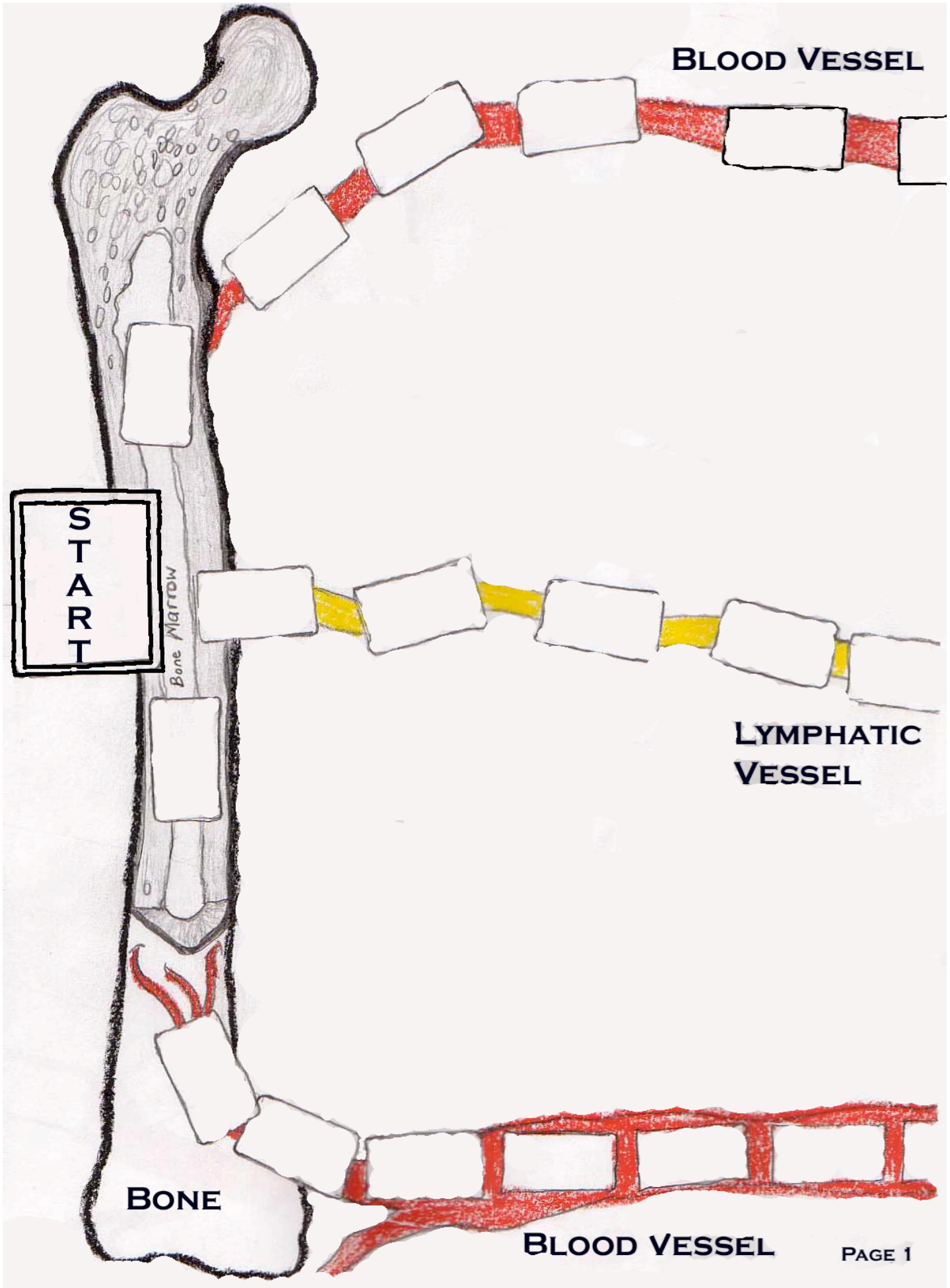
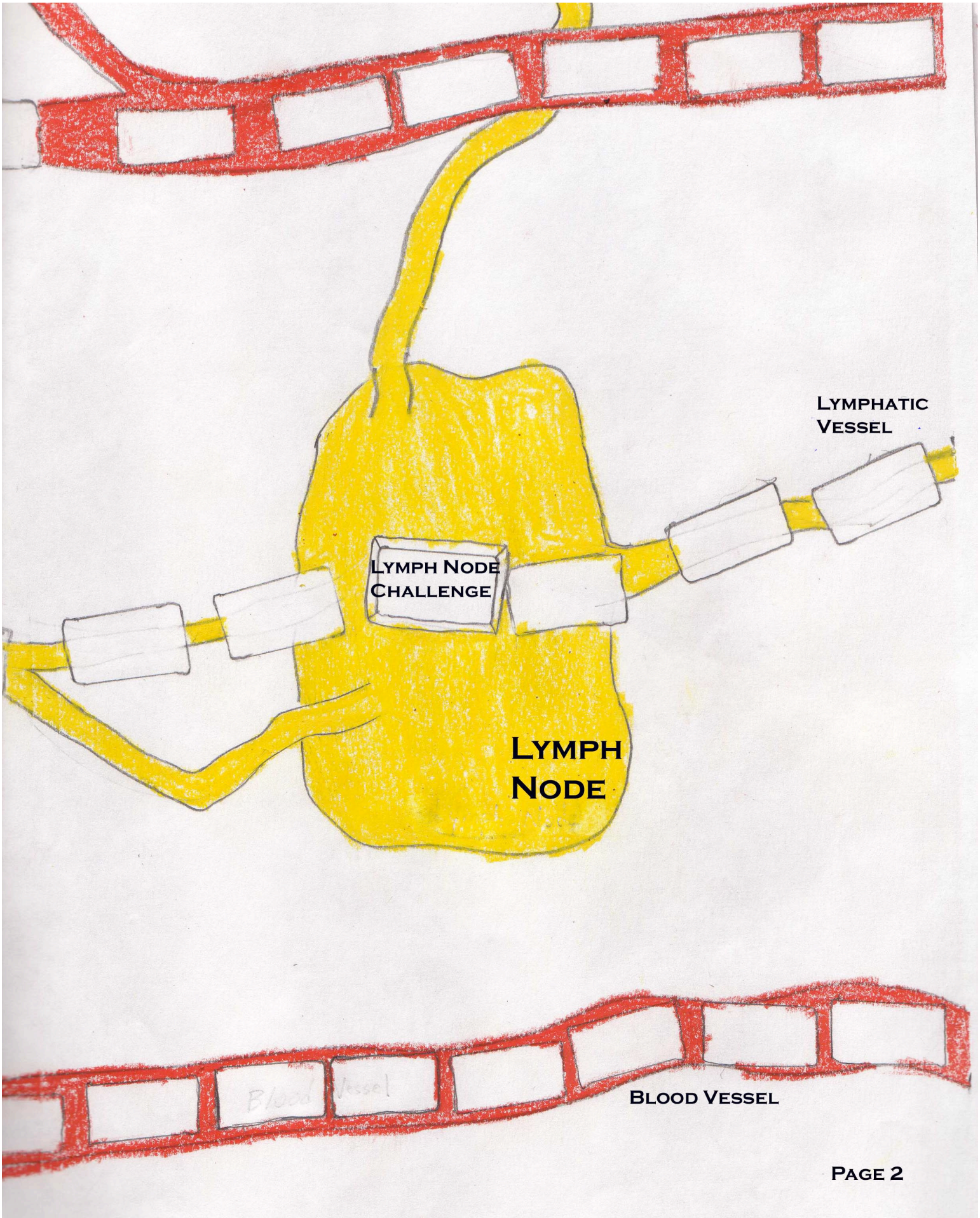
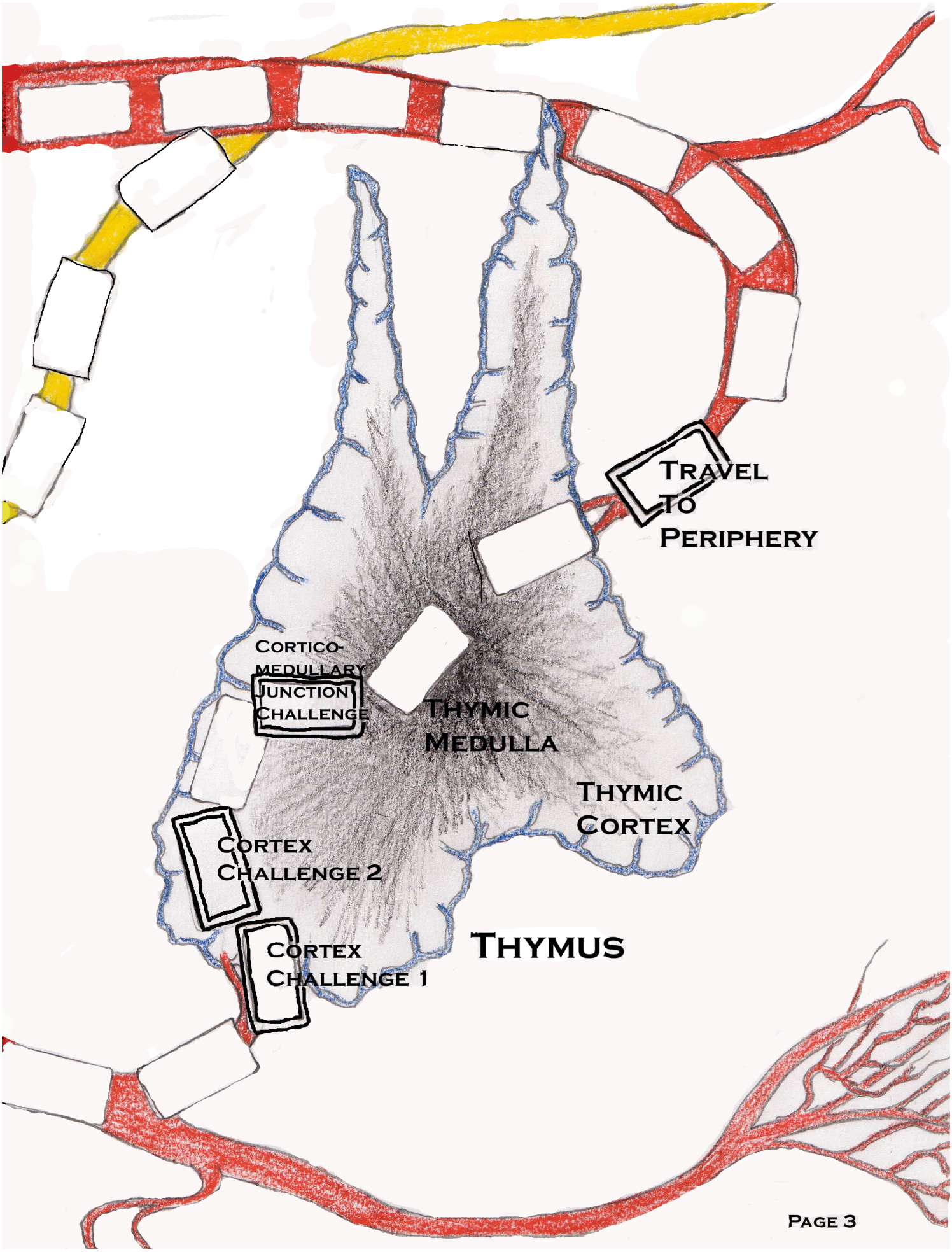

Blood Vessel
START
Bone Marrow
Lymphatic Vessel
Bone
Blood Vessel


Lymphatic Vessel
Lymph Node Challenge
Lymph Node
Blood Vessel
Blood Vessel


Travel
To
Periphery
Cortico-
medullary
Junction
Challenge
Thymic
Medulla
Thymic
Cortex
Cortex
Challenge 2
Cortex
Challenge 1
Thymus

Appendix I: 50 Sets of 400 Randomly Generated Tri-Peptides

1. mdn yqf ntg hyy nhm ctq nff ege svs cef smi vhn syy tsq tps ink tlq tng nlv kgl aqc nqq rqy gsa ccw fsg grg lww vgs qll fmv tyy wdg iss slk ppr tgs eds yck tpw kws ift afc vay tvm syk fkd icf itv ytk trl knp qyr apd vqh flq gdr nqc vff rsy rre fat sfk gqt cpp ldr akm dpk afh pim scs ael thi vsg trv vke nmi ryf dft tkv pas lyi rev hqe gwa nwt nfa npe chn ifa scg sih iyp qhe mnq qtl reh akt wnn nyr dmk lmn drm hna wsm kle qaq egw rtg hqt kkk cip mfr qmi mdk tam drp mmr mry rwi pel hqr tlt ekf amv hlq eni tkc tdw vta ygr cts asp amp ahn hqt rrq dsh adn vfp fvc int ips cyn yhv gle vra cse afq eqh gha yfq fwc sgq ier gma glw ynv hva ehf mtf mee pvl gdi eqc aif gni ivg whr qsw mst hnh dcq ekm hmy pay mpp ege kwd ive stv thn pct dkr hqm mve lgk lyy leq mwn kry hiy tcd dss sii dfc adr rcn wnn ync gig fty gda pnq miw vce vly cnd kdi dqk qgl nse fep plc ahs wkw gfk tdn ntq mtq nis edn nrp gsd icd aes hgt fdm ggg dmd ytw qhf qee qdc ade ftv gil fqq asf avf rec vpc nww vih mey ery epq wcs ekl dlh iip rws avm kiq mpi lws sgs cqn whc dlr asp cpq lys nsa hly kef itn ent cls ikh gwh mpe gwy mwl agy waa cwe mwh gic ykq knw mmi qmk atd ynq pdt cpm eir qqd rnw fcn iye tgw nal yqd tla eck ady knw hat pfk glf ipy nfk qfg yai ldq fgd nqr rsg iye ede tcg pni yil dgm nvh yav fwg yfw dim akh gnt wwv dwm vdd pdr pvv gpa myi wmy grn lee snm hal syl tsd aiv irp hhf dth dwe mfm fec mym mii hgp lyl vwt kag rey vpw tww aie ggg mfa dad pee epm inl qrh dvd pyp dpd wva pqa pft egc elt qld mwk flf tfg qff hci avv eql gmw dgn edw vpa tfa vvp pav fcf vkm nls age vnt ymq pqa gek yfd ked hkq lhp isl kpn ckl ppl

2. yfe mky ykt qme hsw ead hrh lys kaq qed fsm kme qnd twp rec lir tkg wfe swt nml ams nwe arm kca ccd yif aik twh mhv pmi few afp fne kep sqa fmt lpy rwh nyy npa lnr gil khw ahs ykc avl lkh qan hks lmg nvi hev ydg anv pct wys fte yqh sfn mhc nei wdl cin siq dkw nwr qqa sgv dsd lsm amp agk eww skl ccp war kmk cgi fiv elc cyg iep yen lei ash clq yfp ewt tfa nlg mkv ciw mpa yci mtk ylv avt adi apq rpg nla dsl wgv ydn drl svr rkt ttm knk rrg yrq kkt adv qkg vfr qka plh dmk kls hvc akn ptk iks cll lna chi fpw ekc pnv vst csc dkf chs pfy nvn spf fkl drk wsd sta yte ndc mgd nyp cwd svm kyd ssy eng wsk clk rps qpg nyq fra nit efq wph rhs ldd ftv gsv qsr fsn swy ywt qvk wap ltg nms amg ptc tkm qmi wmh pww lkm adr gds stl qfh lnt wen yci qhk fkt pka gfi hme hft sml iyc ede gvn svg wef rtt tdk wrs fhn nve qnk yeq ntk glk ksh ewq dsd hym idw ire nyv yee vif twc mgg wsg gaf iws afm hnq lln fsp kwn ggs csm ets nsg vvh tlp vvv mwg sss eyi gqs ytp mdm iif hyy dkt nvw wgh yac ewa vqr qay khc vcc vlg ycl hqr kew pcy ems emw ftc cmn amk kec pnm iqi mcr mvv wmh dwc mgy fsy gpv ffs wcw wek qfl trm gfl qty wkn lnq dpq hrr vhr ggq ipa dlp hgi dns tpk rgl qmc vlh tmi fky npv dcr dks teh pvc ncm vrr mct slw mid kwk yag ldd lse stm fpf nha rpm wrv pld raa edm fhc wcl eli nhl egy alv fmh vsh sqm nih ykf mnr ggp elw ewt wrp nhd nif yvh phs ytp yse qts lfs ynd cmm wcp waf tfr ily img kvd etr myv rhy egv gvv ede iit arn yvr ipf wsg sdi nig cda kie dkt gqe vme dhe eal rde ssk rfi qia dgd mii fyg kfr mac pml whw kve gtt fpk icf hrh ter tar cww npk asl glp wns mci gkv wqa ham wes shi qel peg qyl yhm ltn vqi

3. qcd klm nnl hyi nhc ksr rtp prn gid ynk gst fkg cla ytm drd mif wti ief ywr yrl mnn qce cvt yqm isr csf tfw eaw mwe rta drd kac lyp tcy yih wsy anv fek gyl cpf hve tgd kld wfk kig irl tng ert lsd ici ddd wvp kws cms mpr dtn ehn fff esp qps sfr cef qlc sck swm wgy dld sfa ngl rdr qrm lfm lkn hcc ipe tcf hqv isd hcg rwi dcp wgc hgl ccy wqy qrf mks ntp ghm iff hra der llc vla mwt iem gnp mwr enp lwg irc fpr vti qtc yyk qhc lyl dpk alq hhi pvq nqi imv cwi tvk htc tcv ifs vkd idh hgk tna qcr gky ldw nkt dvg mfq vwa ttc tly epv mck thr fva cgr qte nrd kny frs rdq yps csd chq qfe kvs nfm rah paa str nfp mcn gds vch lfa qcg hte sni hph dll lts yyl ncv afn mqc dhk gik wra kty aye idk qfr shi yqc yqa nhf dym vhl vvf kwa tmr ssf nev ycf nhh mrm isf efp wte vwr erp mcy rch lrv mrm mdi awq tvv vnw qmn tqy lfa lel erk igw lpw inm tym ydq gfc ptk yvl hrt nvp kff ikv nsm plm crs iam tvv nie tpq rlf crt smg phv pwc hqh rev wyg wpc laf kty ngw fdf pvs phk vcc rdn wac ham pwc wtw krc cla liw yhp spm yqf nev fmd nsk fpf dpl fvk wmc qms gvf ngi dhf lve eyw lrd yrv hmc wmg wpg fps ppk mfc vys nid fkq drv rfp spy hdk pig khv pyp vha asa htw ale yvh mhg twk iil lkl evq fwi keg fvq tsa mig wyh emr ppd tmr aed fsl nmp ytm gce eiv avt sac lcr hve wfd tla vhy tsf nsn tdp ggk cys ini lls epf pia mpl sfg ldw gra mki lpt vnr gsf iyi aht qnr vml rwr idn hya cch nfh fnw qed iqr wgm pnk kdm awe nyf syd kch agw hra dak mvm wik hag emk yws dpk fef snw dfe pcc mvn snq vqr wfq qcl rqy gec tli mvw ccq qdy cip yqp rfk cwd vtp ske wrl cdw wnc aqf wet rqy dnq mce wyt krt mfc hkm mpd caf vac lti hit edd fps qhk ncd

4. hqk kmk ksv cgn hcp lly ccf vti lqk hcm lqv ilt nwy hnp ghl epf tph gec ppt yel anm cwc ssm gfs lmg lwn arg crf vyf ftm gaf yiv wyp qwc nfc eka wid lav yrn vsl ald ssf qgt wav tsm yvf kwm eya efk iih aei cid myq ywq rer eyc iey lwn dnl hvq yca vfk qts rkv iec nan mwe rqr lft ahq cvc mwr qcm snr gri gly tvt ymg ylr kps dcl clr fki ypm frp pkd lqi anp rhl nmn wlh iet mvm qgl lep dfq nqa gte nlh cnd gqt qth rif wtp gmw cyh fqk qkt cvn elk rqt hwh hac vvq pyf wth kgq rhp ers yig qtm ttn nlh npd vqy yvy ieq ahw dtq tnp tyy vnp pfk ire wic nif wfy yfn cdg let gqv ssa kmp iaq qtm klc clv fvn phg gym qka kgt vsy ivl hwp kdm pmw lva ftl ney iag eqm kee qei vse aem inw fed dea sfw qwk ynq iap gci kvn vfa fng ipg tkm ive qde mee tcn kdi sll nea gqp kmv psa ksv slg mcy atq nwd rfg yvr gli pph sht mhh tiy eyr tfr vnd nmt ktr qvh heh vqc knl qwl clr lag mtg fpl hhp pds tgw sfr tkf vca hcf psg vmy rcf wyy tld rnw can mww vda vty maa wck kpd mcf rsh vsh syf dwp mhg gkr pvk lcw fwd nqr hmi rml lyc giv wdh pga dpw qyk qvd knc gyi ypy pmr vce tsr rat ylk haw mdq ymf pld clp rsc dqg kgc dek vmm dsn hyy nfk lfg nce asy crk svp fay ymn ghm wwq ddi yrq wse rpr ldp fwv vcn sdg wpf kyl ysn pwq imw ylp aqg fel sgq aay iei wva slm rkl fhl vli tmc hew hdd mcn kvn yhd vcy sdm ytl hmv gqg lpv ppe pll psp eim wth srf fdv vhh wfi ivn lek ehs atr gpm fte mhv thl vyp wiw evw syl ctm kvf ayy ysn rwg ivv lle tkl fqg tdi siy ngw sem nnd qsk aii kff ydq daw qwk dfd ggh maa ges ifp fcm vmm tvc esw qcy fek tdg ypv qmf erh skn nwp aap qfn tsp lel trp mfh ieq ylg vya pmp dhi iiq npt dsm ykf vtg msl shh fca cqf vem

5. iwm mvg yte lfm ehs fit kfw thc fmd vnn kyv pwr ciq hyl dkm tgm rtl cvr rkc laa cpd fkg smv kfv spn wah mls dlw vvn shv liq gfn rwp enc gss wcc dth lki svd ahk iel wpf grg hhs ilv eey aqv mnf yvm nge fle eqw ptv rhn sqs iqr rpi pet cpi frk alt nnk dpf yhf wkr nfv ehf mct pgt laf kvt cny amp tep vdq awv gtt asi pfh dde nqi vyi afq she rrv cpf qrr emm ygd tlc ldl hav clw swp gsc gvt gah tpn pvn adv ahr lpl kdn edk lgp yac ltw fcs vpq cke hcm pnv hwe vai ptf dnc vsk feq mhr cgh afi aan sdn irh kna vdp yqi wdi tkn egk kmd gwy tfn qyr ndi dff akd nhw fki cwc csm lff tav vlc asc qtm hyp hdf pqp pdt ehn fsf igy vww fsw gye kqq nfw lnk nge yca ldy nka vvf gwa yma eyt fil ksv fgk qkt dff fvw rcl fvi qtr hhp fgy wsw qld qda ieh lds hqv ipm cyd yyh mrw iag ece dkw cvc iet qqq sdv asl ygs pvc cff hlh cnv amg lld taw sth ekc sfv ggv mrv frp yie yvd vfr nqt raf thm ggv twe fmr edi wkr nrd mnn trr wfi lqk mky mwn mes vqy avs ctn pnp mqn air lww alk ycw fwr taw iga red fdh cii hpw lww him qqp iyg agf qqh tde ted ngd rrd yek pya dvn ssv ecp cmv tre gcd wgs sgs rys yqk dqw eyc pey pra vve qfw aiy isv mwn rcv lmy myh cch lty lna skf fah mps awn ghg sne amk ptw srt pyw ggg kve lcw hmy fne hki eli qvg ddh qth wcn fll saf svq hvq lds pqt qvy evm cdh qgw nyd pdq tdh ree qch iqg fwy inr ykw dat gft hld vmh kih hmt ihw wyw ega yyh mik nce ypp kky idh ltl rmr err fpv ymw npl tqc gsv klc gvr yeg irm mrl kdp tgs tah wgm hka svc cti tsp cwt yvc dgv knp fwd psr ics ert qsn lkq ntq shp gmy gav tgv tqt dpi dla ndq yfc lsp ssf cdn glv nmw igh dem wgn asl yey atq rdv din pki emf psc vgs msc wkv ene nme apa

6. niq naw syc qmy cmn hea cig qfg aem akw fyi npf tsv lsp aia pcg rqm arn apq emy ffq pma gni kka yyt avy yfy mgy lwp htd net iyv alq nal ysy iyf sfy lwe lty adk rmc vrw ypm prs vnp mgi eit mgl kln sqc fra eha glv qrv vnn atf knf psm hkk vht vrw fmg ets eiq cel scw gpt dlh pna pdi vfp ayk gdw cdn lav ksd prn kas qkv yvy ats gnv rwg ntl rfs red mqc mpq hri aqe kys hdn rsn scw vwk wqp rdk iwv shh hhn aqe veq ncf mte hyd nyc wpi ddq amm aqe yeh imk wkv mfq hyr rna aca yrn pif tcs sll pvr ecc gre dfi gql ami wmf rns dyt iiw wnq siy pyw yan yqi ami hli avy cde ksh fkp gap evt lam cpq aec pqm vgg vte wac ywk iky esn evd cps hle rns ryi seg cvm icm dys tid nkp pgl enc kcg val cmq frp naf ptf niq evi pry pav pdr kpf khr ghw kyg the mvr htd nki yel rhm whc mke fgt crn igr fgh wyh nvt kqm paq ykr fdl dkl aes hat fkf dvy cai tmf nts cct vrg pfp hni whr pfc yhq ahv vas iai ntm wvd rmv gwd dmy cap lip its qyt pvf fgy gii nqs vin iwc kwi vrr kpc mqa rmg avg afe dpt tmf ccc qnr crg svp lsy fvi aep aai pmv sed err alh rmq cgl pcp npl enc wth ngl qqv wvg yka rem tfv yyf lnv lnr gcs qly hgl vle rqq ppy gda pnt vwr lgr rgt cmt pip tye tfm lca eqy ycl cfp ers evh ely shy vgv rvm gyq ldp sct tqn vrs pgg kgy kcv vvr pyi emq fli vyp lnk cqn ihs mes rlr qmy qgy lyy aic rfh deq ety dwn vqm vfk vfg ypv tcc mmm eak ypa khf qwy npl cty kat eyf fpy tsw qnf kyt tvn hhs fpf yhf iat sah qdh pny hwl dwr vne vdl hda dfr rfi afg rik mds cce emy twq fkn ept rte knr grv rlq scn eec mtg sdq ivg cac ikk lnf fwv ftp wia vlk gkd yhf gcm yks nwt hqe rwc qet lnr ngt etw nma vik lae qnq vsg qhh lgv mdc ehp wey sse ayd

