**Virologica Sinica**

**Supplementary data**

**Rapid identification of full-length genome and tracing variations of monkeypox virus in clinical specimens based on mNGS and amplicon sequencing**

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**Figure S1.** Sequencing depth of four mpox cases. **A** Sequencing depth of MPXV using amplicon strategies. NGS, Next Generation Sequencing; BF, blister fluid swabs; OS, oropharyngeal swabs; BJ, Beijing; GD, Guangdong; IM, Inner mongolia; SH, Shanghai. The red line indicates 100× sequencing depth. **B** Sequencing depth of the mpox case from Shanghai using metagenomic sequencing strategies.

**Table S1. Annotation of homozygotic SNVs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Pos.*** | ***Ref.*** | ***Alt*** | ***Alt-depth*** | ***Ref-depth*** | ***Annotation*** |
| *1262* | G | A | 3349 | 0 | A|missense\_variant|MODERATE|OPG001|NBT03\_gp001|transcript|NBT03\_gp001|protein\_coding|1/1|c.314C>T|p.Ser105Leu|314/741|314/741|105/246 |
| *2591* | G | A | 2821 | 1 | A|missense\_variant|MODERATE|OPG002|NBT03\_gp002|transcript|NBT03\_gp002|protein\_coding|1/1|c.161C>T|p.Ser54Phe|161/1050|161/1050|54/349 |
| *3111* | G | A | 2959 | 2 | A|synonymous\_variant|LOW|OPG003|NBT03\_gp003|transcript|NBT03\_gp003|protein\_coding|1/1|c.1497C>T|p.Ile499Ile|1497/1767|1497/1767|499/588 |
| *3522* | G | A | 2732 | 0 | A|synonymous\_variant|LOW|OPG003|NBT03\_gp003|transcript|NBT03\_gp003|protein\_coding|1/1|c.1086C>T|p.Ile362Ile|1086/1767|1086/1767|362/588 |
| *3818* | C | T | 3044 | 2 | T|missense\_variant|MODERATE|OPG003|NBT03\_gp003|transcript|NBT03\_gp003|protein\_coding|1/1|c.790G>A|p.Asp264Asn|790/1767|790/1767|264/588 |
| *7771* | C | T | 1525 | 0 | T|synonymous\_variant|LOW|OPG019|NBT03\_gp005|transcript|NBT03\_gp005|protein\_coding|1/1|c.192C>T|p.Ile64Ile|192/429|192/429|64/142 |
| *14000* | G | T | 1299 | 1 | T|missense\_variant|MODERATE|OPG025|NBT03\_gp010|transcript|NBT03\_gp010|protein\_coding|1/1|c.1268C>A|p.Ala423Asp|1268/1893|1268/1893|423/630 |
| *15428* | G | A | 2059 | 0 | A|upstream\_gene\_variant|MODIFIER|OPG022|NBT03\_gp007|transcript|NBT03\_gp007|protein\_coding||c.-4570C>T|||||4570|,A|upstream\_gene\_variant|MODIFIER|OPG023|NBT03\_gp008|transcript|NBT03\_gp008|protein\_coding||c.-2528C>T|||||2528|,A|upstream\_gene\_variant|MODIFIER|OPG024|NBT03\_gp009|transcript|NBT03\_gp009|protein\_coding||c.-2200C>T|||||2200|,A|upstream\_gene\_variant|MODIFIER|OPG025|NBT03\_gp010|transcript|NBT03\_gp010|protein\_coding||c.-161C>T|||||161|,A|downstream\_gene\_variant|MODIFIER|OPG027|NBT03\_gp011|transcript|NBT03\_gp011|protein\_coding||c.\*497C>T|||||497|,A|downstream\_gene\_variant|MODIFIER|OPG029|NBT03\_gp012|transcript|NBT03\_gp012|protein\_coding||c.\*1174C>T|||||1174|,A|downstream\_gene\_variant|MODIFIER|OPG030|NBT03\_gp013|transcript|NBT03\_gp013|protein\_coding||c.\*1785C>T|||||1785|,A|downstream\_gene\_variant|MODIFIER|OPG031|NBT03\_gp014|transcript|NBT03\_gp014|protein\_coding||c.\*2450C>T|||||2450|,A|downstream\_gene\_variant|MODIFIER|OPG034|NBT03\_gp015|transcript|NBT03\_gp015|protein\_coding||c.\*3933C>T|||||3933|,A|downstream\_gene\_variant|MODIFIER|OPG035|NBT03\_gp016|transcript|NBT03\_gp016|protein\_coding||c.\*4623C>T|||||4623|,A|intergenic\_region|MODIFIER|OPG025-OPG027|NBT03\_gp010-NBT03\_gp011|intergenic\_region|NBT03\_gp010-NBT03\_gp011|||n.15428G>A|||||| |
