

Drawing and interpreting quasi-median networks with EMPOP

- Directions for Use -

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Introduction

MtDNA data tables can be depicted as quasi-median (QM) networks to enhance the understanding of the data in regard to homoplasy and potential artefacts. Highly recurrent mutations are removed from the dataset (filtering) to help detect data idiosyncrasies that pinpoint sequencing and data interpretation problems. A detailed discussion of the method can be found in Bandelt and Dür (2007) and its application in Parson and Dür (2007), an adapted filter for West Eurasian population data is described in Zimmermann et al (2011).

This instruction manual gives an insight in quasi-median network basics and leads you through the three steps of a quasi-median network analysis:

- 1) the **calculation** (and filtering) of the network via the network software in EMPOP
- 2) the **drawing** of the network via the drawing software to be downloaded from EMPOP and
- 3) the **interpretation** of the quasi-median network.

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1. Quasi-median network (QMN) basics

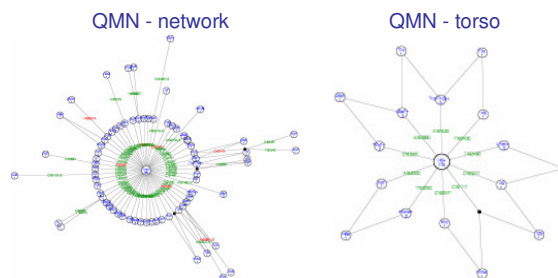
- 1.1. How does a quasi-median network look like?
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- 1.5. Quasi-median network drawing

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1.1. How does a QMN look like?



HVS-I network and torso of 202 West Eurasian mtDNA haplotypes, filtered with the appropriate West Eurasian filter, all available on EMPOP [Zimmermann et al. FSJG 2011]

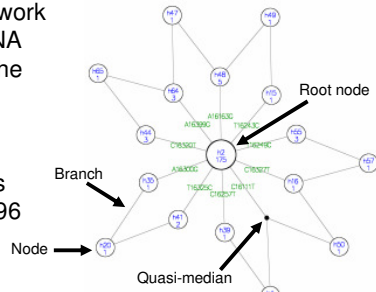
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1.2. Elements of the network

The elements of the quasi-median network torso of 202 mtDNA haplotypes from the example data set WE_Etalon (to be downloaded from EMPOP), analysis range 16024-16596



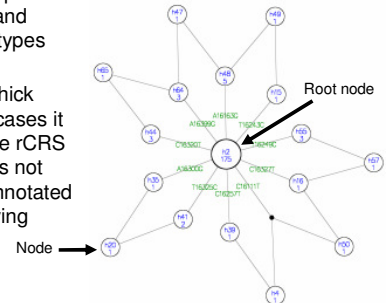
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1.2. Elements of the network Nodes

- The **nodes** correspond to filtered, reduced and condensed haplotypes
- The **root node** is displayed with a thick border; in most cases it corresponds to the rCRS haplotype; if this is not the case this is annotated in the accompanying report file



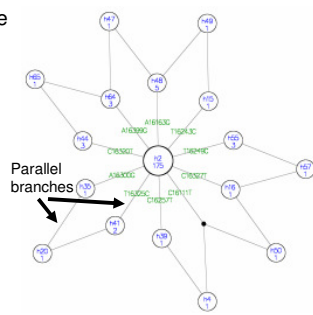
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1.2. Elements of the network Branches

- The **branches** represent mutational events and are to be read starting at the root node
- Mutational events given on the branches are colored either in **green** for **transitions** or in **red** for all **other mutations** (insertions, deletions and transversions)
- Parallel branches carry the same mutation: e.g. T16325C



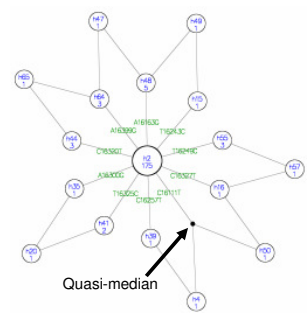
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1.2. Elements of the network Quasi-medians

- The small black node is a **quasi-median** which represent a virtual haplotype
- Quasi-medians are required to link haplotypes within a QM network and are calculated by the network software



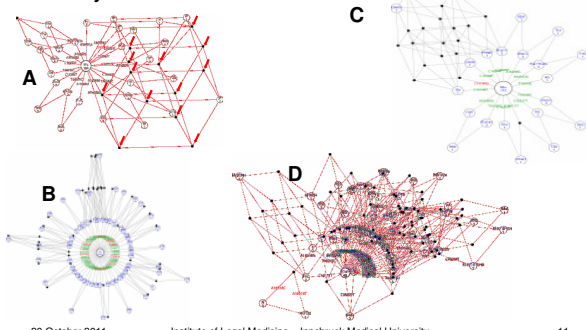
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1.3. Why is my QMN too complex?

