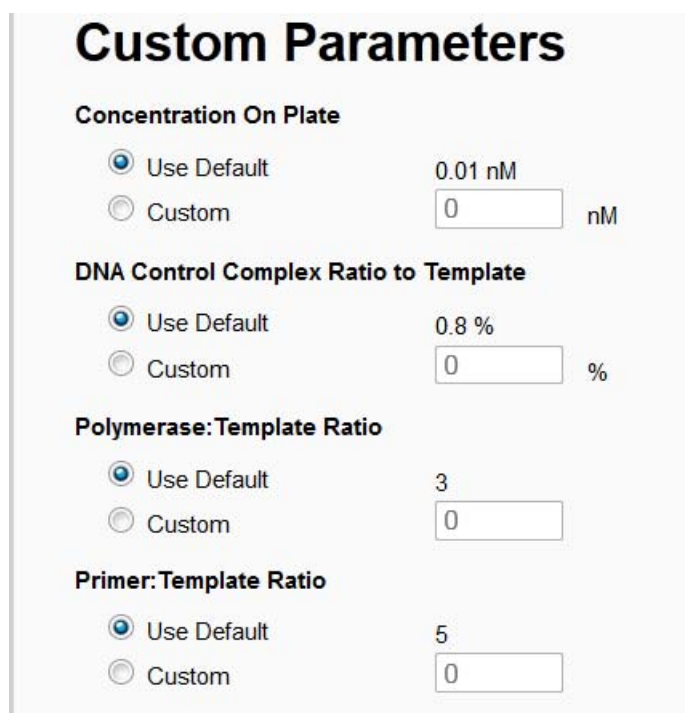


Binding Calculator Parameters Quick Reference Card

The Binding Calculator assists in sample preparation by providing instructions for primer annealing, polymerase binding and sample loading based on the available sample volume, concentration and insert size. This Quick Reference Card summarizes default coefficients that the binding calculator uses to generate a reaction worksheet.

Loading Concentration, DNA Control to Template, Primer to Template, and Polymerase to Template

In the “Custom Parameters” section of the Binding Calculator, either default or customized parameters can be selected (see figure below). The default parameters, summarized in Table 1, are recommendations to help achieve maximum sequencing performance per SMRT® Cell.



Custom Parameters

Concentration On Plate

Use Default 0.01 nM

Custom nM

DNA Control Complex Ratio to Template

Use Default 0.8 %

Custom %

Polymerase:Template Ratio

Use Default 3

Custom

Primer:Template Ratio

Use Default 5

Custom

Table 1: Insert Size, Primer to Template Ratio, Polymerase to Template Ratio and Loading Recommendations

Diffusion vs. MagBead	Insert Size Range (bp)	Primer: Template Ratio		Polymerase: Template Ratio		P6v2 Loading Concentration (pM)	
		Small Scale	Large Scale	Small Scale	Large Scale	Small Scale - Size Selected	Small Scale - no Size Selection
Diffusion	101 bp - 300 bp	20	2	2:1	2:1	Not Tested	112.5
	301 bp - 750 bp	20	2	2:1	2:1	Not Tested	187.5
	751 bp - 1,500 bp	20	5	3:1	3:1	Not Tested	97.5
	1,501 bp - 3,000 bp	20	10	3:1	3:1	Not Tested	150
	3,001 bp - 7,500 bp	20	5	3:1	3:1	Not Tested	Not Tested
	7,501 bp - 50,000 bp	20	5	3:1	3:1	Not Tested	Not Tested
MagBead	751 bp - 1,500 bp	20	5	10:1	3:1	Not Tested	Not Tested*
	1,501 bp - 3,000 bp	20	10	10:1	3:1	Not Tested	10
	3,001 bp - 7,500 bp	20	5	10:1	3:1	Not Tested	10
	7,501 bp - 15,000 bp	20	5	10:1	3:1	40	10
	15,001 bp - 50,000 bp	20	5	10:1	3:1	100	Not Tested

*PacBio recommends using MagBead loading for insert sizes > 1 kb.