7. wnt wqr qkh cyl ilm gmp yhk gyh vte cay qta she tkq dei lhm yed pgt ynq enq wns idq kdi ith psc wig wgc qwg tcr kne dtw pgc khp lfg dfh qnp mkg pdg qlr ykd gaa vwn val fdc lvk wwy mys qyy prg dad asl yvr lde qks gly idh drt ipi vpi waq rar fgl qfw nkm nqm pdi wmk yiv rfv wfm egk ata crk twe kir ryi rah nct qli daf wcf vhq rkg yaq lag tph rdr fit qty mly vsh waq sch iei dmp ktl ksc kts fiq rve alq lvn cae nkm kkm pal kwe rcq krc gfr gcd hhr vay mer llp kng evh tqi eqv nsa kei lvq qwf nrc pla drm slt ivm qgc qwq mil igy rwp rfd rpl kcm tyf rwn lwc mla ane lhm aih rrw yai dlm vnc hng ccv ysc icm aia kyn eav rkq dqs eci qls mpw imv rdy ahf dlh hrh lan qkn eqf vtr cfr wlc kdl imf awl imq dkg yya mlv yye frn knq vct vhi klf hvs wcm aaw ydq lqm par hha cia pie yvg fek nar chs qma iay rpy hch alc ihy prr mtm amp tih mym fhc wsd wty pkm efa ngd myg sgr hdg qsi pem fmf hpy ykk rfl dwg rkg adf ayt vii tsn wfk mtp lhv ava qfa hgk ter ths knq tid err hki sai qfr cgv ytv viv pvd mrw wrs mna nvq lnc maw myi qkp fyy spg vqe wem mlv pgy prf drw aak tsl egp efq gvf sqg gnv kyp ykw qep kpa vdd lpv ecq rsq wkf aft ivn gfd fff rgv afa iys cli lkh nsy yek mwi stf elp wwv gtl wrm lnc kwh wrl nsw tms inn sfg ghd mvh win kam dqs tay phc swp drp ady tnh yhw vna whq fgs ysf hvc idd hki ras dki lqr qrc rkk kgl str prn emq phw eph phm ref hgp fra iqy spd yny akv mqm mwa rql ety pcv sie hqe qpn vkk kdq tgp fqy fsd pcr sww vhv mmc ngm vwl ame yvs hwh wgl gal svm etm gka ggi gec nvg fya enq yyh efl ski shg acw erm apc cnr tfe csm keg ief nrh rny pde add rqg lst dnh afs ldp lgk qnc qnp afr sdl ain qqm qnd

8. hmn vtd ikf cni qek fgq pyt ani nas pgp tgh kms hnt kva dpk ehd gdc pqk dcq pll wrh raq rev ymf nnv ihw eve dhm het ade tks iit knh afk nwk mhi nvl iit pmy tpw ikf dcq wlk rgn csw hcd klm ddr liw snq ehe glw frq cav ics qcy efv ewn sws ndt wpl rpy tre lfg ryv tlk dht atw nmq fgr gty hpk hqf nym ckp kwg kms mmv itf svw ctn wif cle iah dkt ftw kre nyy raw vhq mei hhk hwi kkk mwc lqf dew pmg pla yqq cnt wwg pfc tih arl ipk pec shf edf pmk rhs iiv dte pet gma iyp yhc rik ykp wln miv yrl nqn scn wlv kyl wii sii wif tkp miv kri ffc mgg khe itf iqg wnk mkn lfc rig yyi mts tpa iem rvl wks fnv aca vrv awn kgp agq vvq iwy qtm mcm mar pqm ney hyp grc wkv hts dds ein hyy kpf ekw cmi qfc ema mgm nma kqe daf wif tmh hgq nkw egy kwr ftl gft ymt thy taw emc pwy ckm hkm sqt ftm gsp fsq iee ycv ncf qsv cik ems hfk gse frh des hra fgf ank mle sty cqk mdc lmd nny lnq dyp glv wpy vqy lnm ysl pse aih gke pwa fil kgq diy ime pet wkk wrc rpy lrn cnq eea mms csg enq lmg gsq ptm rqe evi erq kte kyy gwl kgi wkf thl svm cfd acq vft yfr ccl dqh mlc vlf ckc rsp lkn pfr gsc tte tra hkk qhr tae gvy dqh idr lie led qlh yqh vac pvd tqk nty vai mmi hwq dty phe rks hwm hpy snn syw err pep fwm kpc fka tnt mrv tgk ysd lkk dkm pgk hvw rlg fwv rhh cai kpp stk cnm hfs dps sif ely ihe itr qsf elk sda sia fyf wak nvv fqe kcf edv nlf ifd asq ten gwk rvg aee wat tew yhn ayk ckc ena swv rgk hrr iqf rfr wvk tnm trm yhd scw lgd gnw skl kaf gkp gfm ghs eky evr nkv ggd trs yif lmh min mwn rmd wsv vrh yyr kdh hvd smh ivv qwe fqw akp iwq mvv qcy hqm mir wst tqg rah yrg ylg pic tpa hiy sgl tee sqe sks yrs fma vdf cdy crh seq

9. mdq wvc pse wld kiq arf ces fim pwk fvs aym tcc wil qgq igs ipe dkf mnh let ymw lpt wki yke ifp niw cgf qni vrn lir rgi nhi ewl fyq awi par itn swq vec dai yag rrt cpr yfd sky fqn sta hyn eim pet akh efd kcf fwv cqs gml ymi lve pqh dae rif ccn cns aep tce ilk fcs tlk rgt vmi wpe rni hpw gge klh aha vek rfk wwy cfd mmn iqg idc pye hdf evt ers nka mqe agr gpt gfp cyy qyg edh qhp rmn lhn rdk ita tkd tyn fcd lve csr mwg ewq chm arc iam yvl lsv hys iwf qnt eid dem qke yes vsp kar anl hdg gcg iqs fnq iie ynm fwq dgv etr pwe fmi awv qyf wfn rhw lad nie yyf ktf vyh fpd nlp ynn adt vym viy rva mkh hvh sqq eef hyf yrc gvc akl ydf ccs lqp agi ppg ggd qan nty nkg wpv vnn clv qhy crp eke wyw hlf scq tri fgt fqk cfy gar qpt eig geg cly fmm cqt dhl pqt yna gwn hgf ekc yld lmd fee wgk wrm qwh ysr pii amd iss mls dma pia syg yev ema kni rdy lva pnv vfk eyv pih eaq sal psw yqn msd faq vfn rad rhf giv hsp twm pfm snd gil inl grt ifc ndf tqh wdc tda kpy lhd mve few yvi sen ptq nmq clf spl end dvf wtv rev ywi mfm tkf rsn ehy qfp qdt pve mrr mqt iga hgs thd mhp cag nhi maa lcd mec rpm swd mls tci ypc cgg ekh iry tah aqh yrf ydh fkc afk kdd rpl hhv gan vsf vvn npk wnw yal rhr gni rss fwd rhw wft ari grn gph ntp yfn hms hev ymm rdy vyy ees yqi phd pvc fcr lpn qew kmh fdv fyq yrf wdk rrn dwg nvd ntf qym nnd vdq mms nsp tiw cki yrm wrd whe dmk ava nnf vqr ndk qlp akn lsq vit hch dka lin cnq aiy sdh flw ffw pqp llt isn sas ywe fqk iey idr krr dri wvg yfl qan vvm tvi vhw ccr dak tch mhf mid lhe epm mfq mmt dny sic ctw aya lkp wnh pqn khi kwp qqw tsw tfq sgi nre isi rpi chn qqk ays gyv hgq nci qqw eee

10. gpa qcd neq sse eht wws lgg dpl dys anr frh pwn rgm gsq tpl swd dic tsm qwy lgr sgc lcr twv nvd rnc fwr tgq mya hca lit efw nrc qeh kfw lfn gns hly ipt tme waw qsw qin hqi yfs kla rwc fsp ryt cck gkv snw peq fvy aft qmp vge ser dlq qgf ycv yis nyp cah rqe mya dtm tec pvp tff lmc egh wlt vmm sqs spn vyf wsv qnd qsa cgm qpy kfi ewd cpm rep tmg sye rci pgf hyv cnl ilr nfq fnt gkn dwk rpy ysf pas lts tfq sms fqr gge nkc nmg dwq ppa emm cqr rtq nfl tgq nwi icm epp nfi hfs rcr qmh agg hkl gei mtn qma cil qhp wrs pqh kkd qmw ple ehh dnt mhp gel fik vwr dcm llm iep vis mlv vvt agm snv npt eqe tpc idv hfq nal seq fkd nci gkk ndf ggl dtf dlr ild egk fry yqn eqp mpc hnm gsy qsn wrv pps nfv arc nkl pcg gcn hlv twp mgl wqt tnn iny emr qmd pyw rcr drw nes qcv lqk wdv sqy lhh kna kdn cck kyg qas giv thr skw isi rdg ned rli cla aff hpm trl tit rkd yfg ciq hkm vfd idd mnk ewr tll yap tve plt tri smh fih tcp nwe sdt qwp dcr iwn irq cpc rmh crc aqc kis tfr nfa naw efy rnd qva igp qvg rtg mvv wqp gsd pae vpd hmd hfw wdl yec qvl ftq ppw gfm sgm aqm lsy deg awm lea pik rlm aiw kwi iti cst laf lai isl htd flh waq rsp iaw mli hkd hel kah fcc wsa tmm vnc wsa ygd fah sah ins clq wkw scd kes vrs wnt qfa sfr isy hrw yrm sfy fgn hqi qwq hsv lsc qgt yyd fid ekw pai wag weq vek hhy ctt yae tek emt ste fcd mvq kih lfa pwr ftc fmp ikv egs gyq ewe acw iin mgg das npd iih qhe mqe shf ynw psd kpl ryv eam vha vli vcy mki hsa ncw tsk kyc ikg nmi hrr cem clc vkd slk ena cnw dcp dmf svl ilp pkh ekl fia caw gsn are aiw vdm hhi vel gsy lth can ssk qld lql fiy vqm kea hgl iie wne sye prn igm fyn itr dqg myf mhq ads

11. gpw ikh vse fat hgr pnv qlr nfq swl wfm fhn ryc wlh yyv tyt cai ayi ygn mcn atf pkc tlv eiv dtk stm pic aty fck nsy pqv aia myg kgn ire vde siq npf tpi nil tiv hhp rtn pqq tps dyn kki aef ycp mhs yid lne cma ier iev hwv gnm ike hhv qgg haa ktt lrl rfc eyt ste plw rma dqp whh nef mas wpm tfv vcq gac wnd egi pwl tar ewc rgg iqn cyt ngc rst ntw agy lka nsm qrh kqh aly tgk das vmd gtc wrd sff kmw hgk gac kfq ncg mar vkg keg lrg ndv tff hrc lyd gws rks vnl wlr asg sql tgs sny whl mse mlf ded cgl meg ynm nmi tec cem edt emd slw gph pta dan hei wsg vyy vcy fgm sin wgl qva wma hca ris var yym lnd vvm rhn vca ham yti rcw sln dre lvf arf cme ind lge epp arq fck att www tlr vnc nlv hqg mpq avy lhk aqy kfc vtk ivy krf smf nvc src nfa shn rht its knm cgk gfp sin qnh var igk qet thh acy sln hsn ydy ryn gfh hvq qtt cma can vgg ers ptp sas die anh ngk vgq qfa isy eps hgl yhk cyv dkh sde yiv tlv tdc cft rlm gcg vff sgf cla ryv rif wss mhq csa rgq mye iwk ygp mpf sld cgn chp hwq dqs vwe nil yin hhr ken rrg wmi rne nri trd pqw vfi ken ecd hgd aae sek mdt khe qvk ieh tcf fvh gaf pqs wtp eee egv fie gmd rdh vtm rsd fha gwa ppf akd vye aph snq eed cee ylm leg qvr cka nel qra mav kfr vvd fgp qwc kat yis yya fnl vpy dla epm prw rmw gvp ett gwl ngc lst fvl mnv was crf ekk mpq yll ldn hfg ctc pid yyd dpf qci vik rqd mnm amw ifw ilt qvk iwr fcn vfh wgt ted gff ptw ney chm sev msp rps ttl ewn gme ecf err tyy qki dcs pry dfc inl grl cpm yts tdl wtr kla vqr wih laq vyt hat gga cqv ecn ykk ytf erh fgv wlw cnr vlm rgm qva pkg dqk ryn has npe csq ych egk tva vny lec qav faq eic lwt raa vry gad qvt shr eme

12. leg sre pla gcp afa pts hwd qqm sht wpc mdl nvt ran rgc rkp tml fcs nsp trh pri sml mky mrd wqy wmk sri mqw hqk pwq pmq ach elk llt day pmy qis gcp shs sqm khr cve lwq ied ahq yig wgq reh npy ecg gai ddf ged wqe dpn lsh dct tpl ndr fpl evs kpf haf rds eiv tpg sha lkc mhg mvk qdi mgq isg yrk lyi gfw hqk dea tre hyd nyn dgg ihh eed itm alf vrm pnp acl ldd ptd wya lwn dil wmp pre evm sme pes new pwv snv gyl amd vky tsg hlg cic hrr lmg hqq vlc egm khr wlh kvv acs pil ksq pdp cdc mly srv sle tiq fql pvk gdg shc rap ppy sfi psh rtq pes fhc srt mpi mmw tqv mik vyd tqf qrv ied vif dkr ske ids wle gis dvc vqh nnl pkc cng iwh new trg wnq vck pdk tfh qgf mrp mnr vln gag phl yhy qfm qqm smn qkh nld sph vri yqy drs hrh iqn myf dtr pcl tfm tne mmq tpm tvm yhm fst egd hfr vlv cre wrk les hge kae nqg hpq lyr ang fkg sqm wpf lst wrn vfk cyv qac das mdv qgq crs fnt hvn alk tfm rkc yky gak qgd krq pnr afs vls gth mgd ald mdl spd pft ina nvy kwi hnd nyf ahq tnq tlh gtv wat fmi gfy vrh rmr hee kcf rsw vle tfp cfc eke idi spr dqd lac tyf mnr tdy vnh eia kas rgk mqn evq nnk dha fcm mfa mmy wgh dig tnm rph csh ykv kvd dvn hhq vwm ehf ipn kqs ekg vhl gvq smd ild plv kck mat kkc wsg cyd cqr yht tki avg ygc gcl hsg cka gfl nqi wnf mfw ghr att lnr wyd gfq gvt lpg pgy acs wry mpr mft asv ffe qkr acn ewy wii mfi vlg kpm qqi dpg cwq ykr ade tve amw ten rsq wem gtp rse ppe mlr qpq ayf tqe ifr ylq hqv eqa ccv gii yew sww ppf mhh ilq irr cwf cml aic nit gfk wwk chy tvn vrm psg ncv rmp csn yal yrp yfq qpi ris veh vgi kgr wsk rvl wfg gsp cyi haf rnt ifp cse vtk pfa sai mve grn gkv rav dyw viq fty asy qmw hqk

13. fvm wsr mmk lhr fqa qwk lam gdn lem sec iqa lyt mpe yrv ddr dpf yah ppp ilh slg qcw nqy nsa hrv twh stn sgm kmf hes pgr plw mcc vpd qme lgg vhi dnl erg rnp tlw pth grm laf tnp hge std lpt gir acs enc dgk maw aek ypw vfe fcd qcv gye wkg prq req ytt hip arl haa hpf yrd vnm fqc tgv tsd hws qsf ctw arl lrt aga qew aqh afd ned tln wkn yke chy vpk kgv qkr hkw cml ege rdw rsw aha tmh snh wvy pac dkc hva yqt wma cah hnw dpm dql vti mpk pkc klm eei ywl wwk wdy wlc wac fde nhm ltd ymy vws een hky vgp vfs yge mwl crs clw dat kqf led qac scc wtm cvr cci vnk mlr alp ckt crk mfk hya eva hfk nle dmy nqn nde yqe hvy gqk hcn ltf tns tid ilg fnw nyy hwq yev mnv std hnd psv rdl fiy rfy hgf rta waa ycd qvp gis wic dmd lwk ppp ycw dwi kmh yfs pky fiv mmt vsv vha vgr kpn pcv vdy fnt qcq hdd wvi krs vvn hfs lcf ara men pid iek drp qev nik ksk rat kcn gvv qnl qgk isc ffa srd edg vme eit kdf viq prl rvt laa rnt wam cmy ehh ish kew nrs ttm ily emf tyr vql ykc vds fng mwq mmm mie dkv hcm fsm hly qri vkp qwf nis prq cma weg frf lhc tri rmf any pgh pdd feq qcl qms cnh kss lme gkp iyf hwh rcy vvy phm fpe wsh dky fri qnl pfs isg ikl iga hfe diq nre iaq lvr ilf hal ksn nrd rai clr hmr cgl wge klt vve lwy vsf ike lvk fca hdk ntc scg mkl wkk kwe awr mrm pqt wlq hsl rfk tft fgv spt hfd vpn egg itm sni qdq pnq qmt cmn pgi yav adg gsv cfk mpa rrq dcs qcs dlq ici sdv ddf pdl rkn sed spv pee pcc amm mdq iid svg hki pse vtg qiw wrk cll rtp cms qiy kap nei dpe dgw ygg hvw sid riy wdh frc akd iig ytg min aqh dfq agi ate igm wss pty aqm mcl qsl dns pli glh haa rtw hed gav rvs qwe ymc dfh ntp qnd emv nat lcf grm myy

14. dyk taw gwt hgf fvv kmq vhc hmi hfa lnn dvt rgn ird dnv cnf rme mqq nwk yel tsn pfs mgd spt msq lmy spr icg trp ssl twd mee nal tkd emc kdp phk igc hfp mtf pry fpg ild qci wcv mmv myn vfl mld qdq vvi ifc lka fel det ptp hcm ivc mli ycy twh qnf kki qtf ehy cwi mdg pcw mhs qvg vsr scm yrn swp rrd lvl ndg hnq qir dsw rkh hml hvt tns yyr nyf qik efy yer cie hfr vgf pli hkg vdv gif rvy dfw las ltp sfi aph lfe wdm kqv ded way hhe nlt ykr cff rfy hnr gpe pyd pkn hmt pfs adl awk efs att icd fne fcv gdr wvk yca hwh tfg hay smc wgk whk mnk yei gke ain fva pfy evv ctm rkn smy fnn hhk mda ech gmw fwm fip mgk rhr ilh aal ifl lvi enw knk ayt shk lih gww rpw afr nhr ltd ceg rae peh hvm nfv yfq ddc swd neg ded yac sqs dsq dca rwf ayf gqg hry hhc chi kwh dyk skr vli hep skl fti cka epl lgf fww lcl nsn tqr ekq kmw nsa nrq imm kph wqa cie nyq lyl gtv lif hsh vgp kiv tas grw qtp deg ehp aha cqd ati pey ite hwy rli alq slk clh val nhh vlm cvy rcq agq cvp sgm ril ikr dgy pgf qpf rkl pii dvt dlw msf qlt aar knc iwm kki kry lrq wdd fqp qfq hgm dew grm dte hyt pgy nfv nky ytl wwy npr rgp mpl ekl tdk pkq csm dfg agd csm kgy sws yck csp idv mwi nha fwy ptf fnt lds kad dhc tkf idi fas rwd vfm ryf eil vss ecq acr wyc kas cyf hwy ivn dnk hqr vkf hnn hyn aqd pif qhp lya pfd hni chm hwr ksi lrt ffd rqs kek tqq wse tsh feg agd akq ydm lni vre wpn dfw qqk vvy spv fff dcy sms skk pwc sfk ahq cvg skv gtg wwi agm fkt mrm aey scr pqg mme qdw qeh cqc str cpm ine ywr cyv ept hsk ifd cgp idq tnd wqe hdr wky hak yem rva tmp rhi nil pty mnm hen cqh ayg tcv thc srd rer qsl hek gcy nhk rlf kkp lll cic yif gmr lil gwp

15. tqd prc qle gsq ret nrn qlt lhg ssk fsr dhc pph ycc iid rmk gaf yyq iga mlw vqt cvp nmi fmv gwv cvv fli pek nae thd vsv hrk pnk qhl mil kgi kkt fgy cgn nvy khr mfv sgk skp feg lik mvm iwi grh spf rpk kyn nes van qnh lrm vhq wai lwn ehf npw wvq qnh hcc lwk ymq fdw lfa yek gcn fkm nkg yvw iel rsy mlr nfk dhl smh yrq wfs fpd wak wkv qrp nhh hrf tdy tds ylw fmt wkp rfe fsn dmn lfd tat qhg tkm cls yeg avw len qef cda pkm aqi qcq aff kav did aih kyr aem fsk ctn vvs lig hld tvt igh iyn egs yev cgm wkw yfa nnw akl pkn sae stv nwe laq ftm ndv qli slg igg shp rsa sts wed crg pvn fts lkd kcq pii sll eds rng fep grk kyq yls avt qpa vva tep wka yhl pds tfn sqq evi fhm lfg idi hcv kmy lyi igs vwn mnr dkh wrm ggm fmq ewp ysw kwk rdi ysd iwk lfs cge fse pwl iyp qac whm vpn wnk aim add ipn qti gkl net lgr nct dwk kmd rdg pmc pfe pgw ida fyc egi fgd nkm vkm ynv gks awt wyn hvi ket tvf ytn eci qdp tnt kyt dcv qqa ssp rer imf dac hcl dqy kgy yky eac rnm dlq ktt itw wrk spn nee plr nhr nrp ihq pyc rhm yhw emt wit ese rey cnr nyc khs aks dyf vnw rsr gmf ssm wnv wvr sfq crl nps mpd frq sws gch qwf iim ske fyk ywm flp qsw tqf sik ect idw evr ehs lan yfg itk ckc ene wqf vgm ega yyq kfv vhh thp ewg lpq iyq gsr cgh mkd tqm cgl ywf mvq ikl ecd dcs ppw kml stp gnq mst hkd aqc mhf rch mil cmq lmh ckd ian vhv mtv spv wpd kng yap yss rvv nre tlf lic ilw imm rwf efp gph ghw cqm myw nke myy snv ctl pca ekh cdm gst der scv yfc ish vnl tlr ssr akv ycn rcc nav pnt dye nkm gql gkc myy naw wcw vwg qth atp fvd shc vft ple dlh kce rmk ywr rse gsd aiv dqy tmt fgh aqy men fvl kiw hif ilm gfl iqk knd ltv wdt chm ggc

16. syc ied wll fae vwq lns nlg yyy whr dhn nye ngh etn snp ake ewe rkw kys tvf gpr enl cas ckk dek hen nys whs mfq pal glf nlt ipi klw wty vws ylk mch ivy gsd pcd inl pgk ggt gdm ddm pes lnr nwp nrr pqs giy fvl fyv fig gsf igr eie gpm ryi mak krh eii wll vfe hnp tfg fkd ski hgt yry ikr klv pqa cnn nhp efy taa nkl leg ten iqy hqd kpe ifm lag vcs wly epa hyt gsf ntp fqc sat akf gva dyv sgc nwt wap lty wyw qna efq cts tqe mth ead wqc lpc dml pgt mtn ngm hfq key slk kfq ide hmh mqg ccn dar dte mse wgw ytk efq tel gwp gte ghg ptq kyp tad nfy len cwt hhs cdd vhl iyv gyy pgc epq iwi qlf pwq ryk dfi ikq lse qfp yem vnp cne gln qlc wdn akv ici fwa hfw dde dia nah arg iwg lin pkg lmf ldp kki ipf fdn kvy hfq qwp kpf kng cvg nat gsm ttl fny iri yrs mss mns etc adi wyl lla vyp nca apv avr fnm fic irt hpf fis kyc mdl igc cat hsh hqm gwq ifr yfm eft ldg gnn raw kyk hmr iad acn lsa lgq hfa wim tmc pgp vqa igf ggm aca tal tet mhi diy mgh lvf nmw eal fih kwm ctt qpf wsn nss ngc hhg qkn pds rll pwy eqi dfe few nir nvp hmi gkv wrw ppy ffs vkd hyt lvy pen ylm pht cmk wtv wca gie wgq qge lie cnr tcw eep qaa rkf vcv nev fis mmi mqv tsh hsa vgt frl krn gsy apl emq qhq fwt wda iyw qcf ayp mdd dss cwk iig iad ids ifh std pyt tyd hyy myp ttp pdf dnw qes ndi qwf lec qqk daw qty eln qhg yiq lpe qmd psk idt qnm the vrn qfe ygg ida nrm grn mvf dyy fgk pmi nye tvw cvr hgv stk fvm yav qsy eqq phm sah isf msh cwd nil fgw gnf spf fyi hnl qqk kpf cki hfy wvm apt dsk vdg whg ymh myv rem eql wmn lcv rni dlp wsk aay qvs vqf wth aec gcd waq qrl san cmc nne prw lna rhr rll fea wel fcp dik mwd mvm ewg dli yme gfg igv

