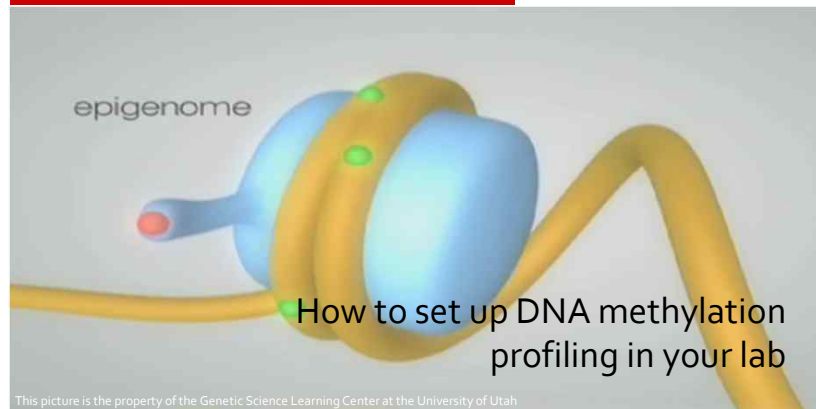
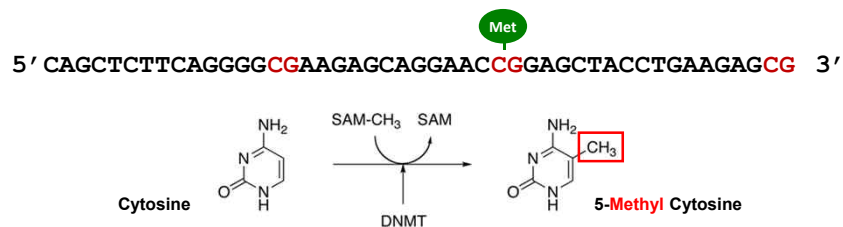


DNA 메틸레이션의 소개와 미래



Forensic application of DNA methylation variation

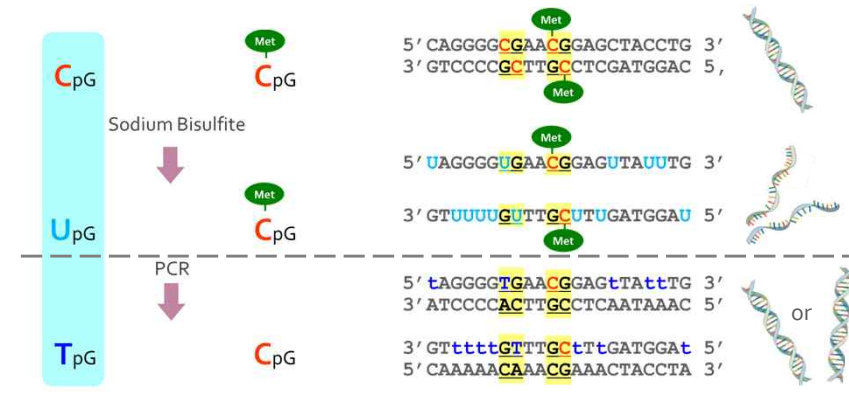
1. Tissue-specific DNA methylation changes
 - Body fluid identification for a clue on activity level of the evidence
2. Age-associated DNA methylation changes
 - Age prediction for the unknown suspect



DNA methylation and bisulfite modification

DNA methylation detection

- Bisulfite treatment converts non-methylated C to U, which changes into T by PCR



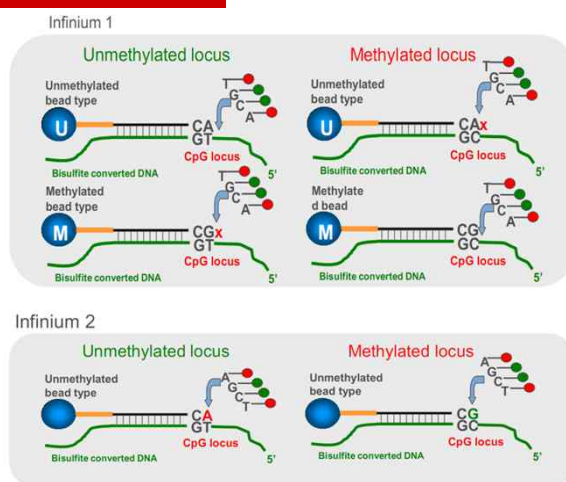
Illumina methylation beadchip array chemistry

Array

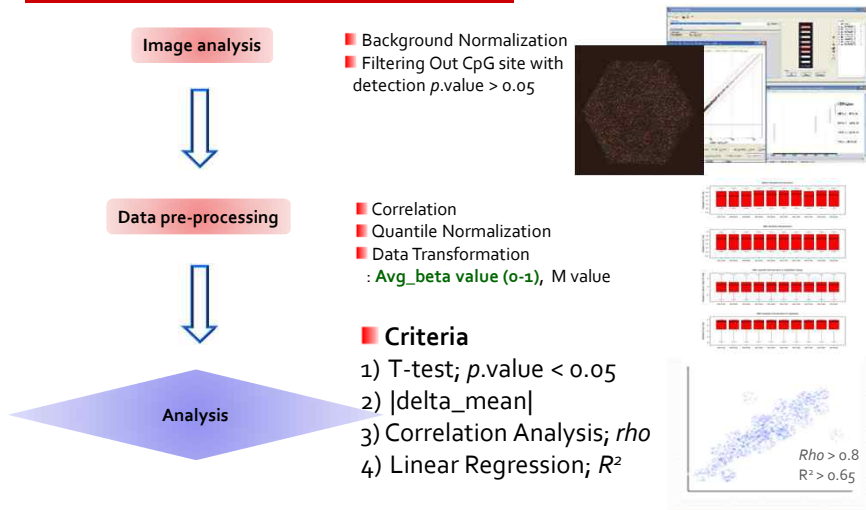
- 27K
- 450K
- 850K: Epic array



$$\text{Beta}_i = \frac{\max(y_{i,\text{methyl}} - 0)}{\max(y_{i,\text{unmethyl}} - 0) + \max(y_{i,\text{methyl}} - 0) + \alpha}$$



