Deep shotgun metagenomic analysis of the oral microbiome identifies certain bacterial plasmids associated with IgA nephropathy

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Ryousuke Aoki[1], Kei Ogiwara[1], Nozomi Kadota[1], Yusuke Fukao[1], Hitoshi Suzuki[1],
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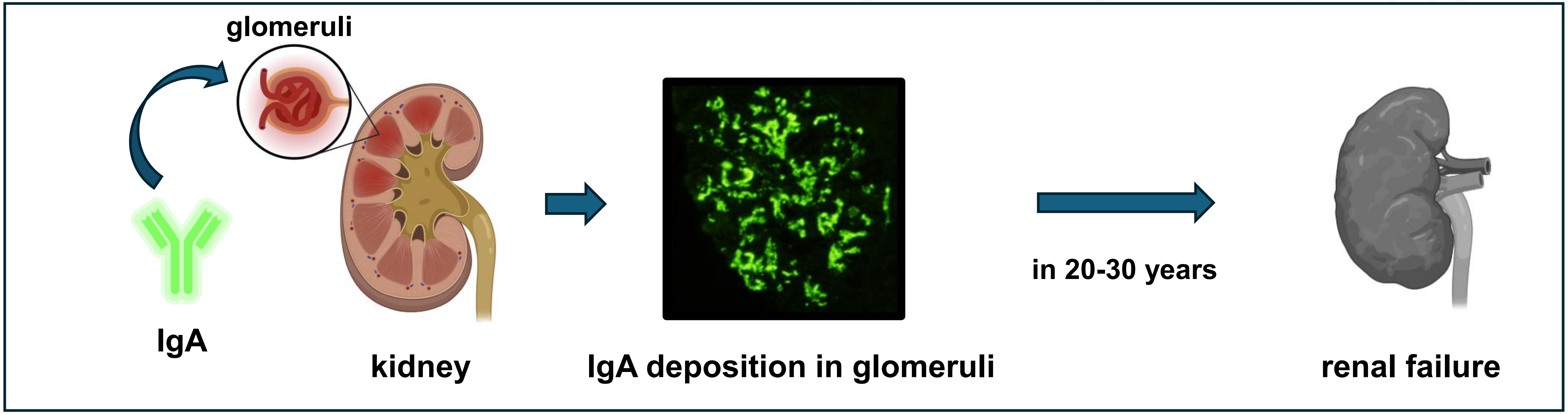
COI disclosure

presenter：　Sho Hamaguchi

Completed training courses on research ethics and medical ethics

I have no relevant financial relationship to disclose any COI for this research presentation within the period of 36 months.

Oral microbiome is involved in the pathogenesis of IgA nephropathy (IgAN)



