CoolMPS™ Sequencing Reagent Sets

Cleaner, Brighter and Longer Sequencing

CoolMPS, the first antibody based massively parallel sequencing chemistry for DNBSEQ platforms announced in Oct 2019 is now available commercially in sequencing reagent sets. This innovative chemistry utilizes non-destructive base recognition for clearer base identification.

CoolMPS chemistry principles

The CoolMPS is the sequencing method used in DNBSEQ technology (Fig 1). It uses

- Four cold dNTPs with an extension block (Fig 2)
- Four antibodies that are both base specific and block dependent (Fig 3). The A, T, G and C specific antibodies have almost zero cross reactivity. Each antibody has a specific dye (label) molecules attached to it.

The CoolMPS chemistry is compatible with all commonly used library preparation methods

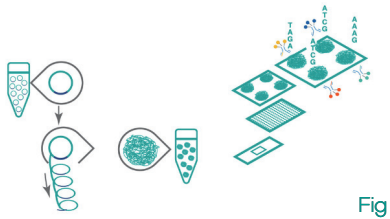


Fig 1

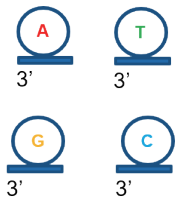


Fig 2

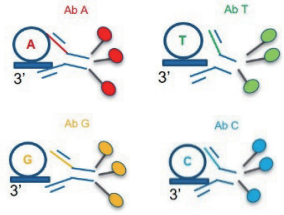


Fig 3

CoolMPS sequencing steps

- The cold unlabeled dNTPs are polymerized using DNA polymerase on the flow cell
- The incorporated base is recognized by a fluorescently labeled antibody that binds specifically to the incorporated cold dNTP
- The flow cell is imaged
- A regeneration agent then cleanly removes the block and the antibody. No scarring of bases.
- The newly added bases are completely natural without any modification
- The sequencing cycles are repeated for necessary read length

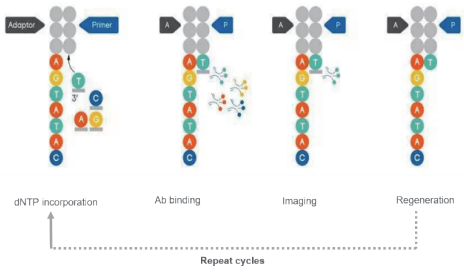


Fig 4

Shows four discrete steps of the chemistry

Benefits and applications

- Strong signal and higher signal to noise ratio
- No signal quenching and scarring
- No systematic sequence-based errors
- Significantly better runon and lag performance due to quantitative reaction

Higher signal

The Fig 5 compares StandardMPS and CoolMPS signal. The CoolMPS shows higher signal and it stays higher than starting signal of standardMPS even after 200 cycles in a PE 100 run.

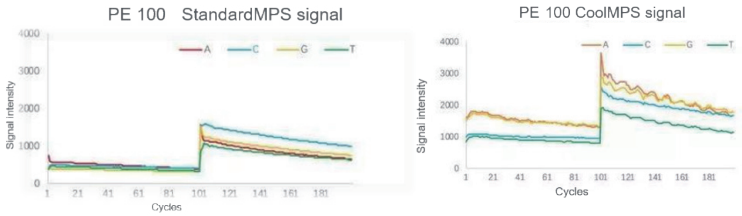


Fig 5: Shows signal comparison of StandardMPS and CoolMPS in PE 100 run

No signal quenching

Quenching can occur in StandardMPS due to G signal suppression and scarring of bases. The G suppression occurs due to the proximity of the dye to ring structure and the scarring occurs due to molecules of the linkers left behind on the incorporated bases. Both these modalities are a non-issue in CoolMPS. The Fig 6 shows incorporated bases with scars in StandardMPS.

