## Assigning forensic body fluids to DNA donors in mixed samples by targeted RNA/DNA deep sequencing of coding region SNPs



Jack Ballantyne, University of Central Florida (UCF), USA

Erin Hanson, UCF, USA

Sabrina Ingold, Guro Dorum, Cordula Haas, University of Zurich, Switzerland Rob Lagacé, Thermo Fisher Scientific, USA

# The Problem: can cell types and DNA donors be associated?

- ID of body fluids can be crucial to an investigation
  - Presumptive, biochemical, RNA, epigenetic, protein
  - Need specific body fluid markers and simultaneous analysis
- Need to associate body fluid with a donor DNA profile (needed to evaluate source versus sub-source level propositions)
- "If there is uncertainty about the association of the body fluid with the DNA profile, then the strength of the evidence is always reduced" (Gill, 2014)
- "Association fallacy" (Gill, 2014)
- "discourage" associating cell types and donors from peak heights when performing RNA and DNA profiling (Sijen et al 2013)

| to a                                    | NANANA ONAL   | MANANA DNAL  | RI<br>Tomaz ta<br>Ri   | rgeted<br>NAseq<br>assay   | Two-Donor<br>mixture                                |
|---|---|--|--|--|---|
| SA WORK                                 | MANNONAL ON C   | WARNANAN ANA   | 𝚱<br>₂ ✓   | Identification of mixt<br>Identification of body<br>Source attribution: w                            | ure<br>/ fluids present<br>/ho deposited what fluid |
| Ó                                       |   | MAMMAN DNAI G WANNA  | W <sup>M</sup> onai ass<br>J W                                   | ociate b<br>ith DNA  | ody fluid<br>\ profile                              |
| Case:<br>Observed                       | DNA<br>13,15,17,18<br>13,15 17,18<br>13,17 15,18<br>13,18 15,17 | RNA-SNP <sub>blood</sub><br>1200 (A allele count–<br>(B allele count–<br>Blood present<br>Donor Genotype: AB | RNA-SN<br>650) 800 (A a<br>550) (B a<br>Saliva pres<br>Donor Ger | P <sub>saliva</sub><br>allele count–10 ^<br>allele count–790<br><sup>sent</sup><br><i>notype: BB</i> | ~ background noise)<br>)                            |
| <u>Reference:</u><br>Donor 1<br>Donor 2 | DNA<br>13,15<br>17,18   | RNA-SNP <sub>blood</sub><br>AB<br>BB   | RNA-SN<br>AA<br>BB   | IP <sub>saliva,</sub> Body<br>Bl<br>Sa   | ood<br>aliva  |

# Early Work – 35plex cSNP panel

- off-the-shelf assays (MiSeq<sup>™</sup> System)
- 35 cSNPs
  - 11 blood, 8 semen, 3 saliva, 3 vaginal, 3 menstrual and 7 skin

| Sample/<br>Marker         | MUC7_1                   | MUC7_2                   | SEMG1                        | SEMG2_1                      | SEMG2_2                  | TGM4_1                  | TGM4_3                  | TGM4_4                    |
|---------------------------|--------------------------|--------------------------|------------------------------|------------------------------|--------------------------|-------------------------|-------------------------|---------------------------|
| Donor 1 (♂)<br>(DNA)      | СС                       | СС                       | ΤT                           | СС                           | AA                       | TT                      | GC                      | AA                        |
| Donor 2<br>(DNA)          | СТ                       | CG                       | TA                           | СА                           | AG                       | GG                      | GG                      | GG                        |
| saliva-semen<br>mix (RNA) | CC<br>C: 50'613<br>T: 59 | CC<br>C: 69'115<br>G: 14 | TA<br>T: 48'537<br>A: 35'771 | CA<br>C: 25'139<br>A: 11'172 | AG<br>A: 3625<br>G: 9056 | GG<br>G: 39'208<br>T: 5 | GG<br>G: 20'696<br>C: 1 | GG<br>G: 63'476<br>A: 100 |

- donor 1 (saliva but not semen); donor 2 (semen but not saliva)
- proof-of-concept support for 'association assay' that uses cSNPs to associate DNA donor with body fluid
  - Didn't include all of our best target genes (e.g. HTN3 , PRM1)
  - Some cSNPs  $\rightarrow$  reduced DP (poor inherent PD, LD)