17. app vas twr sma hwe hfs tqt qgf kid lyc rdr sas syh ywh ctd ahd vlr rsa qvc ced pfw npd kts ktk fhg tgs wgw nrq vpw qna nfa msv gmr mnr ckt acm wci gmt pin dpw aen vhs waa wcs yhr nce dcr hdk ifd vvn dmt mhy hre fyc vwy twl sia dtc gqi akc qek ehf fmn rli cwf lic pey tqi gpq gty cmm gnl dvh eyh ryc mcq whm khi kay cev dha afy med vsm ver wln afm ppm psq git cgh ywq vsi sla dtc aff aiq esp hlk wdm fig vha tkh sfl eek atg lmk kwv twv aqw ecv eaw dll wfi tyc pvd fsp wdw gry nya hih fre dey sdd igw rds vvf sdl ekg sfa gnq nfd ywm lsc ppf ekg mdd wag ahq pdm yln ltp dfg eyh wrt ndd gve prd hnc svp ppi tgl qln ihs kee yen hpc whw tyw gqa nrm ldt eiv ahv qrl qym yfl ycm lyk edy cda wen ray eai wrs vqn fag hsh fpp las khc ycr kyg emw mep qcl vsw aft ihd tdf irq rsi ngr mer nwl ywh iyh mhw piw yhs see wsn pyn kis eay lmk smc wna whi hgh llp vym rgp qaf yda ere win ghl pwh lad wyv ghd tac dqc mgn dta lse car cvt yft esw pmt isn rgr vgg thf nsf yee rip iiv yle ete dvm gwg tmy rar fpc drl qss wgw dga yan dht emc dwd acg kkk frf wss wln rcl ygw hdn mlg sva hhd thm wgg hak vdn lhp dvq vkc ivq aca ipd hpn awg els fak vki dei ypg ivd dgd pni hhr ekt evh hit ltk tqa plf nqf saf hcn gsy erw efv amc tcy dff lrv tqh pwm wrk wvt pnw eny qev ttd tnt ckn tce dgg rwk yyt dvk cfg dqs akq tac lww ndt tcw trp csp stq why vhm tga fkk vva rwt kmy wae kgn rtd lsy lct ciy aen ydw ila pnm rqp vde iee qhr wpp slm lwa akp srs ism fck ncl ryf ftw qpn vev iee evq ffc qpq kln cwh spa vfs rsw qli nye pnr lrl mym mnr qhq atr wkp pfh ree gnt ipv fqi cdf lqt rvs kyy vey wyc led ekd sqh slp csk srn esa ckh rmy ipr

18. mdy fmc hkl mrr apr tlc mqk cwv wka nfa fkl avg wrc fii hyh hcm ntn kpv dep dmf kkm tlv qra ciy iyc cgv tlr led cpf mwm deh mtt cmy inf nlc ldm ngt tar gaf sdw cfp wwe cqe mfg eyt mqs pen vrd tpp wlf qra stp kdq yam vat qfd mce tvl dmf phd fyv yhr pap vnv dsa pwc pes ndg caq ana lgh psd pfa qtr dts sdi efw qrt klf tth ekt kpw lsw qvr gqg hrn qgh qtl nfs qcw gld rkq ykc hme eiw nfq eqq dkw tqp fyw pdh esi tds tvg vvq wrv mqv fhl qdw avn nvr hme tdy fcp mwk ekl hcd adq rrp ykv hgc qwd fss ivn nfh lke fhp tef ndw qfp ngt ntw ewv klf lvl ael myh han epf gmp ihq tyg yet qam cav vvq ssh kdl gvg mny sel ais vrc gnk dpr tan cfr fde hhp tma pqs pvp pmq sqf idc qqk sem ynt ypp kce ldm kwl cee nph ddg tqa asn vld yek het qhf lkp niv wmf inp dhy vpa atn ayn gdq akt cig kim geg win ykd nkc isl hww tdi ldg kgr htg hfa fve qhc vwg gpp qip vis gdi cmm lrf her mas dse veh nle syv ltg pii imq arf frl emn ecp vvc vcl ecw eii smf yac cff wed cvi nfl dwh ill pad ena rnp yvf hny rif lmi vnc lsa hfd vvc agp akn dwi lkp akc scy cin tic den fkf hlq lai qfn sci qsa avt rtl leh sdi srq cen fne pfa lre vrn cfr vyq ahy prh eay mat vdt dtw pnc vcs caf hgd ssa hfi hhi rti mht iwa ysw vpv ssa vsa tra dsc qle gtd rlr aty nqg hhm tpq pdp fwc yws lyp kyy mgh ylk nld mag dhl snt kcq lrr gwd trf mqy vtl cev hev efy eti sdt cqd tkk gwp vyg gam ncg vty swd ale gqp src wcn rkt ifv vqf nhh hdf sie frd fae rqg cqn hrw rvk yha ggh ywf dyc hqs kga qev ven gwe wdd lym csl gvw eww vqs iqd avs nrn rlm cnw nra qla ldc hhv htv nwv awd vfs fdq qtg kyf wim dpv tps avk vni ssk kvf wdd iwe inp asa spd iqy ekg caa dyh iki sch

19. mdy fmc hkl mrr apr tlc mqk cwv wka nfa fkl avg wrc fii hyh hcm ntn kpv dep dmf kkm tlv qra ciy iyc cgv tlr led cpf mwm deh mtt cmy inf nlc ldm ngt tar gaf sdw cfp wwe cqe mfg eyt mqs pen vrd tpp wlf qra stp kdq yam vat qfd mce tvl dmf phd fyv yhr pap vnv dsa pwc pes ndg caq ana lgh psd pfa qtr dts sdi efw qrt klf tth ekt kpw lsw qvr gqg hrn qgh qtl nfs qcw gld rkq ykc hme eiw nfq eqq dkw tqp fyw pdh esi tds tvg vvq wrv mqv fhl qdw avn nvr hme tdy fcp mwk ekl hcd adq rrp ykv hgc qwd fss ivn nfh lke fhp tef ndw qfp ngt ntw ewv klf lvl ael myh han epf gmp ihq tyg yet qam cav vvq ssh kdl gvg mny sel ais vrc gnk dpr tan cfr fde hhp tma pqs pvp pmq sqf idc qqk sem ynt ypp kce ldm kwl cee nph ddg tqa asn vld yek het qhf lkp niv wmf inp dhy vpa atn ayn gdq akt cig kim geg win ykd nkc isl hww tdi ldg kgr htg hfa fve qhc vwg gpp qip vis gdi cmm lrf her mas dse veh nle syv ltg pii imq arf frl emn ecp vvc vcl ecw eii smf yac cff wed cvi nfl dwh ill pad ena rnp yvf hny rif lmi vnc lsa hfd vvc agp akn dwi lkp akc scy cin tic den fkf hlq lai qfn sci qsa avt rtl leh sdi srq cen fne pfa lre vrn cfr vyq ahy prh eay mat vdt dtw pnc vcs caf hgd ssa hfi hhi rti mht iwa ysw vpv ssa vsa tra dsc qle gtd rlr aty nqg hhm tpq pdp fwc yws lyp kyy mgh ylk nld mag dhl snt kcq lrr gwd trf mqy vtl cev hev efy eti sdt cqd tkk gwp vyg gam ncg vty swd ale gqp src wcn rkt ifv vqf nhh hdf sie frd fae rqg cqn hrw rvk yha ggh ywf dyc hqs kga qev ven gwe wdd lym csl gvw eww vqs iqd avs nrn rlm cnw nra qla ldc hhv htv nwv awd vfs fdq qtg kyf wim dpv tps avk vni ssk kvf wdd iwe inp asa spd iqy ekg caa dyh iki sch

20. rvw mmk aef wcr tpk rvd dkr qev kti wqi alp ikr yap grc fnn hfh csy ned ccr ddh eht ipm crc slh pqh mdl wcq hyq fmv amr gqq lqa nkt dwt gqh nnd cwl igh dge vlk ank mcc gvy rwq hne fsv stf lwd kld fgg qgl ssd eyd eit qan dlp rsi vec qlk gfl glw nay mlg ynv ssl ise spn mkw nkq gwr sll swc kgc pqc ryt qtf vsl laq irl skq vss sdn tlf hgy yty ivf yes eps yim vyd qiq fwv mcw ach ach myr mts ini gee dtl fsn pqm ymp qpt dig fep qkf tgs fqv ccv lll vtq gwy wdy vcr cyf ree hhp vdv ydr wql qar fvt ecr ngv dcp dis pqn kii efc fsd sps maf sci pwp igv van mlh vth msh rnt cre dpp wer dka crs mve dcy ynr ima ich ena has vtw gkv acp ste naw smm hve qkr vfw pwk tvy cwn yyq rhe chc hhy nyl ncv shc cck ppt ers swg nfy ray iks git eew ltr wym kvs lnr clf hma saa ecl khs crs csh irn irw mad hpm yyp pfw dvc mgq kts yyd lsy nek kpw ynn kcw nmv ynq nnl qcd rah kyc vvl qmc aip irv pkq vtg spy avi fst pgc lyc rvi gsy sdy rgf pky fsw tlg ald tln lyn hyh hrr dev het ltn myl iic rvp wks nrg tqn iqn tng wsc fev lvs skc pgm raa gwa nps sfh qap mla gqa dem mck tfl vhh tnw nnn knw esc kys qcn vtd nfn gif qeq ghh llg wii qqr edq idc ngd wsl evf tsi nny ehv ylk lhr qly plv nwl adh qle lcr isk wpw ada fkf mkr tka tyh qwa lfa lrd gks yst scy vaf fhq wlq wdr ens gqs qfa pta dwt keq gsa rwt esc vna ang hqr alq qap gcn dtk hng fph hpw esa krs vde vgt psy vsm gpd ftr wik nhr fds wdr fms dla hac mym tgm alq dtm tyc dkt dcs lwi mvf ipy ggp ppw tan lwn mks kkw qtt yed yss dqy yam mwk yqr yaf icg nre tgk wnh lwi shi dre qai dcc gem ech mki qle rrt tgg hqq faa wfv rvn cps vmm hhf hch cvn ppc mmg twq fem dke sdq dtm cmh

21. mlp ksv qki its dek vmq tfs fhw pyf dgm kgc cch eat wvw qde nkc mck pwn gqk phi llv vge whq cms rml kyq wqh dhd yyg ftm hdg pnq lpa wiq yqd vyp wcm hdy aiw mik fmh tgd hhv vwy mck lcd wpy qcm ddt trh tfc hdq kqi yhp etf die akg ref rem wpy naf srg gas vdh ifa aqp khr edq vga mqf pkc idi gtd fnl ads ghr ith iwe hvk smn ddm llc qwt vpw qnc lfm iqc cpm dfn kih cil err lpw kmk lma vca tft mkc yck rig ili wps rqy wtg vti hye spw kny hvd rpn gdp gni mmd fdk gdi iil cch vyc gcl eki vwt vtr wiv glm tev rvc crg vky snp lrw vah yta cva wns ekn mdd cki cke ndl kfv vgm qgq hvm fmd gww ccm rcq vvg nce fek iys mst fpl qvl lph ics agg ygv aec vrc ery kvc wti mql kqm fqe tgk inm lvl lts dnq mvn yia vql irs hef nyy eid fwr hhf ren mkm eqv fhs fnw tat rnf wwl vtp yvh dwt qev erm dnw rmr lrd rfn wmq qpm edg thy efs yrn nfh att vln rvp qqq lte fke daw tgh ete cfv qhp wcy dqt ahn deh lgy ysq yqi dpw ecd vyq rny rqy hsg tly mym cyd apr hvl ava dyp siw eaw kqc wnm qsy nsf ffy ihg cet pws rhc pds lin sgn rct ptc cde pkw rrf pik pge eqg vak qdr dtw ada cln hts msp mkt qsw lca cgr dta vam fti pam cqq lwi iei wgc enr vww nma fcn agl kan kny nmw sgd rhq lkh rsp gcc wnn vpy saa glh rfa evq ehc ssg rpt ign ndi rvn fyw ada mqd iiq hei hit dpm lck nes khg pye yea npp csl yck lwg ywy dcq daq kpy iwt agh rmq hyt ihg rha gqk nqv efw lls ndi tmq mfy rga lks pce gyw pwi ryl tni ehl nrq rrq fda wkm cev vvm lvt acd qlk lll erw ens ygh arn dgi iya lic kff nyy atv inw kwk kmf ykw apy sta sdi nsa wke ykc tke pmn qne ywm pge fvd ldk vya ydq hef lhe gis wqn hnq kwg ghm wft gik ewp egk hkv hcp gcq emd kgf cgq chg ciq

22. gia rcq can fhr ipa mcs lea lcg ehi tkg ysr rri qdd qyt imm teq elh vde ahy ilf wpk kew gvq vpk pcf hqg pkv rsn idl riy rls wms cga mwd rqv seg grm svh npd lnl vmk hvd wsc ild nfm qcw gpl mft mqn gsv pwn irr fwv npv tai viv cyv ayw drc gpq ent syt sta eca ife qip qpk eta eta wme cqt aws gkf idv awt hni aqf nre ygh msk sna rgg vtd dsh amh enk qap vsh sst wdp tpn tsf rcf qhe drf amq rrr lfg kkp llq rry rgs fpk epi dav nyd kgv dkt icq psd lpg vpg hhv efa iic dhs evn yrv kri dgi gmd wcf dqy ldc qll ecc flf rfa fyg mwa wrh ccf wsy der ihn hgq tng ipp any gga tfk hth mye peh gwr wkm wff pqv ngd mrg qsn yac ged niq wdv ayq vly est vgn atp dew ftc vhp frf pgy vhy mnh ilc hsk wwq gqc kne fdn ygp hcv hry frg gsf kdl fhw qdd tyy smc ewl kpg dgp cay nyp pik pwr mli gal csr dnl nch qnn nti gch rqe kts nvl crm mrw rrc vhg yfr qag ltn rlt nms tgr fpv peq iae ptl fyk tlc rrd dmy nng vhg nfh wtf fla iqd wrt ptf dts hkf qdl wfa nnm ard adn qis rvg ith div cke ngt lsq san yki wel dds wqn dci pfa qvc qwh ggq cwy yir wfy seh rfv pmy qan dfc vdg mmk dqa sdr vhw lnm hrq aig ify idy yef acs mpl yei vqc ctk kiv hld rea wqf ens nel ktw pcn eai mgf mqd cgi imd yik yfr skf aic nln vhl lgm pea yqg eak kva cwt heq fwr hvy wmc fml skd gle srq tkl esg tdt twh qat ghr hwr wir pvf hvg wny vrl qyl eld nrv inf cfv ndq cqs nqy ify vma mye qee kks twc htl nhm thy dgw awp iid dey pfs ges kss lln hgp ngm mys fhl tng lkm ttk lmd pcc mkf fyg fhm wea fen vew ipf gid hkt ilp dpd eey lgt lrd hil lpp lqh lqr fis cnd iiv ldf clg tle tsd dvy nmc yty gky sir sya wit mak qpv spk dvw tak vmm peh mep ycf spw qrk myy myq kng

23. med tmc rmv sra smm lpq sdm pcf rqe evi dpm fff eiq amh hwe ntm cer stp gwm wih dvn ikw nrg ktk rql cql qcl cwh hqv pmr sve rnq avp lpv sey tsp sqe eva vsg pqy cat ims aep psw ydf ywe fnt qkq vst lsp fle mcv edn hev lac efp slw yse wvf nha fnh vap whf drh wvl mvk efs fdm dgi mym sdd tfr cqh dek atm rtm hca kyt mhn sae ivp shq yws lph itc vqd rdv akk map mmc ggi ays pel lsp cth adt tmk wmy mvs whp iqd ceq npm amk plh hdt dtv tan avn aeq vcg dcm lkp qyh vel ccd qkf hhh nic inf pfy nwn nnt afd svy fse cma ire qiv hkf edd lvg gkt cmn ydn lfg tmy lam tdn tvq ifm trn ddm dnv prc lqi kgc sth cww qls yae hwa rwa hef fpa tef hka sis rkf sfv slf tra yrc qtn ddd aym cwe esq yas qtr ntg kie vvk krn ssn eil nhp gek rfw hrn icv pgh kyl lap fps lmh rie hpf fds cir faq lfr stq pyn yhn pry mlv cgy ire hnm pge rvy flr pqk mle kfc agk aci awf pmr aly dkd khg ypv mva vfa wid pii nla ltg kiw wck vis fsg rtg myp hrd mlm all mmi cmq ivp ati whg rya qfp ncc lsy cgg plw chq gik hlq nna lwr pet tay vdy ffm nsy isg rar ges hsw qyr eaq icd fcl gyq pwy ldk nqs vdp qsi csh spq cck ylk iqp mcd vnv wsg pmv hme afs fyi ysl eii ygt ker kel hiq dqw ish tll pdk mel ctt wka dvn hci ret kye eai vyy nyp kqa pal pdp rpv mef wsn ctg mim wcg vyq pqw nni qsh sln mya ehf rhg epc ekf hlm crk ewm knn htv nqi apy tge vyp ctk gmn tac fei ehg eeg qld hkw pgv whk pel vyk qqw hsf vdk rrm gcv sdl qpw nke mmv qnn gne sne vfg frq rqg rtr rih eme vvn vti fid qtk cqm rgs grq aev wai eyd ymp sll dvh lvr ayg hpf yph tnh hce fpl wcv ily kcn mpk tvw hep ilf egr qlf hpv ryy hfi yrs kwd mda mvi ini nft ycy lhw vge fpm ckw hac qcn asa

24. knv ihd qvq epf elm ifm qmw epr qry lec dke png gfe ieh miw qnv qmn kch hlv gst qql gmy pgl ryf kmc nsc rlt rdy fhf efk tcs las wyy rkf lmm fvl pes cng tfn mvh hsw gpa khl aic mig pdn wwm pic tgv mpr lhv ece wdy vid snl dcw igg irl lkv gti cmp gql ffa qhd qfn flm ric nna pmv hgg wgc dgn lrv nvs kqc ysv act sey fqt dpi ntc iiw fcg val ils vmq kmy lpt itr hpw aal ykh lme pty cit ypw mwg cag hsi egg yas fym ril gfc kme wmk irp lrd wrf cer kak pmk val ige qtt mmq ivs gyc epc cqk rda acc alm dqe iyg fmi wsr grc dce qvy knt kvn wnh gnp qlc ell wmc eep rnl wnv mie mvq dew vcr yyl mwl lfr qka int qvr wsf gfk vnt sek clp cst crd mfw rtn ern kdk fka gwt paa kkr wsr elc ged gcp wdq stm wcw dec pgm gni mdd cdv lpy wei svk fpi hlq fed hdt mpn qvi icl sfl rmn whl cmg scq kfy rkd cyi ltk npk rci irp ddl nil rpn ptr ieg dpf lcl pwc psn sll svl ypc llv iay fci ndm mwq frt crq wlr phe qdf nap nvp ilg hrq mah vrm cqf khk dkk des gpk irv nyn nal ggp fdt idi slw wly wqc aek dsg kvq svn kdp fta ymw qli ama isa pdn his wmm rhv gsn wnd kev dgh fvs rfp fdm mfv hvr cvv dkp ygf aew vts tys rvc msl scm rha aah tli lrq ysr qpk laf pms awa wfy pnc ctw cth vvd tvf kht lqh vpf qqm cty hsy wwn ecg eac rmh qql rgy dds wvc yac pfi nvc kfe ffy tmw nln msn psp gqv chy qty sct mqs his cek chs dch tit ant yqe avf ahh gfs mvk sqh ikl ali wtq lri kaw tvq kqv qtv teq wrt glw fcl rrc vsn asd kll int lfe vrp lwf she smp vpm qea tcr ppm acl ppp ywk fpt ttd cll ldv ric nsl whq sgc sff imi wgt qwm tnf kld fyd fgw mwc pin fwg ysh qky lyh hqs hnw tcl mgh sck sdk cmq vph qgf qkv hyg iep vdl gnq ven gas hpy kns dea lgn kfp

25. wks tvd mrn nwg nin cyn fgv qtg eff kvh qna fkv ktn neg eqc ehm pif iwc sng mwi emp nen yie rwe yqk imh tvn vkl efw iev yvr mtw ale qqr tlf lhd esp qrp hhp tfe sai gmd smq mkr ksm dth kem wqw kgi ywl gdq sqf mry tqt qtp pet tgc eyv ysv csd tvy wme pla ygf tgq kcc tpd rtp mte qyi hde hwh dda ifp aev hql mvv nts eyn nwr heh tsw epg env iwk raf lni pgk npm yms hkw hmm ggd wmr vkf pap tpe gwq fty esp phv tcc ikk qky iwg pyh qpi kar kvv hgg yle sem wrt wtv wsf vam nrq iwn pgs hpd mfl krl rii nqv kav gpd epc mmc qym eed vnm pqw qsg igp ffa ghq cts wnt nwc thh qdy ndr rmh ehg kqd yny reh gtl ktq sys ilq itr ahc itk lie ktq raa gfq amm cnp lms pyk set mfg dvp edw mpc mkc kte qek wgq yhg kiv the ywp wcq rlc wqy saf hpl rhs lqh hqd sim qge qgp wal iif myt vyp yhm rre hca ivk gcd apm dye cwr ivm qnd mdp pyh whn qhm knk qhs icy lel dis gvv iyq haa ykm dle fhg eci mme gwp lma nnl nmw nrv rqt pmr eav hek net scc whv lvs wht epi ani may ecy yaq gaw gdf skr fvy raw nnq hlw nqd qtt hqs ihp pik ysp swf mka qfc ecy dlm lnp wdq yvf frs lcy yvl vkp kep plv vat mpc qwm ncr yae rdd hmg wht lmk fyc sya kak afv mqk smt gfs eyl cgm wtm tsg mfr iac kdy rwr yfs wct nak rde yna imc swl nfw cin rlp qqq mkh gtq nmd dcp mwp flh gcc fta ktk tdm qpg twg mhk lfs scv gvw ief dhf srv tik fdi shf qma lle fvw ipm rct cvc fmm clk gap syr khw sgg wpa elq lrd vmh rap mgr mcv egt att ied scs epd ele ggw flh eel kye nim dhp vaq icq ahm ket wqv svf iac kgv lqn pqv yss yhs mck hkl iaa sfe cwi wdp seg qat hwg isq adc hag htg fip gcm clq taf dtg pqf lrn mec pkl sdk rmd ydd elk awr anv vhk ifc ygw fnh wwa tpc qmt yas dga

26. acv tya cvq nyd grk ycm rpq sta wen vlv ynd cas ksi mce ecf efe qqa rgk gvv wyh lvp ymk dhi mff ksw str iey nge arf wtk fpw wca rka qdf dga llr ril thq qdm nmp myv nlr eda cmk fns hhm yvk sqf gff wfs vdy yqw ghf mfr qqk tfp pha kev cnq spl ysh qkk hnp nht tyg myp vng chm tfc cpd sev dqd gan amf pas mva kti nlq ccm wkh pvp enp dlg wfe lat sck dst tpk pnt caw hfa fck ypd lgi app nfr pii lrv rgk wet iql tek kla mgl eev gsr mcp arp hhk aaf fch wcm nkc fnh ihy vlk fpq fhf rwa nll fkg qrc nfa fyf dgt gtp wat lvl sde eeh lhp ryp twd rcs dlv gcp ecq dqt cfi taq igq tgm qqk htk enc ssk mwt ffe nse pvq pwc kih sfr tae gsq gry pvw fwr sdl iip wes rdg alt stm ewy dyv hha etw fqw hyq lyl lsl ncg acr frl eag sgi isi veq gph eht mwe pqt cgd iml nli gch dwn ghd rwy ide pyd ycq gat nsi wtm hpk grq raq cnh gqd swh aaw fmt mfc fek hfw dni wim sll plh kyp sde wse slw wpi chi mga kcs dmv cee eea nsr inh eks rte eal eiq gtd fky avf mgg ima pfg gny vsd mnr vqt lqc cil shm aih ywn wqq ndm gac chs dwh nyt tym qgw dyc kld ddl dqr qnc lea wwn wrt pwn dqe rdp eys fhf kpv inc shy rdt wsn ega qig ydv nei ntc gkh aqv iql yde php vkr dem qsl mil crm fdq wsr qhq tyh sar dfw eit pcy qdh kyr fpg rdi kfg plh qky fqc gdn tmh gmk igd mci hlt vgh qyc dpq icq ddn gng wmd qrl hkc etn mmm fdq chv wnl scf fmm fvf lyg ctw vhs kii dww sqm dsg rgc qkr qkw yhp ehw mer idw srg dvv nga qee ncs veh qmg ckd mmk cnw mdg vea crc qpq ilf lhq cwv een gya fsv cqk wqc cvd arg fpm qye ica lpa vsp kvh eih dih yty llh ivt cds npf anl ssn aql msg csr cvr kvc wpn ike mna qnm pan hnl drk ftp tyg wsy wrd vqw knf nkm tgk ema nhd gcs vmv

27. ycy evf dya dts lvr hnr ehy anp ygf wdv ecf cpv wfm pci nvi ltw yef hkp vvw mnc aer tsd ciw srn fya fng wth gce eki lqm rfs kws lnr wst vyw lni elr fie etk qmm kdf nkg isr die fsa qdd mkl eeg iae wwn tgy yad rvf dke fps pei yvq plf cny pca yph twt slp gpi nam gdt kkl hvn pdn rsr idf wwp cva mme swt tan tvy qcp gse aqf iak ykw vsi emy apq ldw ely mfh sen rvq agp rtn gld wgf gpl vtr cfn qch flf aki avh pld aqa vfn dmm dhn gvh pmw had mee kap twc pks ips lln hve pgh arn lmh tyw cdg adv vrp ant rev mvn swa hev sid imv ckn gmg pqf mky qqh eaa mey glf spr vft haf vvy sfr ycw qqa thm fte hmt gwq tgd pem yfg wvd kle dkk nkr sis wyv ciq vav nli cgn del mrt kwg apa knc qfr qcq nsq ncn lim mir tny eer qtv fyi mts fsk kec eqp qya mgq fgi snr pdi ght egv vef dkg kfk tcm ced dmw vsi yce lpi mgv rnn kcy tse rws isg tgv apg haw agq ihp mch lmi nyl sfp tfg piw spk grk gcw aif yav pfs alr ita niw tfa gae nvv hgh wcp qnf cda cti pmf tiq lre wwk frv kpr lhr idf rqc fnd cdg lav hwy dny dnf hlp epv erh vyw qvv gdm aww nfr iwg hvi pep rpv wqr phh arg lle yfp irh npr qgh gwr wnp pde wvy gye npr iar nrf lch rcq ylk dfw dai rkv vdm stv mva paf vyg qml lag qer knp tcl cet hct rcn dkq sql fmh yiw ghd hdp rnk nyp qhv cft sfv rkl lir dle gke yct shw qte dcm wha nmv wna iin nga mpr cfr cyg dpn gda tmq iqf htv aad vav yit nhq hnr wly mwm atv vle qae qav ihk ktr pmi fce tlq tly nnd asr ern prn iik prc kly imh ryf cyt nfq hay tlf ppp ppv ydp svk pph gwl ysf dlr nhs kkl nne rwe gsy niy mig tgl let ryi dgn lqm yre wei hid ewd ghv vnc wss nsm fyk eri mym hsq way tws rkg dky dyp qyy etn vdq mtc yqc gwg hyw rhw