BeadChip array data analysis



DNA methylation and PCR primer design

PCR primer design

- Targets bisulfite-converted DNA

```
GGGAGTGAATGGAATTCGAGACCACCTTCGCTAACAAATCGCAATTATGAACCGAAAGACATGTCAGGT
ATTAGCAATTTTTTCCTTAAAAAATAAAAAAATTTCTGGGACTCGCGGGACACCCAGCTGGCGAC
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CTCTGCGCCCCCGGGCTCGCGTGCCTCAGGGCCGCTCAGGCGGGCGGGCTCGCGGGCGGGC
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CTCTGCGCCCCYGGGCTYGGTYGTGAGGGYGYCTCAGYGGGGYGGCTCYGGYGGGGYGGY
GGYGGCAGYGGCTYGGYGGYGGYGGCAGCAGYGGCAGYGGYGGCAGYGGYGGCAGYGGGCTCTGCG
```

Genomic DNA sequence



Converted DNA sequence

DNA methylation and PCR primer design

PCR primer design

- MethPrimer program (<http://www.urogene.org/methprimer/>)
- PyroMark Assay Design program (Qiagen)

MethPrimer

Genomic DNA sequence

Product Size: Min: 100, Opt: 200, Max: 300
 Primer Tm: Min: 50, Opt: 55, Max: 60
 Primer Size: Min: 20, Opt: 25, Max: 30
 Product CpGs: 1, Primer Poly X: 5
 Primer non-CpG 'C's: 4, Primer Poly T: 8

Primer non-CpG 'C's: The minimum number of non-CpG 'C's in a primer. This is important for discriminating between the bisulfite-modified DNA and unmodified or incompletely modified DNA.

DNA methylation and PCR primer design

PCR primer design

- MethPrimer program (<http://www.urogene.org/methprimer/>)

MethPrimer result

Please cite MethPrimer: Li LC and Dahlby R. *MethPrimer: designing primers for methylation PCR*. Bioinformatics. 2002 Nov;18(11):1427-31. PMID: 12425312

Sequence Name: 475

CGI that is used: 141, 462, 524, 582, 588, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000

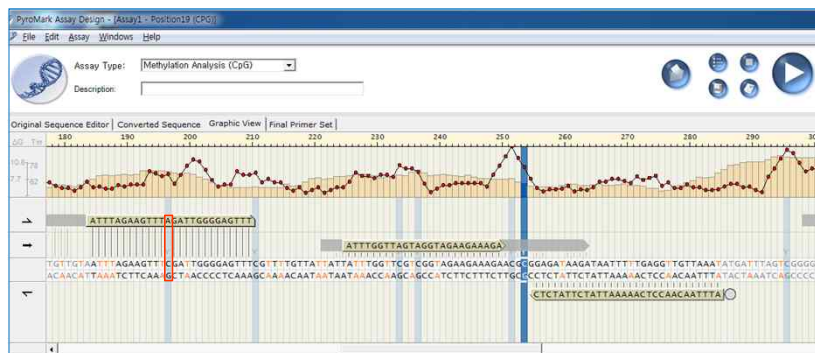
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AGGtTtTtTAAACAtGAtTtAGtYGGGGtYGGYgTtYAGGGGGTgTtTtTGGtTtYGGGtYGGGt
tTtTgTtYgTtTtYGGGtTtYGGTgYGTtAGGGYgYGTtAGGGGGtGGTtTtYGGYGGYGGY
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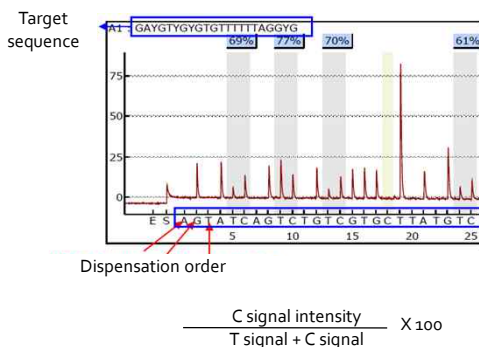
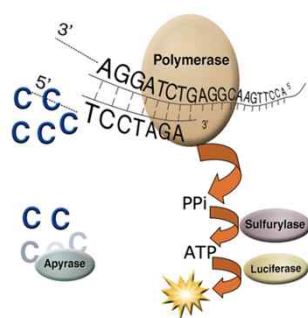
DNA methylation and PCR primer design

PCR primer design

- PyroMark Assay Design program (Qiagen) allows mismatch at primer binding site
- YG → AG (forward primer) & CR → CC (reverse primer)

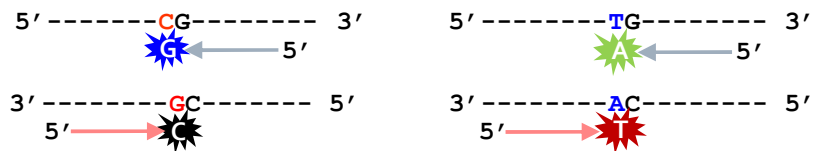


Targeted bisulfite sequencing: pyrosequencing



Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
cp6-1	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-2	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-3	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-4	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-5	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-6	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-7	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-8	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-9	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
cp6-10	T	C	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T

Targeted bisulfite sequencing: methylation SNaPshot



FORWARD



$$\% \text{methyl} = \frac{\text{C intensity}}{(\text{C} + \text{T}) \text{ intensity}} \times 100$$

REVERSE



$$\% \text{methyl} = \frac{\text{G intensity}}{(\text{G} + \text{A}) \text{ intensity}} \times 100$$

Development of an assay for body fluid typing

1. Identification of body fluid-specific CpGs

- Genome-wide screening with Illumina's beadchip array or searching [NCBI GEO database](#)
- 12 semen, 12 blood, 12 saliva, 6 vaginal fluid, 9 menstrual blood samples
- Selection criteria: CpGs having methylation signal only in a target body fluid with completely non-methylation in other body fluids
- Candidate marker validation with targeted bisulfite sequencing, such as pyrosequencing and methylation SNaPshot