| *18769* | A | G | 1732 | 1 | G|synonymous\_variant|LOW|OPG031|NBT03\_gp014|transcript|NBT03\_gp014|protein\_coding|1/1|c.60T>C|p.Val20Val|60/951|60/951|20/316 |
| *21723* | G | A | 2262 | 0 | A|synonymous\_variant|LOW|OPG037|NBT03\_gp018|transcript|NBT03\_gp018|protein\_coding|1/1|c.711C>T|p.Phe237Phe|711/1329|711/1329|237/442 |
| *23105* | C | T | 2075 | 1 | T|missense\_variant|MODERATE|OPG038|NBT03\_gp019|transcript|NBT03\_gp019|protein\_coding|1/1|c.55G>A|p.Glu19Lys|55/663|55/663|19/220 |
| *23564* | C | T | 2064 | 0 | T|synonymous\_variant|LOW|OPG039|NBT03\_gp020|transcript|NBT03\_gp020|protein\_coding|1/1|c.555G>A|p.Ser185Ser|555/855|555/855|185/284 |
| *25661* | G | A | 1991 | 0 | A|upstream\_gene\_variant|MODIFIER|OPG036|NBT03\_gp017|transcript|NBT03\_gp017|protein\_coding||c.-4597C>T|||||4597|,A|upstream\_gene\_variant|MODIFIER|OPG037|NBT03\_gp018|transcript|NBT03\_gp018|protein\_coding||c.-3228C>T|||||3228|,A|upstream\_gene\_variant|MODIFIER|OPG038|NBT03\_gp019|transcript|NBT03\_gp019|protein\_coding||c.-2502C>T|||||2502|,A|upstream\_gene\_variant|MODIFIER|OPG039|NBT03\_gp020|transcript|NBT03\_gp020|protein\_coding||c.-1543C>T|||||1543|,A|upstream\_gene\_variant|MODIFIER|OPG040|NBT03\_gp021|transcript|NBT03\_gp021|protein\_coding||c.-183C>T|||||183|,A|upstream\_gene\_variant|MODIFIER|OPG044|NBT03\_gp024|transcript|NBT03\_gp024|protein\_coding||c.-2430G>A|||||2430|,A|downstream\_gene\_variant|MODIFIER|OPG042|NBT03\_gp022|transcript|NBT03\_gp022|protein\_coding||c.\*162C>T|||||162|,A|downstream\_gene\_variant|MODIFIER|OPG043|NBT03\_gp023|transcript|NBT03\_gp023|protein\_coding||c.\*1464C>T|||||1464|,A|downstream\_gene\_variant|MODIFIER|OPG045|NBT03\_gp025|transcript|NBT03\_gp025|protein\_coding||c.\*2942C>T|||||2942|,A|downstream\_gene\_variant|MODIFIER|OPG046|NBT03\_gp026|transcript|NBT03\_gp026|protein\_coding||c.\*3613C>T|||||3613|,A|downstream\_gene\_variant|MODIFIER|OPG047|NBT03\_gp027|transcript|NBT03\_gp027|protein\_coding||c.\*4085C>T|||||4085|,A|intergenic\_region|MODIFIER|OPG040-OPG042|NBT03\_gp021-NBT03\_gp022|intergenic\_region|NBT03\_gp021-NBT03\_gp022|||n.25661G>A|||||| |
| *28175* | T | C | 1624 | 0 | C|synonymous\_variant|LOW|OPG044|NBT03\_gp024|transcript|NBT03\_gp024|protein\_coding|1/1|c.85T>C|p.Leu29Leu|85/450|85/450|29/149 |
| *30367* | G | A | 1715 | 0 | A|synonymous\_variant|LOW|OPG047|NBT03\_gp027|transcript|NBT03\_gp027|protein\_coding|1/1|c.828C>T|p.Leu276Leu|828/1449|828/1449|276/482 |
| *31053* | G | A | 1775 | 0 | A|missense\_variant|MODERATE|OPG047|NBT03\_gp027|transcript|NBT03\_gp027|protein\_coding|1/1|c.142C>T|p.Arg48Cys|142/1449|142/1449|48/482 |
| *34459* | G | A | 1479 | 0 | A|missense\_variant|MODERATE|OPG053|NBT03\_gp033|transcript|NBT03\_gp033|protein\_coding|1/1|c.232C>T|p.Pro78Ser|232/639|232/639|78/212 |
| *37202* | G | A | 1958 | 0 | A|synonymous\_variant|LOW|OPG056|NBT03\_gp036|transcript|NBT03\_gp036|protein\_coding|1/1|c.1833C>T|p.Phe611Phe|1833/1908|1833/1908|611/635 |
| *38360* | G | A | 1501 | 0 | A|synonymous\_variant|LOW|OPG056|NBT03\_gp036|transcript|NBT03\_gp036|protein\_coding|1/1|c.675C>T|p.Ile225Ile|675/1908|675/1908|225/635 |
