

# Clinical consequences of whole-genome sequencing v.s. SNP methods of noninvasive prenatal screening

Counsyl

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## Introduction

Noninvasive prenatal screening (NIPS) for aneuploidies via next-generation sequencing of cell-free DNA has gained wide clinical adoption due to its high accuracy<sup>1</sup>. The most widely used NIPS offerings employ either whole-genome sequencing (WGS) or SNP-based methods to determine whether patterns deviate from those expected in a euploid pregnancy. Although published outcome studies and clinical guidelines indicate that both approaches have comparable performance, a key point of contention has been how the two methods address low fetal-fraction (FF) samples.

Both methods have excellent sensitivity in the vast majority of patient samples; however, aneuploidies become more difficult to detect in low FF samples. One approach to addressing such samples is to not report results (i.e., “no-call”) samples below a preset threshold<sup>2</sup>, followed by either retesting after pregnancy has progressed or recommendation to undergo invasive testing (per ACOG and ACMG<sup>3,4</sup>).

However, in realistic clinical settings, not all patients submit to redraws, and only a fraction of women consent to invasive testing<sup>5</sup>. In this study we use published methods and clinical data to directly compare the performance of the two methods at low FF in order to determine whether reporting results for all samples (WGS method) leads to a higher rate of detection of common aneuploidies than no-calling low FF samples (SNP method).

