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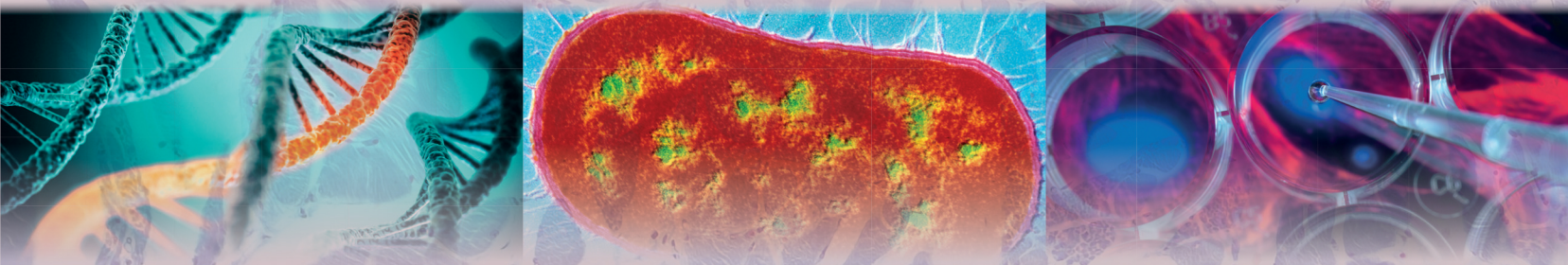
Practical Skills in BIOMOLECULAR SCIENCES

Sixth Edition



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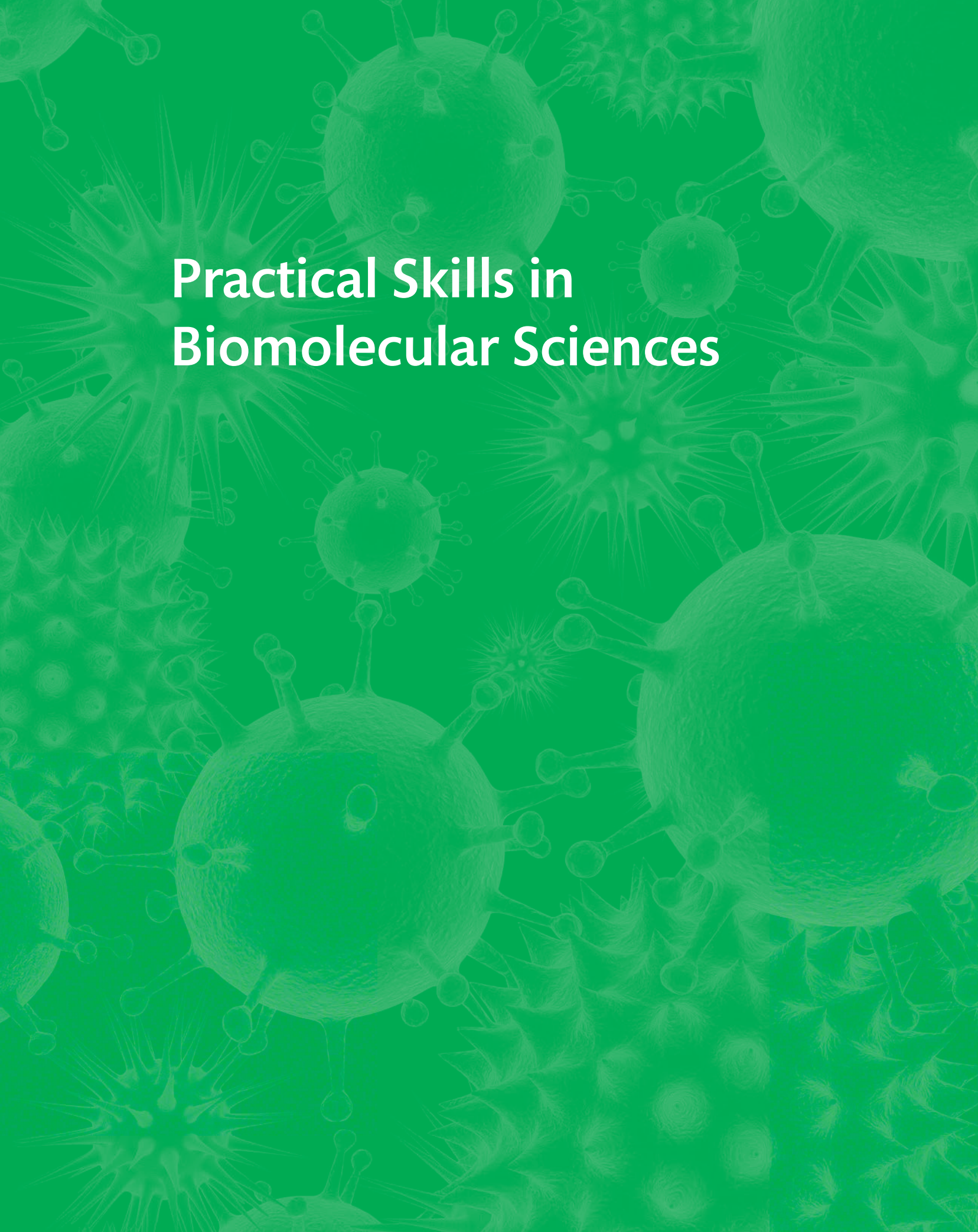

