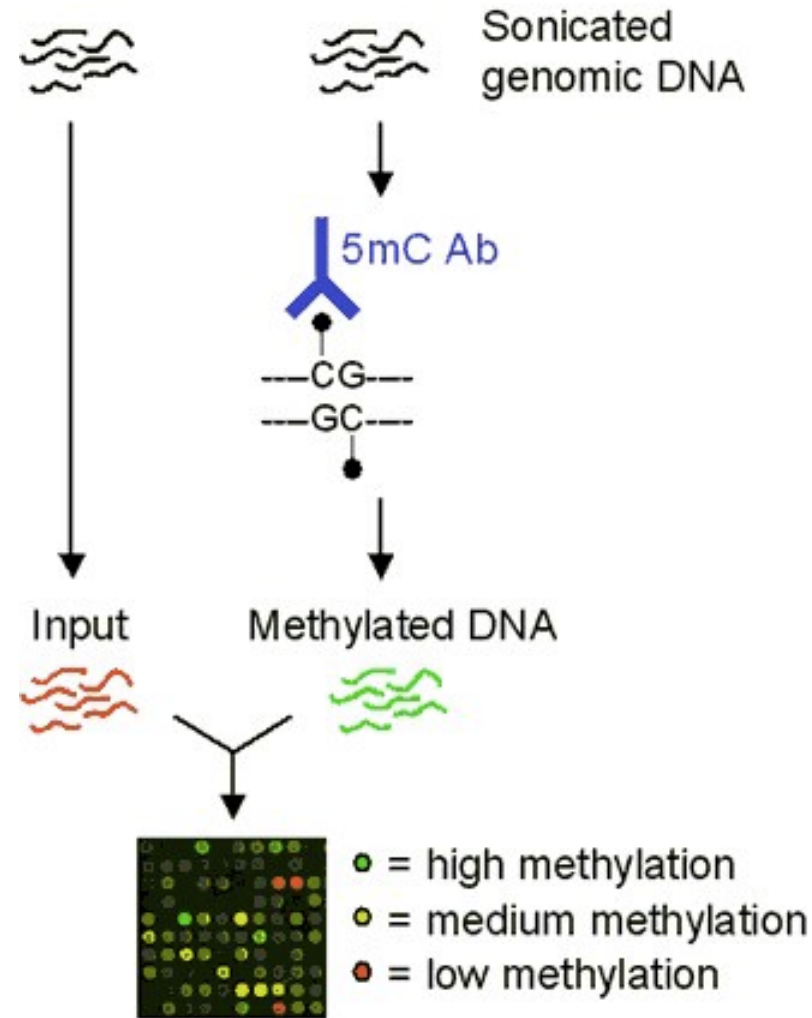
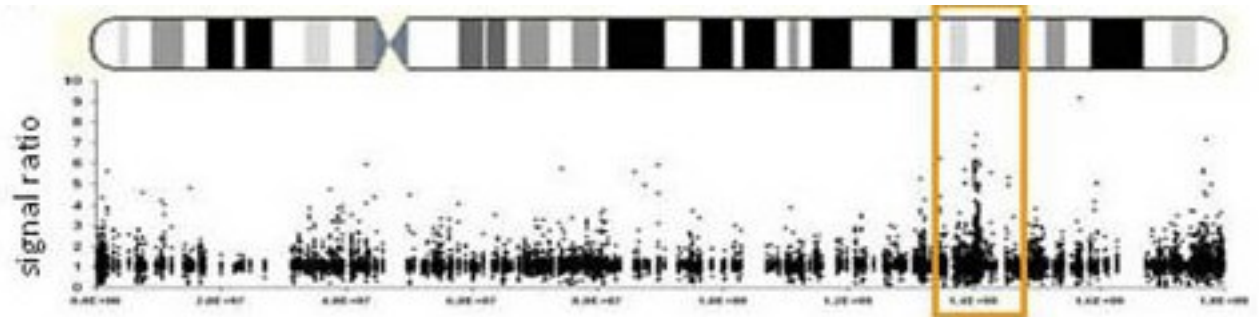
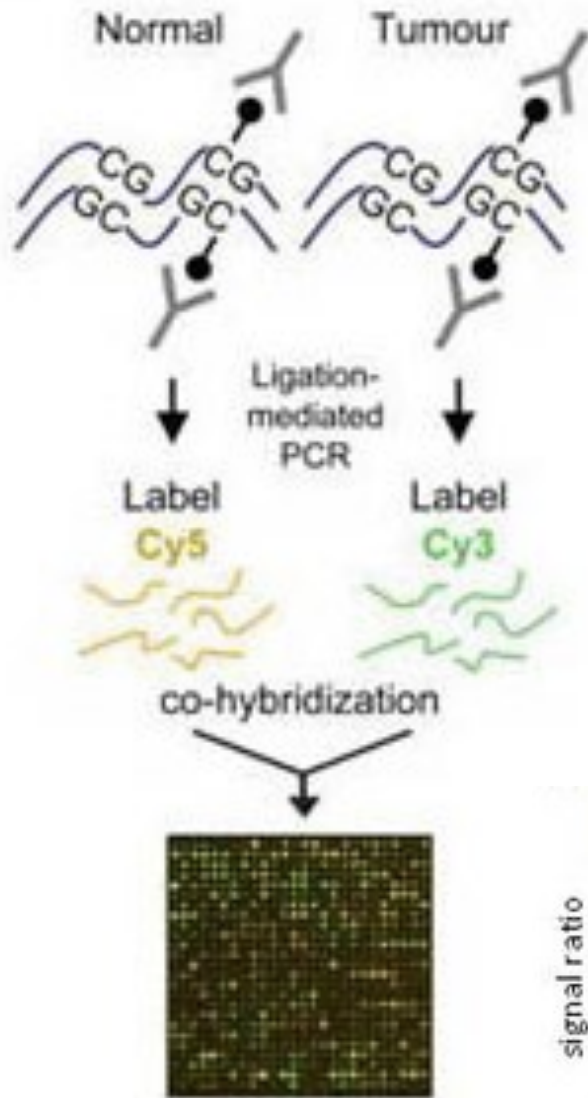