## Overview of NIPS methods

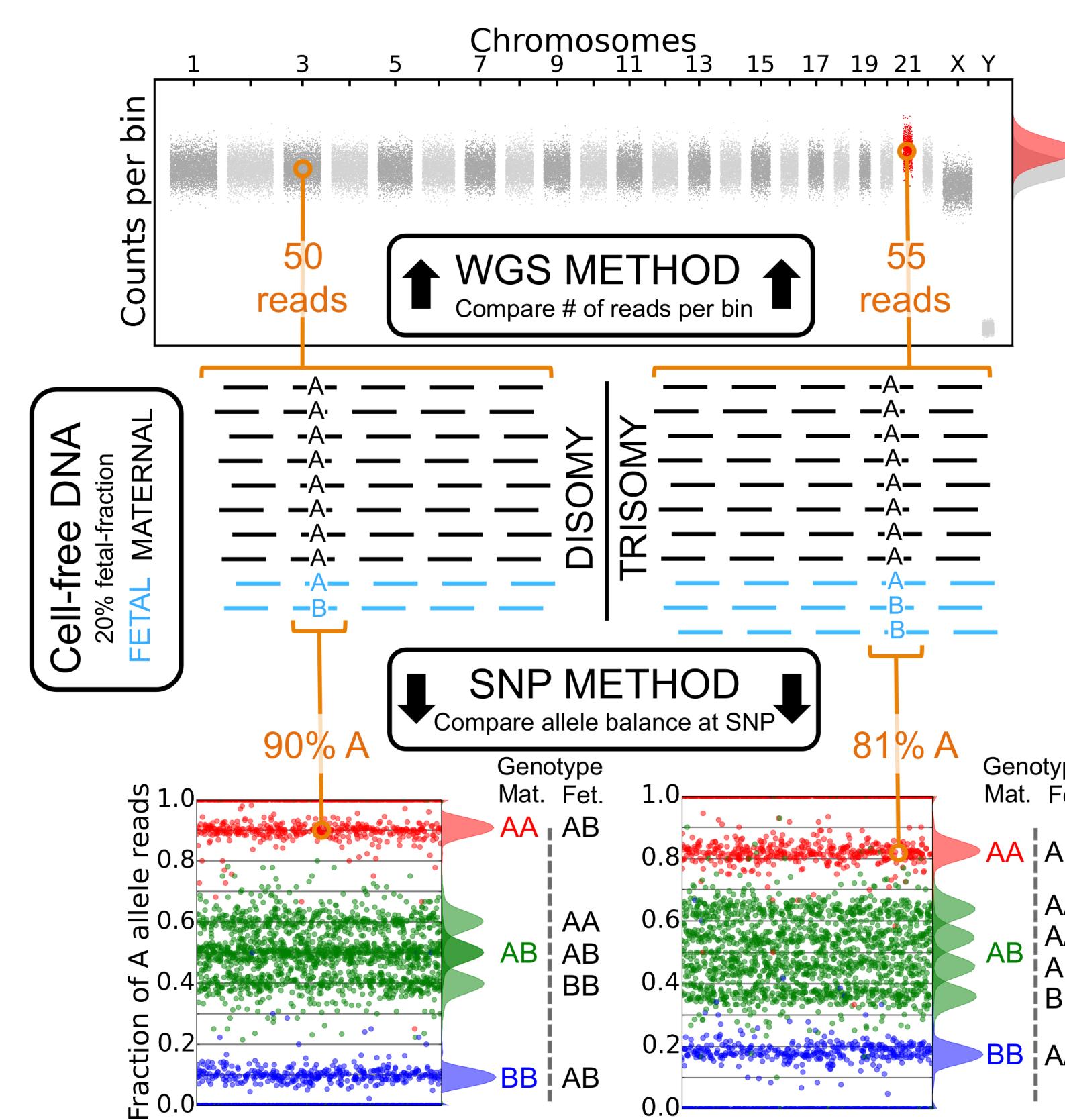


Figure 1 Overview of the WGS and SNP methods

Both methods take advantage of NGS reads originating from the mixture of maternal and fetal cfDNA (20% of the pool). The WGS method (top) divides the genome into equally-sized bins and counts the number of reads originating from each. As illustrated, a fetus with trisomy 21 leads to an increase in the distribution of counts-per-bin originating from chromosome 21 relative to the disomic background. Alternatively, the SNP method (bottom) measures the relative abundance of alleles at variable sites in the cfDNA. Fetal aneuploidies lead to predictable shifts in the frequency of allelic counts based on the possible combinations of maternal and fetal genotypes. By aggregating the signal across many SNPs on a given chromosome, it is possible to assign a numerical likelihood to whether the overall pattern of allele frequencies is more consistent with disomy or aneuploidy.

