**Troubleshooting**

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| **Issue** | **Potential Cause** | **Solution/Tips** |
| Inconsistent specificity and titer of the crosslinked Ab-MNase or PA-MNase | When crosslink, antibodies, ProteinA or MNase contain impurities with primary amines, such as Tris. | ・Dialyze antibody, ProteinA and MNase well against PBS with EDTA in separate plastic bucket to remove the impurities.  ・Try to dialyze at cold temperature to protect from denaturation of the proteins.  ・Try using freshly crosslinked products for testing. |
| Non-specific reads are produced when sequenced | Non-specific bindings of the crosslinked antibodies with MNase to the genome. | ・Try using lower concentration of the crosslinked antibodies with MNase when adding to the formaldehyde fixed cells.  ・Try incubating the cells with the crosslinked antibodies with MNase for shorter period.  ・Rinse out well from the cells the excess crosslinked antibodies with MNase using washing buffer.  ・Try cutting out agarose gel with the targeted PCR products for sequencing at larger DNA size (>140 bp).  ・Try reducing PCR thermal cycles to amplify sequencing products.  ・Try using fresh reagents to avoid DNA from microorganism contamination. |
| Low sequencing reads | Low recovery of purified DNA samples for sequencing | ・Use glycogen to increase the recovery of DNAs by alcohol precipitation.  ・Do not over-dry DNA samples at any steps in this protocol.  ・Try avoiding CaCl2 contamination during any steps until activating MNase to initiate digestion. |