| *38662* | C | T | 1675 | 1 | T|missense\_variant|MODERATE|OPG056|NBT03\_gp036|transcript|NBT03\_gp036|protein\_coding|1/1|c.373G>A|p.Glu125Lys|373/1908|373/1908|125/635 |
| *39119* | C | T | 1659 | 0 | T|synonymous\_variant|LOW|OPG057|NBT03\_gp037|transcript|NBT03\_gp037|protein\_coding|1/1|c.1077G>A|p.Glu359Glu|1077/1119|1077/1119|359/372 |
| *39139* | C | T | 1581 | 0 | T|missense\_variant|MODERATE|OPG057|NBT03\_gp037|transcript|NBT03\_gp037|protein\_coding|1/1|c.1057G>A|p.Glu353Lys|1057/1119|1057/1119|353/372 |
| *52885* | G | A | 1658 | 0 | A|synonymous\_variant|LOW|OPG071|NBT03\_gp050|transcript|NBT03\_gp050|protein\_coding|1/1|c.1554C>T|p.Val518Val|1554/3021|1554/3021|518/1006 |
| *54117* | G | A | 1706 | 0 | A|missense\_variant|MODERATE|OPG071|NBT03\_gp050|transcript|NBT03\_gp050|protein\_coding|1/1|c.322C>T|p.Leu108Phe|322/3021|322/3021|108/1006 |
| *54635* | G | A | 1715 | 1 | A|missense\_variant|MODERATE|OPG072|NBT03\_gp051|transcript|NBT03\_gp051|protein\_coding|1/1|c.166G>A|p.Asp56Asn|166/288|166/288|56/95 |
| *63921* | C | T | 1880 | 0 | T|missense\_variant|MODERATE|OPG083|NBT03\_gp062|transcript|NBT03\_gp062|protein\_coding|1/1|c.796G>A|p.Glu266Lys|796/1272|796/1272|266/423 |
| *64297* | G | A | 1776 | 1 | A|synonymous\_variant|LOW|OPG083|NBT03\_gp062|transcript|NBT03\_gp062|protein\_coding|1/1|c.420C>T|p.Ile140Ile|420/1272|420/1272|140/423 |
| *72362* | C | T | 1677 | 1 | T|missense\_variant|MODERATE|OPG092|NBT03\_gp071|transcript|NBT03\_gp071|protein\_coding|1/1|c.586G>A|p.Asp196Asn|586/1116|586/1116|196/371 |
| *73066* | C | T | 1830 | 1 | T|missense\_variant|MODERATE|OPG093|NBT03\_gp072|transcript|NBT03\_gp072|protein\_coding|1/1|c.89C>T|p.Ser30Leu|89/783|89/783|30/260 |
| *73239* | G | A | 1743 | 0 | A|missense\_variant|MODERATE|OPG093|NBT03\_gp072|transcript|NBT03\_gp072|protein\_coding|1/1|c.262G>A|p.Asp88Asn|262/783|262/783|88/260 |
| *74205* | G | A | 1651 | 0 | A|missense\_variant|MODERATE|OPG094|NBT03\_gp073|transcript|NBT03\_gp073|protein\_coding|1/1|c.426G>A|p.Met142Ile|426/1023|426/1023|142/340 |
| *77383* | G | A | 1480 | 0 | A|missense\_variant|MODERATE|OPG098|NBT03\_gp077|transcript|NBT03\_gp077|protein\_coding|1/1|c.484G>A|p.Glu162Lys|484/756|484/756|162/251 |
| *81275* | G | A | 1515 | 0 | A|synonymous\_variant|LOW|OPG105|NBT03\_gp084|transcript|NBT03\_gp084|protein\_coding|1/1|c.150G>A|p.Lys50Lys|150/3861|150/3861|50/1286 |
| *81977* | A | G | 1672 | 2 | G|synonymous\_variant|LOW|OPG105|NBT03\_gp084|transcript|NBT03\_gp084|protein\_coding|1/1|c.852A>G|p.Gln284Gln|852/3861|852/3861|284/1286 |
| *82373* | C | T | 1863 | 0 | T|synonymous\_variant|LOW|OPG105|NBT03\_gp084|transcript|NBT03\_gp084|protein\_coding|1/1|c.1248C>T|p.Phe416Phe|1248/3861|1248/3861|416/1286 |
| *82451* | G | A | 1779 | 0 | A|synonymous\_variant|LOW|OPG105|NBT03\_gp084|transcript|NBT03\_gp084|protein\_coding|1/1|c.1326G>A|p.Thr442Thr|1326/3861|1326/3861|442/1286 |
| *83326* | C | T | 1638 | 0 | T|missense\_variant|MODERATE|OPG105|NBT03\_gp084|transcript|NBT03\_gp084|protein\_coding|1/1|c.2201C>T|p.Ser734Leu|2201/3861|2201/3861|734/1286 |