**MBD-Chip**

# Workflow: Identify methylated regions



# Workflow: differential methylation



# Oyster DNA Tiling Array

- 3 x 720k design



- Each feature  $\sim 100\text{bp} \times 720\text{k feature} = 72\text{Mb}$  for the microarray

# Oyster DNA Tiling Array

- Reducing the genome to most 'informative' parts (100Mb)
  - 28k oyster genes (227Mb)
  - 13k with evalue  $<1e-20$  (total: 138Mb)
  - 12k on scaffolds that cover 90% of genome (132kb)
- Generate intervals that include the gene and 2kb upstream (promoter)
  - Randomly select 6500 (of the  $\sim 12k$ ) intervals =100Mb

genes and flanks (macgavery:oysterv9\_9 scaffold100

348,927 - 365,328



350,000

360,000

||| oyster\_v9\_M\_fuzznuc\_CG.gff\_BED

||| mRNA GFF to BED

||| mRNAs on90%scaffold evaluateLT1e-20 BED

||| Get flanks on data 25

||| Concatenate on data 25 and data 42

||| Merge on data 43

||| 6500random intervals from Merged 2kb flanks and mRNA w evaluate <1e20

||| Array\_OID40453\_probe\_locations.gff\_BED

||| Array\_OID40453\_annotation.gff\_BED

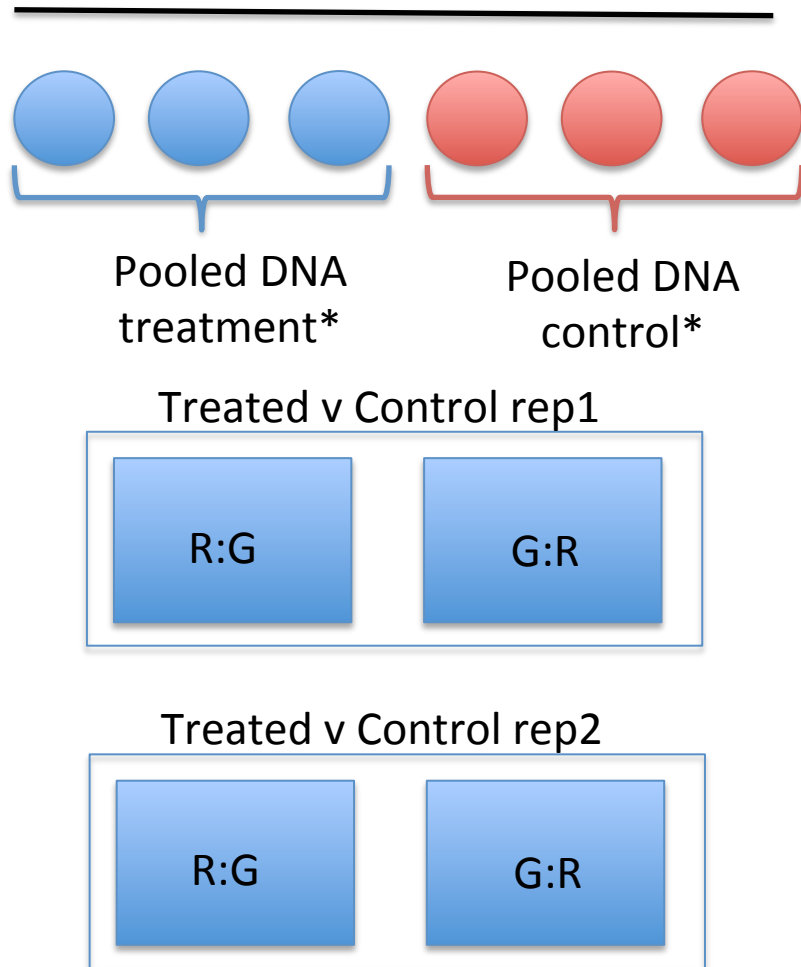
||| oyster\_v9\_M\_RepBase\_inv.bed

||| CDS\_BED

- QC:
  - Dye swap
  - Input v. Input (CNV)
- Validation
  - MBD-PCR
  - pyrosequencing
- Example..

