


Is There a Brain Microbiome?

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ABSTRACT: Numerous studies have identified microbial sequences or epitopes in pathological and non-pathological human brain samples. It has not been resolved if these observations are artifactual, or truly represent population of the brain by microbes. Given the tempting speculation that resident microbes could play a role in the many neuropsychiatric and neurodegenerative diseases that currently lack clear etiologies, there is a strong motivation to determine the “ground truth” of microbial existence in living brains. Here I argue that the evidence for the presence of microbes in diseased brains is quite strong, but a compelling demonstration of resident microbes in the healthy human brain remains to be done. Dedicated animal models studies may be required to determine if there is indeed a “brain microbiome.”

KEYWORDS: 16S rRNA, metagenomics, RNA-seq, “kitome”, neurodegenerative disease

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Introduction

The development of high-throughput sequencing has enabled researchers to overcome the requirement to culture microbes (bacteria/archaea, fungi, viruses, etc.) in order to assay their distribution.¹ This has led to dramatic advances in the characterization of the gut microbiome and other niches of the body colonized by microbes.^{2–4} There is now compelling evidence that the gut microbiome has significant effects on human physiology,^{5–7} and these studies have provided mechanistic insights into how this can be driven by metabolites produced by bacteria⁸ or by microbial modulation of the immune system.⁹ Conceivably, microbes could also influence physiology more directly if they can escape their normal niches and populate blood or organs. Here we consider the evidence as to whether this does occur, and specifically whether microbes enter the brain.

What is a “Brain Microbiome”?

By analogy to the gut microbiome, a brain microbiome would consist of a set of microbes that perdure, although not necessarily actively replicate, in the brain. This is in contrast to brain abscesses or encephalopathies, which clearly involve the growth of (often specific) microbes. A brain microbiome would certainly have orders of magnitude fewer microbes than the gut, and would need to be maintained by either a low level breaching of the blood/brain barrier (BBB) by microbes balanced by their active removal, and/or the continuing presence of largely quiescent microbes. While there is evidence that the gut microbiome has co-evolved with the host¹⁰ this would be very unlikely in a putative brain microbiome, as the brain is presumably a “dead end” niche for any microbes that reside there.

Why Even Consider a Brain Microbiome?

A seminal study published by the Power group¹¹ provided intriguing evidence for the presence of microbes in the human

brain. These researchers set out to determine if the brain injury observed in HIV/AIDS was accompanied by microbial infiltration into the brain. Using high-throughput sequencing of total RNA from autopsy-derived cerebral white matter, these investigators found non-human sequences aligning to 173 different bacteria and phage. Critically, these researchers found similar distributions of microbial sequences in both the HIV and control brain samples. α -proteobacteria was the predominant phylum of bacteria identified, and was found in all brain samples tested. These observations were validated by both 16S rRNA gene amplification and *in situ* staining for peptidoglycan and bacterial 16S rRNA sequences. Importantly, these researchers also demonstrated that bacterial sequences detected in a human brain sample by 16S RNA amplification were present in the brains of immuno-compromised mice (Rag^{-/-}) 7 weeks after transplantation of the human brain tissue into the mice. In contrast, parallel transplantation of heat-treated brain tissue into immuno-compromised mice resulted in no or minimal detection of the targeted 16S RNA sequence, suggesting that the 16S RNA sequences detected in the human brain samples were derived from viable bacteria. While this early study used relatively small sample sizes, it provided the types of validation studies needed to counter the common assumption that the brain is sterile.

The Caveats

Microbial sequences seem to be invariably detected in RNA-seq data from human tissue, accounting for 1.4% of total reads in a pooled analysis of samples across 2630 individuals from 54 diverse human tissues.¹² Similarly, all human tissue RNA seq data that my research group has analyzed (eg, from Refs.^{13–15}) contain sequences aligning to identified microbes, typically bacterial rRNA. Multiple research groups have raised the possibility that microbial sequences detected in human tissue by metagenomic sequencing or 16S rRNA-directed PCR result