glomeruli

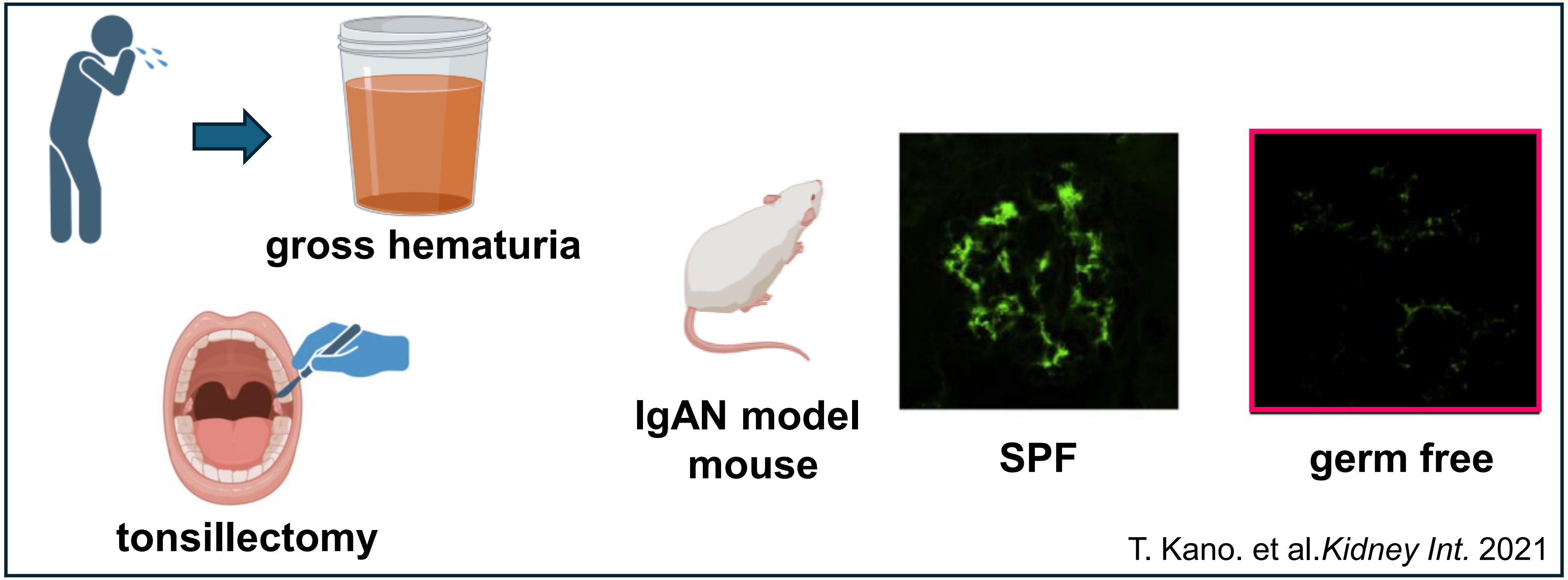
IgA

kidney

IgA deposition in glomeruli

in 20-30 years

renal failure



gross hematuria

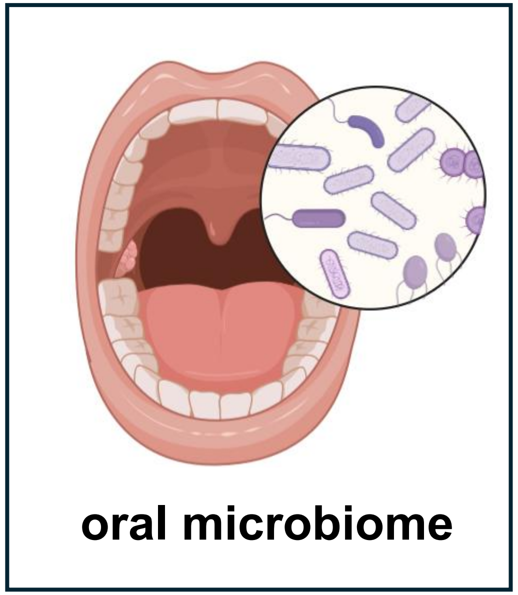
tonsillectomy

IgAN model mouse

SPF

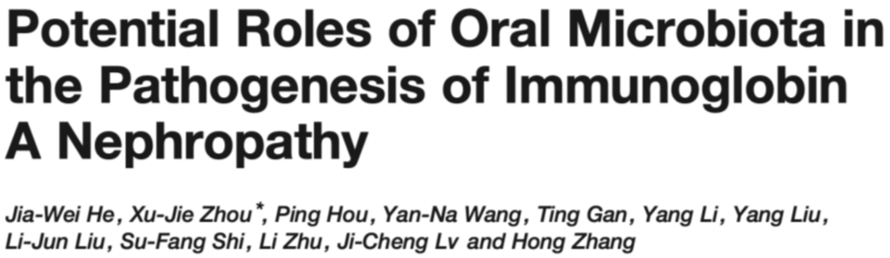
germ free

T. Kano. et al. *Kidney Int.* 2021



oral microbiome

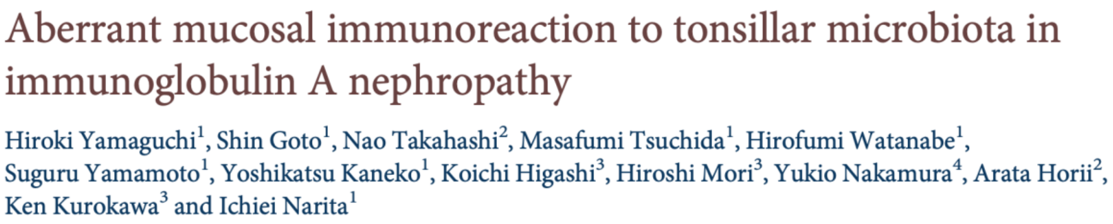
Previous oral microbiome studies based on 16S rRNA analysis have shown inconsistent results



Potential Roles of Oral Microbiota in the Pathogenesis of Immunoglobin A Nephropathy

Jia-Wei He, Xu-Jie Zhou, Ping Hou, Yan-Na Wang, Ting Gan, Yang Li, Yang Liu, Li-Jun Liu, Su-Fang Shi, Li Zhu, Ji-Cheng Lv and Hong Zhang*

***g_Neisseria* ↑**

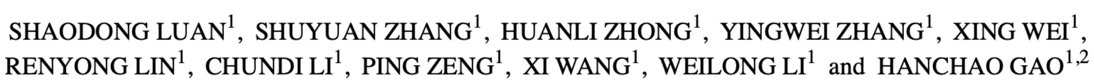


Aberrant mucosal immunoreaction to tonsillar microbiota in immunoglobulin A nephropathy

Hiroki Yamaguchi[1], Shin Goto[1], Nao Takahashi[2], Masafumi Tsuchida[1], Hirofumi Watanabe[1], Suguru Yamamoto[1], Yoshikatsu Kaneko[1], Koichi Higashi[3], Hiroshi Mori[3], Yukio Nakamura[4], Arata Horii[2], Ken Kurokawa[3] and Ichiei Narita[1]

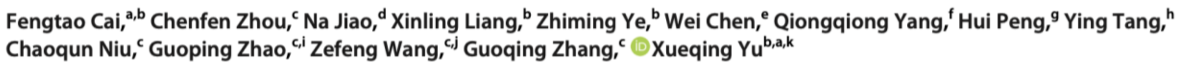
***g_Porphyromonas* ↑**

Is 16S rRNA sequencing truly comprehensive ??



SHAODONG LUAN[1], SHUYUAN ZHANG[1], HUANLI ZHONG[1], YINGWEI ZHANG[1], XING WEI[1], RENYONG LIN[1], CHUNDI LI[1], PING ZENG[1], XI WANG[1], WEILONG LI[1] and HANCHAO GAO[1,2]

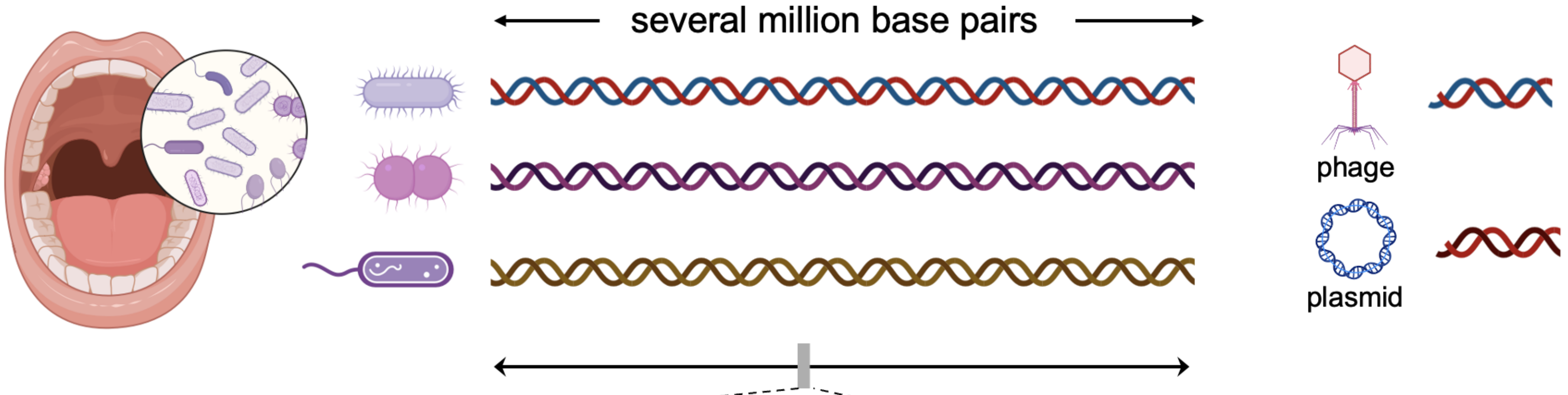
***g_Granulicatella* ↑**



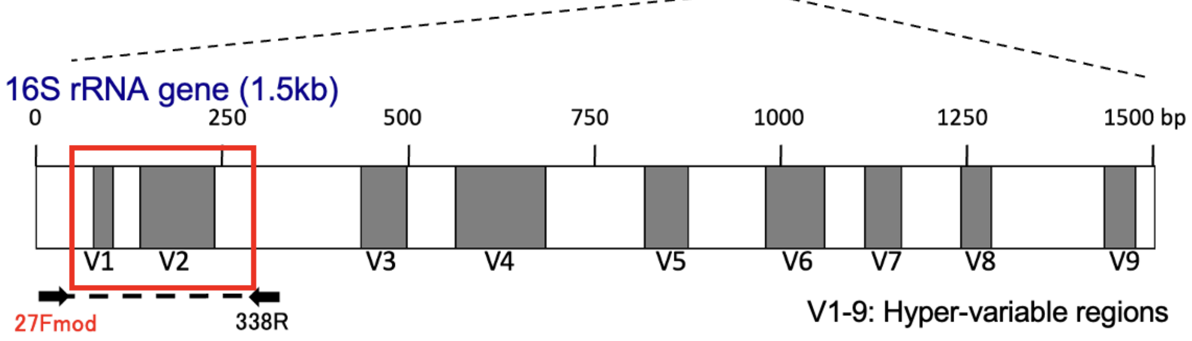
Fengtao Cai,[a,b] Chenfen Zhou,[c] Na Jiao,[d] Xinling Liang,[b] Zhiming Ye,[b] Wei Chen,[e] Qiongqiong Yang,[f] Hui Peng,[g] Ying Tang,[h] Chaoqun Niu,[c] Guoping Zhao,[c,i] Zefeng Wang,[c,j] Guoqing Zhang,[c] Xueqing Yu[b,a,k]

