

# A modular method for high-throughput measurement of ion channel currents in cardiac myocytes

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# A modular method for high throughput measurement of ion channel currents in cardiac myocytes

## Supplementary Information

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## Supplementary Method 1

### Protocol transfer to other planar patch clamp devices

Currently available planar patch clamp instruments operate under one of two standard recording chamber geometries. This protocol utilizes the open or 'fixed' well geometry which describes an open chamber into which the cells and various solutions are pipetted into and diluted and removed (mix and read). Other available devices including the Patchliner (Nanion Technologies), the QPatch line and the Qube (both Sophion Biosciences) use microfluidic 'flow-through' approaches for continuous fluid perfusion with a caudal waste reservoir into which old solutions are deposited **Supplementary Figure 1**. Fixed well and microfluidic geometries both contain unique advantages and disadvantages which are highlighted in **Supplementary Table 1**.

When adapting protocols from this work to other planar patch clamp devices, it is important to consider how fluid is handled. Due to the different chamber formats, the solution composition and the method of addition require attention. Because microfluidic chambers have constant flow-through into a waste reservoir, the cells are directly exposed to the exact concentration of the applied fluid. Therefore, no dilution calculations need to take place. This contrasts the fixed well option used in the present protocol, which requires careful thought and in-well dilution steps to achieve the desired concentrations. A large advantage of this open well method is that there is no limit to the number of addition or dilution steps that can be undertaken because fluid is always removed and replaced, keeping a constant volume. Some microfluidic devices have waste reservoirs of finite volume and no option for removal, limiting the amount of fluid that can pass through the system, and therefore the number of concentration steps during a given experiment.

All steps regarding experimental patch clamp aspects involving capacitance compensation, liquid junction potential correction, voltage protocol design and data export and analysis must be manually input to the user's specifications into the respective software. Different devices will harbor unique software, for example the Patchliner, which utilizes modified PatchMaster NEXT software (HEKA, an affiliate of Harvard Bioscience Inc). Following instructions in respective software manuals, the specifications outlined in the present protocol can be applied and input into the software package corresponding to the device of choice.

## Supplementary Method 2

### Action potential recording (current clamp configuration)

This section describes an additional method to measure action potentials in hiPSC-CM. We caution the measurement of action potentials using typical patch-clamp amplifiers due to circuitry constraints that induce a voltage drop across the cell-amplifier series resistance which requires the injection of large quantities of hyperpolarizing current to the cell to maintain a stable resting potential. This can subsequently alter action potential morphology.<sup>1,2</sup> We recommend sharp microelectrode methods paired with voltage follower or bridge amplifiers for more accurate measurements of true action potential characteristics.<sup>3</sup>

This section can be applied as an independent experiment or applied inside the resting and upstroke toolkit after the  $I_{Na}$  measurements. The following section continues after step 49. It summarised in **Supplementary Figure 2**.

**CRITICAL** The final solution composition exposed to the cells during these experiments is as follows: Pipette (in mM): EGTA 10, HEPES 10, KCl 10, NaCl 10 and KF 110, pH 7.2 (with KOH). Bath (in mM): 10 HEPES, 60 NMDG, 80 NaCl, 4 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 glucose, pH 7.4 (with HCl).

1. Add a 'Set Amp. Mode' step and select current clamp.
2. Add a pause step and input 10 seconds.
3. Add a start test pulse step.
4. Add a CC Amp Cmnds step. Select low frequency voltage clamp and input desired holding potential in the Vtarget (mV) field. -90 mV is recommended.
5. Add a pause step and input 30 seconds.
6. Add a start protocol step.
6. In the 'start protocol' step, open the protocol editor to design a current clamp protocol.
7. Add a holding current step for 10 ms. Select the checkbox 'use IHold'. Add a second step with a 3 ms pulse of 100 pA. Enter 100 ms into the 'I increase field'. Add a third step for 587 ms at 0 current. Select the checkbox 'use IHold'. Lastly, input 15 sweeps into the sweep settings box. This will apply iterative current injections in sequence. In the sweep settings box, select the desired interval to determine stimulation frequency.
8. AP online analysis or 'cursor' steps are pre-loaded. Action potential duration at a desired percentage of repolarisation can be chosen here. This is calculated using inbuilt algorithms.