Practical Skills in Biomolecular Sciences

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Contents

Preface to the 6th edition

The primary aim of this revision of *Practical Skills in Biomolecular Sciences* was to update the text, but we also wished to respond to the helpful comments of several anonymous reviewers of the 5th edition, and in so doing, to reorganise the chapters and include significant new material. The main structural changes we have made are to (1) reorder and rewrite several chapters in the first two sections; (2) add three new chapters on working with bacteria, eukaryotic microbes and viruses; and (3) revise the material on use of software and online sources, to reflect the greater level of knowledge and experience of today's students. In terms of the text itself, we have sought to use more positive phrasing throughout, to emphasise the active nature of learning in this discipline. Some details of further changes and additions are listed on the back cover. The text references and sources for further study have been updated, while the popular study exercises have been retained.

We thank everyone who helped us with earlier editions, and for this one acknowledge in particular the assistance of Professor Gary Black, for feedback on the chapters dealing with molecular genetics, Jill Muller of CQUniversity in helping to revise the material on finding and citing sources, Lou Attwood for her work in copy editing the text, and Indrasena Mukhopadhyay and Nikhil Kumar in coordinating the production of the text and images, together with other staff who were involved in the book's production. We also recognise Rufus Curnow for his enduring support of all of the *Practical Skills* titles. Although this revision has largely been the work of two of the original authors (RHR and JDBW), we thank Allan Jones and Dave Holmes for their contributions to the *Practical Skills* series throughout the years. Finally, we thank staff at all institutions who have adopted this text. Practical education across the life sciences has come under increasing pressure in recent years, with diminishing resources and timetabling allocation. Yet such changes cannot alter the fundamental fact that subjects such as biochemistry, biomedical sciences, genetics, microbiology and molecular biology are practical in nature, where students learn most effectively through 'hands-on' experience in the lab and the field. We hope that this book will help students to prepare better for practicals, projects, lectures, seminars, examinations and assignments, to gain greater enjoyment from taking part in them and to learn more about the nature of living systems at the cellular and molecular level.

The book is divided into several sections:

- **Chapters 1–8 cover general skills**, including self-management and personal development; how to learn; teamwork; and how to locate, evaluate and cite sources.
- **Chapters 9–18 deal with assessment,** including written assignments; practicals and projects; oral and poster presentations; revision and examinations.
- **Chapters 19–70 cover a broad range of specific practical skills and analytical techniques**, ranging from basic laboratory procedures to more advanced techniques.
- **Chapters 71–77 explain data analysis and presentation,** ranging from the presentation of results as graphs or tables through to the application of statistical tests, with worked examples.
- **Study exercises and problems** are provided for each chapter. They enable you to check your understanding and to practice key calculations, either on your own, under the guidance of a tutor, or working with other students. Numerical and text-based answers are provided at the book's website at: go.pearson.com/uk/he/resources.

