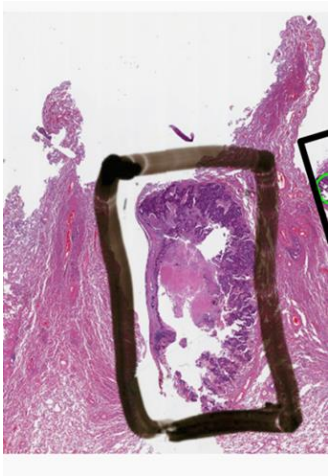
AvanSci Bio Technical Application:
Lung Tumor Tissue Sample Enrichment Using Mesodissection
Assayed by Sequenome Mutation Screening

This technical application presents the results of an experiment to investigate the correlation between dissection precision and tumor tissue enrichment.



In an earlier experiment, the black Sharpie marked area shown below was macrodissected using a hand held scalpel. DNA was isolated using the Qiagen QIAamp FFPE tissue spin column kit, which was assayed using the Sequenome OncoCarta system. The results showed that 31 +/- 5% of the recovered DNA carried a Gly12Cys missense mutation in the KRAS gene.

Goal of current experimental efforts was to determine if mesodissection at a higher resolution would increase the percent of tumor tissue recovered and thus the percent of the DNA sample that contained the G12C mutation.

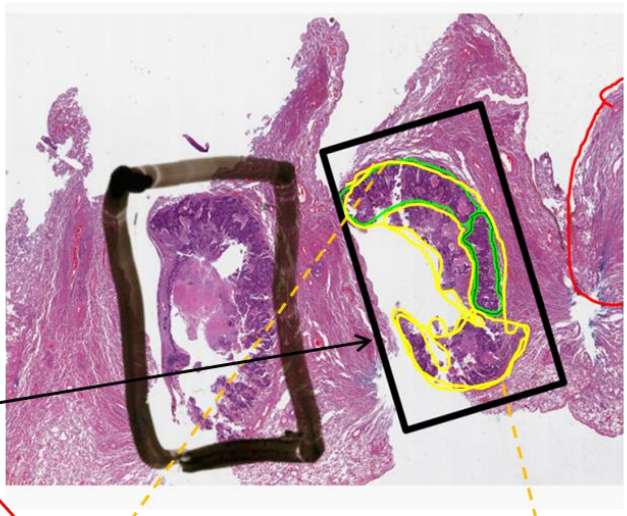
Dissected Areas

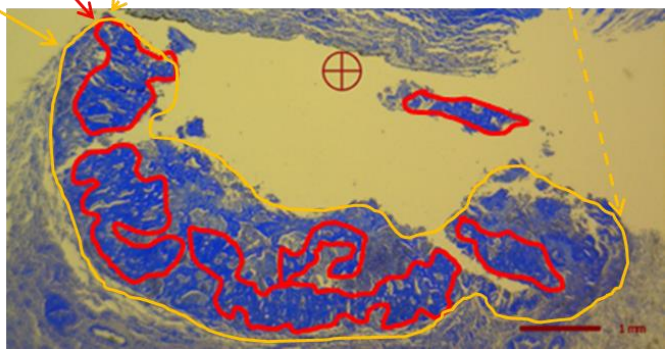
The following areas were dissected from seven serial section:

