PCR Amplification during Library Preparation Confounds Variant Discovery in Whole Genome Sequencing

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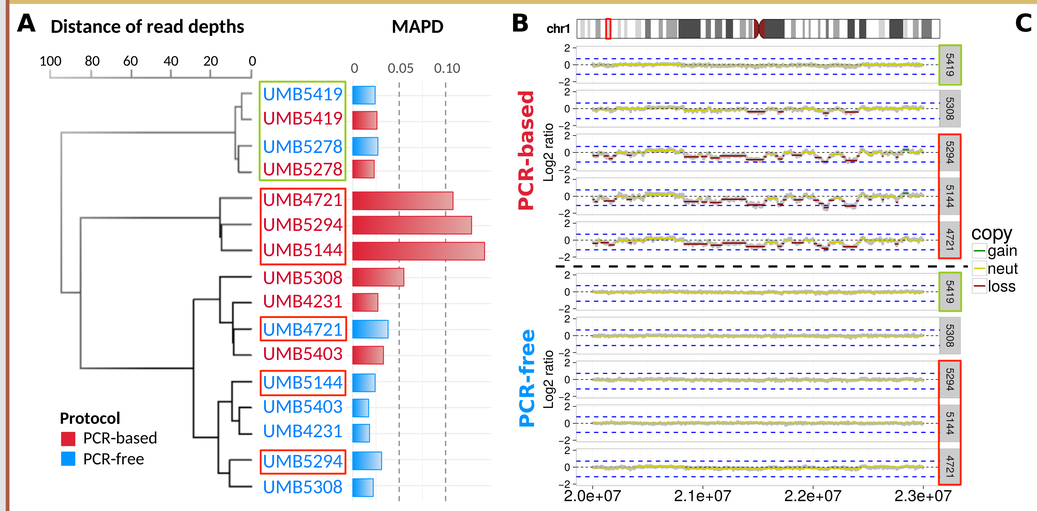
Introduction

The vast majority of publicly available whole genome sequencing data were prepared using PCR amplification during library preparation. Here we directly compare **PCR-based** to **PCR-free** libraries from the same samples and find that PCR-based preparations have the potential to:

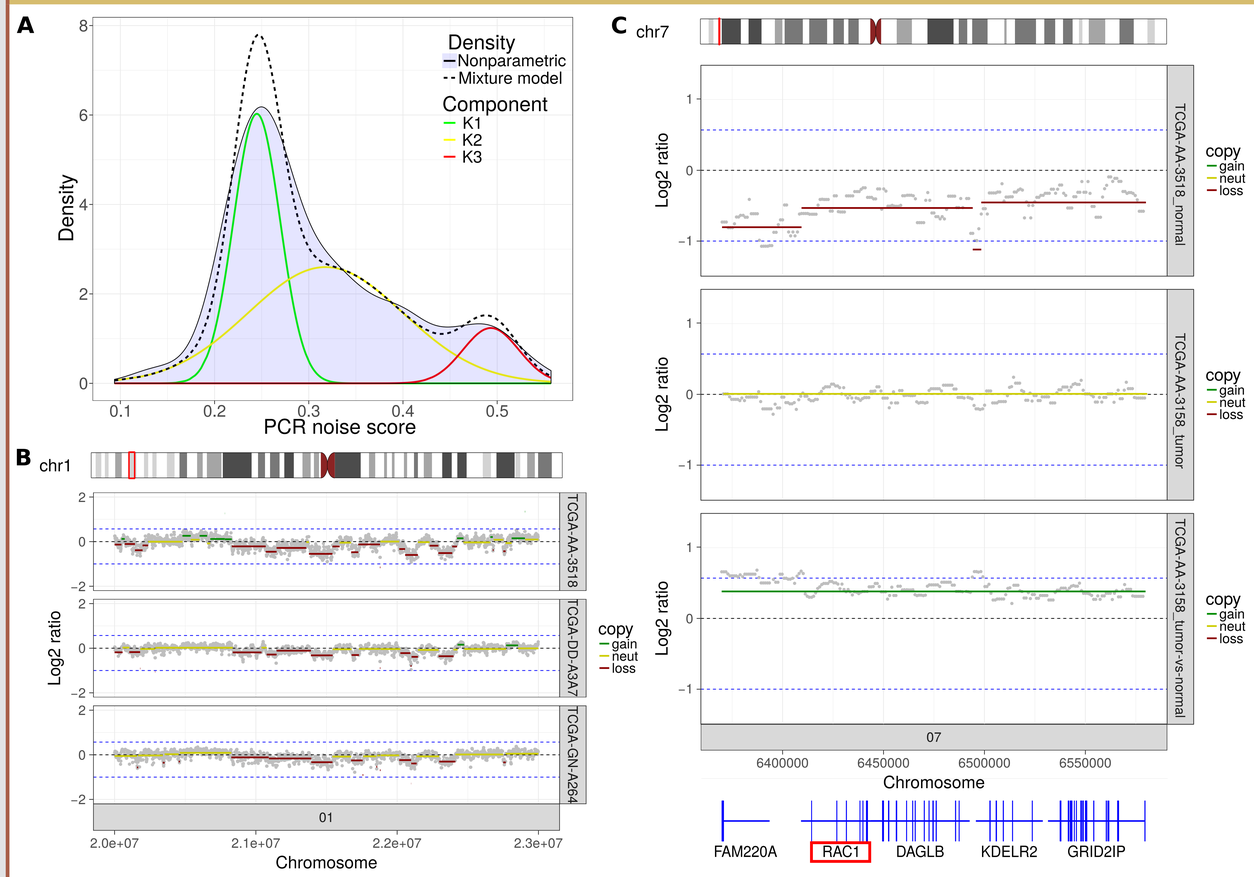
1. Induce systematic non-biological read depth variation, possibly leading to false-positive copy number alteration calls.
   - Analysis of 697 high coverage WGS normal tissue samples from TCGA revealed the PCR noise pattern in at least 10% samples.
   - The noise pattern enriches read-depth CNV calls across thousands of genomic loci, covering hundreds of reported disease-associated genes.
2. Mask thousands of true-positive SNVs while introducing thousands of false-positive SNVs.
   - SNVs called only in PCR-based libraries are characterized by a distinct mutational signature.

