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CWBIO Single Cell WGA Kit Performance test

Purpose: To validate the product performance of CWBIO Single Cell WGA Kit

- Take CW2843(MDA) as an example:

- Experiment 1

1. Using single-cell gDNA as a template, using CWBIO Single Cell WGA Kit to compare the amplification effect with the same kit of Company A, the data are as follows:

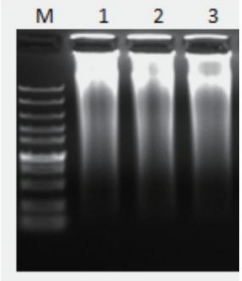


Figure 1. Amplification product

1) A company kit.
2) Single cell genome amplification with CW reagent.
3) CW reagent genome as template for amplification.

2. Using gDNA as template, the sensitivity amplification test (reaction time 2 h) was carried out, and the concentration of amplification product was detected by qPCR for 2 h.

Template input	1 ng	0.5 ng	0.1 ng	0.05 ng
Concentration of amplification product (ng/μL)	656.5	624.0	614.3	624.0
Amplification product yield (ug)	32.8	31.2	30.7	31.2

	1 ng	500 pg	100 pg	50 pg	SC-NTC	Positive	Negative
Primer 1	25.2	25.7	26.1	26.4	37.5	25.0	N/A
Primer 2	26.5	26.4	27.7	27.0	39.7	26.0	38.8
Primer 3	26.4	26.3	26.7	27.1	N/A	25.7	N/A
Primer 4	26.9	27.3	26.9	27.8	34.5	26.0	34.7
Primer 5	25.6	25.7	26.3	25.7	39.1	25.3	N/A
Primer 6	25.9	25.8	26.2	27.7	32.4	24.5	32.7
Primer 7	25.1	25.1	25.9	25.4	N/A	25.0	38.8
Primer 8	27.2	27.2	28.2	27.4	N/A	25.6	N/A
Average CT	26.1	26.2	26.8	26.8		25.4	

Figure 1. Amplification product

3. Using the tumor cells as the template, the whole genome was amplified by CWBIO Single Cell WGA Kit compared with company A, and the CWBIO library kit was used to build the library. The comparison of product 50 gene reconstructed library is as follows:

1) Tumor cell gDNA is the template.

chr	Start	End	Ref	Alt	DP	AD	Freq	MuType	Gene	Accession	Exon	CDSMutation	AAMutation
chr4	55141055	55141055	A	G	124763	124556	99.83%	synonymou	PDGFRA	NM_006206	exon12	c.1701A>G	p.P567P
chr17	7579472	7579472	G	C	237655	236767	99.63%	nonsynony	TP53	NM_001126118	exon3	c.98C>G	p.P33R
chr5	112175770	112175770	G	A	560217	557656	99.54%	synonymou	APC	NM_000038	exon16	c.4479G>A	p.T1493T
chr12	25380275	25380275	T	A	67244	66547	98.96%	nonsynony	KRAS	NM_033360	exon3	c.183A>T	p.Q61H
chr19	1207021	1207021	C	T	28104	26461	94.15%	stopgain	STK11	NM_000455	exon1	c.109C>T	p.Q37X
chr11	534242	534242	A	G	15577	14552	93.42%	synonymou	HRAS	NM_001130442	exon2	c.81T>C	p.H27H
chr3	178936091	178936091	G	A	25851	12673	49.02%	nonsynony	PIK3CA	NM_006218	exon10	c.1633G>A	p.E545K
chr5	170837552	170837552	T	G	195706	3009	1.54%	nonsynony	NPM1	NM_199185	exon10	c.781T>G	p.W261G

2) The single expansion product of tumor cell A company kit is used as template.

