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Sample requirements for DNA sequence capture libraries

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Sample requirements for NimbleGen SeqCap EZ DNA sequence capture libraries

• The following amounts of **unamplified** genomic DNA are required for each sequence capture:

Sequencing platform	Capture method	DNA quantity
Illumina	NimbleGen SeqCap EZ - Standard input	2000 ng in 50-100 µl of 1xTE buffer
Illumina	NimbleGen SeqCap EZ - Low input	200 ng in 50-100 µl of 1xTE buffer

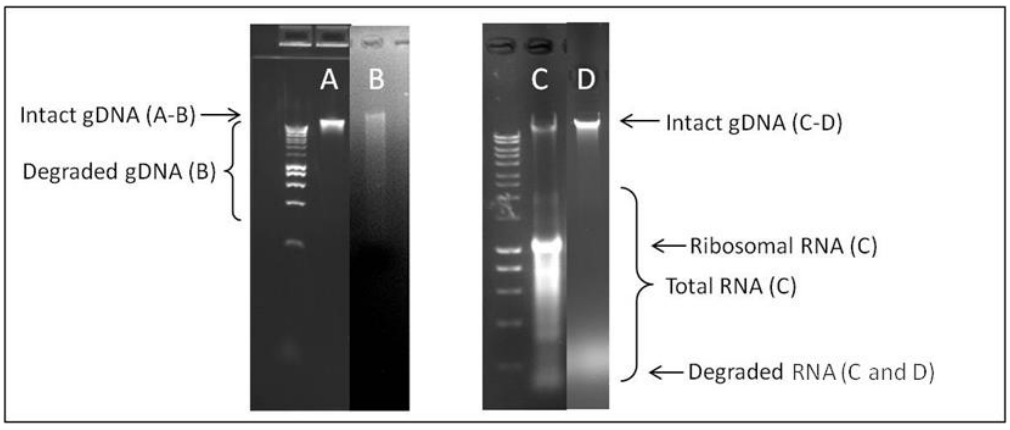
• DNA concentration should be determined by a fluorometry-based assay such as Qubit or PicoGreen.

• The A_{260}/A_{280} and $A_{260}/_{230}$ ratios (which are obtained by NanoDrop) must be ≥ 1.8 and ≥ 1.9, respectively, for all samples (although this may not be possible for the samples that are to be processed through the low input protocol).

• The DNA sample should not contain particulate matter.

• The DNA must be high quality, high molecular weight and free from RNA contamination. This should be assessed by electrophoresis of 50-100 ng of each sample on a 1% agarose gel alongside molecular markers of the appropriate size. Genomic DNA should be visible as a single prominent band >12 Kb. If there is more than one band or a smear, the DNA may be degraded or contain a contaminant that could affect the library preparation. RNA will appear as a smear <200 bp. If RNA is present, please treat the DNA with RNase A (DNase-free) and then purify, preferably using a column based method.

The gel image below illustrates intact, degraded and RNA contaminated genomic DNA run alongside Hyperladder I (Bioline). If a smear is in the range between zero and ~2000 bp, RNase treatment may be required to reveal whether the degraded material is gDNA or RNA.



For projects that involve ≥32 samples, please submit samples in a well sealed 96-well plate to match our workflow.

For projects that involve ≤31 samples, please label the samples and tubes 1, 2, 3 etc. in order to ease our sample identification. Please remember to underscore numbers that can be read upside-down such as 6, 9, 16, 91, 69, 96 etc.

