Quality Assessment of Hybrid Selection Experiments

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02/04/10

Broad Capture Process

Solution hybrid selection with ultra-long oligonuc
for massively parallel targeted sequencing

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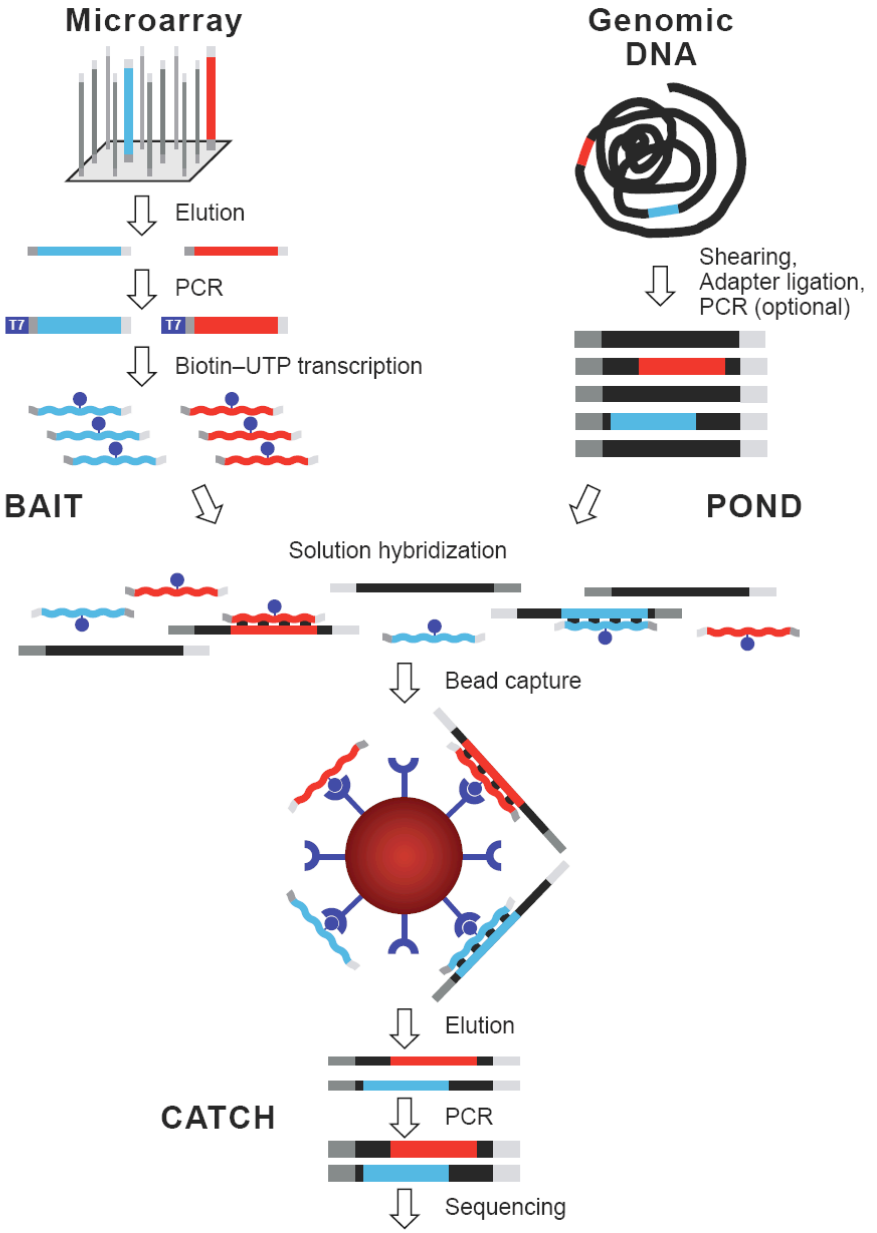
VOLUME 27 NUMBER 2 FEBRUARY 2009 NATURE BIOTECHNOLOGY