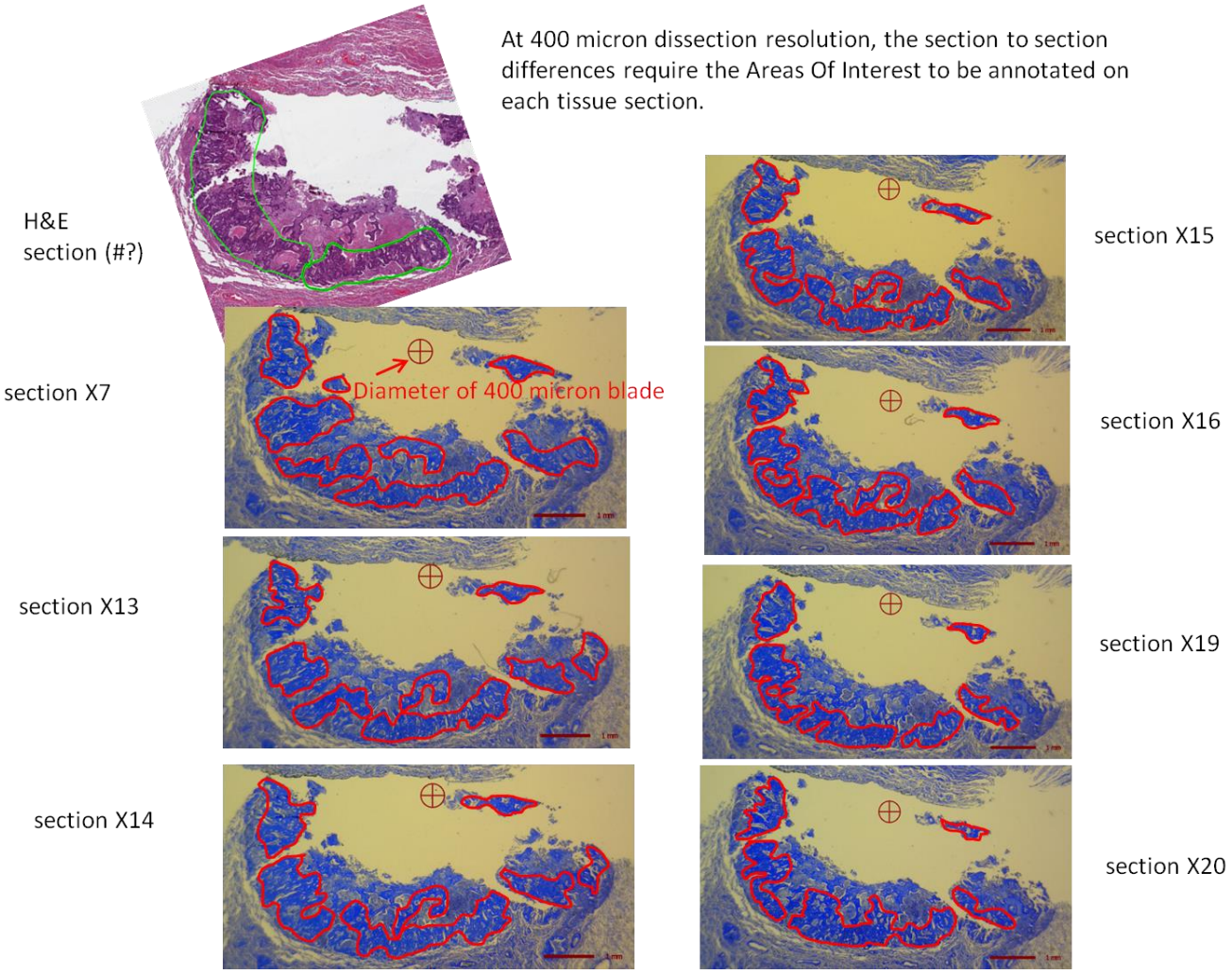
A1 = red areas (highest conc. tumor)

Remainder A1 = orange, after red removed (some tumor)

Outer A1 = black, after red and yellow removed (surrounding tissue that would have also been recovered in manual macrodissection)







Crude Lysate Preparation and Sequenome Biochemistry

In the previously mentioned study, we had to dissect at least 15 mm^2 total tissue in order to obtain a sufficient quantity and concentration of DNA from the Qiagen kit, as we thought the Sequenome chemistry requires at least 10 ng DNA in 2 ul. This requirement would have made it difficult to recover a sufficient quantity of tissue from higher resolution dissections. Fortunately we discovered it is not necessary to use purified DNA as the input, in fact, crude preps appear to work at least as well if not better that purified DNA.