2. Design of a multiplex assay

- Methylation SNaPshot
- Semen (2), blood (2), vaginal fluid(2), saliva (1), menstrual blood (2)

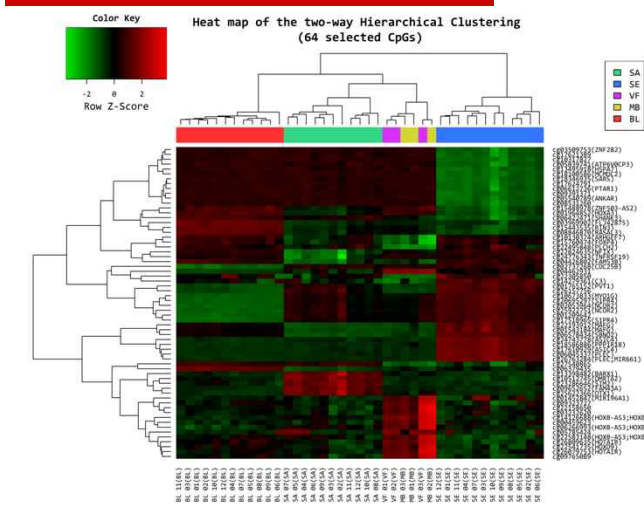
3. Validation of body fluid-specific CpGs

- Independent samples used for marker identification
- Set-up interpretational guidelines

Identification of body fluid-specific CpGs

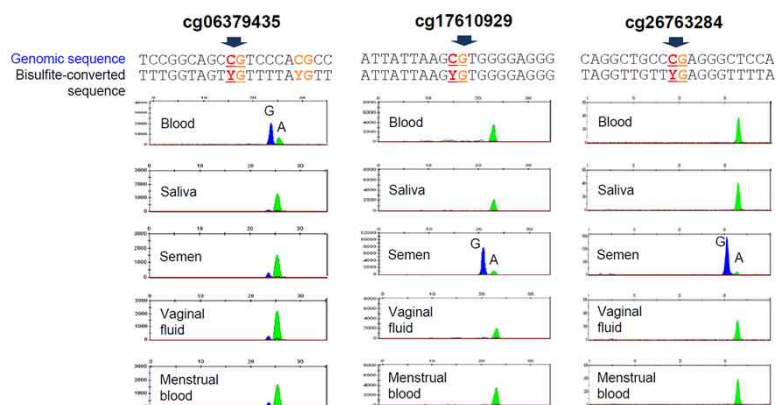
TargetID	(BL,SA,VF,MB) Mean	SE Mean	SE (BL,SA,VF,MB) delta_mean	SE (BL,SA,VF,MB) sd	20M-05-SA	20M-06-SA
cg00000905	0.10390212	0.724695401	0.62079328 hyper	0.022301318	0.046387535	0.176771347
cg17610929	0.025744487	0.924979074	0.899234587 hyper	0.012295329	0.057049908	0.056512882
cg26763284	0.020395829	0.899585963	0.879190133 hyper	0.004682332	0.073013894	0.026326755
cg00001099	0.728387302	0.201444779	-0.526942512 hypo	0.083000945	0.091289046	0.776519405
cg00001364	0.834765427	0.327240531	-0.507524896 hypo	0.024344463	0.096325351	0.782998045
cg00001854	0.886799913	0.335297462	-0.515024511 hypo	0.024161934	0.088355211	0.871253589

Identification of body fluid-specific CpGs

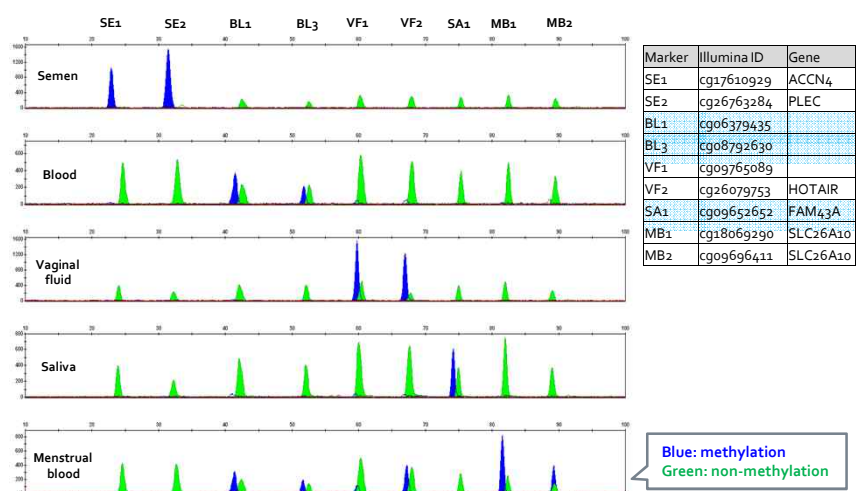


Candidate marker validation

Targeted bisulfite sequencing using methylation SNaPshot



A SNaPshot multiplex for body fluid identification



Lee HY et al., Forensic Sci Int Genet (2016)

SNaPshot multiplexes for body fluid identification

forensic.yonsei.ac.kr/protocols.html

The image shows two overlapping windows. The background window is a web browser displaying the website forensic.yonsei.ac.kr/protocols.html. The foreground window is a Microsoft PowerPoint slide titled "Multiplex SNaPshot for Body Fluid Identification".