| *84587* | C | T | 1827 | 0 | T|synonymous\_variant|LOW|OPG105|NBT03\_gp084|transcript|NBT03\_gp084|protein\_coding|1/1|c.3462C>T|p.Phe1154Phe|3462/3861|3462/3861|1154/1286 |
| *87230* | G | A | 1863 | 0 | A|missense\_variant|MODERATE|OPG109|NBT03\_gp088|transcript|NBT03\_gp088|protein\_coding|1/1|c.2218C>T|p.His740Tyr|2218/2388|2218/2388|740/795 |
| *87297* | G | A | 1892 | 0 | A|synonymous\_variant|LOW|OPG109|NBT03\_gp088|transcript|NBT03\_gp088|protein\_coding|1/1|c.2151C>T|p.Phe717Phe|2151/2388|2151/2388|717/795 |
| *91728* | G | A | 1570 | 0 | A|upstream\_gene\_variant|MODIFIER|OPG108|NBT03\_gp087|transcript|NBT03\_gp087|protein\_coding||c.-4669C>T|||||4669|,A|upstream\_gene\_variant|MODIFIER|OPG109|NBT03\_gp088|transcript|NBT03\_gp088|protein\_coding||c.-2281C>T|||||2281|,A|upstream\_gene\_variant|MODIFIER|OPG113|NBT03\_gp092|transcript|NBT03\_gp092|protein\_coding||c.-3G>A|||||3|,A|upstream\_gene\_variant|MODIFIER|OPG115|NBT03\_gp094|transcript|NBT03\_gp094|protein\_coding||c.-2932G>A|||||2932|,A|upstream\_gene\_variant|MODIFIER|OPG116|NBT03\_gp095|transcript|NBT03\_gp095|protein\_coding||c.-3633G>A|||||3633|,A|upstream\_gene\_variant|MODIFIER|OPG117|NBT03\_gp096|transcript|NBT03\_gp096|protein\_coding||c.-4321G>A|||||4321|,A|downstream\_gene\_variant|MODIFIER|OPG110|NBT03\_gp089|transcript|NBT03\_gp089|protein\_coding||c.\*1464G>A|||||1464|,A|downstream\_gene\_variant|MODIFIER|OPG111|NBT03\_gp090|transcript|NBT03\_gp090|protein\_coding||c.\*519G>A|||||519|,A|downstream\_gene\_variant|MODIFIER|OPG112|NBT03\_gp091|transcript|NBT03\_gp091|protein\_coding||c.\*41G>A|||||41|,A|downstream\_gene\_variant|MODIFIER|OPG114|NBT03\_gp093|transcript|NBT03\_gp093|protein\_coding||c.\*2499C>T|||||2499|,A|intergenic\_region|MODIFIER|OPG112-OPG113|NBT03\_gp091-NBT03\_gp092|intergenic\_region|NBT03\_gp091-NBT03\_gp092|||n.91728G>A|||||| |
| *95034* | G | A | 1409 | 0 | A|synonymous\_variant|LOW|OPG115|NBT03\_gp094|transcript|NBT03\_gp094|protein\_coding|1/1|c.375G>A|p.Val125Val|375/702|375/702|125/233 |
| *100261* | A | G | 1570 | 2 | G|missense\_variant|MODERATE|OPG118|NBT03\_gp097|transcript|NBT03\_gp097|protein\_coding|1/1|c.1816A>G|p.Lys606Glu|1816/1914|1816/1914|606/637 |
| *118161* | C | T | 1475 | 0 | T|missense\_variant|MODERATE|OPG136|NBT03\_gp115|transcript|NBT03\_gp115|protein\_coding|1/1|c.1427G>A|p.Arg476Gln|1427/2676|1427/2676|476/891 |
| *119296* | C | T | 1678 | 0 | T|missense\_variant|MODERATE|OPG136|NBT03\_gp115|transcript|NBT03\_gp115|protein\_coding|1/1|c.292G>A|p.Asp98Asn|292/2676|292/2676|98/891 |
| *120262* | G | A | 1869 | 3 | A|missense\_variant|MODERATE|OPG137|NBT03\_gp116|transcript|NBT03\_gp116|protein\_coding|1/1|c.661G>A|p.Asp221Asn|661/957|661/957|221/318 |
| *121320* | C | T | 1783 | 0 | T|missense\_variant|MODERATE|OPG139|NBT03\_gp118|transcript|NBT03\_gp118|protein\_coding|1/1|c.49G>A|p.Ala17Thr|49/213|49/213|17/70 |
| *124130* | G | A | 1696 | 0 | A|missense\_variant|MODERATE|OPG145|NBT03\_gp124|transcript|NBT03\_gp124|protein\_coding|1/1|c.184G>A|p.Glu62Lys|184/1479|184/1479|62/492 |
| *124674* | G | A | 1397 | 0 | A|missense\_variant|MODERATE|OPG145|NBT03\_gp124|transcript|NBT03\_gp124|protein\_coding|1/1|c.728G>A|p.Arg243Gln|728/1479|728/1479|243/492 |