PCR Amplification Creates an Artificial Read Depth Pattern



A. Libraries display PCR-specific read depth patterns, with high noise PCR-based samples clustering together.
B. Example 3 MB region on chr1 illustrating the PCR read depth noise pattern.
C. High noise PCR-based libraries share >50% of CNV calls between libraries. Corresponding PCR-free replicates share <9% of CNV calls.

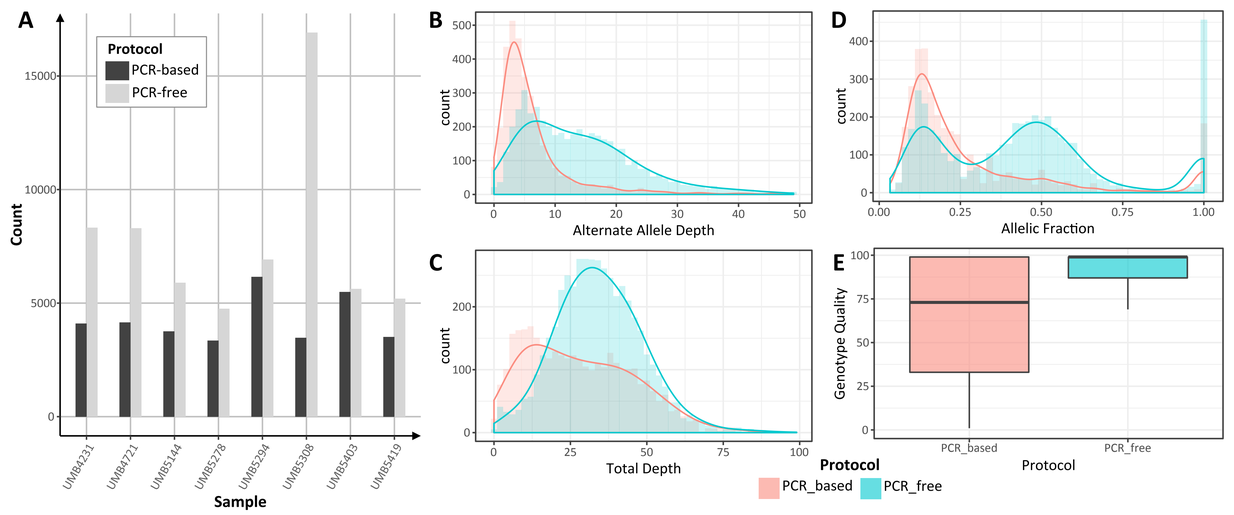
PCR Read Depth Noise Pattern is Present in TCGA Samples



A. Three-component Gaussian mixture model used to detect the PCR noise pattern in 697 normal TCGA tissue samples.
- 73 (10.5%) of samples belong to the most extreme component, indicating presence of the PCR read depth signature.