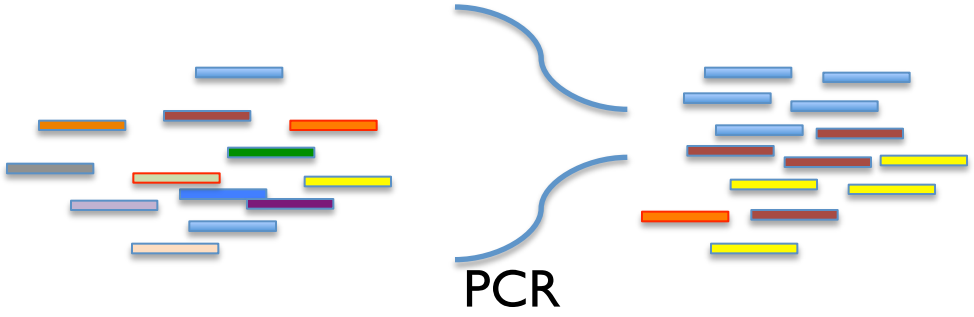


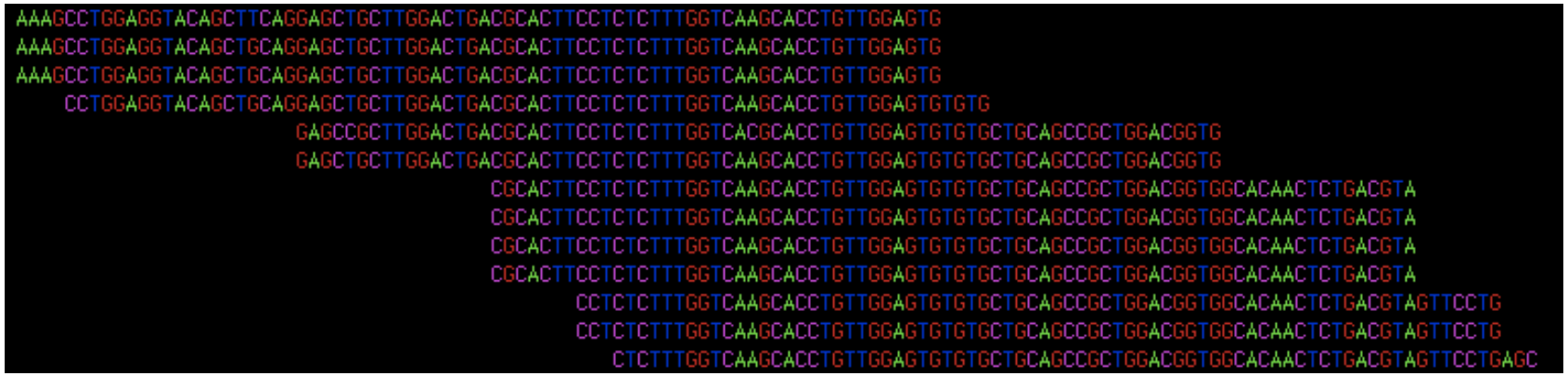
Molecular Duplicates

ISSUE: Molecular Duplicates

Problem

- PCR-free protocol yields small amounts of DNA
- Bottlenecks decrease library complexity





Running MarkDuplicates

- *MarkDuplicates*
  - Part of the Picard toolset and pipeline
  - By default mark, not remove, duplicate molecules
  - Automatically run on every Hybrid Selection library
  - Results located in the per-library directory below your aggregated BAM file:
    - E.g. /seq/picard_aggregation/C278/18325/v1/Solexa-16035/Solexa-16035.duplicate_metrics

```
java -Xmx2048m –jar /seq/software/picard/current/bin/MarkDuplicates.jar
    INPUT=<Solexa-16035.bam>
    OUTPUT=<Solexa-16035.duplicates_marked.bam>
    METRICS=<Solexa-16035.duplicate_metrics>
```