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April 2021
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List of abbreviations

A	absorbance (e.g. A_{260} = absorbance at 260 nm)
AC	affinity chromatography
ACDP	Advisory Committee on Dangerous Pathogens
ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BSA	bovine serum albumin
CCCP	carbonylcyanide *m*-chlorophenylhydrazone
CE	capillary electrophoresis
CFU	colony-forming unit
CGE	capillary gel electrophoresis
COSHH	Control of Substances Hazardous to Health
COVID	coronavirus disease
CRISPR-Cas	clustered regularly interspaced short palindromic repeat-CRISPR-associated
CTP	cytosine triphosphate
CZE	capillary zone electrophoresis
ddNTP	dideoxyribonucleotide triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
d.p.m.	disintegrations per minute
dsDNA	double-stranded DNA
ECD	electron capture detector
EDTA	ethylenediaminetetraacetic acid
EI	electron impact ionisation
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EMR	electromagnetic radiation
EOF	electro-osmotic flow
ESR	electron spin resonance
F	Faraday constant
FIA	fluorescence immunoassay
FID	flame ionisation detector
FITC	fluorescein isothiocyanate
FPLC	fast protein liquid chromatography
FT	Fourier transformation
g	acceleration due to gravity
GC	gas chromatography
GPC	gel permeation chromatography
HEPES	*N*-[2-hydroxyethyl]piperazine-*N*'-[ethanesulfonic acid]
HIC	hydrophobic interaction chromatography
HPLC	high-performance liquid chromatography
IEC	ion-exchange chromatography
IEF	isoelectric focusing
Ig	immunoglobulin
IMAC	immobilised metal affinity chromatography
IR	infrared (radiation)
IRGA	infrared gas analyser
IRMA	immunoradiometric assay
IRMS	isotope ratio mass spectroscopy
ISE	ion-selective electrode
K_m	Michaelis constant
K_w	ionisation constant of water
LCB	lactophenol cotton blue
LDH	lactate dehydrogenase
LSD	least significant difference
MEKC	micellar electrokinetic chromatography
MPN	most probable number
M_r	relative molecular mass
MRI	magnetic resonance imaging
MS	mass spectrometry
NAD^+	nicotinamide adenine dinucleotide (oxidised form)
NADH	nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	nicotinamide adenine dinucleotide phosphate -(oxidised form)
NADPH	nicotinamide adenine dinucleotide phosphate -(reduced form)
NH	null hypothesis
NMR	nuclear magnetic resonance
PAGE	polyacrylamide gel electrophoresis
PAR	photosynthetically active radiation
PCR	polymerase chain reaction
PDP	personal development planning
PEG	polyethylene glycol
PFD	photon flux density
PFGE	pulsed field gel electrophoresis
PFU	plaque-forming unit
pH	$-\log_{10}$ proton concentration (activity), in $\mathrm{mol\,l^{-1}}$
PI	photosynthetic irradiance
PPFD	photosynthetic photon flux density
PPi	pyrophosphate (inorganic)
PVA	polyvinyl alcohol
PY-MS	pyrolysis-mass spectrometry
R	universal gas constant
RCF	relative centrifugal field
R_F	relative frontal mobility
RIA	radioimmunoassay
RID	radioimmunodiffusion
RNA	ribonucleic acid
RP-HPLC	reverse phase high-performance liquid -chromatography
r.p.m.	revolutions per minute
RT	reverse transcriptase
SARS-CoV	severe acute respiratory syndrome-coronavirus
SDS	sodium dodecyl sulfate

