The University of Louisville can trace its history back to the establishment of the Jefferson Seminary in 1798.

During the early 19th century, the growing importance of water transportation, coupled with a strategic location at the falls of the Ohio River, made Louisville a commercial hub for the South and West. As the city's population and economy advanced, so did its institutions. In 1837 the Louisville Medical Institute opened for its first class, and the next year, the city created the Louisville Collegiate Institute. The two schools had no official connection, but they both manifested the city's dream of intellectual as well as mercantile leadership. In 1844 the Collegiate Institute inherited property from the now-defunct Seminary.

The Louisville Medical Institute attracted large enrollments and prospered financially, but the Collegiate Institute struggled to remain open. Proponents of grassroots democracy wanted to combine the schools and divert a portion of the medical school's resources to the college. They won a partial victory in 1846 when the General Assembly merged the Medical and Collegiate Institutes as the University of Louisville. The new charter offered complete vindication to neither interest. It created a common board of trustees, but each division retained financial autonomy. The medical school continued to prosper, but as the liberal arts division struggled and failed to remain open, the newly added law school brought in a respectable number of students.

Most of the professors in the medical and law schools were drawn from the ranks of local physicians and attorneys who considered teaching a part-time vocation. By the 1880s and 1890s, however, the University felt some pressure from the educational reformers who not only believed schools should employ full-time instructors, but also advocated for well-enforced, national standards for academic training. In 1907, this trend contributed to the revival of the College of Arts and Sciences, which had been all but forgotten for most of the 19th century. As the university added new programs such as the Graduate School (1915), School of Dentistry (1918), Speed Scientific School (1925), Louisville Municipal College (1931), School of Music (1932), and Kent School of Social Work (1936), conformity to accreditation guidelines became increasingly important. Expanded academic programs and the adherence to higher educational standards led to the appointment of full-time administrators early in the 20th century.

World War II and the postwar era brought major changes to the University of Louisville. Shortly after the war, a movement began to close the all-black Louisville Municipal College and desegregate the University on all levels. This was accomplished in 1950 and 1951. The School of Business was established in 1953. Perhaps the most dramatic development of the postwar period was the movement of taxpaying citizens from the city to the suburbs. Since the University of Louisville was municipally-funded, this caused a damaging drain on the school's revenue. As early as 1965, a governor's task force suggested the possibility of the University of Louisville joining the state system of higher education, which it did in 1970.

Another major development occurred in 1994, when the Kentucky Council on Postsecondary Education designated University of Louisville as a research university, assigning it a statewide mission in medicine, dentistry, law and urban affairs.

In 1997, the state legislature revised the university's mission statement. University of Louisville was charged with becoming a "premier, nationally recognized metropolitan research university." The state also established the Research Challenge Trust Fund, which pumped more than $118 million into research at University of Louisville, helping the university attract and retain a number of nationally recognized researchers and their teams. The fund, also known as "Bucks for Brains," has helped University of Louisville attract almost $400 million in extramural funding, created 1,500 new jobs and had an economic impact of almost $850 million.

One of the oldest metropolitan universities in the United States, University of Louisville has a long history of dedication to the needs of the Louisville urban area. In 2007, the university reaffirmed that mission through implementation of its Signature Partnership initiative, which formalizes its commitment to improving the lives of residents of some of Louisville's most disadvantaged neighborhoods. University of Louisville faculty, staff and students now support the city through hundreds of programs and partnerships in the areas of health, economic development, education and social and human services. In 2019, the university's presence in West Louisville was expanded to include a newly remodeled building in the Portland neighborhood, dedicated to additional space for the College of Arts & Sciences' Fine Art and Anthropology programs.

Recent years have seen the university set new marks for total degrees awarded, graduation rates, minority enrollments, and average ACT score for entering students. UofL is regularly recognized for its commitments to social justice, diversity and inclusivity, with the university named a national leader for its programs to support and promote veterans, GLBTQ students, minority students, environmental sustainability, and more. The university is the state and national leader in producing Fulbright scholars, with over 120 UofL student-winners since 2003.

The university continues to grow and innovate to meet the changing needs of the Commonwealth. New academic programs and innovative forms of instruction further UofL's place in helping meet the needs of students and of the 21st-century economy. Expanded and improved campus facilities include new housing options, the Student Rec Center, updates to the Floyd Street corridor, expansion and remodeling of the Student Activity Center, expanded and updated facilities on the Health Sciences Campus, and the Belknap Academic Building—over 150,000 square feet of new space including new state-of-the-art classrooms and laboratories, group study areas, gathering spaces, and a Student Success Center—which opened in fall 2018.

2018 also saw the inauguration of Dr. Neeli Bendapudi as university president. Dr. Bendapudi is the university’s 18th president and first female permanent president. She leads a university community made up of over 2,500 faculty, 4,400 staff, and 22,000 students all working together to make UofL a great place to learn, work, and invest.