SureSeq

Capturing Fusions in Hematological Malignancies Through Targeted RNAseq

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What binds us, makes us.

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Introduction

Large chromosomal rearrangements resulting in fusion gene formation are implicated in tumorigenesis and cancer progression in multiple hematologic malignancies.

While karyotyping, FISH, RT-PCR and microarrays are routine research techniques to detect fusion genes, they all have limitations. With improvements in NGS-based methods, DNA- and RNA sequencing are rapidly becoming established as the method of choice. NGS panels facilitate the simultaneous discovery of novel alterations alongside known mutations and structural alterations in genomic research. The rapid growth in the development of this mutation detection capability has moved beyond SNVs and indels to now include translocations.

Acute myeloid leukemia (AML), characterized by myeloid cell overproliferation, is typically underpinned by a range of fusion genes. In this study, we tested OGT's SureSeq™ Myeloid Fusion Complete NGS Workflow Solution V2 to detect known fusions in AML research samples.

Methods

Workflow

100ng - 500ng RNA was converted to cDNA followed by library generation and hybridization enrichment, using SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 (Figure 1).

Panel

The SureSeq Myeloid Fusion panel allows the detection of 30 common myeloid fusions in addition to novel fusion partners. Specific gene targeting (bold text) allows accurate partner gene agnostic identification of fusion genes (Table 1). Moreover, inv(3)/t(3;3)(q21.3q26) detection is achieved through analysis of *MECOM* overexpression.

Sequencing

Sequencing was conducted using 2 x 150 bp reads on an Illumina MiSeq® V2 300.

Samples

We tested 69 RNA samples (20 commercially available cell lines and 49 research samples) previously characterized by FISH and/or qPCR, including 52 samples with known fusion events: *BCR-ABL1* (n=8), *ETV6-RUNX1* (n=10), *RUNX1-RUNX1T1* (n=7), *PML-RARA* (n=11), *CBFB-MYH11* (n=5), *KMT2A-partner* (n=8), inv(3)/t(3;3)(q21.3q26) (n=3) as well as 17 fusion-negative samples.

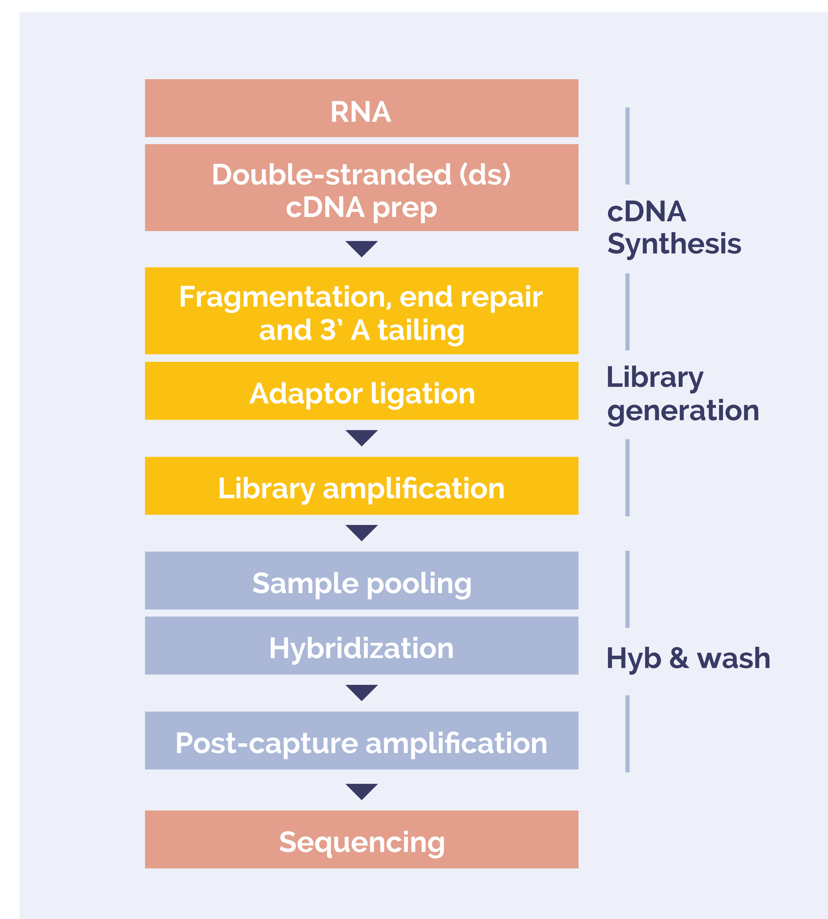

Figure 1: Universal NGS Workflow RNA to sequencer in 3 days with minimal handling time.

