

VTEC NGS sequencing activities at the HPA



- Strain Characterisation
- Outbreak Investigation
- National Surveillance

We use WGS for two main questions:

- What are you? / What have you got?
- How similar are you to others?



- **London 2012 Olympic Preparedness**
- **Scenario based exercise based O104 outbreak**
- **How quickly can we characterise 'unknown' VTEC?**

Case 1

vtx gene present ✓
Serotype O26:H11
Known virulence genes



Case 2

vtx gene present ✓
Serotype ? :H9
Known virulence genes



Case 3

vtx gene present ✓
Serotype O77:H39
Known virulence genes



in silico or “virtual” multiplex PCR on assembled genomes

	aggR	ipaH_1	eae	Stx1A	Stx1B	Stx2A	Stx2B	Stx2a_f	bfpA	espT	rfbE (O157)	wbdI (O111)	wzx (O26)	ihp1 (O145)	wzx (O103)	MLST
O26			X										X			ST21
O77				X	X	X	X									New ST
O103			X	X	X										X	ST386
O111	X					X	X					X				ST40

ID From Clinical Sample



7 month female - HUS

Epidemiologically linked to a nursery outbreak of VTEC O26

No bacteria isolated.

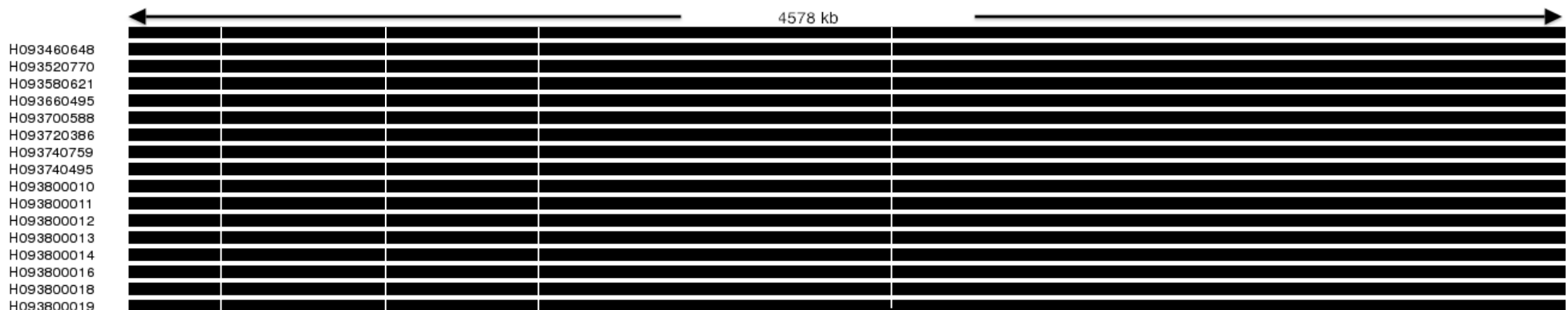
Can we link this case to the outbreak?

Map reads against pathotype panel (key virulence genes & serotype specific genes)

TARGET	aggR	ipaH_1	eae	Stx1A	Stx1B	Stx2A	Stx2B	Stx2a_f	bfpA	espT	rfbE (O157)	wbdI (O111)	wzx (O26)	ihp1 (O145)	wzx (O103)
No of Reads	-	-	8	-	-	20	5	-	-	-	-	-	14	-	-

