



# IgG sequencing of rat hybridoma

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## ABSTRACT

The purpose of this protocol is to amplify IgG antibody variable regions derived from rat hybridoma RNA, using RT-PCR and Sanger sequencing.

## Materials needed:

- RNA extraction: Qiagen RNEasy mini kit (74104)
- Reverse Transcription Kit: SMARTScribe Reverse Transcriptase (639537)
- DNA Polymerase: Invitrogen Platinum SuperFi II PCR Master Mix (12368010)
- Invitrogen's PureLink™ PCR Purification Kit #K310001

The layout of this protocol was adapted from a protocol composed by Andrew McGuire's laboratory at the Fred Hutchinson Cancer Center, which in turn was adapted from Meyer et al 2019 "A simplified workflow for monoclonal antibody sequencing." See attachment

for the respective manuscript.

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sequencing of rat hybridoma.

protocols.io

https://dx.doi.org/10.17504/p

rotocols.io.x54v9ppw1g3e/v1

**Primers**

- TS pF is ordered as RNA oligo; the remainder as DNA oligo  
  
 universal TS pF AAGCAGTGGTATCAACGCAGAGTACATrGrGrG  
  
 ISPCR pF      aagcagtggatatcaacgcagag  
  
 rat IGHG RT pR GGACAGGGCTCCAGAGTTCC  
  
 rat IGHG PCR pR GACTGGCTCAGGGAAATAGCC  
  
 rat IGKC RT pR CTGATCAGTAACACTGTCCAGGAC  
  
 rat IGKC PCR pR CACTGATGTCTCTGGGATAGAAGTTG  
  
 rat IGLC1 RT pR GGGAGATAGGTGCACCATTTGC  
  
 rat IGLC1 PCR pR GGCCACTTCCACATCACTCG  
  
 rat IGLC2 RT pR TCCACACCCTGAGTGATAGGG  
  
 rat IGLC2 PCR pR CTTCCAGACCACTGTCATAACACC

**Procedure**

- RNA extraction:** Extract RNA from the hybridoma sample using RNeasy total RNA kit, according to the manufacturer’s instructions.
- Reverse Transcription:** On ice, prepare the 1<sup>st</sup> reaction mix in PCR tubes for gamma chain and kappa chain (or lambda chain) cDNA synthesis according to the following recipe:

3.1      **Gamma**

Component	Volume per rxn

IGHG RT pR (10 uM)	1 uL
dNTP (10 mM)	1 uL
RNA sample (50 ng/uL)	2 uL
<b>Total volume</b>	4 uL

### 3.2 Kappa

Component	Volume per rxn
IGKC RT pR (10 uM)	1 uL
dNTP (10 mM)	1 uL
RNA sample (50 ng/uL)	2 uL
<b>Total volume</b>	4 uL

### 3.3 Lambda *(include later if Kappa does not amplify)*

A	B
Component	Volume per rxn
IGLC1 (or C2) RT pR (10 uM)	1 uL
dNTP (10 mM)	1 uL
RNA sample (50 ng/uL)	2 uL
<b>Total volume</b>	4 uL

### 3.4 On ice, prepare a 2<sup>nd</sup> mastermix in Eppendorf tubes. This recipe is for one reaction regardless of chain type. *Scale up for the number of samples as needed.*

#### 2<sup>nd</sup> reaction mix

A	B
Component	Volume per rxn
5x SMARTScribe buffer	2 uL
DTT (20 mM)	1 uL
Universal TS pR (100 uM)	0.3 uL
H2O	1.70 uL

A	B
<b>Total volume</b>	5.00 uL

- 3.5** After preparing the 2<sup>nd</sup> mastermix, incubate each of the 1<sup>st</sup> reaction mixes in a thermocycler for **3 minutes at 72°C**.

- 3.6** While the incubation reaction is proceeding, add the following to the 2<sup>nd</sup> reaction mix:

A	B
<b>Component</b>	<b>Vol. per rxn</b>
RNAse inhibitor (40 U/uL)	0.50 uL
SMARTScribe Rev. Transcriptase (100 U/uL)	0.50 uL
<b>Total volume</b>	1.00 uL

- 3.7** Once the incubation of the 1<sup>st</sup> reaction mix (from step 3.5) finishes, add 6 uL of the 2<sup>nd</sup> reaction mix to each tube of the 1<sup>st</sup> reaction mix.

- 3.8** With the 2 reaction mixes now combined, incubate each according to the following conditions:

A	B	C
<b>Temperature</b>	<b>Time</b>	<b>Cycles</b>
42°C	60 min	1
70°C	5 min	1
4°C	hold	-

Proceed to PCR amplification step immediately after incubation has finished (once samples reach 4°C hold step).