Bioinformatic Analysis

Sequencing data analysis including detection of fusions as well as relative expression of *MECOM* was performed using OGT's Interpret NGS Analysis Software.

***RBM15**-MKL1* t(1;22)(p13.3;q13.3)	***KMT2A**-ELL* t(11;19)(q23;p13.1)	***FUS**-ERG* t(16;21)(p11.2;q22.2)	***FGFR1**-ZMYM2* t(8;13)(p11;q12)
*GATA2-**MECOM** (GATA2-EVI1)* inv(3)(q21.3q26.2) **via *MECOM* overexpression**	***KMT2A**-MLLT1 (ENL)* t(11;19)(q23;p13.3)	***CBFB**-MYH11* inv(16)(p13.1q22)	*FIP1L1-**PDGFRA*** del(4)(q12q12)
*RPN1-**MECOM** (RPN1-EVI1)* t(3;3)(q21q26) **via *MECOM* overexpression**	***KMT2A**-AF6 (MLLT4; AFDN)* t(6;11)(q27;q23)	***RUNX1**-RUNX1T1 (AML1-ETO)* t(8;21)(q22;q22.1)	***PDGFRB**-EBF1* del(5)(q32q33)
*DEK-**NUP214** (DEK-CAN)* t(6;9)(p23;q34.1)	***KMT2A**-MLLT11* t(1;11)(q21;q23)	*ETV6-**RUNX1** (TEL-AML1)* t(12;21)(p13;q22)	***PDGFRB**-TNIP1* t(5;5)(q32;q33)
***NUP98**-NSD1* t(5;11)(q35.2;p15.4)	***MT2A**-NEBL* t(10;11)(p12;q23)	***RUNX1-MECOM** (AML1-EVI1L)* t(3;21)(q26.2;q22)	***PDGFRB**-ATF7IP* t(5;12)(q33;p13)
***NUP98**-HOXA9* t(7;11)(p15.4;p15.2)	***PML**-RARα* t(15;17)(q24;q21)	***BCR**-ABL1* t(9;22)(q34.1;q11.2)	***PDGFRB**-ETV6* t(5;12)(q33;p13)
***PICALM**-MLLT10* t(10;11)(p12.3;q14.2)	*KAT6A-**CREBBP*** t(8;16)(p11.2;p13.3)	*NPM1-**MLF1*** t(3;5)(q25.1;q35.1)	
***KMT2A**-MLLT3 (AF9)* t(9;11)(p21.3;q23.3)	*PCM1-**JAK2*** t(8;9)(p22;p24)	***FGFR1-BCR*** t(8;22)(p11;q11)	

Table 1: SureSeq Myeloid Fusion panel content. Gene targets are highlighted in bold text.

Results I

A. Detection of Fusions

We achieved 100% concordant detection for all 69 samples tested (Table 2-3). Sample-cohort comprised 52 fusion-positive and 17 fusion-negative samples previously characterized via FISH and/or qPCR. Reciprocal transcripts are detected for a number of fusion-positive samples.

	Fusion or Overexpression	Cell lines	Donor Breakpoint (NGS)	Recipient Breakpoint (NGS)	Reciprocal Transcripts?
Commercial Cell lines	*ETV6-RUNX1*	REH	Chr 12: 11869969	Chr 21: 34887096	Yes
	RUNX1-RUNX1T1	SKNO-1; KASUMI-1	Chr 21: 34859474	Chr 8: 92017363	Yes
	PML-RARA	HT-93; NB-4; AP-1060	Chr 15: 74023408	Chr 17: 40348316	Yes
	BCR-ABL1	K-562; CML-T1; LAMA-87; SUP-B15; MOLM-1; HT-93	Chr 22: 23290413	Chr 9: 130854064	Yes
	CBFB-MYH11	ME-1	Chr 16: 67082308	Chr 16: 15721051	No
	MECOM overexpression	MOLM-1; HT-93	–	–	n.a.
	KMT2A-MLLT3	NOMO-1; THP-1	Chr 11: 118482495	Chr 9: 20365744	No
	KMT2A-MLLT4	ML-2; SHI-1	Chr 11: 118482495	Chr 6: 167864551	No
	DEK-NUP214	FKH-1	Chr 6: 18236452	Chr 9: 131159383	Yes
	FIP1L1-PDGFRA	EOL-1	Chr 4: 53425965	Chr 4: 54274925	Yes
	FUS-ERG	YNH-1	Chr 16: 31186836	Chr 21: 38383923	Yes
	PICALM-MLLT10	U-937	Chr 11: 85974708	Chr 10: 21586294	Yes
	FGFR10P2-FGFR1	KG-1	Chr 12: 26957743	Chr 8: 38418373	Yes
	BCR-ABL1	UHRR (Agilent)	Chr 22: 23290413	Chr 9: 130854064	Yes
	PICALM-MLLT10		Chr 11: 85974708	Chr 10: 21586294	Yes
	NPM1-ALK	SUP-M2 (untargeted fusion control)	–	–	n.a.
	TCF3-PBX	697 (untargeted fusion control)	–	–	n.a.
	None	Normal Human Lymphocyte RNA (fusion-negative control)	–	–	n.a.