***g_Capnocytophaga* ↑**

Differences between 16S rRNA sequencing and shotgun sequencing



16 rRNA sequencing

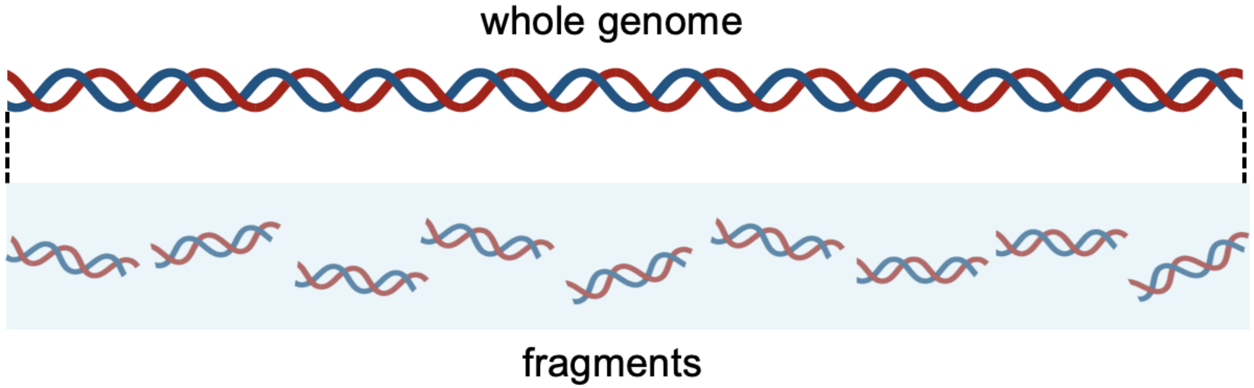


MiSeq (illumina)

Output range

～15Gb

shotgun sequencing

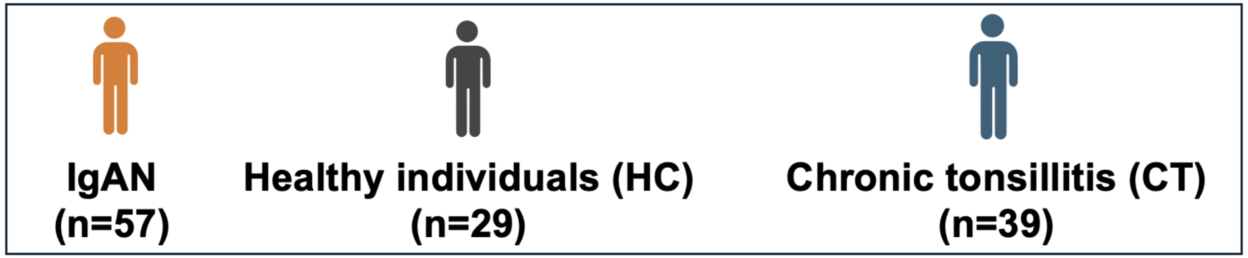


NovaSeq 6000 (illumina)

Output range

～6000Gb

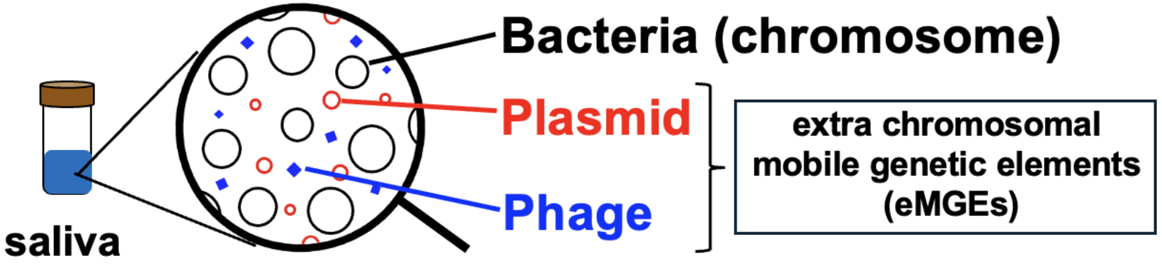
Materials and Methods



IgAN
(n=57)

Healthy individuals (HC)
(n=29)

Chronic tonsillitis (CT)
(n=39)



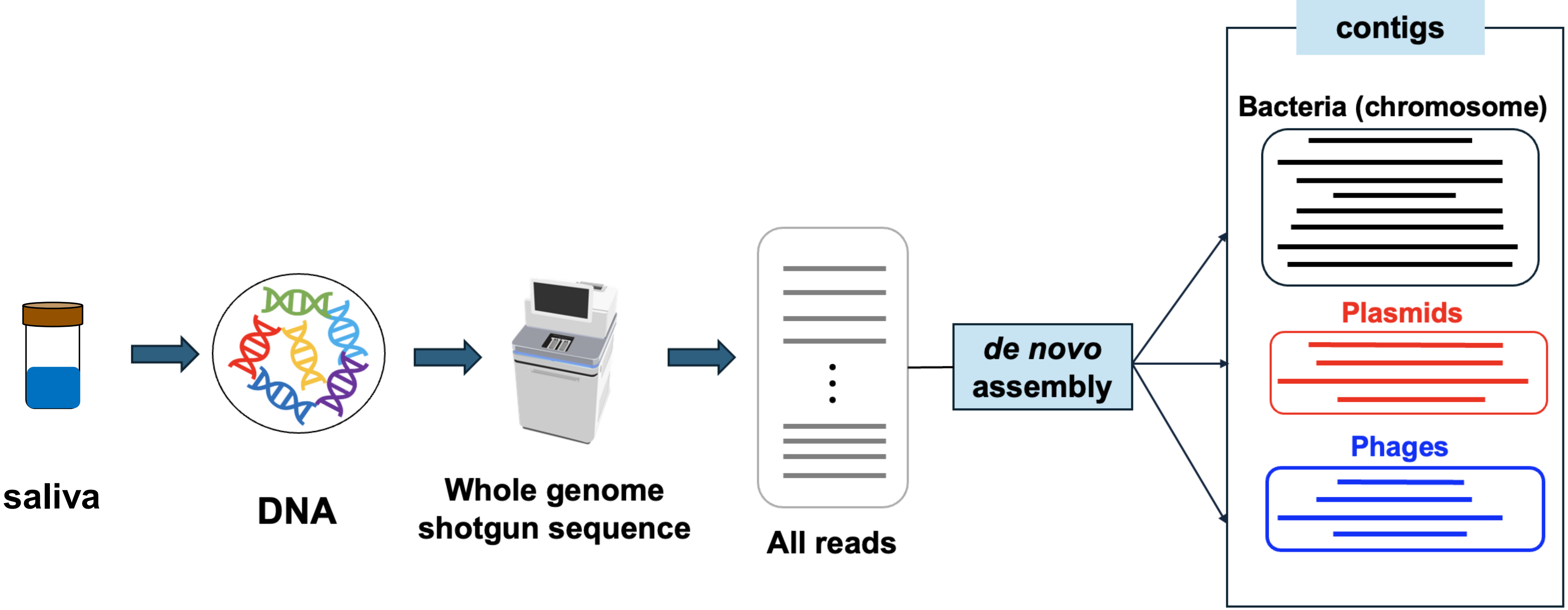
saliva

Bacteria (chromosome)

Plasmid

Phage

extra chromosomal mobile genetic elements (eMGEs)



saliva

DNA

Whole genome shotgun sequence

All reads

***de novo* assembly**

contigs

Bacteria (chromosome)