**^CRITICAL STEP** Upon data export, this data column can be used directly. There is no need for further calculations or functions in spreadsheet software.

9. Save this protocol file.
10. Select the 'wait for recording' checkbox in the 'start protocol' step.
11. Verify the method sequence. If further protocols are desired, always add a start test pulse step followed by a 30 second pause.
12. If a switch back to voltage clamp is desired, add a Set Amp. Mode step and select voltage clamp.
13. Continue the resting and upstroke protocol from step 50 in the main manuscript.

## Supplementary Method 3

### Addition of an $I_{Ca,L}$ measurement into the resting and upstroke protocol ( $I_{Na} \rightarrow I_{Ca,L} \rightarrow I_{K1}$ )

This section can be applied inside the resting and upstroke toolkit after the  $I_{Na}$  measurements. The following section continues after step 49.

**CRITICAL** The final solution composition exposed to the cells during these experiments is as follows: Pipette (in mM): EGTA 10, HEPES 10, KCl 10, NaCl 10 and KF 110, pH 7.2 (with KOH). Bath (in mM): 10 HEPES, 60 NMDG, 80 NaCl, 4 KCl, 2  $CaCl_2$ , 1  $MgCl_2$ , 5 glucose, pH 7.4 (with HCl).

1. Add 3 separate 'QC Commands' steps. For each of them select RSeal, RSeries, and Capacitance, respectively.
2. Add a 'Start Protocol' step. Click the 'Wait for recording' checkbox. When checked, the method will not resume until all sweeps have been carried out. Next, open the protocol editor.
3. Input a voltage protocol for peak  $I_{Ca,L}$  measurement. Start with a 100 ms holding step at  $V_{mem}$  (-80 mV). This is followed by a 100 ms depolarising ramp to -40 mV. Hold for 10 ms then input a step pulse to +10 mV for 100 ms. Subsequently, return to holding potential at -80 mV. Set interval to 2 s. Input 10 sweeps. **Troubleshooting.**

**^CRITICAL STEP** In order to make an IV curve using this protocol: Change the test pulse voltage to +60 mV. Add a 5 mV interval number into the 'V increase' box. Input 21 sweeps. This means that an IV curve will run from -40 mV to 60 mV in 5 mV jumps. This step is fully customizable based on the experimenters needs.

4. Add an item as a cursor set in the lower online analysis area. Place the left cursor 0.5 ms after the depolarising step pulse. Place the right cursor ~20 ms into the depolarising step. Set the y-analysis value to minimum. Name this item 'Peak'.

5. Add a second cursor set after channel inactivation during the step pulse. Place the left cursor at ~10 ms before the end of the step, and the right cursor at 0.5 ms before the end of the step. Set the y-analysis value to mean. Name this item 'Baseline'.

**^CRITICAL STEP** This method of analysis assumes that the rate of channel inactivation will remain constant during an experiment. An alternative option is to use a pre-pulse portion of the trace as baseline, assuming the trace has been corrected for leak (leak correction is applied as standard).

6. Add an item as a formula. Input Peak – Baseline to correct peak current amplitude relative to baseline. Name this item 'Corrected Peak'.
7. Add an item as a parameter and select capacitance. Name this item 'Capacitance'.
8. Add an item as a formula. Input Corrected Peak ÷ Capacitance to acquire current density. Name this item 'Current Density'.

9. Save this protocol file and exit the protocol editor.
10. Add a single 'QC Commands' step. Select Protocol Result and the 'Peak' item of the Online Analysis result.
11. Continue the resting and upstroke protocol from step 50 in the main manuscript.

