

## **SUPPLEMENTARY INFORMATION**

for

### **Individualised multiplexed circulating tumour DNA assays for monitoring of tumour presence in patients after colorectal cancer surgery.**

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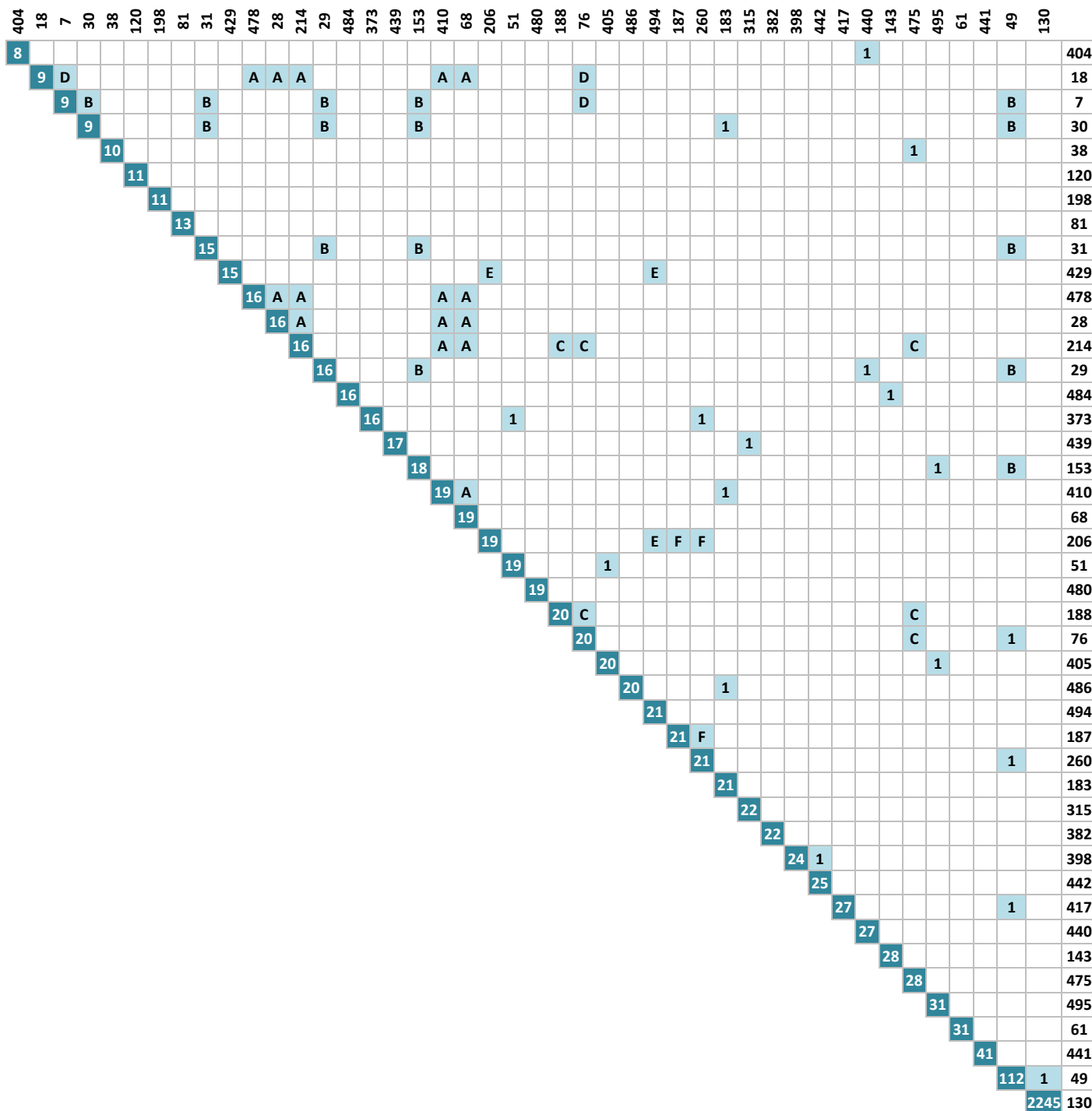
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**Supplementary Figures S1-S4**