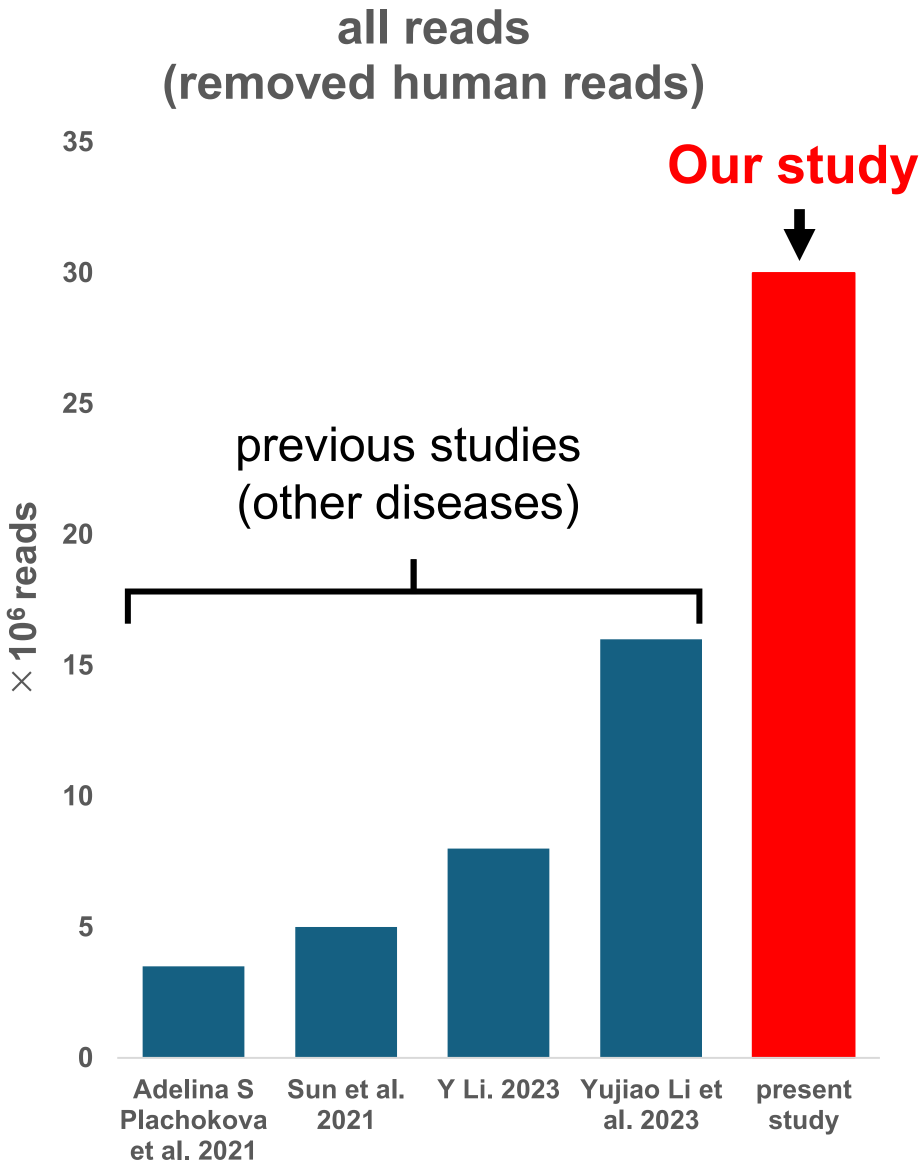
Plasmids

Phages

One of the deepest oral metagenomic sequencing was conducted

Average number of reads	Average number of reads (removed human genomes)
6.7 × 10^7 reads	3.0 × 10^7 reads

human genomes ratio
56.1 %



Our study

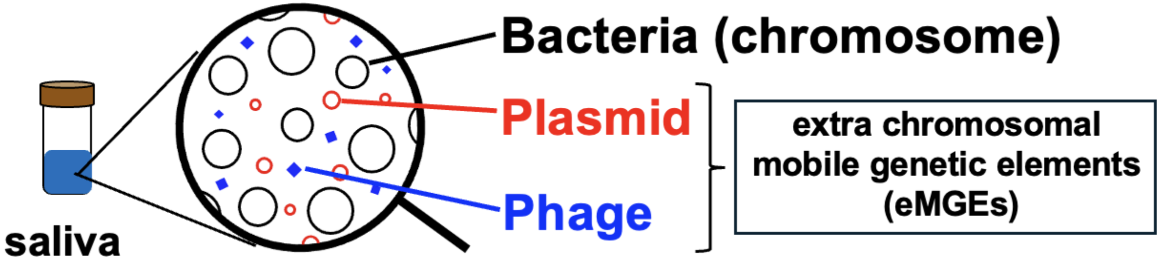
previous studies
(other diseases)

Materials and Methods

IgAN
(n=57)

Healthy individuals (HC)
(n=29)

Chronic tonsillitis (CT)
(n=39)



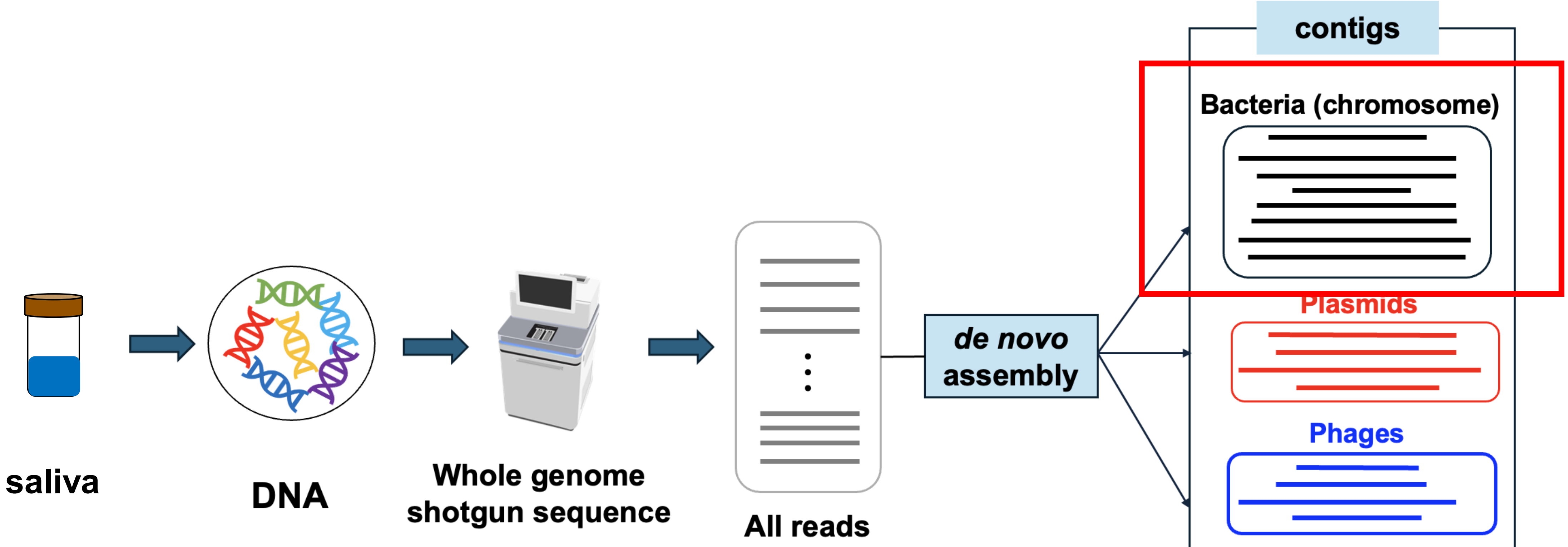
saliva

Bacteria (chromosome)