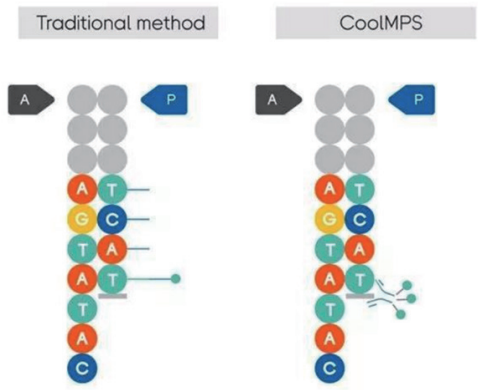


Fig 6: Traditional chemistry leaves behind scars as shown on the left while CoolMPS has clean bases

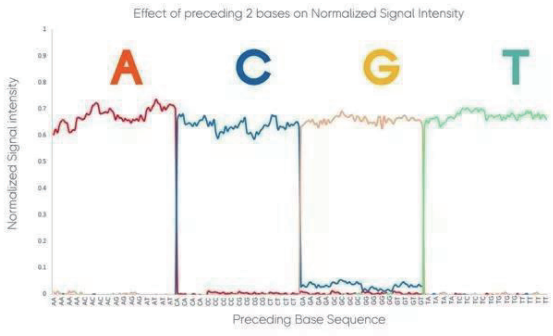


Fig 7: shows very little impact of preceding bases on the signal intensity

In CoolMPS, the fluorescent molecules are attached to the antibody and the Ab's are cleanly removed in the regeneration step. The bases of the newly synthesized strand in the CoolMPS method are natural bases and do not affect the signal. This is evident in the signal intensity plotted in relation to the preceding two bases. As can be seen Fig 7 there is no impact on signal for any of the bases ultimately delivering higher accuracy and longer sequencing reads.

More random errors

The base preceding the base called during sequencing can have significant impact on the errors. The Fig 8 shows more randomness of base calling errors in CoolMPS. The figure shows errors in the called bases related to preceding base.

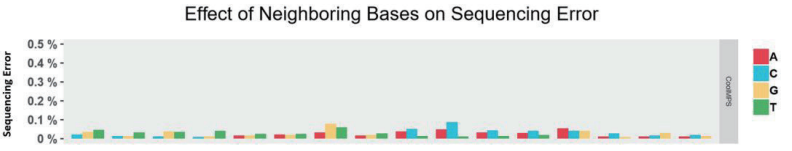


Fig 8: shows very low error rate and bias

Significantly better Runon and Lag performance

The efficiency of labeled dNTP incorporation by a DNA polymerase is less compared to cold dNTPS (Fig 9). The lag and runon are the indicators commonly used to determine the completeness of reaction.

- Lag refers to the proportion of some copies of the reaction to the N-1 position when sequencing proceeds to the N position
- Runon refers to the number of copies that have been reflected to the N + position

As can be seen in Fig 9, the avg lag and runon values of 0.2% and 0.06% respectively in CoolMPS are much lower than 0.7% and 0.24% for StandardMPS. The low lag and low runon of CoolMPS technology will enable longer reads.

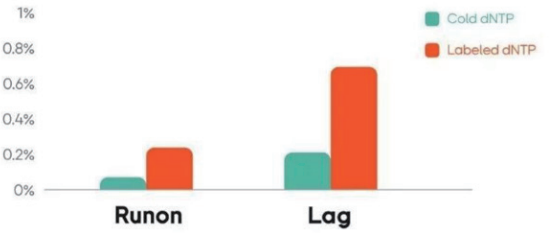
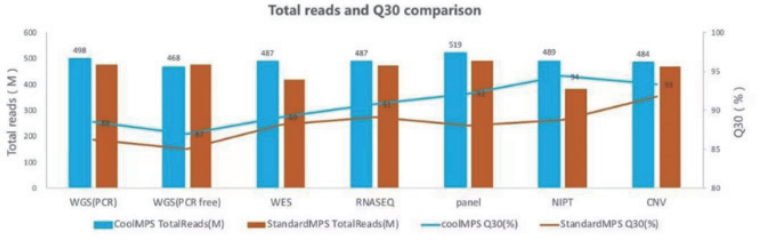


Fig 9: shows lag and runon value for cold and labeled dNTPs

Applications

Wide range of applications were evaluated using CoolMPS chemistry and performance compared to Standard-MPS. The PE100 sequencing comparison for WGS (PCR), WGS (PCR-Free), WES, RNA-Seq, and targeted panel shows (Fig 10) that the data output of CoolMPS is 6% higher than StandardMPS, and Q30% is 1 ~ 9% higher than StandardMPS.