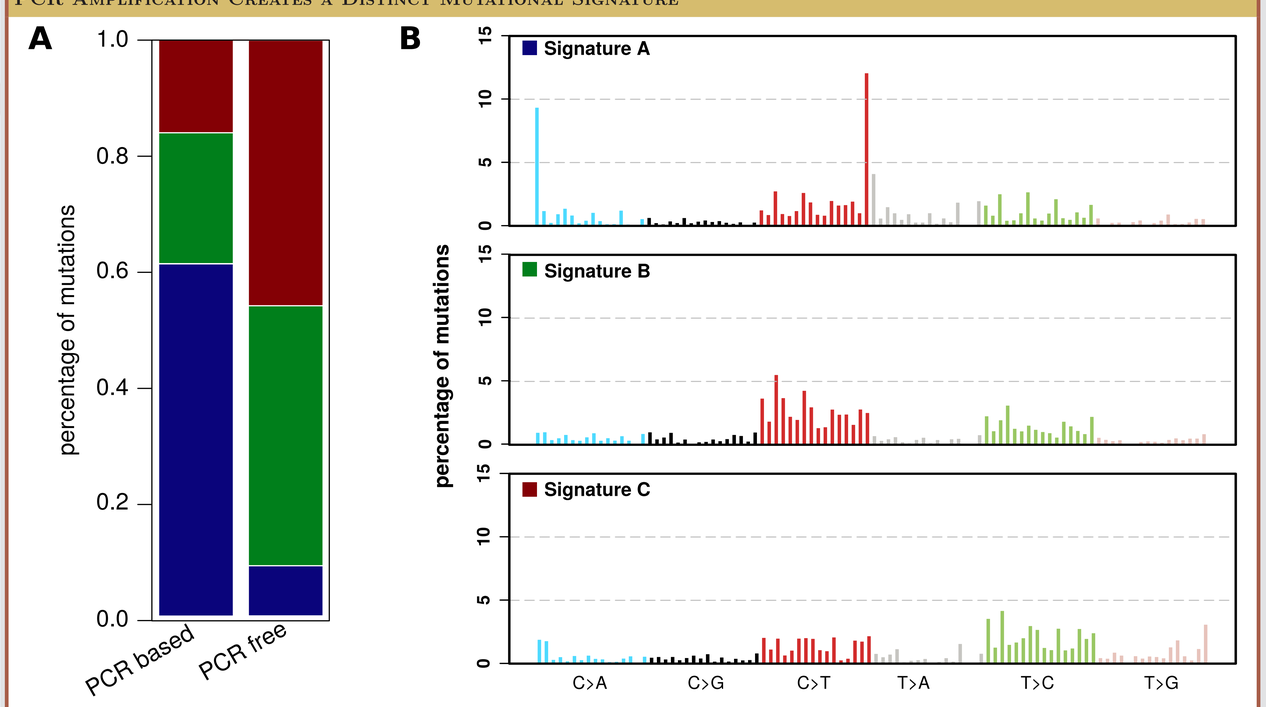
B. The 3 MB example region of three representative high noise TCGA samples exhibits a similar pattern as the samples in the above panel.
C. The PCR noise pattern can induce false-positive CNV calls when performing tumor-normal read depth comparison calling.
- 7389 genes (32% of human genes) are enriched in high noise samples compared to noise-free samples.
- Includes 55% of genes in COSMIC, 58% of genes in SFARI, 49% of genes in AutWorks, and 44% of genes for intellectual disability in the HPO.

PCR Amplification Masks True-Positive and Induces False-Positive SNV Calls



A. SNV calls private to PCR-based and PCR-free libraries from the same tissue. One average 7742 ± 3938 calls are unique to PCR-free replicates and 4251 ± 1028 calls are unique to PCR-based replicates.
B. Alternate allele depth of private SNV calls reveals an excess of PCR-based calls with few alternate reads.
C. Total depth at the private SNV call sites shows PCR-based calls have skewed coverage compared to PCR-free calls.
D. Allele fraction of private SNV calls reveals an excess of PCR-based calls with very low allele fraction, indicative of false-positive calls.
E. Overall genotype quality is lower in PCR-based calls than PCR-free calls ($p < 3e\text{-}16$, Wilcoxon rank-sum test).
- Combined evidence from **B-E** suggests that PCR amplification potentially misses true-positive calls and induces false-positive calls.
- We hypothesize this is due to amplification imbalance and PCR polymerase error during the amplification process.

PCR Amplification Creates a Distinct Mutational Signature



A. Three mutational signatures contribute to private SNV calls. PCR-based calls are dominated by Signature A while PCR-free calls are mostly Signature B and C.
B. Signatures A, B and C.
- **Signature A** is dominated by AC>AA and TC>TT mutations; this feature is distinct from all COSMIC signatures.
- **Signature B** is similar to COSMIC signature 1, which is common in cancer samples.
- **Signature C** is similar to COSMIC signature 5, which is common to most samples.

Conclusions

1. PCR Amplification during library preparation can introduce an artificial read depth variation.
   - The signature is found in at least 10% of TCGA WGS normal tissue samples.
   - The signature enriches for thousands of genes, many of which are associated with disease states including cancer, Autism, and intellectual disability.
2. PCR masks thousands of true-positive SNVs and induces thousands of false-positive SNVs.
   - Masking of true variants may occur due to amplification imbalance.
   - We suspect the introduction of false-positive SNVs occurs due to PCR polymerase error.
3. PCR amplification exhibits a unique mutational signature.
4. WGS data should be checked for the presence of the PCR amplification read depth signature prior to performing read-depth based CNV calling.
5. Care should be taken in curating SNV calls from PCR amplified data.

Methods

- **Aligner**: BWA-mem to GRCh37d5
- **CNV caller**: BICseq2 / CNVnator
- **SNV caller**: GATK HaplotypeCaller

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