| *125249* | G | A | 2128 | 0 | A|missense\_variant|MODERATE|OPG145|NBT03\_gp124|transcript|NBT03\_gp124|protein\_coding|1/1|c.1303G>A|p.Glu435Lys|1303/1479|1303/1479|435/492 |
| *128698* | C | T | 1642 | 0 | T|missense\_variant|MODERATE|OPG150|NBT03\_gp129|transcript|NBT03\_gp129|protein\_coding|1/1|c.920C>T|p.Ser307Leu|920/1149|920/1149|307/382 |
| *133163* | GCAATCTTTCT | G | 471 | 0 | G|downstream\_gene\_variant|MODIFIER|OPG150|NBT03\_gp129|transcript|NBT03\_gp129|protein\_coding||c.\*4237\_\*4246delCAATCTTTCT|||||4237|,G|downstream\_gene\_variant|MODIFIER|OPG151|NBT03\_gp130|transcript|NBT03\_gp130|protein\_coding||c.\*746\_\*755delCAATCTTTCT|||||746|,G|downstream\_gene\_variant|MODIFIER|OPG153|NBT03\_gp131|transcript|NBT03\_gp131|protein\_coding||c.\*2965\_\*2974delAGAAAGATTG|||||2974|,G|downstream\_gene\_variant|MODIFIER|OPG154|NBT03\_gp132|transcript|NBT03\_gp132|protein\_coding||c.\*4545\_\*4554delAGAAAGATTG|||||4554|,G|downstream\_gene\_variant|MODIFIER|OPG155|NBT03\_gp133|transcript|NBT03\_gp133|protein\_coding||c.\*4878\_\*4887delAGAAAGATTG|||||4887|,G|intergenic\_region|MODIFIER|OPG151-OPG153|NBT03\_gp130-NBT03\_gp131|intergenic\_region|NBT03\_gp130-NBT03\_gp131|||n.133164\_133173delCAATCTTTCT|||||| |
| *148412* | G | A | 1839 | 0 | A|synonymous\_variant|LOW|OPG174|NBT03\_gp150|transcript|NBT03\_gp150|protein\_coding|1/1|c.984C>T|p.Ile328Ile|984/1041|984/1041|328/346 |
| *150469* | C | T | 1539 | 0 | T|missense\_variant|MODERATE|OPG176|NBT03\_gp152|transcript|NBT03\_gp152|protein\_coding|1/1|c.661C>T|p.His221Tyr|661/723|661/723|221/240 |
| *151461* | A | C | 1861 | 0 | C|upstream\_gene\_variant|MODIFIER|OPG170|NBT03\_gp146|transcript|NBT03\_gp146|protein\_coding||c.-4683T>G|||||4683|,C|upstream\_gene\_variant|MODIFIER|OPG174|NBT03\_gp150|transcript|NBT03\_gp150|protein\_coding||c.-2066T>G|||||2066|,C|upstream\_gene\_variant|MODIFIER|OPG178|NBT03\_gp153|transcript|NBT03\_gp153|protein\_coding||c.-48A>C|||||48|,C|upstream\_gene\_variant|MODIFIER|OPG180|NBT03\_gp154|transcript|NBT03\_gp154|protein\_coding||c.-1231A>C|||||1231|,C|upstream\_gene\_variant|MODIFIER|OPG181|NBT03\_gp155|transcript|NBT03\_gp155|protein\_coding||c.-2951A>C|||||2951|,C|downstream\_gene\_variant|MODIFIER|OPG171|NBT03\_gp147|transcript|NBT03\_gp147|protein\_coding||c.\*4072A>C|||||4072|,C|downstream\_gene\_variant|MODIFIER|OPG172|NBT03\_gp148|transcript|NBT03\_gp148|protein\_coding||c.\*3444A>C|||||3444|,C|downstream\_gene\_variant|MODIFIER|OPG173|NBT03\_gp149|transcript|NBT03\_gp149|protein\_coding||c.\*3200A>C|||||3200|,C|downstream\_gene\_variant|MODIFIER|OPG175|NBT03\_gp151|transcript|NBT03\_gp151|protein\_coding||c.\*1642A>C|||||1642|,C|downstream\_gene\_variant|MODIFIER|OPG176|NBT03\_gp152|transcript|NBT03\_gp152|protein\_coding||c.\*930A>C|||||930|,C|intergenic\_region|MODIFIER|OPG176-OPG178|NBT03\_gp152-NBT03\_gp153|intergenic\_region|NBT03\_gp152-NBT03\_gp153|||n.151461A>C|||||| |
