



NGS Library QC

General Information

Order Number	1308KFT-0010	Name of Customer	Katherine Hornick	Date of Order	2013-08-16
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Final QC Result of DNA sample(s)					
Arrival Date	Experiment Date	Sample count	Pass	Fail	Hold
2013-08-16	2013-08-30 11:03	16	10	6	0

Final QC Result of RNA sample(s)					
Arrival Date	Experiment Date	Sample count	Pass	Fail	Hold
N/A	N/A	N/A	N/A	N/A	N/A

* **Pass** : Proceed with the library construction.

* **Fail** : Further processes are on hold until the replacement samples received.

We do not recommend in proceeding further steps until a specific instruction was given from the client.

* **Hold** : A specific instruction should be given by the client for further processing as the QC pattern may be triggered by the sample nature.

** MacroGen does not proceed the next step until we have received your permission.

Library QC Result of DNA

Arrival Date	2013-08-16	Experiment Date	2013-08-30 11:03	Tested by	
Comment					

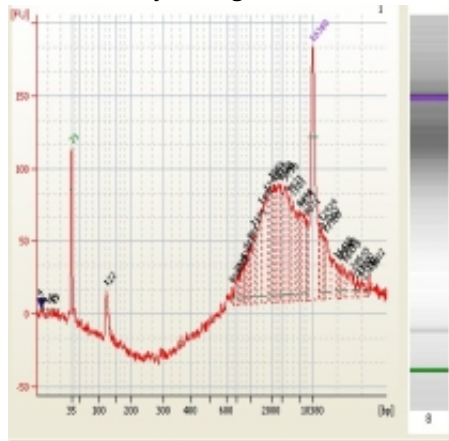
#	Library Name	Library Type	Conc. (molecules/ul)	Size (bp)	Result*	
1	Traigon_Control_1	Amplicon library (Lib-L Type)	-1006348767		Fail	no peak
2	Traigon_Control_2	Amplicon library (Lib-L Type)	30117273188	600	Pass	
3	Traigon_Control_3	Amplicon library (Lib-L Type)	-1200340426		Fail	no peak
4	Traigon_Impact_1	Amplicon library (Lib-L Type)	-556578879		Fail	no peak
5	Traigon_Impact_2	Amplicon library (Lib-L Type)	18567727633	600	Pass	
6	Traigon_Impact_3	Amplicon library (Lib-L Type)	47522459238	600	Pass	
7	Quenec_North_Control_1	Amplicon library (Lib-L Type)	40173946164	600	Pass	
8	Quenec_North_Control_2	Amplicon library (Lib-L Type)	14021871570	600	Pass	
9	Quenec_North_Control_3	Amplicon library (Lib-L Type)	23311664172	600	Pass	

#	Library Name	Library Type	Conc. (molecules/ul)	Size (bp)	Result*	
10	Quenec_North_Impacted_2	Amplicon library (Lib-L Type)	4188947755	600	Pass	
11	Quenec_North_Impacted_3	Amplicon library (Lib-L Type)	-1209426686		Fail	no peak
12	Quenec_South_Control_1	Amplicon library (Lib-L Type)	31535184119	600	Pass	
13	Quenec_South_Control_2	Amplicon library (Lib-L Type)	-1199658957		Fail	no peak
14	Quenec_South_Control_3	Amplicon library (Lib-L Type)	50158383371	600	Pass	
15	Quenec_South_Impacted_1	Amplicon library (Lib-L Type)	1715667683	600	Pass	
16	Quenec_South_Impacted_2	Amplicon library (Lib-L Type)	-1223964703		Fail	no peak