DNA Controls

DNA Internal Controls are samples pre-bound with DNA polymerase and ready to be added to a sample for sequencing. The controls (available from PacBio) provide a means for independent determination of any problems that may occur during binding and/or the sequencing run.

Table 2. DNA Control Based on Insert Size

Diffusion vs. MagBead	Insert Size Range (bp)	P6v2 (Small Scale)	
		Size Selected	No Size Selection
Diffusion	101 bp - 300 bp	Not Tested	0.3%
	301 bp - 750 bp	Not Tested	0.6%
	751 bp - 1,500 bp	Not Tested	1.2%
	1,501 bp - 3,000 bp	Not Tested	2.4%
	3,001 bp - 7,500 bp	Not Tested	Not Tested*
	7,501 bp - 50,000 bp	Not Tested	Not Tested
MagBead	751 bp - 1,500 bp	Not Tested	Not Tested
	1,501 bp - 3,000 bp	Not Tested	1.25%
	3,001 bp - 7,500 bp	Not Tested	1.25%
	7,501 bp - 15,000 bp	0.5%	1.25%
	15,001 bp - 50,000 bp	1.25%	Not Tested

* PacBio recommends using MagBead loading for insert sizes > 1 kb.

Sample Volume Requirements

Sample volumes required in the sample plate vary depending on the loading method and number of SMRT Cells. The following tables summarize volume requirements for MagBead loading and Diffusion loading.

Table 3: Sample Volume Requirements for MagBead Loading (for Calculator Standard Protocol)

Number of SMRT Cells	Final MagBead Volume
1	19
2	28
3	37
4	46
5	55
6	64
7	73
8	82

Table 4: Sample Volume Requirements for MagBead Loading (Calculator OneCellPerWell Protocol)

Number of SMRT Cells	One Cell Per Well	
	Final MagBead Volume	Volume in 96-Well Plate
1	45	45 μ L to 1 well
2	95	45 μ L to 2 wells
3	140	45 μ L to 3 wells
4	185	45 μ L to 4 wells
5	230	45 μ L to 5 wells
6	275	45 μ L to 6 wells
7	320	45 μ L to 7 wells
8	365	45 μ L to 8 wells
9	415	45 μ L to 9 wells
10	460	45 μ L to 10 wells
11	505	45 μ L to 11 wells
12	550	45 μ L to 12 wells
13	595	45 μ L to 13 wells
14	640	45 μ L to 14 wells
15	685	45 μ L to 15 wells
16	730	45 μ L to 16 wells

Table 5: Sample Volume Requirements for Diffusion Loading (Calculator Standard Sequencing Protocol)

# of SMRT Cells per Sample Well	250 bp and 500 bp Libraries		Greater Than 1 kb Libraries	
	No Complex Reuse	Complex Reuse	No Complex Reuse	Complex Reuse
1	9 µL	9 µL	14 µL	14 µL
2	13 µL	9.8 µL	23 µL	17 µL
3	17 µL	9.8 µL	32 µL	17 µL
4	21 µL	13.8 µL	41 µL	26 µL
5	25 µL	14.6 µL	50 µL	29 µL
6	29 µL	14.6 µL	59 µL	29 µL
7	33 µL	18.6 µL	68 µL	38 µL
8	37 µL	19.4 µL	77 µL	41 µL
9	41 µL	19.4 µL		41 µL
10	45 µL	23.4 µL		50 µL
11	49 µL	24.2 µL		53 µL
12	53 µL	24.2 µL		53 µL
13	57 µL	28.2 µL		62 µL
14	61 µL	29 µL		65 µL
15	65 µL	29 µL		65 µL
16	69 µL	33 µL		74 µL
17	73 µL	33.8 µL		74 µL
18	77 µL	33.8 µL		74 µL
19		37.8 µL		
20		38.6 µL		
21		38.6 µL		
22		42.6 µL		

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