

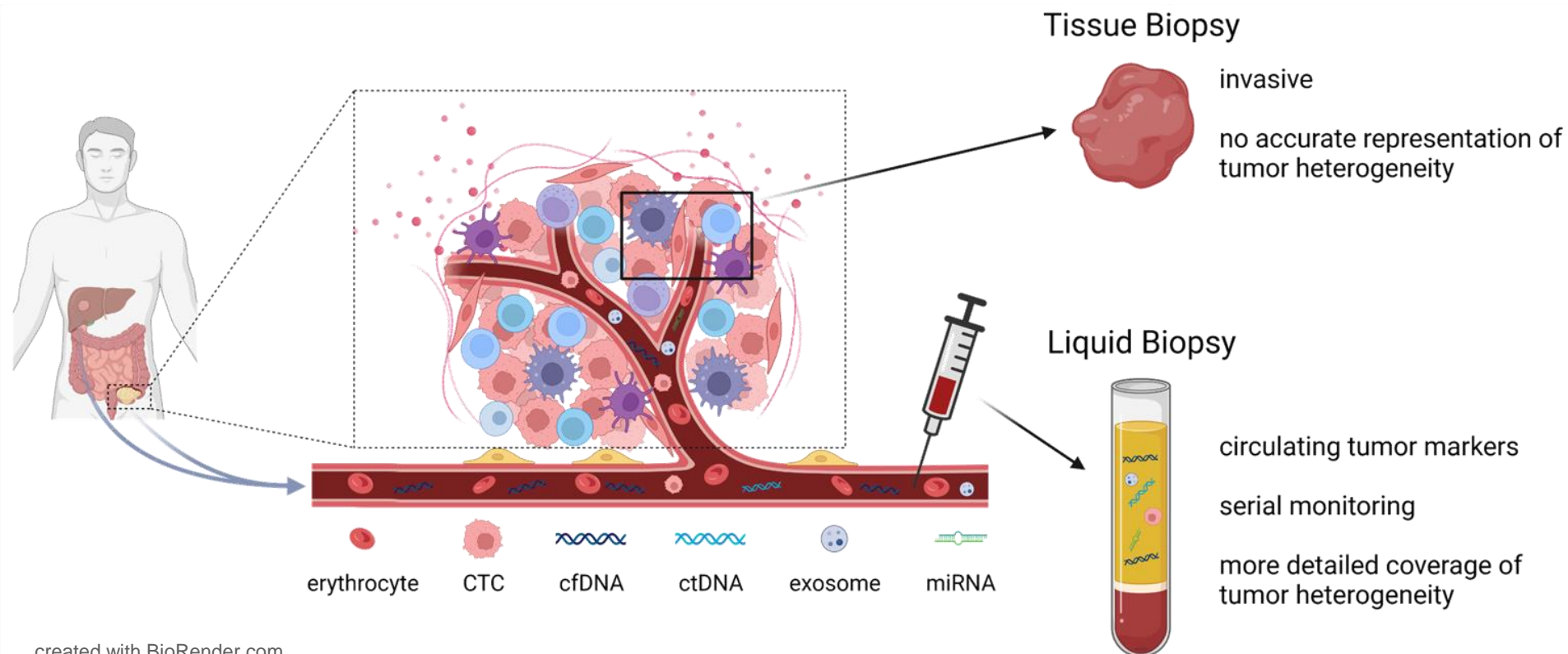
# Impact of reference materials for analytical performance evaluation of liquid biopsy NGS assays