Table 2: Characterization of commercial samples using SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software.

	Fusion or Overexpression	FISH (%)	RQ-PCR ratio (fusion: ABL1)	Donor Breakpoint (NGS)	Recipient Breakpoint (NGS)	Reciprocal Transcripts?
Research Samples	*ETV6-RUNX1*	11 – 93	0.4 – 3.6	Chr 12: 11869969	Chr 21: 34887096	Yes
	RUNX1-RUNX1T1	45 – 96	0.5 – 5.3	Chr 21: 34859474	Chr 8: 92017363	Yes
	PML-RARA	72 – 100	0.21 – 0.92	Chr 15: 74023408	Chr 17: 40348316	Yes
	BCR-ABL1	31 – 90	0.74 – 1	Chr 22: 23182239	Chr 9: 130854064	Yes
	CBFB-MYH11	51 – 97	0.26 – 1.3	Chr 16: 67082308	Chr 15: 15721051	No
	MECOM overexpression	74	n.a.	–	–	n.a.
	KMT2A-MLLT3	70	n.a.	Chr 11: 118482495	Chr 9: 20365744	No
	KMT2A-MLLT10	95	n.a.	Chr 11: 118482495	Chr 10: 21670449	Yes
	KMT2A-AFF1	84	n.a.	Chr 4: 87047594	Chr 11: 118484183	Yes
	Untargeted fusions	n.a.	n.a.	–	–	n.a.
	Fusion-negative	n.a.	n.a.	–	–	n.a.

Table 3: Characterization of research samples using SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software.

B. Detection of *MECOM* overexpression

OGT's SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 successfully detects *MECOM* overexpression, which is especially important for atypical translocations involving *MECOM* inv(3)(q21q26) and t(3;3)(q21;q26) that result in *MECOM* overexpression rather than fusion gene formation (Figure 2).

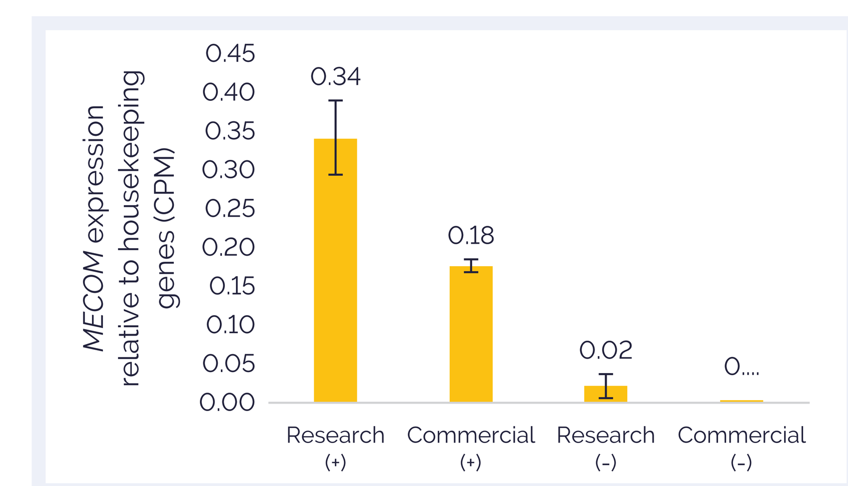

Figure 2: *MECOM* expression detection in research and commercial samples.

C. Exon-level Resolution of Breakpoints

SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 provides exon-level resolution of chromosomal breakpoints for fusions and identifies multiple breakpoints within the same experiment. *BCR-ABL1* (e14-a2, e13-a2 and e1-a2), *CBFB-MYH11* (exon5-exon34), *PML-RARA* (typical isoforms: bcr1 (exon6-exon3), bcr3 (exon3-exon3) and atypical isoforms: bcr3 (exon4-exon2).