Does your QMN look like these?



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1.3. Why is my QMN too complex?

- There can be several reasons for complexity of QM networks. The most common parameters that cause complexity are
 - ERRORS
 - SAMPLE SIZE and
 - PHYLOGENY.
- Errors** often produce quasi-medians that are required to connect the haplotypes in the network. Lots of quasi-medians lead to complex reticulation as you can see in **EXAMPLE A**.
- An **oversized sample set** also leads to a confusing network, see **EXAMPLE B**.
- The phylogenetic composition of a network is also crucial for the output structure. In **EXAMPLE C** you can identify one African haplotype in 202 West-Eurasian haplotypes. This **phylogenetic distance** (manifested in mutations) leads to the production of lots of quasi-medians accompanied by a complex reticulation.
- Also the application of an **inappropriate filter** can cause high complexity. This is shown in **SAMPLE D** where East Asian data was filtered with a West Eurasian Filter.

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1.3. Why is my QMN too complex? What can I do to reduce complexity?

- Example A
 - Errors: check mutations that connect quasi-medians with nodes
- Example B
 - Sample size too large: reduce sample size and try again
- Example C
 - Phylogenetically distant data: remove those and try again
- Example D
 - Inappropriate filter: try the appropriate filter; the first step to reduce the complexity in your data is to use an appropriate filter as you can see in the following section

1.3.1. The appropriate filter for my data Finding the right filter

- On EMPOP there are several filters available. Depending on the samples' origin and/or purpose you can select an appropriate filter.



1.3.1. The appropriate filter for my data Why using a filter?

- MtDNA data include evolutionary hotspots, recurrent and homoplastic mutations which all make the QMN complex.
- To reduce complexity data are filtered according to their phylogenetic background.

1.3.1. The appropriate filter for my data Data and filter are a team

- EMPOPspeedy
 - Filters hotspots based on the worldwide phylogeny
- EMPOPspeedyWE
 - Filters hotspots based on a West Eurasian dataset
- EMPOPall_R*
 - Filters all mutations observed in the current EMPOP release
- A filter for African and Asian phylogeny is in preparation and will be made available soon.

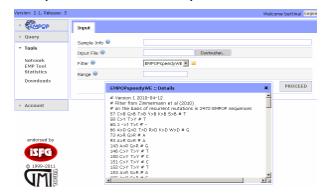
1.3.1. The appropriate filter for my data EMPOPspeedy filter

This filter removes highly recurrent mutations based on the lists provided in Bandelt et al (2002 and 2006). This filter is typically used for the analysis of mtDNA population data within the hypervariable segments HVS-I (16024-16569) and HVS-II (1-576).



1.3.1. The appropriate filter for my data EMPOPspeedyWE filter

This filter removes highly recurrent mutations as presented in Zimmermann et al (2010). This filter is typically used for the analysis of west Eurasian mtDNA population data within the hypervariable segments HVS-I (16024-16569) and HVS-II (1-576).



1.3.1. The appropriate filter for my data EMPOPall_R* filter

This filter includes all mutations found in the forensic haplotypes of EMPOP and is updated with every new release. This filter provides a very quick check on the data by highlighting yet unobserved mutations.



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1.3.1. The appropriate filter for my data Unfiltered QMN analysis

When using the unfiltered option, none of the mutations are removed from your dataset. This is useful for the analysis of very short sequence stretches in the mtDNA CR (see below). The complexity of the network will increase rapidly if no filter is applied to the analysis of larger sequence regions.