Web Page Content:

- DNA methylation analysis**
 - Body fluid identification: SNaPshot multiplex for 7 CpGs (Semen, blood, vaginal fluid); SNaPshot multiplex for 9 CpGs (Semen, blood, vaginal fluid)
 - Age prediction from saliva: SNaPshot multiplex; Age calculator
 - Age prediction from semen: SNaPshot multiplex; Age calculator
- ABO genotyping**
 - Rapid direct PCR for ABO blood typing
 - ABO blood typing for degraded DNA
- PCR product purification**
 - Using the QIAquick® PCR Purification Kit
 - Using the ExoSAP-IT® for PCR Product Cleanup

PowerPoint Slide Content:

Multiplex PCR

Reagents Needed:

- 4 X Primer Mix
- AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
- QIAGEN® 10 X Buffer (Promega, Madison, WI)

4 X Primer Mix for Multiplex PCR:

Target ID	Sequence (5'-3')	Conc. (µM)	Amplification rate (%)	
S11	gg17618628	TTG TTG AGA TGG TTT GAA TTA TTA AG	2.4	174
	ATA ACT TGC CTT ATC AAC AGC AGC	2.4		
S22	cg26192324-1366	TGA TTT ATA ATT ATT AGG GAG GGA AAT AG	0.9	100
	CCG AAA AGA AGC AAT TCC GAA CT	1.8		
S13	cg46176428	TTT ATT GGG GAA TTT TTA TTG GGT AG	0.9	187
	AAA ATA GAA CTT ACT ACT AAA CAC C	0.9		
S13	cg46176326	TGT TTT AAG AAG AAA AAG AA	2.4	220
	GGA GCT GAA TCC AAA GTA ACT AGA	2.4		
V11	cg26192329-2216	TTG GTA GTT TTT GGA TTT TGG AG	2.4	137
	AAA GTC GAA AGC AGC GAA AC	0.9		
V12	cg46176123-76	TTT TGT GAG TGT GAG AGA TTT TTA AGA	1.8	176
	AAA AGC TGC AAA AGA AAA CCT GTA	1.8		
S41	cg46192323-24	GGG GAT TGG TGT TAA GGT	0.9	143
	GGA TTT GGG GGT TCC TAA AA	0.9		
M8	gg17618229	GTT TTT TAG GGG AGA AGA GTA GGA AT	0.9	160
	ATA ATA AAA GGA GGA AAA CAG	0.9		

PCR Mixtures:

PCR Component	Vol. (µl)	Final Conc.
10X	10	1.0
4 X Primer Mix	1	0.1
AmpliTaq Gold (2.5U)	0.7 (2.5 U)	0.125
Bisulfite converted DNA	1	100
Total	20	

Thermal Cycling:

Step	Time
95°C	15 minutes
95°C	30 seconds
55°C	30 seconds
72°C	30 seconds
72°C	7 minutes
4°C	hold

*Please be aware that you should use more than 10 PCR cycles of bisulfite converted DNA when using Sigma's InSight® PCR modification kit because, in our experience, it may cause PCR failure.

Introducing DNA methylation profiling in your lab

1. Refer to previous articles e.g.:

- Jung SE et al. A collaborative exercise on DNA methylation-based body fluid typing. *Electrophoresis*. 2016;21:2759-2766.
- Lee HY et al. DNA methylation profiling for a confirmatory test for blood, saliva, semen, vaginal fluid and menstrual blood. *Forensic Sci Int Genet*. 2016;24:75-82.

2. Prepare reagents and kits such as:

- Bisulfite modification Kit:** Imprint® DNA Modification Kit (Sigma-Aldrich Inc. Cat. No. MOD50) or EpiTect Fast Bisulfite Conversion Kit (Qiagen, Cat. No. 59104)
- Primers (bisulfite converted DNA-specific):** multiplex PCR and multiplex SBE
- PCR product purification: ExoSAP-IT® (USB, Cat. No. 97067-402)
- SBE kit: SNaPshot™ Kit (Applied Biosystems, Cat. No. 4323159)
- Post SBE: SAP or CIP
- CE: Hi-Di Formamide, GeneScan™ 120 LIZ™ Size Standard, Matrix Standard Set DS-02, Run Module GS STR POP4 E5, POP4 (Applied Biosystems)

DNA methylation and bisulfite modification

Each modification kit has a different capacity in the optimal or minimum DNA quantity that it can deal with

- Procedure takes 2 to 12 hours depending on the kits
- Most kits provide less than 5 hours of processing time



Kits and manufacturers	Recommended Input	Minimum Input
Imprint® DNA modification kit (Sigma)	50 pg-200 ng	> 50 pg
EpiTect Bisulfite kit (Qiagen)	1 ng-1 ug	> 1 ng
EZ DNA Methylation™ kit (Zymo Research)	0.5 ng-2 ug	> 500 pg
EZ DNA Methylation-Direct™ kit	DNA, cells, tissue	> 50 pg

The above characters are from Kakao Friends own by Kakao corp.

Introducing DNA methylation profiling in your lab

3. Bisulfite conversion of genomic DNA

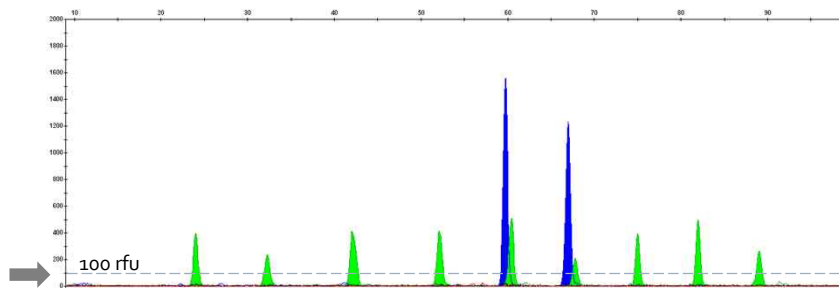
- For various body fluids: semen, blood, saliva, vaginal fluid and menstrual blood
- Extract and quantify DNA
- Select optimal input DNA amount (> 50 ng) and perform bisulfite conversion
- Bisulfite converted DNA is present as a single strand
- The converted DNA is stable for one day at room temperature, one week at 4 °C, and two to four months at -20 °C
- Recommend on storing your converted DNA below -70 °C whenever possible

4. Perform multiplex PCR followed by multiplex SBE

- Determine optimal amount of bisulfite converted DNA (> 10 ng)
- Perform multiplex PCR followed by multiplex SBE according to the protocol
- Analyze on CE