SE	standard error (of the sample mean)
SEM	scanning electron microscopy
SI	Systeme International d'Unités
ssRNA	single-stranded RNA
STP	standard temperature and pressure
TCA	trichloroacetic acid
TCD	thermal conductivity detector
TEM	transmission electron microscopy
TEMED	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine
TLC	thin-layer chromatography
TRIS	tris(hydroxymethyl)aminomethane
TTP	thymidine triphosphate
UNG	uracil-*N*-glycosylase
URL	uniform resource locator
UV	ultraviolet (radiation)
V_{max}	maximum velocity
z	net charge on an ion

Study strategies

1 Developing your skills

Using skills terminology – different phrases may be used to describe skills and associated personal qualities, depending on place or context. These include: 'graduate attributes', 'transferable skills', 'personal transferable skills' (PTS), 'key skills', 'core skills' and 'competences'.

A degree in the biomolecular sciences covers both knowledge and skills. Both are integral to your future qualification: along with your personal attributes, they are what your future employer will be looking for when they hire you. There are many possible classifications of skills and your university may favour a specific approach. One possible division relevant to all biological sciences is into generic and practical skills (Fig. 1.1).

Generic skills are, by definition, those applicable in a range of study and work scenarios. For example, self-management, cognitive (thinking) and interpersonal skills are central to the notion of university education and becoming a graduate. Practical skills have a hands-on aspect and a direct relevance to study, research and employment in the biomolecular sciences. For example, the skills involved in measuring pH (Chapter 24) are relevant at all levels, in fields as diverse as cell and tissue culture, assaying metabolites and molecular genetics.

The phrase 'Practical Skills' in the title of this book implies a focus on laboratory skills, and the text covers a broad range of these in some detail. However, many generic skills are also covered, because they too are essential in any future career as a biomolecular scientist. A good understanding of these skills will help you to succeed and to place your studies within a wider context.

KEY POINT All biomolecular sciences are essentially practical subjects, and therefore involve highly developed laboratory skills. The importance that your lecturers place on practical skills will probably be evident from the large proportion of time you will spend on practical work in your course.

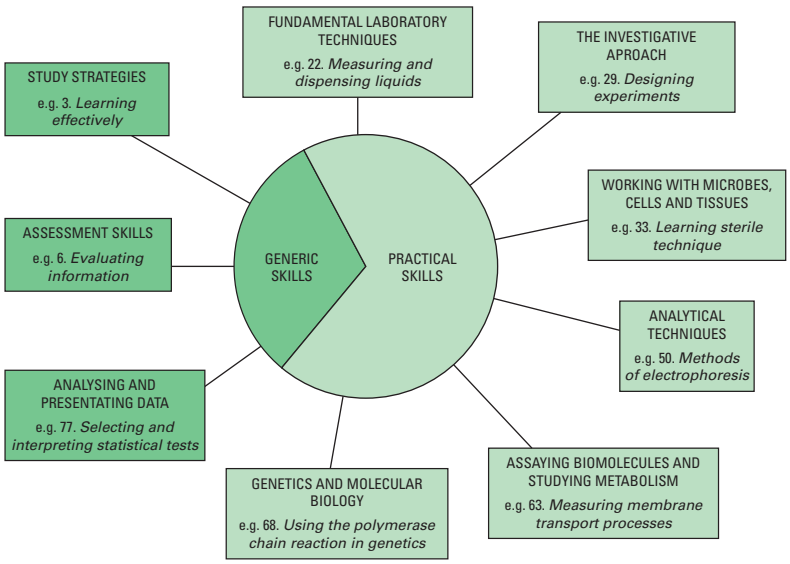

Fig. 1.1 An outline of skills relevant to biomolecular scientists, as covered in this textbook. The skill sub-categories (rectangular boxes) on each side correspond to the book's section headings, and representative chapter titles are shown for each section. Generic skills are mainly covered in the first two sections and in the last section (a total of 25 chapters), whereas the bulk of the book (comprising six sections and 52 chapters) covers practical skills. For the full section and chapter listing, see pp. v–vii.