Manuscript submitted for publication

| Gene      |         | cSNP    |         |
|-----------|---------|---------|---------|
| AMICA1    | cSNP-1  |         |         |
| ANK1      | cSNP-2  |         |         |
| ANK1      | cSNP-3  |         |         |
| CD3G      | cSNP-4  |         |         |
| CD93      | cSNP-5  |         |         |
| CD93      | cSNP-6  | cSNP-7  |         |
| SPTB      | cSNP-8  | cSNP-9  |         |
| SPTB      | cSNP-10 | cSNP-11 |         |
| SPTB      | cSNP-12 |         |         |
| PRM1      | cSNP-13 |         |         |
| TGM4      | cSNP-14 |         |         |
| TGM4      | cSNP-15 |         |         |
| TGM4      | cSNP-16 | cSNP-17 |         |
| SEMG1     | cSNP-18 |         |         |
| SEMG2     | cSNP-19 |         |         |
| KLK3      | cSNP-20 | cSNP-21 |         |
| HTN3      | cSNP-22 | cSNP-23 | cSNP-24 |
| PRB3      | cSNP-25 |         |         |
| PRB4      | cSNP-26 |         |         |
| PRH2      | cSNP-27 |         |         |
| MUC7      | cSNP-28 |         |         |
| CYP2B7P   | cSNP-29 | cSNP-30 |         |
| CYP2A6    | cSNP-31 | cSNP-32 |         |
| CYP2A6    | cSNP-33 |         |         |
| DKK4      | cSNP-34 |         |         |
| FAM83D    | cSNP-35 |         |         |
| MMP10     | cSNP-36 | cSNP-37 |         |
| LEFTY2    | cSNP-38 |         |         |
| MMP11     | cSNP-39 |         |         |
| MMP7      | cSNP-40 |         |         |
| SFRP4     | cSNP-41 |         |         |
| SFRP4     | cSNP-42 | cSNP-43 |         |
| LCE1C     | cSNP-44 | cSNP-45 | cSNP-46 |
| IL37      | cSNP-47 | cSNP-48 |         |
| SERPINA12 | cSNP-49 | cSNP-50 |         |
| COL17A1   | cSNP-51 |         |         |
| KRT77     | cSNP-52 | cSNP-53 |         |
| KRT77     | cSNP-54 | cSNP-55 |         |

|    |                    | Ion S5 <sup>IIII</sup> System   |
|----|--------------------|---|
|    | 12 cSNPs           | Assay Development   |
|    | 5 genes            | <ul> <li>Previous assay based on off the<br/>shalf markers</li> </ul>   |
|    | 9 cSNPs<br>5 genes | <ul> <li>Custom primer design available<br/>for Ion S5 assay</li> </ul> |
| 24 | 7 cSNPs            | (thanks Rob!!)  |
|    | 5 genes            | <ul> <li>Now includes more cSNPs and<br/>also possibly more</li> </ul>  |
|    | 7 cSNPs            | discriminating ones   |
|    | 4 genes            | Some overlap with previous  |
|    | 8 cSNPs<br>5 genes | <ul><li>assay</li><li>16 of the 55 cSNPs</li></ul>                      |
| 46 |                    | Could check concordance   |
|    | 5 genes            | Several iterations after this first design                              |
|    |                    | • v1.4.2 – 45 plex 5  |

#### Original S5 assay = 55 plex



| cSNP | B7489 | B7480 | B7481 | B7486 | B7479 | BL1-Z |
|------|-------|-------|-------|-------|-------|-------|
| 1    | AA    | AA    | AG    | AG    | GG    | AA    |
| 2    | CG    | CG    | CG    | GG    | GG    | CG    |
| 3    | CC    | СТ    | СТ    | ст    | ст    | СТ    |
| 4    | тт    | ст    | TT    | TT    | TT    | TT    |
| 5    | AG    | GG    | AG    | GG    | GG    | GG    |
| 6    | AG    | AG    | AG    | AG    | AG    | AG    |
| 7    | ст    | СТ    | СТ    | ст    | СС    | СТ    |
| 8    | AG    | AG    | AG    | AG    | AA    | AG    |
| 9    | ст    | СС    | СТ    | СС    | ст    | СТ    |
|      |       |       |       |       |       |       |