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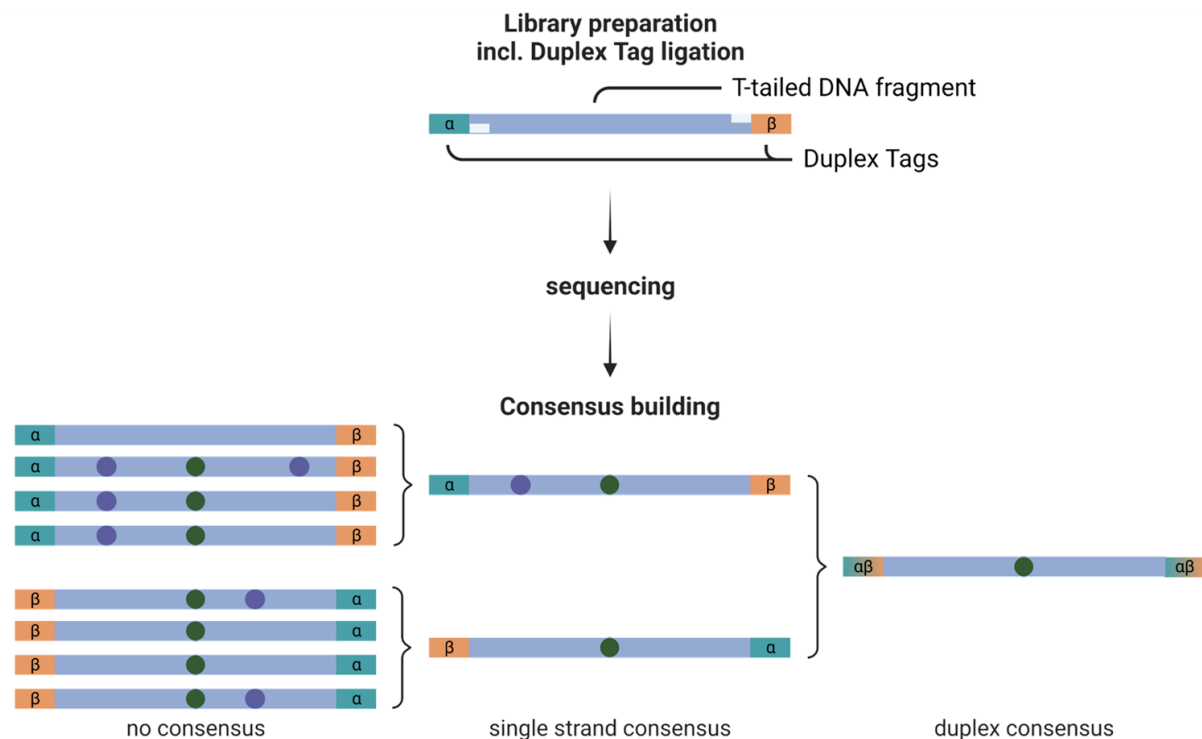
## Background

Liquid biopsy (LB) is a promising complement to tissue biopsy for the detection of clinically relevant genetic variants in tumor and mosaic diseases.