from contamination.¹⁶⁻¹⁸ There is unquestionably a “kit-ome”: bacterial sequences present in the reagents used for the preparation of DNA and DNA sequencing libraries that can subsequently contaminate data from tissue samples.^{17,18} Combined with a possibly unavoidable low-level contamination occurring in the recovery of the tissue sample itself, it may be impossible to generate brain sequence data that does not contain some artifactual microbial sequences. Experimental¹⁹ and computational²⁰ approaches have been established to minimize consideration of artifactual microbial sequences in RNA or DNA sequence data. A limitation of mining published RNA-seq or DNA datasets for microbes is that these studies were directed toward other goals (eg, gene expression or tumor DNA detection) and typically lack the controls or approaches needed to optimally look for *bona fide* tissue microbes. A recent study that did apply rigorous controls concluded that the human placenta lacked a microbiome, as all microbial sequences identified in metagenomic or 16S sequencing data could be accounted for as batch variation or presence in reagents used.²¹ However, as pointed out in a review of this study,²² demonstrating that contamination can account for identified sequences does not prove that it does, and it remains possible that microbial species that appear in the kitome (eg, *E. coli*) are also present in human tissue.²⁰

Another factor in contemplating a brain microbiome is the consideration that probably all recovered human tissues contain some blood, including the brain white matter²³ analyzed in the Branton et al studies described above.¹¹ The presence of microbes in (healthy) human blood was initially suggested by microscopy studies,^{24,25} but controversy remained as to whether bacteria were truly being visualized.²⁶ However, a large number of 16S rRNA and metagenomic sequencing studies have now reported the presence of bacterial sequences in healthy human blood (recently reviewed in Castillo et al²⁷), and the evidence for bacterial sequences (if not viable bacteria) in blood is now compelling. A quantitative 16S rRNA study done by Paise et al²⁸ reported that healthy human blood could have 10⁶ to 10⁷ bacterial genomes per ml. These estimates readily exceed any reports of what can be cultured from blood, although this could be a result of the limitations of culturing combined with the presence of dormant or inviable microbes. Interestingly, the predominant blood bacterial phylum in this study was α -proteobacteria (as observed in the Branton et al study), not the Firmicutes and Bacteroidetes that predominate in the gut. The likely presence of microbial sequences in blood suggests that even if microbial sequences are identified in brain tissue, additional studies or analyses will be required to determine if these sequences are truly “in” the brain. One such approach is to look at different brain regions in the same subject, with the expectation that if different microbes are identified in different brain regions, it is unlikely they are being recovered from blood. Given the heterogeneity of the BBB,²⁹ and the differential breakdown of this barrier with age³⁰ it would be predicted that

if a brain microbiome exists, the overall “microbial load” may also differ between brain regions.

Microbes in Diseased Brains

Given the possibility that infection plays a role in neurodegenerative diseases, significant efforts have been undertaken to look for microbes in postmortem brain samples, particularly in the context of Alzheimer’s disease (AD).³¹ These studies have used a variety of approaches, including immunocytochemical detection of bacterial and fungal antigens (eg, lipopolysaccharide (LPS) and the *E. coli* K99 pilus antigen,³² gingipains,³³ *Candida albicans* epitopes,³⁴ 16S RNA amplification^{33,34} and analysis of large scale datasets for viral sequences.^{35,36} An important consideration is that detection of microbial antigens is not in and of itself compelling evidence for the presence of intact (live or dead) microbes. For example, macrophages infiltrating a diseased brain could conceivably bring with them phagocytized antigens or microbial sequences they encountered in the periphery. Many of the studies described above report an increase in the target species in the AD cases, as well as finding evidence of microbes in some of the control cases. In the context of a putative brain microbiome, it is hard to discount the increased microbial load in AD as a result of artifactual contamination if control and pathological samples were processed in the same way. At face value, the immunocytochemical studies also demonstrate microbial antigen reactivity in CNS tissue rather than associated capillaries.^{33,34} Unresolved is whether the increased microbial load in AD brains results from a secondary “sick brain” effect. There is strong evidence that the blood brain barrier is compromised in AD and other neurodegenerative diseases³⁷ and thus the presence of microbes in these brains may not reflect their association with healthy brains. Nevertheless, it cannot be ruled out that the increased microbial load in diseased brains is contributed to by an expansion of endogenous microbes, possibly resulting from compromised brain immune functions (eg, altered microglial surveillance).