Mutation frequency detection in tumor panel

A tumor detection comparison was done between two chemistries. Fig 10-A shows correlation between measured and expected mutation frequency for standardMPS and Fig 10-B shows for CoolMPS. The CoolMPS mutation frequency detection was comparable to standardMPS but closer to expected mutation frequency. Other quality metrics shown in Table 1 are also comparable.

A FFPE HD 200 (Horizon) panel (ATOPlex)

A PE 100 sequencing on DNBSEQ-G400RS

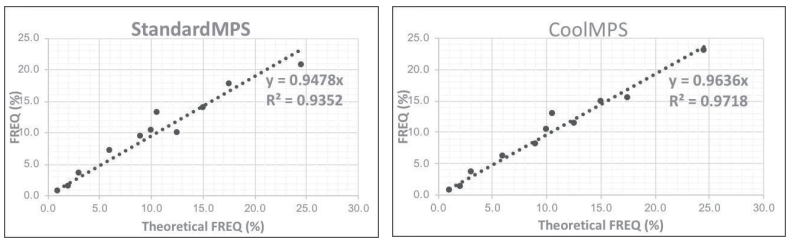


Fig 10 -A (left- StandardMPS) and 10-B (right-CoolMPS)): shows correlation with expected mutation frequency

Metrics	CoolMPS	StandardMPS
Clean Q30	92.10%	87.20%
Mapping rate	99.88%	99.95%
Capture rate	97.05%	97.26%
Coverage(>=100X)	99.36%	99.71%
Uniformity(>0.2x)	97.21%	95.43%

Table 1: The key quality metrics are comparable between the two chemistries

RNASeq performance

CoolMPS RNASeq performance was evaluated using UHRR libraries and compared with standardMPS, Novaseq AND qPCR (200ng input). Q30% was 90% for both StandardMPS and CoolMPS and total reads 477M and 487M, respectively. The mRNA correlation was plotted between different platforms and qPCR (Fig 11). Good correlation was seen between qPCR and three sequencing methods. The reproducibility of CoolMPS was also good.

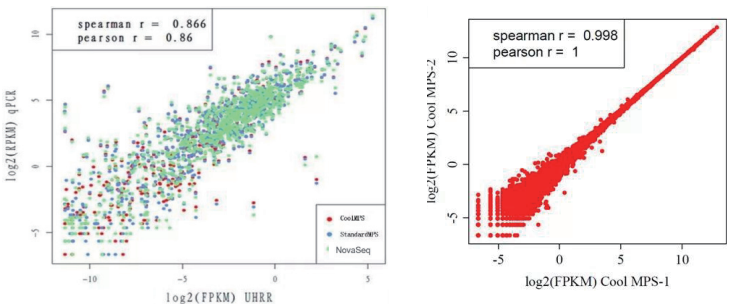


Fig 11 Left graph shows good correlation between all methods evaluated. The right figure shows reproducibility of CoolMPS

Conclusion

The CoolMPS sequencing reagent sets designed for MGI DNBSEQ platforms offer unique benefits over standard-MPS and support wide range of applications without the need to change any established library preparation protocols.

Ordering Information

CoolMPS DNBSEQ-G400RS Sequencing Sets

1000017992	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE50)*
00016933	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE100)*
00016935	CoolMPS High-throughput Sequencing Set (DNBSEQ-G400RS FCL PE100)*

*Unless otherwise informed, all sequencers and sequencing reagents are not available in Germany, USA, Spain, UK, Hong Kong, Sweden, Belgium and Italy.

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