Plasmid

Phage

extra chromosomal mobile genetic elements (eMGEs)



saliva

DNA

Whole genome shotgun sequence

All reads

***de novo* assembly**

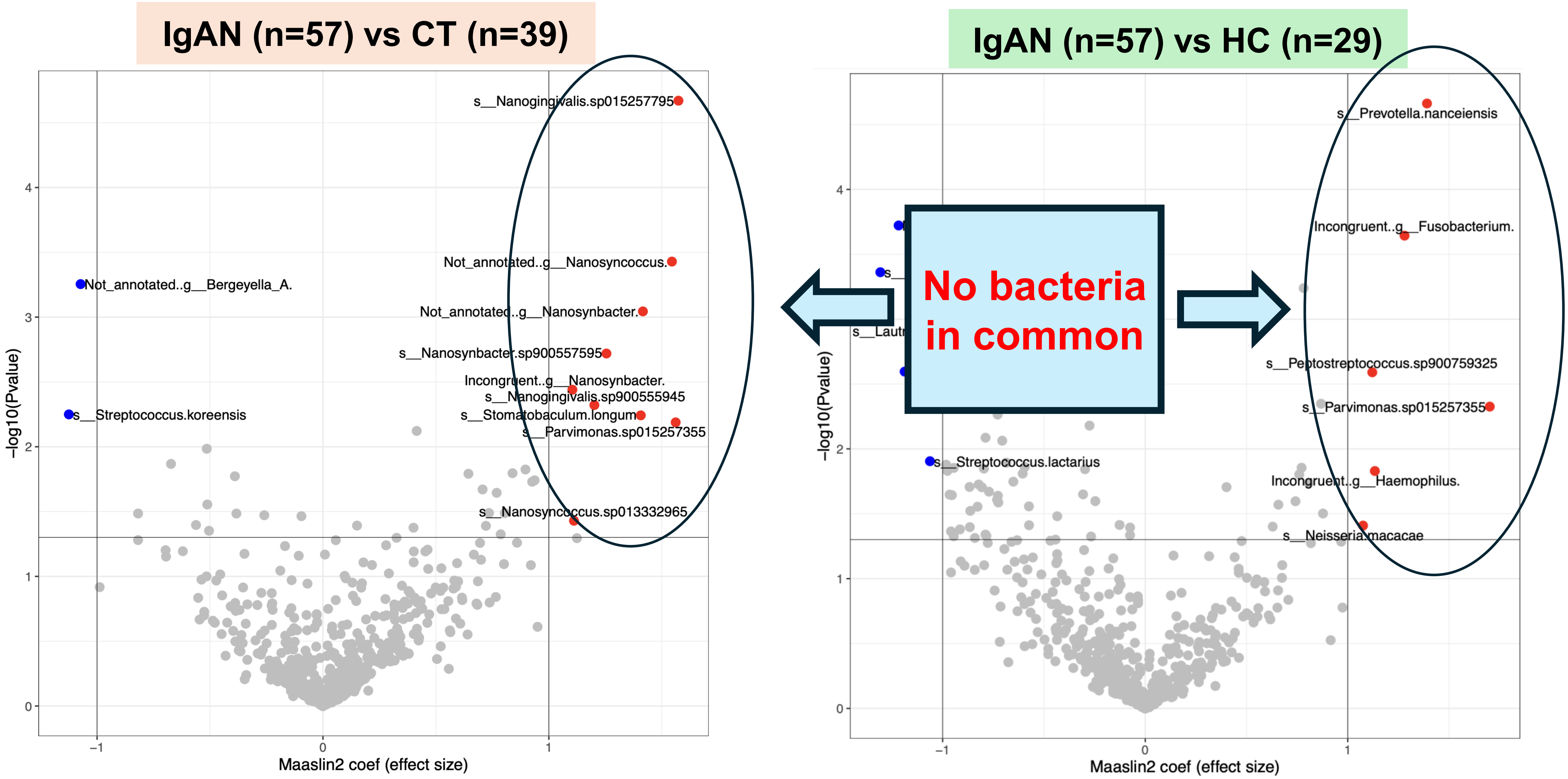
contigs

Bacteria (chromosome)

Plasmids

Phages

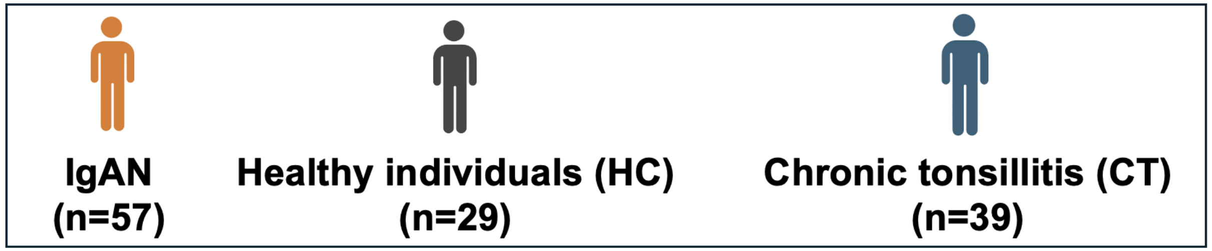
There was no bacterial species which is consistently enriched in IgAN patients



No bacteria in common

Bacterial compositions were calculated using mOTU v3.0.1.
Statistically compared using MaAsLin2.

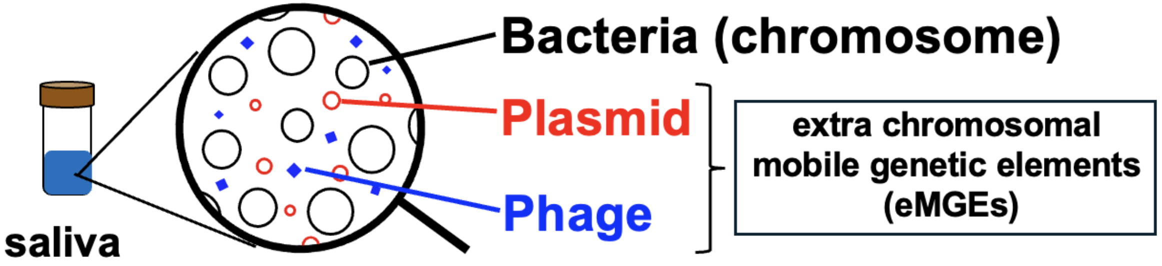
Materials and Methods



IgAN
(n=57)

Healthy individuals (HC)
(n=29)

Chronic tonsillitis (CT)
(n=39)