Brain Viruses

Arguably, there is already a consensus that a human “brain virome” exists, in that sequences from latent neurotropic viruses such as herpes virus have been routinely detected in human brain samples. Although the fraction of healthy brains reported to containing genomic sequences from HSV-1 (28-100%,³⁸⁻⁴⁰) or HSV-6 (2%-70%,^{36,41-43}) varies widely, it appears that in a significant fraction of people these viruses have migrated into CNS tissue.⁴⁴ The role of these latent viruses in pathologies such as AD is currently unresolved,^{36,45} although reactivation of latent HSV-1 infection in mice can replicate pathological hallmarks of AD.⁴⁶

A Dark Brain Microbiome?

Current approaches for identifying microbial sequences in the brain largely rely on searches based on known microbial

sequences (eg, PCR amplification of bacterial/archaeal rRNA using primers to conserved regions) or the unbiased recovery of sequences (eg, by metagenomic high throughput sequencing) that subsequently show nucleotide similarity to previously identified microbes. However, the potential exists that there could be novel brain microbes that are not recovered or identified by standard approaches. While this might seem far-fetched, there is a striking report of novel microbial sequences recovered by *de novo* assembly of sequences obtained in the course of sequencing human cell-free blood DNA.⁴⁷ These researchers identified >3000 novel, long (>1 kb) sequences, and provided convincing evidence that they were neither artificial nor contaminants. They concluded that the human microbiome was significantly undersampled. I note that analysis of human transcriptome data in my lab has also led to the recovery of long non-repetitive sequences that lack significant nucleotide matches in GenBank.⁴⁸ Further characterization of instances of DNA or RNA “dark matter” will be required to determine their biological relevance.

Future Studies

To my knowledge, no studies have been published that look specifically for evidence of a healthy human brain microbiome using unbiased and appropriately controlled methods. It might be particularly informative if this could be done using brain samples representing a range of ages. Although multiple studies have suggested the possibility of microbial sequences in control brains, since these controls are often for age-related neurodegenerative diseases, these control brain samples are from older subjects. If a parallel analysis of brain samples across the lifespan indicated an increasing brain microbial load with age, this would both counter contamination claims and make biological sense, given the established decrease in immune function⁴⁹ and increase in blood brain barrier permeability⁵⁰ with age. Similarly, by analogy with the gut microbiome, it seems likely that brain microbiomes would show significant differences between people. Thus if individual brain samples analyzed in parallel (ie, using the same reagents and run together on a sequencing machine) identified different microbes, this would also argue against contamination. *Disproving* the existence of a brain microbiome is probably a more difficult task, particularly given the background of contaminating sequences (as argued above) that may be hard to avoid. However, if putative brain microbial distributions do not track with any of the factors (age, sex, genetic background, environmental exposure, etc.) known to broadly affect biological outcomes, this would strongly argue against the presence of a *bona fide* brain microbiome.

Human studies preclude interventional approaches that may ultimately be required to convincingly establish both the reality of a brain microbiome and the mechanisms leading to its maintenance. *A priori*, there is no reason to suspect the brain microbiome would be human- or primate-specific. In theory, rodent experiments could be employed to (relatively)

quickly check the correlations between the factors described above and the presence of brain microbes, or to determine if treatment with a brain-penetrant antibiotic (eg, minocycline) altered the recovery of microbial sequences. Brain material from animal models could also be obtained with better control for contamination. An obvious experimental approach would be to look at brain tissue from germ-free mice, which minimally should provide a baseline for the detection of artifactual microbial components. However, the evidence that “germ free” mice (particularly from commercial sources) are really germ free is not compelling - they are not tested by rigorous genomics-based analysis of different tissues (eg, dental tissue), and it remains unclear if the preparation of these animals (ie, by using sterile environments and antibiotics) really depletes all associated microbes. Nevertheless, rodent models might be a better source material for attempting to culture brain microbes. More powerfully, it might be possible to “tag” microbial sequences in rodent tissues, providing additional internal controls and possibly measurement of brain microbial physiology and turnover. One could imagine treating mice with BrdU (as is done visualize brain neurogenesis) and then using BrdU immunoprecipitation to recover newly synthesized DNA. As this label should be incorporated into microbial DNA as well as newly synthesized rodent DNA, this would preferentially recover resident microbial sequences (as opposed to contaminants, which would not be enriched by the immunoprecipitation). By pulse-chase type experiments, it might be possible to measure brain microbe turnover and possibly microbial replication, as inferred from the fraction of microbial reads recovered near origins of replication. Lastly, this approach would allow co-staining experiments (eg, for BrdU and LPS) that could more convincingly demonstrate that microbial epitope staining seen in CNS tissue reflects the true presence of microbes.