Interpretation

- Determine detection threshold for methylation, non-methylation signal, e.g. 100 rfu
- Put peak height values into the excel sheet and calculate methylation percentages
- Determine analytical threshold for methylation percentage at each marker, e.g. 10%
- Compare with reference DNA methylation profiles to determine body fluid type



Interpretation

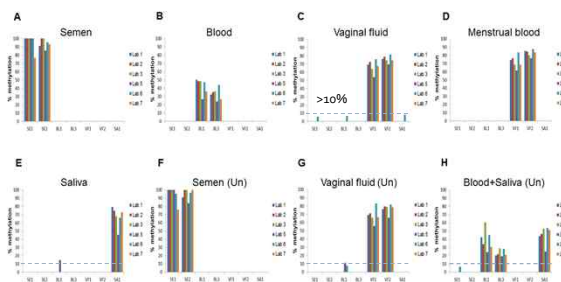
- Put peak height values into the excel sheet and calculate methylation percentages : $100 \cdot \frac{\text{nucleotide G}}{\text{nucleotide G+A intensities}}$
- Determine analytical threshold for methylation percentage at each marker, e.g. 10%
- Compare with reference DNA methylation profiles to determine body fluid type

Dye/Sample Peak	Sample File Name (Size)	Height	Area	Data Point
G,8	BF_SE_BOM1315 (149.76)	133	1047	2554
B,1	BF_VF_20P1502: 59.78	1658	12680	1326
B,2	BF_VF_20P1502: 66.97	1232	10123	1422
G,1	BF_VF_20P1502: 24.01	394	4163	840
G,2	BF_VF_20P1502: 32.25	238	2167	947
G,3	BF_VF_20P1502: 42.01	412	4847	1074
G,4	BF_VF_20P1502: 52.99	418	4352	1217
G,5	BF_VF_20P1502: 60.43	509	4777	1335
G,6	BF_VF_20P1502: 67.74	212	1866	1432

Sample	Body fluid	B	G	METHYL (%)	A	G	METHYL (%)	C	G	METHYL (%)	T	G	METHYL (%)	M	G	METHYL (%)	Q	G	METHYL (%)	S	G	METHYL (%)	W	G	METHYL (%)	Y	G	METHYL (%)	Meig16285200	Meig0696431			
10	20M1315	Semen	1262	0	1887	1993	0	100	158	0	395	0	0	683	0	0	704	0	0	793	0	0	813	0	0	814	0	0	457	0	0		
11	40M1306	Semen	1190	0	130	189	0	100	154	0	154	0	0	627	0	0	824	0	0	845	0	0	844	0	0	1112	0	0	544	0	0		
19	80M1313	Semen	1129	0	100	320	172	89.03	0	345	0	0	699	0	0	817	0	0	899	0	0	876	0	0	1117	0	0	569	0	0			
12	20M1308	Blood	0	382	0	0	495	0	0	147	192	64.64	278	220	0	132	0	0	688	0	0	439	0	0	441	0	0	244	0	0			
13	60M1306	Blood	0	424	0	0	180	0	0	348	337	49.43	296	311	48.84	0	580	0	0	672	0	0	747	0	0	549	0	0	339	0	0		
14	40M1307	Blood	0	493	0	0	312	0	0	170	234	63.64	218	229	48.71	0	560	0	0	732	0	0	367	0	0	489	0	0	129	0	0		
15	20P15053	Vaginal fluid	0	193	0	0	143	0	0	326	306	48.78	143	111	44.11	111	117	51.38	0	325	0	0	411	0	0	134	0	0	134	0	0		
16	20P15023	Vaginal fluid	0	24	0	0	161	0	0	476	418	48.74	162	162	50.00	162	162	50.00	162	162	50.00	162	162	50.00	162	162	50.00	162	162	50.00	162	162	50.00
18	30M1302	Saliva	0	654	0	0	510	0	0	118	556	17.28	0	593	0	0	341	793	45.39	582	742	44.28	0	582	0	0	1127	0	0	545	0	0	
17	20M1302	Saliva	0	338	0	0	419	0	0	249	0	0	179	0	0	271	0	0	340	0	0	128	249	66.39	0	347	0	0	215	0	0		
17	20M1308	Saliva	0	483	0	0	516	0	0	119	709	14.88	0	585	0	0	361	0	0	836	0	0	194	712	67.41	0	848	0	0	427	0	0	
18	30M1301	Saliva	0	473	0	0	754	0	0	0	687	0	0	395	0	0	0	0	0	1122	0	0	893	110	76.29	0	893	0	0	302	0	0	
18	20P15023	Menstrual Blood	0	347	0	0	409	0	0	151	0	0	144	0	0	411	327	44.08	303	339	51.44	0	311	0	0	278	0	0	197	0	0		
19	20P15029	Menstrual Blood	0	464	0	0	444	0	0	471	379	34.34	239	211	48.38	102	434	34.09	533	454	48.39	0	454	0	0	1231	342	76.29	605	140	77.87		
19	20P15029	Menstrual Blood	0	618	0	0	712	0	0	148	324	19.36	112	213	29.34	123	612	22.89	187	684	34.42	0	603	0	0	468	278	49.88	195	141	47.48		
20	20P15029	Menstrual Blood	0	737	0	0	737	0	0	0	499	0	0	225	0	0	1081	404	71.14	411	473	52.98	0	373	0	0	327	0	0	119	0	0	

Interpretation

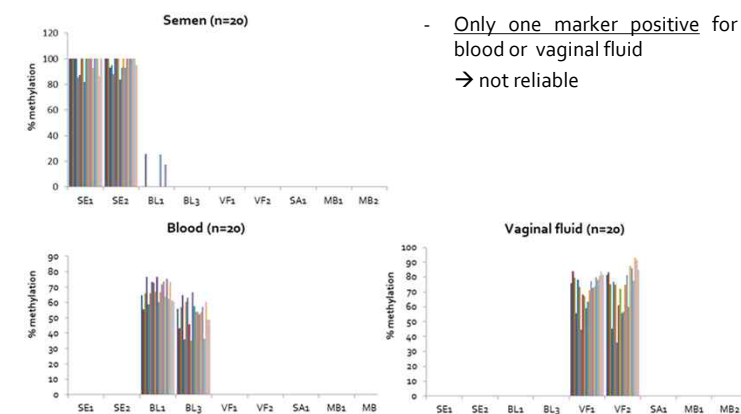
- Determine minimum threshold for positive methylation signal, e.g. 10%
- Compare with reference DNA methylation profiles to determine body fluid type



