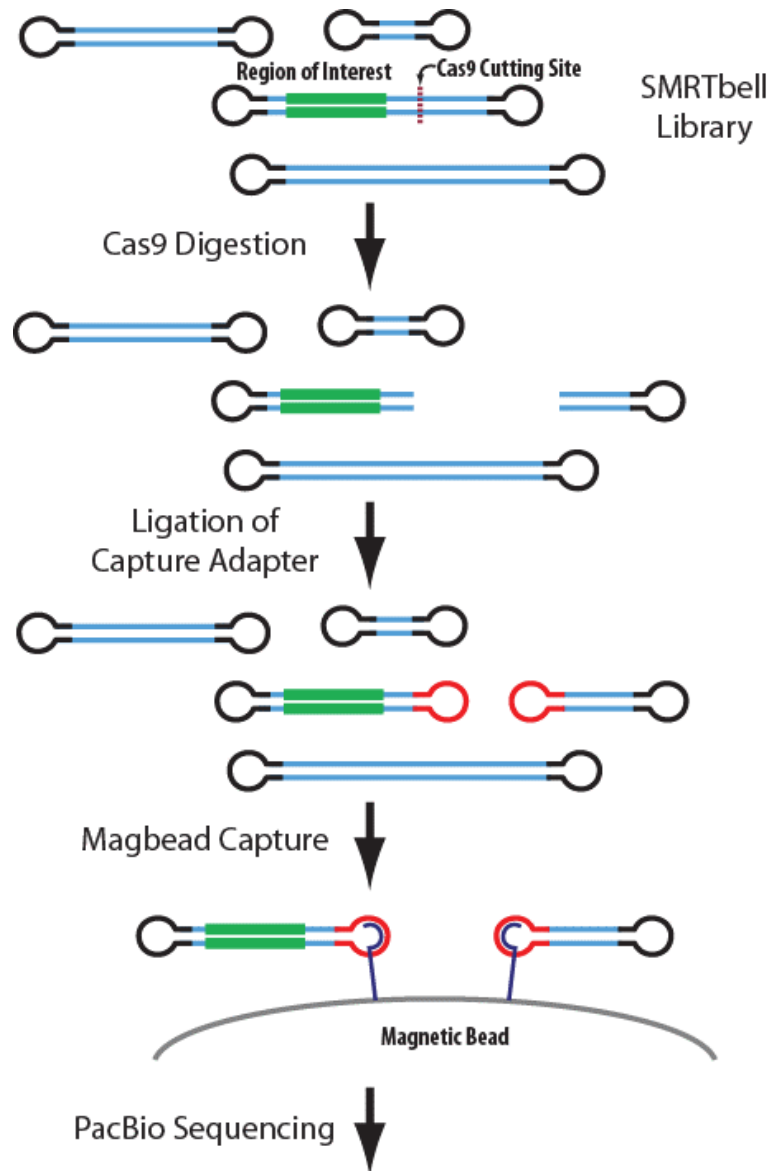
## NO-AMP TARGET ENRICHMENT – CRISPR/CAS9 SYSTEM



Some *in vivo* applications:

- Gene silencing
- Homology-directed repair
- Transient gene silencing or transcriptional repression
- Transient activation of endogenous genes
- Transgenic animals and embryonic stem cells

