

Index mis-assignment

Customers have reported higher levels of index mis-assignment, commonly known as “index hopping”, on HiSeq systems that use pattern flow cells relative to those that don’t. Typical levels of Index hopping on patterned flow cell systems range from 0.5 - 2% depending on the type, quality and handling of the library. Libraries with higher levels of free adaptors will see higher levels of “index hopping”. “Index hopping” causes a specific type of mis-assignment that results in the incorrect assignment of libraries from the expected index to a different index (in the multiplexed pool). Illumina’s internal investigation and the investigations of customers, have shown while index hopping can occur, it has a limited effect on the majority of applications.

Illumina is continuing to investigate the issue and is in the process of developing a permanent corrective action. This is one of our highest priorities. In the interim customers should follow the best practices listed below to reduce the potential levels and effects of “index hopping” in their libraries.

If you have any questions or concerns please contact your local field team or Illumina Tech Support, techsupport@illumina.com

Best Practices

- **Remove free adaptors from library preps**
- **Store libraries individually at -20°C**
 - Pool libraries prior to sequencing
- **Use unique dual indexing strategies (unique i5 and i7 indexes)**
- [Click here for additional information on TruSeq Library Preparation Best Practices](#)
- [Click here for Nextera Sample Prep: Best Practices](#)
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