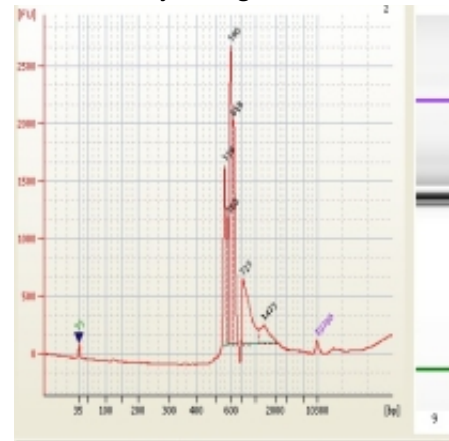
Experiment
Condition

Aglient bioanalyzer & Caliper Labchip GX DNA High Sensitivity

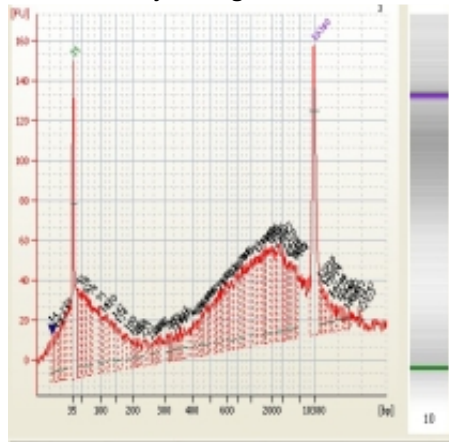
Library : Traigon_Control_1



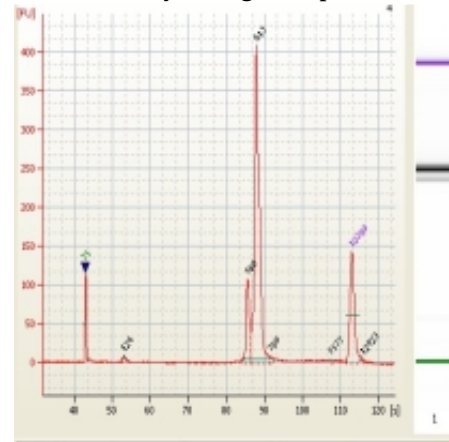
Library : Traigon_Control_2



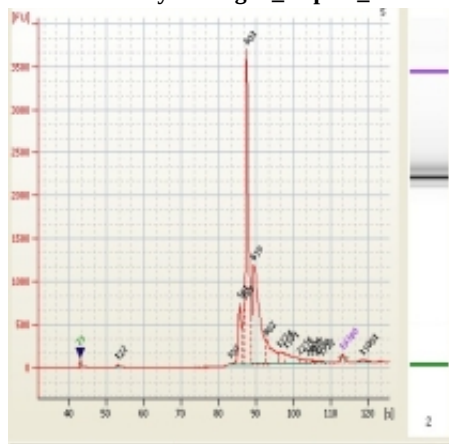
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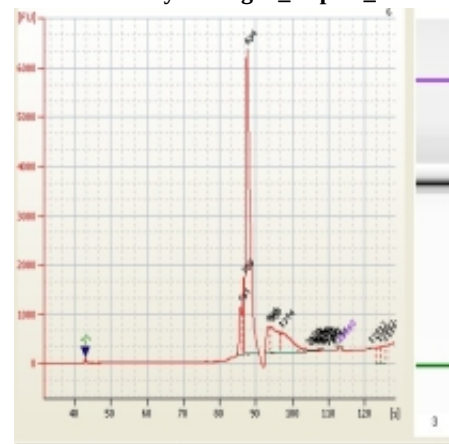
Library : Traigon_Impact_1



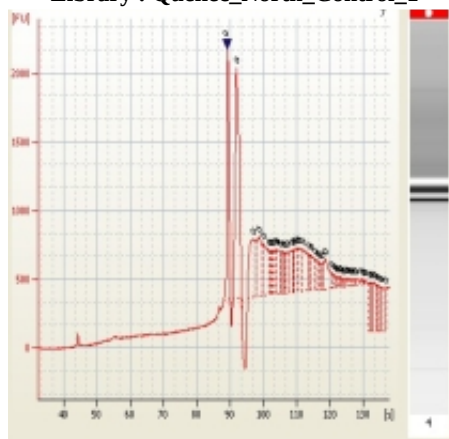
Library : Traigon_Impact_2



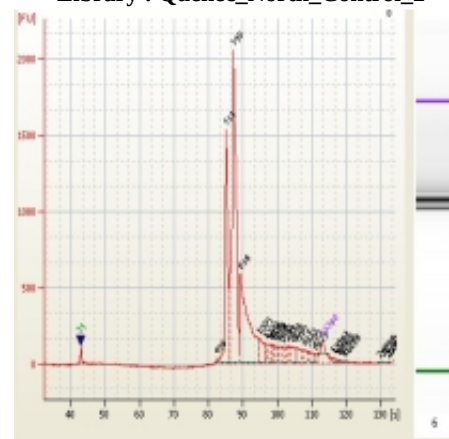
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Library : Quenec_North_Control_1