## Supplementary Method 4

### Rearrangement of plateau and recovery protocol to $I_{Kr} \rightarrow I_{Ca,L}$ ( $\rightarrow I_{Na}$ )

This protocol describes a rearrangement of the plateau and recovery protocol. This describes the measurement of  $I_{Kr}$  in equimolar  $Cs^+$  conditions first, and then a switch of bath and pipette solution to measure  $I_{Ca,L}$ , and if desired,  $I_{Na}$ . This switch prolongs experiments up to 20 minutes and therefore may compromise final success rate. This alternative may be desirable for those who wish measure  $I_{Ca,L}$  under more physiological solutions. It removes the high  $CsCl$  in the bath and replaces it with a more physiological  $NaCl$  for  $I_{Ca,L}$  and  $I_{Na}$  measurement. The following alternative experiment will measure  $I_{Kr}$  first and then  $I_{Ca,L}$  and  $I_{Na}$  from the same cell. The following steps continue from the very start of the plateau and recovery protocol (step 73).

1. Prepare a minimum 50 ml pipette solution of (in mM): 10 EGTA, 10 HEPES, 10  $CsCl$  and 125  $CsF$ , pH 7.2 (with  $CsOH$ ). This is pipette solution 1.
2. Prepare a minimum 50 ml pipette solution of (in mM): 10 EGTA, 10 HEPES, 10  $CsCl$ , and 110  $CsF$ , pH 7.2 (with  $CsOH$ ). This is pipette solution 2.
3. In addition to the solutions listed in the main protocol, Prepare an additional sodium solution of (in mM): 10 HEPES, 135  $NaCl$ , 2  $CaCl_2$ , 1  $MgCl_2$ , 5 glucose, pH 7.4 (with  $NaOH$ ).
4. Cell harvesting will happen soon. Warm FBS, Versene and 0.25% Trypsin-EDTA to room temperature (22-24°C).
5. Add 50 ml Divalent free solution to a 200 ml reservoir on the deck. Add 50 ml high calcium solution to a 200 ml reservoir on the deck. Add 50 ml reference solution to a 200 ml reservoir on the deck. Add 50 ml E4031 solution to a 200 ml reservoir on the deck. Add 200 ml of the new sodium solution to a 200 ml reservoir on the deck. Ensure all reservoir locations are input into the B-Deck step.
6. Add a minimum of 50 ml Pipette solution 1 into a container at internal position 1.
7. Add a minimum of 50 ml Pipette solution 2 into a container at internal position 2.
8. Using the liquid handling software on PC2, continue customizing the method file. Ensure all reservoir locations and names are input into the B\_Deck step.
9. For a full plate experiment, select 'full chip' in the measurement mode section of the 'D\_Experiment Definition' step. For a partial plate experiment: settings can be adjusted here if desired.

**CRITICAL** Steps 10-14 describe the setup for customized bath solution exchange (**Figure 2**)

10. Add a 'D2\_Avanced settings' step directly after D\_Experiment Definition and input 25  $\mu l$  rest volume into the field.

11. Add a 'Remove and Wash tips' step after step 'FillChip'. In settings, choose Remove: yes, Wash: no.
12. Add a 'Remove and Wash tips' step after 'AddCells'. In settings, choose Remove: yes, Wash: no.
13. Replace the 'Sealing' step with a Sealing group from the advanced tab. Input a volume of 50  $\mu$ l inside the 'add SE' step.
14. Replace the 'WashCells' step with a WashCells group from the advanced tab. Input a volume of 37.5  $\mu$ l inside the 'add solution' step. Input End =1 in the loop settings step.
15. In the 'VC Amp Commands' step, set the LJP to +4.1 mV.
16. Set Vmem to -80 mV in the 'Amplifier settings' step.
17. Add 3 separate 'QC Commands' steps. For each of them select RSeal, RSeries, and Capacitance, respectively.
18. Add a 'Start Protocol' step. Open the protocol editor.
- ^CRITICAL STEP** Leave the 'Wait for recording' checkbox unchecked.
19. Input a voltage protocol for  $I_{Kr}$  tail measurement. Start with 100 ms at Vmem (-80 mV), Followed by a 3000 ms step to +40 mV. Revert back to -80 mV for 2000 ms before a small 50 ms hyperpolarising step at -100 mV. Lastly, add a 100 ms step back at -80 mV. Set interval to 6 s. Input the desired number of sweeps (>200).
20. Add an item as a cursor set in the lower online analysis area. Place the left cursor at the start of the -80 mV step. Place the right cursor ~400 ms after the left cursor. Set the y-analysis value to minimum. Name this item 'Tail'.
21. Add an item as a parameter and select capacitance. Name this item 'Capacitance'.
22. Add an item as a formula. Input Tail  $\div$  Capacitance to acquire current density. Name this item 'Current Density'.
23. Save this protocol file and exit the protocol editor.
24. Add a single 'QC Commands' step. Select Protocol Result and the 'Peak' item of the Online Analysis result.
25. Add a pause step for 120 s after the QC Commands step. Pause steps allow the protocol to run for the designated time without progressing to the next step. This pause can be extended as desired.
26. Add a 'Compound Addition' step after the pause. Select the reservoir with the E4031 solution.