**Supplementary Table S1**

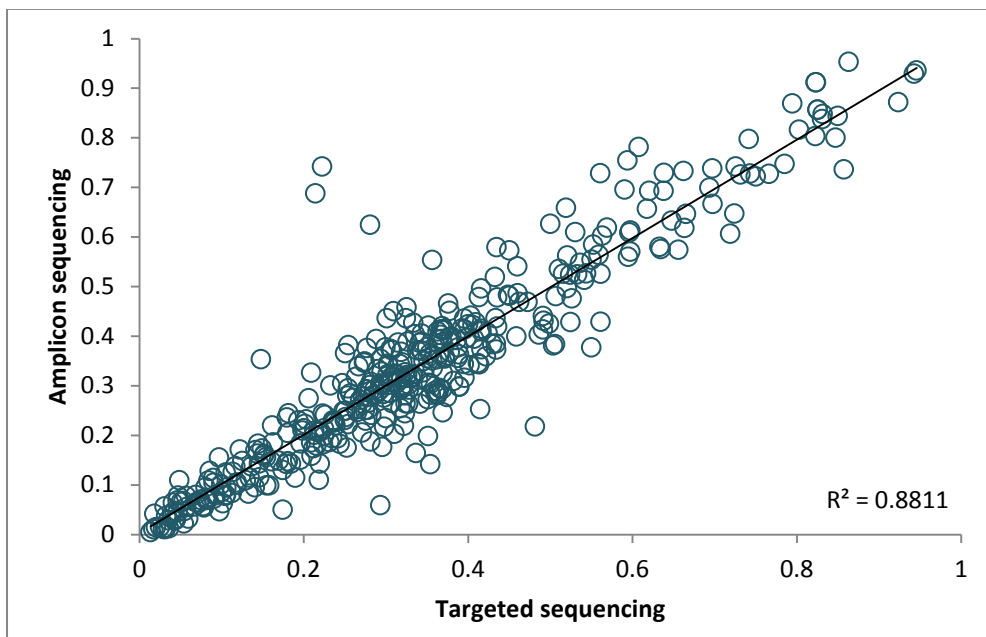
**Supplementary Figure S1: Number of somatic mutations identified in 44 patient tumours.**

The number of mutations in each sample is given on the diagonal (range: 8 – 2245), and as well as the number of mutations any pairwise samples share (lightly shaded boxes, maximum is 1). Mutations which are shared among at least three samples are specifically identified (A-F).

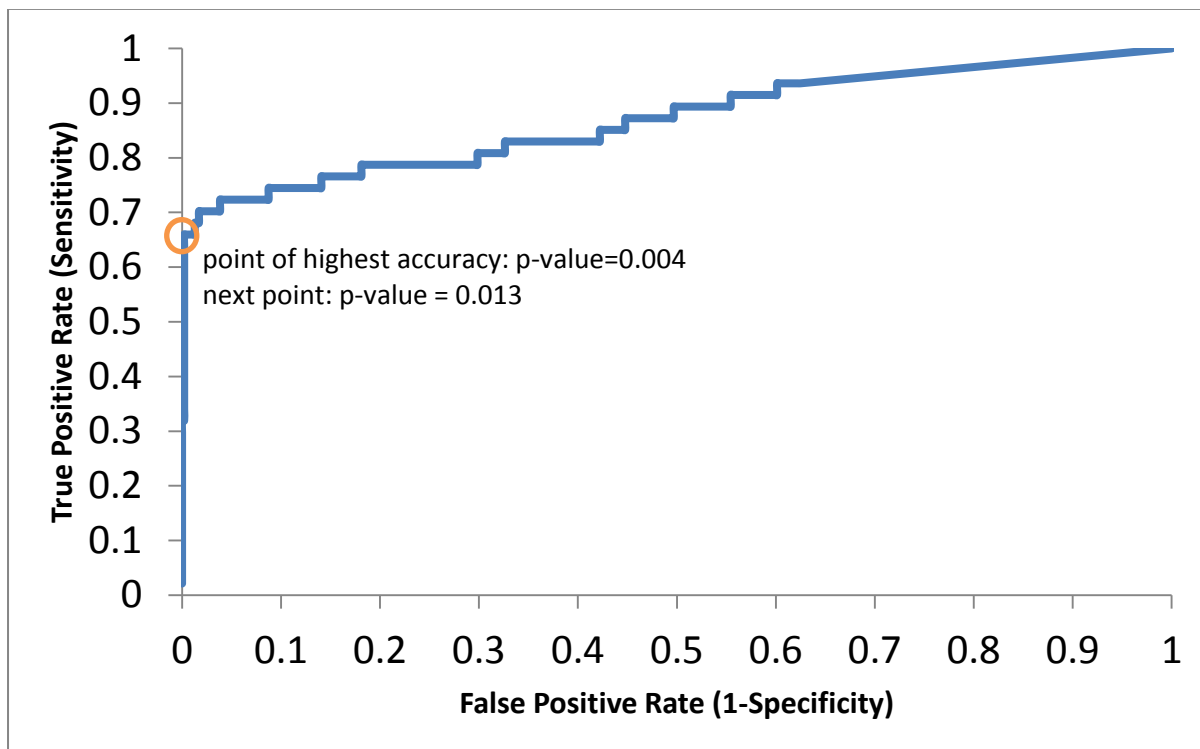


	Gene	Mutation	Samples with mutation
A	KRAS	G12V	6
B	KRAS	G13D	6
C	TP53	R273H	4
D	APC	R232X	3
E	TP53	R273C	3
F	APC	R876X	3