## Origins of nondisjunction

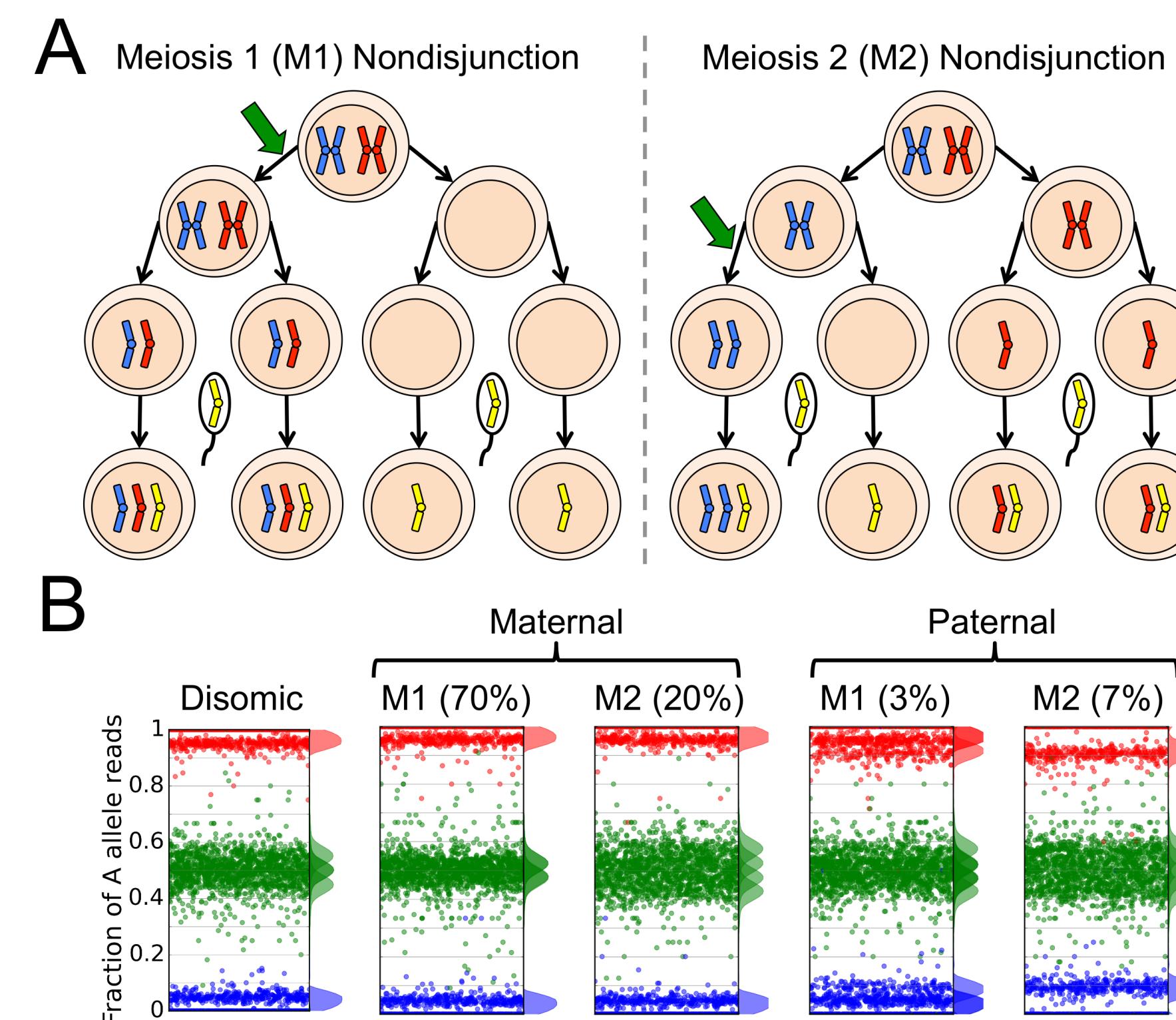