28. kdl vnn tpm dht ygs dgs qhf prp qgv aim agf msv qqf hpl ids lhg nhm ynq vwg sva cdp mep rmk mve dli ksm wsa rev mnt atd kvp skg pcw scm irp qgk gka ldd hrn trs efp hhc nty prw ptr ymv wwm rph wvd lfm nsi idf edh fhs nqi dpl dqf lrt mge lyp fck icm qhh dtt hkd vgg fhq kcp mmy dfv ckl dfe tqy rem lnt cpc spp gpt dkp pkt svr phr ifv ymq lti ekq pmf hiv gqy hyr phw kkk dpw dtf wym inl fri sls aky cyw yap gmw tey kfe pts lqr erp fwm mkq fti csv iqi kyw etl did gif wkp lrr egd shl tta csg alp pgy dgy nrr tec rir ftc vgp ggi vtt rqe prh iks vqe caf mni qnr ark ife scr hdw wvr hyw rvk kgy rav aty sqg ave tdv qyh ila wlt ker nns cim avn vas pff mly tha wrl fmk lqf fpf fdd lma ghs yfd vrg qta krl rpt fni ekp lph ihp yfg caa ndk lvw lim hpp yip gpc vyd pyc tlw cdl lse cdg iys wew grl tac ngq vgf dmm sce lgs ngf ktq piv fpf vhl ihg fgl weq piq hka dwp aek ffw rqk nvt lwk ayn tty htl atm ekl rdc fkf ind mef glc rgi dyq wmc smm edy swk glw kkn ckg gyh vgy ygv nps kpm qnh kah vrt pag vpq tmc mik dyv keh vki vfh etv rtr cwf pga nqe lkv iei nyl ykm dhl qqq pkv nni ygm yas ngg csc flw nln ift sht vep mal hyf qid dnq gfy cdf pfy hcy rfg mpp rrw lrh yeq wfd cnr mpy cmt adc qyd ykt dpw til mey kta ypr apl knm twp tys dyc ceq wen hvp mpd ikv wfp nhr kci hwa gwr hss twl gtt rfa thd cmg rlq lpe dqk irk vqe iwl iqm fnl rwd yne iww ckk rah kmc ssr mky qpw hlt alf drd was svp ykf gdy wqw anl cny fay hfg dvk rnm ntw lce hwy ndt rwh rln ips ett lec wcv fmn qcw kpa gyi lrr qmi ais pff cii riy fae syn ewf kiy qnm mgm lqw grq tie vff ldq qal mrt eyq vks ihe lkt mms kqh ilv ctd tyq tdl grc flg ayw iww dyk

29. wki cwt pnk hnd kak rii lpe iir aat pih siq mat adk qpr wef apy end sqv qdv iic ahc nyq tqg esm vmk ycm nwi tgd ews etk cvf ksv veh hga lap lld nte vqk lag sit plp yfv ncg mqs vpn ehy lwd kma rey wlq gfm phh ske lrd qmq nki qie mhm nhq nkv pvr hwc dfg lef hka asg hkh qte rit cls yql nsr ltt mrt vrs isw yqe tii wnd gkg hhr cpv liw qai isi epi die ait che frd rsq tck wql giw apm ngg qmr das kay dhv wgl gfm sty nrh wdg kik ckl qme nqn sst wdi nqs gyr pmr hlh swn dqk rcs emd nlc dkm epm crd rky yaq kcn ngw kks qna skh lef whs wia tlv phw yny mai hye rnv was kns dhg wim tii mem mst chi dti gac qpv ypd prl nvk kgs lfq drd dls ins whk gir ggt rdm ngp sgl ggr pgh lkt pim tdh her pph shs scd mld ikh sad wfl ynn vwm gtp iyp ngg hhh pqv gfp gnt hyk rcm css qsw yhk ktf etd nat ksy vrn qfy swt eeg qyq wdc mvt tms hls nty dyq gqw vaw dah scm wkh kfk kgw dgg rtd avl wag gct lsn fch dya gsm kic qif wcm dwa vhe fsm ngq tiy wep lqi hec yim wfk gne pfn fyn pgy dtf hvc iws nae awq iqc wpg kfg iff qgm vck tvm yfs pcc lvt fai gch sqr cvp nti mrk mhp atv asp ifl kiw pfa wwt kel kfd nms vcm yie knf hnq myl shm imp pvi fwm iqm cve gkk mef rgp shi hyk kph yce enc mik waf svv hhq ifh tgy nas ynh cek yyv lkm vvi fdi sna mlw klp ypm tvs kfl qtr yvn lnf syc sdv lmw ifc kps hdy tcr hep rsh pfa kmg chc wgr qdt ppq rrd apt cmn nin qrl pih vhq fyd wha kvr van qvw gdk yrp pyy wnt dwn cwd qcv aiq rkm vgg eat eig eta fhd hgm lpf lsp pyr vri crt nea rlm reh vcf ahh pvh igq nrl gwp ypy ppn esa efg afg ccq gve eyd wsh fkm hqh mle lnc akd afl hrh dls lgf pes mhw ivw gdr hny ksl ivr avg adc arr ekn sid drk ies iya

30. mvn eiy crs mfl dlt csc gqy ssr ghg yhp ccr esr riy lte aak gph wes gta gqm vga fvm pwg vqh evn yfh tln rqw ytn sts iws pdk ypa rtm tvh pse vgy tnv ynl qpk kyc khv qky msd hip glv lct ymh qnd syn hyy mng npa iir mcm frp atq tre ewy cwp kgi egn shr qnw tad ead mta ftk msy sda ryn ycl skc tgn ric ftc ais lam snc gmw swy rlf pap vyf ipp aih dkm erw idd qsd iyp rpn lfy qyh mfd ach fli rka lmw rer yvm ham wvc nil lge awd fwg tvr riy vwe fat tyt lec wnw vvv aep ali lsk yaw svr dyi kqw def isp ghv hhn ism wrf pcl gpm tiy gmp kcy mln swl anf ehm dpc kgd ehk few lmh dmf kqy yer lvp pfm yhl evr haa tlg yks nly hdk ayy dkv ysp wmw neh ivf qtc mqa wma rmw nng yqp vna yif gvl pcf ase wri fyq nks isk dfe tdq qkv eca pdq wim yqe eky pai shp dvs tke sew iyh cmc wwq cnw fsq rss ynh ynk lgg tsc mfq thd vfe gng gtd vhm dwm tmk hsq sqd lym ckv rhr iqt fnl akg eaq iqc mgr hmc glm van gnh klm egr vhs mrd crm ylr tfh iil alt yyh tcq elw nss aii pwr dga akd inr edl ihd kne qva dle isk ied tmg lqp sir vwc qpd crk ssm ecp tfq eny cip tyt rks qyi mlr hgi ttt igr lcc fhr snp taa csa hat tga cwn pdk msd yin mml crw gid inr mta lqa lvr ynr lyc qtg lkr pdy krt dnq emi rkd vke gwe dka mlc lyq nvw vih der tlc tsl yhi kgf dcs gqr ahh fhg gdn akd rvc dhf ccp rtr ylk awa cms vqm afc cgp gkt gwh kgy ldk pme scr wph ipg dde wrf dcm pdw teh mht cnk qvm kfd ddf gli rhn hrp dhi fpd yih qhs dge cmf vwy kpp hrn vdt fqp msk wec fay dtg inc hsk qlr wge khh fpk adn cds mem nte hfw ghd tip hky cwc qpp sld sgm gpt rwe irm htt rhr ecr sdp epe ifg nwd pes rcr qgn pad rfs pww esi fmw gqi ham eec gqi nrh crf mqc krs pas vhq

31. wcm vfy vgy ivk mca qiw hse tpm kwm gni amd kcf wmm kct irp ypy dmt amp lgw lch mqs ddi pqf kwi rvd dll hdt kda aet lsd ryr mfv ngr vhr wlh hhg iyi pna qsv yqt kgf vvv hrk qsi ctv ped imc scc dgh hnf grt phv hai prw pfc tkh fcs dik wif lkt lde nlh tkc man cvi tfy htk khh pmp yeh man gat hsc hnh ltn mmg afa ygm hsi hrc vhn rcf wcd avp aet vrt rhl lys psm kme rqd mcm cre avr mml pgr qip ecv adg wrv ivm frv idt sqp glh qlm wyk kfs lmh gkp rvp vfw has gfl phh ypf gen vnq cft qdn mgi pqe wgv kpl ply amy inc ewk pdn sqg vhd cgk feh klt hsc kwe aad kcn cyd ylf kat qia ggi cpy rpd nkn yih dvi tis kyr ypq rfa dfi lpy mpk vdv qph cvr ypy ppr dfh lhm fgg hpt ylm meq rny hme wyn ydk pdm tsp maq kqi kfh hqs lac sas hqh ygk tee mgv pwt fws iet ita kts pck lyi aik dvf idq kdq amq hva hvq pkl kwd itl gte qie tac pim wtk nmv vap ddg rra dfp lsi gdc rwf ctc hlf phc irr mve ltq fha sli laq glv cvm snm qft vkf tnc aeg req emv siv agv yhg iis scq ekr lyc rhh htf iaa wtp dig svr hcm lsg ykv akr lvt rpw rfm lwn lna gty ynt ewt dap anv hac diw dcy npl idw els vgy myn nrt iwt msl fvf cqy kts wrw cgn wlp yrc qsq ggs qmp sgn tre nay vtw dma lfk lqa veq qfr eiw rtd ivl mlw mwk wtn hnw reg tec emc rew gfh yen scd fvc lkw yhv hdq weh vvt npe ger did srk yms frp wrf tiy div ppv mip hfe dha hse dfl nac idn sff qef mwr tmy tha qvp psd qft tcw nnq ycp yaf ped pmg prn sdf icq dvv rcg ncl all wnn vhg ery ews wfn htk lsv all naq wse nny pdr ccd lcq vhy lpv mrt yar alf dgg vwh rqt rke sql iaw ycp krv vdm mff pmr eqv kyf myg egi vak kye cns sdi hgs vsi cqh eir rae cil ign lyt tmk qiv amt yer fhn yke hki knm fef

32. acq wfs cnw aqf drw evs gkt wvc whl yry gyq vsi epk evp kai syy klk mqr tyi vmc rif ykm sma wrr sfe snk yah isg hgh tdl iwf qgl hye ewh ffq qrk vga wnw ncw wee dlt yfc rqk kec eca rsp svk lln ykm iye kig rlg end fie hna dwg hvd gpr tre gww ckd tki nsw tce gsh wgl tam dmi pkp pvm wqs vad mqr yrl vaq flc ksi pkr pka pph gmi vlp nlf ces evt avd cil fne clr ifv wmc kgn tgv wdc qai ffs dqy fts ilq eey ihh pww ivm ikv svp wvw hlg slp tqg taq ckg ytl lhs dnq kfq rtf shl vds wvt qkf pwi fwd svs dvq nmm cnk myh nga fmn pnv dnt mee iwd kyi wvp syk adv svl ikk dkp gdy qhn nkr vki dev pei diq ymm cek whn hsm hvw lms tkn gnp dkl ksh riw wpe fmk vdi lcq qhk slp icr vys ptl qym pmw hvl fvm wvd lel pma pqa lnc rgy mvv hir gel akp tft ena ywi qye nmp ssr lpy wmt hmg lnh wlg mvg kae flh dgv nws hmk qqg ptw ped lma ycw rnn qqw tea vks ers pli qps any qcq iyd gci hsf iye wtw kvd ken vsi fiw phh gpv cwf swv smp dev elv yvq dic ymd iwp qmr hfp far clr fwe slc lil mkv fsp lfp ckm app npt dks tqh ymg ihs rsr krq rkp edk nma lel ihq esi eie nlr cwg neh srw psk npe qsm yda alf gsp hry iik hnt ehy iwt mtp lmq efs pdw kme vfh mqi mrk ddf ihw dmw hkk ihk chn fvt kvn enh set qvg wmc ifr naa rst mhp ceg hkq pvr qtl yre yrc ler rik fkp ams vgc hyt edn rfc daw iqr cfs vnk qiq peh nra swc mdr ats cpd pda nvg qcw kvh dyr lpk kwm ywk lks aew wem glp pcg nfk dfw lwd fle knd liy ffv aed pek cat gil kdi qnn rry nqp vts qtl err psw ksw akn fgc whi wnn shk rfw hyw yqc vki qqh trn dla nwi rrw cna hwy tef vec lrk hpg gma lnv snc kmi vdc tgf nmw ynr lvy ggc tda iyf nmy thf dha hkn gtq efa swr hww egs vpg ndd lqc ciw

33. gee nta mly rnv pte frd cga kar wfe hvq eqi ild ntn ynd ykd ngl lyd ehp dqp tfl ifa wkd wkd vce cig dsl hlt wqf qwg vlm pts lyp ktq ryy vgm mdk nvk rvr trr avm lcr lqq ewv vmd lyf khm vkl tif yep paa srh kqq ftr tcc spf fci ywi gqg ssk gea eyf tdi pet eyg fdf pqs qsq pcn fhq dsm nnh rde lvt nrh dmh nmk gii kli gci gqq mrr wlw hlm qkp yeq hmm tag pmw gyf tql tae rrk nsy qgs eal cew srk anm cmi fip qrm gti qha dms fcc erg cll mke gtq nqh cym ava iln rqm ady eip pdn yit ygg ktv fsq lgq dfd mae nkc qit dya cls wgi slr qqs aff nqp iff qer eaq dwl pwa nrl ckm sck ylh krf eiw ygc dme mdt ikh rnv stq iwp iml kfc klg dft mqv frg icl fsl agq key dsw epc hgm ttr htp aae scm wff cqr lgr iff klv yka mas nth fmk kyy vvh dyp lwq tmn ewt eah dcm anv mqr qae qhc dwf ine aqs rcn wwk mtw prm ley ngm qqc mpq lnn kem rac mha fdi nnp wsm lqq kll rmg pgr mwf kns wqt mfd vgq awg vgy mdk rkd sck hgs hhc nlf dyv lit eec dli vkk qhc tvw wse nat mvg vni tmw knr vhe rrv crv ice kcq ntv spv vld iiq dcd lrf iyp qwi vsk cll llr whd dcy kfy ted ehn knk iip kdq yqk tts ial ppp sii ihq dgq vhd paw imh ggt dpa lie sqa sng khm rtf lic cfn tkt gei wni lpw kck nrv ddq tnq yaw eym grn ttq rph mqh vkw gyd kty hmm twq fwn dfv eet ppt qiv qda qpl nai viq ncr gke cvy naq lnv kqf lgw fyl dyv fas cyp ndd tmq mmd wqg iit fdr pft ade isr qni ege fqk vnh kqt stg gem cys dek sfk awd gls gfg ifw spn iwe dsq ard vqh ifh aws irt vdh wii cns tlt pki hgw pre sgp vhp did gdf scl yfe qcv edt wac tdt fin qcy tka dyv qtf rnf fak kwa miv sph pqp gay fpn idk dnm lvc idg twt yts tkp qtl ekw lim fvy dlq gkr ahe tgr ksr nps aph llm hvd

34. iit cls gyy yrh sep mgf nmr pys ndd mfn hqc tvh drq knn lpw pga pha epk nri iic nkd hqn vmy cld ymq dlc kwk vym dte qqy led gyn vcw wlg lkv eis tqr qhe qwe aee eqq mhl hcv kdq wvq fea edi nyf aav vwm elt wnm lsh emi tya hlv tgr tkr kmm cks ghe spp hee lme wka eny dhh ieh ipp ahc mlg gkq hay dhy hey day sfd gkf gmv vqn swv sfy gig rys kpl dev mwv gpy mtc eys vam iye fnv mcs src vcy glq rwq wet glw rie wfm igt fqh mlp qat imy rss lkf rfq kgh hqy swa yck esa pwd mcn ili knw qna gir nng dei tge kpm cli cld fki yvh kty hnn dml rcf eii gev fvs ygg mrm liv kaw yny knt kam dny qfn tgh yyn hmc tqn ffe ges vsc efs miv hns yyt sqt psi nkt lqt ngn lvk gla qcn qqr qyg sac cwf sgg rgl wwv gsq llf mvt yyw dgi sss lkp prt gse dsy ckt pwk wsi fta fcv ayt vms ykf kdi cfi tvy tkt rlk npq yra pdv qhf lqg drn qcv ffr syr ref ats fkg qtc ppl dra kmd pka fhp ims csa elt hiv sgt rvv ken mir yfd ftt kvp asl yir evh qeg wan wtn fan pec hni fas cgs ihi ypq hem nqq tlr eha ktw nnn yrd mkl mnd rmh naw smk leq aqe gwr tgh qtk kkc ipm frq mim dkt amd rpm mrm ryr wip elh qrt lte kfa mvh lqe nit kct dwv tay grc tsa spa mpa hnh qtr rqd sha dnv fpf vci nti psc yny hvg lev rac sea fqe qhg hay fqf wcs gqi erg mag lwy nvh vae hfm tfm eep vlg die qca ifg nii vgw hrq swm sth nhd cqe kmh myp aym ppe gek wdi ves tvt hnp ctd krh qmm vel gkl yyl eqm wnk ryh iyt cdd sss iid fca drr cnw nny ncg igf tyg dap nqm iwv cev lyc ndk did qkq gln sef epp lca hsw kwg fkt dnn tqm tqn ise dnn rpg sdi cda esh gtf dmg ggd mdy twq hfq eeq dca def tvg ilf qqa sfa vfr ecv pcn wwv ihm eye rye pqw cse kld lhi ipr wsa vmk iqm pil wss gsc

35. qnw hcd lra spc pql ysn sah lyk efi tne ntc lsi khg whm peg wym wla gyw aac wem whg icy kqk nwm miq mka tdy nqp dtc pme irq qdr nwf cni yet gwn aly qdt lae cka dyk nwh atp fkg lfi pyn fkq fgy ail dqf gdn qrr sgi yee det cgp niy ild gmn vhy avf wgy fwd ima khg ntw wvd wmv ddp drp vvg pam yiw myg rnl ees nmh als ylt sda ivi rph hfd thd wem fmf ecm ymm iih gpl ylv wtk agr eyv pwt wwa ieg ggm fad adp eee pmi nlq rhd vdp ypd pdw vap qap fte gce ltq ldm wkf lft fgq ffh cia vve qlg mwd gne dwv pat fav vpp avf cfv kmn lsa gev nty mqp qag eky fdk edh kql hpi slk pnc klp ply fem kyy ktq meh swe adh rhl ysk aqq edf smk meq ndt wpr ecl irt kgw fes wtn mla msn wea rmk cac cdy ifa ikt whm hvp mif ewa fpf nek eps qaf mtl pyr whn yyn pal nrg ilk hwa hsy kca vll khq anh ksl mgn vih evy dga nvp ctw ysf tey qhs fnm hcn eiw dlc ins iqd kwn wrq qas gvd sdl sma mpa gke wws klc cpw ark mkc gif ive lcc ygi epy enl eia shc lqy fpe wtt fan lgm kvc iwm pay cim tky lva vta dia pqr pgn lad slw gvy dnd rls vrr ktt tmk nkr rgy rqk kta dvq kgv frq kap lhd mkk lsh vca knp tki ksc lll nac hif pwe kcp nvv stc scd kfc hsp fyn vns pff kld rkw sds tay ten dcm gdn ypc wds vmk yds sye ngw skc lkr psq pgn yqf ran ite fqw phr hsl ddf tvg svq srf sns wyy wtq vkw apl tgn msa mgp tct kmq miw mhp wwl kma drg dss tlq fhl ntw eny ciq hkf ktp kag fih meh fts mli yce deg vns vgw efr ttt dkw rsf hnn veq nky eqn tkg lkk she wqd sdh ymi dwi ren yvy eev ift wcm ggw sgg afi wsa fmh nql lnf spk wng gsc sme tsn sgv vht lpv vvm wgs sse yig qsy tpm dmi ifh yyd ktn vww ghy ace wav qrq ayk hcv ccv lgy clh qrk ewp cye mse mwf tcc mna

36. mkk ecp nmi qim crm vvw mhd wcm gyf syg pvf fsw cww ekq flt rmg flq tyw knl nqd pqh rrv hrg gqi pad lph gid nst pkr glq mcv lht mif kyn pvd crd kst ehp vcn cmv rrm cts lwm idk wky agl ddl ses tmf pfn har pmw rvp ldr aae dmf hcw cle lin hle gya lvf mhv shs qmn ihy kfm nrg gpe lwe wtw rpn hdn ify vhp hsy tpy seq tsl fsy ndc mmw cpw aft fri lyi mgk vde trm yvr hye gvg vve dei ita rny vri pfw sgs din igc dak ied ktg qev med hee alr des skr fiq iad gdm iif ygk frm acp mlw hwk veg ttf pki cfh rht ert arc wwn pka slg lpw nsm cig kvw qah amw ess hiq elp egq yly hml tnv qiq cdk lmn nlh yin hck srr tpp rng idy nkg stf kgc lay tmd rdm ifw tii efy wry rlm nnq cec vty qmi src sft fwe awm wer tad rdk acl ypt cyy ihw sya nvf ekg ylc pfh vet gdk ldw fkk igi rlt nge rtl sdi cid ddw vpk wsc msm prd tne hnf ffe spq pss frc efq lcs cks wmw gyd lds fan glr drq rml fml knh cci pet cfh qvi sdh cgr wid cpw gch glc cyw qyq rfm ksn tpg hmi ffh rad erl lcv lam wti emg npm wre npl wgi rcf prv tiq tcy ykq hcl yld pka lqh hip vqn qii mvc wit vkh tct cvi fsv kdi dhh gkt naq crg kyl dwn ktd vgm fqv wat tct lye pvm ckt hrf vac grq ten rdk nyf rsr dqy psc sdc hqq fek vsn fmr ahp aas trn fpm cng dsv chl faq cgh tes nih phd lll tsy yln cva fnm qcd hkg ikw rak tya yei dkq frs hiy qcy qan hfd ymv hlv vfk wat mrs sfn evy cfn hhm rmi sfe fpw tev wre rpm cyr chl rvm rmm dia wqt vvv nwq mnt qyl fal ele rki gwl pwi nmt ymy dqg fcp tky vlh rtn vpk ffi kvn smp lmc rsi amt vvn iet pqr lfc rts mgp hvp wch qhr evw ygw pcl afk tyn gwf dfp vsp hkv ccr dnw ach amp wcw twk rcc lal iwy hps pmy qfn evf mdn skf pfd plf vkw mcq

37. msg vfn gid hfl vee ywl rdy rvh mcw mgw pgf psp pkm fcv ysn idf kqd rvr fps pyh dkp igk hvp ypv haa sah twa ley vhm hgt wki ill kle vqf wik egf vqt sam igw yhe mrp pdt mra edf sln mpy tmg cee iva vts acl crh vew fdt lav hyt sfn snt dpg gkc ysi nil lse pfp iam pls fgl dwg ram kil ptv nrg sfi yia htq nrv mlr wri dnh yac chn fhf nwq edi qrw gmp nkk dma wen yfs ydk cha gwh rad akm vmw ikh age mky wsd pkf efs nwd fep ccm vns nqv qrw fqq clr qyg ect lim vwc cqq dyc ipy qpr fkc wdv tps kew rlc dww nca qfw etr qyd nqm cew ytk rtm fch kmm pdc afv acl tih ite ddf psq hkn cdh qkk mkk svc gnh cpl lyc cfv til qkh cml qvi ltn wyh npg hle pft phg ecp pty ela nmc wcs smg fsl mgl wna rgc rfv yff tmg afy ivw ypq wcn fce kaw idl avy rnv sla lds sfq gtw avt smy vfk wme yae fki iha eic idm yqy wqr ere yci eyl wnd nlh vqy cav fkq tsr kvi ecn anm wer qrl fta hqc vcm wrq cms nrg rig lyt vty mgy lrk psd clc lrf kiy pmf rpp kdl qia npr hln mnw lhi etm vmq gll epd fqn qag ten lhc ndg qtq thr ifw tpg mwc yhf qkq ktc vne lkr qth whh acv vqp yfw thk gqr hpe rsy igq tmt tnn lhn pdv qyy vyi eqa hwd tqt npt yyv npp fki rew icn ifw fyy fnc dgl etg qvs sak mpi aqq tmk lcc lvf vnp hgg ymq kak gtv syi vlh wpk dmp mwl vaf tln eyi age qmk eeq eiv sea emi nwf edd eas fwq wky nqi apg cik vnv faf ngi pgt kmi veq dem eet cnk dis lln eag qpk mvp sak svs lgm cya tqn wdr fgy vrg lip phs htm hht iye yrt frv ndn mtk trq vhh ehv qck nlq wlc lrl agm tgf plh hpp dst gws frt kfv cah cfp sgv myr cfw yyt ldr nwc anm wwv dav tym aaw ckk pdm cfr shv shs yfd wpm hgg krp vkl cwf wdn qrh mir mll ycg ivw dhp gad pwq ykq vdk ncg yiy