Output Metrics text file with metrics about the duplicate marking process

Output BAM with duplicate records marked

See http://picard.sourceforge.net/command-line-overview.shtml#MarkDuplicates for more information

Interpreting MarkDuplicates

- **Percent Duplication**
  - *Seems* easy to understand and communicate
  - **Increases with sequencing depth**

- **Estimated Library Size**
  - Estimate of number of unique molecules in the library
  - Goal changes with target size
    - Exome - ~150M
    - 6000 Genes – ~50M
  - ***No increase with sequencing depth***

Metric	Value
LIBRARY	Solexa-16035
UNPAIRED_READS_EXAMINED	948,391
READ_PAIRS_EXAMINED	46,752,523
UNMAPPED_READS	17,451,483
UNPAIRED_READ_DUPLICATES	621,011
READ_PAIR_DUPLICATES	6,929,586
PERCENT_DUPLICATION	15.3%
ESTIMATED_LIBRARY_SIZE	141,711,971

See http://www.broadinstitute.org/~prodinfo/picard_metric_definitions.html for more information

Evaluating Your Selection Event

Running Hybrid Selection Metrics

- *CalculateHSMetrics*
  - Part of the Picard toolset and pipeline
  - Automatically run on every HS lane, library and aggregation
  - Results located next to your BAM file:
    - E.g. /seq/picard_aggregation/C278/18325/v1/18325.duplicate_metrics

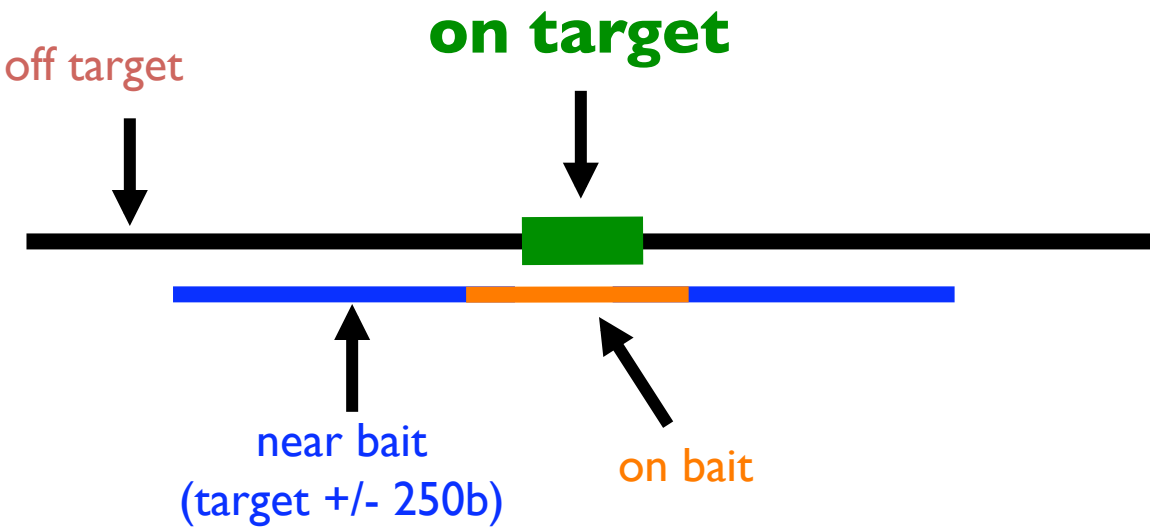
```
java -Xmx2048m –jar /seq/software/picard/current/bin/CalculateHsMetrics.jar
    BAIT_INTERVALS=<whole_exome_agilent_designed_120.baits.interval_list>
    TARGET_INTERVALS=<whole_exome_agilent_designed_120.targets.interval_list>
    INPUT=18325.bam>
    OUTPUT=<18325.hybrid_selection_metrics>
```

Output text file with metrics about this Hybrid Selection event

Target and Bait definitions for the selection to evaluate

Interpreting HS Metrics

- **Percent Selected Bases**
  - Percentage of the PF aligned bases were on or near the target
  - Varies by target size
    - Exome: good selections > 80%