- 4 PCR amplification:** Prepare mastermix for PCR reaction according to following 2-step recipe:

#### 4.1 Add the following components to each PCR tube.

##### Gamma

A	B
Component	Volume per rxn
Platinum SuperFi II PCR MM	25 uL
ISPCR pF (10 uM)	2.5 uL
IGHG PCR pR (10 uM)	2.5 uL
cDNA from RT step (5-100ng)	3 uL
Water, nuclease-free	17 uL
<b>Total volume</b>	50 uL

#### 4.2 Kappa

A	B
Component	Volume per rxn
Platinum SuperFi II PCR MM	25 uL
ISPCR pF (10 uM)	2.5 uL
IGK PCR pR (10 uM)	2.5 uL
cDNA from RT step (5-100ng)	3 uL
Water, nuclease-free	17 uL
<b>Total volume</b>	50 uL

#### 4.3 Lambda *(if Kappa doesn't amplify)*

A	B
Component	Volume per rxn
Platinum SuperFi II PCR MM	25 uL
ISPCR pF (10 uM)	2.5 uL
IGLC1 (or C2) PCR pR (10 uM)	2.5 uL

A	B
cDNA from RT step (5-100ng)	3 uL
Water, nuclease-free	17 uL
<b>Total volume</b>	50 uL

- 4.4** Cap each tube, then mix and briefly centrifuge the PCR tubes.  
Place PCR tubes in thermocycler, and run according to the following conditions:

**Gamma**

A	B	C
Temperature	Time	Cycles
98°C	30 sec	1
98°C	15 sec	35
63°C	30 sec	
72°C	25 sec	
72°C	5 min	1
4°C	hold	-

**Kappa/Lambda**

A	B	C
Temperature	Time	Cycles
98°C	30 sec	1
98°C	15 sec	35
56°C	30 sec	
72°C	25 sec	
72°C	5 min	1
4°C	hold	-

**5 Verify the gel bands**

After PCR reaction completes, set up 1% agarose gel according to the following conditions:

In a flask, add the following components:

0.5 g – Agarose powder

50 mL – 1X TAE buffer

Put flask, with added reagents, in microwave and heat up until all the powder is fully dissolved (about 1 minute).

After microwaving is finished, remove flask with hot pad; add 5 uL of Sybrsafe to flask, swirl to mix. Pour into a tray with appropriate plastic comb, preferably one with wells that accommodate 20uL volumes.

Prepare a fraction of each PCR products to verify the band size on the gel. Aliquot 5 uL from each PCR tube into a new PCR strip, and add 1 uL of DNA Loading Dye to each sample.

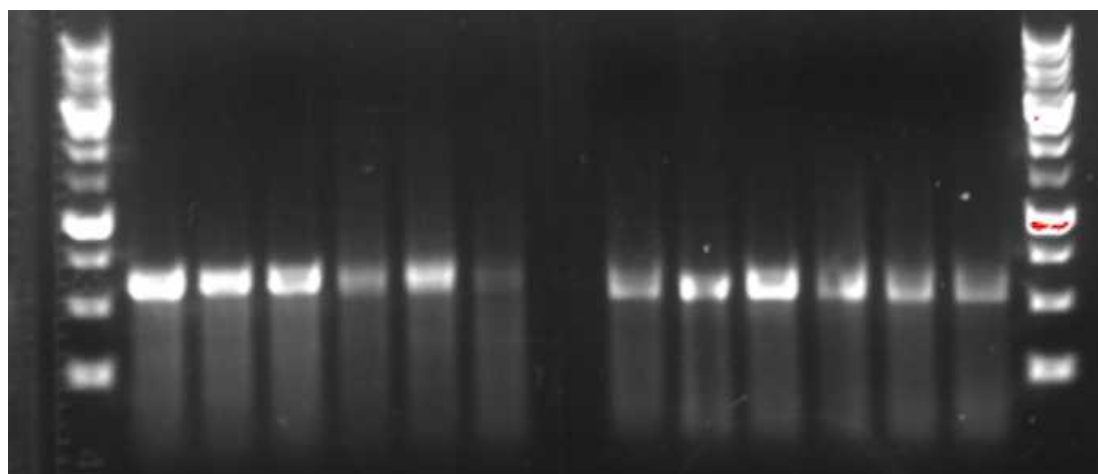
Load the gel and run it for 25-30 minutes at 110 V. *Modify these settings if needed.*

Remove gel, image, and save an image copy for the records.

Note: There should be a gamma chain, and either a kappa or lambda chain.

Amplified rat antibody products: 550-600 bp.

A significant majority of mouse antibody light chains will be kappa; if kappa not present, repeat with IGLC1 and IGLC2 samples.



## 6 PCR cleanup

PCR samples that produce a band of the expected size can then be finalized using a PCR purification kit (e.g. Invitrogen's PureLink™ PCR Purification Kit #K310001), and eluted in 25 uL volume. Verify cDNA concentration by nanodrop.