38. pyp mrv cet srr aty lkh awm dqy mfp ldc lpr scd qgk gcd ekv mmd snh yyn fkl fgn cea syc rks vpf ayy mng hmw wqd diy rqw ser prl dpf wvv cns dgw pfk yly snp wqi mwy lpa qgf els gqa ayi eiw vas lmr klf hlv lit mch ewh ddm cnk vny hdv cys dmy tlh mvg qgl pvp pep llp spe imw ths rff dvv hhw fii vnl eke hsa trg pmf tem hvt hlv ypw iwe vws ylc tmk vfa yyy snr wgi vvl let klf qgt dis iyn gws emn ndq ska iik ffy dqd awq wkd fdg ghm aag esi fpf cmv mmt vce swq cyf ekt dgy pvq mfe rhs knn wpa apq fnt spl elt rpm fhi eqy lgv yap mpd hii iqn ptd smy kfv tgm sls hhf cac qfv emi wmm vgy tel fme hsf itk fwt hcf mdv nnk yvp wrc iqh yld kmt gmr tlc vrr kcl aac pdf kgs mvk fvs pnw ahm lsd lwv vns hvl iqg fnr wpe ncg ssw ccd thl kis vda hki elw pfg rgh hsi lve eya qvm nfy vmn gef lee qwp tvr hns qsi qrr pip etc pif rka ltn nkd pfy hfw krn fve hfm ctp gtl afk vtc nya mpt epv dqa wvg tta sip fhd den qiv yia fqs her rvc pfq rre mmy gdt lcl dlh avc lws wpg scg vtg aht pnp vna dva hrl plk dne dkl gpy acl twf csv pqc keh cmp nvh wev aip tfd ncv skf eqm hrc gha fia ans dni rhk nav dpy qiw dit kne gkk mdg wyt fnq yrn did ffa kdn hwf kic wcc sml fft avv lca scq tmh yph dfp qpp dte hnf sfi gyv wwf swg yek qqn fwl nkn gey cal dyn kav vfg way mae ytf ilk svf gkq ktd fff vwr clf viq trh hpf gyw swq ldq dai ehl dsh qvi pmc ydy yhm rwi age ced kwc vci etq qqs dva sly gsp vcc ffh lhc nva mgl ldt aws the kcs fvg gvm rvf rpy iey vdv frn qtr aft hmg gvt wef mre diw krn rdm nnt rrw fil qkm kym wnm esv qya vsc tnp npm qna irl wwa lky cwf wrt awi gar edf dhc iih pwl wwh imq qpi yga gfq qht det edn gdr rdy ekp yad

39. vns sve cpc mvt reg cdw gss gsr ysy qkd qwe ycp eyp rav veq fwa iyi svm wnr cvl mym yhc chl tyl nas kff ahm psa wng hgs nea mkp tws rtp ywg ggk vel cwh myf neh kie liq vgd dhq thw cnf lls afs vqh vql dsp qtq vye vmc dhq gwn ydp dqt dhr eeq chi qgf wyi nry kwd atg fth ldv mhk ihh mti hww ywe gay yhm ikn cey ppk kyi dhl tlr mre rrf pvy mwn plt qcg svk lcg vry egi rmm rlk dpt gst ahw gmh kas vcc tit spc wty vcd gvk npf wdp sri cse rtq snl kqn tqs hpg myg avt gvt qtd pid lan dqy fcl sps sfc dng lvn mwi ghd emw gna sly eya tqr dvd inp kie mfp scv gsm scw kve nen mea pan iqn aws ycq myc mnh eac igq fga ema kwf yin pft svl spa iap cgr qma rna pqe myf fqp mag nik kay yta vyy fym gyl wph tdn eti yva lqn aly syi yfs fyl wel tya dkr mcv rwy pmp rsv npm gie itm glk lns qcf rae hag lvq rvv nna tfk nfp smh kkv htv rwf mge tse iqc els cwg ptd lhp nap div fpa ykg dwc dnl avk sdp rnk asq kvy vya tsg nvr wgn tlr fsr edm qcm pqh ria qek ysh dnr sns cwv wkw qpr dki wvs hhh hna qev eqn cfm teh ydn ycw pid dqa mma qey ehi mkw kvm fqh yrr naa cay rnp ift css llp vre ccg red fig qla miw mfr nsd yti iww nqs iyp ywy any qia mih lia vyc dek shf kpg ape vtl amc pqa ecp qmv ggv tew acy wki kye sne vdc psh ler nsr yis egc vmi cmd yst idn kpp gle nck cgv alc mqf rpn afp tfn iqe vip ryp avp drk pfk hrg hwk ygt hem vrh tdn kiy elr hmw hcm kef gtc rni grf ghw yhn vtk qmp aqy krf dld kla esh atf kfd vyc ait mfn tsc ctv rgp fph niw hrp fcy hqa hvv asi ain tmw vdr mvg wdd myc apl ipi tsq ytp vff gyg iin qsv ini wck wiv rrk pcm qar mga vga fed ptt mfc ccq nrc rgs vpl syf via epa aip mvs ede rra lhr mqc glp cpn

40. ple ncw thn glq qvw vgy kar emt fvy yfl nvl nrg csq lyh glv ler qqp pyg dap ntv wrl grr gtc mtp ipt yet fml cae qyy clc fpe rse vhe lys hyv gvr vmg yql dps ctt qnv rsp ggk gyk cvv vrp yie mqf liv ypl nkc qni hsm esr lrq myq gyl yya icr fhd eqe tyd wnv qmv fkv fgy pvt ccm mme aky pak hfq wyn plc tyk ate yff pyt swq nfk ytt vnh hsf pfy hfi ats ahq dhp nyh wld wrv nev dlh dad frr fia fgr ikm dsc cee myt wqf kne ptr tek nrg rvr lqs cne ecm tgs dqi vgc aci kkl nff wvf tpw iav lkg kdy hfg cmy ksn wth qer wcq etl nrn gte twn mav ikl aeq nqv sgq hhl gvm dce hpw eys sea ydm vne iyc rsm fld ltc scg qys srg hgy hpc cre srr iyl tea akg phw esg tag qmv gaf vmp wgv qhe vny fht lnr qwy tns tsi wsp dky par tmt vhp sev gyt nvy nlq pkk yck hvq kym sdh ipg lvl cty mhq nds ynh yym ngn pai irm cmf rpa tqt ree wyc wpk gie gns hrq nwt ade adm taf tkm sys dar yny cls kct gnr icf tca isl ams ncg mws wyr lfp apv yfi ppa ihd kme rwi ddq sdi ypr pnl fyq yhm fda chf lir kal mwr ery vmh amw vcn ill gea wdf wgt vrr iyv wef att ytl ecw nwv vva epa lil sky aws vrd yik qwd efi spg hvh hni smw rfp clg pmt iyg mpk cym lns wla nfe hmd pck gde hkf ewl mhd mfk qyy erl vpp fmy hle vrh aat lgy ksn lyh dka yyd kvy spw mwn ehi vfq tcm qaw mar mwn ngy qpv nay ifg vlp cfa sew rif yqn ksi skd fet dqq kve cap dqw imy qee kyp ais hpd vst kes ewi yhc mcw wqc nwf sqr ssy nhy nkl ggt scm fqt hdv feg ngg tdv hmd wmt mkh sqs qdl ymc kvr hri qtf nla kge aqi qcm grh mcg lmv ang nhk lme grv hsm rdc rmy lrt fhi ila lty yht cqe lwn ssa iip wrd gaa kdi nre dli hdk neq vad lei ski edt mgl qps irv wcq pdc rks sme cpt fqe nyc ipt ytr

41. ksq yim lrh git tti grl ccf hrs npt aac sah att gac wnp dkm sdy inm mlc rwg idi nrm tal qal vry nrl ycq tgl krp dyk rtd nqe mir kdv keg wed kam lcl yqn vwv ihd ert lct sly hik gfd csg qra hhf hgg dna kdr vcd hfc cpr try lka wac msv qma fcc gpg ktg whk gyl dkp mes crw phi pgd dew rfd cmp dwt ehm htc nkq vmk fdd dfg lir hnh rpd hif pdy ihq hsd gec mfv wyk pph rnv dtf qpm skw ecf ayd tgi nch skq lrw gek hhf pka dnc dsm emn teh fwg hdt iph kyc wcq pps lds gmg ptr wei rmh ttr hre crs dpe pei fgn wdp esr crq gnp adr fsp wwe sif mwg qih ame ecg qin rhc rfm qck rsp asv hqm dyf mch klm rra prt lcm qkc wvw kan faf kla vgw rcf iih yhh cmn tnk pvd epd mfk kmt lvq rac iyi ycc gvt lrl edc pfm wmd ehm ttc myi nfn lcl dmn gtt arg afs dwc fpw wec qem fge ytm qsp env rdt ppw lfq ras tpk dqy amv atq fdm cet vld mfp hdf yvy hrp apv nvd sap wcp esn dgc aqa nal ghp sdp faq trd tss die fwq rtk lft the ktk pwl swq vrg qgh rnq ghq tln fsq cwg ldr kqy kch mee iwn fqe qqd kwt qpf ywp dhe sit dst vgv vqw rvm qvf hlq dwa vnn vrh met dyf cpm wke klh cda dqr rpy kvh gcq wdf ssi vnn fhl kef hpt efn dwq fpn gtn twe wvk lfl vla elm yhh ane pfg mpi hqt ygy etq amc avv vqs shk dlg vgm nys ela isv rcg nkd prt anc frf deh hpt map qsp vpp mqs qfi dcq qks emy nty ppk cel dmk wlc een phd dgt qaa snv ldy ekh etq hfl kpn ivw mfi npd hyv paa tna yng dqa ktc igk img egw iny kdn kci slh wwt dil dgk grh tgh faf veq hcv wgg ppq ipv isg dic mml rfh erm asd sev ehn les yvl tgp iii mqa rff rle mne cpv vcv cle cwe iis mfy acc ffw edc vin fld whi llp ade nar npy vfh nyr ifl miv ncl sah fdv vca gpa knd wil kpa kcs cyc int icd

42. enf kfh lql aiq fns ciq saa vtr tll ehs dis rqc enf nep fal rev rnc frv yqa hyp rhe aym atv dtd twp ncv csc afh gds sah fih hir tim hti way swv pvs sav sat rad scq leg tdr lra tyn qgh hmt pqp dpf wcy wsl ypk yym ghy lkn ldm agd hls ntk cql rrg wdt rfm qyv tlc evh eve fye tis dtc qdt kkg wpv ygg amn cgv tys wda leg qps rcw cnr kti fvv qfn hhh dfs ief rdf aer qgc qnh rwr vky hag ghy wfd ych qsk gaq evv eng wew ddl ymc slg vwe wwv qsi qda vsn rnr lmc nwn raq lal dch hvh tvn wva wdv fsf dqq tgk yfw imd pvt psa vkv nis skk vfw ddi wei npa sas pdi qye kgc aad yhi kis chs wln wae mgs afl lnn vql lyq egn qee ntq hrp ayl pgl mlh qqe dyi ekw kkm fni gli lqw tes pcf sid dvm pgg klh nnr cei lpv iyv ynk skw gnv nnq his ths mhn krh fng vsm acr giv qlf mha nsd khr yiq wng rqw fen deg ewe rhv crl pqn ysr ass cle skk ylf ihe gcn mpe idt hqt ynh qrd rqg eww ssl twc vyd lid yrr vsq png rtg qiy ypy kpv lyq llw wmq tgd niv slv vpt fan yms kwr amw elk qpd cwg gag kqv gwa nle tiq vtg qtv dlr nhc ffv qgk pdp wsy snl qqm rrc mdm cne san ecs dhw rcd hyw yak tvf tyw mas eks wgi kdm vdk fep ght pnm kcm khr eec qrv aqm ite phr nde syl kwa vqn wqq niw gyc hhh ygq gtt wfs apm qtq gsd qnm vnv fdp aff cdd lmk wvh avy par dky ptv vic tac anm aae lml lfg tcc sfc qwq cra ssw rie dic ctq snc ncc clt cll arr pev tdp dpt tmg smf rph cnt ewe gip ear wyt mct lmy drh rvd cmr flq kpi gls hfq itm iwm dsh hlr fgg hei cty ppn apf dqs anv egc crs rrt cay tkw krk ace dke dls ipc prm ncf rvq wsi rfq yhs nfs tdq mna kck vpe iev wyp sai lir pgd pth scn kws arq kfl emh rek vfv tmm cgr pdi wss qpw yci wvh mdi nnk cwt skh ndc

43. cmk mde ecp qal pan yym mkg aeh qtk rad sge qsw mlg wsc iet hsk ivd ekd rtn nvq aeq hqr tft vcv hrv wlk twl tvs wai esv ypq wyl kif mdy kew iii kqp wcy drv ete kth pca iaa qpl kif dwp sah pfn fqs nwd cil kld ivh ier faq lnn icn dvq edc yis mte whq paa svy gvm alf eel lqy hqn qcs whw gna yhm dsl kav fhg tcd ilk dlv pyp ahn yqy ads whw myt hti amr nvr ysd fdv ewa lwr evs idk man pcs tcl grw fes hfn gtl vkt kei ihd hnv qkc ctn qpk mmr mfl pey vwv scc idw pna ple trw fiv gqr vpf nip hih plw vmr epi avw yes mhn grs pqd lss ivv prp dgh hqc yck tiw qqw ikq hif cnp ryd cca ppv ast wrs mah weh fst qtq gfk idl ycr drs ass yhy whc tda hdv lrr saq vcc edp fwn pdk tsk tkf hgt gsw gwn rqv pwq nan fam svg mrm nrc kta cmw cig mtp ind pwa env hsw aaw csy hrn ced crh dki fdv vnd mtm hyh ref ycv wyt wls iad tcg qia kcq eke hff mnr lic wfl icp eyt qig pqg tyc mmg nld vhe yhr ycm cqw hmk hik ayc evd haa fym edv smv erw lna fmp pmp sqg itc ghy wqv sis lad tca ffa iqe sph lkw dmf igv hat khs fle eka tgl mkk wvt wva qwe cve awd llw fit ycp vdf spw dwg ryn yah ihf red eys ddi gwr dsv vfs dle kgs fag nqn fdy wml scp pfe kgm ddw aga hqp dmy lnl tpd fge yhw rtn ddg vep rdh ncs vpp pit glq lni hsk eey enh pcw hwt ywg qan rml dte iva hvq rlq ymy fly cml yke dyc daw enr aye aiw rsv qnf agh shf ppl ask hcy crk yge mwm epq clv swa fti hdt dfi rqr sin grm ern wly whi yhm hwp iwy hss eew snp ynk ise ayl mks mcw naw hih ghl lpv ymr gpq afy dae rew ing hlp whl adw yvg hdt acd qcm gnd tal sec arc vty fte swp mti snr grv ggt hfn sfy eer ipi ivy lee ted vmg wgt myr arf pcd rlq ssw gwd gay and hte mcd wda cgk kkf

44. rfw ssw lnr cly gwh dnm lgs vah hdt hmw ggh akv dnl hpd vqv kci vqa cai pdh pna wge lsf akv kid eiy pgi vdd gdp nih hre kte vhh itl tkt qap lfn qfs afh cng sye rwe fva mct cyd ffl rvt qhp wmw rkw vtp nwe nyq evt tdt ntc knt ced ggr wky ytd vkc fgd qsa kqt acl wwn dtt cwt rpc sps tqw hyv hmt gaf kkv var wtk myw aek gnr tdl syl ctc iya eny dwi lap nmr qpv dei eeq hrw pps lml waa kps rsi smf ckn clr yff twq pnv evi eee vqf fcq pqk lnc whs pav fsr swq lin yep nrl rlm ymm nrq hqa trw kpp fhr eeg nti pvf qic dfl qtr vsk yyv eyw ycl ede kds qhs lpc sks rne sac khr myi prs yci edw llf aev wql nsn lgy yyw hrd hnn yen ghe tns npa kee wer kwk yst vfg pre nlc asc kkd ekh enn ysw hsm fqp alg lfn lti pik lww tyv wsy lkm chi vyg sdp cdi nlp gkg gtg dmd dmp esl nrn wpn rrp qsg iyf vlf yvf igg sfi gre ieg pmr yim akk rhe iiw llv feh npt fgf kds kih gty ryi krk lvp qac nnn hpe fyt aan kll egt eni qyh qdk pei fml agv csw lye pah ytg sfn tpf qcs ata kfg vad yvs gcn wtw apl tyw ywq nae fqc tst qem the adw qcl pcd mlp gtm tnn gmh fqk eys lkk fqi deh mhm qgc cnd ard tem sew gwy tke fqt elg wpg teg hgp tqk ypt adn fyl enc wth hsc ddv hli yvg yyp gce pqi wiq lfp wqr ykd fii kql seq fpy emv npc neg lnq lcw dna kvi cif wah fwd ded ian aha rgi wgl inp kgl mfl dpk kii pff dnk vyh fqq wpk pfk ngc vgn atg smt tlf nyi riy rsm ssm nse tca diw yll lav ypn caa pva vrf nmf ici rth pff isk ycm dli gcc ath shh qmg wqi fry fme ftl dgg nnr awk ykh mri ada cnl sal gqh faw imt mcp gpv qai gfg gma cat alt etm hid iym ghl vfn mwe alf ihk wmc ttq pfw snn ssn gch hgq knp dsr llp wye qid fef ewn irn vph mig kvw rwp pyf

45. fsv kdh ytl vyp eny lmp htc mkw tvw cag iew gqq gel iec nrt cwe epq aar kfv cvn evf ism mim qvt shh sav gtf rlk rng sya ple mqq hqf wtw dai ywq cfa ypm ddd ssc wki igi adi dsi fhs tdp ytt ydh yym ypt tpp dfd nwq esn diq wfl ecq qkd awq tye lnq hgy iql peq mdp ski dtq nmt hev rnq fey ggi dan rmg rnm vfd yyf gkp min yet vwc vrh gvs tkf vmy avq sye qqp hms ahi sfm shc wdn ilf gwg nfs pff yih nlq qkk pfc kih fyw vma ptd skv dgw hgy mhm yvr eme qlw mnl cvr nid lpw ska ayq vsv qfw tha ecg cdw aqq rls anc mcn nep rwl nar hrr llf eaw elf cpd ikm wdm vme wgd liy meg fgi gvt qdp rcq leg sqr etn rnq ltl hgs skf srd hcp phy cci idr mkg afy yqi gam lwv qtc vpn mif mvg wvc vvf lip ekn aet hdv svh rkp nkq hlm ilk gik ktf gyc gnn vyk hrm fvs gks kpf egl ikm vmi wig rhs pfr pkk ynn esv anq nhl rmv hqw ail wne hfn pww vqq nhh ccl wky mqf dwl fay ekg cnf kmn kgy vwi elr sym lrn fkd hls mhy rqw fsf pdw akw kvq rpn hhh rft dyt dsy lwf mtw kpr fef snd mnl fdt atq hgt kmc lsy ega vwl enq efc dap kma qiq cqa ffk avd ida ihk yra emf skc tnv vsl igh ldt vti ghi yne gsy evc gmw kwy fan nwa klp kns aes tvn wel aqf tmn dvq lis lgi ggs hpr sas tsw edc rgp vnf tsl kdk cqp iis lle dsr ngf epg rkk yqy lsa vtq pav vat epw kay hlp dst fns qqe vif hml fgi dih cvk myl yii gsv wnm nrd khw rmg gmf mqe wpy swk wkr diy sdi wkl fsc gef sep wli ypq acw hmv pnw nka ima ddi pnq tig kln etl grn ctd wkk mdr dgp mcp fep gwi daf yce gif gdn kmv kmy nvg ksa wtw ynh vik ett vfy tne ciq dpt ntk ytd cvq qas spr eri mfd ach cld qyk gyy kye acr nmd lqk tti tww rks pnn eep lrn hrn rpi hqp ycr hmy hwe mtw ite ser eyc nrn yck hsa

46. ksd yfv nwr srg mfs smw nvw vrs fqc rln psm pdf rqs wsg chq wfi ims kef yky wmf lpq swt qfs ike cti dwe vre hsl any fgi tkc kce new qfv gme gay yqk fvv hht hpe wgl pqi yqg src ghm kdt qmc gly wfm vqi kle cdd csp pwk mls tpg nqm sth kda qcm hfr chm ilc mql mhc kdi anv hvm tvs pvw pdk ngy apy ssr vvn ret lfl ici lwi mmr wfe fpg phg hwc qmm ywn kem yys nvc tlp cae khc dmg std ers cvy fci shv nlt lrs sra kvy cnr ccn avp ntd yen kmg qlg kcm yyn aww cwv wgq tha tpf vds hcv ftp led lhk cer mky wrr seg sda ivd qyt mtf gha qym enf vlk iwh ifi lmg fli qkk ndl tvw dtc hmg gcd ykt awg wth gff vvk mqv hch mih fal nnd vtr gni rdd nvc nfr mem qqn wky elt snp fsm gds ptm sql mys pri cgt rps slt wdm een alt kde mck dpp hki gch fpm tfp ryf pgi ldq ciw cvm mvm ynv flm ldq dqv vii fcl kaf eld etp tph cmi vcm liy cyt whq nfk kiq tfe hyc wim dgp cwm hsq vgv srs cmy rns wpr rdl vln dgh nqq ird swr khh mlh vtt nsy yrn yfq ike fyy erc ieh frv gfp lih kgv dvg ifr vyd fwl asl tps fia phl few dmk qvd edw ayh hen lty krc ffr fyh nrg pep ydp knh mtp fsa dla wke fsa tti cdf nef cvg drw vky cah wht fgh ays mcw gkw hkm nky eig kea inf vap fye vvc tmr kns myf nnh hkm dae chg mwf wmf ipm gkr hri lha ali fll vie nwk nka yts hkl ihg wwr pwa frn hrl tdc egr aep ehh vmn fvy fqd dcs wdn egd edy acs qsd sqd car wfa yfg qgh ryh hcc hik whd yks krv lih eps klf tic kae pll gff wwl cln snt qre kqk mwn san rqi mmk phw qac ien yql ylg tvl ifh shv gpk ivt asg rwq tpd ege hpa hac qda tip eyi teh wyr lia lqs lkc lhv aln hhv lmc vyr cqa gqc vps gmr ili krd gyp gfq pfr klp iid vtd lwm sfq lta ark nci wmk kik ryl rqw ddf qpq

47. fqh gmd ewg rmd teh ytp gyn fvn kyy tlw wyn prr gpm ple klt dkp kqc smd fga gdc smk gys wsy ckc spi dvm win haf wyp tff ntl dsk add hct kfi dif asr wdv fmr yfe ilv sse cqa crw yck asc yfh wyi vnd nkh qrv kfh nnh yna qdp ifq hpl yap fdh nic hmh wrk sil rtf fdr qsk ekt qqw set shf ega gda kqy dml niv rew pnd fwq qkv vys pvf ffd cys mss kkp wcs fka hqc vgs kvg tgw wia gmf ktm rma eys crp qgm meq dwq ehc qcs trc pmi ney wrc yve pth ski fdc gpi dqt ndw qeh drw kyh aky emr vat mpr hin ilp tym nmh enc qha ygt cvt hcs rdr erq slh ekg cyn hkr lfk kpl llc icy ifg mrl ilg wpf vmw srm mkl hrf qaq wkl amg dnl ems eci qal ytm pey rvd drd pfy ahp ppi lhs lgq cwn qyn sah rvt whs tns gmk mfh esp grp lwm ccv pdq mel ggv hid nle rgr npt lwp thg rml aft nph ges tdl ptg ira cse ncd gkm awa eky pwv fef cdq cvg yew kgp rqr eqy tth ipa rlh aah pfy rdv nmf qct gvt sdh wsq sfc twa rll rta gaq ewa qha fdn edt lnw kny kec hyv pkk gvq krh kwc mle ger dwr swa hat mhs nhw vyp acd kch vay qtw mac ahh nwd pmd qlv tim pkp kck lme eiy wlw wkw dyw lcw acf den hml tdy myv wse enh kyv gpv fsy gem wlc rsc lwd atk qfl edq acs ccw tmc vrc civ nkm lra lpc ktc rkm fkh yae vah fkn led myn qnn dey qeh vyg qkh cnl tft nst idi lgf nwn yyh wqy evm nvs tdh ndp svr dlf iyr fyh gfr taw aay cdq vpg isw icd mdl wkp ppy cwd wik mhy fsp kyf ivm mtv svv hav grk pnp cvv dyf ntq cqh ddw vik rsv vnh fsl cfa ram enp idi ekd rpq evn ikk skr atk cng vvq nlq gki scf fas rde dgv mee itk dfv iqp rlr vtl aar ntw amc mye hhi shk ewn rst tmi lye mft yrv qly kcv dsf ngm wqm mmm ied kvh cmf smh lyq riv kpq wfn isp rqc maw egf rfl hct rir mfa

48. nyp ghp ddf eqq clq msc nhk ssl meg kpi yfh whr cyv vyp hmf pew shd kyf riq nlp fsi sgi kli gah fed iqn rei aql vri lfh alk snn rdr aic lrh mrc glw gek ltv vel wyv sfi kel vkf cah dkn tcs cgm klw kkk wea wrm rmp qtw lqh slr fkt ftf gvs pth fdv pne ggi tms niq dqp nqq mtc mnp giy ava dgg svc fkm par rqd csq csd lqi cis dvd dfp dlr kns eds pvp eep cca mmm dqi ids vgh kip sev tgq iww rkc llr tpc msq iyk apn eid ped gwy ggh vws idr iyw dhf rca kdi igy tgm ina qhd fqa gia tei gmw ssp tya qmm clq sld nsp lig lhh aar twh edg avr vsq wey mcd fhn tpq nde mvn atl cfg rmm yyl egs rep lag cpa fap tsh wdq qms htw pcm dln vtr anr gcr kpt mlf csn spt rhp ris mlm kym rdw qyw mks rim qwh qkp wqp mqa che lkl ltd ayp myq isg cps hss qmk hrc vel wqi eda hqy igw gqr ehn pye cgg aid dfg edw qed pnl shd ctt pln drf ple vsk pfh afr dse ivt pgs hal kcm hgm vkq dim gqi sgy rkl yig fwh qkd eat reh ydn ynd ggi hhe edi tma lfv rmp npa cll ddp tdw yal wnd ilw mpp ree vms mep esn ewp wvs nvg yla mdv kyt sgh lgc ich rrl mgh qqv lce gmk hrw lhk vva csp ilk sqp dpc dcm lys rvs let iqf qlp vkg dgs hcr app pys fip shr tqp esf hcs rtm pim mwt qvm ikv ydt qfq rvi edv ifd krs kei dsw leg isd vcv qhn nlp kcc ngi whn ewt rgw nqv ckp dkt fhq gfm rpm nrv lng agp hly hyq fmq qms mnq khn lds phv riy qyd rsh rhi qnm yfd trp clt fmt nem mqt pmt vmy hmf ste gdh frv lvc rew rkl esh gek aen qgh pql yra ngf kgs qmw pfl stw rnv fkc yvq acd asm dvq gqc rsf nth vna lkt fmr kcy kyg akq gdk rqp nra fsv lsg thm gda ldm dls pdp fti nan vyk wih ndn yfa hqt nqt lhg tvw atf mig fyv rhr mrh eek cfr swv let fpc fce kei dis prd qdl act