Fusions	Breakpoints deteced by FISH/qPCR			Breakpoints detected by NGS		
	Number of breakpoins	Donor Gene	Recipient Gene	Number of breakpoints	Donor Gene	Recipient Gene
CBFB-MYH11	1	*CBFB* ex:5	*MYH11* ex:34	1	*CBFB* ex:5	*MYH11* ex:34
PML-RARA	2	1. *PML* ex:3 2. *PML* ex:6	1. *RARA* ex:3 2. *RARA* ex:3	3	1. *PML* ex:3 2. *PML* ex:6 3. PML ex:4	1. *RARA* ex:3 2. *RARA* ex:3 3. *RARA* ex:2
BCR-ABL1	1	1. *BCR* e14	1. *ABL1* a2	3	1. *BCR* e14 2. *BCR* e13 3. *BCR* e1	1. *ABL1* a2 2. *ABL1* a2 3. *ABL1* a2

Table 4. Breakpoint detection by SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software.

D. Partner-agnostic Fusion detection

OGT's SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 is capable of partner-agnostic fusion detection. In this study, we detected 8 *KMT2A* fusions involving 4 gene partners: *MLLT3*, *MLLT4 (AFDN)*, *MLLT10* and *AFF1* (Tables 2-3). Examples of these fusions are presented in Figures 3-4.

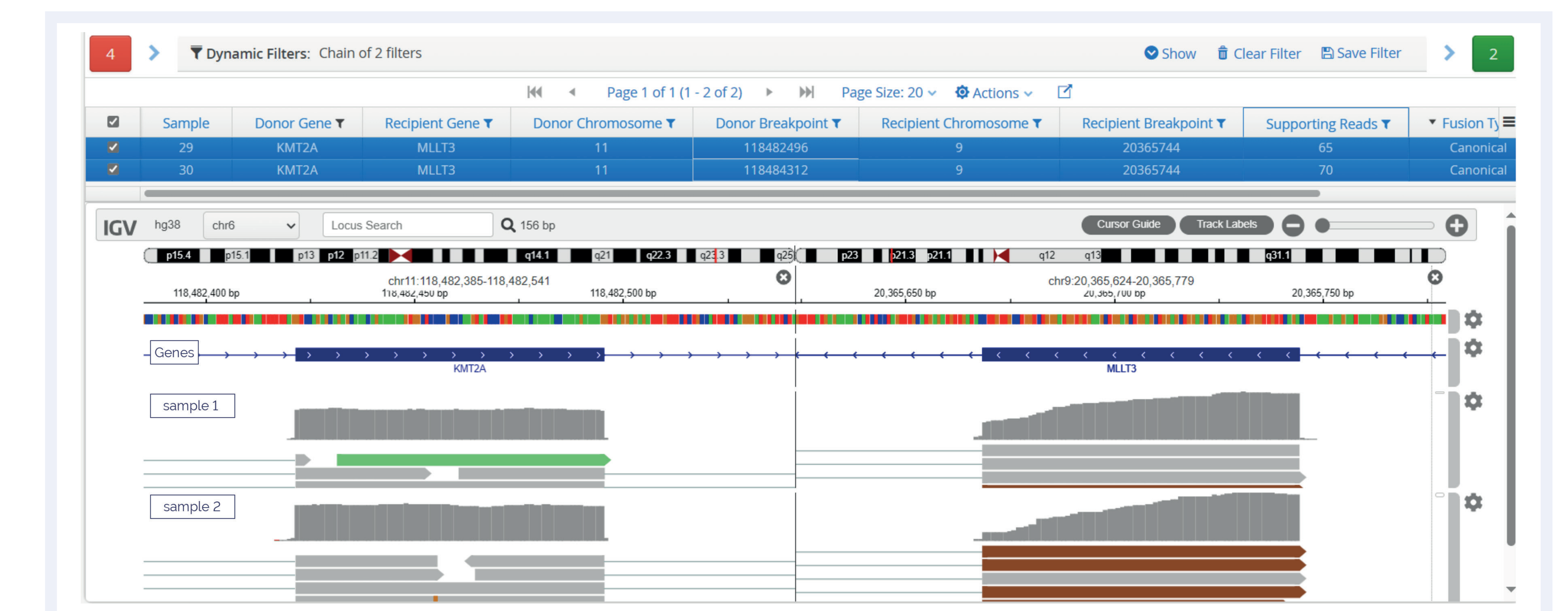

Figure 3: SureSeq Interpret NGS Analysis Software displaying *KMT2A-MLLT3* fusion in 2 research samples

