

Training Exercises

CAS-1200™ automated sample setup

The Corbett Robotics Operating Software was originally designed for PCR setup. While this is still most prevalent, over time the CAS-1200 has been used for a variety of other applications.

The following exercises have been compiled from a variety of real-world situations and have been collated to act as a step-by-step guide to learn more about the CAS-1200's capabilities.

It is expected that the user already has some familiarity with the software to calibrate plates, change plate types and change the layout of the robot's deck. If not, it's recommended that the user consult the CAS-1200 manual.

Some of the exercises provide hints on how to set up run files. Note however that there are often several ways of achieving the same end result and the solutions presented in these exercises provide just one way of achieving a desired result.

Exercise 1

Prepare a PCR setup to combine a pre-made master mix with samples for the Rotor-Gene 6000 (RG-6000).

Plates/Tubes

- Samples are stored in 200 µL unskirted PCR plate
- Reactions are to be set up in 0.1 mL Rotor-Gene Strip Tubes
- The master mix has been prepared in a 1.5 mL tapered tube

Set up

- 16 samples in positions A1, B1, C1, ... ,H2
- Set up in reaction plate, in duplicate with 20µL master mix and 5µL sample
- Minimise tip consumption

Hints

- Start by setting up the robot's deck with the appropriate plate types
- Set up your pre-made master mix
- Create a sample bank to group all of your samples
- Use the "Add" button to create your reactions

Exercise 2

Prepare a PCR setup to combine a pre-made master mix with samples for the RG-6000.

Plates/Tubes

- Samples are stored in 200 µL flip cap PCR tubes
- Reactions are to be set up in 0.1 mL Rotor-Gene Strip Tubes
- The master mix has been prepared in a 1.5 mL tapered tube

Set up

- 24 samples in columns 1, 3 and 5 of a 96-well plate
- Set up in reaction plate, in triplicate with 15 µL master mix and 5 µL sample
- Maximise pipetting precision

Hints

- Create a single discontinuous sample bank to group all of your samples
- Pipetting precision can be maximised by not re-using tips

Exercise 3

Set up a PCR run to combine a pre-made master mix and standards with samples for the RG-6000 using a Gene-Disc 72.

Plates/Tubes

- Samples are stored in 200 μ L flip cap PCR tubes
- Reactions are to be set up in a 72 well Gene-Disc
- Sufficient master mix has been prepared in a 2 mL free standing tube
- Standard is to be created by the robot in 200 μ L strip tubes

Set up

- 24 samples in columns 1, 3 and 5 of a 96-well plate
- Set up in Gene-Disc, in duplicate with 22.5 μ L master mix and 2.5 μ L sample
- Reduce tip usage by re-using tips 4 times
- Standard is to have 4-fold dilution with 8 orders of magnitude, do not change tips between each standard. The standard is to be pipetted in triplicate.

Exercise 4

Create a run file for a PCR setup to combine a pre-made master mix with samples, standards and NTCs for a 96-well ABI cyclor.

Plates/Tubes

- Samples are stored in 1.5 mL tapered tubes
- Reactions are to be set up in a half-skirt 96-well ABI plate in a horizontal fashion
- The master mix has been prepared in a 5 mL tube
- Standard is to be created by the robot in 200 μ L strip tubes

Set up

- 22 samples in positions 1 – 11 and 23 – 33 of a 33-well plate for 1.5 mL tubes
- Set up in reaction plate, in triplicate with 20 μ L master mix and 5 μ L sample
- Standard is to have 10-fold dilution with 6 orders of magnitude, change tips between each standard. The standard is to be pipetted in triplicate.
- Pipette the NTC in triplicate into the last three wells on the plate (H10, H11 and H12)
- Reduce tip usage by re-using tips 4 times

Hints

- Use the horizontal version of the 96-well ABI plate
- To force the NTCs into the last wells, skip tubes by using the “Add” feature without actually selecting anything other than the number of wells to skip.

Exercise 5

Write a PCR setup in which the robot prepares the master mix and standards as well as using samples and NTCs to set up a full 96-well plate for a 96-well cyclor.