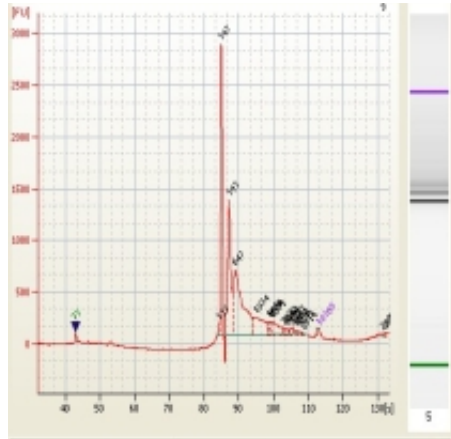
Library : Quenec_North_Control_2



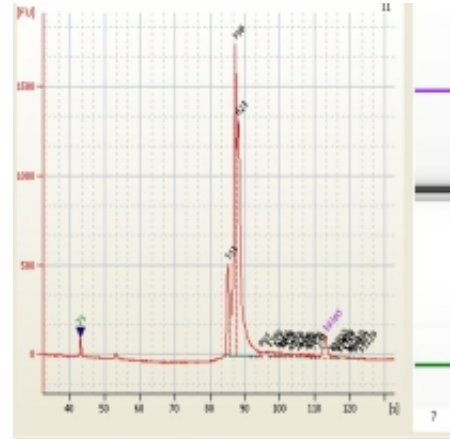
Experiment
Condition

Aglient bioanalyzer & Caliper Labchip GX DNA High Sensitivity

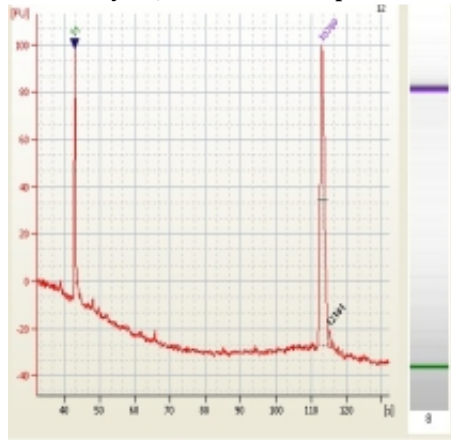
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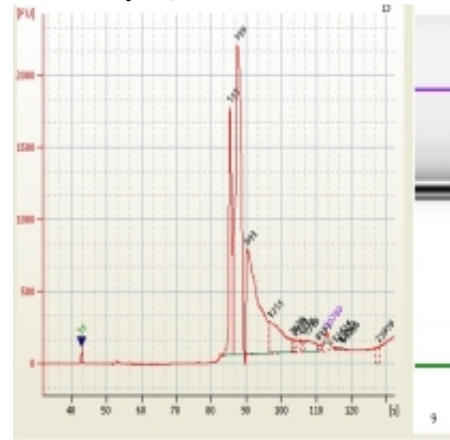
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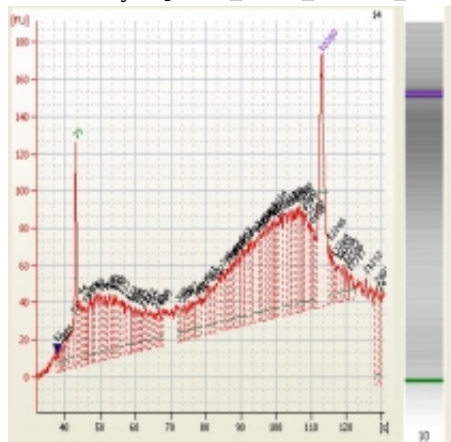
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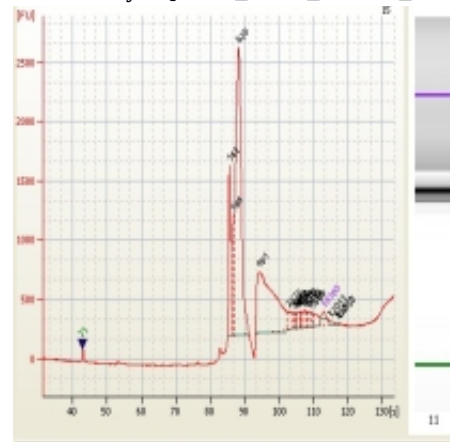
Library : Quenec_South_Control_1



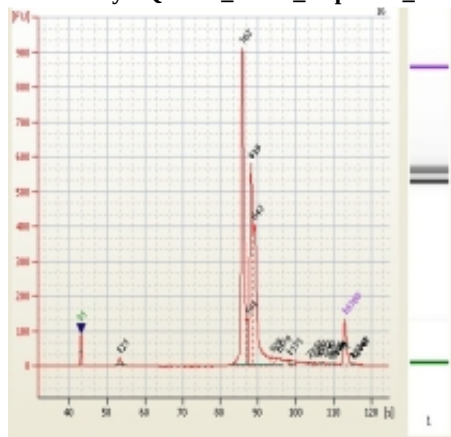
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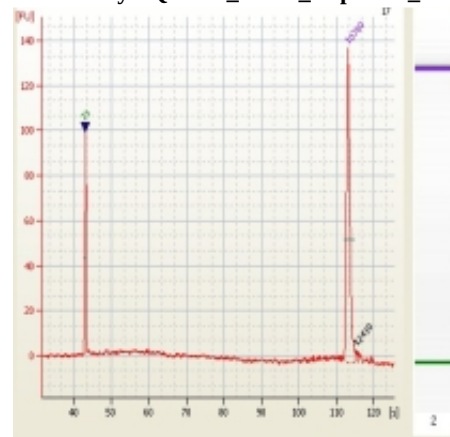
Library : Quenec_South_Control_3