49. yfm nrt dyv nhe iak asr gkm qne vqn nkd haf cmm fam myw ghd igt nmr phc shy kvk vdd vnh hqv wme hfi pnk qse kgv hlg vqs mdi ldp lvk ckm atk kcw sgc ydc qry htt kia vgy gcg clh sgc kag fln qiw nfm fwg hra ttl nrw ydg fqg vtl pgp gya csw rym prm fta svf feq kra cne wyw iim fiv lgk pmq qid pgc wqy kra det vea mwt enr sqw emg tpr sep pem lrq pqa yft qei fry lqh qve qac cvg iiy ews wwp pfm hhi lqi rrc wfc mla icn itg fkw wkc hyt vnv rmp sgn cvr mpc sny aly rpy fqq pir isv ehv gik grw skr vlw fgg spc yih afr nti fpc sev tkp fas aim veg rng kvr avd ywv iqf tya syq mwh qkg pwi khv sef ath grp nvq lrn fqs wlw fmf hnr ycw lhy yvt ytc aia yiy gnm cna tfp kct lve ivd tks tmp ica tyf ckn syp qva iam ygk gni rev des iqn pft pin ilt ivh hpr tnp qqt psd ynk kia efy cpm hsy idl nec mai eri evh wvg nmi keh hvq ggh aak ttl rlr fce yts tep lwr mad qpw hhn efm asw pmt fvv cqg acw nde gip wlt are wcr ggi qnc ytn gcr stn twa gyl kan smq rhk qha lyt gkd asv mdg tcw rds ffk mwh gkg ack fqn cgm arv kgk egl rgn dvt ffh rcl ydg wsr ksv nlw lra sgs qlt gss nyw hlm sem lfd edc glm egy nmn mit ecc eme dte mds lwg php tad anh eiw sgv yyv cyf gms inw glq vaw mah car isv ary yml ndv vmr hnv cah amy tir cws lnd rel vfa rfc mei ndl gee ppa rqf cka ttw wwt lrv ncn lvh qgm pqa vyl hka qyk fcv tki vyk rfs mfn vcs rcn fas hnr hti tsk nmc gkg fps inq nhv ari gkq ett hha cys lnh sny dyr yng fhh vqq ttc mac anv gge rsp tps asd iea nhn gkv gqq fai sye psh gly hkc yvd khs dey ivt lvt dcc ftr lmg cgv ffs gfc lar yvr ifw ssm hqc sar gqm yei wky gpm pfs ldc gnc hph wqd qsv wen ily inh hrk enr rgw mir nen rit rdp qwv fik

50. ene cdh gda aes ekm dtk heq vki eht cff vhg afp qsw tpe eee gvf ieg mdr dhv tmr sdf hag wap pfa kdv yea phs nqe edc eey lml egq vrc kan elq ram avk frv vdf gpq wck aty isy yaf nlv pyd lae pmp rwr mwg vpe ttg wln gcl stf vlm nvw asc rfe kkm pqy lll dnh fgc tcp idy ydd pfq civ ikr qdm nma mwi fwi ltq vki wrf cnv fhw gtt edg ffp twn eyc hms evm spr pst tle wng mee cfe rrt yyq kid csp ryd fci nlg rlc pmy tst dlw trk lav qrw ihl aqv yth atg gac qve cny kky tfe rhf gvw lwc nrv lmr gmq vap kgd qkr ynh asn pec sqy che gkt vav nyl ecq avf aqe icl wtr aav ryg adq vts hre meg paq cdn eqs see htw wsl ggd pld ysa nrf rhp wnr gmg sqt pls wdd ict smq wyl grs gcl crt wvn vdr ncf wrt gqm yah cal ite fwn rcq ehk fwl fng nsh lyi ptt mew awq swq inh qiy fsk lar wcf spr ytc ckg kvs nwp eqf vya ftq mpv ges erd lqq gfy cvy isn ypc ahr qem yad tmt ecp vpt ffl mce ghw ltv mms qss pnv yfw svq ndq sac gmq pyk fie wdc pmr ept mgs yer cip gfh yvc nli lrn fqf ntg knd wkr pyy sfp asl tst fqs msf qrg gen kcn mgd wqp pae mmc qrr tqn flt gqn wii cme ppn fih fsr crq mha ggh klg eim tnq mac ilq hpw rsp qhk kdq mwp lee hhd ntm hpg elf ikv wrd cml lmi epv ism lvv vta gms nvn pte qet pci dvh fqn als eqf kdn cig kkn dfg gld tfd lri lde gkf ryy qne qpm pch nmg syq snw rvp psn fva rcn klp cgg cnh lvt wpm glw qtt nni nye mrq mdp ywr crd rwn esq cvl qkw dvs qyl hhk nak dnc ckk ygq asg ivt hrs kwi sir dgn edr lic laa ffh pmt rlt itr kdy fgc iqh kmv fdi ddm nke wrt lly apt vep ltt ris mhf iht cpn wes dtq wpd cri wni rqc pcr mhc rca qck ist frn fan awe fyr ndq vai gpq vgr tgm vvw qpg sdp aev pdh mdh fww dly ecq vlf tqp

Appendix II: Single-letter Abbreviations for the twenty amino acids

The amino acids used in proteins can be refered to by a single letter. These abbreviations are given below. So, in a sense, the amino acid sequence of a protein can be spelled using a 20 letter alphabet (acdefghiklmnpqrstvwy). (That it, all the letters in the English alphabet except b, j, o, u, x, and z)

Nonpolar Amino Acids
A = Alanine
G = Glycine
V = Valine
L = Leucine
I = Isoleucine
M = Methionine
F = Phenylalanine
W = Tryptophan
P = Proline

Polar Amino Acids
S = Serine
T = Threonine
C = Cysteine
Y = Tyrosine
N = Asparagine
Q = Glutamine

Negatively Charged Amino Acids
D = Aspartic Acid
E = Glutamic Acid

Positively Charged Amino Acids
K = Lysine
R = Arginine
H = Histidine

Understanding Immunity By Tracing Thymocyte Development
Activity I: Simulating The Generation of a Diverse and Self-Tolerant T-cell Repertoire

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Middletown, DE 19709

Introduction:

This activity is intended as a homework assignment. To complete this activity properly, students must have internet access. If internet access is not feasible, Appendix I can be used instead. Appendix I contains fifty sets of 3-letter sequences, with four-hundred three-letter sequences in each set. Before you begin this activity you will need to have carefully read the scientific background section of this unit.

In this activity we will simulate the production of immunological diversity by generating random letter sequences. The central idea in this activity is that the amino acid composition of various antigen-binding sites can be represented by sequences of letters. These letter-sequences are generated using a random-letter-generator. The randomness of letter selection is loosely analogous to the diversification that takes place during the gene-segment splicing that occurs during genetic recombination in early B-cell and T-cell development (See Scientific Background). It is important to keep in mind that the number of amino acids that actually make-up an antigen-binding site can vary. In this activity 3-letter sequences are used to represent the amino acids that make up different antigen-binding sites. However, the number of amino acids needed to make a realistic antigen-binding site is more than three. Using three-letter sequences to represent antigen- binding sites is a simplification. Unfortunately, this simplification is a practical necessity. If longer amino acid sequences were used, the diversity of the antigen-binding-site pools would become unmanageably large. Trying to determine whether a particular antigen-binding site is present in the pool would be like looking for a needle in a haystack. In order to make this simulation more practical, the size of the haystack had to be drastically reduced. This was achieved by limiting the size of the antigen-binding site sequence to only three amino acids.

There are twenty letters in the random letter alphabet because there are twenty amino acids typically found in proteins. With only three letters in each antigen-binding-site sequence, the number of possible sequences is 20^3 or 20x20x20, which equals 8,000. If thirty students are in a class, and each generates 400 sequences, then the total number of sequences generated would be 12,000. With this number of sequences, there is a reasonable chance that any of the 8,000 possible sequences will be represented. Of course, there is no guarantee that every one of the 8,000 possible sequences will be represented among the 12,000 generated by twenty students. This is because the sequences are generated randomly and could include some repeats. With a smaller class, the chance of having gaps in the sequence set increases.

One other simplification that should be kept in mind is that the antigen-binding site sequences would not tend to bind to matching (i.e. identical) antigen sequences. Indeed, some antigens are not even peptides. Instead, there needs to be molecular complementarity between the two partners in a binding interaction. However, there is no simple rule for predicting complementarity. So, identical matching is being used as a proxy for the more biologically accurate event—molecular recognition between antigens and antigen-binding sites. Those TCRs with the right amino acid sequence to produce an antigen binding site with high affinity for a particular antigen are said to recognize that antigen.

Step 1: Using Appendix II answer the following questions:
What amino acid is represented by the single-letter abbreviations below:

G _____ **P** _____ **Y** _____

D _____ **R** _____ **H** _____

Step 2.

Visit the random letter generator provided at the web site
http://www.creighton.edu/~davereed/Nifty/randSeq.html
a. In the box labeled "Letters to choose from" set the alphabet to acdefghiklmnpqrstvwy. (That is, the English alphabet except b, j, o, u, x, and z).
b. In the box labled "Length of each random letter sequence" enter the number 3.
c. In the box labled "Number of random letter sequences to generate" enter the number 400.
d. Generate the 400 3-letter sequences.
e. Copy the results produced by the random sequence generator and paste them into a word-processing program.
f. Print the 400 sequences. These will be used in class during Activity II.

Step 3. Repeat Step 2, but this time, in the box labled "Number of random letter sequences to generate" enter the number 1. Write the result here _____.

Step 4:

Answer the questions below.
Question 1: Why were the letters b, j, o, u x, y and z not included in the "letter to choose from" field in step 2a above?

_____

Question 2: If each student uses the random letter generator independently, will any two students be likely to come up with identical sets of the same four hundred three-letter words? If so, why? If not, why not?

_____

_____

Question 3: If each student uses the random letter generator independently, will any two students be likely to come up with any of the same three-letter words? If so, why? If not, why not? (Read this carefully, it is not the same as the question above).

_____

_____

Question 4: Write down the first four triplets in your set of 400 using the full name for the amino acids, not the single letter abbreviations. For example, if you obtained the triplet "aar" write "alanine-alanine-arginine."

_____ _____

_____ _____

Question 5: Why is the use of a sequence of only three amino acids a simplification of the more complex biological reality? Explain.

Question 6: Why does it make sense to use a random-letter generator in order to derive a diverse set of sequences? Surely, the immune system does not have a random-letter generator. What does happen in the immune system that is analogous to the random-letter generator?

Question 7: You may recall that triplets of RNA nucleotides are used to specify the amino acids incorporated in proteins during translation. Are the three-letter sequences used here the same? Explain. What are the three-letter sequences here representing?

Question 8: Scan your set of 400 sequences (generated in step 2) to see whether any of the four hundred matches the one sequence generated in step 3. Did you find a perfect match? Yes No (circle one).

Question 9: Should you have expected to find a match? If so, why? If not, why not?

Question 10: A person with a healthy immune system possesses many millions of different, mature T-cells. The most crucial difference between these different T-cells is that each possesses a different TCR and therefore recognizes different antigens. Over a person life, his or her immune system is likely to encounter only a few hundred, or perhaps, a few thousand different pathogens. Given these numbers, would you expect a person with a healthy immune system to have a T-cell that specifically recognizes each pathogen encountered in a lifetime? Explain.

AAI Curriculum Unit: Understanding Diversity By Tracing T-cell Development

TEACHER MATERIAL

Understanding Immunity By Tracing Thymocyte Development
Activity I: Simulating The Generation of a Diverse and Self-Tolerant T-cell Repertoire

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Introduction:
This activity is intended as a homework assignment. To complete this activity properly, students must have internet access. If internet access is not feasible, Appendix I can be used instead. Appendix I contains fifty sets of 3-letter sequences, with four-hundred three-letter sequences in each set.

In this activity we will simulate the production of immunological diversity. The central idea in this activity is that the amino acid composition of various antigen-binding sites can be represented by sequences of letters. These letter-sequences are generated using a random-letter-generator. The randomness of letter selection is loosely analogous to the diversification that takes place during the gene-segment splicing that occurs during genetic recombination in early B-cell and T-cell development (See Scientific Background). It is important to keep in mind that the number of amino acids that actually make-up an antigen-binding site can vary. In this activity 3-letter sequences are used to represent the amino acids that make up different antigen-binding sites. However, the number of amino acids needed to make a realistic antigen-binding site is more than three. Using three-letter sequences to represent antigen- binding sites is a simplification. Unfortunately, this simplification is a practical necessity. If longer amino acid sequences were used, the diversity of the antigen-binding-site pools would become unmanageably large. Trying to determine whether a particular antigen-binding site is present in the pool would be like looking for a needle in a haystack. In order to make this simulation more practical, the size of the haystack had to be drastically reduced. This was done by limiting the size of the antigen-binding site sequence to only three amino acids.

There are twenty letters in the random letter alphabet because there are twenty amino acids typically found in proteins. With only three letters in each antigen-binding-site sequence, the number of possible sequences is 20^3 or 20 x 20 x 20, which equals 8,000. If thirty students are in a class, and each generates 400 sequences, then the total number of sequences generated would be 12,000. With this number of sequences, there is a reasonable chance that any of the 8,000 possible sequences will be represented. Of course, there is no guarantee that every one of the 8,000 possible sequences will be represented among the 12,000 generated by twenty students. This is because the sequences are generated randomly and could include some repeats. With a smaller class, the chance of having gaps in the sequence set increases.

One other simplification that should be kept in mind is that the antigen-binding site sequences would not tend to bind to matching (i.e. identical) antigen sequences. Indeed, some antigens are not even peptides. Instead, there needs to be molecular complementarity between the two partners in a binding interaction. However, there is no simple rule for predicting complementarity. So, identical matching is being used as a proxy for the more biologically accurate event—molecular recognition between antigens and antigen-binding sites. Those TCRs with the right amino acid sequence to produce an antigen binding site with high affinity for a particular antigen are said to recognize that antigen.

AAI Curriculum Unit: Understanding Diversity By Tracing T-cell Development

TEACHER MATERIAL

Step 1: Using Appendix II answer the following questions:
What amino acid is represented by the single-letter abbreviations below:

G	Glycine	**P**	Proline	**Y**	Tyrosine
D	Aspartic Acid	**R**	Arginine	**H**	Histidine

Step 2.

Visit the random letter generator provided at the web site
http://www.creighton.edu/~davereed/Nifty/randSeq.html
a. In the box labeled "Letters to choose from" set the alphabet to acdefghiklmnpqrstvwy. (That is, the English alphabet except b, j, o, u, x, and z).
b. In the box labeled "Length of each random letter sequence" enter the number 3.
c. In the box labeled "Number of random letter sequences to generate" enter the number 400.
d. Generate the 400 3-letter sequences.
e. Copy the results produced by the random sequence generator and paste them into a word-processing program.
f. Print the 400 sequences. These will be used in class during Activity II.

Step 3. Repeat Step .2, but this time, in the box labeled "Number of random letter sequences to generate" enter the number 1. Write the result here _____.

Step 4:

Answer the questions below.

Question 1: Why were the letters b, j, o, u x, y and z not included in the "letter to choose from" field in step 2a above?
Because there none of the standard twenty amino acids has a one-letter abbreviation using any of these letters.

Question 2: If each student uses the random letter generator independently, will any two students be likely to come up with identical sets of the same four hundred three-letter words? If so, why? If not, why not?
No each student is expected to have a unique set of sequences. Since there are 8000 possible three-letter sequences, there are many sets of 400 that can be made by selecting from this pool. It would be extraordinarily unlikely that two would happen to produce the same set of 400. One way to assure yourself of this is to look at Appendix I. Notice the appendix includes fifty sets of sequences and each set is different from every other.

Question 3: If each student uses the random letter generator independently, will any two students be likely to come up with any of the same three-letter words? If so, why? If not, why not? (Read this carefully, it is not the same as the question above).
Yes, it is expected that the same three-letter word will come up more than once. This is becauseeach student has four hundred opportunities to find a match with a classmate and presumably, there are many classmates. So there are many opportunities for matches. One way to convince yourself this is the case is to look at Appendix I. You can see that "mdn" is the first sequence in the first set. The sequence "mdn" also occurs on the last line of the 36th set. To further convince yourself, look at the next few sequences from the first set: yqf occurs twice in the fifty sets. "ntg" occurs three times and hyy occurs six times.

Question 4: Write down the first four triplets in your set of 400 using the full name for the amino acids, not the single letter abbreviations. For example, if you obtained the triplet "aar" write "alanine-alanine-arginine."
methionine-aspartic acid-asparagine tyrosine-glutamine-phenylalanine
asparigine-threonine-glycine histidine-tyrosine-tyrosine

Question 5: Why is the use of a sequence of only three amino acids a simplification of the more complex biological reality? Explain.
Because the three-letter sequences is much shorter than the biological reality. In reality, a TCR is a protein composed of hundreds of amino acids and its antigen binding site might include dozens. We use three here to keep from having an overwhelmingly diverse set of sequences.

Question 6: Why does it make sense to use a random-letter generator in order to derive a diverse set of sequences? Surely, the immune system does not have a random-letter generator. What does happen in the immune system that is analogous to the random-letter generator?
During lymphocyte development various gene segments are joined together to construct TCR and antibody genes. The variety of possible genes that can be formed by this recombinatorial process is large. Also, the proteins produced by these genes have unpredictable combinations of amino acids. Likewise, the random-letter generator puts together letters randomly. This makes random-letter generation an imperfect, but useful analog for genetic recombination.

Question 7: You may recall that triplets of RNA nucleotides (called codons) are used to specify the amino acids incorporated in proteins during translation. Are the three-letter sequences used here the same? Explain. What are the three-letter sequences here representing?
Absolutely not. The three-letter sequences here represent tri-peptides. The three letter codons of mRNAs specify the amino acid sequences of proteins, but it would take a nucleic acid sequence of nine nucleotides to specify the three amino acid sequence represented by the tri-peptide three-letter sequences used here.

Question 8: Scan your set of 400 sequences (generated in step 2) to see whether any of the four hundred matches the one sequence generated in step 3. Did you find a perfect match? Yes No (circle one). Either answer is possible.

Question 9: Should you have expected to find a match? If so, why? If not, why not?
No. There are 8000 possible tri-peptides. In a set of 400 it is unlikely (although by no means impossible) that any one of the 8000 would be found.

Question 10: A person with a healthy immune system possesses millions of different T-cells. The crucial difference between these different T-cells is that each possesses a different TCR and so recognizes different antigens. Over a person life, their immune system is likely to encounter only hundreds, or perhaps, a few thousand different pathogens. Given this, would a person with a healthy immune system probably have a T-cell that specifically recognizes each pathogen encountered in a lifetime? Explain.
Remember, the immune system generates an enormously diverse populaition of T-cells. Of course, most of the T-cells that are made are lost during positively selected or negative selection. Still, the number different mature T-cells is estimated to be of the order of tens of millions or more. So, it is likely that there will be a TCR capable of recognizing antigen from almost any of the thousands of possible pathogen. Although it is possible an antigen will exist for which no TCR is a good match, the sheer diversity of the TCR repertoire makes this unlikely.

Understanding Immunity By Tracing Thymocyte Development
Activity II: The Thymocyte Game of Life
Complete Rules

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The Object: The object of the game is to be the first player to recognize an invading pathogen and undergo T-cell activation. By playing this game you will be challenge to demonstrate understanding of the generation of immune diversity, negative selection, positive selection and T-cell stimulation by antigen. Before you begin this activity you will need to have carefully read the scientific background section of this unit.

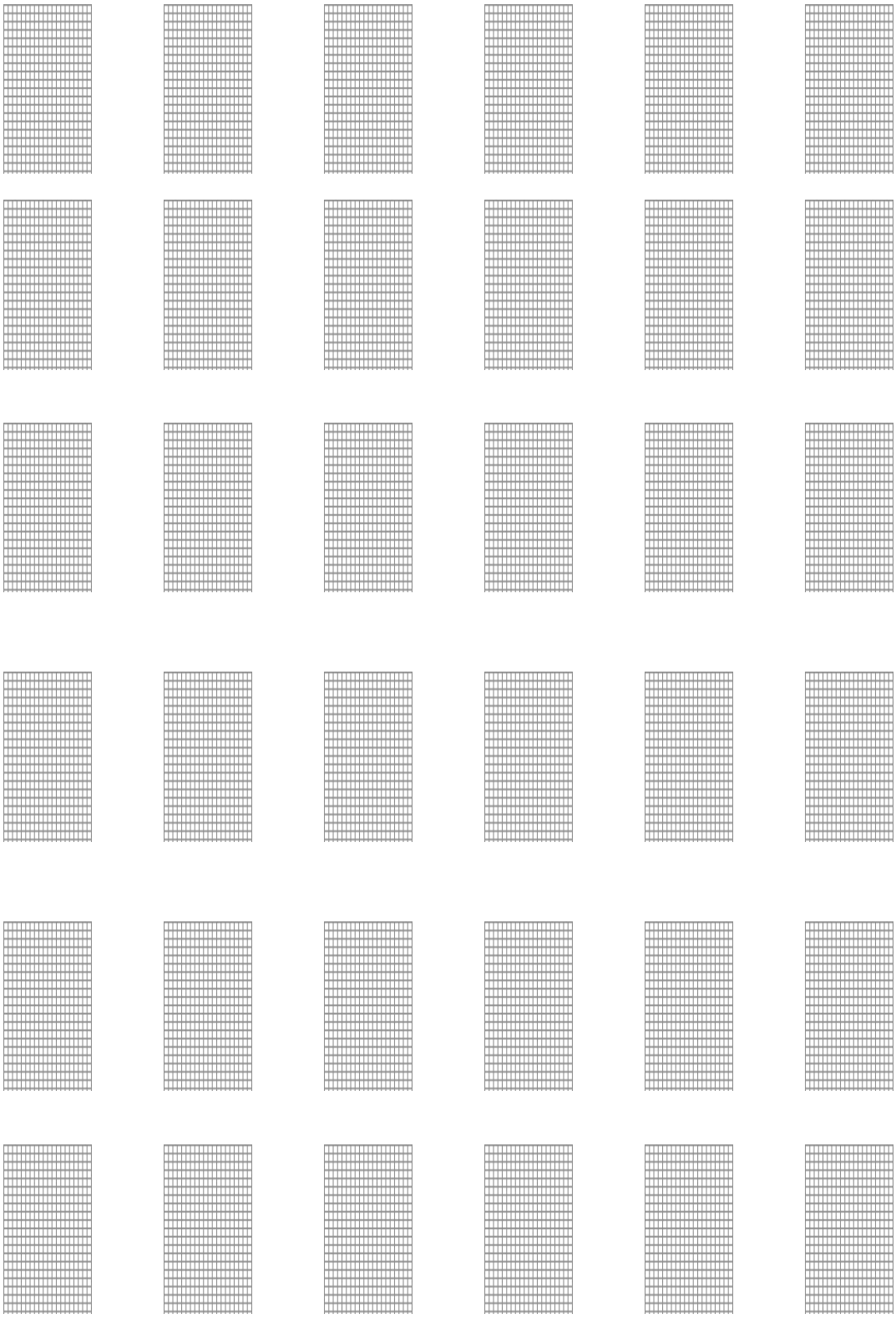
Materials Needed:
Game Pieces
Dice (one pair of six-sided dice for each group of students)
Game Board
Scientific Background Document
Random-letter generator or Appendix I
Random-letter generator or Scrabble® tiles

Note: It will probably be best if there are no more than six to eight game pieces on any one board, so students may be assigned to teams, and/or more than one board may be used.

How to Play: Play begins by players cutting out game pieces and labeling them with their names. Each game piece contains 400 squares in a grid. The game piece represents your repertoire of immature thymocytes. To win the game you must successfully overcome five key challenges in the life of a thymocyte. Each of these challenges is set apart on a different part of the board. To move from one part of the board to another, you roll a pair dice and move the corresponding number of spaces. If you roll more than the required number, you may stop your movement at the desired location. The board permits movement freely in several directions. You must start at the space labled "start" (in the bone marrow) and complete the challenges on the spaces marked with a bold double-rectangle. Each player is responsible for choosing the correct path in order to complete each of the five challenges of T-cell maturation in the proper sequence.

Game Pieces:

Each grid contains 400 quadrants. Each of these 400 quadrantss can be thought of as individual thymocytes in your immune repertoire. You should cut out one grid, write your name on it and use it as your game piece.



The Five Challenges: The requirement for each challenge is slightly different. Each challenge has its own set of rules. In order to complete a challenge you must land your game piece on the specially marked spaces. These special spaces are marked "Start", "Thymic Cortex", "Cortico-medullary Junction", "Travel to the Periphery", and "Lymph Node".

The Thymic Cortex 1 -- The Generation of Immune Diversity:
Each player begins the game in the bone marrow because this is where the precursors of T-cells are first formed. In the bone marrow, hematopoietic stem cells divide to give rise to T-cell precursors, called thymocytes. Later, in the thymus, thymocytes undergo somatic genetic recombination to produce a highly diverse population of cells. The thing that makes this population of cells so diverse is that the vagaries of recombination cause different cells to have different TCR genes. The three-letter sequences, generated in Activity I, represent the different antigen-binding sites of the TCRs. These TCRs are found on the surface of the thymocytes produce in the bone marrow. **In order to proceed to the second challenge in the thymic cortex, you must:**

1. **Complete Activity I, and thus, have a set of 400 hundred three-letter sequences, and;**
2. **Explain what your set of three letter sequences represent.**
3. **Correctly describe roughly how many different types of thymocytes the human body can produce through genetic recombination.**

The Thymic Cortex 2 —Positive Selection:
Before thymocytes can be of use they must be able to interact with other cells in the body. One interaction of particular importance is the binding of T-cell receptors to "major histocompatibility complex" (MHC) proteins. Only those thymocytes that are capable of performing this interaction are able to mature further. The process by which thymocytes are signaled to mature by engaging the MHC of thymic cortex cells is called positive selection. All the cells that are not positively selected for their ability to engage MHC, quickly die. The death of these cells is sometimes called "death by neglect" and is accomplished through apoptosis. Positive selection and death by neglect ensure that all mature T-cells have TCRs that can interact with the MHC. Typically, only about 10% of the thymocytes generated in the bone marrow succeed in being positively selected. The remaining cells succumb to death by neglect. To simulate this, each player must select one amino acid from the list of 20 (acdefghiklmnpqrstvwy). Selecting this amino acid can be easily accomplished by using the random-letter generator, setting the length of the sequence to one. (See Activity I). Having selected one amino acid you must now cross out all three-letters sequences (in your set of 400 sequences) that do not include the selected amino acid. For example, if the selected amino acid were alanine the three-letter sequence "gci" or "kde" would have to be stricken from the repertoire but the sequence "nna" would not. This process should result in about 90% of the three-letter sequences being deleted from the repertoire. The three-letter sequences that did happen to possess the selected amino acid survive. They have succeeded in passing the challenge of positive selection and can continue to mature. The cells remaining in the repertoire at this stage are said to be MHC-restricted.