27. Add a pause step for 120 s after the compound addition step

28. Add a 'Stop protocol' step. This will terminate the protocol, regardless of the sweep number

**^CRITICAL STEP** This marks the end of the  $I_{Kr}$  measurement. The final solution composition during this measurement is (in mM): 10 HEPES, 135 CsCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 glucose, pH 7.4.

29. Add an 'Internal exchange' step. Select position 2. This will perfuse new pipette solution during the experiment.

30. Add an 'Add Solution' step. Select the reservoir with the sodium solution. Input 3 washes at 50  $\mu$ l each.

31. Add a 'Start Protocol' step. Click the 'Wait for recording' checkbox. When checked, the method will not resume until all sweeps have been carried out. Next, open the protocol editor.

32. Input a voltage protocol for peak  $I_{Ca,L}$  measurement. Start with a 100 ms holding step at  $V_{mem}$  (-80 mV). This is followed by a 100 ms depolarising ramp to -40 mV. Hold for 10 ms then input a step pulse to +10 mV for 100 ms. Subsequently, return to holding potential at -80 mV. Set interval to 2 s. Input 10 sweeps.

**^CRITICAL STEP** In order to make an IV curve using this protocol: Change the test pulse voltage to +60 mV. Add a 5 mV interval number into the 'V increase' box. Input 21 sweeps. This means that an IV curve will run from -40 mV to 60 mV in 5 mV jumps. This step is fully customizable based on the experimenters needs.

33. Add an item as a cursor set in the lower online analysis area. Place the left cursor 0.5 ms after the depolarising step pulse. Place the right cursor ~20 ms into the depolarising step. Set the y-analysis value to minimum. Name this item 'Peak'.

34. Add a second cursor set after channel inactivation during the step pulse. Place the left cursor at ~10 ms before the end of the step, and the right cursor at 0.5 ms before the end of the step. Set the y-analysis value to mean. Name this item 'Baseline'.

**^CRITICAL STEP** This method of analysis assumes that the rate of channel inactivation will remain constant during an experiment. An alternative option is to use a pre-pulse portion of the trace as baseline, assuming the trace has been corrected for leak (leak correction is applied as standard).

35. Add an item as a formula. Input Peak – Baseline to correct peak current amplitude relative to baseline. Name this item 'Corrected Peak'.

36. Add an item as a parameter and select capacitance. Name this item 'Capacitance'.

37. Add an item as a formula. Input Corrected Peak ÷ Capacitance to acquire current density. Name this item 'Current Density'.

37. Save this protocol file and exit the protocol editor.

38. Add a single 'QC Commands' step. Select Protocol Result and the 'Peak' item of the Online Analysis result.

39. Add a 'Stop protocol' step. This will terminate the protocol, regardless of the sweep number.

40. Add a stop protocol step. Verify the method sequence.

**^CRITICAL STEP** This marks the end of the  $I_{Ca,L}$  measurement. The final solution composition during this measurement is (in mM): 10 HEPES, 130 NaCl, 5 CsCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 glucose, pH 7.4.

**CRITICAL** A further addition of an  $I_{Na}$  protocol is possible. If this is not desired please continue the plateau and recovery protocol from step 108.

41. To add an  $I_{Na}$  protocol, add 3 separate 'QC Commands' steps. For each of them select RSeal, RSeries, and Capacitance, respectively.