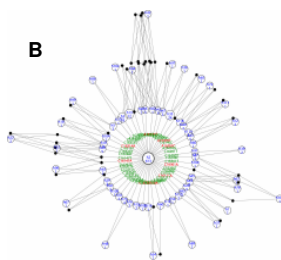


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1.3.2. Useful sample size and phylogenetic aspects



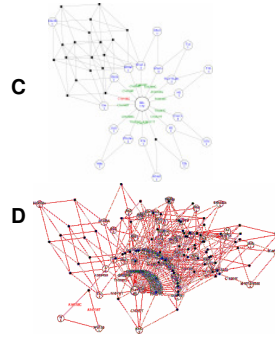
- As we can see in Example B sample size plays a major role in the formation of a quasi-median network.
- Here more than 700 haplotypes were calculated in one QMN analysis. We recommend to **analyze 300 haplotypes at maximum** per query to get useful results.

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1.3.2. Useful sample size and phylogenetic aspects



- As we can see in Examples C and D also phylogeny plays a major role in the formation of a quasi-median network.
- Example C: the dataset includes different phylogenetic lineages that cause complexity. A QMN query should be performed with **related phylogenetic lineages**.
- Example D: the inappropriate filter was applied. A West Eurasian filter does not fit East Asian data; choose the **appropriate filter** (see section 1.2.1.)

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1.4. Quasi-median network calculation

- This is a hypothetical DNA data table to explain the principle of data reduction prior to quasi-median network calculation:

filter data: filter position 3 (for example)

reduce data: condense identical haplotypes

merge equivalent partitions

Ref.	1	2	3	4	5	Freq.
h1	G	T	A	A	A	1
h2	A	A	A	G	A	1
h3	A	A	A	A	G	1
h4	A	A	G	A	A	1
h5	A	A	G	G	G	1
h6	A	A	A	A	A	1
h7	A	A	A	A	A	1
weight	1	1	1	1	1	

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1.4. Quasi-median network calculation

- The resulting filtered, reduced hypothetical DNA data table.
- The condensed haplotype h4 shows a frequency of 3, positions 1 and 2 are merged to one weighted character (with the weight of 2). This reduced table is the basis for further calculation of quasi-medians. For detailed information see [Schwarz and Dür 2011].

Ref.	1/2	4	5	Freq.
h1	G/T	A	A	1
h2	A	G	A	1
h3	A	A	G	1
h4	A	A	A	3
h5	A	G	G	1
weight	2	1	1	

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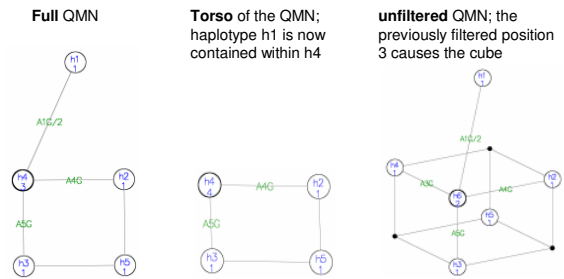
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1.5. Quasi-median network drawing

- The vector graphics output from a QMN analysis can be drawn with the software downloaded from EMPOP (see section 2.1.2.) recently published in [Schwarz & Dür Discrete Appl Math 2011]
- Good to know:
 - Draw the **torso** of the network to get a quick **overview** of the genetic structure of your data
 - Draw the **full network** that provides deeper insight into your data for a **detailed analysis**

1.5. Quasi-median network drawing

The resulting networks from our hypothetical example:



2. How to perform a QMN analysis?

- Necessary preparations
 - Registration and login at www.empop.org
 - Download of the drawing program and an example file (optional)
- Performing the QMN analysis
 - Network tool on EMPOP
 - Preferences input
 - Downloading the result files
- Drawing the QM network
 - QM network and torso of the network
 - How to read a network
 - Drawing the QM network/torso
 - Configuring the network/torso (illustration facilities)

2.1. Necessary preparations

- Good to know:
 - Registration and download/installation of the drawing program is required only once
 - The calculation and drawing of a network can be repeated any time directly via EMPOP

2.1. Necessary preparations

2.1.1. Registration and login

- Register and login at <http://empop.org> for access to the network tool



2.1. Necessary preparations

2.1.2. Drawing program

- Download the drawing program DNWsetup.zip [Schwarz and Dür 2011] and the example file Etalon_WE.emp (optional)
- Unzip DNWsetup.zip and install the software for drawing networks by executing DNWsetup.exe



2.2. Perform the QMN analysis

2.2.1. Network tool on EMPOP

- Before the QM network can be drawn it must be calculated using the Network tool of EMPOP
- Navigate to the network tool on EMPOP Tools - Network