Library : Quenec_South_Impacted_1



Library : Quenec_South_Impacted_2



Library QC Method

1. Library Size Check

To verify the size of PCR enriched fragments, we check the template size distribution by running on a Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip.

2. Library Quantity Check

1) illumina library

In order to achieve the highest quality of data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. This requires accurate quantitation of DNA library templates.

So we quantify prepared libraries using qPCR according to the Illumina qPCR Quantification Protocol Guide.

2) GS FLX library

To generate a standard curve of fluorescence readings and calculate the library sample concentration, we use Roche's Rapid library standard Quantification solution and calculator.

Library DNA QC Criteria

Platform	Requested library type	QC Criteria				etc
		Conc (molecules/ μ l)	Conc (nM)	Conc (ng/ μ l)	Size (bp)	
GS FLX Plus	RL library (>1.5kb)	1.46×10^8			1400~1800	
GS FLX	RL library (>1.5kb)	1.46×10^8			600~900	
GS FLX	PCR product (LMW)	1.46×10^8				
GS FLX	Amplicon library	1.0×10^9				
GS FLX	Mate paired End library (3kb)			>0.2ng/ μ l	500~700	
GS FLX	Mate paired End library (8kb)			>0.2ng/ μ l	500~700	
GS FLX	Mate paired End library (20kb)			>0.2ng/ μ l	500~700	
HiSeq	TruSeq DNA library		5	>1.5ng/ μ l	400~500	
HiSeq	Mate paired End library (2~5kb)		5	>1.5ng/ μ l	350~650	
HiSeq	Mate paired End library (6~10kb)		5	>1.5ng/ μ l	350~650	
HiSeq	Mate paired End library (11~20kb)		5	>1.5ng/ μ l	350~650	
HiSeq	Nextera Mate Pair (Gel-free)		5	1.5 ng/ μ l	600~900	
HiSeq	Nextera Mate Pair (Gel-plus)		5	1.5 ng/ μ l	600~900	
HiSeq	Truseq DNA PCR-free (350bp insert)		2		250~1000	
HiSeq	Truseq DNA PCR-free (550bp insert)		2		450~1200	
HiSeq	Library for Nimblegen EZ Whole Exome	146		>35ng/ μ l	300~400	
HiSeq	Library for Sureselect	67		>17ng/ μ l	250~500	
HiSeq	Library for TruSeq Exome Enrichment	58		>15ng/ μ l	300~400	
HiSeq	ChIP seq library*	5		>1.5ng/ μ l	400~500	
HiSeq	Nextera library	2		-	200~1000	
HiSeq	Nextera XT library	2		-	200~1000	
HiSeq	TruSeq HT library (dual indexing)	5		-	400~500	
HiSeq	Whole Genome Bisulfite treated library	5		-	400~500	
HiSeq	MBD enriched library	2		-	400~500	
HiSeq	Library for nextera Rapid exome capture	58		>15ng/ μ l	250~350	
HiSeq	Amplicon library	2		-	-	

Library RNA QC Criteria

Platform	Requested library type	QC Criteria					etc
		Status	Conc (molecules/ μ l)	Conc (nM)	Conc (ng/ μ l)	Size (bp)	
GS FLX	cDNA Rapid library	Total RNA	1.46×10^8			600~1200	
GS FLX	cDNA Rapid library	purified mRNA	1.46×10^8			600~1200	
HiSeq	mRNA library	total RNA		5	>1.5ng/ μ l	200~400	
HiSeq	mRNA library	mRNA		5	>1.5ng/ μ l	200~400	
HiSeq	Strand-specific RNA library	total RNA		5	>1.5ng/ μ l	300~500	
HiSeq	Strand-specific RNA library	mRNA		5	>1.5ng/ μ l	300~500	
HiSeq	small RNA library	total RNA		2	>0.6ng/ μ l	140~160	
HiSeq	small RNA library	smallRNA		2	>0.6ng/ μ l	140~160	