42. Add a 'Start Protocol' step. Click the 'Wait for recording' checkbox. When checked, the method will not resume until all sweeps have been carried out. Next, open the protocol editor.

43. Input a voltage protocol for peak  $I_{Na}$  measurement. Start with 10 ms at  $V_{mem}$  (-80 mV). Followed by a 900 ms hyperpolarising step to -110 mV. Deselect record data for this step. This is followed by a 30-ms test pulse to -20 mV before returning to  $V_{mem}$  for 90 ms. Set interval to 2 s. Input 10 sweeps.

**^CRITICAL STEP** In order to make an IV curve using this protocol: Change the pulse voltage to +20 mV. Add a 5 mV interval number into the 'V increase' box. Input 21 sweeps. This means that an IV curve will run from -80 mV to 20 mV in 5 mV jumps. This step is fully customizable based on the experimenters needs.

44. Add an item as a cursor set in the lower online analysis area. Place the left cursor 0.5 ms after the depolarising step. Place the right cursor ~20 ms into the depolarising step. Set the y-analysis value to minimum. Name this item 'Peak'.

45. Add a second cursor set after channel inactivation during the step pulse. Place the left cursor at ~10 ms before the end of the step, and the right cursor at 0.5 ms before the end of the step. Set the y-analysis value to mean. Name this item 'Baseline'.

**^CRITICAL STEP** This method of analysis assumes that the rate of channel inactivation will remain constant during an experiment. An alternative option is to use a pre-pulse portion of the trace as baseline, assuming the trace has been corrected for leak (leak correction is applied as standard).

46. Add an item as a formula. Input Peak – Baseline to correct peak current amplitude relative to baseline. Name this item 'Corrected'.
47. Add an item as a parameter and select capacitance. Name this item 'Cap'.
48. Add an item as a formula. Input Corrected  $\div$  Cap to acquire current density. Name this item 'Density'.
49. Save this protocol file and exit the protocol editor.
50. Add a single 'QC Commands' step. Select Protocol Result and the 'Peak' item of the Online Analysis result.
51. Continue the plateau and recovery protocol from step 108 in the main manuscript.

## Supplementary Method 5

### Macro-enabled MS Excel file to automatically extract readouts from large Result Tables

This supplementary method describes how to use the macro enabled extractor file included in the supplementary files (**Supplementary Software 2**). It describes an alternative to the 'Organizational analysis with MS Excel' section. The following steps continue after step 159.

**CRITICAL** In the analysis software, ensure that the result tables are customized in such a way that there is no content selected in the Export Header tab. The Well Header tab should only include the 'Sweep Result Block' and the 'Time of Sweep (header)' options (step 158).

1. Open the file entitled Supplementary Software File 2 using MS Excel. Ensure that content is editable, and macros are enabled.

**^CRITICAL STEP** Instructions are included in this file. Click the button with the red instructions text to access a pop up information textbox which will reiterate the following.

2. Copy the contents of the clipboard into cell A1. Ensure that column A contains the names of successful wells.

3. Ensure that the first named well is in cell A4.

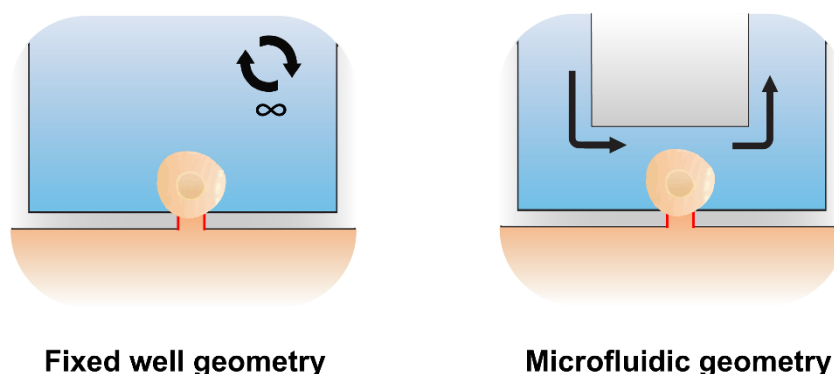
4. Ensure that Row 2 contains the names of all the readouts included in a single sweep.