Sample: 18325 (Microcephaly Exome) Project: C278 Sample Status: Complete	
Bait Territory	37,640,396
Target Territory	28,646,006
Bait Design Efficiency	76.10%
Total Reads	111,904,920
PF Reads	96,322,338
% Unique Reads	81,842,155
% PF Reads	86.08%
PF Unique Bases Aligned	5,310,101,316
% Selected Bases	80.38%
Mean Bait Coverage	95.71
Mean Target Coverage	104.50
% Zero Coverage Targets	2.68%
Fold 80 Base Penalty	4.35
Target Bases >= 2x	94.68%
Target Bases >= 10x	88.02%
Target Bases >= 20x	80.95%
Target Bases >= 30x	74.60%
HS Penalty 10x	8.63
HS Penalty 20x	8.97
HS Penalty 30x	9.76

See http://www.broadinstitute.org/~prodinfo/picard_metric_definitions.html for more information

Interpreting HS Metrics

- **% Zero Coverage Targets**
  - Percentage of targets that don't have at least one base >= 2X
  - Often are targets that are difficult to ***sequence***, as in whole genomes these are missing also (repetitive, high gc)
  - For exomes, typically < 3%

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Interpreting HS Metrics

- **Mean Bait / Target Coverage**
- **% of Target Bases > 2x, 10x, 20x, 30x**
  - Measure of project completeness
  - Typically 80% of target over 20x
- Difference between these two illustrates non-uniformity in coverage…

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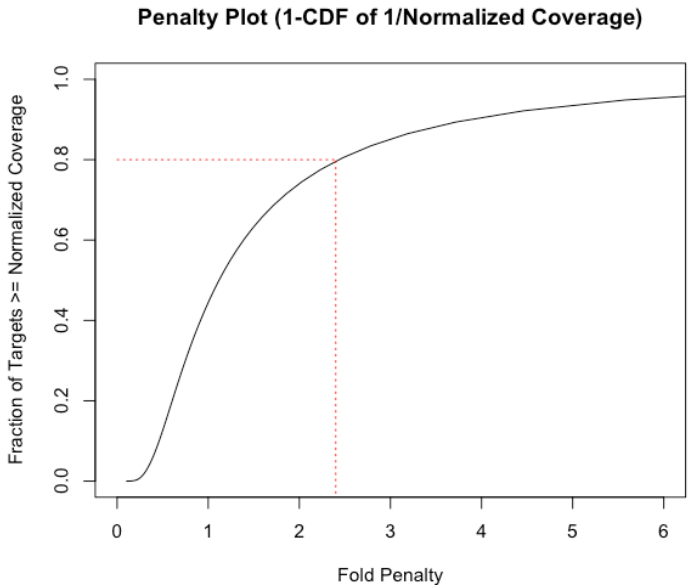
Interpreting HS Metrics

- **Fold 80 Base Penalty**
  - Measure of non-uniformity
  - # of units of sequencing to raise 80% of the bases to the average coverage
  - Lower is better, less than 5 is good and exomes are often between 3-4



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Interpreting HS Metrics

- **HS Penalty 10x / 20x / 30x**
  - Overall penalty to get 80% of bases to 10x
  - Captures all targeting inefficiencies
  - In other words… how much input sequence do I need to get a target base to 10x
  - Example:
    - 30 mb of target
    - 10x desired coverage
    - 30mb * 10x * 8.63 -> 2.59 GB
  - *A good value is < 10*
  - ***Sometimes this is empty, meaning it's impossible to sequence this library to the desired depth***

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References

- Bait / Target Information
  - /seq/references/HybSelOligos/…
- Picard Tools
  - http://picard.sourceforge.net
  - /seq/software/picard/current/bin
- Picard Documentation
  - http://picard.sourceforge.net/command-line-overview.shtml
  - http://www.broadinstitute.org/~prodinfo/picard_metric_definitions.html