## 7 Sanger sequencing should be performed (e.g. GeneWiz or Genomic Core).

## 8 Analyze the antibody nucleotide sequence

Open the website: [https://www.imgt.org/IMGT\\_vquest/vquest](https://www.imgt.org/IMGT_vquest/vquest)

Copy and paste sequence into the IMGT webpage, in the section "sequence submission".

Select "Rattus norvegicus" for species, and "IGH" as receptor type or locus.

Click on "Start".

### Your selection

Species

Rattus norvegicus (Norway rat)

×

Receptor type or locus

IGH

×

Sequence submission

Type (or copy/paste) your nucleotide sequence(s) in [FASTA](#) or in [FASTQ](#) format

>1G7 GH|

NNNNNNNTGGNAGCTGGTTGNANACACATCCNGGAGCCAGTGGATAGACAGATGGGGCTGTTGTTGGGCTGAGGAGACAGTGACTGAAGCTCCTTGACCCAGGCAGTCATAACATAGAGTTGCCCTGTCTTGCACAATAAAGTGCCGTGTCTCAGACTTCAGACTGTTTCATTTGCAGGTACAGG GTGCTTTTTCATTATCTCTGGAGATAGTGAATCGGCCCTTCACGGAGTCTCCATAGTAAGTGCCACCACTCATGACTAATGGATGCGACCCACTCC AGACCTTCTTTGGAGCCTGGCGGACCCAGGCCATGTAATAGTCACTGAAAGTGAATCCTGAGACTGCACAGGAGAGTTTCAGGGACCTCCAGGCT GTACTAAGCCTCCCCAGACTCCACAGCTGCACCTCACACTGGACACCTTTATGAAAAGGACAAGGAAACCAAGCTGAGCCTGGTGTCCATGGTG AGTAGTCTGTGCAGTGTCTGAGTACTGATTACTGAGTGGGAGAGCCTCAGAGTCCAGGACTGGGCTCTCGCTCCCCCANGNACTCTGCNNNNN

Or give the path access to a local file containing your sequence(s) in [FASTA](#) or in [FASTQ](#) format

Choose File

no file selected

Start

Clear the form

## A. Detailed results for the IMGT/V-QUEST analysed sequences

Number of analysed sequences: 1

1. 1G7\_GH

 This release of IMGT/V-QUEST uses [IMGT/JunctionAnalysis](#) for the analysis of the JUNCTION

 Hyphens (-) show nucleotide identity, dots (.) represent gaps

Sequence: 1 1G7\_GH

Nb of 5' trimmed-n: 8.  
Nb of 3' trimmed-n: 5.  
Analysed sequence length: 570.  
Sequence analysis category: 3 (complementary reverse\_no indel search).  
Complementary reverse sequence compared with the [Rattus norvegicus \(Norway rat\) IG set](#) from the [IMGT reference directory](#) (set: F+ORF+ in-frame P)

>1G7\_GH (complementary reverse)  
gcagagtncntgggggacgcaggagccagtcctggactctgaggtctccactcagta  
atcagtgactgcagctgcacagactactcaccatggacacaggtcagcttggttttc  
ctgtgctctttcataaaagggtgcagtggtgagggtcagctggtggagctcgggggaggc  
ttagtagacgctggagcggcctggaactctctgtgcagtcagagattcacttccagt  
gactattacatggcctgggtccgcaggctccaagaagggtctggagtggctgcacatcc  
attagtcagtgaggtggtggcacttactatggagactccgtgaaggccgattcactatc  
tccagagataatgcaaaaagcaccctgtacctgcaaatgaacagctggaagctcaggac  
acggccacttattattgtgcaagacagggcaacctctatgtatgactgcctgggtcaa  
ggagcttcagtcactgtctctcagcccaaacacagccccatctgtctatccactggct  
cnggatgtgngtntcaaccagctncca

Result summary: 1G7_GH		Productive IGH rearranged sequence (no stop codon and in-frame junction)	
V-GENE and allele	<a href="#">Ratnor IGHV5-22*01 F</a>	score = 1359	identity = 96.88% (279/288 nt)
J-GENE and allele	<a href="#">Ratnor IGHJ4*01 F</a>	score = 225	identity = 90.74% (49/54 nt)
D-GENE and allele by IMGT/JunctionAnalysis	<a href="#">Ratnor IGHD3-4*01 F</a>	D-REGION is in reading frame 2	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.38.11]	[8.8.11]	CARQGNLYVMTAW
JUNCTION length (in nt) and decryption	39 nt = (11)0(3)-15(5)-3(1)-4(19)	(3'V)3'(N1)5'(D)3'(N2)5'(5'J)	