Finding out about the skills covered in your studies – programme (degree, course) and module (unit) handbooks for your subject will draw attention to the skills elements of your course. Usually the learning outcomes (objectives) will summarise the skills that are covered (Chapter 3). While the precise topics and related skills covered in lectures and practicals will reflect the interests, expertise and experience of lecturers, the curriculum must also adhere to national standards. In the UK, these are laid out in a 'Subject Benchmark Statement' published by the Quality Assurance Agency for Higher Education and they are inspected through rigorous institutional and programme review procedures. Appendix 1 provides a listing of skills from this Benchmark Statement for the Biosciences, showing where these are covered in this book.

Example
The skills involved in teamwork cannot be developed fully without a deeper understanding of the interrelationships involved in successful groups. The context will be different for every group and a flexible approach will always be required, according to the individuals involved and the nature of the task.

Taking opportunities to develop and practise skills in your private or social life – you could, for example, practise spreadsheet skills by organising personal or club finances using Microsoft *Excel*, or teamwork skills within any university clubs or societies you may join.

Identifying the range of skills relevant to biomolecular sciences

The biomolecular sciences cover a wide range of topics, including studies on whole organisms, cells and molecules. Accordingly, the range of skills involved is extremely large. To accommodate this diversity, this book is divided into nine sections dealing with related skill areas (Fig. 1.1) with a total of 77 chapters, each covering a different topic in detail.

The word 'skill' implies much more than the robotic learning of, for example, a laboratory routine. Of course, some of the tasks you will be asked to carry out in practical classes will be repetitive. Certain techniques require manual dexterity and attention to detail if accuracy and precision are to be attained, and the necessary competence often requires practice to make perfect. However, a deeper understanding of the context of a technique is important if the skill is to be appreciated fully and then transferred to a new situation. That is why this text is not simply a 'recipe book' of methods and why it includes background information, tips and worked examples, as well as study exercises to test your understanding.

Reflecting on the transferability of skills

The term 'transferability' is often used in relation to skills to imply that someone with knowledge, understanding or ability gained in one situation can adapt or extend this for application in a different context. In some cases, the transfer of a skill is immediately obvious. Take, for example, the ability to use a spreadsheet to summarise experimental data and create a graph to illustrate results. Once the key concepts and commands are learned (Chapter 72), they can be applied to many instances outside the biomolecular sciences where this type of output is used. This is not only true for similar data sets, but also in unrelated situations, such as making up a financial balance sheet and creating a pie chart to show sources of expenditure. Similarly, knowing the requirements for good tabulation and graph drawing (Chapters 73 and 74), perhaps practised by hand in earlier work, might help you use spreadsheet commands to make the output suit your needs.

Other cases may be less clear but equally valid. For example, towards the end of your undergraduate studies you may be involved in designing experiments as part of your project work. This task will draw on several skills gained at earlier stages in your course, such as preparing solutions (Chapter 23), deciding about numbers of replicates and experimental layout (Chapter 29) and perhaps carrying out some particular analytical method. (Chapters 43–70). How and when might you transfer this complex set of skills? In the workplace, it is unlikely that you would be asked to repeat exactly the same process, but in critically evaluating a problem or in planning a complex project for a new employer, you will need to use many of the time-management, organisational and analytical skills developed when designing and carrying out experiments. The same applies to information retrieval and evaluation and writing essays and dissertations, when transferred to the task of analysing or writing a business report.

Making the most of your graduate attributes

The skills emphasised in biomolecular sciences courses are sometimes considered alongside a university-wide framework of graduate attributes that are intended to summarise the qualities and skills that an employer might expect in those with qualifications from your institution. The associated

Definitions

Employability – the ability to secure employment and follow a long-term career, requiring: (1) a mix of subject knowledge and understanding; (2) the possession of relevant practical and generic skills; (3) suitable personal attributes and attitudes; (4) an appreciation of workplace values; and (5) an understanding of the need for continuing personal and professional development.