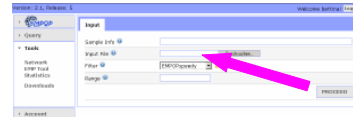


2.2. Perform the QMN analysis

2.2.2. Network tool input parameters

Here you have to input your data in emp format (see section 5.1.) and the required analysis settings

- Sample Info (optional) - for later recognition of the network calculation in your account history
- Input File (mandatory) - select the emp formatted file containing the data to analyze
- Filter (mandatory) - select a filter to remove hotspot mutations from the dataset
- Range (mandatory) - indicate the range for which the network is calculated



2.2. Perform the QMN analysis

2.2.2. Network tool input parameters – examples

- Sample Info
 - e.g. „Etalon WE_16024-16569_EMPOpspeedyWE“
- Input File
 - e.g. „Etalon_WE.emp“ (from Downloads)
- Filter
 - e.g. „EMPOpspeedyWE“
- Range
 - e.g. „16024-16569“ or „1-576“
- Press „PROCEED“ to start the network calculation



2.2. Perform the QMN analysis

2.2.3. Download the result file (zip format)

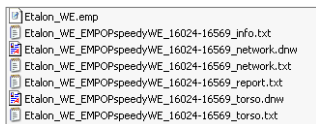
- Download the resulting zip file that includes all result files and unzip the file



2.2. Perform the QMN analysis

2.2.3. Download the result file - example

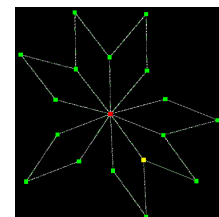
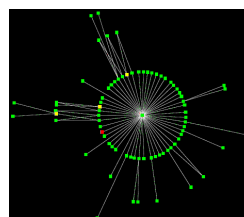
- For detailed description of the individual result files see section 5.



2.3. Draw the quasi-median network

2.3.1. Network and torso of the network

- NETWORK**
 - The quasi-median network displays the filtered, condensed and reduced haplotypes of the analyzed data set
- TORSO of the network**
 - Collapsing all pendant subtrees into their base nodes the torso of the quasi-median network is obtained



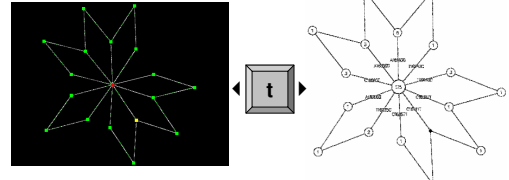
2.3. Draw the quasi-median network 2.3.3. Configure the network

- You can adapt the appearance of the network/torso by applying the following instructions

```
Screen resolution 1680x1050
Use left/right mouse button to move nodes/edges or press
escape key to exit,
directional keys to translate network,
page up/down key to zoom,
home directional key to reset,
h for bounding box,
c/C for scaling circles,
d/D for scaling dots,
e for writing EPS to <datasetname>.eps,
E for writing EPS to <datasetname>.eps with title,
f/F for scaling fontsize,
g for writing FIG to <datasetname>.Fig,
G for writing FIG to <datasetname>.Fig with title,
h for displaying haplotypes/haplogroups,
H for displaying labels,
a for displaying medians,
q/Q for scaling quads,
r/R for rotating (in initial position),
s for saving new layout to <datasetname>.dnu,
t for drawing torso.
```

2.3. Draw the quasi-median network 2.3.3. Configure the network

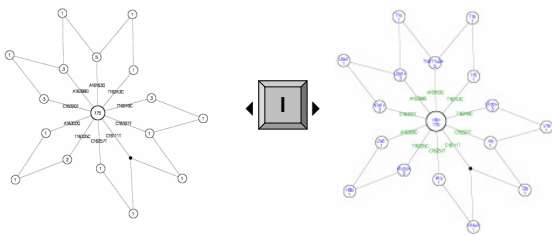
- Change the draft version (left) to the drawing version of the network/torso (right) by pressing „t“



- the draft version provides a fast overview showing the root node in red, samples in green and quasi-medians in yellow
- the drawing version indicates the mutational events on the branches and the number of affected haplotypes within the nodes

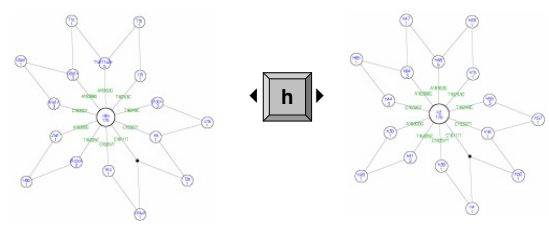
2.3. Draw the quasi-median network 2.3.3. Configure the network