Author Contributions

This minireview was conceived and written by CDL.

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REFERENCES

1. Hamady M, Knight R. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome Res.* 2009;19:1141-1152.
2. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med.* 2016;8:51.
3. Knight R, Callewaert C, Marotz C, et al. The microbiome and human biology. *Annu Rev Genomics Hum Genet.* 2017;18:65-86.
4. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med.* 2018;24:392-400.
5. Maruvada P, Leone V, Kaplan LM, Chang EB. The human microbiome and obesity: moving beyond associations. *Cell Host Microbe.* 2017;22:589-599.
6. Xiao H, Kang S. The role of the gut microbiome in energy balance with a focus on the gut-adipose tissue axis. *Front Genet.* 2020;11:297.
7. Zhang X, Zhao LD, Li H. The gut microbiota: emerging evidence in autoimmune diseases. *Trends Mol Med.* 2020;26:862-873.
8. Yadav M, Verma MK, Chauhan NS. A review of metabolic potential of human gut microbiome in human nutrition. *Arch Microbiol.* 2018;200:203-217.

9. Haase S, Haghikia A, Wilck N, Müller DN, Linker RA. Impacts of microbiome metabolites on immune regulation and autoimmunity. *Immunology*. 2018;154:230-238.
10. Groussin M, Mazel F, Alm EJ. Co-evolution and co-speciation of host-gut bacteria systems. *Cell Host Microbe*. 2020;28:12-22.
11. Branton WG, Ellestad KK, Maingat F, et al. Brain microbial populations in HIV/AIDS: alpha-proteobacteria predominate independent of host immune status. *PLoS One*. 2013;8:e54673.
12. Asplund M, Kjartansdóttir KR, Møllerup S, et al. Contaminating viral sequences in high-throughput sequencing viromics: a linkage study of 700 sequencing libraries. *Clin Microbiol Infect*. 2019;25:1277-1285.
13. Shin H, Shannon CP, Fishbane N, et al. Variation in RNA-Seq transcriptome profiles of peripheral whole blood from healthy individuals with and without globulin depletion. *PLoS One*. 2014;9:e91041.
14. Prudencio M, Belzil VV, Batra R, et al. Distinct brain transcriptome profiles in C9orf72-associated and sporadic ALS. *Nat Neurosci* 2015;18:1175-1182.
15. Gagliardi S, Zucca S, Pandini C, et al. Long non-coding and coding RNAs characterization in peripheral blood mononuclear cells and spinal cord from amyotrophic lateral sclerosis patients. *Sci Rep*. 2018;8:2378.
16. Lusk RW. Diverse and widespread contamination evident in the unmapped depths of high throughput sequencing data. *PLoS One*. 2014;9:e110808.
17. Salter SJ, Cox MJ, Turek EM, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol*. 2014;12:87.
18. Mangul S, Yang HT, Strauli N, et al. ROP: dumpster diving in RNA-sequencing to find the source of 1 trillion reads across diverse adult human tissues. *Genome Biol*. 2018;19:36.
19. Eisenhofer R, Minich JJ, Marotz C, Cooper A, Knight R, Weyrich LS. Contamination in low microbial biomass microbiome studies: issues and recommendations. *Trends Microbiol*. 2019;27:105-117.
20. Zinter MS, Mayday MY, Ryckman KK, Jelliffe-Pawlowski LL, DeRisi JL. Towards precision quantification of contamination in metagenomic sequencing experiments. *Microbiome* 2019;7:62.
21. de Goffau MC, Lager S, Sovio U, et al. Human placenta has no microbiome but can contain potential pathogens. *Nature*. 2019;572:329-334.
22. Bushman FD. De-discovery of the placenta microbiome. *Am J Obstet Gynecol*. 2019;220:213-214.
23. Hase Y, Ding R, Harrison G, et al. White matter capillaries in vascular and neurodegenerative dementias. *Acta Neuropathol Commun*. 2019;7:16.
24. Tedeschi GG, Amici D, Paparelli M. Incorporation of nucleosides and aminoacids in human erythrocyte suspensions: possible relation with a diffuse infection of mycoplasmas or bacteria in the L form. *Nature*. 1969;222:1285-1286.
25. McLaughlin RW, Vali H, Lau PC, et al. Are there naturally occurring pleomorphic bacteria in the blood of healthy humans? *J Clin Microbiol*. 2002;40:4771-4775.
26. Mitchell AJ, Gray WD, Schroeder M, et al. Pleomorphic structures in human blood are red blood cell-derived microparticles, not bacteria. *PLoS One* 2016;11:e0163582.