chr	Start	End	Ref	Alt	DP	AD	Freq	MuType	Gene	Accession	Exon	CDSMutation	AAMutation
chr4	55141055	55141055	A	G	150763	150489	99.82%	synonymou	PDGFRA	NM_006206	exon12	c.1701A>G	p.P567P
chr17	7579472	7579472	G	C	966636	962952	99.62%	nonsynony	TP53	NM_001126118	exon3	c.98C>G	p.P33R
chr5	112175770	112175770	G	A	496937	494415	99.49%	synonymou	APC	NM_000038	exon16	c.4479G>A	p.T1493T
chr12	25380275	25380275	T	A	70831	69765	98.50%	nonsynony	KRAS	NM_033360	exon3	c.183A>T	p.Q61H
chr19	1207021	1207021	C	T	225570	221173	98.05%	stopgain	STK11	NM_000455	exon1	c.109C>T	p.Q37X
chr11	534242	534242	A	G	13870	12133	87.48%	synonymou	HRAS	NM_001130442	exon2	c.81T>C	p.H27H
chr3	178936091	178936091	G	A	27184	14365	52.84%	nonsynony	PIK3CA	NM_006218	exon10	c.1633G>A	p.E545K
chr17	7578408	7578408	C	T	511792	34915	6.82%	synonymou	TP53	NM_001276699	exon1	c.45G>A	p.R15R
chr5	170837552	170837552	T	G	323647	5310	1.64%	nonsynony	NPM1	NM_199185	exon10	c.781T>G	p.W261G
chr5	112175634	112175634	C	G	180247	2812	1.56%	nonsynony	APC	NM_000038	exon16	c.4343C>G	p.T1448S

3) The single amplification product of tumor cell CW is used as template.

Chr	Start	End	Ref	Alt	DP	AD	Freq	MuType	Gene	Accession	Exon	CDSMutation	AAMutation
chr4	55141055	55141055	A	G	107893	107676	99.80%	synonymous SNV	PDGFRA	NM_006206	exon12	c.1701A>G	p.P567P
chr17	7579472	7579472	G	C	309458	308019	99.54%	nonsynonymous SNV	TP53	NM_001126118	exon3	c.98C>G	p.P33R
chr5	112175770	112175770	G	A	370052	368112	99.48%	synonymous SNV	APC	NM_000038	exon16	c.4479G>A	p.T1493T
chr12	25380275	25380275	T	A	60113	59163	98.42%	nonsynonymous SNV	KRAS	NM_033360	exon3	c.183A>T	p.Q61H
chr19	1207021	1207021	C	T	189902	186877	98.41%	stopgain	STK11	NM_000455	exon1	c.109C>T	p.Q37X
chr11	534242	534242	A	G	16959	15834	93.37%	synonymous SNV	HRAS	NM_001130442	exon2	c.81T>C	p.H27H
chr3	178936091	178936091	G	A	67237	32423	48.22%	nonsynonymous SNV	PIK3CA	NM_006218	exon10	c.1633G>A	p.E545K
chr7	55242430	55242430	G	A	28209	3025	10.72%	nonsynonymous SNV	EGFR	NM_005228	exon19	c.2200G>A	p.E734K
chr5	112175634	112175634	C	G	139042	4961	3.57%	nonsynonymous SNV	APC	NM_000038	exon16	c.4343C>G	p.T1448S
chr5	170837552	170837552	T	G	566344	8904	1.57%	nonsynonymous SNV	NPM1	NM_199185	exon10	c.781T>G	p.W261G
chr3	178936092	178936092	A	C	67238	1042	1.55%	nonsynonymous SNV	PIK3CA	NM_006218	exon10	c.1634A>C	p.E545A
chr17	7577049	7577049	G	A	130426	1975	1.51%	nonsynonymous SNV	TP53	NM_001276699	exon4	c.412C>T	p.H138Y

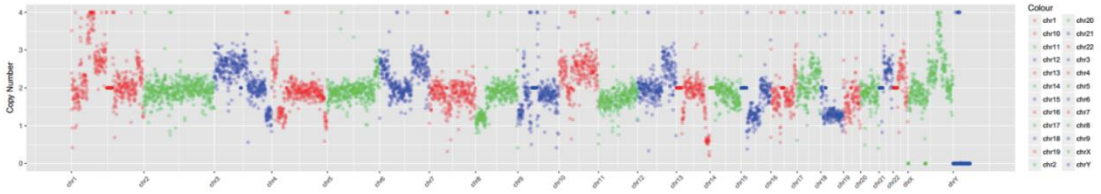
Figure 2. Comparison of multi-library construction

Conclusion: The experimental results show that compared with similar products, CWBIO Single Cell WGA Kit has excellent amplification performance, high sensitivity and high amplification yield, and can effectively detect SNV mutations in tumor cells.