DNA methylation profiling results from 6 laboratories using bisulfite converted DNA from the same batch: a few unexpected signals with <10% methylation

Interpretation

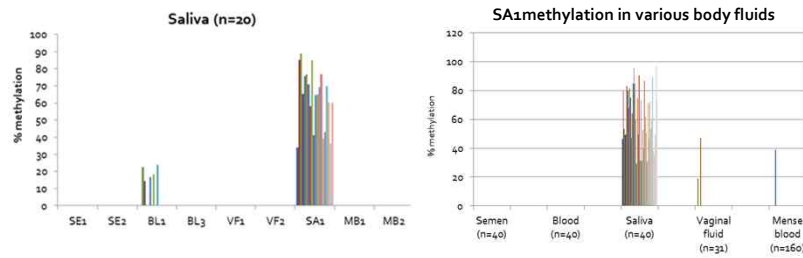
- Compare with reference DNA methylation profiles to determine body fluid type
- Two markers positive for semen, blood or vaginal fluid → observed



- Only one marker positive for semen, blood or vaginal fluid → not reliable

Interpretation

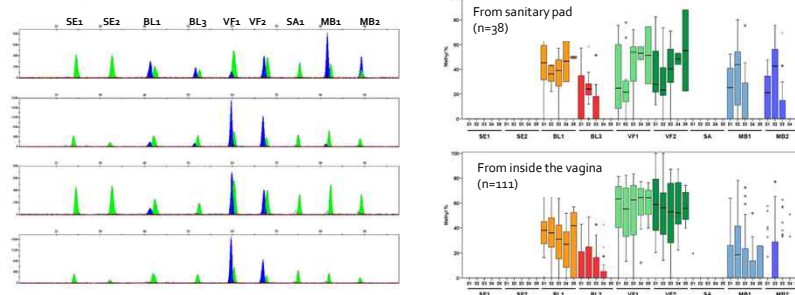
- Compare with reference DNA methylation profiles to determine body fluid type
 - Only one marker positive for saliva → observed
 - One marker positive for saliva but with additional positive signal → need more careful considerations



Interpretation

- Compare with reference DNA methylation profiles to determine body fluid type
 - Menstrual blood samples show different methylation profiles depending on the menstrual cycle and the sampling methods
 - Both of the MB1 and MB2 markers positive → observed
 - One of the MB1 and MB2 markers, BL1 and BL3 markers positive → observed

Various profiles of menstrual bloods



Introducing DNA methylation profiling in your lab

5. Analyze more samples

- More body fluid samples (validation and establishment of interpretational guidelines)
- Lower amount (< 50 ng of g DNA and < 10 ng of bisulfite converted DNA)
- Mixed samples (Joined interpretation with STR and DNA methylation profiles)

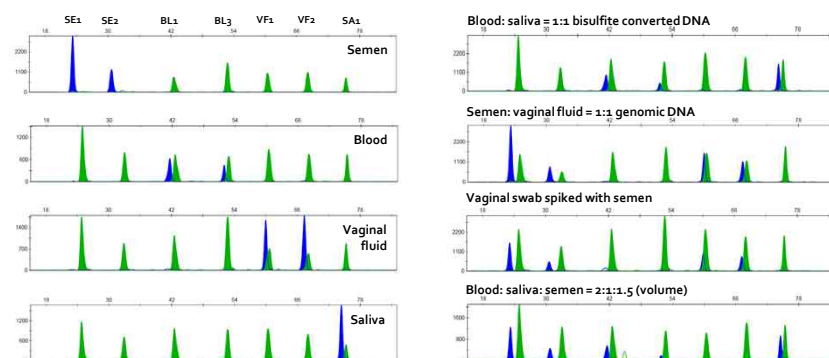
6. Develop reporting guidelines or formats

7. Establish Standard Operating Procedures

8. Inform your clients

More complex samples

e.g. Mixed samples test in a lab of collaborative exercise (Electrophoresis, 2016)

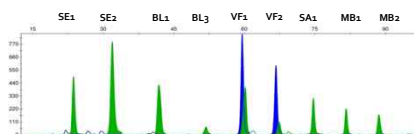


Examples of NFS DNA methylation profiling

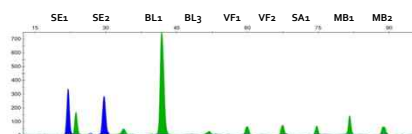
Case 1: Rape

- Vaginal swab from the victim was positive for semen in ACP test and the A-STR profile of the perpetrator was observed
- From a penile swab, STR profile of the victim was obtained
- A stain from the belly of the victim was negative for semen and saliva, but showed STR profile of the perpetrator

A penile swab (victim's STR profile): 0.427 ng/ul



A stain from the belly (perpetrator's STR profile): 0.071 ng/ul

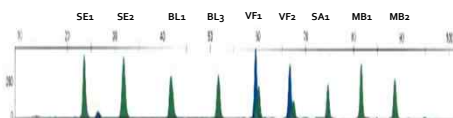


Examples of NFS DNA methylation profiling

Case 2: Digital penetration on the public transportation

- Vaginal swab was negative for semen and showed only STR profile of the victim
- A swab wiped the fingers of the alleged perpetrator showed STR profile of the victim