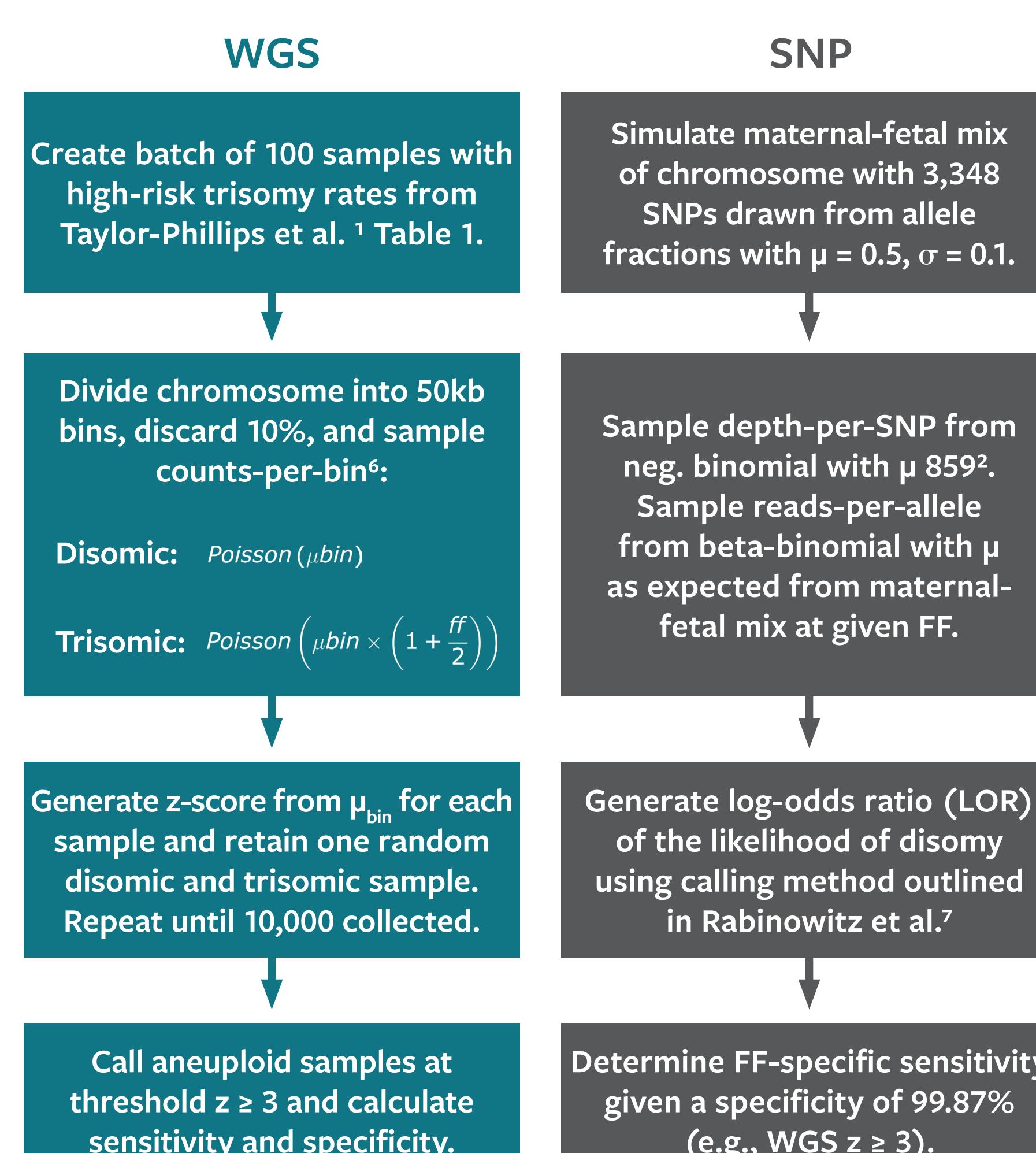