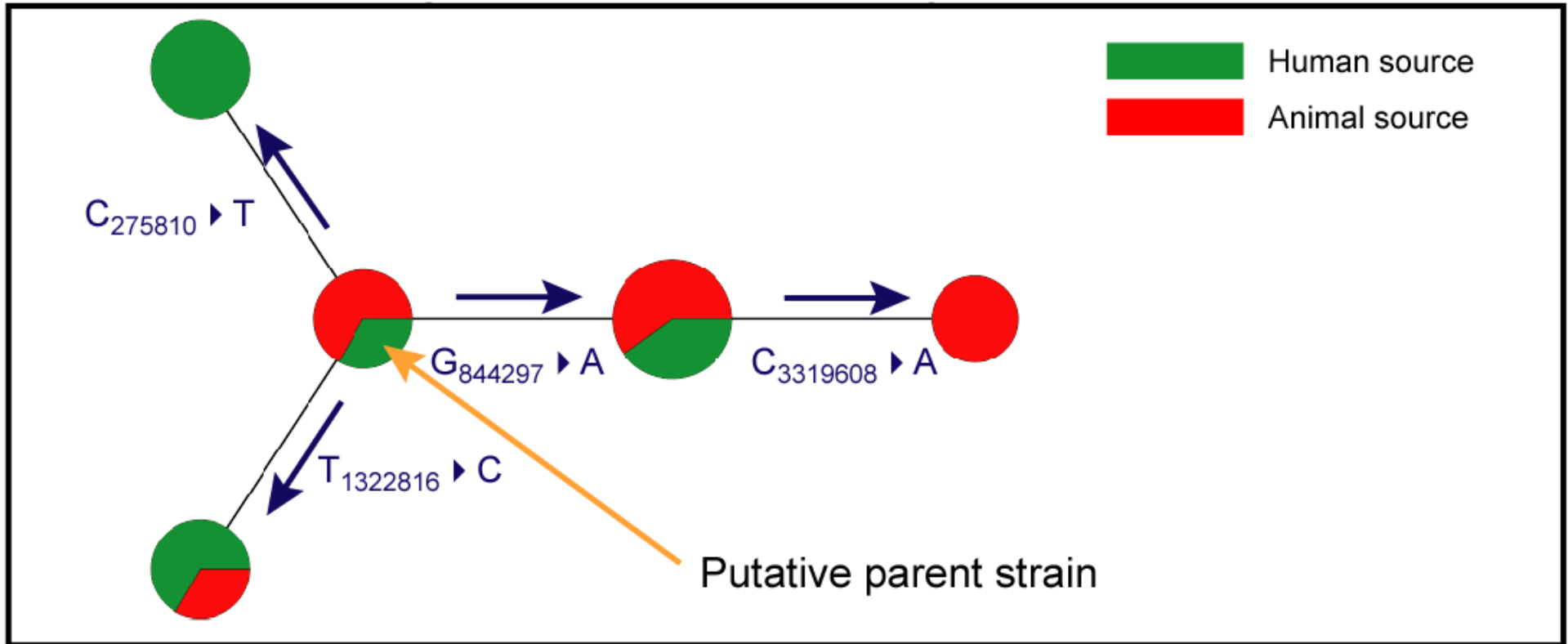
Implicates this case to isolated VTEC O26

- **Open Farm Outbreak 2009**
- **Sequenced 16 genomes (454+Illumina)**
- **Little core genomic variation - just 4 SNPs**
- **The majority of other polymorphism consisted of loss, gain or rearrangement of phage-related sequences.**