| *154408* | T | C | 1865 | 0 | C|upstream\_gene\_variant|MODIFIER|OPG181|NBT03\_gp155|transcript|NBT03\_gp155|protein\_coding||c.-4T>C|||||4|,C|upstream\_gene\_variant|MODIFIER|OPG185|NBT03\_gp156|transcript|NBT03\_gp156|protein\_coding||c.-4509T>C|||||4509|,C|downstream\_gene\_variant|MODIFIER|OPG175|NBT03\_gp151|transcript|NBT03\_gp151|protein\_coding||c.\*4589T>C|||||4589|,C|downstream\_gene\_variant|MODIFIER|OPG176|NBT03\_gp152|transcript|NBT03\_gp152|protein\_coding||c.\*3877T>C|||||3877|,C|downstream\_gene\_variant|MODIFIER|OPG178|NBT03\_gp153|transcript|NBT03\_gp153|protein\_coding||c.\*2285T>C|||||2285|,C|downstream\_gene\_variant|MODIFIER|OPG180|NBT03\_gp154|transcript|NBT03\_gp154|protein\_coding||c.\*37T>C|||||37|,C|intergenic\_region|MODIFIER|OPG180-OPG181|NBT03\_gp154-NBT03\_gp155|intergenic\_region|NBT03\_gp154-NBT03\_gp155|||n.154408T>C|||||| |
| *155795* | G | A | 1371 | 0 | A|upstream\_gene\_variant|MODIFIER|OPG185|NBT03\_gp156|transcript|NBT03\_gp156|protein\_coding||c.-3122G>A|||||3122|,A|upstream\_gene\_variant|MODIFIER|OPG187|NBT03\_gp157|transcript|NBT03\_gp157|protein\_coding||c.-4827G>A|||||4827|,A|downstream\_gene\_variant|MODIFIER|OPG178|NBT03\_gp153|transcript|NBT03\_gp153|protein\_coding||c.\*3672G>A|||||3672|,A|downstream\_gene\_variant|MODIFIER|OPG180|NBT03\_gp154|transcript|NBT03\_gp154|protein\_coding||c.\*1424G>A|||||1424|,A|downstream\_gene\_variant|MODIFIER|OPG181|NBT03\_gp155|transcript|NBT03\_gp155|protein\_coding||c.\*379G>A|||||379|,A|intergenic\_region|MODIFIER|OPG181-OPG185|NBT03\_gp155-NBT03\_gp156|intergenic\_region|NBT03\_gp155-NBT03\_gp156|||n.155795G>A|||||| |
| *160702* | C | T | 1888 | 0 | T|synonymous\_variant|LOW|OPG187|NBT03\_gp157|transcript|NBT03\_gp157|protein\_coding|1/1|c.81C>T|p.Phe27Phe|81/912|81/912|27/303 |
| *162243* | G | A | 1870 | 0 | A|synonymous\_variant|LOW|OPG188|NBT03\_gp158|transcript|NBT03\_gp158|protein\_coding|1/1|c.648G>A|p.Leu216Leu|648/1512|648/1512|216/503 |
| *162331* | C | T | 2013 | 1 | T|synonymous\_variant|LOW|OPG188|NBT03\_gp158|transcript|NBT03\_gp158|protein\_coding|1/1|c.736C>T|p.Leu246Leu|736/1512|736/1512|246/503 |
| *164832* | C | T | 1816 | 0 | T|synonymous\_variant|LOW|OPG189|NBT03\_gp159|transcript|NBT03\_gp159|protein\_coding|1/1|c.1500C>T|p.Leu500Leu|1500/1686|1500/1686|500/561 |
| *168120* | C | T | 1653 | 0 | T|missense\_variant|MODERATE|OPG193|NBT03\_gp163|transcript|NBT03\_gp163|protein\_coding|1/1|c.787C>T|p.Leu263Phe|787/804|787/804|263/267 |
| *170262* | G | A | 1852 | 0 | A|synonymous\_variant|LOW|OPG198|NBT03\_gp166|transcript|NBT03\_gp166|protein\_coding|1/1|c.225G>A|p.Arg75Arg|225/849|225/849|75/282 |
| *178133* | G | A | 1791 | 0 | A|upstream\_gene\_variant|MODIFIER|OPG208|NBT03\_gp171|transcript|NBT03\_gp171|protein\_coding||c.-1133G>A|||||1133|,A|upstream\_gene\_variant|MODIFIER|OPG209|NBT03\_gp172|transcript|NBT03\_gp172|protein\_coding||c.-2385G>A|||||2385|,A|upstream\_gene\_variant|MODIFIER|OPG210|NBT03\_gp173|transcript|NBT03\_gp173|protein\_coding||c.-3223G>A|||||3223|,A|downstream\_gene\_variant|MODIFIER|OPG204|NBT03\_gp169|transcript|NBT03\_gp169|protein\_coding||c.\*2546G>A|||||2546|,A|downstream\_gene\_variant|MODIFIER|OPG205|NBT03\_gp170|transcript|NBT03\_gp170|protein\_coding||c.\*114G>A|||||114|,A|intergenic\_region|MODIFIER|OPG205-OPG208|NBT03\_gp170-NBT03\_gp171|intergenic\_region|NBT03\_gp170-NBT03\_gp171|||n.178133G>A|||||| |
| *181980* | G | A | 1957 | 0 | A|missense\_variant|MODERATE|OPG210|NBT03\_gp173|transcript|NBT03\_gp173|protein\_coding|1/1|c.625G>A|p.Asp209Asn|625/5643|625/5643|209/1880 |