v1.4.2

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6 donors  $\rightarrow$  6 unique genotypes

|       | Gene         | cSNP | Semen-1 | Semen-2 (vs) | Semen-3 | Semen-4 | Semen-5 | Semen-6 | Semen-7 | Semen- |
|-------|--------------|------|---------|--------------|---------|---------|---------|---------|---------|--------|
|       | PRM1         | 1    | AA      | -            | AA      | AA      | CC      | CC      | AA      | AA     |
|       | SEMG2        | 2    | AC      | AC           | AC      | 20      | CC      | AC      |         | 00     |
| Somon | <b>VIV</b> 2 | 3    | Π       | СТ           | Π       | ст      | СТ      | СТ      | СТ      | Π      |
| Semen | NLKS         | 4    | AA      | AG           | AA      | AG      | AG      | AG      | AG      | AA     |
|       |              | 5    | 33      | CC           | СТ      | 33      | СТ      | CC      | CC      | Π      |
|       | TGMA         | 6    | GG      | GG           | AG      | AG      | AG      | GG      | GG      | AA     |
|       | 101414       | 7    | CC      | CC           | СТ      | 20      | СТ      | CC      | Π       | -      |
|       |              | 8    | GG      | GG           | AG      | GG      | AG      | GG      | AA      | -      |

#### 8 donors → 8 unique genotypes

| Gene | cSNP | Saliva-1 | Saliva-2 | Saliva-3 | Saliva-4 | Saliva-5 | Saliva-6 | Saliva-7 | Saliva-8 |
|------|------|----------|----------|----------|----------|----------|----------|----------|----------|
|      | 1    | 23       | СС       | СС       | СС       | СТ       | CC       | CC       | CC       |
| HTN3 | 2    | π        | Π        | Π        | π        | ст       | СТ       | СТ       | СТ       |
|      | 3    | 23       | СС       | CC       | CC       | CC       | 23       | СТ       | CC       |
| PRB3 | 4    | 23       | CC       | CC       | CC       | CC       | 23       | CC       | CC       |
| PRB4 | 5    | 23       | CC       | -        | CC       | CC       | CG       | CC       | CG       |
| PRH2 | 6    | 00       | СТ       | -        | CC       | CC       | CC       | CC       | СТ       |
| MUC7 | 7    | 23       | CC       | СТ       | CC       | CC       | 00       | CC       | CC       |

#### 8 donors $\rightarrow$ 7 unique genotypes

| Gene    | cSNP | Vaginal-1 | Vaginal-2 | Vaginal-3 | Vaginal-4 | Vaginal-5 | Vaginal-6 | Vaginal-7 | Vaginal-8 | Vaginal-9 | Vaginal-10 |
|---------|------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|
| CVD2D7D | 1    | CC        | CC        | СТ        | СТ        | СТ        | Π         | CC        | CC        | Π         | CC         |
| СТРЕВЛР | 2    | AT        | Π         | Π         | Π         | Π         | Π         | AT        | Π         | Π         | Π          |
|         | 3    | GG        | AG        | AG        | AG        | -         | GG        | AG        | GG        | GG        | GG         |
| CYP2A6  | 4    | AG        | GG        | AG        | AG        | GG        | AG        | AG        | GG        | GG        | AA         |
|         | 5    | GG        | CG        | CG        | CG        | GG        | GG        | CG        | CC        |           | GG         |
| DKK4    | 6    | CC        | 23        | CC        | 00        | CC        | CC        | CC        | 00        | CC        | -          |
| FAM83D  | 7    | CC        | CC        | СТ        | Π         | Π         | CC        | CC        | <b>CC</b> | CC        | CC         |

#### 10 donors $\rightarrow$ 10 unique genotypes

| Gene   | cSNP | Menstrua-1 | Menstrual-2 | Menstrual-3 | Menstrual-4 | Menstrual-5 | Menstrual-6 |
|--------|------|------------|-------------|-------------|-------------|-------------|-------------|
| MMP10  | 1    | СТ         | Π           | СС          | СТ          | СТ          | СТ          |
|        | 2    | Π          | Π           | Π           | Π           | ст          | Π           |
| LEFTY2 | 3    | Π          | Π           | Π           | Π           | Π           | Π           |
| MMP7   | 4    | CC         | CC          | CC          | CC          | CC          | GG          |