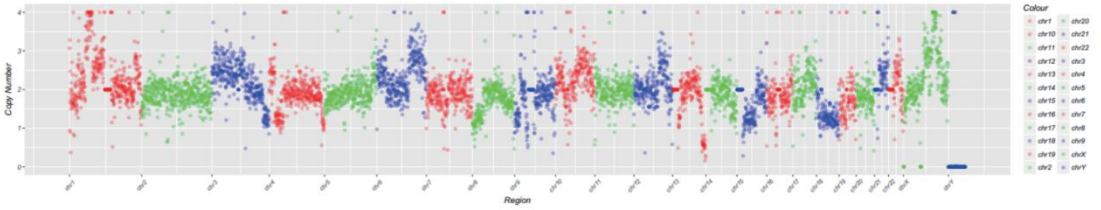
Experiment 2

1. Using cultured cells (293T) as template, after amplification by CWBIO Single Cell WGA Kit, the products were collected and the library was constructed after three interruptions. CNV detection was as follows:

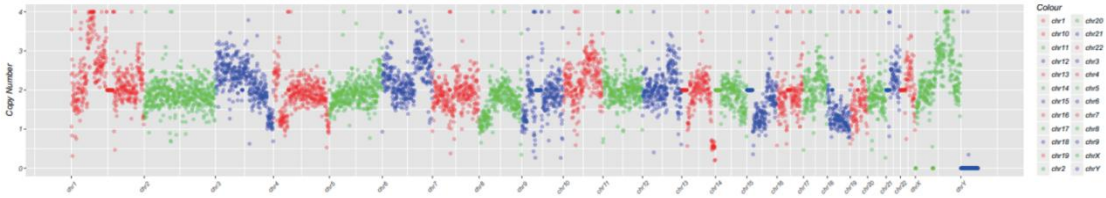
1) Ultrasonic interruption library construction.



2) Construction of restriction endonuclease interrupted library.

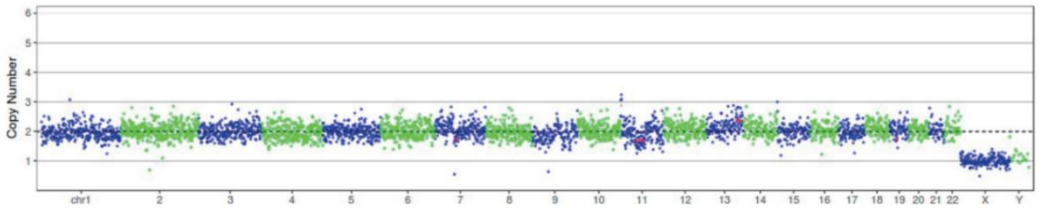


3) Construction of transposable enzyme library.

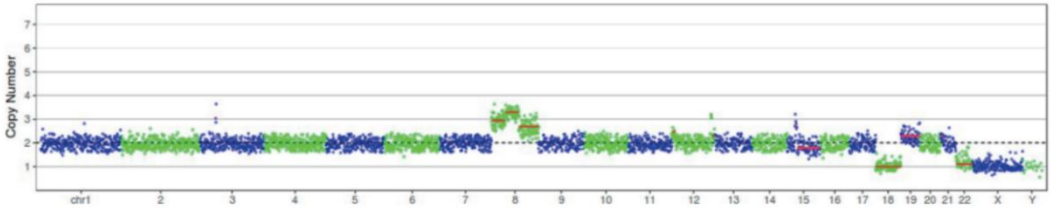


2. For different types of samples, CWBIO Single Cell WGA Kit and Library Construction Kit were used for amplification, and the CNV detection was as follows:

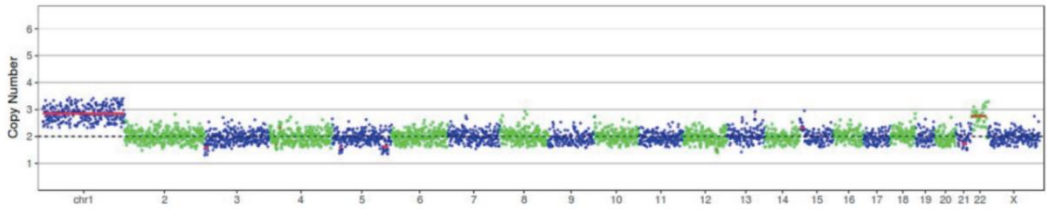
1) Normal sample.



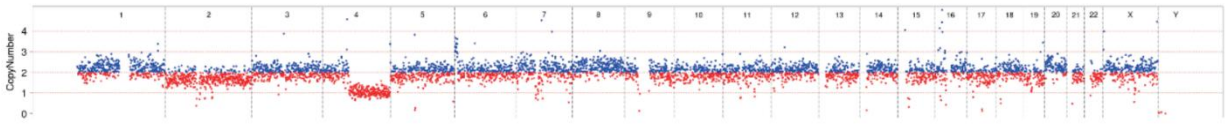
2) Abnormal sample 1.