- The appearance of the network/torso can be further changed by pressing „l“ for displaying the corresponding haplogroups and coloring transitions in green and all other mutations in red



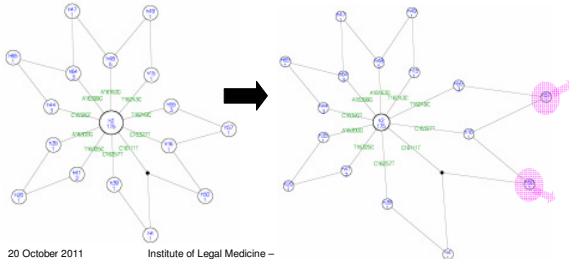
2.3. Draw the quasi-median network 2.3.3. Configure the network

- By pressing „h“ for displaying the corresponding haplotype designation instead of haplogroups. Haplotypes underlying the designations h1, h2, h3,... are given in the result file *_report.txt



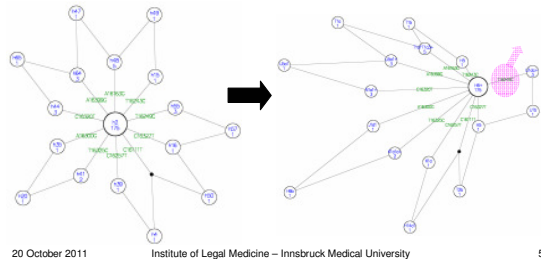
2.3. Draw the quasi-median network 2.3.3. Configure the network

- Nodes can be moved by clicking and holding the left mouse button on a node. The software ensures that parallel edges remain parallel.



2.3. Draw the quasi-median network 2.3.3. Configure the network

- Branches can be moved by clicking and holding the right mouse button on a mutation

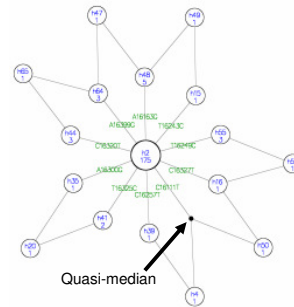


3. Interpretation

- 3.1. Basics
- 3.2. Example Gednap 39

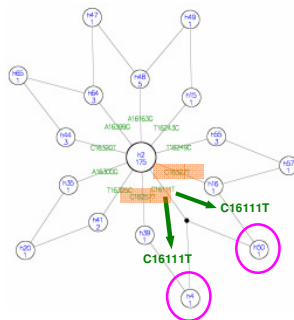
3. Interpretation

- How can this QM be interpreted?



3. Interpretation 3.1. Basics

- Two samples of the Etalon data set contain the transition **A16111T**
- In the network torso the two samples are named **h4 and h50**
- The two samples are separated by the mutations **C16257T** (h4) and **C16327T** (h50)



3. Interpretation 3.1. Basics

- The two samples h4 and h50 can be found in the *_report.txt file

```
*_report.txt-File:
42 Haplotype designation in the netwo
43
44 h1: haplotype001 (H1a1)
45 h2: haplotype002 (H1) haploty
46 h3: haplotype004 (H1a2)
47 h4: haplotype005 (H1a3)
48 h5: haplotype008 (H1a) haploty
49 h6: haplotype016 (H1k) haploty
50
51 h47: haplotype124 (T1a)
52 h48: haplotype125 (T1a) haploty
53 h49: haplotype130 (T1b)
54 h50: haplotype134 (T2b)
55 h51: haplotype135 (T2b)
56 h52: haplotype139 (T2b6)
57 h53: haplotype144 (T2a)
58
59
```

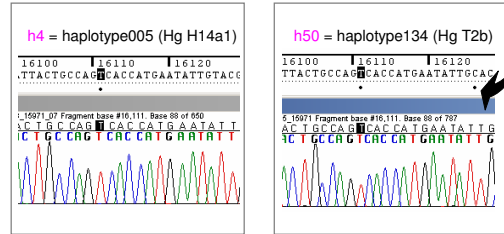
3. Interpretation 3.1. Basics

- The corresponding haplotypes 005 and 134 can be found in the *.emp file (both haplotypes harbor the transition C16111T)