#### 6 donors $\rightarrow$ 5 unique genotypes

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Saliva

Vaginal

Menstrual

v1.4.2

#### cSNP v. 2.1 (combined BFID/DNA association assay)

| Body Fluid | Gene     | cSNPs         | Body fluid | cSNP |
|------------|----------|---------------|------------|------|
|            |          | cSNP-1        |            |      |
|            | ANKI     | cSNP-2        |            |      |
| Rload      | cD3G     | cSNP-3        |            |      |
| Biodu      |          | CSNP-4-5      |            |      |
|            | SPTB     | cSNP-6        |            |      |
|            |          | cSNP-7        |            |      |
|            | PRM1     | cSNP-8        |            |      |
|            | SEMG2    | cSNP-9        |            |      |
| Semen      | KLK3     | cSNP-10-11    |            |      |
| Semen      |          | cSNP-12       |            |      |
|            | TGM4     | cSNP-13       |            |      |
|            |          | cSNP-14-15    |            |      |
|            | HTN3     | cSNP-16-17-18 |            |      |
|            | PRB4     | cSNP-19       |            |      |
| Saliva     | PRH2     | cSNP-20       |            |      |
|            | MUC7     | cSNP-21       |            |      |
|            | STATH    |               |            |      |
| Vaginal    | CYP2B7P1 |               |            |      |
| Vaginai    | CYP2A6   |               |            |      |
| Monstruct  | MMP10    |               |            |      |
| Wenstrudi  | LEFTY2   |               |            |      |
|            | LCE1C    |               |            |      |
| Skin       | COL17A1  |               |            |      |
|            | IL37     |               |            |      |

Focus on association for blood, semen saliva (most relevant). Reduced cSNPs due to specificity issues, PD etc

25ng total RNA

- Ion Chef™ System
  - Library prep/24 cycles
  - Templating/50 pmol library
- Ion S5 sequencing
  - 510-520-530 kit
  - 520 chip/200 bp reads
- 19 BFID genes (5 BF + skin)
- 11 DNA association genes
  - 21 RNA cSNPs
  - Blood 3 genes, 7 cSNPs
  - Semen 4 genes, 8 cSNPs
  - Saliva 4 genes, 6 cSNPs
- 4 MHs

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- Primers mainly in different exons
  - 20 of 24 primer sets
  - 4 across exon-exon
- PD (blood) > 0.99 (Cauc/Afr)
- PD (semen) > 0.99 (Cauc/Afr)
- PD (saliva) > 0.95 (Cauc/Afr)

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## <u>Genotyping – v 2.1</u>

| Gene | cSNP      | B7489 | B7480 | B7481 | B7486 | B7479 | BL1-Zurich | <b>BL2-Zurich</b> | <b>BL3-Zurich</b> | <b>BL4-Zurich</b> | <b>BL5-Zurich</b> |
|------|-----------|-------|-------|-------|-------|-------|------------|-------------------|-------------------|-------------------|-------------------|
| ANK1 | rs504574  | CG    | CG    | CG    | GG    | GG    | CG         | CG                | CG                | GG                | CC                |
| ANKI | rs7816734 | CC    | СТ    | СТ    | СТ    | СТ    | СТ         | CC                | CC                | СС                | СС                |
| CD3G | rs3753059 | Π     | СТ    | Π     | Π     | Π     | Π          | Π                 | Π                 | Π                 | ст                |
|      | rs229586  | AG    | GG    | AG    | GG    | GG    | GG         | AG                | GG                | AG                | GG                |
| SDTD | rs1741487 | СТ    | СТ    | СТ    | ст    | CC    | СТ         | Π                 | СТ                | СТ                | π                 |
| 3FTD | rs1741488 | AG    | AG    | AG    | AG    | AA    | AG         | GG                | AG                | AG                | GG                |
|      | rs229592  | СТ    | CC    | СТ    | CC    | СТ    | СТ         | Π                 | СТ                | Π                 | Π                 |