# Guerrero-Bosagna et al 2010 (vinclozolin treated sperm F3 gen)

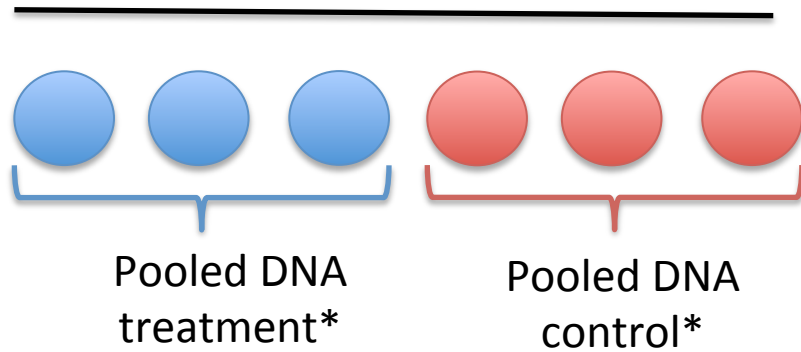
Experiment 1





# Guerrero-Bosagna et al 2010 (vinclozolin treated sperm F3 gen)

Experiment 1



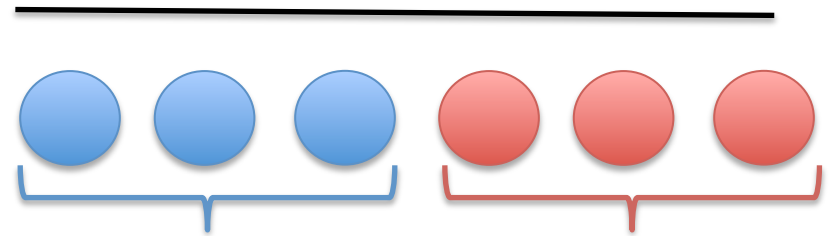
Treated v Control rep1



Treated v Control rep2



Experiment 2



Treated v Control rep1