5. Click the extract button. A pop-up message will appear asking the user which item should be extracted. Input the corresponding number and click OK. Wait up to 20 seconds.

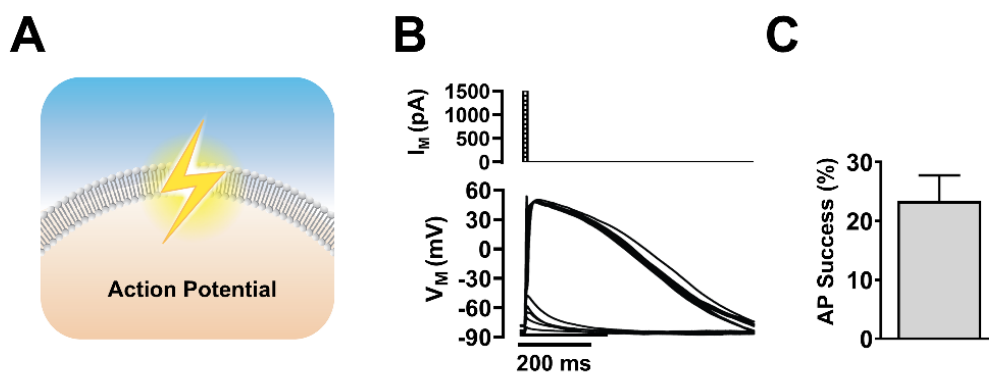
6. The process will be finished when a final pop-up box appears announcing task completion.

7. A new sheet will be created. It will be named according to the item that has been extracted for every sweep. Continue the protocol outlined in the main manuscript after step 165.

## Supplementary Figures



**Supplementary Figure 1.** Simplified cross-sectional schematic of planar patch clamp with a fixed well geometry recording chamber (left) and a microfluidic chamber (right). A cell is attached to a single patch clamp aperture in both examples. External fluid is highlighted by the color blue. Due to constant fluid removal and addition, the fixed well geometry is able to accommodate unlimited amounts of fluid exchange via sequential dilution. In microfluidic systems where the caudal end of the chamber is closed, the number of solution exchanges may be limited.



**Supplementary Figure 2. Current clamp experiments in hiPSC-CM using high throughput patch-clamp.** a, Schematic of the parameters measured in this particular protocol. Under the current clamp configuration, action potential (AP) measurement following a switch of the amplifier to current clamp mode b, Stimulus protocol (upper) to elicit an action potential after achieving threshold potential and a representative action potential trace (lower) c, Absolute success rate of recording action potentials in high throughput ( $n = 3$  experiments). Data is replotted with permission from Obergrussberger *et al.* 2021.<sup>4</sup>

## Supplementary Tables

**Supplementary Table 1.** Comparisons of commercially available planar patch clamp platforms.

	<b>SyncroPatch 384 (Nanion Technologies)</b>	<b>Qube (Sophion Bioscience)</b>	<b>QPatchIII/Compact (Sophion Bioscience)</b>	<b>Patchliner (Nanion Technologies)</b>	<b>Port-a-patch (Nanion Technologies)</b>
<b>Active wells</b>	384	384	8-48	4-8	1
<b>Substrate</b>	Borosilicate glass	Polymer	Silicon	Borosilicate glass	Borosilicate glass
<b>Well geometry</b>	Fixed well	Microfluidic	Microfluidic	Microfluidic	Fixed well
<b>Liquid handling</b>	Fully automated	Fully automated	Automated (QPII) /Manual (QPc)	Fully automated	Manual
<b>External solution exchange</b>	Mix and read with automated waste renewal	Perfusion with automated waste removal	Perfusion	Perfusion with automated waste removal	Mix and read
<b>Aspects for protocol transfer</b>	–	Solutions, software use	Solutions, software use	Solutions, software use	Software use
<b>Add/change settings/steps while an experiment runs?</b>	Yes	No	No	Yes	Yes
<b>Amplifier make</b>	Tecella	Proprietary	Proprietary	HEKA	HEKA/Tecella
<b>Temperature control</b>	Yes	Yes	Yes	Yes	Yes

## References

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