A finger swab from the alleged perpetrator (victim's STR profile) : 0.051 ng/ul

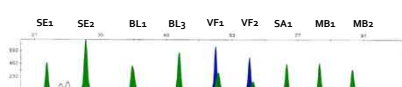


Examples of NFS DNA methylation profiling

Case 3: Murder in 2004

- A female karaoke worker was found to be dead
- A knife and an unknown stain were found near the victim
- From the test in 2004
 - The knife blade was negative for blood, but showed STR profile of the victim
 - The stain was positive for saliva, and showed unknown male's STR profile

Knife blade (victim's STR profile): 6.7 ng/ul



Unknown stain: 10.7 ng/ul



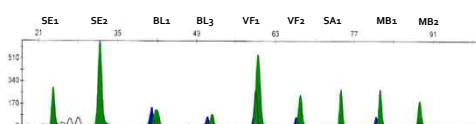
- The victim's body was damaged by cutting the left breast part and vagina with the knife
- DNA methylation profile was consistent with the scenario of the case
- DNA methylation profiling was successfully done with 13 year-old DNA samples

Examples of NFS DNA methylation profiling

Case 4: Murder at the scene of a fire in 2004

- A fire broke out in a room of a cafe at night
- A woman's dead body was found under a blanket with a stab wound to the neck
- A tissue paper with red stains was found at the scene
- From the test in 2004
 - The tissue paper was positive for blood and negative for semen
 - STR profile from the victim was detected on the tissue paper

Tissue paper (victim's STR profile): 2.5 ng/ul



Development of an assay for age prediction

A model-based prediction analysis typically consists of three steps

- Discovery step
- Model-building step
- Model-validation step
- The samples in each step must be independent

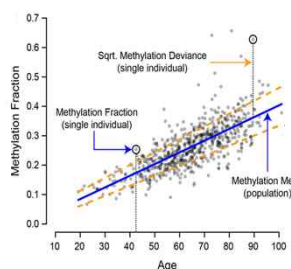
1. Identification of age-associated CpGs

- Regression analysis between DNA methylation and age

2. Model training and validation

- Determination of analysis platform (e.g. array, pyrosequencing, methylation SNaPshot)
- Model training: multivariate regression, random forest etc.

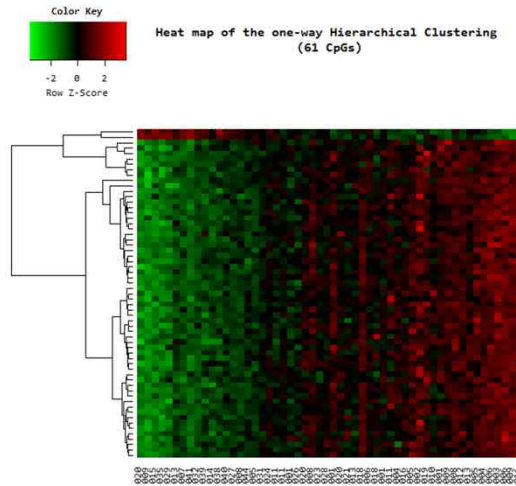
Identification of age-associated CpGs



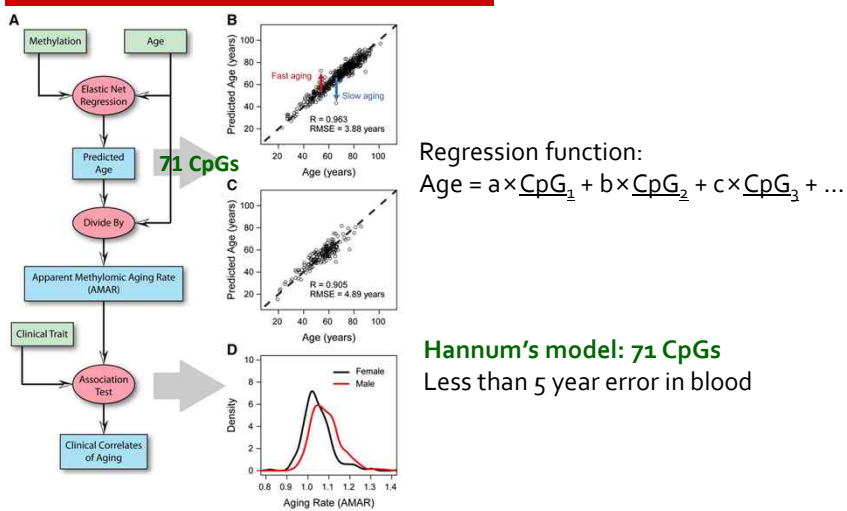
$R^2 > 0.65$

chr	pos	beta	beta.se	r	r.se	p	beta	beta.se	r	r.se	p
1	100000000	0.00012345	0.00001234	0.1234	0.001234	0.001234	0.00012345	0.00001234	0.1234	0.001234	0.001234
2	200000000	0.00023456	0.00002345	0.2345	0.002345	0.002345	0.00023456	0.00002345	0.2345	0.002345	0.002345
3	300000000	0.00034567	0.00003456	0.3456	0.003456	0.003456	0.00034567	0.00003456	0.3456	0.003456	0.003456
4	400000000	0.00045678	0.00004567	0.4567	0.004567	0.004567	0.00045678	0.00004567	0.4567	0.004567	0.004567
5	500000000	0.00056789	0.00005678	0.5678	0.005678	0.005678	0.00056789	0.00005678	0.5678	0.005678	0.005678
6	600000000	0.00067890	0.00006789	0.6789	0.006789	0.006789	0.00067890	0.00006789	0.6789	0.006789	0.006789
7	700000000	0.00078901	0.00007890	0.7890	0.007890	0.007890	0.00078901	0.00007890	0.7890	0.007890	0.007890
8	800000000	0.00089012	0.00008901	0.8901	0.008901	0.008901	0.00089012	0.00008901	0.8901	0.008901	0.008901
9	900000000	0.00090123	0.00009012	0.9012	0.009012	0.009012	0.00090123	0.00009012	0.9012	0.009012	0.009012
10	1000000000	0.00101234	0.00010123	1.0123	0.010123	0.010123	0.00101234	0.00010123	1.0123	0.010123	0.010123