# NO-AMP TARGET ENRICHMENT – OVERVIEW



## REPEAT ANALYSIS WORKFLOW – OVERVIEW

SUBREAD  
FILTERING

REFERENCE-  
GUIDED  
CLUSTERING

TEMPLATE  
ESTIMATION

TEMPLATE  
SCORING

TEMPLATE  
PREDICTION

ASSIGNMENT &  
CONSENSUS

OUTPUTS

Sample

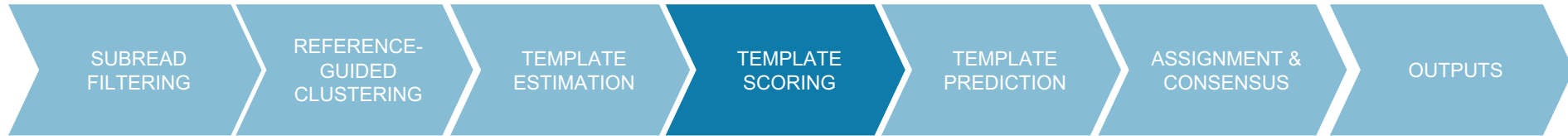
Locus

Allele

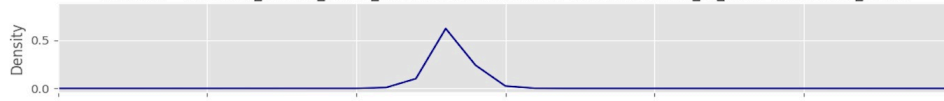
Clean-Up

Report

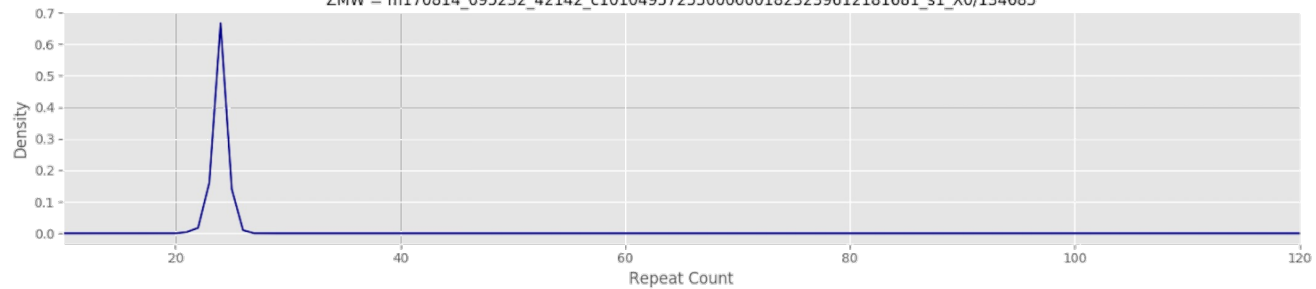
# REPEAT ANALYSIS WORKFLOW – TEMPLATE SCORING BY SUBREAD



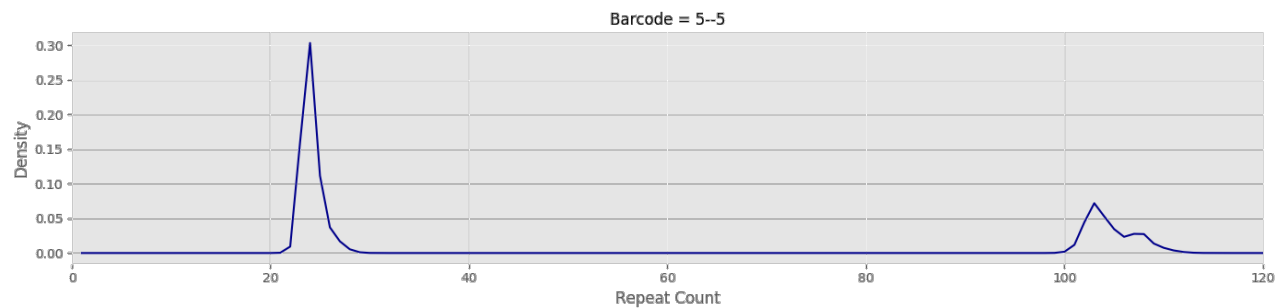
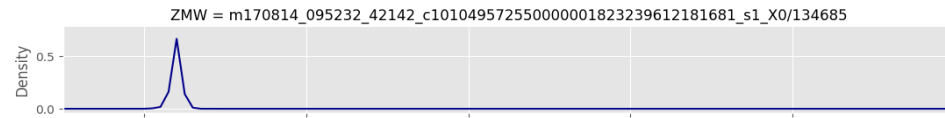
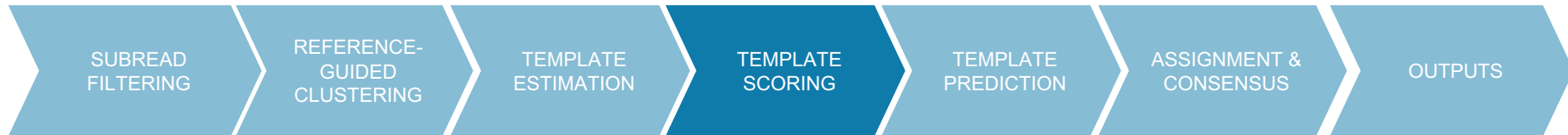
Subread = m170814\_095232\_42142\_c101049572550000001823239612181681\_s1\_X0/134685/61356\_62435