27. Castillo DJ, Rifkin RF, Cowan DA, Potgieter M. The healthy human blood microbiome: fact or fiction? *Front Cell Infect Microbiol*. 2019;9:148.
28. Paise S, Valle C, Servant F, et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. *Transfusion*. 2016;56:1138-1147.
29. Wilhelm I, Nyúl-Tóth Á, Suciu M, Hermenean A, Krizbai IA. Heterogeneity of the blood-brain barrier. *Tissue Barriers*. 2016;4:e1143544.
30. Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron*. 2015;85:296-302.
31. Miklossy J, ed. *Handbook of Infection and Alzheimer's Disease*. IOS Press; 2017.
32. Zhan X, Stamova B, Jin LW, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. *Neurology*. 2016;87:2324-2332.
33. Dominy SS, Lynch C, Ermini F, et al. Porphyromonas gingivalis in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci Adv*. 2019;5:eau3333.
34. Alonso R, Pisa D, Fernández-Fernández AM, Carrasco L. Infection of fungi and bacteria in brain tissue from elderly persons and patients with Alzheimer's disease. *Front Aging Neurosci*. 2018;10:159.
35. Readhead B, Haure-Mirande JV, Funk CC, et al. Multiscale analysis of independent Alzheimer's cohorts finds disruption of molecular, genetic, and clinical networks by human herpesvirus. *Neuron* 2018;99:64-82 e7.
36. Allnutt MA, Johnson K, Bennett DA, et al. Human herpesvirus 6 detection in Alzheimer's disease cases and controls across multiple cohorts. *Neuron*. 2020;105:1027-1035 e2.
37. Sweeney MD, Zlokovic BV. A lymphatic waste-disposal system implicated in Alzheimer's disease. *Nature*. 2018;560:172-174.
38. Jamieson GA, Maitland NJ, Itzhaki RF. Herpes simplex virus type 1 DNA sequences are present in aged normal and Alzheimer's disease brain but absent in lymphocytes. *Arch Gerontol Geriatr* 1992;15 Suppl 1:197-201.
39. Sanders VJ, Felisan S, Waddell A, Tourtellotte WW. Detection of herpesviridae in postmortem multiple sclerosis brain tissue and controls by polymerase chain reaction. *J Neurovirol*. 1996;2:249-258.
40. Gordon L, McQuaid S, Cosby SL. Detection of herpes simplex virus (types 1 and 2) and human herpesvirus 6 DNA in human brain tissue by polymerase chain reaction. *Clin Diagn Virol*. 1996;6:33-40.
41. Cermelli C, Berti R, Soldani SS, et al. High frequency of human herpesvirus 6 DNA in multiple sclerosis plaques isolated by laser microdissection. *J Infect Dis*. 2003;187:1377-1387.
42. Chapenko S, Roga S, Skuja S, et al. Detection frequency of human herpesviruses-6A, -6B, and -7 genomic sequences in central nervous system DNA samples from post-mortem individuals with unspecified encephalopathy. *J Neurovirol*. 2016;22:488-497.
43. Wipfler P, Dunn N, Beiki O, Trinka E, Fogdell-Hahn A. The viral hypothesis of mesial temporal lobe epilepsy-is human herpes virus-6 the missing link? A systematic review and meta-analysis. *Seizure*. 2018;54:33-40.
44. Marocchi ME, Napoletani G, Protti V, et al. Herpes simplex virus-1 in the brain: the dark side of a sneaky infection. *Trends Microbiol*. 2020;28:808-820.
45. Hogestyn JM, Mock DJ, Mayer-Proschel M. Contributions of neurotropic human herpesviruses herpes simplex virus 1 and human herpesvirus 6 to neurodegenerative disease pathology. *Neural Regen Res*. 2018;13:211-221.
46. De Chiara G, Piacentini R, Fabiani M, et al. Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice. *PLoS Pathog* 2019;15:e1007617.
47. Kowarsky M, Camunas-Soler J, Kertesz M, et al. Numerous uncharacterized and highly divergent microbes which colonize humans are revealed by circulating cell-free DNA. *Proc Natl Acad Sci U S A* 2017;114:9623-9628.
48. Melnick M, Gonzales P, LaRocca TJ, et al. Application of a bioinformatic pipeline to RNA-seq data identifies novel viruslike sequence in human blood. *G3 (Bethesda)*. Published online April 29, 2021. doi:10.1093/g3journal/jkab141
49. Nikolich-Zugich J. The twilight of immunity: emerging concepts in aging of the immune system. *Nat Immunol*. 2018;19:10-19.
50. Marques F, Sousa JC, Sousa N, Palha JA. Blood-brain-barriers in aging and in Alzheimer's disease. *Mol Neurodegener*. 2013;8:38.