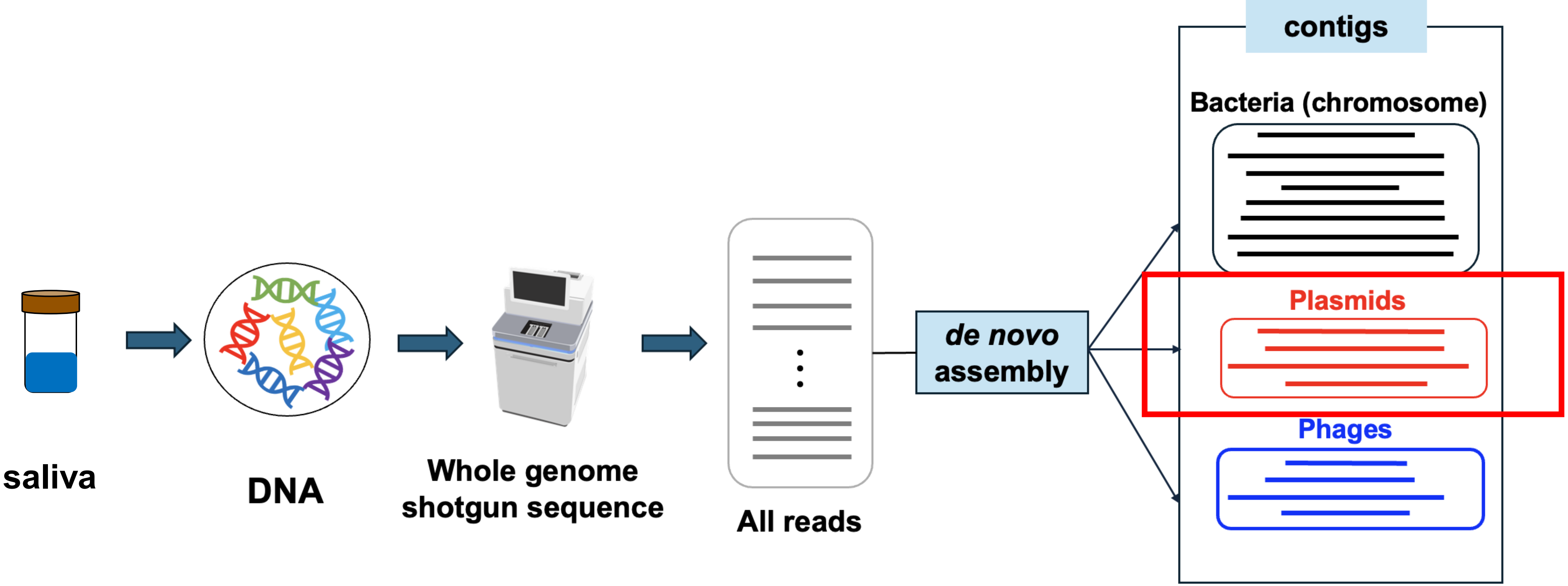
saliva

Bacteria (chromosome)

Plasmid

Phage

extra chromosomal mobile genetic elements (eMGEs)



saliva → **DNA** → **Whole genome shotgun sequence** → **All reads** → ***de novo* assembly** → **contigs**

contigs

Bacteria (chromosome)

Plasmids

Phages

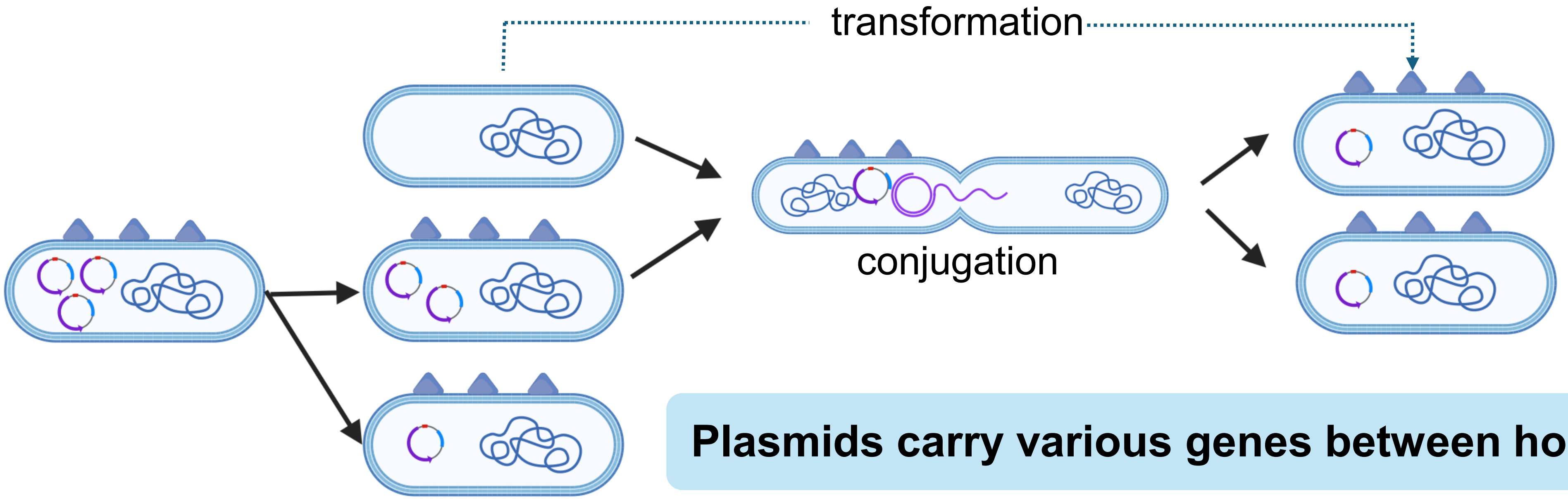
Plasmids horizontally transfer between host bacteria and induce host transformation

◆Mobile elements found in specific genera or species

◆Replicate independently

◆Transfer horizontally between host bacteria

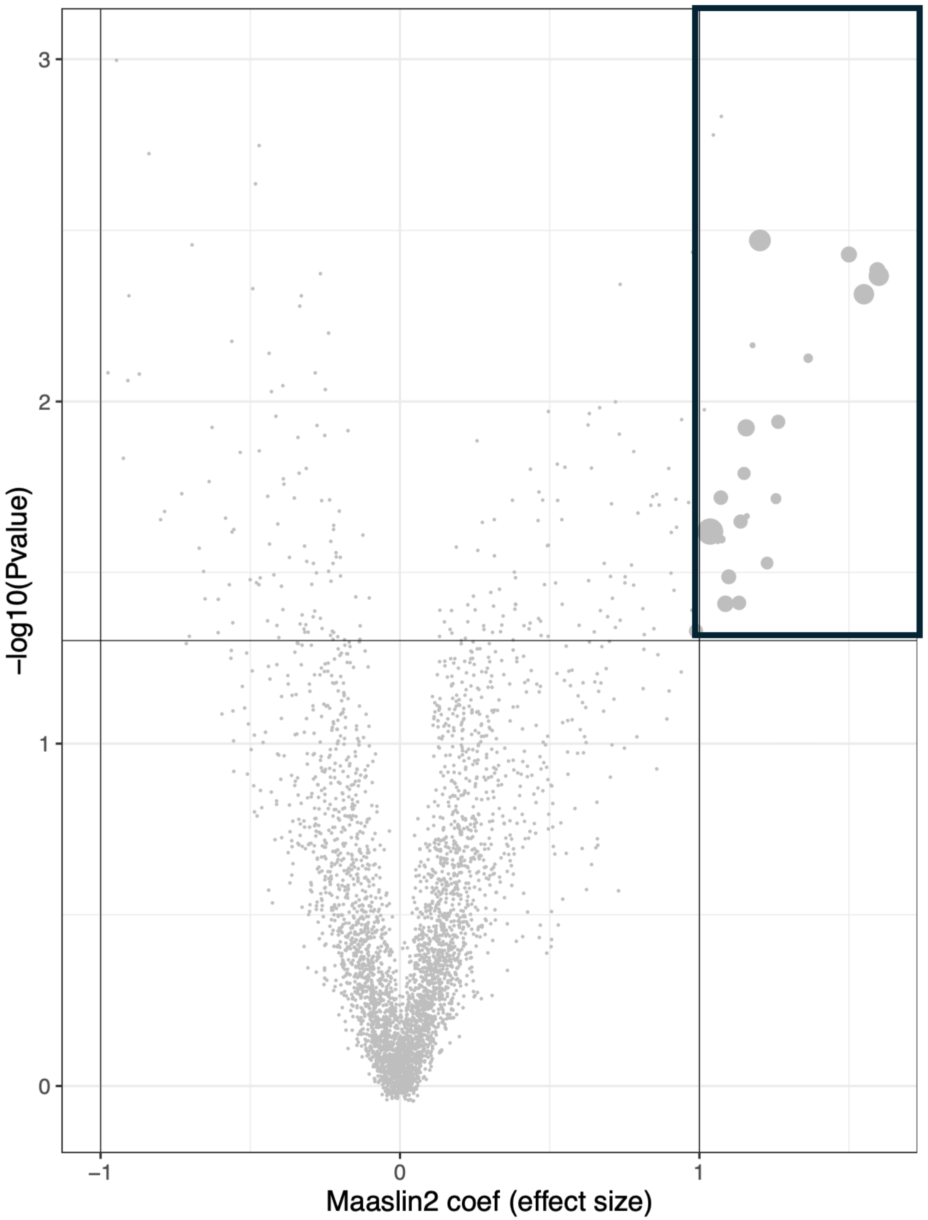
◆Confer antibiotic resistance and virulence genes to their host