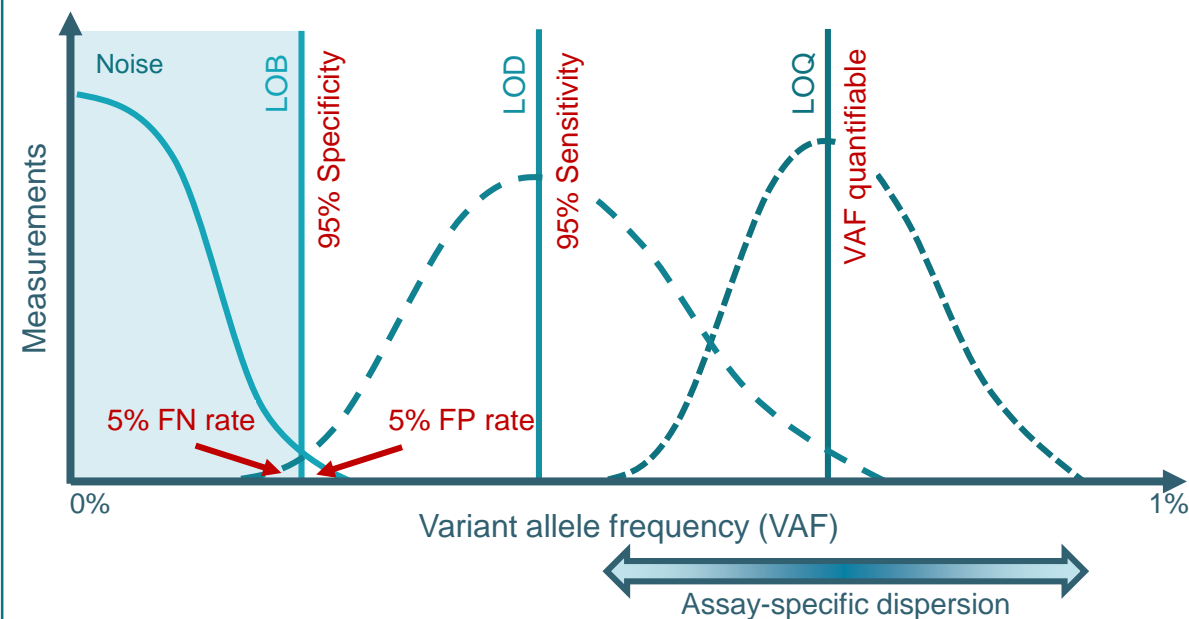
## Liquid Biopsy Duplex Sequencing

For identification of very low frequency variants in circulating free DNA (cfDNA), strand-aware consensus building from sequencing reads is performed.



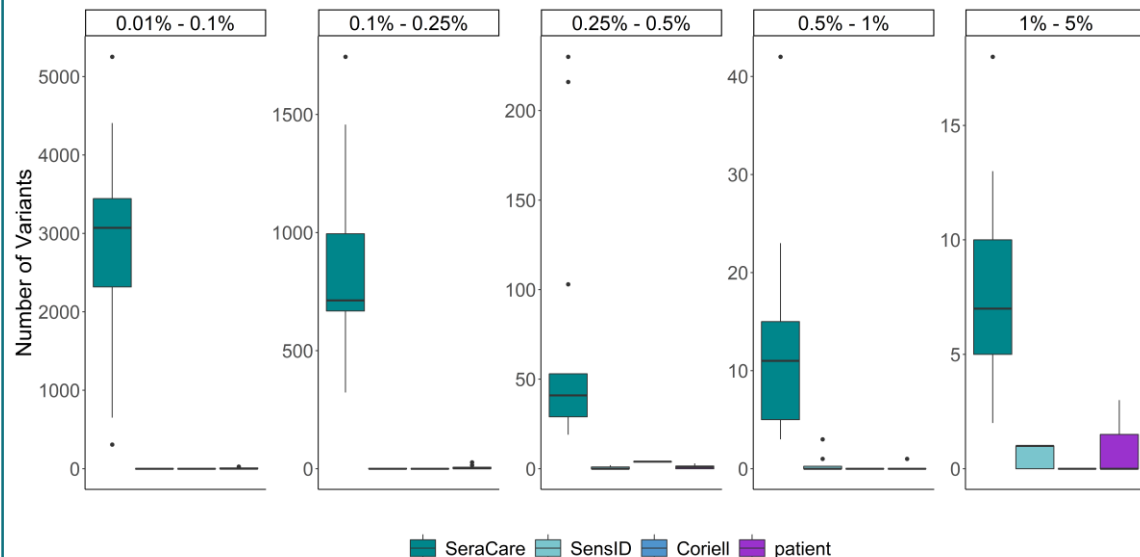
## Performance evaluation of Liquid Biopsy NGS assays

NA24385-based wild-type (WT) cfDNA reference materials from SensID, Coriell, and SeraCare and 15 patient samples were analyzed using our Duplex Sequencing-based Liquid Biopsy NGS assay. Wild-type controls were used to determine the background noise critical for determination of the limit of blank (LOB). Positive controls were used for determination of the limit of detection (LOD) and limit of quantification (LOQ).



