Quantification of the molar concentration of the NGS libraries by qPCR

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MATERIALS TEXT

MATERIALS

Agencourt AMPure XP **Beckman Coulter Catalog #A63880**

KAPA Library Quantification Kit for Illumina® Platforms **Kapa Biosystems Catalog #KK4835**

Agarose S **Nippon Gene Catalog #312-01193**

BEFORE STARTING

Note: use low-binding tubes and pipet chips.

1 Using the NGS library concentration obtained from the Agilent BioAnalyzer, dilute the libraries to 100 pg/µl with 10 mM Tris-HCl, pH 8.0. Then prepare a 1:10000 dilution of each diluted library (final concentration, 0.01 pg/µl) by conducting 10-fold dilution series using 10 mM Tris-HCl, pH 8.0.

*If the library concentration is unknown, conduct a 10-fold dilution series to achieve an appropriate final dilution (Note: for a 1:3 diluted library, we conducted a 1:500,000 dilution with 10 mM Tris-HCl, pH 8.0).

2 Prepare a reaction mix and performs qPCR in accordance with the manufacturer's manual of KAPA Library Quantification kit (KAPA Biosystems).

3 Check the product size and presence of adaptor dimers in a 1-µl aliquot of the PCR products by conducting electrophoresis through a 3% agarose–TBE gel. For example, in the Fig. 1, the lanes marked "×" show contamination with adaptor dimer.

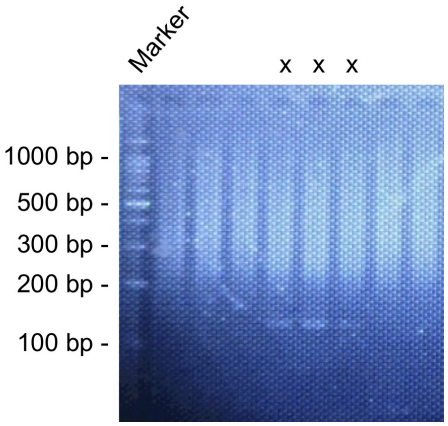

Fig. 1. Example agarose electrophoresis.

4 Further clean-up the library with Agencourt AMpure XP beads (Beckman Coulter), if the library contains adaptor dimer. Refer to the Agencourt AMpure XP beads manual for reagent amounts and clean-up procedures. Elute to the same final volume as the original library and re-quantify and check again (i.e, repeat steps 1–3).

5 Calculate the molar concentration of each libraries from the standard curve.

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