Sample requirements for Agilent SureSelect XT DNA sequence capture libraries

• The following amounts of **unamplified** genomic DNA are required for each sequence capture:

Sequencing platform	Capture method	DNA quantity
Illumina	Agilent SureSelect XT- Standard input	3000 ng in 50-100 µl of 1xTE buffer
Illumina	Agilent SureSelect XT - Low input	200 ng in 50-100 µl of 1xTE buffer

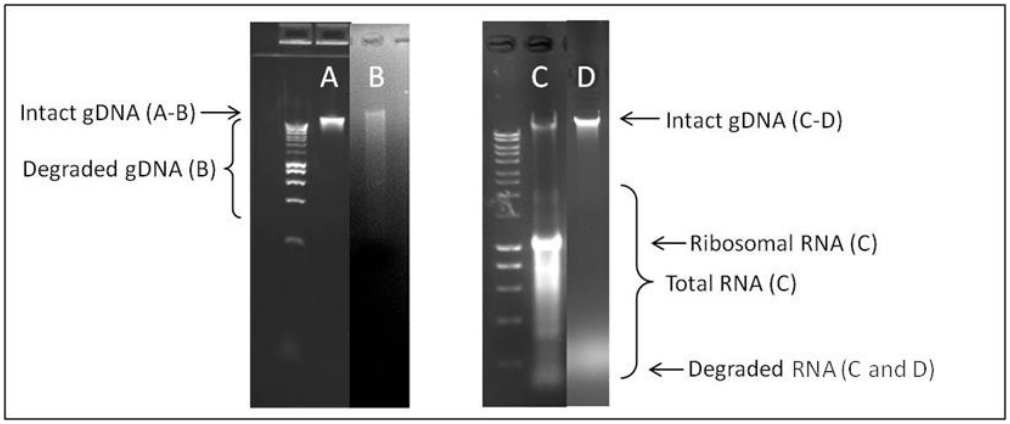
• DNA concentration should be determined by a fluorometry-based assay such as Qubit or PicoGreen.

• The A_{260}/A_{280} and $A_{260}/_{230}$ ratios (which are obtained by NanoDrop) must be ≥ 1.8 and ≥ 1.9, respectively, for all samples (although this may not be possible for the samples that are to be processed through the low input protocol).

• The DNA sample should not contain particulate matter.

• The DNA must be high quality, high molecular weight and free from RNA contamination. This should be assessed by electrophoresis of 50-100 ng of each sample on a 1% agarose gel alongside molecular markers of the appropriate size. Genomic DNA should be visible as a single prominent band >12 Kb. If there is more than one band or a smear, the DNA may be degraded or contain a contaminant that could affect the library preparation. RNA will appear as a smear <200 bp. If RNA is present, please treat the DNA with RNase A (DNase-free) and then purify, preferably using a column based method.

The gel image below illustrates intact, degraded and RNA contaminated genomic DNA run alongside Hyperladder I (Bioline). If a smear is in the range between zero and ~2000 bp, RNase treatment may be required to reveal whether the degraded material is gDNA or RNA.



For projects that involve ≥32 samples, please submit samples in a well sealed 96-well plate to match our workflow.

For projects that involve ≤31 samples, please label the samples and tubes 1, 2, 3 etc. in order to ease our sample identification. Please remember to underscore numbers that can be read upside-down such as 6, 9, 16, 91, 69, 96 etc.

Sample requirements for Agilent SureSelect XT HS and Low Input DNA sequence capture libraries

• The following amounts of **unamplified** genomic DNA are required for each sequence capture:

Sequencing platform	DNA sample source	DNA quantity
Illumina	Fresh biological samples (non-FFPE)	10-200 ng in 30-60 µl of buffer AE*
Illumina	FFPE tissue sections	All that is available in 30-60 µl of buffer ATE**

* Buffer AE composition:
10 mM Tris-Cl
0.5 mM EDTA; pH 9

** Buffer ATE composition:
10 mM Tris-Cl, pH 8.3
0.1 mM EDTA
0.04% NaN_3

• For fresh biological samples (non-FFPE), please refer to page 3 for sample QC requirements.

• For FFPE samples, DNA concentration should be determined by a fluorometry-based assay such as Qubit or PicoGreen. A qPCR-based assay will be used to quantify and qualify these samples at the CGR prior to library preparation.