Graduate attributes – the set of qualities and skills that graduates develop through their academic study and engagement in student life, including the acquisition of subject-specific knowledge, intellectual skills, practical skills, personal skills and digital literacy.

notion of 'graduateness' summarises the effect of degree-level experience and learning on an individual. This in turn is connected with the concept of 'employability' which encompasses those skills and qualities required to gain and maintain employment. An understanding of these concepts is important for every student, as this not only leads to a better appreciation of the value of certain activities and assessments, but also provides a specialised vocabulary and gives insights about your personal development and career potential.

At the end of your course, which may be some time away, you will aim to get a job and start on your chosen career path. You will need to sell yourself to your future employer, firstly in your application form and curriculum vitae (Chapter 8), and perhaps later at interview. Companies rarely employ bioscience graduates simply because they know how to carry out a particular lab routine or because they can recall specific facts about their chosen degree subject. In addition to subject expertise, they will be looking for a range of graduate-level skills and attributes. Typically, for example, they will seek employees who can demonstrate the ability to work in a team, to speak effectively and write clearly about their work. All of these skills and attributes can be developed at different stages during your university studies.

KEY POINT Factual knowledge can be important in degrees with a strong vocational element, but understanding how to find and evaluate information is often rated more highly by employers than the ability to memorise facts.

Most likely, your future employer(s) will seek someone with an organised yet flexible mind, capable of demonstrating a logical approach to problems – someone who has a range of skills and who can transfer these skills to new situations. Many competing applicants will probably have similar qualifications. If you want the job, you will have to show that your additional skills and personal attributes place you above the other candidates.

Text reference

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Sources for further study

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STUDY EXERCISES

1.1 Evaluate your skills. Examine the list of skill topics shown in in the chapter listing on pp. v–vii. Now create a new table with two columns, like the one on the right-hand side. The first half of this table should indicate *five* skills you feel confident about in column 1 and show where you demonstrated this skill in column 2 (for example, 'working in a team' and 'in a first year group project on marine biology'). The second half of the table should show *five* skills you do not feel confident about, or you recognise need development (for example, communicating in verbal form). List these in column 1 and in column 2 list ways in which you think the course material and activities in your current modules will provide you with the opportunity to develop these skills.

1.2 Find skills resources. For at least one of the skills in the second half of the table in Study exercise 1.1, check your university's library database to see if there are any texts on that subject. Borrow an appropriate book and read the relevant sections. Alternatively, carry out a search for relevant websites (there are many); decide which are useful and bookmark them for future use.

Skills I feel confident about	Where demonstrated
1.	
2.	
3.	
4.	
5.	
Skills that I could develop	**Opportunities for development**
6.	
7.	
8.	
9.	
10.	

1.3 Analyse your goals and aspirations. Spend a little time thinking what you hope to gain from university. See if your friends have the same aspirations. Think about and/or discuss how these goals can be achieved, while keeping the necessary balance between university work, paid employment and your social life.

Answers to these study exercises are available at go.pearson.com/uk/he/resources

2 Self-management

The term 'self-management' covers a wide range of skills, from being able to organise your life to understanding how to learn independently, making up an important subset of the broader skills highlighted in Chapter 1. This will become increasingly important throughout your studies, until you reach a stage when you will be expected to be able to work independently –for example, when completing a research project in your final year.

Learning how to learn – this is a vital self-management skill for university and beyond, covered in Chapter 3.

KEY POINT Being able to self-manage is a key characteristic of a successful graduate and being able to demonstrate this is a strong aid to employability (Chapters 1 and 8).

Definitions

Aim (or Goal) – a long-term achievement you want to work towards, reflecting your ambitions.

Milestone – a 'marker' or key event along the way towards an objective or aim.

Objective – a well-defined short-term step towards your overall aim.

Outcome (or Target) – a measurable result from your activities.

Note: authorities differ on the definition of these terms, often depending on context. The above definitions relate to self-management.