Crude prep:

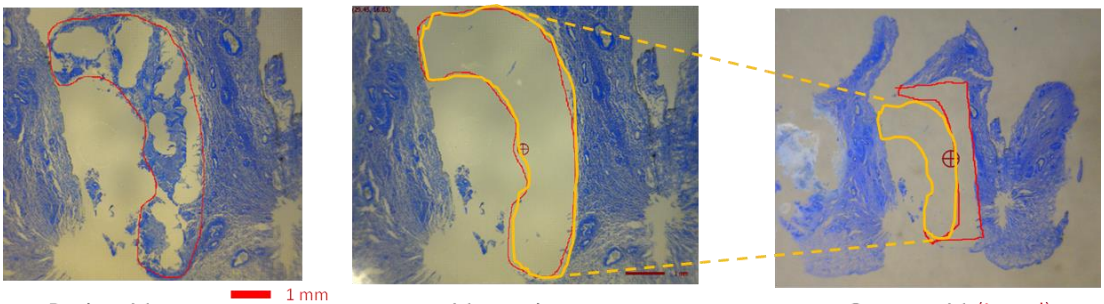
- Mesodissect tissue in 2 mM Tris, pH 8.0, 0.2 mM EDTA, 0.1% Tween 20
- Centrifuge tube containing recovered tissue, remove supernatant to leave 18 ul
- Add 2 ul TET containing 5 ug fresh Prot K and 40 ul mineral oil
- Incubate on Thor 92°C 1 hour 1200 rpm, 60°C 1 hour 800 rpm, then 92°C 15 min 1200 rpm
    - Quick spin, remove mineral oil with wicking strip

The above protocol allows us to dissect just a few square millimeters and obtain sufficient template for the Sequenome chemistry.

Sequenome Chemistry

Step 1: PCR – 2 ul sample template in 5 ul total reaction volume
Step 2: SAP – add 2 ul Shrimp Alkaline Phosphatase cocktail to PCR products
Step 3: Extension using dideoxy nucleotides – add 2 ul extension cocktail

Percent Mutation Determined using Sequenome System



Region A1 A1 remain Outer to A1 (in red)

Sample	Slide	Region	Area dissected (mm²)	Area dissected average and Std Dev (mm²)	Total DNA recovered (ng) by PicoGreen	Total DNA recovered average and Std Dev (ng) by PicoGreen	qPCR Ct	Mutant allele frequency (all KRAS p.G12C_c.34 G>T)	Mutant allele frequency Average / Std Dev	Sequenome Z-score	Sequenome Confidence
2	X7	A1	5.6	**4.4**	76	**84**	32.1	0.631	**0.61**	10	1.High
3	X13	A1	4.7	0.7	63	25	35.0	0.615	0.01	10	1.High
4	X14	A1	4.8		85		26.8	0.614		10	1.High
5	X15	A1	4.2		54		27.6	0.596		10	1.High
6	X16	A1	4.6		129		25.9	0.62		10	1.High
7	X19	A1	3.4		81		26.4	0.61		10	1.High
8	X20	A1	3.7		101		26.6	0.588		10	1.High
10	X7	Remain- A1	6.1	**5.8**	62	**83**	32.8	0.551	**0.49**	10	1.High
11	X13	Remain- A1	6	0.5	71	16	27.8	0.48	0.03	10	1.High
12	X14	Remain- A1	5.8		89		28.6	0.478		10	1.High
13	X15	Remain- A1	5.1		97		27.6	0.507		10	1.High
14	X16	Remain- A1	5.3		64		27.2	0.475		10	1.High
15	X19	Remain- A1	5.5		97		27.1	0.468		10	1.High
16	X20	Remain- A1	6.5		100		27.7	0.472		10	1.High
18	X7	Outer-A1	11.4	**10.8**	50	**61**	32.0	-	**0.06**	-	-
19	X13	Outer-A1	10	1.2	51	10	28.0	0.049	0.01	5.214	2.Medium
20	X14	Outer-A1	10.7		70		27.3	-		-	-
21	X15	Outer-A1	12.5		61		27.7	0.059		5.51	2.Medium
22	X16	Outer-A1	9.8		54		26.4	0.062		4.746	2.Medium
23	X19	Outer-A1	9.2		62		26.9	-		-	-
24	X20	Outer-A1	11.9		78		27.3	-		-	-

These results demonstrate that mesodissection at higher resolutions can be used to significantly increase the percentage of mutant DNA in a tumor sample.

Region	% tumor
A1	61%
A1 remain	49 %
Outer to A1	6%

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