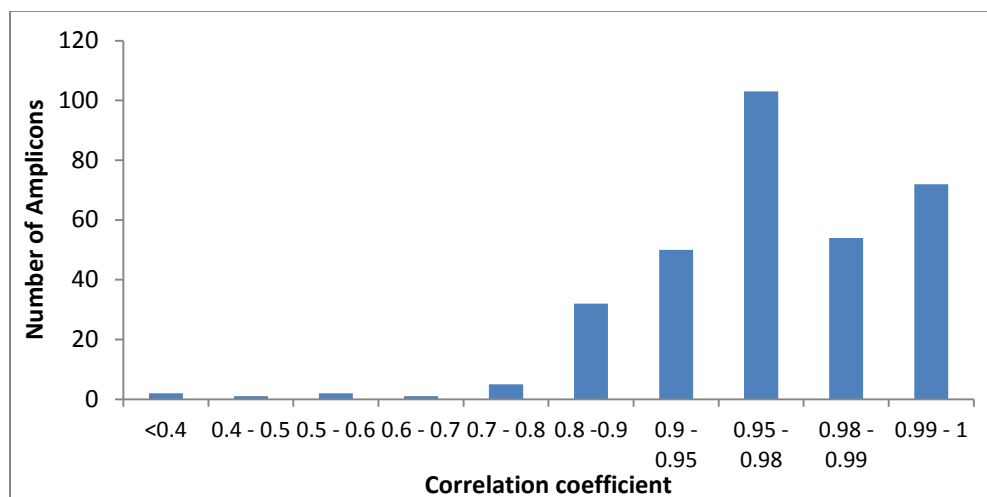
**Supplementary Figure S2: Concordance of somatic mutation allele frequency as estimated by targeted sequencing and amplicon sequencing**



**Supplementary Figure S3: ROC curve of positive and negative amplicons based on plasma samples from patients and healthy volunteers respectively.**



**Supplementary Figure S4: Histogram of Pearson correlation coefficients between observed and expected variant frequencies over 326 amplicons in 44 tumour dilution series.**



**Supplementary Table S1: Patient characteristics including availability of plasma samples and ctDNA detection at selected clinical events.**

Patient	Total number of plasma samples	Age	Gender	Stage of primary tumor (T Stage/N stage [no. of lymph nodes involved/no. of lymph nodes harvested])	Site of primary tumor	Pre-primary-surgery plasma available?	ctDNA detected in pre-primary-surgery plasma?	Post-operative (within 1 week) plasma available?	ctDNA detected in post-operative plasma?	Did patient develop recurrence?	Site of recurrence	Plasma samples prior to/at recurrence after primary surgery available?	ctDNA detected pre-recurrence?	Metastectomy / surgery for recurrent disease performed?	Was there recurrence post-metastectomy?	Site of recurrence post-metastectomy	Plasma samples available prior to/at recurrence after metastectomy available?	ctDNA detected pre-recurrence?
417	2	68y	Male	T1N0 [0/8]	Sigmoid Colon	y	n	y	n	n	NA							
441	14	77y	Male	T3N1 [1/11]	Sigmoid Colon	y	n	y	n	n	NA							
494	3	69y	Male	T4aN2 [4/16]	Sigmoid Colon	y	y	y	y	metastatic at diagnosis	NA (metastatic to liver at diagnosis)							
440	2	77y	Male	T3N0 [0/19]	Ascending Colon	y	y	y	n	n	NA							
478	2	64y	Female	T3N0 [0/21]	transverse colon	y	y	y	n	n	NA							
480	2	61y	Male	T3N0 [0/19]	Sigmoid Colon	y	y	y	n	n	NA							
495	2	61y	Male	T3N0 [0/15]	Descending colon	y	y	y	n	n	NA							
130	10	59y	Male	T3N0 [0/12]	Rectum	y	y	y	n	n	NA							
206	9	63y	Male	T3N1c [0/11+ satellite nodule]	Rectum	y	y	y	n	n	NA							
486	4	59y	Male	T4bN0 [0/14]	Sigmoid Colon	y	y	y	n	n	NA							
405	13	62y	Male	T2N1 [2/13]	Rectum	y	y	y	n	y	Lung	y	n	y	y	Liver + Local	y	y
442	1	67y	Female	T3N0 [0/12]	Descending colon	y	y	n		n	NA							
410	1	60y	Female	T2N2 [5/21]	Rectum	y	y	n		n	NA							
76	11	55y	Male	T3N1 [3/21]	Sigmoid Colon					y	Liver	y	y	y	y	Liver	y	y
28	12	66y	Male	T3N2 [4/24]	Rectum					y	Lung and Bone	y	y					
120	23	64y	Female	T4aN2 [9/15]	Rectosigmoid Colon					y	Peritoneum and Lung	y	y					
214	13	66y	Male	T3N0 [0/12]	Transverse Colon					y	Lung, Liver and Peritoneum	y	y					
475	9	56y	Male	T3N1 [2/17]	Rectum					y	Lung	y	y					
30	1	47y	Female	T4aN2 [6/14]	Sigmoid Colon					y	Right and Left Ovary and Tube	y	y					
429	1	60y	Male	T2N0 [0/8]	Rectosigmoid Colon					y	Liver	y	y					
153	11	66y	Male	T3N1 [1/18]	Sigmoid Colon					y	Lung	y	n					
382	1	64y	Female	T3N2 [7/24]	Ascending Colon					y	Brain	y	n					