3) Abnormal sample 2.



4) Abnormal sample 3



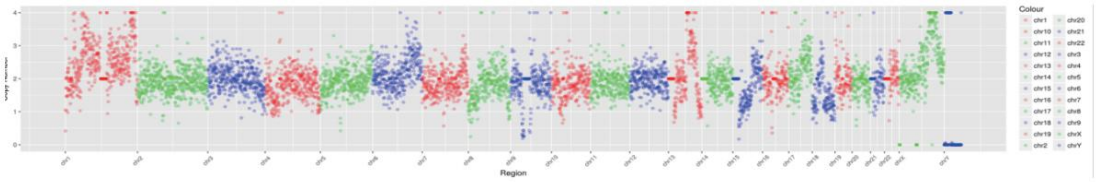
- Take CW2844(DOP-PCR) as an example:
- Experiment 1

1. Using tumor cell gDNA and single cell amplification products as templates, using CWBIO library building kit to build the database, compared with B company, the copy number variation was analyzed by MGI, the data analysis is as follows:

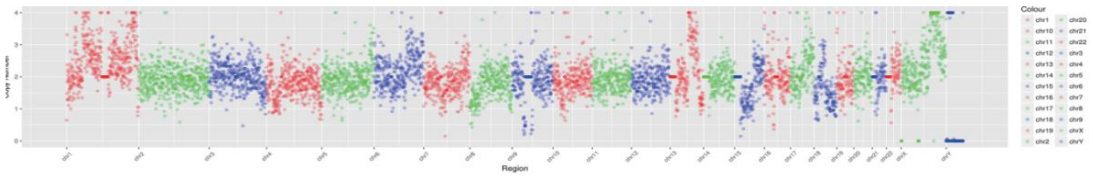
1) Comparison with B company's reagent.

SampleID	raw_data	Q30	R1_gc	R2_gc	reads number	mapped	rmdup	perfect
gDNA, CW-1	1935700200(1.94G)	86.54%	42.77%	42.05%	18458220	92.45%	99.05%	80.67%
gDNA, CW-2	1237126600(1.24G)	87.19%	42.14%	42.19%	11792100	93.83%	99.26%	81.84%
gDNA, Company B	2076679800(2.08G)	87.20%	42.04%	42.26%	19732120	93.45%	98.90%	77.88%
cell, CW-1	1881487200(1.88G)	86.89%	41.82%	42.09%	18006210	93.88%	98.69%	81.70%
cell, CW-2	2022866400(2.02G)	87.39%	41.92%	42.24%	19319880	94.03%	98.81%	81.73%
cell, Company B	1307065400(1.31G)	87.48%	41.55%	41.73%	12466744	94.32%	98.85%	81.31%

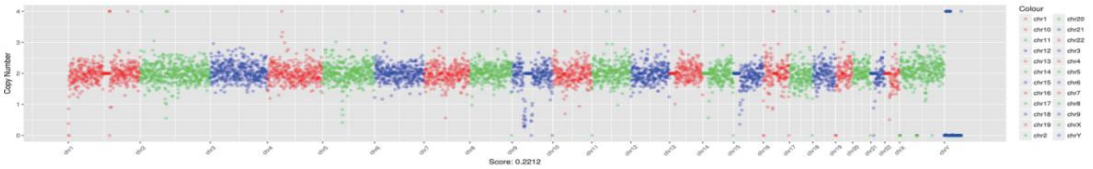
2) Analysis of the number of copies in the kit of company B.



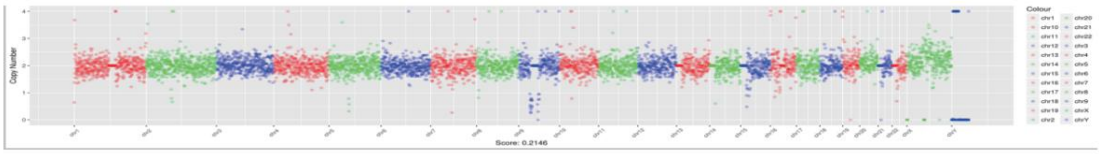
3) Copy number analysis of CW kit.



4) The amplification reagent of company B is off the machine.



5) CW amplification reagent off the machine.



Conclusion: The experimental results show that compared with similar products, the copy number of CWBIO Single Cell WGA Kit can meet the requirements of detecting the insertion or deletion of fragments > 4Mb, which has high reproducibility and accuracy.