Highlights

- Microbial sequences have been recovered from human brain tissue by PCR amplification of 16S rRNA DNA and by high throughput sequencing.
- Low level contamination of brain samples occurring during their preparation can potentially account for the recovery of microbial sequences, although the presence of microbial sequences is not *de facto* proof of contamination.
- The association of increased microbial sequences or epitopes with pathological (eg, Alzheimer) brains is unlikely to be a result of sample contamination.
- The contention that the human brain is sterile has not been rigorously tested.

Outstanding Questions

- Would a well-controlled analysis of a cohort of healthy brain samples (ideally representing a wide age spectrum) be able to discredit the presence of a brain microbiome?
- If there are brain-associated microbes, can they be accurately quantified, and their viability assayed?
- Are microbial sequences associated with brain tissue actually in the brain, or are they from associated blood vessels?
- Can brain tissue be screened for microbes that do not have a nucleotide match (ie, significant BLASTN score) to previously identified microbes?
- Can rodent models be used to confirm the presence of a brain microbiome in mammals, and if so, can the physiology of the brain microbiome be characterized?

- If there is a brain microbiome, does it matter? Specifically, if there are low numbers of transient or dormant microbes in the brain, does this actually have any affect on brain function?

Glossary

16S rRNA - In this review, this refers to the gene sequences encoding the small subunit ribosomal RNA of bacteria. PCR amplification of this DNA with primers directed to highly conserved regions of the 16S RNA genes allows recovery of even low levels of diverse rRNA sequences present in a biological sample.

high throughput sequencing - Use of massively parallel DNA sequencing (eg, on instruments developed by Illumina) to decode sequences present in complex DNA mixtures. This can be used to determine the origins of 16 rRNA

sequences recovered by PCR, or of unamplified DNA purified from samples.

metagenomics - The study of gene sequences from a community of organisms. This often involves computational *de novo* assembly of complex DNA mixtures from a community of microorganisms (eg, the gut, environmental samples) to identify the resident organisms.

RNA-seq - High throughput sequencing of DNA prepared by reverse transcription of complex RNA samples (eg, all RNA present in a cell or tissue). Typically used to determine transcript abundance and infer levels of gene expression.

α -proteobacteria - a diverse phylum of gram negative bacteria that includes free-living, endosymbiont, and pathological members.

cell free DNA - DNA extracted from biological specimens (typically blood) after removal of all cells. Used for liquid cancer biopsies.