| *183519* | C | T | 1674 | 0 | T|missense\_variant|MODERATE|OPG210|NBT03\_gp173|transcript|NBT03\_gp173|protein\_coding|1/1|c.2164C>T|p.Pro722Ser|2164/5643|2164/5643|722/1880 |
| *186578* | G | A | 1627 | 2 | A|missense\_variant|MODERATE|OPG210|NBT03\_gp173|transcript|NBT03\_gp173|protein\_coding|1/1|c.5223G>A|p.Met1741Ile|5223/5643|5223/5643|1741/1880 |
| *187428* | C | T | 1972 | 0 | T|upstream\_gene\_variant|MODIFIER|OPG005|NBT03\_gp174|transcript|NBT03\_gp174|protein\_coding||c.-1771C>T|||||1771|,T|upstream\_gene\_variant|MODIFIER|OPG016|NBT03\_gp175|transcript|NBT03\_gp175|protein\_coding||c.-2982C>T|||||2982|,T|upstream\_gene\_variant|MODIFIER|OPG015|NBT03\_gp176|transcript|NBT03\_gp176|protein\_coding||c.-3626C>T|||||3626|,T|downstream\_gene\_variant|MODIFIER|OPG210|NBT03\_gp173|transcript|NBT03\_gp173|protein\_coding||c.\*430C>T|||||430|,T|intergenic\_region|MODIFIER|OPG210-OPG005|NBT03\_gp173-NBT03\_gp174|intergenic\_region|NBT03\_gp173-NBT03\_gp174|||n.187428C>T|||||| |
| *193392* | G | A | 3085 | 0 | A|missense\_variant|MODERATE|OPG003|NBT03\_gp177|transcript|NBT03\_gp177|protein\_coding|1/1|c.790G>A|p.Asp264Asn|790/1767|790/1767|264/588 |
| *193688* | C | T | 2782 | 1 | T|synonymous\_variant|LOW|OPG003|NBT03\_gp177|transcript|NBT03\_gp177|protein\_coding|1/1|c.1086C>T|p.Ile362Ile|1086/1767|1086/1767|362/588 |
| *194099* | C | T | 2982 | 4 | T|synonymous\_variant|LOW|OPG003|NBT03\_gp177|transcript|NBT03\_gp177|protein\_coding|1/1|c.1497C>T|p.Ile499Ile|1497/1767|1497/1767|499/588 |
| *194619* | C | T | 2839 | 2 | T|missense\_variant|MODERATE|OPG002|NBT03\_gp178|transcript|NBT03\_gp178|protein\_coding|1/1|c.161C>T|p.Ser54Phe|161/1050|161/1050|54/349 |
| *195948* | C | T | 3241 | 0 | T|missense\_variant|MODERATE|OPG001|NBT03\_gp179|transcript|NBT03\_gp179|protein\_coding|1/1|c.314C>T|p.Ser105Leu|314/741|314/741|105/246 |

**Table S2. Annotation of intra-host SNVs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***Pos.*** | ***Ref.*** | ***Alt*** | ***Alt-depth*** | ***Ref-depth*** | ***Annotation*** |
| *16940* | C | T | 1156 | 916 | T|missense\_variant|MODERATE|OPG029|NBT03\_gp012|transcript|NBT03\_gp012|protein\_coding|1/1|c.130G>A|p.Ala44Thr|130/468|130/468|44/155 |
| *41495* | C | T | 751 | 1102 | T|synonymous\_variant|LOW|OPG061|NBT03\_gp041|transcript|NBT03\_gp041|protein\_coding|1/1|c.390G>A|p.Leu130Leu|390/696|390/696|130/231 |
| *54262* | C | T | 1168 | 847 | T|synonymous\_variant|LOW|OPG071|NBT03\_gp050|transcript|NBT03\_gp050|protein\_coding|1/1|c.177G>A|p.Pro59Pro|177/3021|177/3021|59/1006 |
| *150584* | T | TG | 369 | 706 | TG|upstream\_gene\_variant|MODIFIER|OPG170|NBT03\_gp146|transcript|NBT03\_gp146|protein\_coding||c.-3807\_-3806insC|||||3807|,TG|upstream\_gene\_variant|MODIFIER|OPG174|NBT03\_gp150|transcript|NBT03\_gp150|protein\_coding||c.-1190\_-1189insC|||||1190|,TG|upstream\_gene\_variant|MODIFIER|OPG178|NBT03\_gp153|transcript|NBT03\_gp153|protein\_coding||c.-925\_-924insG|||||924|,TG|upstream\_gene\_variant|MODIFIER|OPG180|NBT03\_gp154|transcript|NBT03\_gp154|protein\_coding||c.-2108\_-2107insG|||||2107|,TG|upstream\_gene\_variant|MODIFIER|OPG181|NBT03\_gp155|transcript|NBT03\_gp155|protein\_coding||c.-3828\_-3827insG|||||3827|,TG|downstream\_gene\_variant|MODIFIER|OPG171|NBT03\_gp147|transcript|NBT03\_gp147|protein\_coding||c.