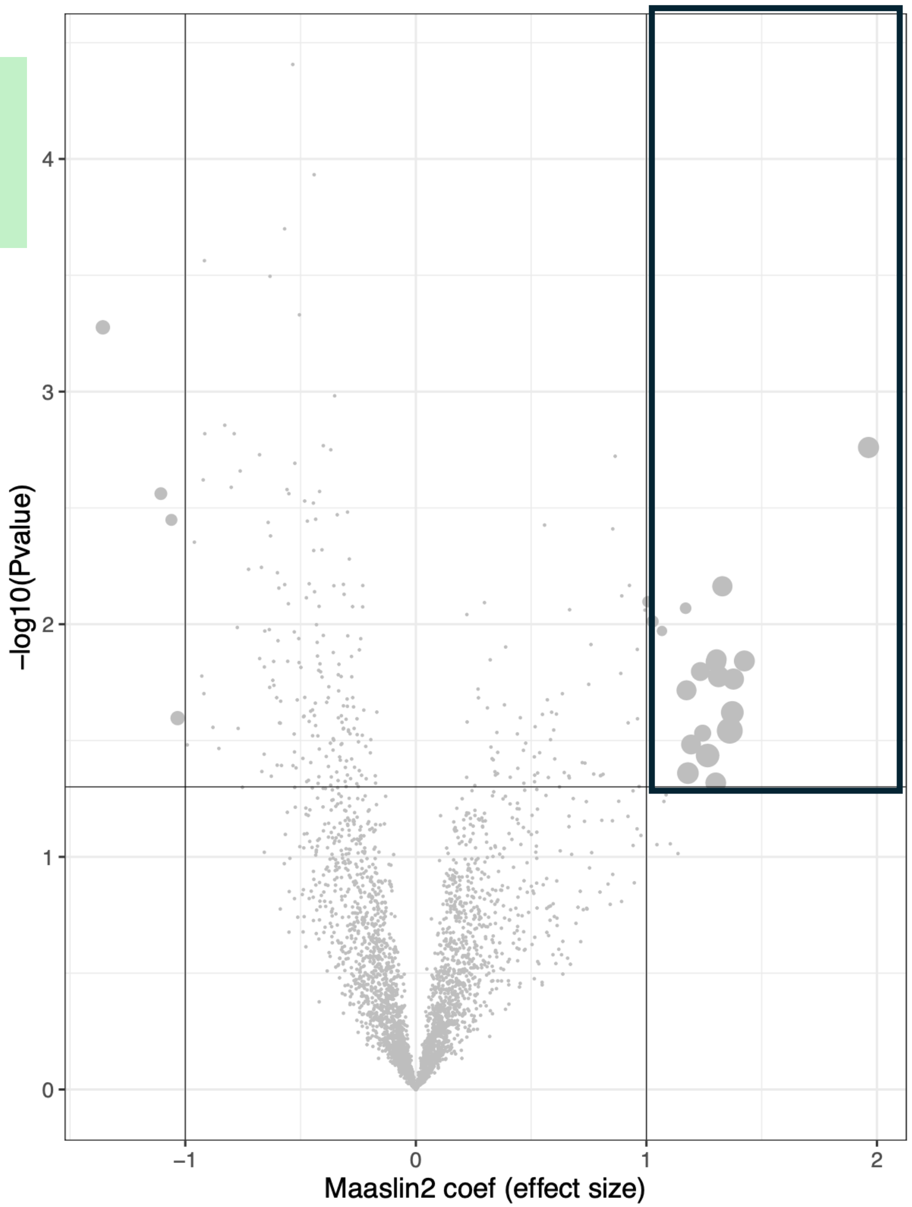
Plasmids carry various genes between host bacteria

Some plasmids were significantly increased in IgAN patients

IgAN (n=57)
vs
CT (n=39)



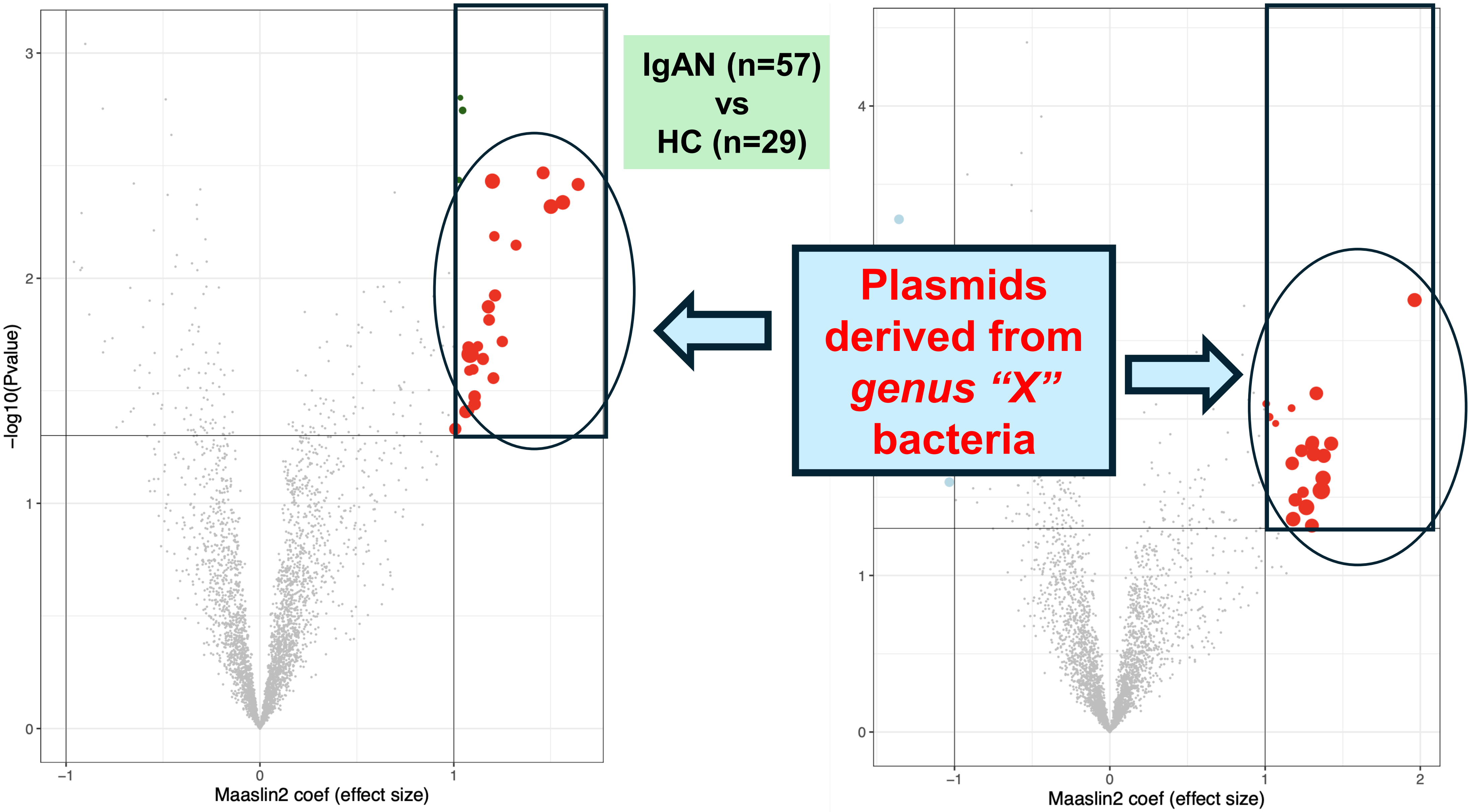
IgAN (n=57)
vs
HC (n=29)