In order to leave the thymic cortex you must:
1. Reduce your thymocyte repertoire through positive selection.
2. Explain the purpose of positive selection.

The Cortico-medullary Junction-- Negative Selection:
The MHC-restricted thymocyte repertoire must be screened yet again. This time the thymocytes that need to be eliminated are those that happen to possess TCRs that interact too strongly with self. The way we simulate this is by scanning the remaining three-letter sequences for any that spell actual words in the English language. For example, if your MHC-restricted repertoire possesses three-letter sequence, such as, dad, age, egg, or pee. These would have to be crossed out. These sequences represent those TCRs that would recognize self. Failure to eliminate all such thymocytes could result in auto-immune disease.

In order to leave the Cortico-medullary Junction you must:
1. Reduce your thymocyte repertoire through negative selection.
2. Explain the purpose of negative selection

Travel to the Periphery:
After completing positive and negative selection thymocytes are now called T-cells. These cells are mature but are so-called "naïve." These T-cells migrate through the medulla of the thymus and enter blood vessels. T-cells move out to all parts of the body where they enter the bodies tissues and recirculate to the blood via the lymphatic system. There is no special challenge at this stage of the game, just moving from the thymus to the blood to the lymph.

In order to exit the thymus and travel to the periphery you must:
1. Simply move your game piece.
2. Explain what would happen if an immature thymocyte traveled directly to the periphery without first undergoing maturation in the thymus.

The Lymph Node-- Antigen Recognition and T-cell Activation:
It is within lymph nodes (and other secondary lymphoid tissues) that T-cells typically encounter pathogens. The challenge at this stage is, in a sense, a waiting game. It is quite possible that a particular T-cell will live its entire life without ever encountering a pathogen whose antigen has just the right properties to be recognized. Indeed, almost all T-cells will live dormant lives, never being called to actually do anything. However, we are constantly encountering pathogens so at any given time there will be at least some small fraction of the T-cell repertoire that is actively toiling to protect the body. When a T-cell's TCR binds tightly to an MHC presenting the right antigen, this binding helps trigger activation of this T-cell. To simulate this process, set up the random-letter generator as follows:

Visit the random letter generator provided at the web site
http://www.creighton.edu/~davereed/Nifty/randSeq.html
a. In the box labeled "Letters to choose from" set the alphabet to acdefghiklmnpqrstvwy. (That is, the English alphabet except b, j, o, u, x, and z).
b. In the box labeled "Length of each random letter sequence" enter 2.
c. In the box labeled "Number of random letter sequences to generate" enter 1.
d. Generate the three-letter sequence.

e. Compare the three-letter sequence generated in step d above with all the three-letter sequences that have survived positive and negative selection.[1]

The first player to successfully match a three-letter sequence in his or her mature T-cell repertoire with the two-letter sequence generated in step d, above, is the winner of the game. If there are no matches, repeat the steps above until a match is found. In order to have a match between the three-letter TCR sequence and the two-letter antigen sequence their can be no gaps in the matching sequence. So, for example, aga would be a match for the two letter sequences "ag" and "ga" but not for "aa."

In order to win you must achieve T-cell activation. To do this you must
1. Match one of the three-letter sequences in your mature T-cell repertoire with one of the two-letter sequences representing an antigen.
2. Describe the additional developmental events that are triggered by T-cell activation.
3. Briefly explain how a differentiated T-cell, such as a cytotoxic T-cell, helps rid the body of pathogens.

[1] If using the random-letter generator is inconvenient, it is simple to generate a small number of random-letter sequences by pulling letters from a bag. Scrabble tiles work well.

Activity II: The Thymocyte Game of Life
Concise Rules

The Object: The object of the game is to be the first player to recognize an invading pathogen and undergo T-cell activation.

How to Play: To win the game you must successfully overcome five key challenges in the life of a thymocyte. You roll a pair dice to move your game piece. You must complete the challenges of T-cell maturation in the proper sequence. You must start at the space labeled "start" and complete the challenges on the spaces marked with a bold double-rectangle.

The Five Challenges: **After leaving the bone marrow, you must move through the board completing the following challenges:**

The Thymic Cortex 1—Generating A Diverse Thymocyte Repertoire

1. Complete Activity I, and thus, have a set of hundreds of three-letter sequences;
2. Explain what your set of three-letter sequences represent; and
3. Correctly describe roughly how many different types of thymocytes the body can produce through genetic recombination.

The Thymic Cortex 2-- Positive Selection:

In order to leave the thymic cortex you must:
1. Reduce your thymocyte repertoire through positive selection.
2. Explain the purpose of positive selection.

The Cortico-medullary Junction-- Negative Selection:

In order to leave the Cortico-medullary Junction you must:
1. Reduce your thymocyte repertoire through negative selection.
2. Explain the purpose of negative selection

Travel to the Periphery--

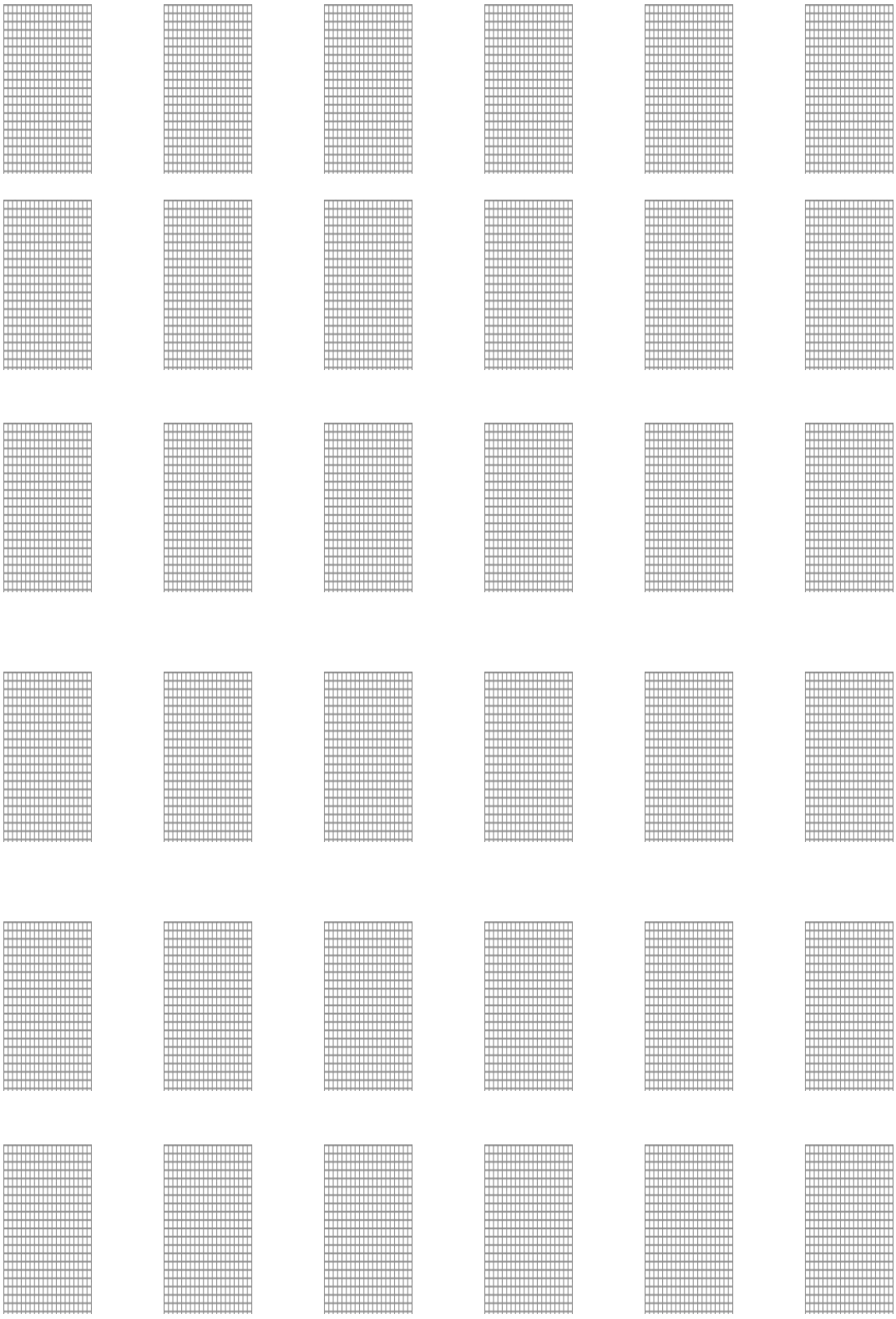
In order to exit the thymus and travel to the periphery you must:
1. Simply move your game piece.
2. Explain what would happen if an immature thymocyte traveled directly to the periphery without first undergoing maturation in the thymus.

The Lymph Node-- Antigen Recognition and T-cell Activation: In order to win you must achieve T-cell activation. To do this you must

1. Match one of the three-letter sequences in your mature T-cell repertoire with one of the two-letter sequences representing an antigen.
2. Describe the additional developmental events that are triggered by T-cell activation.
3. Briefly explain how a differentiated T-cell, such as a cytotoxic T-cell, can help rid the body of pathogens.

Game Pieces:

Each grid contains 400 quadrants. Each of these 400 quadrantss can be thought of as individual thymocytes in your immune repertoire. You should cut out one grid, write your name on it and use it as your game piece.



Activity III: Why do Mature T-cells Respond To stimulation Differently From Immature Cells?

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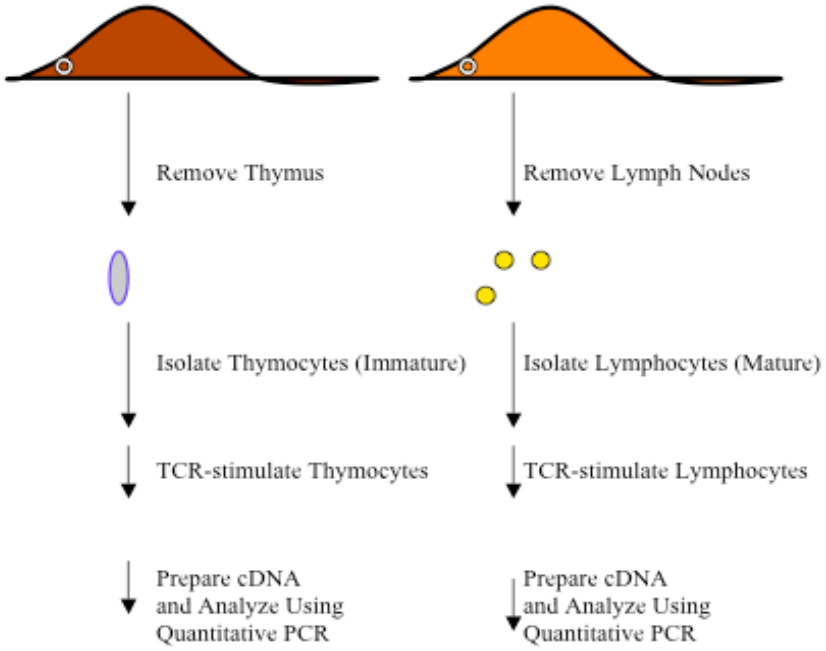
In studying the scientific background section, you read that TCR-stimulation of immature thymocytes causes those thymocytes to undergo apoptosis, yet, the very same stimulation, will, cause a mature T-cell to proliferate and differentiate. What could be more unexpected—in one case stimulation caused a cell to kill itself and in another case, causes the cell to create many copies of itself. Of course, this is exactly what is needed for the immune system to function well. If a mature T-cell experiences TCR-stimulation it is likely the T-cell has recognized a pathogen-derived antigen. It makes sense that this very cell would make many copies of itself. That would give the body a whole arsenal of cells that recognize a known invader. In contrast, an immature thymocyte is likely to be stimulated by host-derived antigen. So, in this circumstance, it makes sense that the cell kill itself. A self-reactive T-cell would have only caused auto-immune trouble if allowed to survived to prowl the periphery.

Just because these cells are responding in ways that make sense, functionally, the question remains. How do these cell know how to do the right thing? Dr. Punt, and others, have wondered how T-cell maturation could cause it to respond so differently to stimulation. What is different about the mature T-cell? How has the apoptosis signal been cancelled? How does the signal to differentiate and proliferate get communicated?

Something must have changed within the immature cell to account for such radical change in the way it responds to TCR binding. The full details of this change are not yet known. Instead, this question is being actively investigated. in Dr. Punt's laboratory, and others. One reasonable hypothesis is that the molecules that convey signals from the cell surface differ in some important way in mature verses immature thymocytes. Several such signal-transduction molecules are being investigated for their role in triggering apoptosis and/or proliferation. One molecule of special interest is Nur77. Nur77 is a steroid receptor that acts as a transcription factor—a protein that triggers the transcription of genes. When Nur77 is over-expressed in an immature thymocyte, the cell undergoes apoptosis. It has also been shown that expression of Nur77 in transgenic mice results in a sharp increase in the quantity of several mRNAs. Since Nur77 is a transcription factor this is exactly what one would expect. It may be that the proteins encoded by these mRNAs are important in causing apoptosis.

One way of examining the importance of transcription to apoptosis is to purify the mRNAs from mature and immature cells after each has had its TCRs stimulated by binding to antigen-like molecules. If some mRNAs are highly expressed in immature cells but not in mature cells, these mRNAs may be involved in bringing about apoptosis.

Figure 1: Investigating Gene Expression in Thymocytes at Different Developmental Stages



Together with Dr. Punt, I investigated differences in the quantities of various mRNAs in TCR-stimulated mature T-cells compared with TCR-stimulated immature cells. Activity III presents some of the data obtained in this investigation. It is intriguing to consider that changes in the abundance of certain mRNAs might lead to the radically different responses of T-cells to stimulation at different points in their maturation.

One technique that is used to examine this is called "Quantititative RT-PCR." See Figure 1. The first step in this process is to isolate immature and mature thymocytes. Since mature T-cells exit the thymus and congregate in lymph nodes, it is possible to isolate mature T-cells from lymph nodes. Likewise, isolating immature thymocytes is fairly straightforward, since these cells are localized to the thymus. Once these two types of cells have been isolated they are exposed to molecules that simulate TCR binding partners. This is called "stimulation." Shortly after the two types of cells have been stimulated they are broken open and their mRNA is purified. The mRNA is fragile and so is reverse transcribed to produce a hardy DNA copy, called complementary DNA (or "cDNA"). This DNA is then amplified using the polymerase chain reaction. If, in fact, stimulation of immature thymocytes caused in increase in the transcription of certain genes, there should be more of the mRNA for these genes in the immature cell RNA preparations than the mature T-cell preparations. Consequently, there should be more DNA for these genes in the cDNA preparations. If so, amplification of these cDNA preparations should reveal DNA being produced by PCR in greater quantity in the immature cells than in the mature cells.

One way of measuring the quantity of DNA produced in a PCR experiment is to separate the DNA on a gel. This technique, called gel electrophoresis, causes DNA molecules of different sizes to migrate to different positions on the gel. The smallest molecules find it relatively each to squeeze through the gel matrix, and so, migrate the farthest. The larger molecules migrate more slowly.

Below is a image of the electrophoretically separated DNA molecules produced by the quantitative RT-PCR of three genes: actin, NDG1, and TCF4. Answer the question at the bottom of the figure legend—What do these data suggest about the relative abundance of the three mRNAs (actin, TCF4 and NDG1) in mature verses immature TCR-stimulated cells?

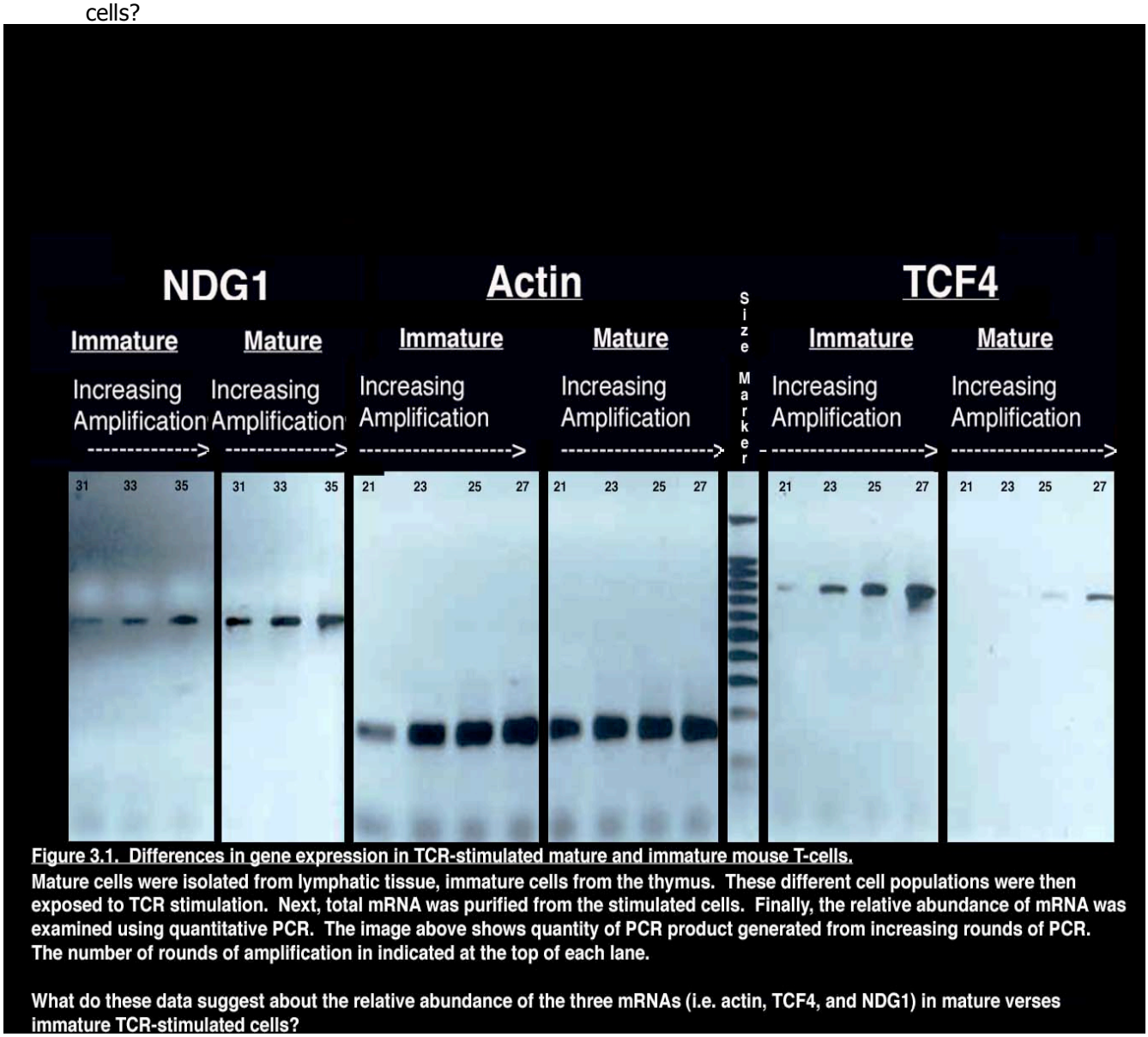


Figure 3.1. Differences in gene expression in TCR-stimulated mature and immature mouse T-cells.
Mature cells were isolated from lymphatic tissue, immature cells from the thymus. These different cell populations were then exposed to TCR stimulation. Next, total mRNA was purified from the stimulated cells. Finally, the relative abundance of mRNA was examined using quantitative PCR. The image above shows quantity of PCR product generated from increasing rounds of PCR. The number of rounds of amplification in indicated at the top of each lane.

What do these data suggest about the relative abundance of the three mRNAs (i.e. actin, TCF4, and NDG1) in mature verses immature TCR-stimulated cells?

Understanding Immunity By Tracing Thymocyte Development

Introduction, Acknowledgements and Scientific Background

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I. Introduction

This curriculum unit is intended as a resource for educators teaching immunological concepts to high school students. The unit is likely to be most appropriate for use in an advanced course, such as advanced placement biology, or for students with a special interest in the immune system. This unit consists of three related activities. Activity I uses a random-letter generator to simulate the generation of immunological diversity. Activity I is intended to be completed as a homework assignment in preparation for Activity II. At the conclusion of Activity I each student has created a set of letter sequences that represent a population of hundreds of genetically distinct thymocytes. Activity II is a board-game in which each player steers this population of thymocytes through their essential developmental stages. The first player to complete these stages and recognize antigen wins. Activity III presents students with a set of data on the abundance of different mRNAs in mature and immature thymocytes. Activity III challenges students to analyze primary data in order to understand the molecular basis for differences in thymocyte response upon stimulation by antigen. Note: **Each student will need a copy of the scientific background section of this document in order to prepare for each of the three activities.**

A. Learning Objectives:

The goal of this unit is to help students understand the following:

1. The immune system plays a fundamental role in everyday life.
2. Infectious disease has tremendous global impact on human health.
3. Advances in our understanding of the immune system may have enormous practical importance, specifically, on public health and the treatment of disease, including:
    a. autoimmune disease
    b. infectious disease
    c. cancer
    d. tissue/organ transplant tolerance.
4. Although scientists have learned a great deal about immune function, the process of scientific inquiry is ongoing. Even as we answer some of the fundamental questions about immune systems, additional questions continually arise.
5. The following fundamental insights into immune system function
    a. adaptive immunity is made possible when somatic genetic recombination gives rise to diverse repertoires of B and T lymphocytes.
    b. specificity in the immune system is made possible by the complementarity of antigens for antigen-binding sites
    c. T-cells recognize antigens that are cradled in a presenting MHC molecule.
    d. the T-cell repertoire is a subset of the thymocyte repertoire. Positive selection ensures that mature T-cells can recognize the MHC.
    e. self-tolerance is achieved through apoptotic purging of self-reactive lymphocytes.
6. Each mature, differentiated cell in a multicellular animal is the product of a particular developmental pathway.
7. Development is regulated both by intracellular events (such as changes in gene expression) and by external cues (such as stimulation of cell-surface receptors by ligands).

B. Important Prior Understandings

Students should already be familiar with the following concepts:

1. Genes encode the amino acid sequences of proteins.
2. Proteins are crucial participants in nearly all biological phenomena. They are, by and large, the actors that perform the molecular events that make life possible.
3. A protein is an amino acid polymer. The sequence of amino acids in the polymer determines the shape and other properties of the protein.
4. The human genome contains about 25,000 protein-coding genes but not all genes are in use in all cells at all times. Instead, genes must be expressed. Gene expression is a regulated process.
5. Bacteria, viruses, and other pathogens cause disease in humans and other organisms.
6. Mammals have several non-specific defenses that complement the specific defenses of the immune system.
7. Eukaryotic cells usually possess receptors on their surfaces. These receptors help relay information about the cell's surroundings to the cell interior.

II. Acknowledgements

This unit was made possible by the generous support of the American Association of Immunologists (AAI). During the summer of 2004, the author was the recipient of an AAI John H. Wallace Fellowship. This fellowship enabled him to spend six weeks performing hands-on research in immunology in Dr. Jennifer Punt's laboratory at Haverford College in Haverford, Pennsylvania. Dr. Punt was an inspiring teacher and a generous mentor.

Understanding Immunity By Tracing Thymocyte Development
Scientific Background

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A. An Overview of Immune Function:

Even in a clean home, viruses, bacteria, fungi and other disease-causing organisms (called "**pathogens**") pose a constant threat of infection. Sadly, pathogens typically kill about 15 million people each year. This is more than one quarter of all deaths worldwide. Indeed, more than twice as many people die each year from infectious disease as from all forms of cancer combined. Recent estimates place infectious disease at the very top of the list of causes of death worldwide. Still, mortality from infectious disease would be very much greater if people did not possess built-in systems for defending themselves from specific pathogens. For most people, pathogens are eliminated not by taking medicine, but instead, by the functioning of their body's own defenses, such as their immune system.

Day after day each of our immune systems engage in silent, invisible battles against an unending assault by invading organisms. In addition, the immune system protects the body from cancer by helping destroy precancerous or cancerous cells in the body. Each day that you experience health is a day that your immune system has prevailed in its battles. After each successful battle, the war continues each day of our lives. If you have lived a life free of disease, your immune system is surely part of the reason.

In contrast, if you suffer from a weakened immune system, everyday battles against infection or cancer are more likely to be lost. One clear illustration of this fact is the great number of people suffering from **acquired immune deficiency syndrome** ("AIDS") who succumb to otherwise controllable infections (such as pneumonia, or tuberculosis) or cancers (such as Kaposi sarcoma). Typically, **human immunodeficiency virus** ("HIV") does not cause much direct bodily harm. Instead, by disabling immune cells, HIV infection makes it much easier for **opportunistic infections** to thrive.

Although it is important to have a robust immune system, an over-aggressive system can also lead to serious illness. Our immune systems must be able to properly distinguish between our own healthy cells, and harmful invaders. The failure to properly draw this distinction can result in immune cells attacking our own tissues. This sort of self-directed attack, analogous to "friendly fire" on the battlefield, is the cause of **autoimmune diseases**. There are dozens of autoimmune diseases, including Type I diabetes, lupus, arthritis, multiple sclerosis and psoriasis.