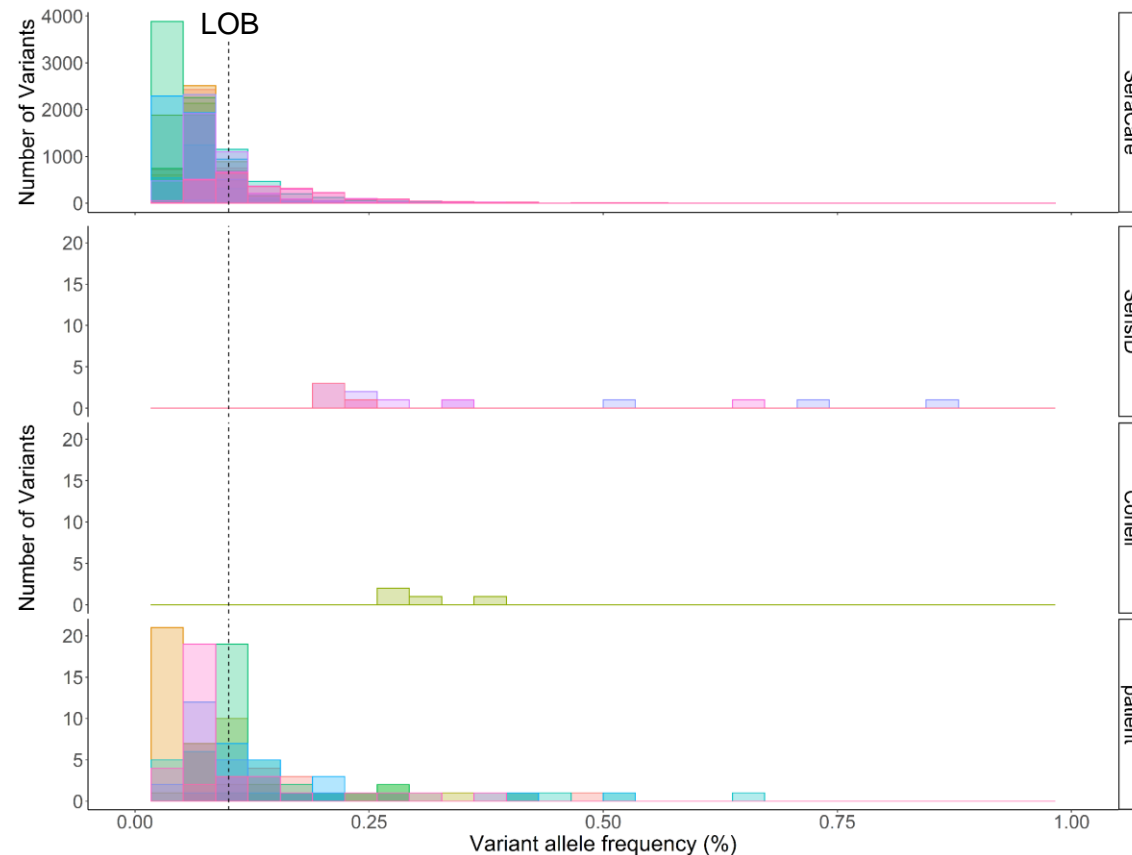
## Variants detected with a VAF <5%

To investigate the background noise of three different commercially available WT reference materials (SeraCare, SensID, Coriell), which are important for the evaluation of specificity, the distribution of variants with a variant allele frequency (VAF) <5% was determined for five intervals and compared to patient samples.



## Distribution of low frequency variants

The reference materials provided by SensID and Coriell showed very low background noise at low VAFs. Therefore these materials are highly suitable for determination of the LOB of Liquid Biopsy NGS assays.



## Performance of our Liquid Biopsy NGS assay

By comparing different reference materials we identified striking differences in the determination of the specificity of the test. The comparable number of low frequency variants in reference materials from SeraCare and Coriell identified those materials ideal for the specificity determination.

	Parameter	SeraCare	SensID	Coriell
LOB = 0.1% VAF	Number of false positives	1512	2	8
	Sensitivity	98.5%	100.0%	98.5%
	Positive predictive value (PPV)	4.1%	95.6%	88.9%
LOB = 0.25% VAF	Number of false positives	117	0	8
	Sensitivity	98.5%	100%	98.5%
	Positive predictive value (PPV)	35.4%	100%	88.9%

## Conclusion

In summary, careful consideration of commercially available reference materials is required for performance evaluation of LB NGS assays. While reference materials with well-defined variants are preferable for determining specificity, reference materials that closely resemble native cfDNA aid in the development of experimental protocols.