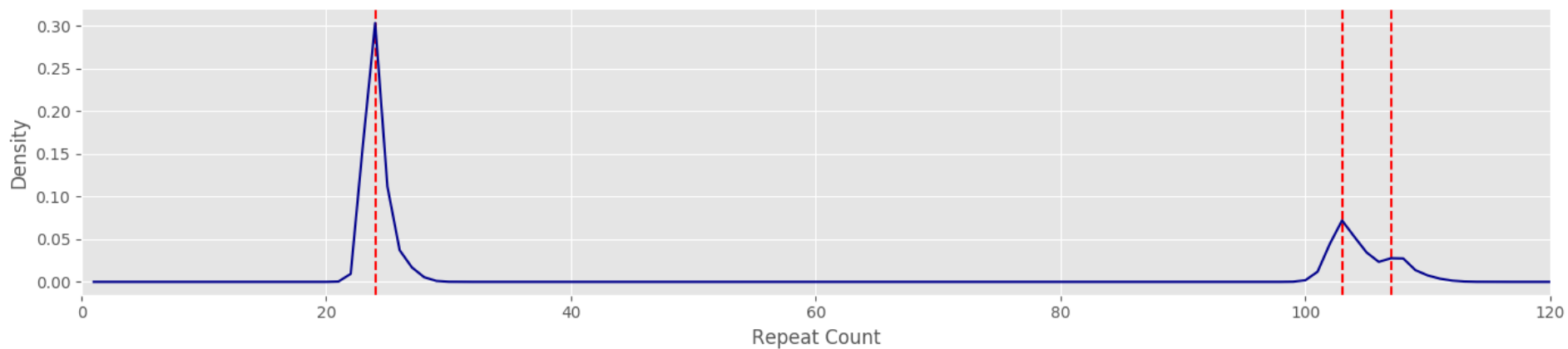
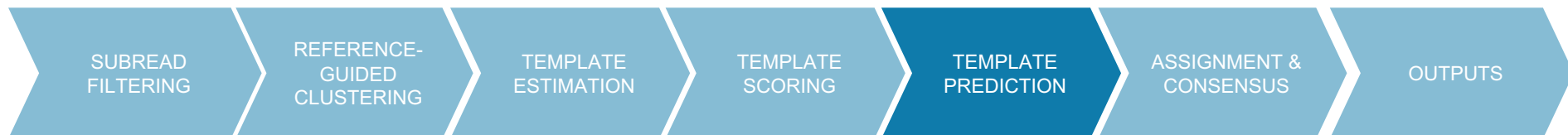
ZMW = m170814\_095232\_42142\_c101049572550000001823239612181681\_s1\_X0/134685



# REPEAT ANALYSIS WORKFLOW – TEMPLATE SCORING BY ZMW

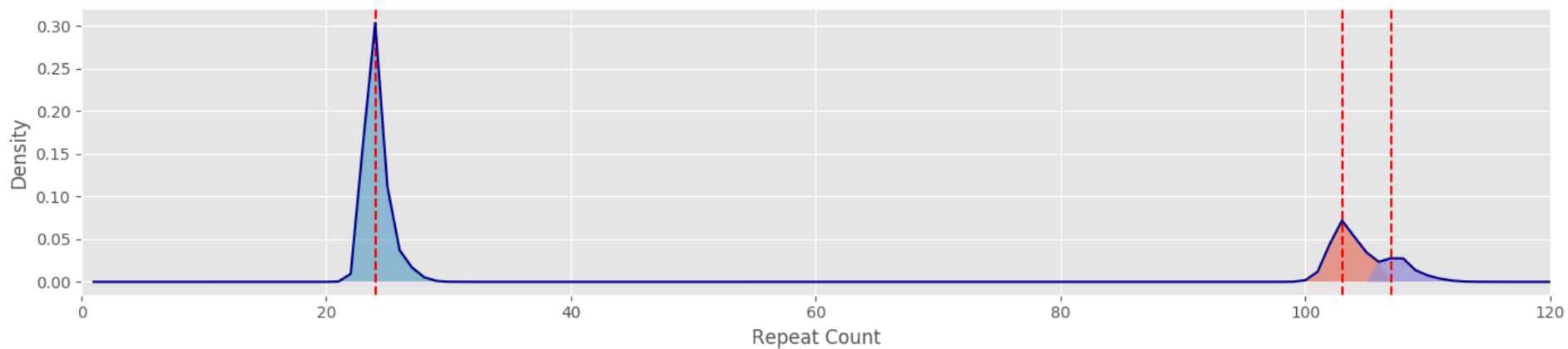
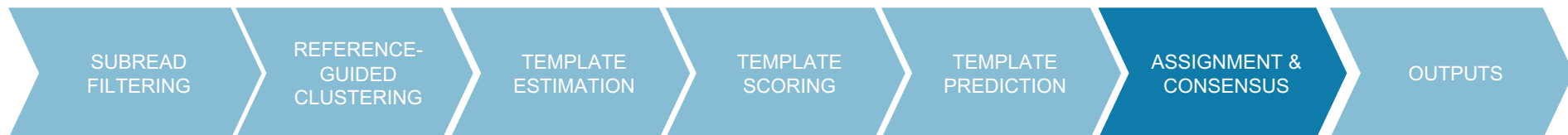