\*3195\_\*3196insG|||||3196|,TG|downstream\_gene\_variant|MODIFIER|OPG172|NBT03\_gp148|transcript|NBT03\_gp148|protein\_coding||c.\*2567\_\*2568insG|||||2568|,TG|downstream\_gene\_variant|MODIFIER|OPG173|NBT03\_gp149|transcript|NBT03\_gp149|protein\_coding||c.\*2323\_\*2324insG|||||2324|,TG|downstream\_gene\_variant|MODIFIER|OPG175|NBT03\_gp151|transcript|NBT03\_gp151|protein\_coding||c.\*765\_\*766insG|||||766|,TG|downstream\_gene\_variant|MODIFIER|OPG176|NBT03\_gp152|transcript|NBT03\_gp152|protein\_coding||c.\*53\_\*54insG|||||54|,TG|intergenic\_region|MODIFIER|OPG176-OPG178|NBT03\_gp152-NBT03\_gp153|intergenic\_region|NBT03\_gp152-NBT03\_gp153|||n.150584\_150585insG|||||| |

**Table S3. Microorganisms detected in multiple clinical samples using mNGS**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Samples** | **Species** | **Coverage rate (%)** | **Depth****(×)** | **Reads stringently mapped in species level (SMRN)** |
| Nasal swabs | *Monkeypox virus a* | 99.75 | 51.86 | 67,208 |
| *Ureaplasma urealyticum a* | 11.89 | 1.08 | 739 |
| *Cutibacterium acnes b* | 4.71 | 1.03 | 1,243 |
| *Rothia dentocariosa b* | 2.66 | 1.03 | 944 |
| *Lautropia mirabilis b* | 4.99 | 1.03 | 853 |
| *Streptococcus salivarius b* | 2.96 | 1.02 | 736 |
| *Campylobacter concisus b* | 1.57 | 1.01 | 651 |
| *Prevotella melaninogenica b* | 1.08 | 1.01 | 338 |
| *Haemophilus parainfluenzae b* | 1.06 | 1.01 | 287 |
| *Actinomyces graevenitzii b* | 0.8111 | 1 | 263 |
| *Veillonella dispar b* | 0.4758 | 1.01 | 70 |
| *Veillonella atypica b* | 0.2397 | 1.03 | 57 |
| Oropharyngeal swabse | *Monkeypox virus a* | 99.91 | 20.76 | 47,275 |
| *Ureaplasma urealyticum a* | 16.09 | 1.15 | 1,980 |
| *Lautropia mirabilis b* | 96.26 | 11.03 | 239,420 |
| *Streptococcus salivarius b* | 79.8 | 5.33 | 110,694 |
| *Campylobacter concisus b* | 53.36 | 1.92 | 60,028 |
| *Rothia dentocariosa b* | 52.31 | 2.01 | 55,706 |
| *Prevotella melaninogenica b* | 47.9 | 1.69 | 28,993 |
| *Haemophilus parainfluenzae b* | 34.26 | 1.49 | 28,229 |
| *Actinomyces graevenitzii b* | 36.26 | 1.42 | 17,955 |
| *Veillonella atypica b* | 28.44 | 1.38 | 12,431 |
| *Alloprevotella tannerae b* | 43.96 | 1.48 | 12,314 |
| *Leptotrichia wadei b* | 35.5 | 1.39 | 10,240 |
| *Veillonella dispar b* | 28.02 | 1.39 | 6,724 |
| *Oribacterium sinus b* | 28.82 | 1.26 | 5,383 |
| *Selenomonas felix b* | 40.29 | 1.53 | 5,334 |
| *Neisseria elongata b* | 6.26 | 1.19 | 3,056 |
| *Fusobacterium nucleatum b* | 6.09 | 1.07 | 3,005 |
| *Achromobacter pulmonis c* | 77.89 | 158.73 | 6,635,215 |
| *Elizabethkingia miricola c* | 66.87 | 23.49 | 380,092 |
| *Acanthamoeba polyphaga c* | 20.93 | 1.20 | 26,566 |
| *Acanthamoeba lugdunensis c* | 12.17 | 1.16 | 24,132 |
| Blister fluid swabse | *Monkeypox virus a* | 99.80 | 80.07 | 122,427 |
| *Achromobacter pulmonis c* | 77.30 | 27.38 | 1,140,164 |
| *Elizabethkingia meningoseptica c* | 29.20 | 1.28 | 18,240 |
| *Stenotrophomonas maltophilia c* | 24.90 | 1.45 | 18,866 |
| *Acanthamoeba polyphaga c* | 0.0872 | 1.00 | 102 |
| *Acanthamoeba lugdunensis c* | 0.0410 | 1.00 | 91 |

a Potential pathogens

b Colonizing bacteria in the respiratory tract

c Contaminant