Planning your approach to university

As in all things, you are likely to achieve more from your time at university if you make purposeful decisions about what you want and how you are going to get there. Thinking about exactly what you hope to accomplish in your studies and personal life will help you to plan and prioritise your activities and make the most of your time. Typically, this will involve the following steps:

1. **Establishing your aims.** These are the broad statements of intent, setting out the end results you would like to achieve. They are sometimes called goals. You need to consider what you wish to accomplish by the end of your studies, for example, 'graduate with a good degree', as well as the other things you'd like to achieve during your time at university, such as 'learn Spanish'.
2. **Setting your objectives.** These are more specific in their focus, when compared with aims. In essence, they are the smaller steps that help you to achieve an overall aim, for example, 'improve my ability to use spreadsheets'. Each objective should also have a measurable outcome (a target).

The process of writing out your aims and specifying your objectives is an iterative one. Both should be reconsidered from time to time to ensure their relevance. Also, once you've completed any particular objective, you should re-evaluate your plans, and, where necessary, establish new ones.

Once you have drafted your aims, it may help to discuss them with a friend or tutor. Then write out some objectives within different time frames, forming an overall plan. Make sure that your objectives fit the 'SMART' criteria.

Using the 'SMART' approach to write objectives. You should ensure they are:

- **Specific** – clear and unambiguous
- **Measurable** – with quantified targets, so you can assess progress
- **Achievable** – attainable within your abilities and resources
- **Relevant** – helping to achieve your overall aim
- **Timed** – so you can work to a suitable timeline for completion

The best objectives will, therefore, have detail on aspects of 'what', 'how', 'where' and 'when'. The objective 'to spend an extra hour each week on directed study in microbiology next term' addresses these aspects and fulfils the SMART criteria, in contrast to a general intention 'to study more'.

KEY POINT There are no hard-and-fast rules around setting out your aims, writing objectives and planning – some people prefer a highly structured approach while others follow a looser path. Self-management involves deciding what works best for you, thereby making the most of your time.

Example
A possible set of objectives for a 1st year biomolecular sciences student:

Short term

- Allocate two hours on Monday, Wednesday and Thursday to research my biochemistry essay.
- Join the tennis club before mid-term.

Medium term

- Set up a revision timetable for the end of semester exam by week 5.
- Find out about volunteering at the local food bank at the start of Semester 2.

Longer term

- Improve my exam skills by attending the learning centre's sessions in Semester 3.
- Discuss with my flatmates options for accommodation next year and search for options.

Using formal organisational systems – those produced by e.g. Filofax, Time Manager International (TMI) or Day-Timer tend to be aimed at the business market, and are often relatively expensive.

Planning complex projects – longer, multipart tasks like research projects (Chapter 32) may benefit from a more organised approach, where you track your progress in elements of the work, taking account of their interdependence and your achievement of relevant milestones. This can be organised and monitored by creating a specialised 'Gantt' chart with the elements displayed on the vertical axis and time (and progress) on the horizontal axis (Chapter 32).

Keeping your decluttering efforts under control – work quickly and effectively on organising your workspace; don't use tidying up as a form of work-avoidance (procrastination, p. 12).

Organising your time and tasks

Being better organised should help you to achieve more. This applies to things such as the tidiness of your desk and the filing of your notes, but also to the focus of your efforts in achieving your objectives. Smartphone calendars allow you to organise your activities and can be used to provide reminders for important activities. As well as these, many more specialised time-management and productivity apps are available (search 'time management [or planner] apps for students'), most of which will sync with your smartphone calendar. Alternatively, you may prefer to use a 'planner' type of diary. Apps and diary-style planners are convenient for recording notes and 'to-do' lists, while post-it notes are a low-tech method of writing down short-term lists of tasks.

A revision timetable (Chapter 17) is a good example of a short-term plan – and a similar approach can be taken to make the most of your time in other situations.