#### **10 DONORS - 10 GENOTYPES**

| Gene  | cSNP      | SE26 | SE4vs | SE5 | SE13 | SE15      | SE16 | SE460 | SE1-Zurich | SE2-Zurich | SE3-Zurich | SE4-Zurich | SE5-Zurich |
|-------|-----------|------|-------|-----|------|-----------|------|-------|------------|------------|------------|------------|------------|
| PRM1  | rs737008  | AA   | -     | AA  | AA   | <b>CC</b> | CC   | AA    | AA         | AA         | AC         | AA         | AC         |
| SEMG2 | rs2233896 | AC   | AC    | AC  | 23   | 23        | AC   | -     | CC         | CC         | AA         | AC         | CC         |
| KI K3 | rs11573   | Π    | ст    | Π   | СТ   | СТ        | СТ   | ст    | Π          | СТ         | СТ         | ст         | ст         |
| RERJ  | rs1135766 | AA   | AG    | AA  | AG   | AG        | AG   | AG    | AA         | AG         | AG         | AG         | AG         |
|       | rs1995640 | CC   | CC    | СТ  | CC   | СТ        | CC   | CC    | Π          | CC         | CC         | СТ         | СТ         |
| теми  | rs1995641 | GG   | GG    | AG  | AG   | AG        | GG   | GG    | AA         | GG         | GG         | AG         | AG         |
| TOWI4 | rs3749195 | CC   | CC    | СТ  | CC   | СТ        | CC   | Π     | -          | CC         | CC         | CC         | CC         |
|       | rs9876921 | GG   | GG    | AG  | GG   | AG        | GG   | AA    | -          | GG         | GG         | GG         | GG         |

#### 12 DONORS - 12 GENOTYPES

\*

| Gene | cSNP       | SA1       | SA2 | SA10 | SA13 | SA70 | SA61 | SA74 | SA75 |
|------|------------|-----------|-----|------|------|------|------|------|------|
|      | rs1849937  | CC        | CC  | CC   | CC   | СТ   | CC   | CC   | CC   |
| HTN3 | rs1136515  | Π         | Π   | Π    | Π    | СТ   | СТ   | СТ   | СТ   |
|      | rs75067954 | CC        | CC  | CC   | CC   | CC   | CC   | СТ   | CC   |
| PRB4 | rs1052808  | CC        | CC  | -    | CC   | CC   | CG   | CC   | CG   |
| PRH2 | rs10772391 | CC        | СТ  | -    | CC   | CC   | CC   | CC   | СТ   |
| MUC7 | rs2306948  | <b>CC</b> | CC  | СТ   | CC   | CC   | CC   | CC   | CC   |

\*

**8 DONORS - 7 GENOTYPES** 

#### <u>Blood-Semen Mixtures – body fluid ID</u>



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### **Blood-Semen Mixtures – association**

|   | Μ         | ixture Sam | ble      | (99% semen, 1% blood) |  |  |  |  |
|---|-----------|------------|----------|-----------------------|--|--|--|--|
|   | Target ID | Genotype   | Coverage |                       |  |  |  |  |
|   | ANK1      | CG         | 1003     | ANK1 CG               |  |  |  |  |
|   | ANK1      | СС         | 1360     | ANK1 CC               |  |  |  |  |
| - | CD3G      | TT         | 1946     | CD3G TT Ref type -    |  |  |  |  |
|   | SPTB      | AG         | 5858     | SPTB AG donor 1       |  |  |  |  |
|   | SPTB      | ст         | 2065     | SPTB CT               |  |  |  |  |
| - | SPTB      | AG         | 2072     | SPTB AG               |  |  |  |  |
|   | SPTB      | ст         | 1094     | SPTB CT               |  |  |  |  |
|   | PRM1      | AA         | 74072    | PRM1 AA               |  |  |  |  |
|   | SEMG2     | AC         | 3471     | SEMG2 AC              |  |  |  |  |
|   | KLK3      | TT         | 13658    | КЦКЗ ТТ               |  |  |  |  |
|   | KLK3      | AA         | 13650    | KLK3 AA Reftype-      |  |  |  |  |
|   | TGM4      | СС         | 9052     | TGM4 CC donor 2       |  |  |  |  |
|   | TGM4      | GG         | 9361     | TGM4 GG               |  |  |  |  |
|   | TGM4      | СС         | 3284     | TGM4 CC               |  |  |  |  |
|   | TGM4      | GG         | 3327     | TGM4 GG               |  |  |  |  |