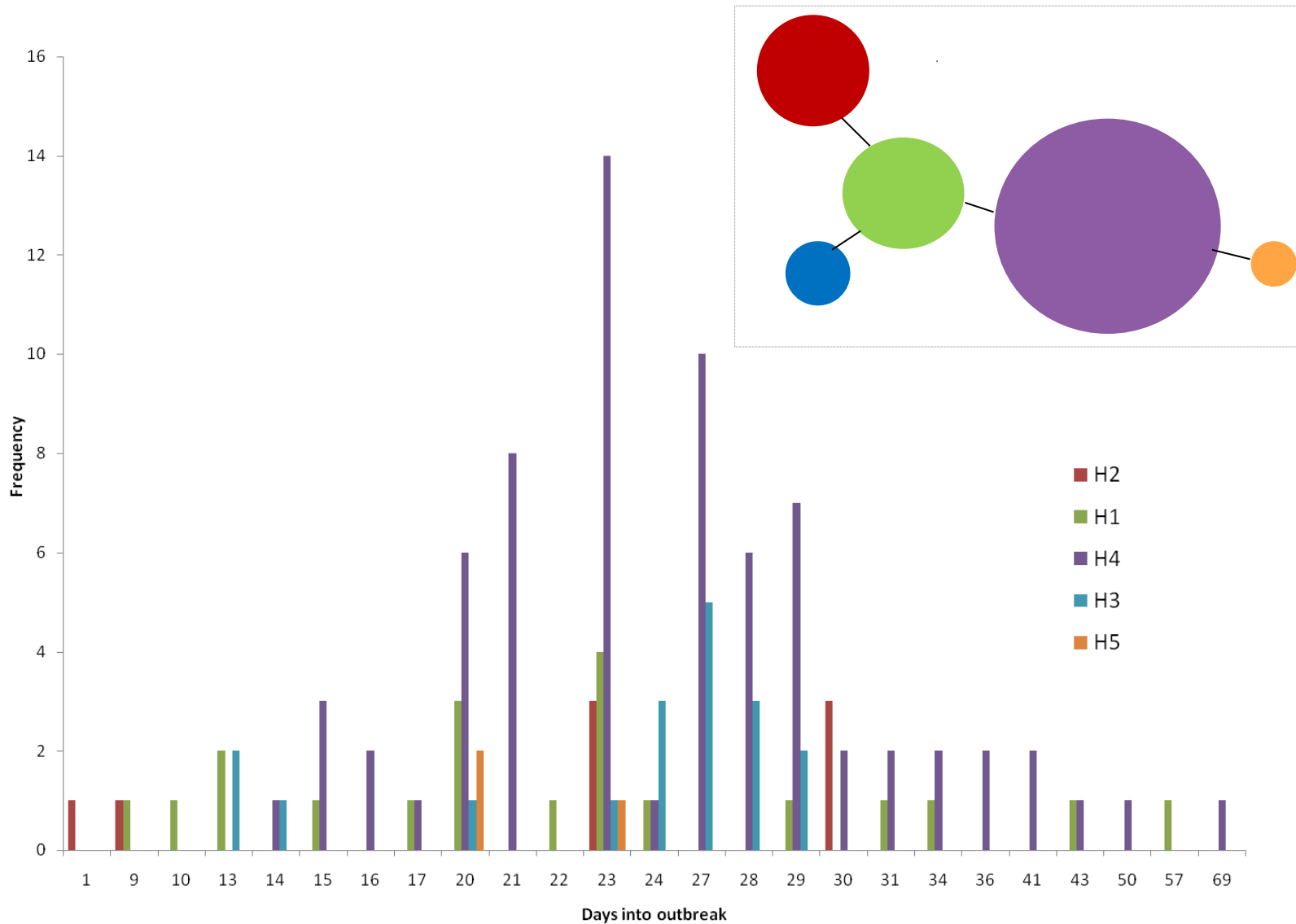


SNP results

Minimum spanning tree of SNPs

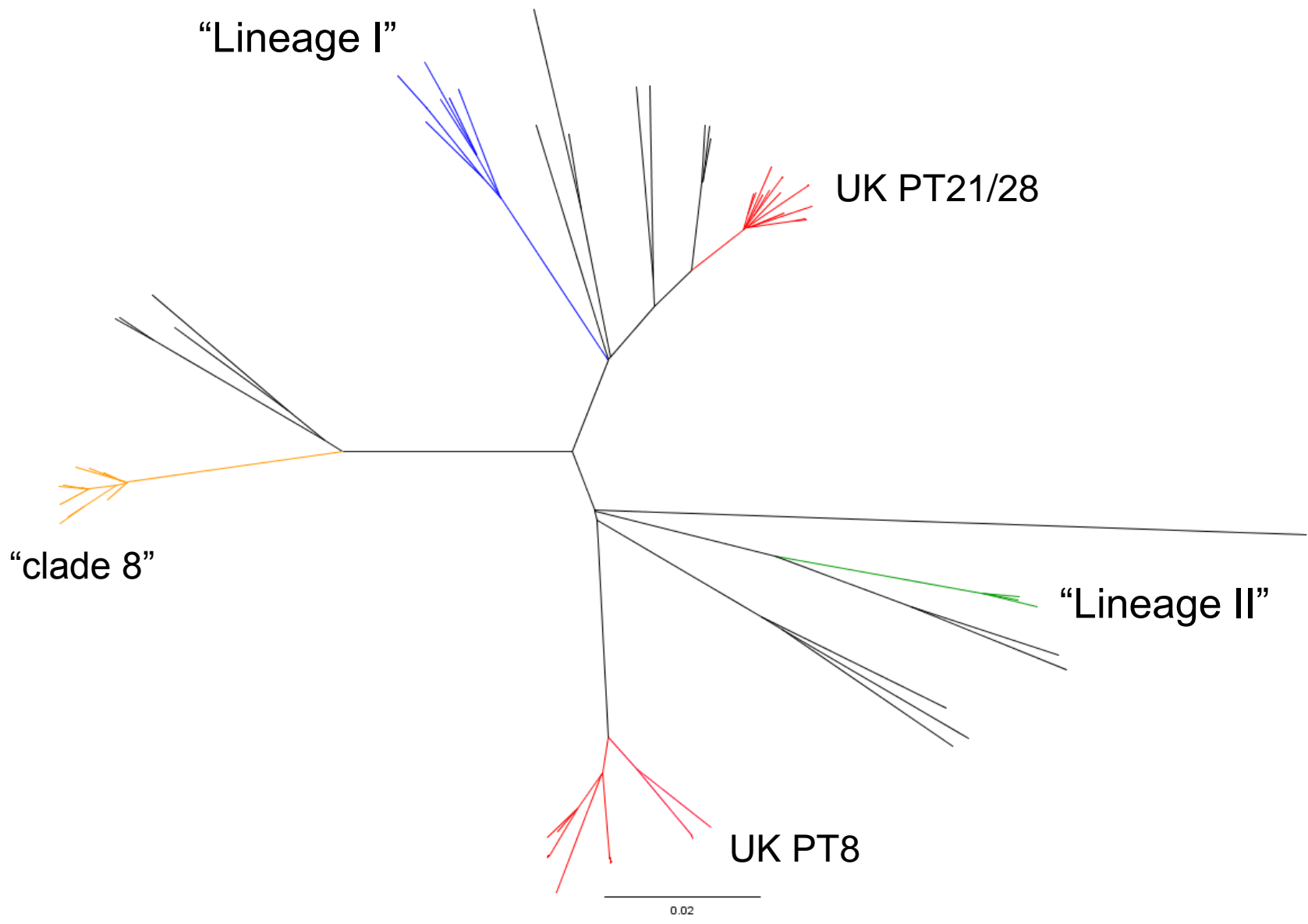