Plates/Tubes

- Samples are stored in 2 mL Axygen Deep Well Plate
- Reactions are to be set up in an unskirted 200 μ L 96-well PCR plate a vertical fashion
- Standard is to be created by the robot in 200 μ L strip tubes
- All reagents are in 1.5 mL tapered tubes

Set up

- 24 samples in positions A1–H3
- The master mix is to contain the following reagents:
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Water	5.0 µL/reaction
2x Buffer	6.0 µL/reaction
dNTP	2.5 µL/reaction
MgCl ₂	3.0 µL/reaction
Fwd Primer	1.0 µL/reaction
Rev Primer	1.0 µL/reaction
Probe	1.0 µL/reaction
Taq	0.5 µL/reaction (tends to be viscous)

- Set up in reaction plate, in triplicate with a 5 µL sample volume
- Standard is to have 10-fold dilution with 6 orders of magnitude, do not change tips between each standard. The standard is to be pipetted in triplicate.
- Pipette the NTC in triplicate
- Reduce tip usage by re-using tips 4 times

Exercise 6

Repeat Exercise 5 with the following differences:

- Set up the entire assay on the Gene-Disc loading block, including all the reagents and master mix. The diluent (water) remains in position A of the master mix block.
- Only prepare the samples in duplicate, all samples are in Rows 1 and 2 on the Gene-Disc loading block.
- Use a pre-made standard positioned in Row 3 of the Gene-Disc loading block.
- Ensure that the reagents are added to the master mix so that the diluent (water) is added last.

Exercise 7

Create a PCR setup in which one template is tested against eight primers sets. The robot prepares the master mix and sets up for an RG-6000 in 0.1 mL tubes.

Plates/Tubes

- Primer sets are stored in a fully skirted 96-well plate
- Reactions are to be set up in 0.1 mL Rotor-Gene tubes
- All other reagents are stored in 1.5 mL tapered tubes
- The template DNA is in a 2 mL free standing tube

Set up

- The eight primer sets of interest are stored in columns 1 and 2 of the plate such that the forward primer is in column 1 and the corresponding reverse primer is in column 2.
- The final reaction is to contain the following:
-

Water	5.0 µL
2x Buffer	6.0 µL
dNTP	2.5 µL
MgCl ₂	3.0 µL
Fwd Primer	1.0 µL
Rev Primer	1.0 µL
Probe	1.0 µL
Taq	0.5 µL (tends to be viscous)
Template DNA	5.0 µL

- et up in reaction plate, in duplicate
- Maximise pipetting precision

Hints

- Add the template to your master mix

Exercise 8

Write a PCR setup to combine two pre-made master mixes with samples for an RG-6000. Samples are split between two 96-well plates.

Plates/Tubes

- Samples are stored in two separate 96-well fully skirted 200 μ L plates (plate A and B).
- Reactions are to be set up in 0.1 mL Rotor-Gene Strip Tubes
- Both master mixes have been prepared in 2 mL free standing tubes.

Set up

- Plate A has 19 samples in row A and column 3.
- Plate B has samples in column 1 and, 11.
- Set up in reaction plate, (no replicates) with 15 μ L master mix and 5 μ L sample so that all samples from Plates A and B are combined with Master Mix 1. Samples in column 3 of Plate A must also be run with Master Mix 2.

Exercise 9

Prepare a run file for a PCR setup to compare two thermal cyclers with a robot prepared standard curve.

Plates/Tubes

- Reactions are to be set up in 0.1 mL Rotor-Gene tubes and in Cepheid SmartCycler tubes.
- Sufficient master mix has been prepared in a 500 μ L PCR tube
- A dilution series is to be created by the robot in 200 μ L strip tubes

Set up

- Set up 12 μ L master mix and 10 μ L standard in both reaction plates
- Maximise pipetting precision
- Standard is to have 4-fold dilution with 8 orders of magnitude, change tips between each standard. The standard is to be pipetted in duplicate.

Exercise 10

Set up a PCR run to compare two thermal cyclers with a robot prepared standard curve. Samples are split between the cyclers after they have been combined with master mix. Repeat Exercise 9 with the following differences:

- Create a reaction mixture of 144 μ L (96 μ L master mix, 48 μ L standard) in 200 μ L PCR tubes
- Split this reaction mixture to prepare 30 μ L reactions, in duplicate in each of the 0.1 mL tubes and SmartCycler tubes.

Exercise 11

Create a run to combine 96 samples in 1.5 mL tapered tubes into a single 96-well plate.

Plates/Tubes

- Samples are stored in 1.5 mL tapered tubes. These tubes are racked into 32 at a time into three 33-well tapered tube loading blocks.
- Target plate is a 650 mL rack of 96 micro tubes (X-tractor elution plate)

Set up

- Transfer 100 μ L of all samples to target plate

Exercise 12

Prepare a run to combine 96 samples in 1.5 mL tapered tubes into a single 96-well plate. However, the same 33-well sample block is to be re-used in the same robot deck position. The user is to be prompted when to replace the plate.

Plates/Tubes

- Samples are stored in 1.5 mL tapered tubes, only **one** 33-well loading block is available.
- Target plate is a 650 mL rack of 96 micro tubes (X-tractor elution plate)

Set up

- Transfer 100 µL of all samples to target plate

Hints

- Create three sample banks of 32 wells each on top of one another on the 33-well sample plate.
- Use the "User Defined Pause" programming step in the "Special" menu to prompt the user to change plates.