# REPEAT ANALYSIS WORKFLOW – TEMPLATE PREDICTION



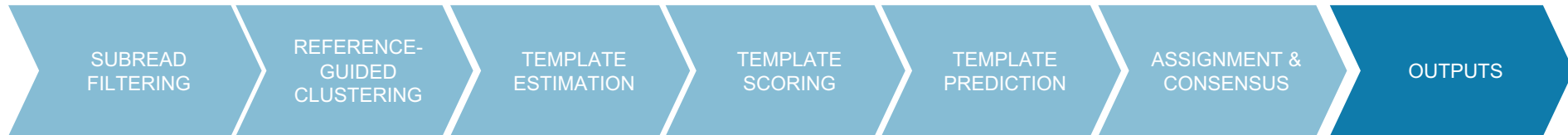


# REPEAT ANALYSIS WORKFLOW – ASSIGNMENT & CONSENSUS

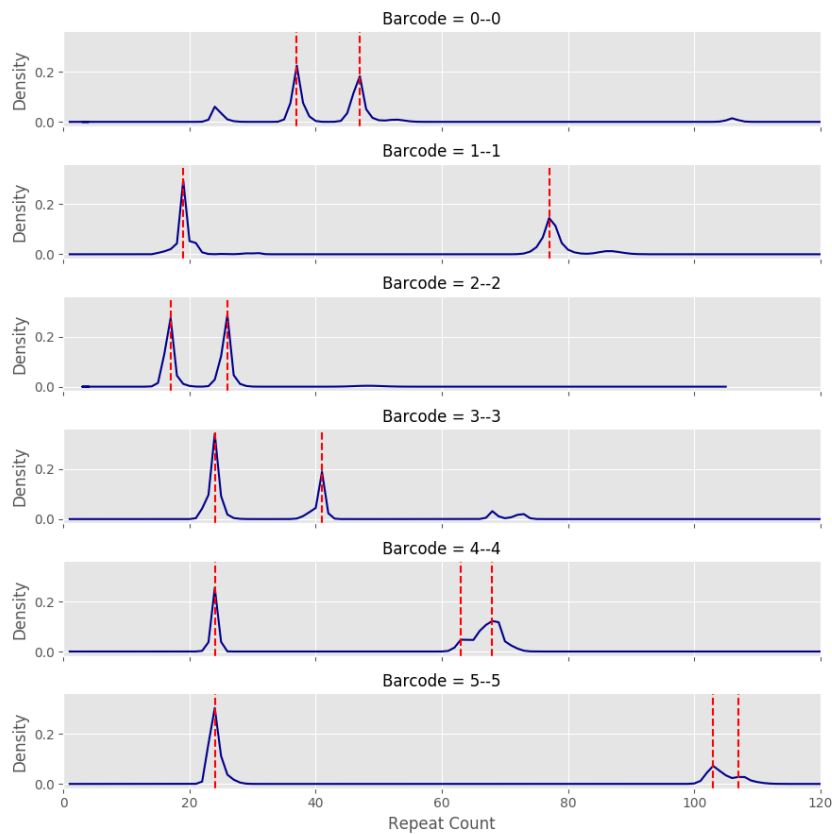




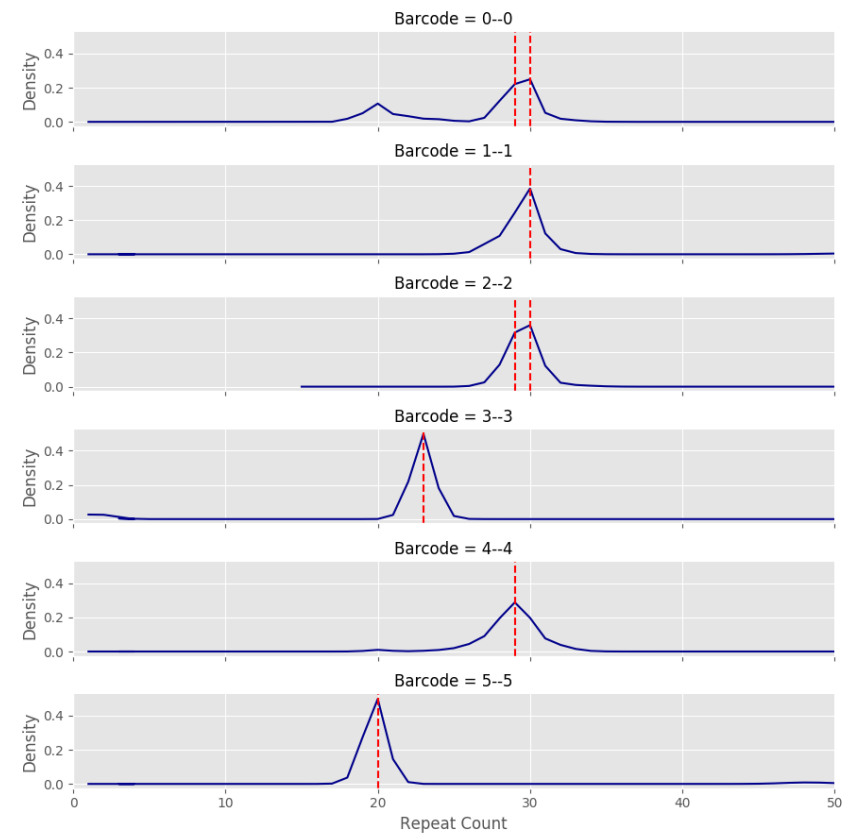
# REPEAT ANALYSIS WORKFLOW – OUTPUTS



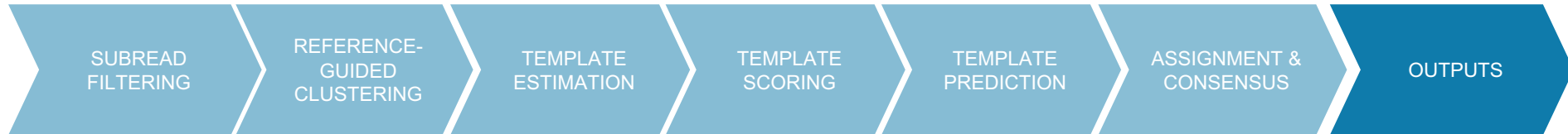
## HTT



## FMR1



# REPEAT ANALYSIS WORKFLOW – OUTPUTS



## repeat\_analysis.fastq

@Barcode5--5\_Cluster1\_Phase0\_NumReads308 CAGx24

```

GATCCTTTCGCATGGGCGCTGCGCCCGCGCTCGGCGCCCCCTCCACGGCCCGCCCGTCCATGGCCCGTCCCTTCATGGGCGAGCCCCTCCATGGCCCTGCCCTC
CGCGCCCCACCCCTCCCTCGCCCCACCTCTCACCTTCCTGCCCGCCCCAGCCTCCCCACCCCTCACCGGCCAGTCCCCTCCCCTATCCCGCTCCGCCCCCTAGCCG
CCCCGCCCTCAGCCGGCTGCCTAATGTCCTCCGTCGCCAGCATCGCCCCGCCCGCCCCCGTCTCGCCCCGCCCTCAGGGGGCTCCCTGCTGTGCCCGCCCC
GGCTCGCCACGCCCTACCTCACCACGCCCCCGCATCGCCACGCCCCCGCATCGCCACGCCTCCCTACCATGCAGTCCCGCCCGTCCCTTCCCTCGTCCCGCT
+
  
```

```

~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:
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~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:~::~:
  
```

...