Luminex SNP detection assay on 124 isolates



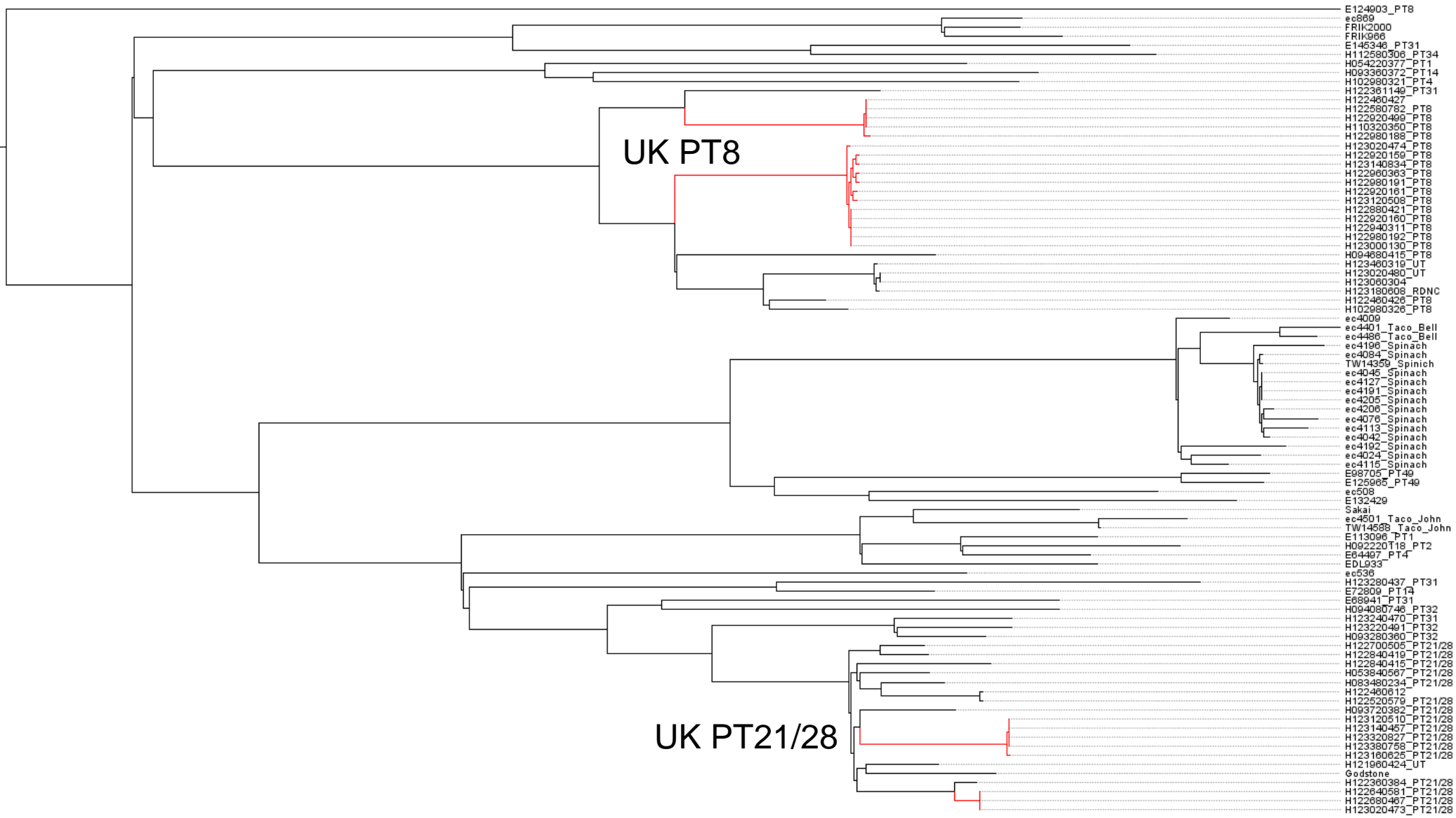
3. Defining NBE for every type only as sequence of all types
 Sequence Everything!