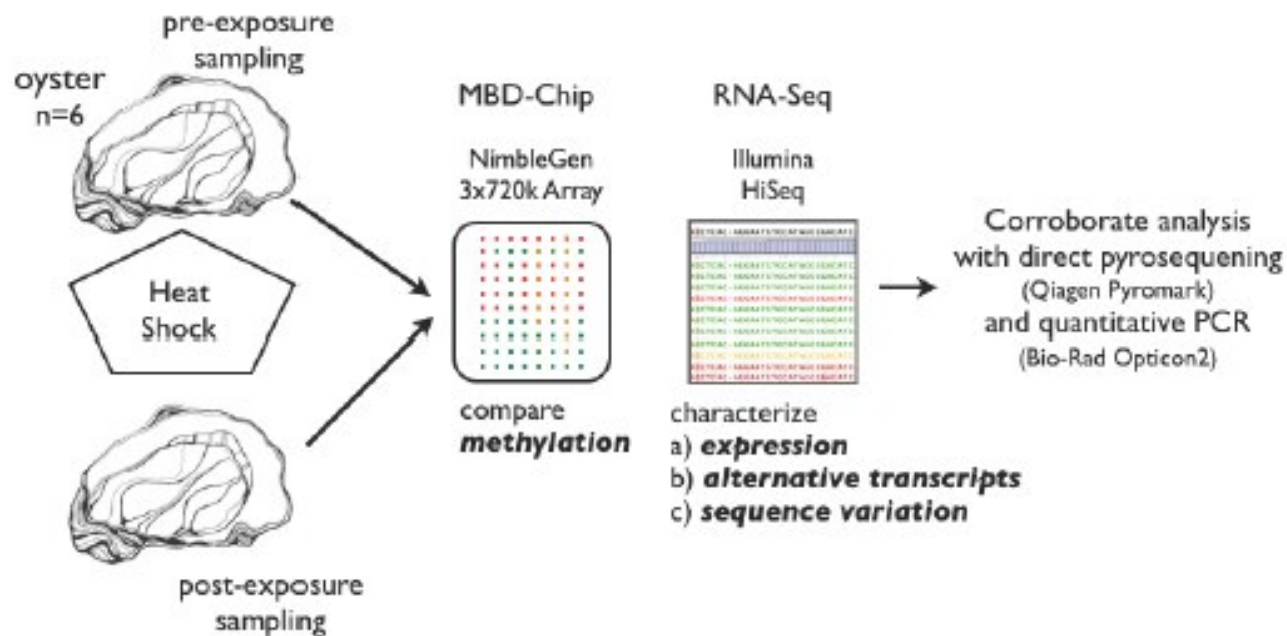


Treated v Control rep2

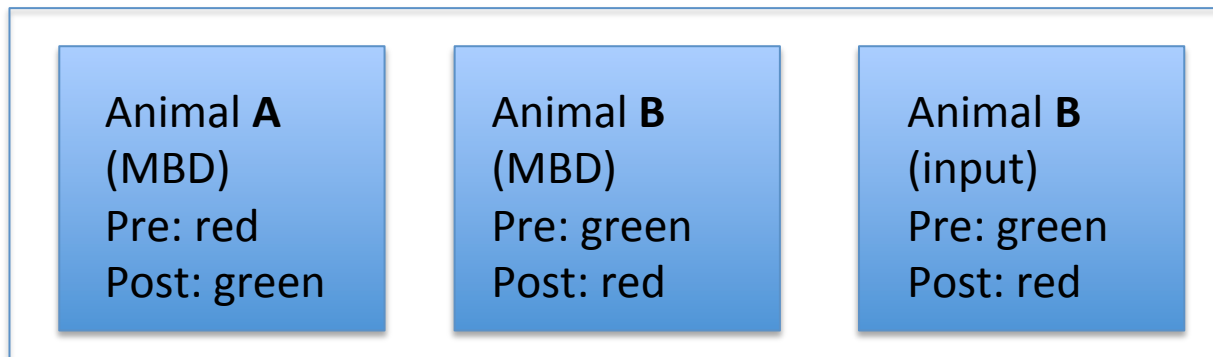




- Chip #1: methylation changes after heat shock



- Chip #1: methylation changes after heat shock



- Chip #2: methylation changes in response to EE2
  - Day 7
    - Control Pool: 4 females (across 3 tanks)
    - EE2 Pool: 8 females (select 4 across 3 tanks)