Donor set 1

cSNP genotype for blood – matches reference of donor 1 → donor 1 contributed the blood RNA

cSNP genotype for semen– matches reference of donor 2  $\rightarrow$  donor 2 contributed the semen RNA

### Semen-Saliva Mixtures – association

| Mi    | xture Sample | :<br>: | Donor se<br>(68% ser | et 2<br>nen, 32% sal | iva)                  |  |
|-------|--------------|--------|----------------------|----------------------|-----------------------|--|
| PRM1  | CC           | 30214  | PRM1                 | СС                   |                       |  |
| SEMG2 | AC           | 2429   | SEMG2                | AC                   |                       |  |
| KLK3  | ст           | 12650  | KLK3                 | ст                   | Ref type –<br>donor 1 |  |
| KLK3  | AG           | 12565  | KLK3                 | AG                   |                       |  |
| TGM4  | CC           | 2951   | TGM4                 | СС                   |                       |  |
| TGM4  | GG           | 4664   | <br>TGM4             | GG                   |                       |  |
| TGM4  | CC           | 567    | <br>TGM4             | CC                   |                       |  |
| TGM4  | GG           | 576    | <br>TGM4             | GG                   |                       |  |
| HTN3  | ст           | 8156   | HTN3                 | СТ                   |                       |  |
| HTN3  | ст           | 8131   | <br>HTN3             | ст                   | Ref type –            |  |
| HTN3  | CC           | 8117   | <br>HTN3             | СС                   |                       |  |
| PRB4  | -            | 0      | PRB4                 | СС                   |                       |  |
| PRH2  | CC           | 918    | <br>PRH2             | CC                   |                       |  |
| MUC7  | CC           | 15281  | MUC7                 | CC                   |                       |  |

cSNP genotype for semen – matches reference of donor 1 – donor 1 contributed the semen RNA

cSNP genotype for saliva–matches reference of donor 2  $\rightarrow$  donor 2 contributed the saliva RNA

### **Blood-Saliva Mixtures – association**

| Mixture Sample |          |          |  | Donor set 1<br>(70% saliva, 25% blood) |    |            |  |
|----------------|----------|----------|--|--|----|------------|--|
| Target ID      | Genotype | Coverage |  |  |    |            |  |
| ANK1           | CG       | 738      |  | ANK1                                   | CG |            |  |
| ANK1           | CC       | 770      |  | ANK1                                   | СС | Deftune    |  |
| CD3G           | TT       | 1694     |  | CD3G                                   | TT | donor 1    |  |
| SPTB           | AG       | 2933     |  | SPTB                                   | AG |            |  |
| SPTB           | ст       | 1092     |  | SPTB                                   | ст |            |  |
| SPTB           | AG       | 1094     |  | SPTB                                   | AG |            |  |
| SPTB           | ст       | 659      |  | SPTB                                   | ст |            |  |
| HTN3           | CC       | 17633    |  | HTN3                                   | СС |            |  |
| HTN3           | TT       | 17266    |  | HTN3                                   | тт | Ref type - |  |
| HTN3           | CC       | 17277    |  | HTN3                                   | СС | donor 2    |  |
| PRB4           | -        | 0        |  | PRB4                                   | СС |            |  |
| MUC7           | CC       | 3142     |  | PRH2                                   | CC |            |  |
| PRH2           | CC       | 3870     |  | MUC7                                   | СС |            |  |

cSNP genotype for blood – matches reference of donor 1 → donor 1 contributed the blood RNA

cSNP genotype for saliva–matches reference of donor 2  $\rightarrow$  donor 2 contributed the saliva RNA



- Prototype NGS combined RNA BFID/DNA association system developed
  - Based upon coding region SNPs (cSNPs) present in gDNA and mRNA transcripts
  - BFID (blood, semen, saliva, VS, MB, skin)
  - DNA Association (blood, semen, saliva)
- In progress
  - mixtures (2 person, > 2 persons, limitations)
  - mini 21-cSNP-DNA assay
  - population studies (e.g. LD, MH)
    - allele specific expression variation
  - probabilistic framework for interpretation <sup>12</sup>

#### Acknowledgements

This work was supported in part by Award No. 2014-DN-BX-K019, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, conclusions findings and or recommendations in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

European Union Seventh Framework Program (FP7/2007-2013) grant agreement nº 285487. (EUROFORGEN-NoE)

Thank you for your attention! 15

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