**Supplementary Table 1 (continued): Patient characteristics including availability of plasma samples and ctDNA detection at selected clinical events.**

Patient	Total number of plasma samples	Age	Gender	Stage of primary tumor (T Stage/N stage [no. of lymph nodes involved/no. of lymph nodes harvested])	Site of primary tumor	Pre-primary-surgery plasma available?	ctDNA detected in pre-primary-surgery plasma?	Post-operative (within 1 week) plasma available?	ctDNA detected in post-operative plasma?	Did patient develop recurrence?	Site of recurrence	Plasma samples prior to/at recurrence after primary surgery available?	ctDNA detected pre-recurrence?	Metastectomy / surgery for recurrent disease performed?	Was there recurrence post-metastectomy?	Site of recurrence post-metastectomy	Plasma samples available prior to/at recurrence after metastectomy available?	ctDNA detected pre-recurrence?	
143	3	59y	Female	T4aN0 [0/15]	Sigmoid Colon					y	Peritoneum , Small bowel and Omentum	n		y	y	Peritoneum	y	y	
187	3	72y	Male	T3N0 [0/33]	Sigmoid Colon					y	Anastomosis, para-aortic nodules and peritoneal disease	n		y	y	Peritoneum	y	y	
68	1	59y	Male	T2N0 [0/10]	Sigmoid Colon					y	Liver	n		y	y	Liver	y	n	
404	1	45y	Male	T3N0 [0/12]	Sigmoid Colon					y	Liver	n		y	y	Liver			
484	4	55y	Female	T3N0 [0/12]	Transverse Colon					y	Liver	n		y	n				
315	4	55y	Male	T4aN0 [0/39]	Rectum					y	lymph node + local recurrence	n		y	n				
398	3	73y	Male	T3N1 [3/21]	Rectum					y	lymph node + local recurrence	n		y	n				
31	2	64y	Male	T3N2 [8/22]	Rectum					y	Lung	n							
38	12	61y	Male	T4aN2 [7/14]	Caecum					y	Liver, LN	n							
51	9	64y	Male	T3N2 [12/21]	Rectosigmoid Colon					y	Lung	n							
260	20	71y	Male	T3N1 [2/17]	Sigmoid Colon					y	Lymph nodes	n							
29	4	45y	Male	T4aN1 [3/13]	Rectosigmoid Colon					y	Liver	n							
183	7	60y	Male	T3N2 [6/24]	Sigmoid Colon					y	Peritoneum and Spleen	n							
198	7	63y	Female	T4aN1 [1/29]	Ascending Colon					y	Ovary	n							
373	4	58y	Female	T3N2 [5/30]	Splenic Flexure					y	Ovary	n							
7	1	73y	Male	T3N1 [1/10]	Rectum					y	Lung	n							
81	6	51y	Male	T3N1 [2/16]	Rectum					y	Lung	n							
18	2	59y	Male	T3N1 [1/13]	Sigmoid Colon					y	Liver	n							
439	13	61y	Male	T4aN2b [10/12]	Rectosigmoid Colon					y	Lymph nodes	n							
61	1	53y	Male	T3N0 [0/15]	Rectum					y	Liver and Lung	n							
188	2	67y	Male	T3N1 [12/46]	Sigmoid Colon					y	Liver	n							
<i>Patient removed from further analysis due to mix-up</i>																			
47	4	54y	Male	T4bN0 [0/12]	Transverse Colon					y	Liver			y	n				