

## SX-8G IP-Star Automated System Specifications for Auto MBD (MethylCap)

AUTO MethylCap		
Factor	Description	Numbers
Protocol Type	Highthroughput	8 IPs
	DNA purification method	Phenol/Chloroform Spin Columns
	Working volumes	150 µl
	Type of Elution	<b>One Elution</b> Fraccionated Elution
Protocol Duration	Diagenode Optimized Protocols	<b>8 IPs protocol</b> EluAll: 5h ElluFrac: 6h
Starting cell amount for shearing		Max cell number: $3 \times 10^6$ 20 to 30 µg of DNA
Starting DNA amount for MBD		1 µg of DNA
Type of Cells	U20S, MCF7, NB4	
Cell lysis and DNA purification	GenDNA Module	
DNA Shearing	Bioruptor® Sonicator conditions	Advised starting parameters: Power: "low" # cycles: 10 cycles Time on/off: 15 seconds: "ON"/ 15 seconds: "OFF"
Tubes for shearing	1.5 ml	Volumes: 300 µl Amount: 30 µg (0.1 µg/µl)
	15 ml	Volumes: 1ml Amount: 100 µg (0.1 µg/µl)
Fragment Size	Analyze in 1% agarose	100 - 500bp
Starting IP Amount	DNA (0.1 µg/µl)	1 µg
MBD protein	H6-GST-MBD fusion protein	2 µg
IP incubation time	Fixed for the customer	2 hours
Capture MBD-DNA complex reaction	Fixed for the customer	1 hour
Control primers	TSH2B cell specific methylated region GADPH promoter unmethylated region	
DNA recovery after MBD (e.i U20S)	dsDNA	Low Fraction: 45 ng Medium fraction: 50 ng High Fraction: 10 ng

<p><b>Real-time PCR</b></p>	<p>% recovery sample/input (in U2OS)</p>	<p><b>TSH2B</b>            Low Fraction: 30%            Medium fraction: 45 %            High Fraction: 5%  <b>GADPH: 0.</b>            Low Fraction: 0.2%            Medium fraction: 0.2 %            High Fraction: 0.15%</p>
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