## repeat\_analysis\_mapping.5—5.HTT.csv

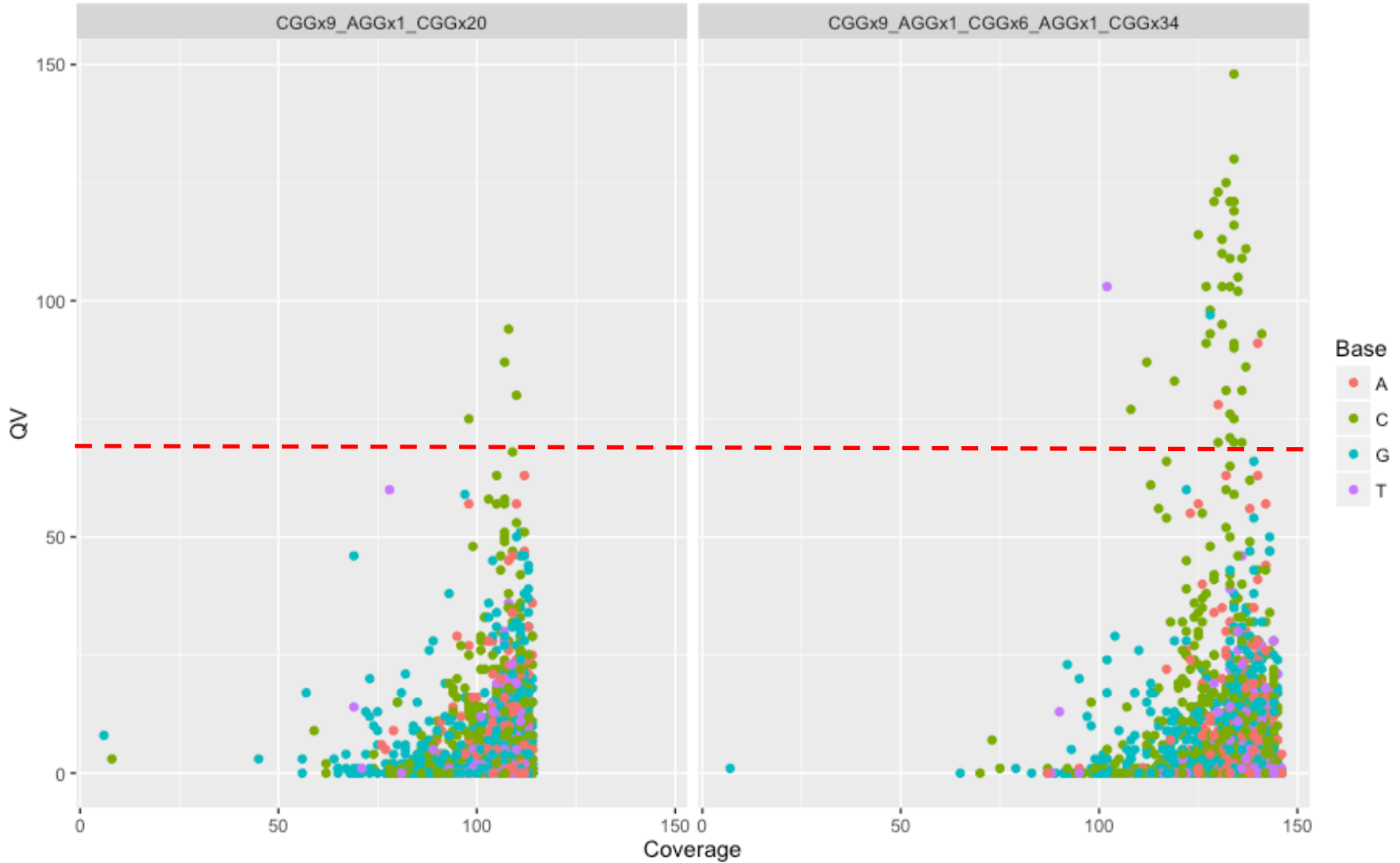
**SubreadId,Barcode5--5\_Cluster1\_Phase0\_NumReads308,Barcode5--5\_Cluster1\_Phase1\_NumReads99,Barcode5--5\_Cluster1\_Phase2\_NumReads55**

```

m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/0_1176,1,0,0
m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/1218_2385,1,0,0
m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/2462_3616,1,0,0
m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/3664_4817,1,0,0
m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/4900_6055,1,0,0
m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/6098_7318,1,0,0
m170814_095232_42142_c101049572550000001823239612181681_s1_X0/629/7400_8522,1,0,0
  
```

...

# ... AND NON-AMPLIFIED TARGETS RETAIN METHYLATION





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