In addition to having a system that is robust in the face of invaders, but does not harm our own cells, it would be advantageous to many if their immune system tolerated the presence of a transplant. Currently, patients undergoing transplants risk **transplant rejection**; that is, they might lose the benefit of the transplants because their immune system attacks it. A common approach to this problem is to administer medication that suppresses the transplant-recipient's immune system. Unfortunately, while this reduces the risk of transplant rejection, it simultaneously increases the risks associated with a weakened immune system.

In summary, the immune system helps protect our bodies from infectious disease and cancer but it also causes autoimmune disease and transplant rejection. **Immunologists**, the scientists who study the immune system, hope that by gaining a better understanding of the immune system, improvements can be made in our prevention and treatment of disease.

By understanding the way our immune systems function, we will be better prepared to address infectious disease, autoimmunity, cancer and transplant rejection. Unfortunately, the complexity of the immune system makes it difficult to understand. The approach taken in this curriculum unit is to follow the development of **thymocytes** and **T-lymphocytes**. In the course of tracing the various possible developmental fates of thymocytes, this unit will explain how our immune system is capable of recognizing such a diverse set of invading organisms. Furthermore, it will teach how **self-tolerance** is achieved and how **differential gene expression** may cause mature T-cells to responding to stimulation very differently from immature thymocytes.

B. The Generation of Immunological Diversity Involves Genetic Recombination:

The immune system can be divided into two components: **innate immunity** and **adaptive immunit**y. The innate system is good at reacting to certain invaders with a generalized response. For example, any of dozens of different bacterial species can trigger the same generic response (such as the complement cascade) because many bacterial species possess the same cell-wall components widely recognized by the same complement proteins.

However, the adaptive system is capable of much greater specificity. Regardless of the invader, the adaptive immune system provides a specially tailored army of cells for responding to the particular invader. These specially tailored armies are specific because the cells that make them up possess proteins on their surface that can vary tremendously. Each different surface protein will bind to a different foreign substance. These foreign substances are called **antigens**. Most often, antigens are small pieces of a pathogen. In mammalian immune systems, antigens are usually fragments of a pathogen's proteins. Some cells, such as **macrophages** are capable of engulfing pathogens, breaking the pathogens apart and then displaying the resulting fragments (i.e. antigens) on their surfaces. When a macrophage does this, it becomes an "**antigen-presenting cell**." Most antigen presenting cells display a protein fragment of nine, ten, or eleven amino acids in length.

Some of the most important immune system components are also proteins. Of particular importance are **antibodies** and T-cell receptors ("**TCRs**"). Antibodies are proteins that are secreted from certain immune cells (called **plasma cells**) and which bind antigens in the blood stream or other bodily fluid. Similarly, TCRs, which are structurally similar to antibodies, are found on the surface of T-cells. Antibodies and TCRs both consist of hundreds of amino acids. However, only a small subset of the amino acids of these proteins are directly involved in binding to antigens. This small sub-region of the antibody or TCR is called the **antigen-binding site**.

Regardless of whether an invader is any of hundreds of different viruses, dozens of different bacteria or dozens of different eukaryotic parasites, the adaptive immune system is able to mount a response specific to that particular invader. If you pause to consider that pathogens are rapidly and continuously being altered by evolutionary processes, the specificity of the adaptive immune system can be seen to be even more remarkable.

How is it that our bodies can achieve specific responses to so many different and evolving invaders? Is it that we are each born with thousands of immunity genes capable of encoding thousands of different defenders? No! As a matter of fact, the number of genes associated with immunological diversity is far too small for this approach to work. Indeed, it has been found that the generations of copious immunological diversity can be accomplished using less than two thousand genes or gene segments. So, the question then is, how does the body generate a repertoire of millions of genetically different immune cell lineages without having millions of different immunity genes?

The answer is "**somatic cell genetic recombination**." Somatic cell genetic recombination is a bit like playing cards. Although there are only 52 different cards in a deck, there are 52^5 = 380,204,032 possible 5-card sequences![1] Similarly, although there are only about two thousand immune system gene segments in a mammalian genome, there are millions of gene products that can be made by joining gene segments in different combinations. These different combinations ensure there will be a diverse repertoire of antibodies and TCRs each with a different antigen-binding site. Because recombination enables millions of different antigen binding sites to be produced, the number of antigens that can be specifically identified by the immune system is likewise in the millions. The details of recombination are complex and vary from one species to another. Among mammals, the process has been studied well in mice. In a mouse, any of about 300 different "V gene segments" can be combined with any of 4 different J gene segments to make 1200 possible V_L genes. Each of these 1200 possible V_L genes can produce any of 1200 different polypeptides. In addition, each of the V_L polypeptides is partnered with any of 50,000 different V_H polypeptides. These 50,000 possible polypeptides are themselves the result of the genetic recombining of a different set of V, J and D gene segments. (In the case of the production of V_H any of about 12 D gene segments, 4 J gene segments and about 1000 V gene segments combine). When the possible V_L polypeptides are paired with the possible V_H polypeptides, we see there are about 50,000 x 1,200 = 60 million different antibodies or TCRs that can be produced using fewer than 2000 gene segments. In fact, the number of possible antibodies or TCRs is even more than 60 million due to the tendency of the gene-recombining mechanism to add or delete nucleotides at the junctions of gene segments. This process, called **joining diversification,** can add to the diversity of the immune repertoire, brining the number of different antibodies or TCRs above 60 million.

The immune system consists of many different cell types, including macrophages—which ingest and kill pathogens and become antigen presenting cells; **neutrophils**—which release chemicals that kill pathogens and themselves; **natural killer cells**—which puncture and kill infected or cancerous cells; various **B-lymphocytes** ("B-cells") and various **T-lymphocytes** ("T-cells"). Only **B-cells** and **T-cells** perform genetic recombination. In B-cells the recombinant gene encodes an antibody. The antibodies

[1] The number of hands of 5-cards is lower if the deck is not "bottomless" that is, if an ace of spades cannot be dealt more than once. However, applying this limit to the generation of diversity would be inappropriate because each cell that undergoes recombination starts with a full genome. If one cell uses a particular gene segment, nothing prevents a different cell from using the same one. In real poker, the number of possible hands is further reduced because the sequence of cards in a hand does not matter. In poker it makes no difference whether you have the cards "king, ace, ace, ace, ace" or "ace, king, ace, ace, ace." Both hands would be counted as "four aces, king high". However, in the joining of gene segments during somatic genetic recombination, the sequence does matter. So the closest analogy to the biological condition is a bottomless deck of cards being dealt to produce hands where the sequence does confer distinction.

produced by B-cells are presented on the cell surface and, when appropriately stimulated, B-cells can transform to become antibody-secreting cells called **plasma cells**. Secreted antibodies bind to invading pathogens or infected cells marking the pathogens or infected cells for destruction by other immune cells (such as **killer T-cells**). Generic recombination in T-cells produces genes for TCRs. These TCRs remains anchored to the T-cell's plasma membrane and enable the T-cell to recognize antigen.

Thus, the diversity of the immune system consists of the millions of different B-cells, each possessing the ability to release a different type of antibody, and the millions of different T-cells, each possessing a different TCR on its surface. Because recombination can make so many different genes, each TCR gene and each antibody gene is likely to be unique. This uniqueness manifests itself in the shape, electrical charge, hydrophobicity and other properties of the antigen binding site of the antibody or TCR. These protein properties give each antigen-binding site its binding specificity. That is, each different TCR or antibody will only interact strongly with a small number of specific antigens. The specificity of binding of antigens to TCRs or antigens to antibodies is an essential feature of the immune system. The system would not function well if antigen-specific binding did not exist. If locks could be opened by any key, locks and keys would be obsolete. Likewise it would be useless to have antibodies or TCRs that bound to everything. To function properly the immune system must be specific-- able to see dangerous pathogens as threats and able to ignore everything else.

However, it is important to keep in mind that when we say an immune cell "sees" a pathogen as threats we attribute properties to an immune cell which it does not have. Of course, no cell ever truly "sees" anything. They have no eyes and live in permanent darkness. (Very little light penetrates more than a couple of millimeters into our bodies). "Seeing" is a useful metaphor for molecular recognition. It is helpful to remember, however, that it is only a metaphor. It would be more accurate to say an immune cell possesses the appropriate protein sequence to enable strong interaction between antigen binding proteins of that cell and the antigens of the pathogen. The strength of this interaction is the primary basis for the specific recognition that exits in the immune system. Molecular recognition between molecules that bind to each other can also be compared to the fit of a key in its corresponding lock. In order for the interaction between lock and key to be productive, the shape of the key must be the complement of the shape of the lock. Likewise, in biology, it is very often the case that shape, charge and other biochemical properties are complementary to the shape and other properties of the thing bound.

Molecular recognition by a TCR has been well characterized for some antigens. For example, the coat of the influenza virus includes a protein called hemagglutinin. When cells are infected with influenza some of the hemagglutinin is broke into smaller pieces and these pieces are displayed on the surface of certain immune cells, such as macrophages. One fragment of hemagglutinin has the amino acid sequence IYSTVASSL (that is, isoleucine-tyrosine-serine-threonine-valine-alanina-serine-serine-leucine). When this peptide is displayed in the peptide-binding groove of an MHC, those killer T-cells with just the right TCR will bind tightly to the antigen and will destroy the infected cell.

C. Positive Selection Ensures That Mature T-cells Can Bind The MHC:

The life of a T-cell begins in the bone marrow. See Figure 1. In the marrow, hematopoietic stem cells divide to produce precursor cells that are released from the marrow, enter the blood stream, and travel to the thymus. After infiltrating the thymus,

precursor cells localize to the outer part of the thymus, called the thymic cortex. Most of these cells simply die. Indeed, only 5% of thymic precursor cells are successful at overcoming the many challenges of thymocyte development. There are several ways an immature thymocyte can fail to make it through the maturation process. One way is if the thymocyte lacks the ability to effectively bind to a structure called the **major histocompatibility complex**, or **MHC**[2]. The MHC serves as a presenter of small polypeptides, such as antigens. Typically, T-cells can recognize antigen only when the antigen is cradled in a crevice of the MHC. It is the combination of MHC and antigen taken together that trigger recognition in a T-cell. It is the TCR of a T-cell that binds the MHC-antigen complex. If, in the course of generating its TCR, a thymocyte happens to be bind MHC very weekly, the thymocyte will soon die. This is called death by neglect. Approximately 90% of thymocytes undergo death by neglect. These cells were unlucky when TCR gene segments were being recombined. Instead of producing an effective TCR these cells produced TCR that do not have enough affinity for MHC.

The alternative to death by neglect is **positive selection**. If a thymic precursor does bind to the MHC effectively, the cell receives a signal to survive and does not die. These surviving cells have been positively selected for their ability to interact effectively with the MHC. Still, these cells might yet be destroyed by another important process in T-cell development—negative selection!

D. Self-Tolerance Is Achieved Through Negative Selection:

Perhaps you have seen depictions of police officers-in-training shooting, or holding their fire as cut-outs of criminals or unarmed people pop into view. This exercise is designed to sharpen the reflexes of trainees. We want to ensure they are careful enough to avoid shooting children, surrendering criminals, pregnant mothers, etc., yet decisive enough to use sufficient force against a real threat. The immune system must perform a similar kind of discrimination. The immune system must distinguish between the body's own antigens, which immunologists call "self" and the antigens of pathogens. When an organism's immune system does discriminate properly between pathogens and self it is said to be **self-tolerant**. When an organism fails to achieve self-tolerance, the immune system attacks the body's own cells. This is the cause of auto-immune disease.

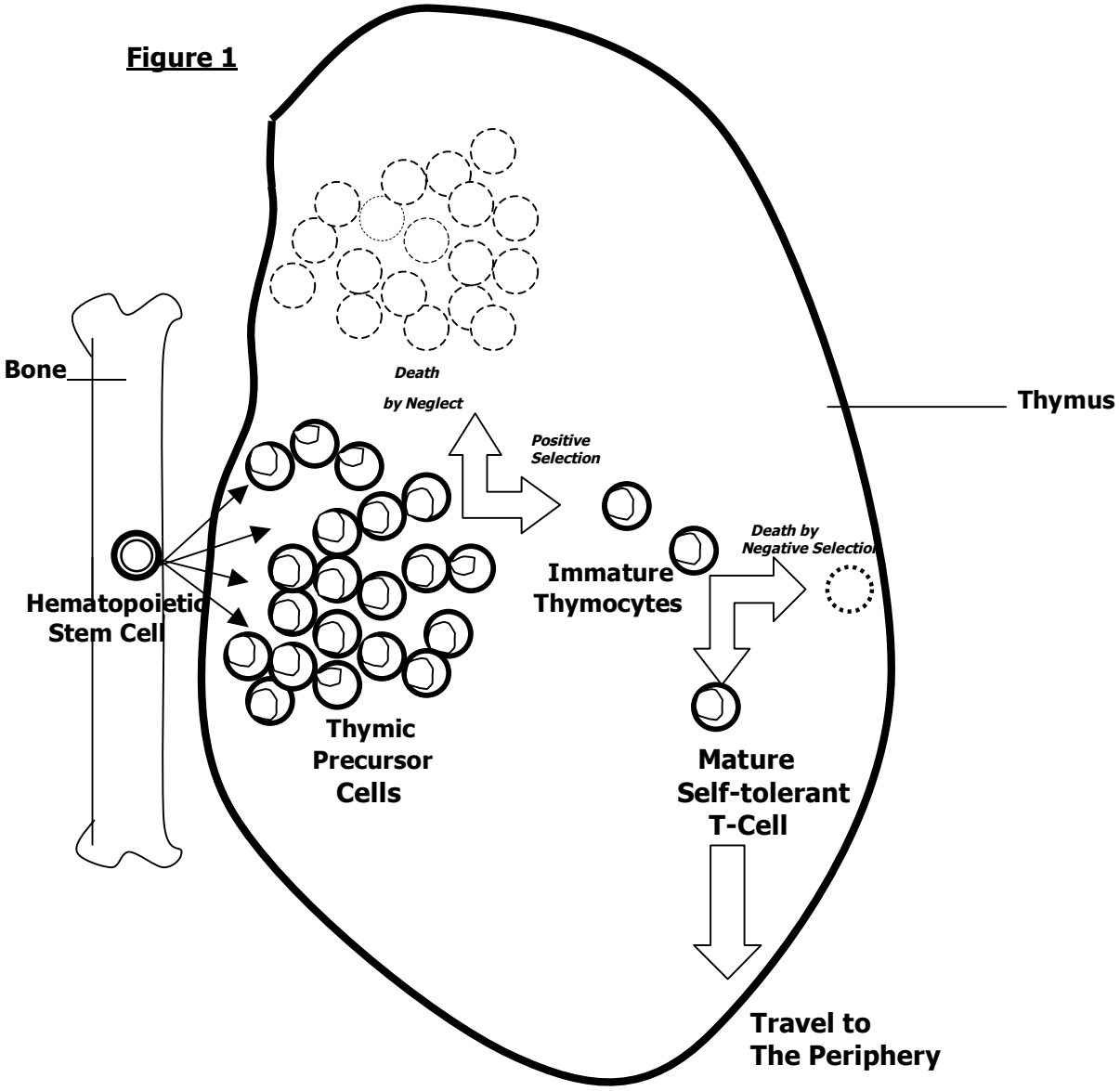
Because the recombining that generates immunologic diversity is somewhat haphazard, there is no guarantee that the immune system will avoid generating antigen-binding sites specific for molecules that are part of one's own body. The immune cells that express such antigen-binding sites are said to be "self-reactive." They experience TCR stimulation in response to the bodies own molecules. Self-reactivity is such a potentially serious problem that an ingenious mechanism exists to prevent it. Before a person is even born, the body begins to destroy developing T-cells that exhibit strong binding to self antigen. This process is called **negative selection.** It is as if the immune system made the assumption that, immature thymocytes have a very small chance of encountering pathogens, but a very good chance of encountering self molecules in the thymus. If a thymocyte does bind self-molecules at this early developmental stage, TCR stimulation causes a series of biochemical reactions that ultimately cause the cell to kill itself. This sort of cellular suicide is called **apoptosis**. Interestingly, a mature T-cell responds in an entirely different manner when it binds to antigens. In this case, instead of killing itself, antigen binding triggers the mature T-cell to rapidly divide, producing numerous clones of itself. This will be discussed in more depth in section F below.

[2] It is more appropriate to apply the term "MHC" to the set of genes that encode the antigen-presenting proteins. In humans, these genes are referred to as human leukocyte antigen (HLA) genes, although people often use the abbreviation "MHC" to refer to HLA gene products, as I do in this document. To avoid confusion some use "Mhc" to refer to the HLA protein molecules and reserve "MHC" for the region of the genome that encodes for this molecule.

Imagine a police academy where the pool of trainees enters with all sorts of bizarre and unalterable personalities. Some always shoot at armed robbers and only armed robbers, some at snipers and only snipers, some at knife-wielding rapists, some at schoolchildren and some at harmless pedestrians. If we expose these trainees to all the various things they might encounter, (such as, robbers, snipers, rapists, children and pedestrians) we can weed out those with an inappropriate specificity. After the homicidal trainees have been weeded out, the protective police can be sent out to patrol. Likewise, after all self-reactive immune cells have been destroyed, a person is said to be "self-tolerant." The immune system of a self-tolerant person should only attack foreign substances.

However, if, for some reason, the process of weeding out self-reactive T-cells is incomplete, surviving self-reactive cells may develop into mature cells. Now, when exposed to antigen (which in this case would be some self molecule) these mature cells proliferate to produce an immune defense against one's own body. This is what happens during certain autoimmune diseases. Likewise, when tissue transplants are performed, the mature immune system of the recipient is full of mature immune cells that proliferate when they encounter transplant antigens. Since the transplanted tissue was not around during immune cell training, the transplant may be attacked as if it were a pathogen. The transplanted tissue could then be damaged or destroyed and the benefit of transplantation lost.

Figure 1

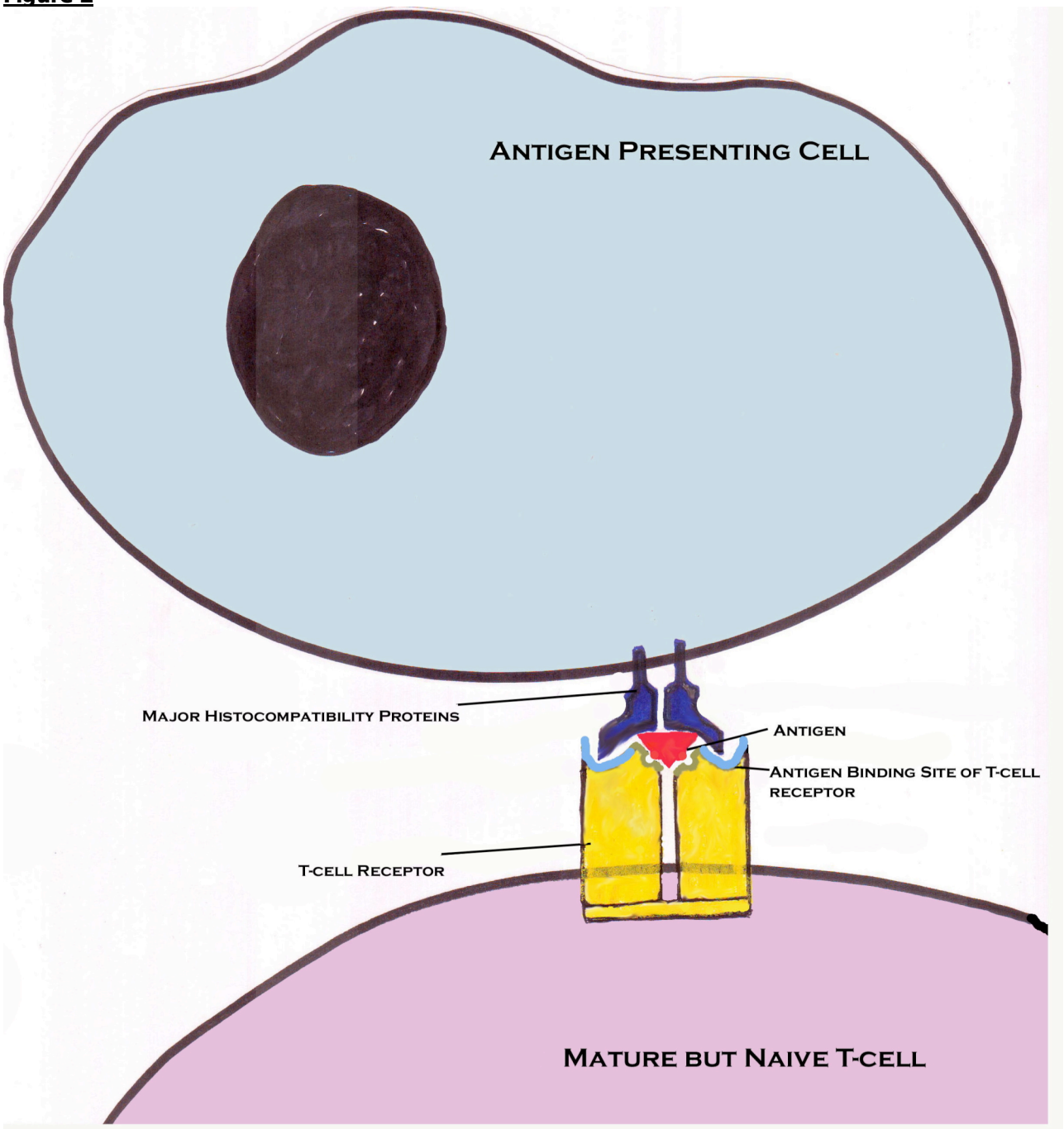

Bone
Death
by Neglect
Thymus
Positive
Selection
Death by
Negative Selection
Hematopoietic
Stem Cell
Immature
Thymocytes
Thymic
Precursor
Cells
Mature
Self-tolerant
T-Cell
Travel to
The Periphery

E. TCR-Stimulation in Mature T-cells Results in T-Cell Activation:

If a T-cell survives the weeding-out processes of positive and negative selection, it will exit the thymus and complete its maturation in the lymphatic system. Most importantly, it is in lymphatic organs that mature T-cell will encounter antigen and undergo activation.

The lymphatic system is an organ system that consists of lymph nodes, lymphatic vessels and the spleen. Fluid and cells that exit the blood stream are recirculated back to the blood by way of the lymphatic system. In addition, lymphatic organs are sites scattered throughout the body that serve as gathering places for immune cells. In a sense, lymphatic organs are where immune cells conduct meetings. When T-cells exit the thymus they are carried to the lymph. The vast majority of T-cells will remain dormant within the lymph. This is because the antigen they happen to recognize is not being displayed by any antigen-presenting cells. However, there will be a tiny fraction of the mature T-cell repertoire that do happen to have the right TCRs to recognize the antigens being displayed in the lymphatic system. If a T-cell does recognize a presented antigen, that cell's TCR will be stimulated and the cell will undergo activation. Upon activation, T-cells can change into various types of effector cells through a process called differentiation. For example, they can become cytotoxic T-cells or helper T-cells. In addition to differentiating, activated T-cells also make copies of themselves—they proliferate. This ensures that there will be many T-cells in the body that are capable of recognizing the particular antigen that happens to be present. Cytotoxic T-cell and helper T-cell are the main effectors of cell-mediated immunity. Helper T-cells help fight infection by activating cytotoxic T-cells, by activating B-cells, and by stimulating the humoral response. Cytotoxic T-cells help fight infection by binding to the body's infected cells and destroying them. This prevents the infection from spreading to uninfected cells. Cytotoxic T-cell may also kill cancer cells.

Figure 2



A T-cell recognizes an antigen. This figure illustrates the most important event in the life of a T-cell-- molecular recognition of antigen by the cell's T-cell receptor. The ability of the antigen binding site of the T-cell receptor to recognize antigen is represented above by the shape complementarity between these molecules. It is this complementarity that is being simulated when, in Activity II, you find a match between the amino acid sequence of the TCR and the amino acid sequence of an antigen. Note how the T-cell receptor has an antigen binding site that fits both the antigen, and the major histocompatibility proteins (MHC) on the surface of the APC. Positive selection in the thymus ensures that only those T-cells who's receptors interact properly with the MHC will mature. The TCR surface interacting with the MHC is colored light-blue. The TCR surface interacting with the antigen is colored light-green. The figure above is a simplified version of T-cell stimulation. Other important participants in T-cell activation, such as CD4, are not shown.

F. TCR Stimulation Produces Entirely Different Responses in Mature T-Cells Compared to Immature Thymocytes:

Negative selection depends on the fact that TCR-stimulation of immature thymocytes causes those thymocytes to undergo apoptosis. Yet, the very same stimulation, will, cause a mature T-cell to survive and even reproduce. Something must have changed within the immature cell to account for such radical change in the way it responds to TCR binding. The full details of this change are not yet known. Instead, this question is being actively investigated, in Dr. Punt's laboratory, among others. One reasonable hypothesis is that the molecules that convey signals from the cell surface differ in some important way in mature verses immature thymocytes. Several such signal-transduction molecules are being investigated for their role in triggering apoptosis and/or proliferation. One molecule of special interest is Nur77. Nur77 is a steroid receptor that acts as a **transcription factor**—a protein that triggers the transcription of genes. When Nur77 is over-expressed in an immature thymocyte, the cell undergoes apoptosis. It has also been shown that expression of Nur77 in transgenic mice results in a sharp increase in the quantity of several mRNAs. Since Nur77 is a transcription factor, this is exactly what one would expect. It may be that the proteins encoded by these mRNAs are important in causing apoptosis.

One way of examining the importance of transcription to apoptosis is to purify the mRNAs from mature and immature cells after each has had its TCRs stimulated by binding to antigen-like molecules. If some mRNAs are highly expressed in immature cells but not in mature cells, these mRNAs may be involved in bringing about apoptosis.

Together with Dr. Punt, and others, I investigated differences in the quantities of various mRNAs in TCR-stimulated mature T-cells compared with TCR-stimulated immature cells. Activity III presents some of the data obtained in this investigation. It is intriguing to consider that changes in the abundance of certain mRNAs might lead to the radically different responses of T-cells to stimulation at different points in their maturation.