Figure 2 The origin of a trisomy leads to different expected distributions of allele-frequencies under the SNP method

The origin of a trisomy leads to different expected distributions of allele-frequencies under the SNP method. A) Maternal trisomies can originate from either from meiosis stage 1 (M1; left) or stage 2 (M2; right) nondisjunction. In M1 nondisjunctions, the embryo inherits one copy of each of the maternal chromosomes (i.e., blue and red chromatids) while in M2 nondisjunctions, a daughter cell inherits two copies of a single maternal chromosome. Paternal nondisjunctions have the same consequences with the parental origins reversed. B) Simulated allele-frequency distributions for 10% FF samples for each of the different origins of trisomies are shown along with their relative frequencies. Note that rare paternal trisomies produce stronger deviations. In contrast, all four origins produce the same elevated read signal in the WGS method.

## Modelling NIPS



## Performance at low FF

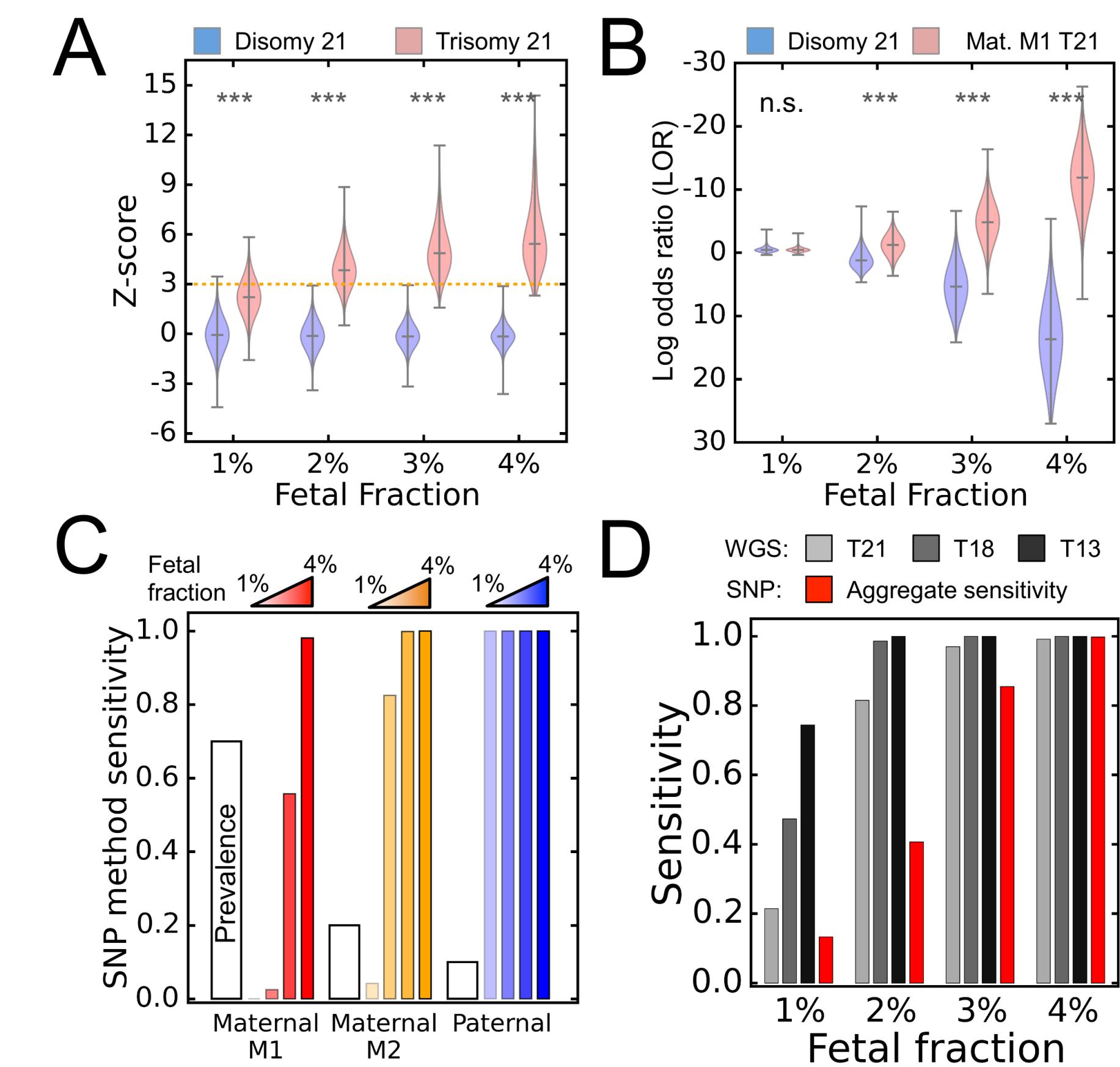


Figure 3 Comparison of performance characteristics of the two methods at low FFs (1-4%)

A) Specificity remains high at the calling threshold (orange line;  $\geq 99.87\%$ ) across FFs in the WGS method. B) In the SNP method, low FF samples (1-2%) show poor resolution in log-odds ratios (LOR), justifying a no-call threshold to maintain high-specificity. C) The sensitivity of the SNP method is greatest for rare paternal trisomies as compared to common maternal nondisjunctions. D) Comparison of the sensitivities of the WGS method for each of the three common autosomal trisomies (grey bars) to the aggregate sensitivity of the SNP method (red bars). The aggregate sensitivity of the SNP method was obtained by summing the sensitivities scaled by prevalence across trisomic origins.