Exercise 13

Write a PCR set up in which the robot prepares multiple master mixes and multiple standards to be combined in a 72-well Gene-Disc.

Plates/Tubes

- Reactions are to be set up in a Gene-Disc
- Standards are to be created by the robot in 200 µL strip tubes
- Reagents are in combinations of 1.5 mL tapered tubes and 2 mL free standing tubes

Set up

- The reagents and master mixes are to be set up as follows:

Master Mix A	Master Mix B	Reagent Vol/Reaction	Reagent Tube
Water		5.0 µL/reaction	1.5 mL tapered
2x Buffer		6.0 µL/reaction	1.5 mL tapered
dNTP		2.5 µL/reaction	1.5 mL tapered
MgCl ₂		3.0 µL/reaction	1.5 mL tapered
Fwd Primer A	Fwd Primer B	1.0 µL/reaction	2.0 mL free standing
Rev Primer A	Rev Primer B	1.0 µL/reaction	2.0 mL free standing
Probe A	Probe B	1.0 µL/reaction	1.5 mL tapered
Taq		0.5 µL/reaction (tends to be viscous)	1.5 mL tapered

- Standard A is to be run with Master Mix A and Standard B with Master Mix B.
- Standards are to have 4-fold dilution with 4 orders of magnitude, do not change tips between each standard. The standards are to be pipetted in duplicate.
- Reduce tip usage by re-using tips 4 times

Exercise 14

Complete Exercise 13 and then make the following additions/changes:

- 24 samples in 200 µL PCR tubes.
- Use Roche Lightcycler capillaries as the reaction vessel.
- Prepare the samples in duplicate, running the first 8 samples with Master Mix A, the second 8 samples with Master Mix B and the last 8 samples with Master Mix A and B.

Exercise 15

Set up eight master mixes with a variety of primers and probes without using them in a robot prepared PCR reaction.

Plates/Tubes

- All master mixes are to be prepared in 2 mL free standing tubes
- Primers are stored in 1.5 mL tapered tubes
- The 2x Buffer is stored in a 5 mL tube
- All other reagents are stored in 1.5 mL tapered tubes

Set up

- 1.5 mL of each of the eight master mixes is to be prepared.
- Eight primer sets are in 16 consecutive tubes alternating between forward and reverse primers.
- The master mixes are to be prepared based on the following reaction mix for 40 reactions each:

Water	5.0 μ L
2x Buffer	6.0 μ L
dNTP	2.5 μ L
MgCl ₂	3.0 μ L
Fwd Primer	1.0 μ L
Rev Primer	1.0 μ L
Probe	1.0 μ L
Taq	0.5 μ L (tends to be viscous)
Template DNA	5.0 μ L

Exercise 16

A dilution series is to be created in 1.5 mL tapered tubes.

Plates/Tubes

- The initial stock concentration is in a 1.5 mL tapered tube
- Dilution is to be set up in 1.5 mL tubes

Set up

- Create a dilution series of 5 orders of magnitude at a volume of no less than 500 μ L of each concentration at a dilution ratio of 10:1

Exercise 17

Repeat Exercise 16 with the following enhancements:

- The template that is used in the first 1.5 mL tapered tube is to come from a 200 μ L flip cap PCR tube at a ratio of 3:1
- In the same run file, create, from the 1.5 mL tapered tubes, three 100 μ L aliquots of each concentration into 200 μ L flip cap PCR tubes

Hints

- Define each 1.5 mL tube in the reaction plate as its own sample bank

Exercise 18

Create three copies of 96-well sample plate.

Plates/Tubes

- The source plate is a 650 mL rack of 96 micro tubes (X-tractor elution plate)
- The plate copies are 96-well half-skirted PCR plates

Set up

- Copy an aliquot of 10 μ L from the stock plate into each of three reaction plates such that well A1 goes to wells A1, A1 and A1 of the three target plates. Do this for all 96 wells.

Hints

- What is your tip usage in this run?

Exercise 19

Repeat Exercise 18 with only using 96 tips. If you managed to use 96 tips in Exercise 18, repeat the setup with using 288 tips.

Hints

- Use the Plate Duplication programming step under “Special”

Exercise 20

Create a 384-well PCR setup for an Applied Biosystems 7900HT with 24 samples. Run the samples in quadruplicate with four separate master mixes, achieving the plate layout as pictured below.