- **For all assignments such as essays and reports, enter submission dates in your diary/planner** – work back in time to the present, entering milestone tasks (see, for example, Fig. 32.2) so that you can complete the work on time (for example, 'complete literature survey by now'). Put reminders in your smartphone in advance of each milestone, ensuring that you give yourself enough time to complete the task.
- **Note down the times of all lectures, tutorials, lab classes and other commitments.**
- **Consult these entries to plan out the forthcoming week or month**.
- **Write down your daily and weekly 'to do' checklists** – keep them in one place.
- **Use your checklists to monitor progress on large tasks** – recognise that you don't necessarily have to complete the component parts in a linear sequence; for example, when writing a project report (Chapter 12).

Workspace organisation is another aspect where people have their own preferences: for example, you might be a person who tidies up as you go, or you might 'declutter' each week or before each major new task. Reflect on your current approach and decide if it needs to be improved, and how this might be achieved. Useful tips include:

- **ensure you have enough folders/ring-binders** – organise the paperwork for modules on your course using these
- **set out your bookshelves so you know where to find key textbooks and sources** – keep them from cluttering your desk when not being used
- **ahead of a big task, make sure you have sufficient paper, pens, printer ink, etc**. so you aren't interrupted by not having these items when needed
- **make sure your working environment is reasonably quiet** – make sure the temperature is suitable, and includes room to spread out sources, space for your computer or laptop and good lighting
- **decide how you will reward yourself for achieving milestones and objectives** – this can be as simple as deciding that you will take some exercise or eat a snack.

Non-academic activities will continue to be important while you are working on a big academic task, which is another reason for good planning.

Examples might include organising shopping for food, clothes, etc., or creating, tracking and sticking to a financial budget. Try to timetable these activities for periods when you feel less inclined to study.

Re-balancing your activities

If you feel that the balance of your working and personal time isn't right, then you could try analysing your current activities, since this is an important factor in being a successful student. However, it is important to recognise that putting time-management techniques into practice is an individual matter: self-discipline and self-evaluation, based on what works for you, will enable you to develop effective working systems.

Analysing and organising your time

Definitions

Committed time – timetabled activities involving your academic objectives, including lectures, practicals, tutorials, work on assessments and associated personal study/revision.

Maintenance time – that spent supporting your general activities, such as shopping, cleaning and laundry.

Discretionary time – time for you to use as you wish, for example, recreation, sport, hobbies and socialising.

Knowledge of how you currently spend your time is key to developing better time-management. To do this, you might keep a log of your different activities over, say, a week. Ideally, this will cover the full range of your activities, so that it is representative. You might, for example, create a table divided into half-hour segments for each day, and note down what you do during each time slot. Alternatively, you could try to recreate a past record of the past few days from memory, though this is likely to be less accurate.

Think beforehand how you should categorise the different things that you do while awake, from the mundane (for example, cooking and eating) to the timetabled (for example, lectures and practicals). For example, it might be relevant to group your activities under broad terms like 'committed time', 'maintenance time' and 'discretionary'. You then could use a spreadsheet (Chapter 72) to summarise this information and present it as, say, a pie chart or a bar chart (p. 575–576), so you can visualise the relative proportions. You can now analyse your data, seeking answers to questions such as:

- **How much time do I spend on each category in a typical week?**
- **How do I divide my time between study, general activities and relaxation?**
- **Am I satisfied with these time allocations, when compared to the importance of each category?**
- **How much of my time is used effectively?**
- **What changes do I want to make?**

The key overarching question for you to answer is: '**Does my present allocation to committed time allow me to fulfil my aims?**'

Analysing and organising your tasks

Not only do you need to decide on your allocation of time across different categories of activity, you need to choose what to do and when. One way of achieving this is to analyse your current tasks according to their importance and urgency (Fig. 2.1). Important tasks are those with significant consequences, such as studying for a test whose results will impact on your final grade, while urgent tasks are those which must be done as a top priority and at short notice, such as work towards an impending essay deadline. One approach is to allocate items on your checklist of current tasks according to their position on a grid showing relative urgency and importance (Fig. 2.1a). Then, organise your tasks by prioritising those in the 'urgent and important' category and downgrading those in the 'non-urgent and unimportant' category, ranking the 'important' and 'urgent' ones as you see fit.