## Consequences of no-calls

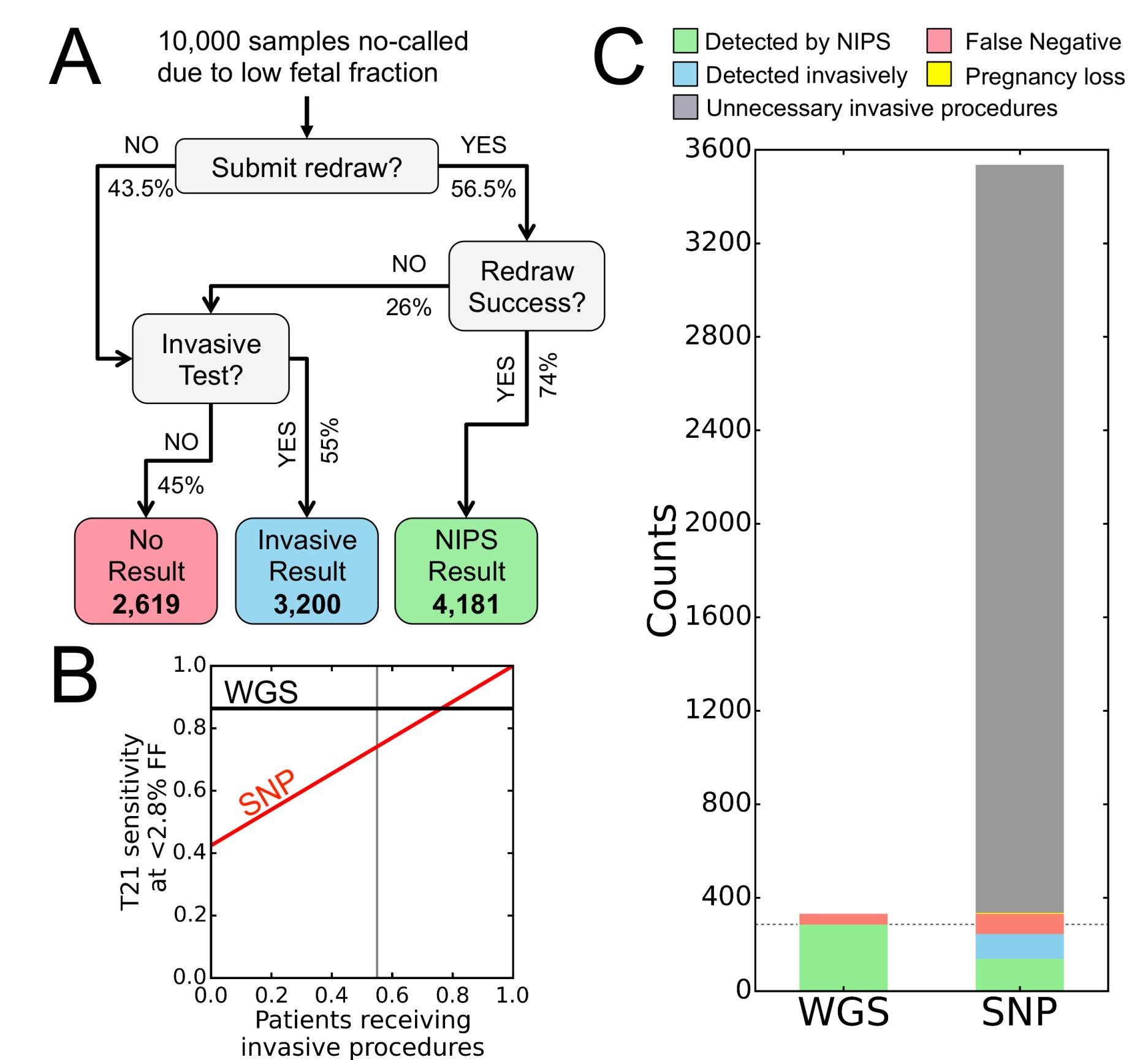


Figure 4 Consequences of no-calling samples at low FF vs. reporting at reduced sensitivity

A) Clinical decision flowchart for samples receiving a no-call from the SNP method. Frequencies associated with each decision branch point were obtained from published literature<sup>2,5</sup>. B) The aggregate sensitivity of T21 detection of the WGS method (86%) was obtained by summing the sensitivity over the frequency of FFs in the range of under 2.8% (black line). In contrast, the SNP method (red line) is expected to detect 42% of aneuploid cases by redraw (the intercept). All further cases must be detected by invasive procedures, which is unlikely to be higher than 55%<sup>6,8</sup>. C) Bar chart illustrating clinical consequences for 10,000 low FF patients. While the WGS method would detect 285 out of 330 expected cases of T21, the SNP method would detect 138 by NIPS (all via redraw). In addition, 3,200 invasive procedures would be required to detect an additional 106 cases (74% total), also resulting in five procedure-related pregnancy losses (yellow line).

## Conclusions

- The WGS method retains high specificity (>99.8%) at low FF whereas the SNP method shows poor resolution in log-odds ratios, justifying a no-call threshold (2.8%)<sup>2</sup>.
- Releasing calls in low FF samples detects more aneuploidies than simply no-calling all samples below a threshold FF given published clinical data showing incomplete compliance with invasive testing recommendations.