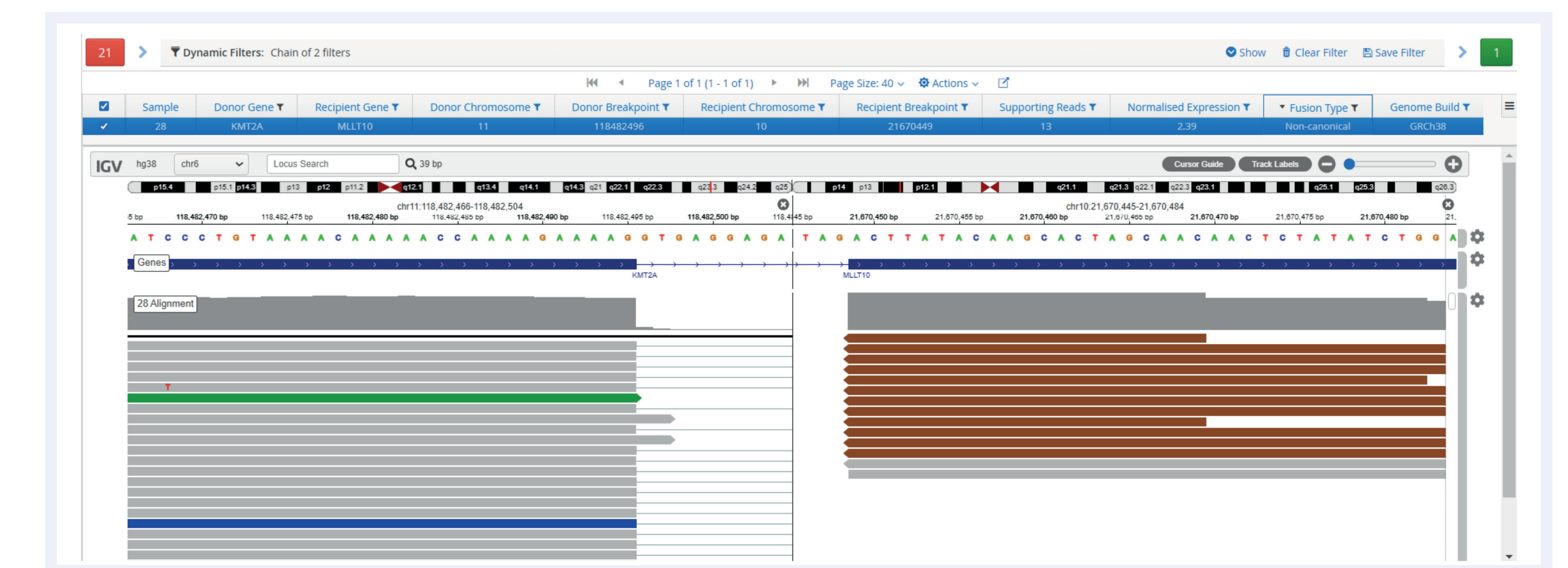

Figure 4: SureSeq Interpret NGS Analysis Software displaying *KMT2A-MLLT10* fusion in 1 research sample

Conclusions

- By achieving 100% concordance with qPCR and FISH for all samples tested, we have demonstrated the capability of the SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 to detect known rearrangements in AML
- SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 detects *MECOM* overexpression, which in turn, allows for the detection of translocation events such as inv(3)(q21q26); t(3;3)(q21;q26) that do not form fusion genes but rather result in *MECOM* overexpression
- The NGS data detected single-exon resolution of breakpoints, multiple breakpoints as well as reciprocal fusion transcripts that would have remained undetected with FISH. Thus, our NGS assay provides a more comprehensive transcriptomic landscape of fusions in Myeloid Leukemias
- SureSeq Myeloid Fusion Complete NGS Workflow Solution V2 allows partner-agnostic fusion detection, which is especially important for promiscuous driver genes like *KMT2A* that have multiple fusion partners.

Further information contact@ogt.com | ogt.com | Oxford Gene Technology Ltd., Unit 5, Oxford Technology Park, 4A Technology Drive, Kidlington, Oxfordshire, OX5 1GN, UK.

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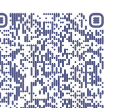
Learn more about the fusion panel



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