Statistically compared using MaAsLin2

Genus "X" plasmids were consistently increased in IgAN patients

IgAN (n=57)
vs
CT (n=39)

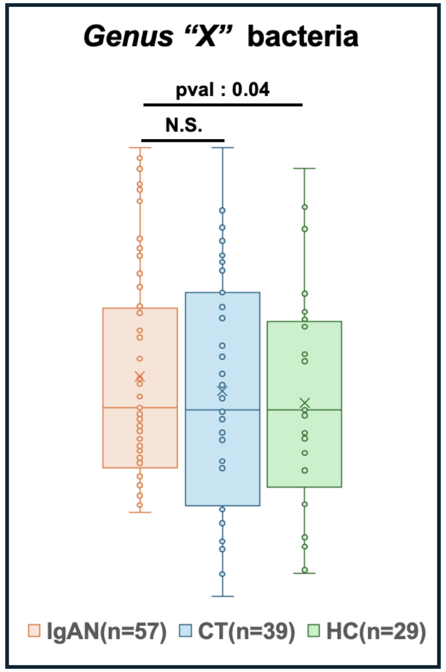


IgAN (n=57)
vs
HC (n=29)

Plasmids derived from *genus "X"* bacteria

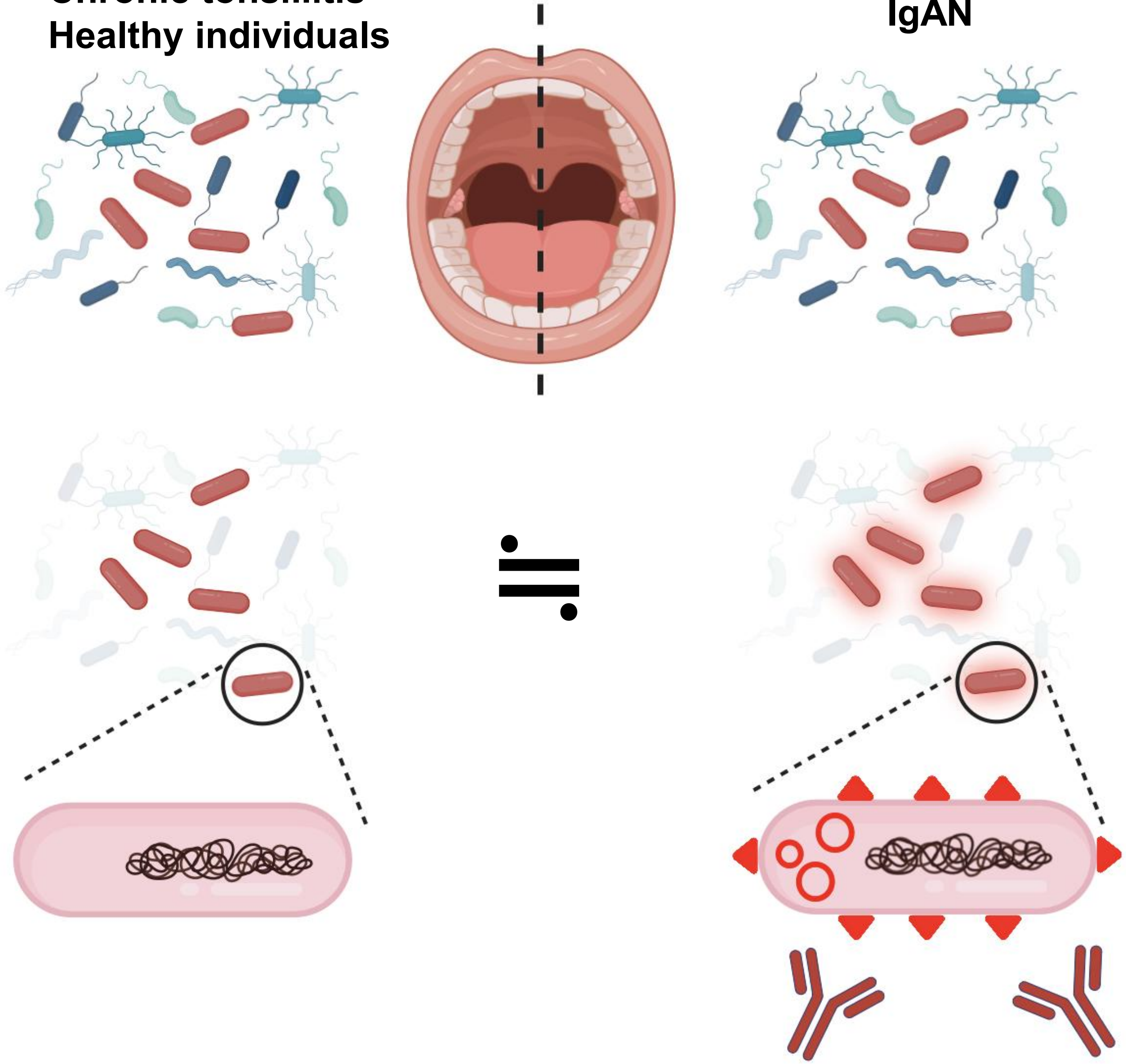
***Genus "X"* plasmids with p-value < 0.05 are colored in red.**

Genus "X" plasmids increased independently of their host bacteria and may contribute to the pathogenesis of IgAN

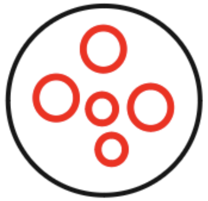


Chronic tonsillitis
Healthy individuals

IgAN



Genus "X" plasmids ↑↑



The relative abundance of *genus "X"* bacteria exhibited minimal variation between groups.

It is possible that the increase in plasmids has led to an expansion of transformed *genus "X"* bacteria.

pathogenic Gd-IgA1