Identification of age-associated CpGs



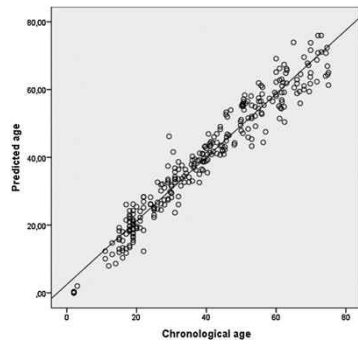
Age prediction model construction



Hannum et al. Mol Cell (2013)

Age prediction in blood

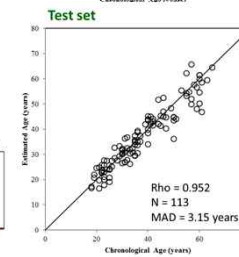
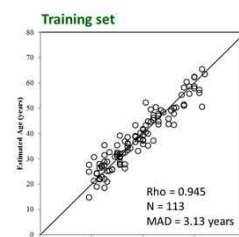
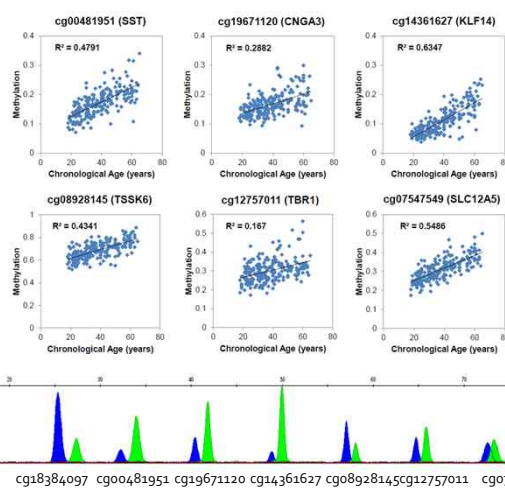
5 CpGs in the genes ELOVL2, C1orf132, TRIM59, KLF14 and FHL2



MAD (Mean Absolute Deviation) from chronological age = 3.9 years

Zbieć-Piekarska et al. Forensic Sci Int Genet (2015)

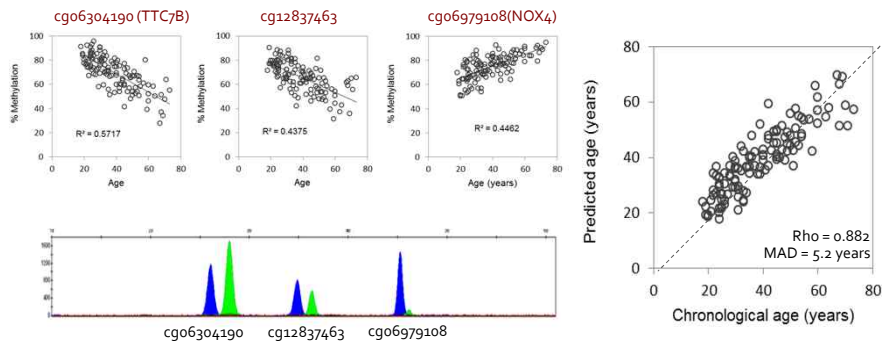
Age prediction in saliva



Hong et al. Forensic Sci Int Genet (2017)

Age prediction in semen

- Age correlation of the 3 CpGs and predicted versus chronological ages of 125 semen samples

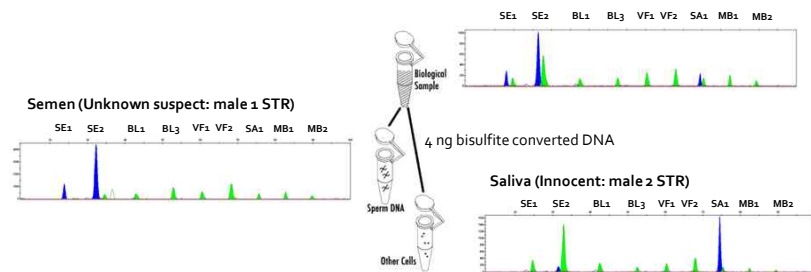


Lee et al. Forensic Sci Int Genet (2015)

Examples of NFS DNA methylation profiling

Case 5: Indecent exposure with no suspect

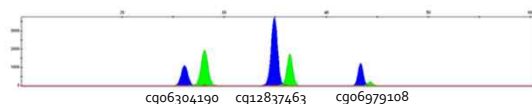
- A stain preliminary positive for semen and saliva
- Two men's mixed STR profile
- Differential extraction and body fluid ID test
 - Supernatant: STR profile of a man
 - Precipitate: STR profile of another unknown man → add. age prediction



Examples of NFS DNA methylation profiling

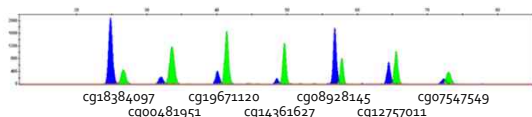


Semen age prediction (ppt)



➔ 61 years old

Saliva age prediction (supernatant)



➔ 30 years old

Future forensic DNA typing

To Get More Information on Suspects and Crime Scenes

Research Networks

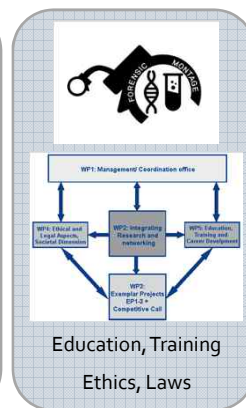
Age prediction

Pigmentation

Biogeographical Ancestry

Body fluid identification

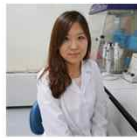
DNA transfer



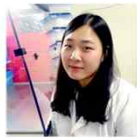
Acknowledgement



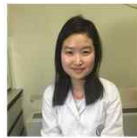
Kyoung-Jin Shin



Sang-Eun Jung



Eun Hee Lee



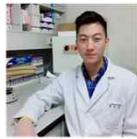
Sae Rom Hong



Bomin Kim



Mi Hyeon Moon



SeungMin Lim



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