*_report.txt-File:	*_emp-File:
42 Haplotype designation in the netwo	# N=402
43 h1: haplotype001 (H10a1)	#! 16024-576
44 h2: haplotype002 (H11) haploty.	haplotype001 H10a1 1 16114T 16244T 16252C
45 h3: haplotype004 (H11a2)	haplotype002 H11 1 16295G 16311C 1585 263G
46 h4: haplotype005 (H14a1)	haplotype003 H10a1 1 16298G 16311C 1585 263G
48 h5: haplotype008 (H1a) haploty.	haplotype004 H11a2 1 16295G 16311C 1585 263G
49 h6: haplotype016 (H1k) haploty.	haplotype005 H14a1 1 16111T 16252T 16257T
50	haplotype006 H15 1 1622C 1522G 1538 263G 309.1C
51	haplotype007 H15a1 1 1622C 1522G 1538 263G 309.1C
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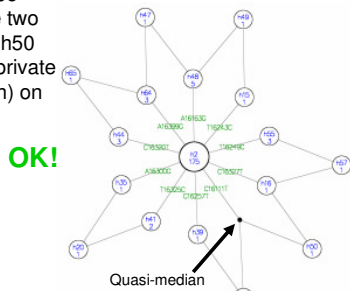
3. Interpretation 3.1. Basics

- By checking the raw lane data we can confirm the transition C16111T in both samples
- The T16126C transition is a signature mutation in hg T.



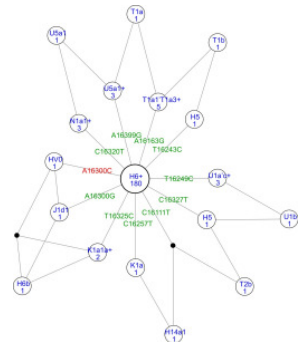
3. Interpretation 3.1. Basics

- The virtual haplotype (QM) connects the two haplotypes h4 and h50 that harbor both a private mutation (transition) on position 16111



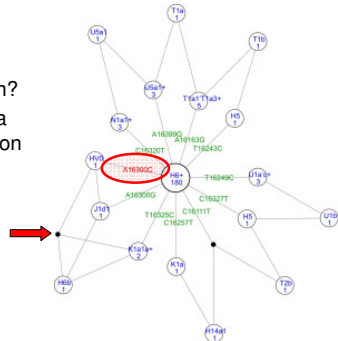
3. Interpretation 3.2. Example (Gednap 39, person 2)

- Network torso of 208 Westeurasian mtDNA haplotypes
- 202 samples from the Etalon_WE and 6 samples from the annual mtDNA trial of the German DNA Profiling Group (Gednap) Gednap 39 [Rand et al. 2004]



3. Interpretation 3.2. Example (Gednap 39, person 2)

- How to interpret this quasi-median and the subsequent three-dimensional reticulation?
 - Origin of the QM is a A16300C transversion found in one HV0 sample

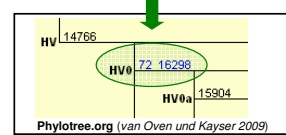


3. Interpretation 3.2. Example (Gednap 39, person 2)

- Reanalysis of this sample reveals the A16300C transversion as erroneous, besides the phylogenetic conflict of a missing T16298C signature mutation of haplogroup HV0

Gednap 39 erroneous haplotype: 16300C 72C 195C 263G 309.1C 315.1C

Gednap 39 correct haplotype: 16298C 72C 195C 263G 309.1C 315.1C



3. Interpretation

3.2. Example (Gednap 39, person 2)

- Corrected network torso of 202 West Eurasian haplotypes (Etalon_WE) and 6 Gednap exercise samples
- The 6 samples from the Gednap 39 exercise are reduced within the Etalon_WE dataset



4. References

- Bandelt HJ et al (2002) The fingerprint of phantom mutations in mitochondrial DNA data. Am J Hum Genet 71:1150-1160
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5. Appendix

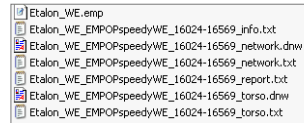
5.1. emp-file

- Input file format requested (a description can be found at EMPOP - Help - EMP Tool)



5. Appendix

5.2. Content of result file: example



5. Appendix

5.2. Content of result file: *.emp

- The input file in emp-format

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5. Appendix

5.2. Result files: *_info.txt

- Identification of network analysis run; using die RID (result identification number) the network calculation can be found in your account history