Plates/Tubes

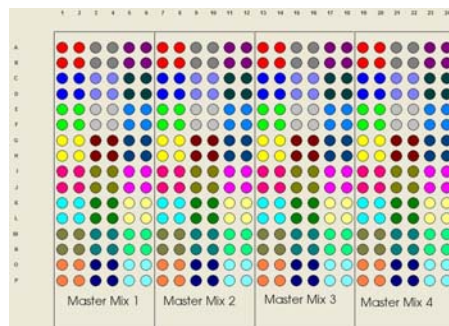
- Samples are stored in a 96-well 200 μ L half skirted PCR plate
- All master mixes have been sufficiently prepared in 5 mL tubes

Set up

- The reaction mixture should be 3 μ L master mix with 2 μ L of sample
- Set up each sample in quadruplicate in the layout pictures below
- Reduce tip usage by re-using tips 8 times
- Ensure that sample/master mix mixing is turned on to flush the sample out of the tip

Hints

- Careful sample bank definitions will make the programming significantly easier.



Exercise 21

Create a 96-well PCR setup with 30 samples, one positive control and one NTC. The required reaction plate layout is shown in the Table below.

Plates/Tubes

- Samples and the positive control are in 1.5 mL tapered screw cap tubes.
- Both master mixes have been sufficiently prepared in 1.5 mL tapered tubes

Set up

- All 30 samples are run in duplicate with the FAM master mix and as an individual reaction with the JOE master mix. The reaction mixture should be 10 μ L master mix with 5 μ L of sample
- At the end of the reaction plate there are to be positive and negative controls as indicated

Hints

- Careful sample bank definitions will reduce the number of programming steps to less than 20.

	1	2	3	4	5	6	7	8	9	10	11	12
A	FAM1	FAM1	JOE1	FAM2	FAM2	JOE2	FAM3	FAM3	JOE3	FAM4	FAM4	JOE4
B	FAM5	FAM5	JOE5	FAM6	FAM6	JOE6	FAM7	FAM7	JOE7	FAM8	FAM8	JOE8
C	FAM9	FAM9	JOE9	FAM10	FAM10	JOE10	FAM11	FAM11	JOE11	FAM12	FAM12	JOE12
D	FAM13	FAM13	JOE13	FAM14	FAM14	JOE14	FAM15	FAM15	JOE15	FAM16	FAM16	JOE16
E	FAM17	FAM17	JOE17	FAM18	FAM18	JOE18	FAM19	FAM19	JOE19	FAM20	FAM20	JOE20
F	FAM21	FAM21	JOE21	FAM22	FAM22	JOE22	FAM23	FAM23	JOE23	FAM24	FAM24	JOE24
G	FAM25	FAM25	JOE25	FAM26	FAM26	JOE26	FAM27	FAM27	JOE27	FAM+	FAM+	FAMNTC
H	FAM28	FAM28	JOE28	FAM29	FAM29	JOE29	FAM30	FAM30	JOE30	JOE+	JOE+	JOENTC

Exercise 22

Recently extracted samples are in a 96-well rack with varying concentrations. It is required that the samples are normalised to an equal concentration.

Plates/Tubes

- The samples are in a 650 mL rack of 96 micro tubes (X-tractor elution plate)
- They are to be normalised into a 650 mL rack of 96 micro tubes (X-tractor elution plate)

Set up

- 16 samples are in the first two columns of the plate with concentrations of (in ng/ μ L): 0.065, 0.057, 0.073, 0.061, 0.063, 0.055, 0.050, 0.054, 0.081, 0.066, 0.069, 0.071, 0.065, 0.077, 0.057, 0.053
- It can be assumed that sufficient sample is available for the normalisations
- All 16 samples are to be normalised to 0.05 ng/ μ L with a final volume of 100 μ L

Exercise 23

The non-normalised samples from Exercise 22 are to be normalised directly into a PCR setup for a Roche Lightcycler.

Plates/Tubes

- There is sufficient master mix in a 2 mL free standing tube
- Reactions are to be set up in Roche Lightcycler capillaries

Set up

- The reaction mixture should be 20 μ L master mix with 5 μ L of sample
- Set each sample up in duplicate
- Normalise the sample concentration into the reaction mixture at an appropriate concentration

Hints

- Choose a concentration that will result in reasonable sample/water volumes

Exercise 24

Three columns of samples are to be pooled into eight wells on a fresh 96-well plate.

Plates/Tubes

- The samples are in a 200 μ L 96-well unskirted PCR plate
- The samples are pooled into 200 μ L PCR tubes with flip caps

Set up

- The samples are in columns 1, 2 and 3 of the plate and are to be pooled such that all samples in row A end up in one tube, the samples in row B in a second tube and so on.

Hints

- Use the "reset" feature in the "Special" menu
- How many tips are used?

Exercise 25

Three columns of samples are to be pooled into eight wells on a fresh 96-well plate, whilst minimising tip usage. Repeat exercise 24 with the following difference:

- Use the pooling function in the "Special" menu

Hints

- Compare tip use to Exercise 24