- **HPA investigating using WGS to replace traditional typing methods**
- **Onsite high throughput sequencing capacity**
- **3 year grant secured to investigate O157:H7 UK population structure and molecular epidemiology**



89 strain (62 UK + 27 International)

5057 polymorphic positions



E124903_PT8
 ec869
 FRK2000
 FRK966
 E146346_PT31
 H112580306_PT34
 H054220377_PT1
 H083380372_PT14
 H102990321_PT4
 H122361140_PT31
 H122480427
 H122580782_PT8
 H122820499_PT8
 H110320350_PT8
 H122980188_PT8
 H123020474_PT8
 H122820159_PT8
 H123140834_PT8
 H122860363_PT8
 H122980191_PT8
 H122820181_PT8
 H123120508_PT8
 H122880421_PT8
 H122820180_PT8
 H122940311_PT8
 H122980182_PT8
 H123000130_PT8
 H094680415_PT8
 H123480319_UT
 H123020480_UT
 H123060304
 H123180608_RDNC
 H122480426_PT8
 H102980326_PT8
 ec409
 ec4401_Taco_Bell
 ec4486_Taco_Bell
 ec4196_Spinach
 ec4084_Spinach
 TW14359_Spinach
 ec4045_Spinach
 ec4127_Spinach
 ec4191_Spinach
 ec4205_Spinach
 ec4206_Spinach
 ec4076_Spinach
 ec4113_Spinach
 ec4042_Spinach
 ec4192_Spinach
 ec4024_Spinach
 ec4115_Spinach
 E88706_PT49
 E125965_PT49
 ec508
 E132429
 Sakai
 ec4801_Taco_John
 TW14568_Taco_John
 E113096_PT1
 H092220118_PT2
 E64487_PT4
 E01933
 ec536
 H123280437_PT31
 E72809_PT14
 E68941_PT31
 H084080746_PT32
 H123240470_PT31
 H123220491_PT32
 H083280360_PT32
 H122700505_PT21/28
 H122840419_PT21/28
 H122840415_PT21/28
 H053840567_PT21/28
 H083480234_PT21/28
 H122480612
 H122520579_PT21/28
 H063720382_PT21/28
 H123120510_PT21/28
 H123140467_PT21/28
 H123320827_PT21/28
 H123380758_PT21/28
 H123180625_PT21/28
 H121980424_UT
 Godstone
 H122360384_PT21/28
 H122840581_PT21/28
 H12280467_PT21/28
 H123020473_PT21/28

- **Infrastructure requirements**
- **Skill requirements**
- **Communication of NGS data**
- **Coordination on an International level**

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